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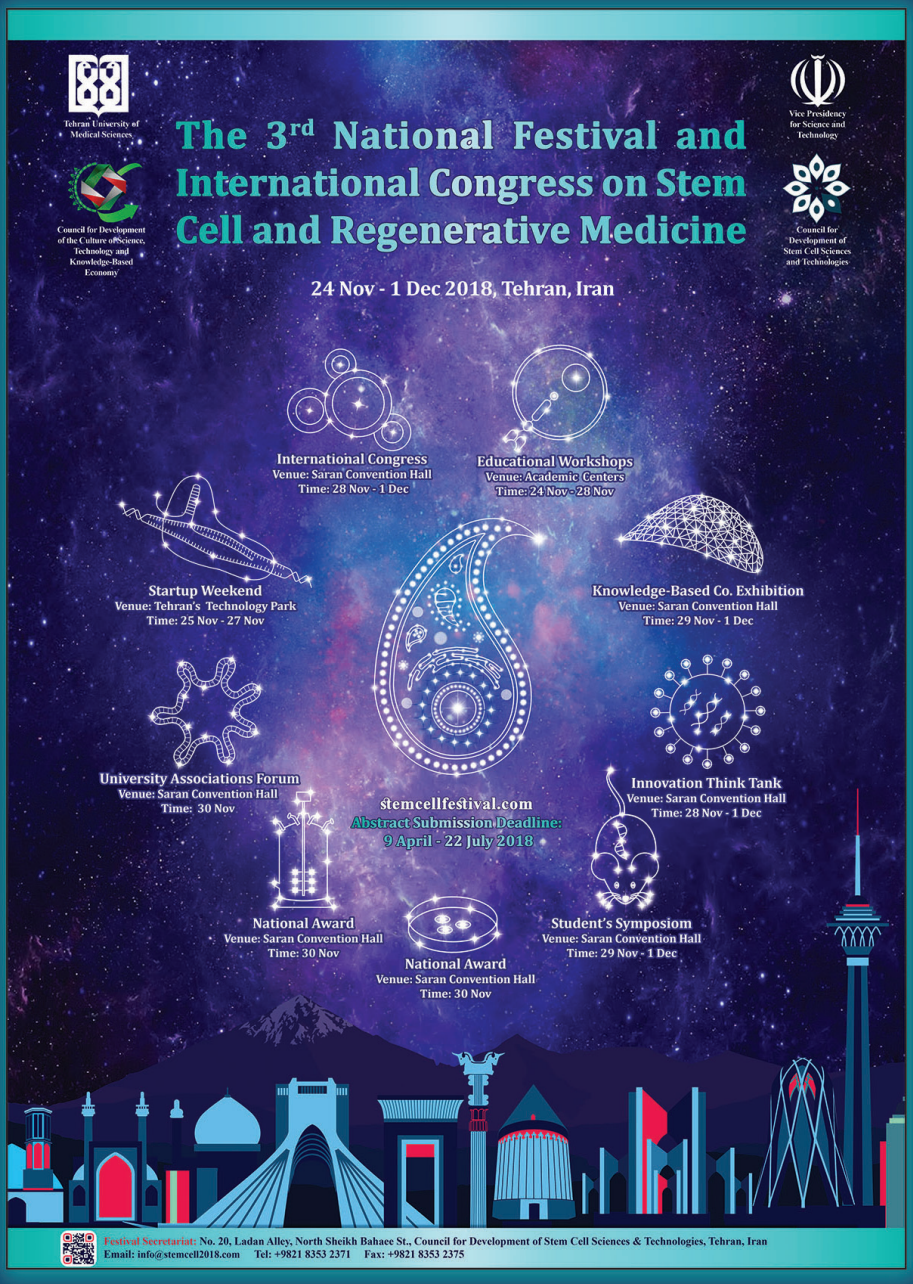
BioImpacts



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"Boteh Jegheh", "Boteh" or "Buta" is an ancient Persian motif, which is known as "Paisley" in western countries.[1,2] This floral design has been used to decorate royal regalia, crowns, court garments, as well as textiles and carpets in Persian culture and heritage throughout centuries.[1] It is generally believed that this pattern is inspired from cypress (or cedar) tree, an ancient Persian symbol of life and eternity. In fact, it is a bent cedar, the evergreen tree that Zarathustra planted in paradise (according to primeval Persian myths, over 3000 years ago). The "bent" cedar is also the sign of strength and resistance but modesty.[2] These attributes remind stem cells, which are evergreen, life-giving, revitalizing and resistant cells in the body.

1. Farrokh S. Transformation of Natural Elements in Persian Art: the Flora. Meio Univ Bull. 2007;13:63-80.
2. Davari N. Iranian Traditional Motif: The Boteh Design. Selangor, Malaysia: Universiti Teknologi Mara; 2009.

Cedar tree in the cravings of the Persepolis

Buta motif on the entrance of a Persian sanctum

The 3rd National Festival & International Congress on Stem Cell & Regenerative Medicine

November 28 – December 01, 2018
I.R. Iran International Conference Center,
Tehran, Iran

Council for Development of Stem Cell Sciences and Technologies

History and Background:

Stem cell research began in the late 1950s, when scientists started looking for treatments to help people with untreatable disorders. Advancement in regenerative medicine, from flaps to tissue engineering have taken place thanks to our knowledge on stem cells. We now know stem cells as the core of tissue engineering.

The ultimate aim of scientists in the field of stem cell research is to be able to build tissues or organs that can replace injured or diseased tissues in the human body. This concept which gives rise to the generation of mature tissues has made adult stem cells the focus of intense research, designed to treat a variety of human diseases.

The history of stem cell research in Iran goes back to the first hematopoietic stem cell transplantation (HSCT) in 1990s. Since 1994, Iranian researchers have published papers in stem cells-related fields in domestic and international journals. By 2004, stem cell studies in Iran were developed to include embryonic stem cell research.

Since early 2005, Iranian researchers have also been engaged in the field of tissue engineering and regenerative medicine. Publishing valuable articles in high ranked international journals in these fields of science, has been a continuous trend among Iranian researchers ever since.

The Council for Stem Cell Sciences and Technologies, affiliated to the Iranian Vice Presidency for Science and Technology was established in February 2009 with the aim of accelerating progress towards stem cell based treatments.

The national document of Stem Cell Sciences and Technologies was also approved as part of the country's national scientific map in September 2013 at the Supreme Council of the Cultural Revolution. Iran's headway towards stem cell sciences and regenerative medicine, despite limited investments, reveals the country's enormous potential to grow in this field.

In terms of published papers in the field of stem cells and regenerative medicine, Islamic Republic is ranked first in the Middle East and among Islamic countries and is second among the East Mediterranean and North African countries.

Stem cell research market in the world has grown exponentially over the last decade. It is hoped that due to this incredible increase in investments in stem cell research, Iran will be one of the world's top 10 countries by 2025 in terms of science and wealth creation in this novel area of research.

Mission

• To improve optimal stem cell mobilization results
• To bring together our internal scientific expertise and our intimate understanding of the fields we support, inventing ways to help leading scientists do their work more efficiently and effectively
• To encourage participation of the private sector, NGOs and foreign investors in the field, with an emphasis on inter-agency cooperation
• To observe the ethical, religious, and social principles for further development of the field
• To keep up with country's 10-year strategic plan by 2025

Vision

• To encourage self-reliance in the production of basic material, laboratory equipment and supplies with the aim of meeting at least 50 percent of the domestic demand
• To enhance national wealth creation through applying stem cells and its products to treat various diseases and to access international stem cell market value
• To obtain the knowledge required in efficient banking of stem cells
• To involve private sector in research, technology development and wealth creation while keeping the policymaking and supervisory role to the government
• To create new knowledge with a view of making our way up to the world's top ten countries in the field, as well as publishing scientific papers in credible international journals
• To engage 700 researchers to the council and to assign them to 25 target-oriented expert committees
• To hold the "annual festival and congress" on stem cells and regenerative medicine
• To set up associations to develop stem cell sciences and regenerative medicine, with universities of medical sciences worldwide

International Cooperation

Regarding international cooperation, the Council for Stem Cell Sciences and Technologies is interested in:
• Collaborating with universities, research centers and companies at the international level to conduct clinical trials in the field of stem cell sciences and regenerative medicine
• Conducting gene therapy studies and clinical trials on genetic disorders such as thalassemia, severe inborn immunodeficiency etc.
• Sharing Expertise in the field of stem cell therapy for cancer, including CAR T-cell therapy

Organizers

Vice-presidency for Science and Technology

Council for Development of Stem Cell Sciences and Technologies

Council for Development of the Culture of Science, Technology and Knowledge-Based Economy

Tehran University of Medical Sciences

Islamic Republic of Iran Ministry of Health and Medical Education

Islamic Republic of Iran Ministry of Science, Research and Technology

Islamic Republic of Iran Ministry of Education

Royan Institute

Shahid Beheshti University of Medical Sciences

Tabriz University of Medical Sciences

Shiraz University of Medical Sciences

Tarbiat Modares University

Sharif University of Technology

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Pasteur Institute of Iran

Kharazmi University

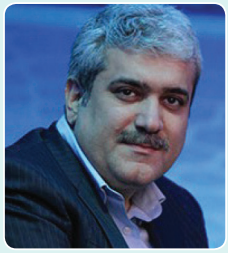
Islamic Azad University

National Institute of Genetic Engineering and Biotechnology

Iran University of Medical Sciences

Amirkabir University of Technology

Kerman University of Medical Sciences



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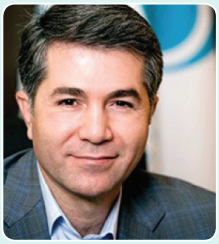
Dr. Sorena Sattari

General Secretary:
Dr. Amir Ali Hamidieh

Administrative Secretary:
Dr. Alireza Daliri

Executive Secretary:
Dr. Mohammad Amir Amirkhani



Scientific Secretary:
Dr. Nasser Aghdami

Chairman Welcome Message

In the Name of God

Development of science and technology is one of the most important policies mentioned by the Supreme Leader, which guarantees country's growth and prosperity.

The Vice Presidency for Science and Technology aims to expand entrepreneurship ecosystem in the field of hi-tech sciences including stem cell sciences and regenerative medicine with focusing on the development and commercialization of ideas and knowledge-based products.

Graduates and researchers are one of the most valuable assets of countries and are particularly in the focus of policy makers. Much of the progress and success in science, technology and economics is made by these important sectors of the society.

Our country's economy has been mostly reliant on oil income by selling crude oil for many years. This type of economy masters us to neglect one of the most important resources of the country "the human capital"

Human capital and economic growth have a strong relationship. Human capital has a pivotal role in economic growth and can help to promote an economy through the knowledge and skills.

Hence, in the past few years, the Vice Presidency for Science and Technology has been trying hard to disseminate this attitude.

We envision that in the next 10 years or so, the infrastructure provided during the recent years, of which the knowledge-based companies are among the top list, will have the potential to generate significant employments.

On this ground and by considering the creativity and entrepreneurship, our young scientists will pave way for nation's progress.

Self-esteem refers to a person's beliefs about their own worth and value. Self-esteem is important because it heavily influences people's choices and decisions.

Lack of self-esteem in a society has widely spread a consequence, that's why it is quite important to help internal entrepreneurs build up self-esteem.

The key objective of "The 3rd national festival and international congress on stem cell and regenerative medicine" is to communicate science, technology and medical research between academia, researchers and industry through inspiring sessions and tons of networking.

Mastering the knowledge of stem cell provides international dignity and brings in many employment opportunities.

At the end, I would like to thank the executive and scientific committees of the festival, and I honor the participation of all activists in the field of stem cells at the 3rd National Festival and the International Congress of Stem cells & Regenerative Medicine.

Dr. Sorena Sattari
Chairman

General Secretary Welcome Message

In the Name of the Creator

The 3rd National Festival and the International Congress on Stem cells and Regenerative Medicine is a scientific and technological event that with the trust in the Almighty Allah and with the help of scholars and experts in the field of regenerative medicine and cellular sciences will be a turning point in the country's developments based on modern technologies.

The holding of this national festival and international congress will provide an opportunity for decision makers of the health sector of the country, professors, researchers in the fields of medical sciences, basic sciences and engineering, Industry owners and capital of medicine and medical equipment, entrepreneurs, producers and other activists in this field to pave the way for the formation of third-generation universities with their effective presence and presentation of scientific and technology-based ideas so that on the eve of the 40th anniversary of the victory of the glorious Islamic

Revolution, another golden event will be added to the honors of the Islamic system.

We celebrate the scientific achievements of the last ten years in the field of regenerative medicine and cellular technology at the 3rd National Festival and the International Congress on Stem cells and Regenerative Medicine, while witnessing a clear horizon for accelerated scientific progress of the country and believe that this moving forward will surpass the boundaries of knowledge in the near future, will illuminate the glow of hope for treating serious illnesses in the heart of the sick and will create new dimensions of scientific achievements in the medical field. We Hope that with the active and effective participation of all those who are interested in the development of science and technology and the progress and excellence of our beloved homeland, in this national festival and international congress, we once again bring the scholarly competencies of our country to the fore and the proud flag of the Islamic homeland on the peaks of scientific advancement and technology development.

Undoubtedly, the strategic knowledge of the stem cells and regenerative medicine that have been gained by efforts of Iranian scholars and students has provided a clear path to reach the peaks of science and technology up to international levels, and can provide excellence to the scientific status of Iranian universities and science centers. We believe that with the perseverance and effort of all the compassionate, decision-makers of this field, we will see a great development in the country's scientific and research area, in the near future, and the outcome of these efforts is the creation of work space for young and sensational researchers, The development of startups and newly established knowledge-based companies and fulfilling the constructive ideas toward the formation of third-generation universities, which will ultimately lead to a constructive and effective development of health in the attainment of these excellent goals.

I once again honor the presence of all loved ones and prominent companions at the 3rd National Festival and the International Congress of Stem cells & Regenerative Medicine, and demand successes for high-minded scholars and people from the only and one creator and universe.

Dr. Amir Ali Hamidieh
General Secretary

Scientific Secretary Welcome Message

Dear lovers of the science and my thoughtful friends,

Researches in the field of regenerative medicine and stem cells including the fundamental studies of science limits, studied over the animal behavior and medical practices through applying the world's cutting-edge technologies and with the purpose of prevention, identification and treatment of diseases has opened up new horizons toward identification, controlling and curing of many degenerative diseases.

Toward this scientific leap and also aside the other countries, in Iran, the headquarters of stem cells, backed up with rich backgrounds and experiences of more than 20 years of research on stem cell and also supporting newly established knowledge based companies in this field, holding seminars and international symposiums had a great impact in this field.

The headquarters of stem cells in science and technology's vice presidential is honored to host well-known scientists and thinkers from all over the world for the third consecutive year in diverse fields of medicine and biology in the 3rd national festival and international congress of stem cells and regenerative medicine on November 24th to December 1st 2018 in Tehran, the capital city of ancient Iran.

The scientific subjects of congress contain a wide range of modern topics including recent advances in tissue engineering and biomaterials, cell banks, cell therapy, immune cell therapy, gene therapy, ethical considerations, genetic manipulations, organoids and their application in disease modeling, strengthening and cellular reprogramming and introducing new technologies used in stem cell researches.

It needs to be mentioned that the crucial aim of this year's festival is gathering young intellectuals in various fields of medicine, biology and engineering, holding workshops, scientific meetings, identifying and introducing product-oriented idea owners to investors and also introducing new domestic and foreign companies which are active in the field of stem cells.

It would be my honor to invite you scientists, researchers, healthcare investors, students and all interested people in this field to augment this congress's scientific richness by your presence.

Dr. Nasser Aghdami
Scientific Secretary

Executive Secretary Welcome Message

Annual stem cell festival, an event for flourishing

The growth and development of stem cell science and technology in our beloved Iran has come to the forefront of other developed countries and has found a satisfactory pace at the international level. The admirable efforts of senior managers of the country's science and technology field, university professors, researchers, industry owners, manufacturers and students have led to significant production of knowledge and technology, biological products and clinical services in this area, which has increased the reputation of the Islamic Republic of Iran in international rankings, particularly in 2016 and 2017.

Over the past three years, the development of science and technology of stem cells of the Vice Presidential Scientific Council has over 30 specialized working groups and widespread use of the wisdom of more than 800 faculty members, researchers, technicians, manufacturers, owners of knowledge-based companies and other professionals.

The annual stem cell festival, which will be held this year with the title of "The 3rd National Festival and the International Congress of Stem Cells and Regenerative Medicine", is the largest stem cell gathering in the country, with the participation of all senior Iranian science and technology directors, professors, researchers, industry owners and manufacturers, and students. Today, the festival is a very promising opportunity to enhance the target and scientific progress of the country by reviewing new global developments in this strategic field and benefiting from the ideas of domestic and foreign scientists.

The annual stem cell festival is an important event for the flourishing of this knowledge at the national level, which has enormously attracted scientists' attention. Therefore, having this opportunity, while appreciating the efforts of all the colleagues of the Strategic Committee, the Executive Committee and the Scientific Committee in organizing this valuable scientific event in the country, I invite all of you to participate in this scientific adventure to flourish this strategic area of knowledge for our beloved country, Iran.

Dr. Mohammad Amir Amirkhani
Executive Secretary of Festival

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Vice-President for Science and Technology Supreme Chairman of the Festival

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The Steps Leading to Development of Stem Cell Sciences and Technologies in Iran: The Path Towards Future

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Background and Aim: Stem cell technology was started in Iran in the late of 1990 by first hematopoietic stem cell transplantation in Tehran University of Medical Sciences and in less than a decade, Royan Institute started stem cell research followed by infertility treatment. Since then, different universities and research centers have focused on stem cells and regenerative medicine which resulted in publishing more than 2500 articles in journals indexed in PubMed as well as creating new ideas. The council of stem cell sciences and technologies in the vice presidency for science and technology started its activity ten years ago, with the aim of expanding education, research and development of human resources. Scientific opportunities in this field have been created by the integration of government and scientific communities.

During this time, by the financial and spiritual support given by this center, research projects were carried out in more than 45 universities and institutes. Four Centers of Excellence for Stem Cell & Regenerative Medicine has been established in Royan, Tabriz, Shiraz, and Tehran to integrate scientific capacities human resources and laboratory equipment. Student Olympiads were held at the high school level, and the establishment of Student Research Centers in three provinces can be considered as the most influential activities for stem cell council. In recent years, recognizing the importance of promoting the culture of a knowledge-based economy, stem cell council also seeks to strengthen the knowledge-based companies by supporting and guidance of researchers and investors, as it has caused the number to increase to more than 75 titles.

The council of stem cell sciences and technologies also organized two national festival and international congress, 70 symposiums, and several workshops to spread stem cell sciences and technology among the public. The first social network and science-specific messenger, across the country, has been launched in 2017 by the stem cell council promotion. The application as mentioned above would be a platform which can provide effective interactions among specialists in the field of stem cell sciences. Animal lab network is the other supported project by stem cell council. It gives an opportunity for the researchers to use various animal models and facilitate in vivo studies. Finally, the collaboration between Iran stem cell council and Iran FDA has led to the development of products regulations. In this regard, four cell-based products were licensed to enter the market. Now Iran is the first rank among Eastern Mediterranean and Middle East countries. The issues mentioned are part of the mission of the stem cell council which has been carried out until now.

Keywords: Stem cells; Transplantation; Stem cell council; Cell-based products

Promotion of Stem Cell Technology in the Islamic Republic of Iran at the International Level

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Stem cell science and technology has grown dramatically in today's world, and the Islamic Republic of Iran is one of the leading countries in this strategic science. Stem cell technology has gained considerable popularity in Iran with the efforts of academics, researchers, student, and managers of knowledge-based companies and also policymakers in recent years. This field has provided significant capacities and opportunities for the country at the national and international levels. The 10-year strategic plan for developing this technology has been provided 30 programs and three areas in 2013 that include: training, research and human resources development, commercialization and marketing, and development of clinical service centers. This comprehensive strategic planning was approved to manage scientific and technical capacities of the country that covers all the country's potential. Thanks to this plan, ten centers of stem cell and regenerative medicine have been designed and developed which located in 10 most important universities in Iran, and so far, three centers have been established and are fully functional. Knowledge-based companies attending at these centers to participate in Ph.D. students and Postdoctoral fellows training course to advance this scientific field. Over the past couple of years, over 100 knowledge-based companies are working in the field of regenerative medicine with the support of the Council towards the development of stem cell sciences and technologies. Now, these companies are ready to export their technologies and products to other countries. Focusing on the development of stem cell science and technology is not specific to Tehran, and more than 20 other provinces have the infrastructures for the stem cell development under the supervision of the Council. What is more, over the past few years, one of the most important steps towards the development of stem cell science and technology in Iran has been the Annual National Festival and the International Congress in the stem cells and regenerative medicine. This Congress has different sessions, including theoretical and practical workshops, the committee of innovation for Iranian diaspora, national awards competition, an exhibition of knowledge-based products, set market meeting, startup event, student tournament, scientific symposium. Also, more than 5000 participants, including highly-regarded professors, scientists, researchers, students, and managers of companies from all over the world attend this event annually. Taken all, it should be noted that the Islamic Republic of Iran aims to collaborate with other scientific centers around the globe to develop this technology and provide valuable services for patients.

Keywords: Stem cell science and technology; Commercialization

Invited Section

IS-001. Cell Therapy Manufacturing Facility: Design and Operation

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Recently a great interest has arisen in the field of cell therapy products (CTPs) manufacturing. According to the current international standards the CTPs should be manufactured under principles of good manufacturing practices (GMP). GMP is fundamental to product safety and quality and to involve multiple elements covering all aspects of production. Although each element is equally important, the facility is the most obvious and tangible aspect of GMP. There are few specific regulatory requirements for CTPs; consequently, GMP for pharmaceuticals and biologics should be applied. The objective of this presentation is to summarize the basic requirements for CTPs manufacturing facilities and provides an overview of facility planning and design, cleanroom classifications, and operation.

Keywords: Cell therapy; Clean room; GMP; Regenerative medicine; Regulation

IS-002. Advanced Approached to Stem Cell Therapy

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Cardiovascular diseases (CVDs) are the major cause of mortality and morbidity worldwide stem cell therapy is an alternative modality in the treatment of CVD especially ischemic heart disease Although safety and feasibility of different cell types have been proved in several clinical trials, the beneficial outcome is not significant. The most successful result of stem cell therapy in human has been achieved by cardiac stem cell (CSC), with the improvement of ejection fraction about 10%. Other cell types in clinical trials led to an average 3-5% beneficial outcome or no positive effects. New strategies for improving stem cell-based therapy in CVD include:

- Preconditioning of stem cells: ischemic induction, application of low energy shock wave and pharmacologic pretreatment with statin
- Enhance engraftment and survival of stem cells
- Using growth factors, cytokines, overexpression of $CXCR_4$ and SDF1 for angiogenesis adhesions and engraftment
- Genetic modification for increasing cell efficiency
- Magnetic cell targeting and ultrasound-mediated improve cell delivery and retention
- Application of biomaterials for cell delivery and retention, an O_2 releasing scaffold for increasing survival and proliferation of stem cells.

Extensive works are still required to increase the beneficial effect of stem cell therapy.

Keywords: Cardiac stem cells; Cardiovascular diseases

IS-003. Chemoattractant and Stem Cells in the Regeneration of Intervertebral Discs

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Background and Aim: Having exploited an organ culture model of induced IVD degeneration, we have reported that the human mesenchymal stem cells (MSCs) could migrate via the endplate of a bovine disc. In addition, the degenerative disc conditioned medium could significantly upregulate the expression of certain chemokine receptors in the MSCs cultured, which indicate the responsiveness of these cells to the degenerative environment. We have also looked at the likelihood of MSC recruitment in the disc *in vivo* using a mouse tail model of induced disc degeneration, even though only a limited number of bone-marrow cells were used in the disc.

Methods: The migration of MSCs through the endplate was further investigated by injecting the chemokine SDF1 into the cavity of a partially nucleotomized disc in organ culture. Having used a hyaluronan-based thermoreversible injectable hydrogel, the SDF1 was delivered to support the disc cell growth, matrix production and MSC differentiation towards the disc-like phenotype.

Results: The SDF1-releasing hydrogel was found to significantly increase the MSC recruitment to the disc, indicating the potential of a chemokine delivery system to accelerate cell homing. The proteomic profile of the conditioned medium of an IVD maintained under induced degenerative settings was analyzed in order to identify disc-derived chemotactic factors. Proteomic analysis demonstrated that the two main chemotactic factors, CL5/RANTES and CXCL6, could be secreted by the degenerative disc. The histological sections of bovine and human degenerative discs confirmed the presence of CCL5/RANTES and its receptors, indicating their key role in cell recruitment.

Conclusion: Clear evidence clarifies that the homing of endogenous regenerative progenitor cells might occur in the disc, which could be amplified by the chemoattractant delivery system. Besides, some recent studies have shown the presence of progenitor cells at different locations in healthy and degenerative IVDs. Take all, mobilization, augmentation and activation of these endogenous progenitor cell populations validate them as attractive targets for future regenerative approaches.

Keywords: Stem cell; Chemoattractants; Bioreactor; Hydrogels

IS-004. Hyaluronan Hydrogel Platform for Musculoskeletal Regeneration

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Background and Aim: Hyaluronic acid (HA) is one of the main extracellular matrix components that is involved in various biological events, including tissue development and regeneration, cell motility and viscoelasticity. HA has been used commonly for the formulation of drug delivery systems and regenerative medicine constructs. Having capitalized on an unconventional conjugation method, in this current study, we report on a series of derivatives providing HA with tunable pseudoplasticity, temperature-induced gelation and dual enzymatic/light-induced gelation.

Methods: In this study, HA was conjugated via DMTMM-mediated amidation with (i) an array of substrates including small, large and functional molecules for validating the method, (ii) short alkyl chains of propyl and butylamine, (iii) thermoresponsive moieties, (iv) tyramine. The formulated products were characterized by means of rheometer, ^{1}H-NMR and UV-vis spectrophotometry. The reaction kinetics and coupling yields were assessed under a spectrum of conditions. To deliver chemokines *in vivo* in a rabbit osteochondral defect model, we used the thermoresponsive derivative. Further, the HA-tyramine derivative was optimized for the additive manufacturing with a dual enzymatic and light gelation.

Results: In this study, we used the thermoresponsive derivative for *in situ* tissue engineering. After one week, an increased cell density was found within the rabbit osteochondral defect upon the delivery of chemokines compared to chemokine-free gel. The current method seems to be an attractive strategy since it can facilitate the endogenous cell recruitment, eliminating the need for tissue harvesting and cell expansion. Besides, the application of HA-tyramine for 3D printing showed that (i) the dual gelation allowed good extrusion properties and reliable shape maintenance; (ii) cells can attach and spread within the material also in the absence of cell-binding peptides; (iii) a low concentration of polymer (2.5%) and low degree of substitutions (6.0% to 14.5%) can be used to obtain gelation. Furthermore, this method is free from the use of UV light

and has no safety concerns regarding the UV-induced DNA damage and tumorigenesis.
Conclusion: We have demonstrated how chemical modification can improve the technological properties of HA including viscoelastic profile, drug delivery and 3D printing capability. These derivatives are valuable tools for fabricating constructs in tissue engineering and regenerative medicine.
Keywords: Hydrogels; Stem cells; Cartilage; 3D printing

IS-005. Exosomes as a New Trend for Cell-Free Therapy

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Graft-versus-host disease (GvHD) is still a major and life-threatening complication of allogeneic hematopoietic stem cell transplantation (AHSCT) with a high rate of incidence, morbidity, and mortality. A wide range of approaches to control the GvHD are currently under research among which the preventive approaches is the most desirable to achieve. Immune-modulation features of mesenchymal stem cells (MSCs) make them as a promising strategy to prevent and treatment of inflammatory diseases including GvHD. A series of ongoing and completed clinical trials on GvHD have reported the great response of patients to MSC therapy with a dramatic decrease of inflammatory factors and an increase in patients' survival. One of the most concerns of the application of MSCs is their plasticity and also poor information about probable long-term side effects. The recent solution is to use the cells' byproduct instead of cells themselves. A most effective byproduct of cells is extracellular microvesicles and exosomes which have some function of cells without concerns of cellular plasticity and self-replicating capacity. Exosomes are naturally occurring extracellular vesicles released by exocytosis from many types of cells and contain lipids, proteins and RNA, which allow them to participate in the intercellular communication processes. Due to the origin, MSC-derived exosomes mostly have immune-suppressive functions and their small size makes them ideal for GvHD treatment. Moreover, some recent studies and patents have claimed the possibility of using exosomes as a preventive strategy in addition to treatment. Growing tendency besides hopeful results of the application of MSC-derived exosomes in GvHD prevention and management implicate the bright future of cell derivatives rather than cell therapy.
Keywords: Exosome; Graft-versus-host disease; Cell therapy

IS-006. Translational Regenerative Medicine: How to Build a Scientific Bridge Between Basic Knowledge and Clinic

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Stem cell therapy has introduced promising hopes for the treatment of various diseases. On the other hand, clinical utilization of stem cells needs to translate basic sciences and protocols before starting clinical phases by bridging stem cell research into clinical trials. Therefore, stem cells translational medicine will open a new horizon in this area of research and practice. Accordingly, there are several risk factors relevant to the safety issues of stem cell preparation and transplantation that must be considered in the translational phase. For instance; transplantation site reactions, immune responses, biodistribution, ectopic grafting, unintended differentiation into another cell type, tumorigenicity, and lack of functional characteristics. In summary, to conduct clinical stem cell transplantation trials, the safety concerns must be carefully weighed against the potential benefits and all preclinical and clinical researches must be designed to elucidate potential safety concerns before translating from the bench to the bedside.
Keywords: Cell therapy; Regenerative medicine; Safety; Stem cells; Translational medicine

IS-007. The Potential of Stem Cell Therapies for Pediatric Neurological Disorders

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Until the middle of the 20th century, regeneration of nerve tissue was considered to be impossible, however, nowadays cell replacement therapy has provided the basis for the development of potentially powerful new therapeutic strategies for a broad spectrum of human neurological diseases. Stem cells are a nonspecialized type of cells that are capable of replicating itself but also differentiate into specialized cells, leading to new tissue formation, repair, and regeneration. Literature review suggests that stem cells can migrate from the site of injection into the injured site. In fact, we must believe that stem cells will be used clinically, not as a curative treatment but as part of a therapeutic management. The most important aspect of cell therapy is applying the right cell type to the right disease and explanation the right amount of expectation to the patient. There are three types of stem cells based on the differentiation potential, including totipotent, pluripotent and multipotent cells. The origin of the stem cells, can be of embryonal, fetal and adult somatic stem cells. Stem cells have been investigated as a treatment for neurological damage since the 1990s. For using neurological trial, neurons and glial cells have been generated from stem cells as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs) and neural stem cells (NSCs) or neural progenitor cells. MSCs are multipotent cells that generate into neurons, astrocytes and oligodendrocytes and can be prepared from various sources, including autologous bone marrow-derived mononuclear cells, adipose tissue-derived stem cells and umbilical cord stem cells. Because of the increasing popularity of postnatal umbilical cord stem cell storage, the most easily accessible current source of stem cells in clinical trials is umbilical cord-derived stem cells. Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, amyotrophic lateral sclerosis and stroke are the main neurological disorders of adults that cell therapy may be effective. There are a number of developmental disorders that are considered targets for stem cell therapy, such as, Cerebral palsy and autistic spectrum disorders. Head injury, spinal cord injury, spinal muscular atrophy, neurological deficits in meningomyelocele and poliomyelitis, Duchenne muscular dystrophy and neurodegenerative disorders especially LSDs are the main neurological disorders of pediatric age group that cell therapy may be effective.
Keywords: Stem cell therapy; Pediatrics; Neurological disorders

IS-008. Cornea Regeneration

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Corneal reconstruction has dramatically progressed in recent years. The cornea is the most important optical element of the eye. It has three main histologic parts; epithelium, stroma and endothelium. All parts especially their stem cells may be destroyed by different offending mechanisms. The type of epithelial regeneration depends on the severity and laterality of the lesion. In unilateral total limbal stem cell deficiency (LSCD) surgical alternatives include conjunctival limbal autograft (CLAU), cultivated limbal epithelial transplantation (CLET), simple limbal epithelial transplantation (SLET) and in vivo cultivation of limbal

stem cells. In bilateral total LSCD surgical options are keratolimbal allograft transplantation (KLAL), living-related conjunctival limbal allograft transplantation (lr-CLAL) and cultivated oral mucosal epithelial transplantation (COMET). Stromal reconstruction includes penetrating keratoplasty (PKP) and deep anterior lamellar keratoplasty (DALK). Tissue engineering is more elementary in stromal reconstruction, however, the only proven clinical trial which is in progress is using collagenous biomaterials. Endothelial reconstruction includes Descemet stripping automated endothelial keratoplasty (DSAEK), Descemet membrane endothelial keratoplasty (DMEK) and endothelial cell culture.
Keywords: Corneal reconstruction; Limbal stem cells; Cornea

IS-009. Gene Therapy: Promises and Problems

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Gene therapy has developed intensely since the first clinical gene transfer experiment in October 1989. This therapy has the potential to cure incurable diseases and significantly change the face of medicine in the future. The dream of treating incurable diseases moves closer to reality with the success of CAR T cell therapies, novel gene delivery systems, and the discoveries of miRNAs, and CRISPR/Cas9. There are a few approved gene therapy medicinal products (GTMPs), but several new GTMPs are currently under review. However, despite recent technological advances, substantial challenges persist, many of which encompass the underlying technologies. This paper will review the landmarks that had a significant impact on the progress of gene therapy. It will also address some concerns that have been related to gene therapy as an emerging therapy.
Keywords: Gene therapy; Gene delivery; CRISPER

IS-010. Genome Editing with the CRISPR-Cas9 System: Principles and Applications

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The CRISPR-Cas9 systems are revolutionizing studies of gene function and possibilities of gene therapy. In bacteria, natural CRISPR-Cas9 systems provide adaptive immunity by cleaving foreign DNA. Cas9 is an RNA-guided endonuclease and artificial guide RNAs can easily be designed to cleave target any DNA sequence of interest. When used for genome editing, the CRISPR/Cas9 system allows making a great variety of DNA sequence modifications (deletions to inactivate gene function or potential regulatory sequences, insertions of reporter genes, point mutations, etc). We have developed online tools for selection of guide RNAs in more than a 300 species (crispor.org) and helped to perform genome editing in many experimental systems. Examples from our lab, in cultured cells, and from collaborations, in model organisms such as the rat and zebrafish, will be shown to illustrate a wide range of applications of genome editing for the study of gene function and genetic diseases.
Keywords: CRISPR-Cas9; Gene Therapy

IS-011. Optimization of Genome Editing with the CRISPR/Cas9 System

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Optimization of genome editing needs to be performed at several steps, from guide RNA design to delivery of CRISPR/Cas9 reagents. In treated cells, genome editing per se takes place during repair of DNA double-strand breaks made by CRISPR/Cas9 nucleases. Several DNA repair pathways can process may be involved and influence the eventual outcome of including classical non-homologous end-joining (NHEJ), micro-homology mediated end-joining (MMEJ) and homologous recombination (HR). Our lab has developed strategies of genome editing that take advantage of these different possibilities in order to increase the efficiency of genome editing with the CRISPR/Cas9 system.
For example, when developing gene editing in *Clytia hemispherica* jellyfish, we observed that unexpectedly deletions made in target genes were often flanked by short stretches of identical sequence. This is characteristic of DNA repair by MMEJ which therefore appears to be the predominant end-joining pathway for introduction of indels during genome editing with CRISPR-Cas9. This unexpected finding is in fact common to many experimental systems and needs to be taken into account when designing guide RNAs.
Gene inactivation by the introduction of small deletions or insertions is highly efficient with the CRISPR/Cas9 system. In contrast, making precise, programmed modifications such as transgene insertions often remains challenging. We have developed several strategies to address this challenge, including homology-independent transgene integration in zebrafish and using chemically modified oligonucleotide donors. We also recently developed a novel approach for increasing the efficiency of transgene integration by homology-dependent repair (HDR).
Keywords: CRISPR/Cas9; Genome Editing

IS-012. Nanomaterials in Stem Cell Regenerative Medicine: The Scaffold-Based Approach

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Background and Aim: Rapid progress in biomaterial engineering has a profound impact on tissue engineering and regenerative medicine. The application of nanotechnology to regenerative medicine is a wide issue. Nanotechnology can provide new strategies for regenerative medicine, including better tools to improve or repair damaged tissues. The adhesion, growth, and differentiation of stem cells are controlled by nanotopography of their surrounding microenvironment or the architecture of scaffolds that forms a network for human tissues. Nanostructured materials such as nanocomposites and nanofibers have attracted increasing interest in regenerative medicine, because these nanomaterials can mimic the physical characteristics of the natural extracellular matrix (ECM) at the nanoscale level. Nanofibers, have been used as scaffolds for musculoskeletal tissue engineering (including bone, cartilage, ligament, and skeletal muscle), skin tissue engineering, vascular tissue engineering, and neural tissue engineering. In this study we will present the preparation and evaluation of electrospun nanofibrous scaffolds for stem cell differentiation and tissue regeneration. We have prepared various biodegradable blends of natural and synthetic polymers for scaffold fabrication with the ability to increase cell survival, proliferation, and migration and provide supporting mechanical properties by mimicking the ECM.
Methods: Biodegradable blends of natural polymers such as collagen, gelatin and chitosan with biodegradable polyesters such as poly(L-lactide) (PLLA), poly(glycolide) (PGA), poly (ε- caprolactone) (PCL), poly(lactide-co-glycolide) (PLGA) and poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε -caprolactone) (PCL-PEG-PCL, PCEC) have been prepared as nanofibrous scaffolds and incorporated with appropriate nanoparticles such as hydroxyapatite (HA), silica nanoparticles (n-SiO_2), magnetic nanoparticles (MNPs), and growth factor-loaded polymeric nanoparticles for the creation of 3D cell-supportive scaffolds. In another approach, different thermosensitive hydrogels, based on poly (N-isopropylacrylamide; PNIPAAm) copolymers, have been developed and used as injectable implants. The biocompatibility, morphology, proliferation and differentiation abilities of human mesenchymal stem cells on these nanostructured scaffolds have been investigated. The applications of these nanostructured scaffolds have been evaluated for bone, cartilage and skin regeneration.
Results: Blends of natural polymers such as gelatin, chitosan and collagen with biodegradable polymers exhibit improved mechanical properties

and provide an effective, non-toxic approach for the fabrication of biomimetic scaffolds for tissue engineering. The incorporation of magnetic nanoparticles and/or HA can promote osteointegration and osteoinduction. The polyester-collagen hybrid nanofibrous scaffold provides a stimulus microenvironment for the chondrogenic differentiation of the MSCs. The PCL-PEG-PCL/ CS/ n-SiO_2 and n-HA with an average diameter in the range of 190–260 nm showed excellent cell adhesion and proliferation properties. Alizarin red S staining and qRT-PCR analysis confirmed the osteogenic differentiation of hDPSCs on PCEC-CS nanofibers incorporated with n-HA and n-SiO_2. Thermosensitive PCEC/ Gel and (PNIPAAm-PCEC-PNIPAAm)/Gel hydrogel scaffolds possesses useful hydrophilic properties for the growth and cell embedding and secretion of extracellular matrix. It can serve as an ideal strategy to promote the formation of cartilage tissue.

Conclusion: Development of biomaterials is important for scaffold fabrication. Electrospun blend nanofibrous scaffolds have a high surface area to volume ratio, controllable diameter and porosity, excellent biological activity and can provide an appropriate architecture for bone, cartilage and skin tissue engineering.

Keywords: Nanomaterials; Electrospun nanofibers; Scaffolds; Tissue engineering

IS-013. Could Autologous Fecal Microbial Transplantation Be a Treatment Option in Patients with Intestinal Graft-Versus-Host Disease: Lessons Learned From an Experience in Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) includes various forms of disorders; however, Crohn's disease (CD) and ulcerative colitis (UC) are two major types of this disease. Ulcerative colitis is characterized by expansion mucosal inflammation in the colon, while Crohn's disease is characterized by patchy, transmural inflammation, which may affect all parts of the gastrointestinal tract. There are some evidence about the role of genetic and immune disorders and environmental factors, such as a change in the fecal microbiota, in the pathogenesis of IBD. Commensal microbiota could promote IBD through changes in the composition of the mucosal-associated bacteria, which is known as dysbiosis; deregulated induction of immune system in the intestine; and genomic instability and induction of mutations in the host by their metabolites and toxins. Dysbiosis increases gut permeability to bacterial products and promotes inflammatory disorders through induction of the immune system. *Clostridium difficile* infection (CDI) in patients with IBD is associated with more severe disease, longer hospital admission, higher treatment costs, higher risk of colectomy and mortality rate. Fecal microbiota transplantation (FMT) is one of the treatment choices in patients with recurrent CDI, especially among those with underlying diseases. In this presentation we report three unsuccessful FMT procedures in IBD patients with recurrent CDI. All the patients were subjected to FMT by heterologous samples. Our results did not support FMT as a treatment option to subside the induced inflammatory response in these patients. These findings could propose usage of autologous instead of heterologous FMT for patients with intestinal graft-versus-host disease (GvHD), when it is suggested as a therapeutic option.

Keywords: Graft versus Host disease; Fecal Microbial Transplantation; Inflammatory disorder

IS-014. The Effects of Autologous Adipose-Derived Stem Cell (ADSC) on Optimizing Achilles Tendon Repair in Rat

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Background and Aim: Tendon injury is a common and challenging problem which often requires surgical intervention to repair the damaged tendon. Most important factor in having more favorable outcome is the strength of repaired tendon and lack of adherence to the adjacent tissues. The aim of this study is to survey the effects of adipose-derived stem cells on optimizing Achilles tendon repair in the rat.

Methods: Thirty-six rats randomly divided into 3 groups. Autologous fat-derived stem cells were prepared from the inguinal fat pad of 12 rats. Achilles tendon was cut in all 3 groups. Autologous stem cell was injected into both cut ends of tendon before repair in the first group. In the second group normal saline and in the third group culture medium without cell was injected in the cut ends of the tendons. Then all the tendons were repaired with the same method. Strength and quality of repair were surveyed by tensiometry and histopathology evaluation after 30 days of repair.

Results: Tensiometry results showed stem cell group had the most tendon strength with a statistically significant difference to other groups (P value = 0.017). However the histopathology indices such as the amount of collagen, fibroblasts, Collagen arrangement, vascularization, the number of inflammatory cells and foreign body reaction were not significantly different.

Conclusion: This study showed that stem cells could significantly improve the biomechanics of tendon repair. However, according to the histopathology results, this conclusion is obscure and needs more studies.

Keywords: Autologous; Adipose-derived stem cells; Achilles tendon; Rat

IS-015. Architecture of the Stem Cell Compartment and Clonal Evolution in Myelodysplastic Syndromes

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Background and Aim: Myelodysplastic syndromes (MDS) are hematopoietic stem cell (HSC) disorders of the elderly characterized by ineffective hematopoiesis and a major risk of evolution to acute myeloid leukemia (AML). The WHO classification recognizes eight subgroups, based on 5q deletion, uni/multilineage dysplasia, ring sideroblasts or *SF3B1* mutation, the percentage of blasts and. The phenotypic diversity of MDS has driven the variegation of combined genetic events. To address the heterogeneity of clonal hematopoiesis at diagnosis and under disease-modifying treatment, we investigated the architecture of hematopoietic stem/progenitor cell (HSPC) compartment.

Methods: Mutation status of 40 genes were obtained in a cohort of 60 non-del(5q) MDS by next-generation sequencing. The hematopoietic hierarchy was analyzed by multiparametric flow cytometry. The clonal architecture was assessed by the genotyping of single CD34+CD38- cell-derived colonies, long-term colony-initiating cells (LTC-IC) and clonogenic progenitors. Disease-initiating cell was identified by genotyping human cells engrafted in immunodeficient NSG mice after 8–16 weeks. Clonal evolution of patients receiving lenalidomide was

followed at evaluation after 4 cycles and further when they responded to treatment (GFM LenEpo2008, clinicaltrials.gov, EudraCT number 2008-008262-12).

Results: In single HSPCs, the founding mutations usually affect epigenetic regulators (*TET2*, *DNMT3A*) or splicing factors (*SF3B1*, *SRSF2*). Mutations accumulate in a dominant subclone and generate few minor subclones, with a linear or branched architecture. The dominant clone is detected in the LTC-IC compartment and repopulates the bone marrow of NSG mice with a myeloid and lymphoid progeny, showing that the cell of origin is a genuine HSC. A refined flow cytometry analysis using a 14-color panel demonstrates that hematopoiesis in low-risk MDS is characterized by a myeloid bias of multipotent progenitor (MPP) compartment at the expense of pre-B progenitors. This results in the accumulation of common myeloid (CMP) at the expense of granule-monocytic (GMP) and megakaryocytic-erythroid (MEP) progenitors, except in sideroblastic anemia where MEP are preserved. We noticed that mutation contents may differ along the hematopoietic hierarchy. For instance, *STAG2* mutations not detected in single HSCs, were present as neo-mutations in MPP-like or GMP-like progenitors that drove the amplification of a clone dominant at the time of AML transformation. Finally, mutations accumulate with time along disease evolution, whatever the therapeutic strategy. We found that lenalidomide by inducing an immune response, transiently reduced the size of the dominant clone in responding patients whereas new mutations could emerge in non-responding patients.

Conclusion: We identified MDS-initiating cells and clones driving the leukemic transformation. Mutation combination and selection during evolution contribute to the heterogeneity of MDS. Defining the hierarchy of driver mutations provides insights into the process of transformation, and may guide targeted therapies.

Keywords: myelodysplastic syndrome, hematopoietic stem cell, hematopoiesis, clonal architecture, lenalidomide

IS-016. Clinical Implementation of the One-Step Surgical Procedure for Craniofacial Dental Implantation in the Maxillary Sinus Floor Elevation (MSFE) Model

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Background and Aim: It is long known that patients with insufficient maxillary bone height might need maxillary sinus floor elevation (MSFE) prior to the placement of dental implant. Currently, the bone substitutes are used as an alternative as the 'gold standard' (i.e., as the autologous bone). However, the bone substitutes only allow the osteoconduction, since viable osteogenic cells are lacking. In the recent years, cell-based bone tissue engineering has been introduced as a promising treatment option to improve the bone forming capacity of bone substitutes.

Methods: In a government-sponsored phase I trial, we assessed the feasibility, safety and efficacy of combining a calcium phosphate (CaP) as a bone substitute using freshly isolated adipose stem cells during a one-step surgical procedure for MSFE. In this line, to avoid costly stem cell expansions and a second intervention, the osteoinductive carriers (i.e., CaP seeded with the freshly isolated stromal vascular fraction of adipose tissue) were generated in an intra-operative procedure in the OR-complex within hours. Furthermore, a 'split-mouth design' (with only CaP scaffold at the contralateral control side) was applied to allow efficacy evaluation, wherever possible. At regular intervals during follow-up, adverse events were monitored, and clinical, X-ray, and Cone-beam CT data were collected. After six months, biopsies were obtained during the placement of dental implant, which were evaluated for the bone formation by histomorphometry and µCT.

Results: In this phase I trial, we included 10 patients. All patients uneventfully underwent an MSFE procedure and no adverse effects were reported during 3.5-year follow-up. Bone as well as an osteoid percentage were higher in bone biopsies taken from study sides than the control sides throughout the complete biopsies, suggesting that bone formation does not only occur from the pre-existing sinus floor, and that adipose stem cells may stimulate bone formation.

Conclusion: For the first time, this present study demonstrated the feasibility, safety and potential efficacy of freshly isolated adipose stem cells with a calcium phosphate for MSFE. Based on these findings, this modality provides the first step towards a novel treatment concept that might offer the broad potential for cell-based regenerative medicine applications.

Keywords: Adipose stem cells; Regenerative medicine; Phase I trial

IS-017. Innovative Mandibular Reconstructions Using Fibula's, 3D-Planning, and 3D-Printed Saw Guides

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Background and Aim: Vascularized and non-vascularized fibular graft transfer for skeletal reconstruction purposes has been shown to be an effective treatment strategy for various congenital and acquired skeletal deformities, including for mandibular reconstructions due to tumor, infection and trauma.

Methods: We present a novel method where free fibula transfer utilizing preoperative virtual surgical 3D planning is used to produce a total autogenous reconstruction.

Results: The virtual surgical planning allows to effectively quantify the bone stock required preoperatively. Moreover, the virtual surgical planning is used to carefully design nylon saw guides that contain also pre-planned drill holes for screw placement, which can be used during surgery to optimally cut the fibula such that an anatomically correct reconstruction can be realized.

Conclusion: This will result in reduced surgery times and improved functional and aesthetic outcomes. In the near future, this type of reconstruction may be replaced by using personalized 3D-printed scaffolds seeded with adipose stem cells to avoid donor site morbidities.

Keywords: Additive manufacturing bone reconstruction; Personalized medicine

IS018. The Stem Cell Niche for Cardiovascular Tissue Engineering

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Background and Aim: To date, three-dimensional (3D) in vitro cell systems have been considered as a promising alternative to the animal models to study cardiac biology and disease.

Methods: In this present study, we have generated 3D in vitro cell cultures of the human heart, "cardiac spheroids" (CSs), by co-cultivation of the human primary or iPSC-derived cardiomyocytes, endothelial cells and fibroblasts at the ratios approximating those present *in vivo*.

Results: Our findings revealed that the microenvironment ("*niche*") of these spheroids presented cellular organization, extracellular matrix and microcapillary network that better recapitulate the typical in the human heart compared to other existing *in vitro* models. These spheroids were employed to study the dose-limiting cardiotoxicity of the common anticancer drug doxorubicin (DOX). Viability/cytotoxicity assays indicate dose-dependent cytotoxic effects, which are inhibited by the nitric oxide synthase (NOS) inhibitor L-NIO, and genetic inhibition of endothelial NOS, implicating peroxynitrous acid as a key damaging agent.

Conclusion: The results obtained in this study indicate that CSs can better mimic the key characteristics of human heart morphology, biochemistry and pharmacology *in vitro*. As a result, we propose this CSs 3D model as a promising alternative to animal models and standard cell cultures with regard to mechanistic insights and prediction of toxic effects in human heart tissue.
Keywords: Cardiac spheroids; Niche; iPSCs, Cardiotoxicity; Nitric oxide

IS-019. 3D Bioprinting of Patient-Derived Vascularized Cardiac Spheroids for Heart Tissues

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Background and Aim: 3D bioprinting of tissue and organs from human stem cells is a promising alternative to promote tissue regeneration.
Methods: We have generated a "bio-ink" for the bioprinting of the human heart by co-culturing in hanging drops human primary or iPSC-derived cardiomyocytes, endothelial cells and fibroblasts at ratios approximating those present *in vivo*.
Results: These microtissues are called "vascularized cardiac spheroids" (or "VCSs") as their cellular organization, extracellular matrix and microcapillary network recapitulate the microenvironment of the human heart tissue. VCSs respond to both physiological and pathological stimulation *in vitro* and have been employed to investigate molecular and cellular mechanisms regulating cardiotoxicity, angiogenesis, fibrosis and regeneration of the heart. Loaded into the nozzle of the 3D bioprinter, VCSs are used as building blocks for the bioprinting of human heart tissues. To bioprint heart tissues with defined geometries, VCSs are deposited layer-by-layer within a biocompatible matrix or "hydrogel" that better recapitulates the human heart microenvironment.
Conclusion: 3D bioprinting of a fully vascularized human heart tissue using VCSs offers a promising alternative to animals and standard cell cultures for drug testing and disease modeling in vitro. Transplantation of patient-specific 3D bioprinted human heart patches from VCSs are currently investigated to promote tissue regeneration for cardiovascular disease patients.
Keywords: bioprinting, cardiac spheroids, bio-ink, personalized therapy, angiogenesis, hydrogels.

IS-020. Stem Cells Therapy: A Review on Approaches That Can Be Used for Treatment of Respiratory Failures in Sulfur Mustard Injured Patients

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Sulfur mustard (SM) as a toxic agent can impose severe abnormal issues in the airway system, including oxidative stress, inflammation, necrosis, chronic bronchitis, shortness of breath, and chronic obstructive pulmonary disease (COPD). So far some possible mechanisms have been considered for the toxicity of SM; however, there is still a great necessity to find a suitable clinically sound treatment modality to decrease chronic lung injuries caused by SM. In this study, a large number of published papers were surveyed. This review aims to discuss the therapeutic effects of MSCs in the treatment of SM-induced pulmonary injuries in both animals and humans. Owing to great advancement and accomplishment in tissue repairing using stem cells therapy (SCT), the importance of cell therapy modalities in lung injuries has increasingly been acknowledged. A number of factors as the most critical criteria should be taken into account in SCT, including types of stem cells, necessary conditions for growth and proliferation of stem cells and their homing into the target tissues. Of the stem cells, mesenchymal stem cells (MSCs) are considered as a class of multipotent stem cells, which possess proliferative and self-renewal capacity and hence can differentiate into different cell lines such as lung epithelial cells. MSCs have great potential in repairing damaged tissues. They also display immune modulatory properties, which make them as a good candidate for the regeneration of bronchioles tract in SM-exposed patients. SCT modality, unlike chemical drugs, possesses differentiation potential and a high-level of safety. Thus, MSCs can be considered as a new strategy for the treatment of SM-injured patients with pulmonary complications.
Keywords: Sulfur mustard, Mesenchymal stem cells, Lung diseases, Airway remodeling, Inflammation

IS-021. Statins as New Targets for Cancer Therapy

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Statins are well-known as cholesterol-lowering medication. They are competitive inhibitors of 3-hydroxy-3- methylglutaryl-coenzyme A reductase (HMGCR) which is the key enzyme in the mevalonate pathway. Mevalonate is the precursor of isoprenoids and cholesterol in this pathway. After statins blockage of HMGCR, the production of isoprenoid pyrophosphates (farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP)) will be abolished. Besides lowering cholesterol, this inhibits the prenylation of small Rho GTPases and blocks their translocation to the plasma membrane which results in attenuated cell growth. Therefore, statins are not only involved in lowering cholesterol biosynthesis but also are involved in the activation of small Rho GTPase. Recently, a large cohort study has been investigated about the possible benefits of statins in cancer patients in approximately 200 000 individuals and showed a beneficial effect of long-term statin use on the survival rate of patients with different types of cancers. In another investigation it has been reported a similar increase in survival time in glioblastoma multiform patients who had been taking statins for longer than one year. In the past five years, my group has shown that statins induce apoptotic cell death in a broad range of human tumor cells with different origins including breast cancer, lung cancer and brain tumor cell lines. Our investigations have shown that statins induced intrinsic apoptosis cell in all of these cells affecting GGPP. In addition, our investigations have shown that statins sensitize GBM cells to temozolomide induced apoptosis via inhibition of autophagy flux. My group continues the investigation on the possible mechanism of statins on cancer cells for possible future application of these medications as combination therapy with other chemotherapy agents in different types of cancer.
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Keywords: Statin; Glioblastoma; Temozolomide; Prenylation; Cancer therapy

IS-022. Autophagy as a Regulator of Cell Fate in Statin Targeting of Rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) which is both occurred in children and adults is an aggressive soft-tissue malignant tumor. Soft tissue sarcomas account for approximately 1% of all cancers and 10% of all childhood cancers and it is worth mentioning that RMS comprises 50% of all childhood soft tissue sarcomas. Based on clinical reports, survival among patients with metastatic RMS has not significantly improved in the past few years, emphasizing an urgent for developing new strategies to treat and prevent this disease. Statins are inhibitors of mevalonate (MEV) cascade which is involved in producing prenylation intermediates (geranylgeranyl pyrophosphate [GGPP], and farnesyl pyrophosphate [FPP]). In the past few years, there are several clinical and basic science reports focusing on the anti-cancer effect of statins. In the current work we have used human rhabdomyosarcoma cell line (RH30) (Human muscle cancer cells) and mouse muscle cell line (C2C12) to investigate the mechanism of cell death which is induced by MEV cascade inhibitor (Simvastatin:Simva). Our results showed that statin induces apoptotic cell death in both RH30 and C2C12 cells, which is depended on FPP and GGPP. Our results also showed that Simva induce differentially autophagy hallmarks in RH30 and C2C12 cells and Simva-induced apoptosis is regulated via the autophagy pathway in these cells. Our 3-dimensional (3D) RH30 and C2C12 model also showed that Simva. induced cell death involves both apoptosis and autophagy mechanism in 3D culture model.

Funding Agency: Saeid Ghavami and Shala Shojaei were supported by Health Science Foundation general operating grant. Shahla Shojaei was also supported by Mitacs Accelerate PDF. Saeid Ghavami and Joseph W Gordon were also supported by CHRIM operating grant. Simone da Silva Rosa was supported by UMGF studentship. Mohsen Akbari and Ehsan Samie thank NSERC (Discovery program) and BC Cancer Foundation for their financial support. Mohsen Akbari also thanks Canadian Foundation for Innovations and B.C. Knowledge Development Fund for supporting this project.
Keywords: Statin; Sarcoma; Prenylation; Mevalonate; 3-dimensional rhabdomyosarcoma culture

IS-023. Chimeric Antigen Receptor T Cell Therapy for Non-Hodgkin lymphoma

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Non-Hodgkin lymphoma (NHL) is the most common hematologic malignancy. Worldwide, approximately 200 000 people die of NHL every year. Recent advances in immunotherapeutic approaches for cancer have resulted in the development of very effective immunotherapies including engineered T cells expressing Chimeric Antigen Receptor (CAR) designed to bypass cancer immune evasion. US Food and Drug Administration (FDA) recently approved axicabtagene ciloleucel (Yescarta) and tisagenlecleucel (Kymriah) both CD19 CAR T cell therapies for the treatment of relapsed refractory diffuse large B cell lymphoma (DLBCL), most common NHL. Approval of these two CAR T cell therapies is the first sign of a big wave that will dramatically change the way we treat cancer in general. Here I review the advances and challenges of CAR T cell therapy for NHL.

Keywords: Chimeric antigen receptor T cell therapy; Non-Hodgkin lymphoma

IS-024. Chimeric Antigen Receptor T Cell Therapy (CAR-T) for Acute Lymphoblastic Leukemia (ALL) and Multiple Myeloma (MM)

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Multiple myeloma is the second most common hematologic malignancy. Worldwide, approximately 160 000 people are diagnosed with MM every year. Multiple myeloma is not curable with current standard treatments and almost all patients will die of their disease. ALL is the most common pediatric cancer and approximately 60 000 children and adults are diagnosed with ALL worldwide every year. It is estimated that 15 000 to 20 000 people die of ALL worldwide every year. Recent advances in immunotherapeutic approaches for cancer have resulted in the development of very effective immunotherapies including engineered T cells expressing chimeric antigen receptor (CAR) designed to bypass cancer immune evasion. US Food and Drug Administration (FDA) recently approved tisagenlecleucel (Kymriah) a CD19 CAR T cell therapy for treatment of relapsed refractory B cell ALL for patients up to age 25. Several CAR-T cell trials are currently enrolling patients with MM with very promising preliminary results. Here, I review the advances and challenges of CAR T cell therapy for ALL and MM.

Keywords: Chimeric antigen receptor T cell therapy; CAR-T cell therapy; Acute lymphoblastic leukemia; Multiple myeloma

IS-025. Trends in CRISPR Animal Genome Editing

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Background and Aim: The CRISPR-Cas9 system is a revolutionary genome editing technology which has impacted almost every field of biological research. During the past 5 years, the research community has realized that generating knock-in and conditional knockout models using the CRISPR-Cas9 system, via direct mouse zygote injections, is extremely inefficient. Even though CRISPR-Cas9 tool is very efficient in creating gene disruptions via nonhomologous end joining repair mechanism, one of the major problems this tool is the very poor ability to insert foreign sequences at the Cas9 cleavage sites via homology-directed repair (HDR) mechanism. Based on the experience of embryonic stem (ES) cell targeting methods, using double-stranded DNA mediated homologous recombination (HR), the research community tried employing dsDNA donor approaches using the CRISPR system. Even tough HR using dsDNA was successful in a very few cases at an efficiency of about 1 to 10%, it has been largely unsuccessful for many loci.
Methods: In order to solve this major challenge of the CRISPR tool (i.e., very poor efficiency of insertion of foreign DNA cassettes) we tested two different formats of guide RNA (such as in vitro transcribed single guide RNA or crRNA+tracrRNA), two forms of Cas9 (Cas9 mRNA or Cas9 protein) along with long single-stranded DNA donors to test what formats produce better knock-in efficiencies.
Results: Our results indicate that crRNA +tracrRNA + Cas9 protein along with long ssDNA donors yield highest and reliable efficiencies of inserting foreign DNA cassettes at the CRISPR cut sites. We call this method as Easi-(Efficient additions with ssDNA inserts) CRISPR. We show for over a dozen loci that *Easi*-CRISPR generates correctly targeted insertion alleles at a very high efficiency.
Conclusion: *Easi*-CRISPR solves one of the major problems of animal genome engineering, namely the inefficiency of targeted DNA cassette insertion.
In my presentation I will discuss how Easi-CRISPR has simplified the process of creating designer animal models. I will also present a few examples of designing animal models using Easi-CRISPR technology.

Keywords: CRISPR-Cas9; Easi-CRISPR; Knock-in mice; Transgenic mice; Genetic engineering

IS-026. Latest CRISPR Technologies for *In Vivo* and *Ex Vivo* Genome

Engineering

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Background and Aim: Methods of creating genetically engineered animals have been developed for over three decades. However, methods to create genetically engineered cells were not well established until the advent of programmable nucleases, particularly the CRISPR-Cas9 system. The CRISPR-Cas9 system offers as a powerful tool for both animal (*in vivo*) and cellular (*ex vivo*) genome engineering methods. Methods to create genetically engineered animals involve three major steps: harvesting embryos from one set of females, embryo microinjection (performed *ex vivo*), and transferring embryos to another set of females. Although tedious, these methods were used for over three decades to create animal models. Regarding cellular genome engineering, even though the CRISPR-Cas9 tool is very efficient in creating gene knockout cell lines, creating knock-in cell lines is still a major challenge.

Methods: We recently developed a completely in vivo method called GONAD (Genome editing via Oviductal Nucleic Acids Delivery), that by-passes the three critical steps. The method involves surgical exposure of oviducts of pregnant mice, installation of CRISPR reagents into the oviduct, followed by electroporation of the oviduct and finally suturing of the surgical incision. We further tested parameters of GONAD such a stage of pregnancy and types of CRISPR reagents. In order to solve the challenge of *ex vivo* genome editing problem, i.e., to create knock-in cell lines, we tested various CRISPR reagents formats and electrophoretic conditions along with long single-stranded DNA or double-stranded DNA donors to test what formats produce better knock-in efficiencies. Human primary T cells were used for these experiments.

Results: The results of our in vivo method development experiments indicate that the 0.7-day pregnancy and CRISPR ribonucleoprotein (RNP) complexes, such as crRNA +tracrRNA + Cas9 protein along with short or long ssDNA donors yield highest and reliable efficiencies of genome editing. The improved GONAD method (*i*-GONAD) is shown to be suitable for the routine generation of knock-out, knock-in and large-deletion models at comparable efficiencies as the microinjection-based methods. For cellular genome engineering, we show that electroporation of CRISPR RNPs along with double-stranded or single-stranded DNA can produce knocking in of DNA cassettes into human primary T cells at as high as 40% efficiency.

Conclusion: *i*-GONAD offers several advantages over the previous methods: it does not require the second set females (embryo recipients) and so also vasectomized males; the females used for *i*-GONAD can be recycled for other experiments; *i*-GONAD *can be easily adapted* at the laboratories lacking sophisticated microinjection equipment, and; *it can be performed by researchers not having embryo-handling skills*. In my presentation I will discuss how i-GONAD method has simplified the process of creating animal models (*in vivo*), and how Easi-CRISPR system can be used for cell genome engineering (*ex vivo*).

Keywords: CRISPR-Cas9; GONAD; I-GONAD; Easi-CRISPR; Transgenic mice; Knock-in Cells

IS-027. Pancreatic Islet Transplantation, Hope and Hype in Treatment of Type I Diabetes

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Type 1 diabetes (T1D) is an autoimmune disease that the immune system destroys insulin-producing pancreatic beta cells, resulted in high serum blood glucose levels with pathologic processes lead to long-term complications. Pancreatic islet transplantation has emerged as an alternative therapy for diabetic patients. This procedure does not require major surgery but still lifelong immunosuppression is required to preserve the transplanted islets from immune system attack. Regarding the vast therapeutic advantages, some practical challenges still remain that affect the wide use of this therapy. The major challenge of islet transplantation is the lack of donor for pancreatic islet. Nowadays brain-dead donors are the major source of islets for transplantation. Although non-heart-beating donors may also be introduced as potential sources for islet isolation in near future. Scientists are working massively on some other approaches such as the differentiation of beta cells from human embryonic stem cells or induced pluripotent stem cells. Many islets are destroyed in the early post-transplantation time due to apoptosis made by damage to islets during islet preparation steps. Thus, any improvements in the steps of pancreas procurement, islet isolation and culture lead to increased islet yield and/or its functional parameters. Furthermore, pancreatic islets are highly vascularized tissue with a huge capillary network. Despite the high demand of islet cells for blood supply there is a 2-week lag phase for revascularization of islet which promotes islet cell loss in this time period. Besides, another most challenging issue about islet transplantation would be the presence of both allogenic immune reactions and previously existing autoimmunity against islets which leads to allograft rejection of transplanted islets. The newly emerged works ideally choose immune-modulation or immune-isolation approach that could target both types of immune reactions. There are several strategies that may cover each of the mentioned problems of islet transplantation, but a combinatory approach may be the ideal solution to shift this therapy to the routine treatments for diabetes.

Keywords: Type 1 diabetes; Islet transplantation; Blood supply; Allograft rejection

IS-028. Overcoming Challenges in the Cellular Therapy Laboratory

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Bone marrow transplantation (BMT) is the treatment of choice for many malignancies and genetic disorders. However, the necessary condition regimens for BMT and the transplant graft itself often leave patients susceptible to viral infection, relapse, and graft-versus-host disease, which are the three biggest causes of mortality post-transplant. Here we will discuss three novel modalities aimed at treating these three complications. We will then evaluate the challenges in moving beyond BMT, what logistical and technical challenges we encounter while trying to manufacture various cellular therapies for clinical use, and finally what solutions we have devised to overcome these challenges.

Keywords: Bone marrow transplantation; Viral infection

IS-029. Cord Blood-Derived Virus-Specific T Cells After Cord Blood Transplantation

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Cord blood transplantation is an alternative method of stem cell transplantation that can be curative for some malignancies and genetic disorders. However, the necessary conditioning associated with the transplant renders patients susceptible to viral infections, such as those from CMV, EBV, and adenovirus. Our group and others have shown that virus-specific T cells are an attractive option for the treatment or prophylaxis of viral infection, while the expansion of virus-specific T cells is challenging when the donor is not seropositive for the virus. Here, we will discuss a new approach to expanding virus-specific T cells from the naive T cell population that is predominant in cord blood. We will discuss new methods of expanding these cells as well as their clinical safety and potential efficacy.

Keywords: Cord blood-derived virus-specific T cells; Cord blood transplantation

IS-030. Chemical Control of Human Pluripotent Stem Cell Renewal

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Background and Aim: The large-scale and cost-effective production of quality-controlled human pluripotent stem cells (hPSCs including human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells) for use in cell therapy and drug discovery would ideally require a chemically defined xenobiotic-free culture system. Towards the development of such a system, costs associated with the use of recombinant proteins as supplements in basal culture media and culture substrate need to be reduced.
Methods: In a hypothesis-driven small chemical library screening approach following prediction of hPSC self-renewal mechanisms based on comprehensive genome-wide gene expression analysis, we have identified novel signaling cascades and chemical compounds that regulate hPSC self-renewal and differentiation. Utilizing the chemical compounds, we have developed a growth-factor-free culture medium that uses just three chemical compounds and a lower number of recombinant proteins than used in a commercially available medium. In addition, by utilizing polymers, we have developed protein substrate-free 3D suspension culture method for hPSCs.
Results: In the culture system, all examined human ES and iPS cell lines could be expanded robustly. These cells maintained their undifferentiated state markers expression and differentiation capacity to derive all major cell types without modification of well-established differentiation methods. Furthermore, the culture system could support the generation of iPS cell derivation from either human dermal fibroblasts or peripheral blood mononuclear cells.
Conclusion: Utilizing these compounds and polymers, we have been developing defined hPSC culture system for their applications. Our findings should facilitate the ongoing development of a completely xeno-free, chemically defined, synthetic culture system for hPSCs.
Keywords: Human pluripotent stem cell; ES cell, iPS cell; Chemical compound; Polymer; Culture system

IS-031. Development of Sustainable Malaria Plasmodium vivax Lever Stage Model

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Background and Aim: Vivax malaria is a global health issue challenged by undetectable dormant forms in the liver responsible for multiple relapses. The absence of suitable models of hepatocytes permissive to *Plasmodium vivax* infection is responsible for the paucity of knowledge for this type of infection and relapse mechanisms and unsuccessful efficient drug development. In addition, genetic diversions and variable infectivity, vector susceptibility and latency periods, which is evident across spatiotemporal geographical locations, skews infectivity studies conducted without accounting for its geographical epidemiology. To overcome these hurdles, we aim to develop a robust *in vitro* liver-stage assay by utilizing malaria patient-derived induced pluripotent stem cells (iPSCs) as a source of donor-specific hepatocytes and *P. vivax* sporozoites obtained from the same geographical location.
Methods: We undertook 5 processes; (1) Collection of blood from *P. vivax* mono-infected patients and generation of induced pluripotent stem cells (iPSCs) from the blood cells, (2) Production and characterization of hepatocytes differentiated from the iPSCs, (3) Production and isolation of *P. vivax* sporozoites from mosquitoes fed on *P. vivax* patient blood through membrane feeding, (4) Infection of the iPSC-derived hepatocytes with the sporozoites and detection of *P. vivax*, and (5) Evaluation of the infectivity and optimization of *P. vivax* liver stage assay with known antimalarial drugs.
Results: We have driven 3 iPSC lines each 3 malaria patients and 1 non-patient. We have developed a new method of efficient hepatocyte differentiation by monitoring hepatic gene expression, protein expression, enzyme functions as well as expression of malaria entry molecule expression. In a combination of the hepatocytes and *P. vivax* sporozoites produced in Anopheles mosquitos, we could develop a liver stage model. In this model, *P. vivax* could infect into hepatocytes and form structures as potential exoerythrocytic forms although the infectivity in our assay is still low as same as other models, and the infectivity of hepatocytes derived from patients' iPSCs and control ESCs or non-patient iPSCs are not significantly different
Conclusion: Although there was no significant infectivity different between patient and non-patient samples, this assay would be highly reproducible and cost-effective in a study of donor-specific drug responses and screening large compound libraries, and would pave way for the development of drugs targeting liver-stage malaria.
Keywords: iPS cells; Malaria *P. vivax*; Malaria liver stage assay

IS-032. Mandibular Reconstructions Using 3D-Printed Constructs, Synthetic Bone Substitutes, and Expanded Stem Cells

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Background and Aim: Despite the existence of isolated reports on the hard-tissue reconstruction in the craniomaxillofacial skeleton, multi-patient case series are lacking. Here, we will review the experience of craniomaxillofacial hard-tissue defects at four anatomically different sites, including (i) frontal sinus, (ii) cranial bone, (iii) mandible, and (iv) nasal septum.
Methods: Autologous adipose tissue was harvested from the anterior abdominal wall. The adipose-derived stem cells were cultivated, expanded, and then seeded onto the resorbable scaffold materials for subsequent reimplantation into hard-tissue defects. The defects were reconstructed by means of either bioactive glass or β-tricalcium phosphate scaffolds seeded with the adipose-derived stem cells (ASCs), while in some cases, recombinant human bone morphogenetic protein-2 was added. The production and use of ASCs were performed according to good manufacturing practice (GMP) guidelines. The follow-up time periods ranged from 12 to 52 months.
Results: We witnessed successful integration of the construct to the surrounding skeleton in 10/13 (76%) cases. Two cranial defect cases, where nonrigid resorbable containment meshes were used, showed sustained bone resorption to the point that they required the procedure to be redone. One septal perforation case failed outright after 1 year because of the postsurgical resumption of the patient's uncontrolled nasal picking habit.
Conclusion: These results will be discussed in a broader view to highlight problems encountered, optimizations possible, and future directions.
Keywords: Three-D printing; Regenerative medicine; Adipose stem cells

IS-033. Nanotechnology, 3D-Printing and Regenerative Medicine: Synergistic Technologies for Bone Tissue Reconstructions?

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Background and Aim: Personalized reconstructive scaffolds used in the treatment of traumatic, congenital, and maxillofacial skeletal deformities or post-tumor resection defects should ideally mimic the structure to be reconstructed, but also preferentially condition the neighboring

environment to activate the regeneration process and/or to eradicate residual tumor cells in case of tumor resections.
Methods: The structural and dimensional requirements may be met by using additive manufacturing technologies ("3D printing"). Conditioning of the environment within the scaffold or the neighboring tissue may be accomplished by controlled release of regeneration-activating agents such as growth factors and cytokines, but also anti-tumor agents such as chemotherapeutic or small interfering RNA (siRNA).
Results: Nowadays, these environment-affecting agents can be effectively packaged in nanoparticles, which can subsequently be incorporated within 3D-printed scaffolds, either or not combined with regenerative cells.
Conclusion: Thus, these "smart" scaffolds can be regarded as individualized treatment modalities that can be fine-tuned according to the needs of the defect to be reconstructed. We will discuss the state-of-the-art, challenges to be met, and future directions.
Keywords: Additive manufacturing; Bone reconstruction, Nanotechnology

IS-034. Development of a Novel Intra-operative Tissue Engineering Concept Using Freshly Isolated Adipose Stem Cells and Synthetic Bone Substitutes

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Background and Aim: The sources of cells used in regenerative medicine can vary. Of the cells used, stem cells or progenitor cells (regeneration-competent cells) have been recruited from the tissue surrounding the implanted tissue construct, or can be harvested from well-known stem cell sources such as bone marrow, skin, muscle, and adipose tissue. Adipose tissue has several advantages over the commonly used bone marrow, such as easy accessibility, minimal morbidity upon harvest, and an abundant, clinically relevant stem cell number which potentially eliminates the need for in vitro expansion. Based on this, we have formulated an innovative surgical concept, the so-called one-step surgical procedure. This concept seems to be cost-effective and beneficial to the patient. In this line, the optional use of expensive recombinant growth factor(s) is reduced to a minimum, and a second surgical intervention is avoided while expensive contamination-sensitive stem cell expansion and specialized laboratories are not required. In addition, this concept might reduce donor site morbidity resultant from the graft harvesting.
Methods: In a parallel process, the surgeon performs the normal surgical procedure while the tissue engineer processes the adipose tissue and procures the stromal vascular fraction (SVF) containing the adipose stem cells (ASCs). Either or not, the SVF can be stimulated fora n ultrashort period (10-30 minutes) with a physiological dose (10 ng/mL) of an inducing factor such as BMP-2. The nucleated cells within the SVF are counted, and subsequently seeded on a suitable carrier such as calcium phosphate bone substitutes, and implanted for tissue regeneration.
Results: After extensive in vitro optimization of the various steps within the concept, the procedure has been verified in vivo for multiple applications, i.e. regenerative strategies for bone regeneration including spinal fusion and maxillary bone augmentation, articular and intervertebral disc cartilage repair, ear and nose reconstructions after burn wounds, and also for cardiac repair after acute myocardial infarction.
Conclusion: The intra-operative procedure is feasible, safe, and shows efficacy in the various animal models, and can be applied in human trials.
Keywords: Adipose stem cells; Regenerative medicine; Animal model

IS-035. Photopheresis, Indications and Mechanisms of Action

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Graft-versus-host disease (GvHD) is a frequent complication of allogeneic hematopoietic stem cell transplantation (HCT). Its severity and frequency depend on many factors such as HLA compatibility, age and gender of donor and patient, bone marrow or peripheral blood or cord blood stem cell origin and its dose, conditioning and prophylactic regimens. The acute GvHD (aGvHD) occurs in about 60-to 80%, and chronic GvHD (cGvHD) occurs in about 50% of patients under standard immunosuppressive therapies and is responsible for the high rate of morbidity and mortality. The extracorporeal photochemotherapy (ECP), so-called photopheresis, is an immunomodulating cell therapy, where the own mononuclear cells of the patient are reinjected to the patient after an ex vivo exposure to the UVA light in the presence of a DNA intercalating agent such as 8-methoxy psoralene (8-MOP). This treatment modality was first used in the treatment of cutaneous T-cell lymphoma (CTCL) successfully. Then, it was examined in a variety of T-cell depending pathologies, including GvHD. Because of the efficacy and safety of ECP, the number of treated patients has been dramatically increased worldwide in three main indications, including acute graft rejection, GvHD, and CTCL. The other indications of ECP are the autoimmune diseases such as rheumatoid arthritis, systemic sclerosis, SEL, *Pemphigus vulgaris*, multiple sclerosis, erosive lichen planus, generalized extensive lichen planus, diabetes, and refractory Crohn's disease. Numerous clinical trials of GvHD treatment by ECP have already been published indicating its safety and efficacy. Mechanisms of action of ECP are not well known. Induction of apoptosis is one of the major consequences of ECP and the reinjection of these apoptotic cells to the patient is the main factor of immunological changes and therapeutic effects of ECP and induction of tolerance by increase of the T regulatory cells (CD4 CD25hi), and shift in the cytokine pattern (increase of IL10, IL4, TGF beta and decrease of pro-inflammatory cytokines.
Keywords: Graft-versus-host disease; Photopheresis; Hematopoietic stem cell transplantation

IS-036. Molecular Approach in Control of Cell Death in Embryonic Stem Cell Differentiation

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We aimed to investigate whether mitochondrial apoptosis and specification employ a common pathway due to various evidence that indicates that the differentiation and apoptosis might share common features. Split luciferase complementary assay showed that delay in apoptosome complex formation contributes to mouse embryonic stem cells cardiogenic differentiation. The contribution of apoptosis hallmarks to human embryonic stem cells (hESCs) cardiogenic differentiation has also shown. Our results indicate the involvement of delayed mitochondrial apoptotic pathway as well as reversible mitochondrial outer layer disintegration in cardiogenic differentiation. We have also found that upon moderate doxorubicin apoptosis induction, hESC showed differentiation hallmarks. Further investigation showed that low level of Apaf-1 expression during early stages of neural differentiation can be considered as a possible regulatory barrier by which differentiating cells control cell death upon a rise in ROS (reactive oxygen species) elevation and cytochrome *c* release from mitochondria. We will show how molecular approach in control of cell death can support human embryonic stem cells during differentiation.

Keywords: Embryonic stem cells; Cell differentiation; Apoptosis

IS-037. An Artificial Intelligence Approach to Develop Tunable Nanoparticulate Delivery Systems for Regenerative Medicine Applications

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Background and Aim: Regenerative medicine plays a definitive role to treat diseases once thought difficult to treat; however, the creation of successful protocols toward widespread clinical application is very challenging. Efforts have been launched to use artificial intelligence (AI) to accumulate skills and experiences of world-leading researchers to ensure quality, low-cost and widespread regenerative therapy. The recent AI applications include: controlled differentiation of iPS cells, cell therapy outcome prediction, and tissue scaffold biofabrication optimization. This study highlights the potential of AI to develop tunable nanoparticulate delivery systems for tissue engineering applications.
Methods: Artificial neural networks and genetic algorithm were applied to fabricate and optimize PLGA nanoparticles with respect to size, zeta potential, polydispersity, loading capacity and release profile. A mechanistic model coupled with genetic algorithm was developed to predict the structural and release characteristics of PLGA/PLLA core-shell bilayer nanoparticles for spatiotemporal control of growth factor release. The nanoparticles were prepared based on the conditions suggested by the AI-based models. Then, the nanoparticles were employed to control co-release of VEGF and bFGF and sequential release of PDGF. Based on the endothelial sprouting assay, the angiogenic response of the nanoparticles was assessed in methacrylated collagen hydrogel with respect to the co-release of VEGF and bFGF, sequential release of PDGF, and the release of VEGF alone. The novelty of this study is associated with the distinct potential of the proposed AI-based approach for rate-modulating PLLA/PLGA nanoparticles for tissue engineering applications.
Results: The developed AI-based models successfully predicted the nanoparticle fabrication conditions which led us to achieve pre-defined growth factor release profiles. The predictability of the AI-based models was confirmed by experiments. Notably, as predicted by the AI-based models, the release lag phase of the core-shell nanoparticles was followed by zero-order release kinetics, which is essential for time-delayed release of growth factors. A combination of growth factor-loaded nanoparticles, which were designed using the AI-model, successfully provided different release scenarios of VEGF release only, VEGF, bFGF and PDGF co-release, and sequential release of PDGF. The sprouting angiogenesis, based on the rat aortic ring assay, indicated a significant difference in angiogenic response between the three release scenarios, suggesting that the designed nanoparticulate delivery system was able to regulate the predefined growth factor release patterns to promote angiogenesis in methacrylated collagen hydrogel.
Conclusion: The proposed Geno-Neural approach offers great potential for the design and optimization of PLLA/PLGA-based nanoparticles for preprogramming of various release profiles for tissue engineering applications including angiogenesis in hydrogel scaffolds. The hybrid AI-model can elucidate the release mechanism of growth factors from core-shell nanoparticles. The AI-based model suggests that despite relatively rapid PLGA core degradation, PLLA shell predominantly controls the overall release from PLLA/PLGA core-shell nanoparticles. The proposed AI-models can be employed for tuning growth factor release from bilayer PLLA/PLGA nanoparticles.
Keywords: Artificial intelligence; Nanomedicine; Controlled release; Tissue engineering; Regenerative medicine; Angiogenesis

IS-038. Towards safe Stem Cell-Based Regenerative Medicine: Implementation of 3D Bioprinting and Microfluidic Systems

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Background and Aim: Stem cells (SCs) are the frontline regenerative medicine source. Efforts are being made to found stem cell-based regenerative medicine onto tissue engineering technologies; however, the safety and immunotolerance of the engineered implants remain critical issues for clinical applications. Ideally the engineered tissue grafts should not only support cell adhesion/ingrowths but also eliminate harmful dividing SCs, be nonimmunogenic and support angiogenesis. This presentation attempts to highlight the significance of Fail-Safe and immuno-cloaking systems combined with microfluidic systems and 3D-bioprinting technologies to address these issues.
Methods: Based on the protocol developed in the Nagy's Lab (Liang Q, et al. A solution for cell therapy safety. *Nature*. In press), the Ganciclovir (GVC)-inducible suicide system was generated for human embryonic SCs (hESCs). The Fail-Safe system was evaluated using the teratoma assay in NSG mice. The Fail-Safe hESCs were genetically manipulated to become immune-cloaked through the modulation of eight genes involved in immunoresponse. The Fail-Safe immuno-cloaked hESCs were differentiated into functioning cardiomyocytes and endothelial cells (ECs). Relying on microfluidic tools, a microvasculature-on-a-chip was developed to create a perfused network of endothelialized microchannels connected to a blood flow mimicking medium containing human peripheral blood mononuclear cells (PBMCs). Integrated with two-photon confocal microscopy imaging, the microfluidic device enabled the real-time assessment of cloaking behavior of ECs. The ECs were bioprinted into 3D hybrid nano-reinforced hydrogel scaffolds made of methacrylated collagen and alginate followed by characterizing the scaffold physical and biological characterization. Also, the cloaked cardiomyocytes functionality on the bioprintable hydrogel was assessed.
Results: The GCV treatment of the Fail-Safe derived teratomas in NSG mice resulted in stabilizing teratoma size. The cloaked ESCs were successfully differentiated into cloaked ECs (and cardiomyocytes) confirmed by flow cytometry analyses, immunohistochemistry and functional assays. The microchannels of the PDMS-based microfluidic device with the vasculature-like network were coated with ECM components which promoted EC attachment and maturation to form perfused lumens *in-situ*. The real-time qualitative/quantitative evaluation of the endothelialized microfluidic device revealed a significantly lower PBMC attachment and EC death for cloaked ECs. The differentiated ECs and cardiomyocytes exhibited positive tube formation and contractility when grown on methacrylated collagen hydrogel. The cloaked cells were successfully bioprinted into a hybrid scaffold consisting of methacrylated collagen and alginate. Electrical and mechanical properties of the 3D scaffold were improved by modulation of bioprinting pattern and incorporation of functionalized carbon nanotube into the hydrogel.
Conclusion: The hESC-based Fail-Safe system enabled us to arrest dividing stem cells in NSD mice. Our real-time assessment of the interaction between human PBMCs and cloaked cells in the microfluidic device showed that the cloaked ECs and cardiomyocytes exhibited immunotolerance behavior. The successful implementation of bioprinting of cloaked cells offers great promise to create 3D hydrogel scaffolds which take advantage of the allogenic tolerance technology for a safe regenerative therapy.
Keywords: Bioprinting; Cardiomyocyte; Endothelial; Immune tolerance; Microfluidic system; Stem cell therapy

IS-039. In Vivo Reprogramming as a New Avenue to Regenerative Therapies in Demyelinating Diseases

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Recent investigations demonstrate that astroglial cells may be directly converted into functional neurons and oligodendrocytes. Here, we report that single transcription factor Sox10 as well as the chemicals could reprogram astrocytes into oligodendrocyte-like cells, *in vivo*. For trans-

differentiation, GFP expressing viral particles caring the coding sequence of TFs were injected into cuprizone-induced demyelinated mice brains, after which we analyzed the presence of specific oligodendrocyte lineage cell markers in the transduced cells using immunohistofluorescence (IHF). As a control, another group of demyelinated mice received GFP expressing viral particles. For chemical conversion, astrocytes were exposed to the candidate compounds and then immediately transplanted to demyelinated mice brains. After weeks, the majority of transduced/treated (GFP+) cells in animals which received control vector were positive for oligodendrocyte lineage markers. We also used human astrocytes or extracted primary astrocytes from mouse pups and purified them. Human or primary astrocytes were used for confirming the results in vitro. These data suggested a master regulatory role for Sox10 which enabled astrocytes to change their fate to OPC-like cells and establish an oligodendroglial phenotype. We also mentioned that chemicals could do in vivo conversion, which promises for the safety of future approaches. We believe this proposed approach might lead to effective myelin repair in patients suffering from myelination deficit.

Keywords: In vivo reprogramming; Demyelinating disease; Reactive astrocytes; Oligodendrocytes lineage cells; Trans-differentiation

IS-040. Comparative Analysis of Different Media Condition for Growth & Maintenance of Induced Pluripotent Stem Cells

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Background and Aim: The discovery of induced pluripotent stem cells (iPSCs) had a great impact on fundamental stem cell studies and diseases modeling; moreover, iPSCs have provided a new perspective on precision and regenerative medicine. Accordingly, several types of xeno-free and feeder-free media and matrixes were developed in the past decade to support culturing of iPSCs. All these media allow continuous growth of pluripotent stem cells (PSCs) in an undifferentiated state representing powerful tools to improve our understanding of disease mechanisms and develop therapies to treat these diseases. Currently, international standards for the validation and characterization of PSCs are based on expression of self-renewal and pluripotency markers. However, in our view there is a lack of in-depth molecular information regarding the true character and uniformity of PSCs.

Methods: To do this, we are reprogramming three different human healthy dermal fibroblasts using CytoTune iPS 2.0 Sendai Reprogramming Kit. Next, transduced fibroblasts will be plated on different matrixes and feed with different feeder & feeder-free media. After the emergence of iPS colonies, four colonies from each condition will be picked using TRA-1-60 Kit for Live Cell Imaging and propagated till passage 8 and will characterize according to general characterizations standard. To gain more insight into differences or similarities between xeno-free and feeder-free media, we will perform comparative transcriptome and methylome analysis of three clones per each fibroblast line and reprogrammed and grown under different media conditions selected based on their characterization and absence of Sendai virus. We recently developed a simple but thorough method to study DNA methylation in detail (Boers et al, 2018). This MeD-seq technology is based on a methyl-dependent restriction enzyme LpnPI, which recognizes 50% of all methylated CpGs, and generates 32bp fragments for sequencing. LpnPI activity is blocked by a short template size preventing over-digestion of densely methylated regions.

Results: We recently applied MeD-seq to compare fully and partially reprogrammed iPSCs, and using unsupervised clustering easily cluster the partially reprogrammed iPSCs apart from fully reprogrammed iPSCs. This underscores the power of this new technology that will now be applied in conjunction with RNA sequencing to interrogate effects of different media conditions on the epigenome and transcriptome.

Conclusion: This study might shed light on the quality of each culture condition and its consequent ability to generate stem cells suitable for use in regenerative medicine and disease modeling.

Keywords: Reprogramming; Induced Pluripotent stem cells; LpnPI; MeD-seq

IS-042. Diagnostic and Therapeutic Application of Menstrual Blood Stem Cells: Direction Toward Clinic

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The source of stem cells, in order to maximize the safety and efficacy of tissue engineering and cell-based therapies, is clearly of great importance. Nowadays, the menstrual blood has been introduced as a non-invasive source of mesenchymal stem cells (MSCs) with several advantages, including (i) easy accessibility without the need for anesthetic, (ii) renewability as they can be sourced on a monthly basis, (iii) high proliferative capacity in culture without inducing genetic abnormalities, and (iv) non-ethical concerns. We showed that the isolated menstrual blood stem cells (MenSCs) have higher proliferative and self-renewal capacities than other MSCs like bone marrow stem cells (BMSCs) and can modulate the inflammatory reaction. Moreover, MenSCs could differentiate into multiple mesodermal and occasionally endodermal and ectodermal lineages, but with different capacity depending on lineage type compared to other MSCs like BMSCs. We also indicated typical repair and/or restoration ability of MenSCs compared to BMSCs in animal models with myocardial infarction, acute liver failure, and cartilage defects without using immunosuppressant. These data make MenSCs extremely attractive and useful for the stem cells therapy even in allogeneic cellular therapies and open a wide perspective of potential clinical applications to other stem cell sources. At present, some clinical research groups including us and companies launched clinical trials using these cells. Based on the preliminary results, these cells sound to be safe and efficient through cell therapy of some disorders like congestive heart disease, multiple sclerosis, Asherman syndrome in women and so on. Another interesting idea is MenSCs banking in order to treatment of probable disorders in especially post-menopause age. Since these cells are well tolerated, with no toxicity or any adverse side effects report, banking and holding this source of stem cells can be suitable for clinical use. Therefore, it sounds that MenSCs banking has a vast scope in the future and is the next big thing in the medical world. Beside clinical application, the possible role of MenSCs as a diagnostic tool especially in the pathogenesis of endometriosis endometrial hyperplasia and endometrial cancer has been suggested. These finding can be achieved through the study of MenSCs obtained from women with pregnancy disease in comparison to MenSCs isolated from donors with uncomplicated pregnancy history. Future research and new evidence would greatly propose MenSCs as a novel and best diagnostic tool in complicated pregnancy. Therefore, MenSCs are extremely attractive and useful for the diagnosis of reproductive disorders and also stem cells therapy even in allogeneic cellular therapies and open a wide perspective of potential clinical applications to other stem cell sources.

Keywords: Menstrual blood; Stem Cell; Differentiation; Cell Therapy; Regenerative medicine; Clinical application

IS-043. Differentiation of Human Adipose Stem Cells in Filament-Like Hydrogel Through Visible Light-Initiated Crosslinking

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Background and Aim: Hydrogel fibers are structurally and biologically useful devices for fabrication of filament-like tissues and differentiation

of stem cells. We established cell-laden degradable hydrogel fibers through visible light-initiated crosslinking to differentiate stem cells and fabricate filament-like tissue.
Methods: The human adipose stem cells (hADSCs) cell-laden fibers were fabricated by cross-linking phenolic-substituted alginate and gelatin (Alg-Ph and Gela-Ph respectively) in an aqueous solution containing cells through irradiating visible light. The crosslinking of phenolic moieties was mediated by ruthenium (II) tris-bipyridyldication (Ru (II) bpy and sodium ammonium persulfate (SPS).
Results: The encapsulated hADSCs proliferated and grew within cell-laden hydrogel fiber. The encapsulated cells maintained their pluripotency ability and could form filament-like tissue. The filament-like tissues covered with an additional heterogeneous cell layer was made by degrading the fiber membrane using alginate-lyase after covering the fiber surface with vascular endothelial cells. The cellular viability is preserved during Alg-Ph and Gela-Ph hydrogel fiber fabrication and filament-like tissue formation.
Conclusion: These results demonstrate the feasibility of alginate-based hydrogel fibers obtained through the Ru/SPS-mediated crosslinking system and visible light irradiation for the engineering of filament-like tissues.
Keywords: Alginate and gelatin derivative hydrogel; Fiber; Visible light irradiation; Filament-like tissues; Biodegradation

IS-044. The Future of Stem Cells in Liver Diseases

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Background and Aim: The liver is the main organ for multiple metabolic pathways and many metabolic and genetic diseases affect primarily hepatocytes. On the other side, infectious diseases like hepatitis B and C can damage liver cells permanently and lead to liver cirrhosis. Liver cirrhosis is a critical medical problem with high morbidity and mortality and liver transplantation is the only acceptable treatment, which affects life quality dramatically. We have investigated the feasibility, safety, and efficacy of using autologous mesenchymal stem cells for the treatment of liver cirrhosis and potential usage in ex vivo therapy for metabolic diseases.
Methods: Eight patients were included in this study after they approved informed consent. Four have hepatitis B, one hepatitis C, one alcoholic, and two cryptogenic liver cirrhosis. All patients have a Model for End-Stage Liver Disease (MELD) score more than ten and liver cirrhosis was confirmed by histology. Bone marrow from each patient was taken from his/her iliac crest and autologous mesenchymal stem cells were isolated. Approximately, 30-50 million MSCs were proliferated and injected into peripheral or the portal vein under ultrasound guide. Liver function tests (e.g., ASAT, ALAT, Bilirubin, alkaline phosphatase) and other liver-related tests like albumin and INR were measured. Clinical symptoms were evaluated at baseline and one, two four, eight and 24 weeks after injection of mesenchymal stem cells.
Results: All patients tolerate treatment without any side effect and hypersensitivity reaction. Liver function improved. MELD score decreased from 17.9+/-5.6 to 10.7+/-6.3 ($P<0.05$), prothrombin complex from international normalized ratio from 1.9+/-0.4 to 1.4+/-0.5 ($P<0.05$). Serum creatinine changed from 114+/-35 to 80+/-18 µmol/L ($P<0.05$). Serum albumin changed from 30+/-5 to 33+/-5 g/L and bilirubin from 46+/-29 to 41+/-31 µmol/L. No adverse effects were noted. All patients had very good psychological intentions.
Conclusion: Our data show that autologous mesenchymal stem cells injection is a potential treatment of end-stage liver disease with satisfactory tolerability and may improve clinical indices of liver function in end-stage liver disease. Since mesenchymal stem cells are considered immunologically privileged, and they have the ability to differentiate to many cell types, they are one the best candidates for ex vivo therapy for single gene and metabolic disease.
Keyword: Stem cells; Liver diseases

IS-045. Novel Insights in Cell Microencapsulation Technology for Mass Production of Stem Cells and Modular Tissue Formation

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To provide clinically enough cell populations for different cell therapies, *ex vivo* mass production of stem cells should be carried out to treat patients with different cancers and other disorders. Microencapsulation of stem cells for their mass production using hydrogels can offer several advantages such as the creation of defined and designed 3D microenvironments to modulate stem cell phenotype, protect from hydrodynamic forces and prevent agglomeration during cell expansion. Microcapsules can also prevent direct contact of encapsulated cells and immune cells after *in vivo* implantation in the host system. Furthermore, the small size of microcapsules allows facile diffusion of nutrients, oxygen, metabolic waste, and therapeutic agents secreted by microencapsulated cells (the ideal size of microcapsules to encapsulate cells is ~400 µm). Recently, an engineering concept called as "bottom-up" approach is emerging, wherein microstructure modular tissues using stem cells are prepared first as building blocks and then assembled to fabricate macrotissues, leading to great promise for generation of 3D constructs with no limitation in size by the assembly of modular tissues. A potential alternative for building blocks can be microcapsules with diameters less than 500 µm. Many features of the micro-hydrogels such as swelling, mechanical properties, degradation, biochemical, and diffusion can be modulated through a lot of processing conditions. Indeed, to effectively use microcapsules for cell cultures, it is desirable to use tissue-specific ECM with desired biomechanical and biochemical properties. In this presentation, we will discuss novel insights concerning architecture of microcapsule composition as well as the systems which enable greater freedom to change microcapsule cores for mass production and survival enhancement of encapsulated cells, recreating 3D microenvironment that mimics the natural tissue. The results of our group regarding mass production of hematopoietic and hepatocyte model stem cells as well as modular bone tissue formation by using microencapsulation technology will also be reported. Finally, we will argue that microcapsules can be considered as an efficient 3D platform to prepare tissue building blocks as well as mass production of stem cells in dynamic cultures.
Keywords: Cell microencapsulation; Stem Cells; Modular tissue formation

IS-046. Kotozukuri' for Regenerative Medicine– Social Development of Regenerative Medicinal Technology

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Regenerative medicine including cell therapy has been developed toward general therapy with the industrial progress of the regenerative medicinal technology. It needs to pursue this endeavor as 'Koto-zukuri (Social development for novel technology)', including three critical factors: 'Mono-zukuri (Manufacturing)' – developing novel techniques; 'Rule-zukuri' (Rule-booking) – making rules aimed at standardization, and 'Hito-zukuri' (Human resource development) – nurturing human resources. It's extremely important to progress the novel technology with these factors in parallel.
In Japan, medical doctors have an allowance to make health care services under Medical Practitioners Act & Medical Care Act, leading double tracks for health care services (approved service with National Health Insurance and non-approved service without National Health Insurance). Based on the background, in 2013, Japanese government make the additional acts for regenerative medicine; Revised Pharmaceutical Affairs

Law (Revised PAL, PMD Act) and Regenerative Medicine Safety Act (RM Safety Act).
PMD Act is applied for clinical trial and approved therapy, similarly to approval tracks for the drug and medical device products by setting the new category for regenerative medicinal products and the approved products are used for therapy with public healthcare insurance. Moreover, in the category of the regenerative medicinal product, the conditioned approval track is prepared to realize fast marketing with the post surveillance to clarify the product efficacy. On the other hand, RM Safety Act is set for clinical researches and non-approved therapy to keep the safety for application of regenerative medicine, which is checked by "certificated committee for regenerative medicine". The Japanese Society for Regenerative Medicine (JSRM) make a big effort to make Hito-zukuri and Rule-zukuri for the stakeholders, including patients (recipients), volunteers (public), basic researchers (creators), medical doctors (conductors) and operators (supporters).
In cell manufacturing, as it is known that the serial processes influence the quality of the cells, the processes in the the appropriate cell processing facility is expected not only to maintain an aseptic environment but also to lead to stable processing, considering the characters of these products such as raw materials that cannot be sterilized. I made the concept of "Cell manufacturability" as follows; Manufacturing design of cell-based products in such a way that they are easy to manufacture through simple, safe and efficient (cost-saving) process with stable product quality and secure to the customer (especially patients) by considering transpiration and hospital preparation outside the factory. This concept will lead to facilitation of the consistency and robustness for the process as well as the reduced cost for the cell manufacturing. In addition, it is necessary to consider whether the risk is manageable not only from the viewpoint of the facility but also from the viewpoint of manufacturing operation such as performance evaluation. Through the national consortium project, JSRM starts the new certification system for cell processing operator and makes the video contents as well as guidance for cell processing using aseptic technique to standardize the operation.
These developments described above improve the status of 'Kotozukuri' in Japan for regenerative medicinal technology.
Keywords: Regenerative medicine; Social development for cell manufacturing; Human resources development; Certification system for cell processing operator; Guidance for cell processing, Cell manufacturability

IS-047. Flexible Modular Platform for Cell Manufacturing

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Expansion culture of stem cells, which is the most critical steps to realize the transplant of cells for cell therapy and regenerative medicine, are accomplished regarding the safety and cost-saving under the aseptic environment in the cell processing facility (CPF). In this line, the triple issues of "safe, cost-saving and secure" (3S) are desired for the industrialization, in large part because of keeping the cost down for cell processing and stability of the final products. Given that the serial processes may affect the quality of the cells, the processes in appropriate CPF is expected not only to maintain an aseptic environment but also to provide stable processing. The "design for manufacturability (DFM)" is long known to be the general engineering approach for the designing products in an easy to manufacture manner. This concept exists in almost all engineering disciplines, but the implementation differs widely depending on the manufacturing technology. The cell production has many features in processes, I would like to make the concept of "cell manufacturability" as follows; Manufacturing design of cell-based products in such a way that they are easy to manufacture through simple, safe and efficient (cost-saving) process with stable product quality and secure to customer (especially patients) by considering transpiration and hospital preparation outside factory. This concept can lead to facilitation of the consistency and robustness for the process and the reduced cost for the cell manufacturing. The system consists of input and output for the process. There are several fluctuations derived from extrinsic noises (environmental errors) against the system, input quality such as starter cells and materials (medium, reagents, vessel and pipet etc.), and intrinsic disorders (in-process errors). Especially, intrinsic disorders cause the difficulty to make consistency and robust process for stable quality because the cells have uncertainty accompanied by time-dependent and tardive properties. Therefore, environmental, material, and operational standardizations are required to realize a consistent process. The cell processing in a closed aseptic chamber can be done using a cell processing isolator system, which can reduce the equipment and maintenance/operation costs and also provide a reliable aseptic environment. A new design of manufacturing facility has been suggested based on the isolator technology. Our proposal system is the flexible modular platform (fMP) that may be seen as the individual aseptic modules that can connect and disconnect between modules (or pods) flexible with keeping the aseptic environment in each module (or pods). Such approach can result in the compactness of aseptic processing area and quick change-over for multi-purposes and patients. To efficiently implement such fMP technology, an interface that can be aseptically detached and attached from one module to another is required, responding to diversified requirements for the cell processing. The interface of the double door system is developed for the flexible connections between modules with shortening of the decontamination process. Thus, our attempts are concluded to build an advanced culture system employing isolator technology as well as large-scale culture in suspension of iPSCs, and the adaptation of the fMP in CPF will lead to easy installation of the new modules for production line addition and/or revision through the clinical phases as well as commercial production, which contributes to the reduction of production costs.
Keywords: Stem cells; Cell processing facility; Cell manufacturability; Flexible Modular Platform; Automation

IS-048. Oligodendrocyte Progenitor Cell Dysfunction in Familial Schizophrenia

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Background and Aim: While schizophrenia is highly heritable, its underlying pathophysiology is yet to be fully understood. The strongest known determinant for developing schizophrenia is family history. Recent genetic and induced pluripotent stem cell (iPSC)-based studies have converged on a model by which neuronal function, and in particular synaptic transmission, is a major pathophysiological mechanism of schizophrenia. However, functional neuronal alterations may arise either by direct cell-type autonomous changes to neurons themselves, or indirectly through a primary pathophysiological influence on other cell types that influence neuronal function.
Methods: We implemented a family-based approach for genetic discovery in schizophrenia combined with functional analysis using induced pluripotent stem cells (iPSCs). Genetic analysis: Linkage and Copy Number Analysis was performed with Illumina HumanCytoSNP-12v2 chip arrays using an affected-only model with an assumption of 99.9% penetrance. Whole-genome exome sequencing was performed twice: initially at 40x, and again at 90x coverage. Exome variants were considered for additional validation if they were rare (MAF < 0.001), predicted to alter coding sequence (missense, nonsense, frameshift, essential splice site), and occurred within the regions of suggestive linkage. Cellular studies: Human iPSCs were differentiated to NPCs and neurons by embryoid body neural differentiation. Electrophysiology was performed in whole cell patch-clamp configuration after 8-10 weeks of differentiation. iPSC-derived OPCs were differentiated according to Monaco et al with modifications. Biotinylation of cell surface proteins was adapted from Huang et al. OPC viability was assessed by quantitative

fluorometric monitoring of resazurin conversion to resorufin. Myelination assay was performed using ex vivo organotypic cerebral cortex slices of Shiverer mice.

Results: We observed familial segregation of two rare missense mutations in Chondroitin Sulfate Proteoglycan 4 (*CSPG4*) (c.391G>A [p.A131T], MAF 7.79 $\times 10^{-5}$ and c.2702T>G [p.V901G], MAF 2.51 $\times 10^{-3}$). The *CSPG4*^{A131T} mutation was absent from the Swedish Schizophrenia Exome Sequencing Study (2536 cases, 2543 controls), while the *CSPG4*^{V901G} mutation was nominally enriched in cases (11 cases vs. 3 controls, P = 0.026, OR 3.77, 95% CI 1.05-13.52). CSPG4/NG2 is a hallmark protein of oligodendrocyte progenitor cells (OPCs). IPSC-derived OPCs from *CSPG4*^{A131T} mutation carriers exhibited abnormal post-translational processing (P=0.029), subcellular localization of mutant NG2 (P=0.007), as well as aberrant cellular morphology (P=3.0 $\times 10^{-8}$), viability (P=8.9 $\times 10^{-7}$), and myelination potential (P=0.038). Moreover, transfection of healthy non- carrier sibling OPCs confirmed a pathogenic effect on cell survival of both the *CSPG4*^{A131T} (P=0.006) and *CSPG4* ^{V901G} (P=3.4 $\times 10^{-4}$) mutations. Finally, in vivo diffusion tensor imaging of *CSPG4*^{A131T} mutation carriers demonstrated a reduction of brain white matter integrity compared to unaffected sibling and matched general population controls (P=2.2 $\times 10^{-5}$).

Conclusion: The results obtained in this study revealed a great consistency with the growing body of evidence, which implicate the integrity of white matter in schizophrenia neuropathology. In fact, recent findings demonstrating the myelination of parvalbumin-positive GABAergic interneurons, which are arguably the most well-established neuronal cell type implicated in the pathophysiology of schizophrenia, may raise an intriguing possibility that schizophrenia might be initiated by neurodevelopmental alterations of PV interneuron myelination. Taken all, these results provide a convergence of genetic and functional evidence to implicate OPC dysfunction as a candidate pathophysiological mechanism of familial schizophrenia.

Keywords: Schizophrenia; Induced pluripotent stem cells; Genetics; Exome sequencing; Oligodendrocyte progenitor cells; Myelination

IS-049. Leveraging Human Induced Pluripotent Stem Cells (iPSCs) to Investigate the Pathophysiology of Mental Illness

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Background and Aim: The interactions among neurons and astrocyte are very important during both early neurodevelopment and in the adult brain. Accordingly, the presence of astrocytes significantly improves the functional maturation of human pluripotent stem cell-derived neurons. Presently, most experiments introduce non-human sources of astrocytes via co-culture using human induced pluripotent stem cells (iPSCs)-derived neurons. Nevertheless, differentiation protocols, which are based on common progenitor giving rise to both neurons and astrocytes, appear to proceed more similarly to *in vivo* neurodevelopment. Having capitalized on the latter approach, we now report on the development of a simplified differentiation protocol for deriving functionally mature neuronal networks from iPSCs.

Methods: Human iPSC-derived NPCs (passages 5–11) were plated on sterile coverslips and coated with poly-L-ornithine. One fully confluent 10 cm dish of NPCs was divided over a 12-well plate. A 100 µL drop of NPC cell suspension was placed on the laminin-coated spot for 1 h to allow for attachment of NPCs on coverslips in neural differentiation medium (Neurobasal medium, 1% N2 supplement, 2% B27-RA supplement, 1% minimum essential medium/non-essential amino acid, 20 ng/ml brain-derived neurotrophic factor, 20 ng/mL glial cell-derived neurotrophic factor, 1 µM dibutyryl cyclic adenosine monophosphate, 200 µM ascorbic acid, 2 µg/ml laminin and 1% penicillin/streptomycin). Cells were refreshed with medium 3 times per week. During weeks 1–4, the medium was fully refreshed. After 4 weeks of neural differentiation, only half of the volume of medium per well was refreshed. Electrophysiology and confocal imaging were performed between 8 and 10 weeks after plating of NPCs.

Results: Our protocol produces a consistent 60:40 ratio of neurons and astrocytes, which arise from a common forebrain neural progenitor. Whole-cell patch-clamp recordings of 114 neurons derived from three independent iPSC lines confirmed their electrophysiological maturity, including resting membrane potential (–58.2 ± 1.0 mV), capacitance (49.1 ± 2.9 pF), action potential (AP) threshold (–50.9 ± 0.5 mV) and AP amplitude (66.5 ± 1.3 mV). Nearly 100% of neurons were capable of firing APs, of which 79% had sustained trains of mature APs with minimal accommodation (peak AP frequency: 11.9 ± 0.5 Hz) and 74% exhibited spontaneous synaptic activity (amplitude, 16.03 ± 0.82 pA; frequency, 1.09 ± 0.17 Hz).

Conclusion: In the present study, we provide a simplified differentiation protocol for the generation of electrophysiologically mature iPSC-derived neuronal networks with no need for astrocyte co-culture or specialized media. Furthermore, these results provide a quantitative basis for considering the variability of distinct electrophysiological parameters for the modeling of iPSC-based disease. This protocol is envisioned to be a considerable utility for implementing cellular modeling approaches towards the study of human neuropsychiatric disease pathophysiology.

Keywords: Human pluripotent stem cells; Neurons; Astrocytes; Synaptic transmission; Electrophysiology

IS-050. Evaluation of Safety of Intradiscal Implantation of Nucleus Pulposus Derived Chondrocytes in Regeneration of human Degenerated Lumbar Disc in Patients with Chronic Low Back Pain, a Phase 1 Clinical Trial

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Background and Aim: Chronic low back pain is one of the most common causes of disability in all societies. Even after invasive treatments, about 70% to 80% of patients will have recurrent pain. Disc degeneration commonly occurs due to changes in disc morphology composition of the extracellular matrix as well as loss of disc cells, therefore, a potential therapeutic strategy would be the augmentation of the disc cell population to restore normal biologic function and matrix insufficiencies. Mesenchymal stromal cells (MSCs) are a source of such cells for regeneration of disc. Our goal in this phase 1 clinical trial was to evaluate the safety of post-endoscopic surgery implantation of nucleus pulposus derived chondrocytes.

Methods: Patients with chronic low back pain who did not respond to conservative treatments for at least 6 months of treatment and who were candidates for endoscopic disc enucleation, were enrolled in this clinical trial. Patients underwent endoscopic disc enucleation surgery. During the operation, the protruded part of the nucleus pulposus, was removed and this sample of nucleus pulposus was sent to the Royan Institute clean room for culturing chondrocytes. One month later, and upon arrival the number of chondrocytes reached a limit of 20 million chronocities per disc, these cells were reimplanted. Patients were evaluated for side effects according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. They were followed up 3, 6, 12 and 24 months after implantation of chondrocytes.

Results: Five patients were included in this clinical trial, including 1 female and 4 men. The mean age and average BMI were 40.3-year and 25.5 kg/m^2, respectively. Meantime when patients had low back pain was: 14 ± 1 months. They were followed up for two years after chondrocytes implantation. No side effects were seen at this period of time. Baseline visual analogue scale (VAS) and Oswestry low back pain questionnaire (ODI) values and follow up data are shown in the Table. The pain of all the patients was reduced according to VAS and

ODI questionnaire 6 months after implantation of chondrocytes, and this decrease remained two years after implantation. According to the results of MRIs, the amount of disk protrusion decreased compared to pre-implantation and remained the same for two years after implantation **Conclusion**: In this clinical trial, we investigated the safety of intradiscal implantation of autologous nucleus pulposus derived chondrocytes for the patients suffering from chronic uncontrolled low back pain. According to the results of this treatment, it can be concluded that treatment of chronic low back pain using of chondrocytes derived from the autologous intervertebral disc, is safe, which has had very good effects on our patient's outcomes. To further investigations for evaluating this implantation efficacies, this treatment should be taken on a larger number of patients and with longer time for follow-up.
Keywords: Intradiscal implantation; Nucleus pulposus cells; Chondrocytes; Clinical Trial

IS-051. Plerixafor Induces Safe and Efficient Mobilization of Hematopoietic Stem Cells in Sickle Cell Disease Patients in the Perspective of Gene Therapy

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Sickle cell disease is characterized by chronic anemia and vaso-occlusive crises, which eventually lead to multi-organ damage and death. Allogenic bone marrow transplantation is the only curative treatment but it is limited by toxicity and poor availability of HLA-compatible donors. A gene therapy approach based on the autologous transplantation of lentiviral-corrected bone marrow hematopoietic stem was shown to be efficacious. However, alterations of the bone marrow environment and properties of the red blood cells hamper the harvesting and immunoselection of patients' stem cells from bone marrow. The use of G-CSF to mobilize large numbers of peripheral blood stem cell has been associated with severe adverse events in sickle cell patients. Thus, the gene therapy approach requires the development of alternative safe and efficient mobilization methods. We have conducted a clinical trial to assess the safety and the efficiency of a single injection of Plerixafor to induce a high mobilization of hematopoietic stem cells with previous red blood cell exchange to decrease the hemoglobin S level in sickle cell mobilized patients. Large numbers of $CD34^+$ cells were mobilized extremely quickly without any severe adverse effects. Importantly, the collected cells expressed high levels of stemness genes, and engrafted very efficiently in immunodeficient mice. These results open up a new possibility for gene therapy in the context of SCD.
Keywords: Plerixafor; Hematopoietic stem cells; Sickle cell disease; Gene therapy

IS-052. Gene Therapy in Patients with Severe Hemoglobinopathies: The French Experience

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Donor availability and allogeneic transplantation risks limit the use of allogeneic bone marrow transplantation in patients with severe β-thalassemia or sickle cell disease. Transplantation of autologous, genetically corrected hematopoietic stem cells represents an alternative therapy for patients lacking a compatible BM donor. The safety and efficacy of transplantation of autologous hematopoietic stem cells transduced ex vivo with a Lentiviral β-Globin vector which encodes adult hemoglobin (HbA) with a T87Q amino acid substitution (HbA^{T87Q}) have been studied through prospective studies. We obtained autologous CD34+ cells from severe β-thalassemia and sickle cell patients. The in vitro modified cells were then reinfused after the patients had undergone myeloablative busulfan conditioning. Efficacy assessments included levels of total hemoglobin and HbA^{T87Q}, transfusion requirements, and average vector copy number. We subsequently monitored adverse events, vector integration, and levels of replication-competent lentivirus. Patients begin to produce the HbT87Q already 1 month after genic therapy and they reach significant levels after 6 months. Patients with long follow-up have been free of chronic transfusions. Treatment-related adverse events were typical of those associated with autologous stem-cell transplantation. No clonal dominance related to vector integration was observed. Promising results of GT in β-thalassemia major and SCD are now available.
Keywords: Gene therapy; Hemoglobinopathies; French Experience

IS-053. Reciprocal Regulatory Loop Between Akt and miRNA301 - Relevance for Tumor Progression

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Micro-RNAs (miRs) have emerged as a new category of genes with regulatory function towards the expression of other genes, mainly at various levels of mRNA maturation, stability and translation. The anomalous expression of a number of miRs has recently been associated with different types of cancers. We have investigated the functional interactions between selected miRs and major pro-survival signaling pathways. We have observed that miR301 inhibition influences PI3K-Akt pathway activity. The overexpression of Akt in MCF7 and MDAMB468 cells caused downregulation of miR301 expression. This effect was confirmed by co-transfection of miR301-modulators in the presence of Akt. Cells overexpressing miR301-inhibitor and Akt, exhibited an increased migration and an enhanced proliferation. Experimental findings also validated that the PI3K, PTEN and FoxF2 could be the regulatory targets for miR301. Furthermore, Akt expression in conjunction with miR301-inhibitor increased nuclear accumulation of PTEN, thus preventing it from downregulating the PI3K-signalling. In summary, our findings highlight the significance of miR301 inhibition on PI3K-Akt pathway-mediated cellular functions. As a result, we envision that it opens new paths for the development of new anti-cancer agents favorably with the potential of targeting the PI3K-Akt pathway.
Keywords: miRNA301; PI3K-Akt pathway

IS-054. Generation of (Corneal) Limbal Cells from Somatic Cells-Comparison of Reprogramming and Transdifferentiation

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Currently used regenerative medicine technologies provide great possibilities for the *in vitro*-production of organs and tissues and their therapeutic replacement. A number of up-to-date methods offer a wide range of natural and synthetic biomaterials to be used as the scaffolds fulfilling the biomechanical requirements for tissue engineering. The cellular component of tissues and organs is typically individually made from the recipient's own cells, and hence, the histocompatibility is impeccably matched – an approach so-called isograft-type transplantation. In the eye, a small pool of tissue stem cells located at the limbus are responsible for maintaining the corneal epithelium. However,

this pool of stem cells may get depleted through certain injuries or diseases, which may result in the visual impairment. To resolve this issue, several standard treatment modalities can be applied, including autologous or allogeneic limbal stem cell (LSC) transplantation. However, the graft rejection and chronic inflammation have been shown to limit the success rate of such procedure over a long time. To date, the use of transdifferentiation technology and induced pluripotent stem (iPS) cells have paved the way towards new treatment possibilities against various diseases with patient-specific cells, eliminating the risk of immune rejection. In the past couple of years, various protocols have been established in order to differentiate the iPS cells into the corneal epithelial lineage through mimicking the environmental niche of limbal stem cells. Nevertheless, most applications from the lab into the clinic have been hindered because of the risk of teratoma formation associated with the use of iPS cells. Our group has developed an optimized protocol for the differentiation of iPS cells into the corneal epithelial cells. Having used such protocol, the obtained cells displayed the expression of corneal epithelial markers showing a successful differentiation. However, the process used appeared to be time-consuming while the level of gene expression for the pluripotency markers did not completely disappear. Therefore, we developed a direct transdifferentiation method to avoid the intermediate state of pluripotency (iPS-stage). The cells attained by the direct transdifferentiation of fibroblasts into limbal cells showed the morphology of the corneal epithelial cells and also expressed the key markers of these cells. Our findings confirmed that the transdifferentiation protocol can efficiently perform in comparison with other reprogramming approaches with the subsequent differentiation into corneal limbal cells. Taken all, the direct transdifferentiation of human dermal fibroblasts into the corneal epithelial lineage is proposed to serve as a reliable source for corneal epithelial cells for the transplantation.

Keywords: Limbal cells; Somatic cells; Induced pluripotent stem

IS-055. Evaluating of Safety and Efficacy of Autologous Adipose Tissue-Derived Mesenchymal Stem Cells (AMSC) Transplantation in Women with Premature Ovarian Failure

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Premature ovarian failure (POF) is a clinical syndrome defined by loss of ovarian activity before the age of 40 years. POF is characterized by menstrual disturbance (amenorrhea or oligomenorrhea) with raised gonadotrophins and low estradiol. The prevalence of POF is 1%. Population characteristics such as ethnicity may affect the prevalence. Primary ovarian insufficiency affects 1 in 10000 women by age 20, 1 in 1000 women by age 30, 1 in 100 by age 40. The diagnosis POF is based on the presence of menstrual disturbance and biochemical confirmation. The diagnostic criteria are (i) oligo/amenorrhea for at least 4 months, (ii) and elevated FSH level >25 IU/L on 2 occasions >4 weeks apart. The causes of POF include genetic disorder (chromosomal abnormality, fragile-X permutation), autoimmune, metabolic abnormalities, infections, surgery, radiotherapy – chemotherapy and idiopathic. The side effects of POF are: cardiovascular disease, reduce bone mineral density, negative impact on psychological well-being and quality of life, reduced life expectancy, largely due to cardiovascular disease and Infertility. The infertility treatments of POF patients are (i) egg donation, and (ii) renewal of ovarian tissue. The survival and function of oocyte require their interaction with granulosa cells, and the number or activity of granulosa cells. The number of newly formed adult primordial follicles is determined by the availability of granulosa cell nets. In ovaries with POF, degenerating intravascular oocytes and ovarian cortical granulosa cell nets are virtually absent. This indicated that the availability of transplanted germ cells in adult human ovaries lacking granulosa cell nets may not be sufficient for follicular renewal. Stem cells are the foundation cells for every organ and can differentiate into the different type of cell. Stem cells are self-renewable undifferentiated cells capable of differentiation and divide into specialized cells. Ovarian stem cells are bipotent stem cells for the formation of ovarian germ and granulosa cells. Many researchers have reported that therapeutic stem cells derived from different sources (human endometrial stem cell, menstrual-derived stem cells, Human placenta-derived mesenchymal stem cells, bone marrow-derived stem cells) can generate oocytes in female rats or mice. Based on previous experimental results in animals, we designed a study to show the safety and efficacy of autologous adipose tissue-derived mesenchymal stem cells (AMSCs) injection into the ovary in women with POF patients. To evaluate the safety and efficacy of autologous adipose tissue-derived mesenchymal stem cells (AMSC) in women with POF. Nine POF patients were scheduled for AMSC transplantation due to inclusion and exclusion criteria. AMSC preparation from the adipose tissue-derived lower abdominal wall, vaginally or laparoscopically injected into the ovary of POF patients. Safety and possible side effects, FSH and AMH levels and return menstrual cycle were evaluated.

Keywords: Efficacy; Autologous; Adipose tissue; Mesenchymal stem cells; Transplantation; Premature Ovarian Failure

IS-056. Patient-Derived Liver Organoids in Study of HCC

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Hepatitis B virus (HBV) infection is the leading cause of hepatocellular carcinoma (HCC) worldwide. However, due to its species and organ specificity, and limitations in model systems to study the virus, the molecular events that drive HBV-mediated HCC remain unclear. We have established and characterized 3D human liver organoids as a primary platform to model HBV infection-induced tumorigenesis. Liver organoids are seeded from infected patient liver biopsies and used in the molecular and functional analysis. We find that patient-derived liver organoids resemble their primary tissue of origin, and display an early cancer gene signature, presenting a patient-specific in vitro platform for disease surveillance and drug discovery.

Keywords: Liver organoids; Hepatocellular carcinoma

IS-057. Pediatric GVHD: An Overview of Cutaneous Manifestations

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Cutaneous graft-versus-host disease (GvHD) as a complex disease process is marked by both acute and chronic alterations. Our knowledge on the basic mechanisms triggering this condition continues to evolve. In fact, the clinical manifestations of this disease are largely dependent on the interplay between inflammation and fibrosis that needs to be fully comprehended. It appears that cutaneous manifestations of GvHD vary in the pediatric population. Further, evaluation by a dermatologist with knowledge of the spectrum of GvHD appearances might be helpful in terms of making a prompt diagnosis. While steroids remain as the first line medication modality to control the disease, a number of investigations are underway to categorize alternative treatment modalities to manage this condition, in particular within the pediatric population. Given that much more investigations into this disease and its management are concluded, it seems that our knowledge of its underpinnings might allow us to provide GvHD patients with more effective treatment regimen in order to circumvent/control the long-term sequelae of this condition.

Keywords: GVHD; Cutaneous manifestations

IS-058. An Overview of Quality, Standards and Accreditation in Bone Marrow Transplantation

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Background and Aim: The HSCT sector has been a pioneer in the area of healthcare quality, standards and accreditation. From the mid-1990s onwards, several organizations in the United States and Europe developed initiatives to improve quality and safety of this complex therapeutic intervention based on standards and voluntary accreditation processes. AABB, FACT and JACIE have set the benchmark and internationally are regarded as the reference organizations.

Methods: Standards are developed by professionals working day-to-day in HSCT. They are evidence-based as much as possible and where this is not feasible, the consensus is reached on the most appropriate approach. More generic standards focus on installations, staff education and skills, quality management, patient and donor management and care while more technical requirements cover specific aspects of the process e.g. stem cell storage, transportation, administration of the product. Accreditation is a tool or mechanism that nowadays is widely accepted in healthcare as a means to drive quality improvement and adherence to standards. Accreditation is usually based on an application process requiring presentation of documentation followed by an on-site visit by auditors or assessors, usually drawn from the professional community (peer-review), who verify compliance with the Standards. The assessors issue a report which is normally reviewed by a dedicated expert group or committee. The committee issues a recommendation on any actions that the center should implement in order to achieve accreditation. JACIE is based in the EBMT offices in Barcelona, Spain from where applications and inspections are managed. Training is also provided both in-person and currently small-scale online content.

Results: Key factors in the success of accreditation in HSCT have been that (i) it is driven by the professional community, (ii) there is evidence of a positive impact on patients and donors, (iii) it complements regulatory requirements, and (iv) it is positively viewed by regulatory agencies. Since 2000, JACIE has received 418 applications from transplant programmes or facilities in 33 countries in Europe, Asia, South America and Africa. Over 350 have been inspected and more than 326 accreditation awards have been issued. JACIE accreditation is also recognized by regulators in 7 European countries and features in numerous guidelines. JACIE inspectors now number 289. Threats to the sustained success of this model are persistent perceptions of an over-bureaucratic process that generates substantial paperwork, ever-expanding requirements and the not insignificant costs required to maintain compliance during accreditation cycles. One initiative to address this issue is the Stepwise project which aims to make the accreditation process more accessible for centers in low- to medium-income countries by breaking the requirements into graded steps.

Conclusion: The HSCT community is to be congratulated for its proactive focus on quality and safety which in fact pre-dates many subsequent initiatives in areas of medicine. In order to avoid complacency, the community must continue efforts to follow and reflect developments in practice so that the model remains relevant and a driver of excellence.

Keywords: Quality; Accreditation; Standard; Transplantation

IS-059. Addition of ATG to the Conditioning Regimen in Unrelated Donor Hematopoietic Stem Cell Transplantation

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Hematopoietic stem cell transplantation (HSCT) from an unrelated donor (URD) is a suitable option for patients in need of a transplant when a sibling donor is not available. Thanks to the recent advances in different aspects of transplantation medicine, such as improvements in the criteria of donor selection, well-tolerable conditioning regimen and progress in managing post-transplantation complications, Outcomes of URD-HSCT have improved considerably. Conditioning regimens are administered as part of the pre-transplant treatment to achieve 2 goals: provide ample immunoablation to prevent graft rejection and eradicate the resistant tumor cells as much as possible. The conditioning regimens include chemotherapeutic agents most commonly, cyclophosphamide, based on its antineoplastic and immunomodulatory properties. Because of the high probability of developing acute graft versus host disease (GvHD) with URD-SCT, anti-thymocyte globulin (ATG), is often administered as a part of the conditioning regimens for these transplants to reduce this risk of GvHD. ATG is a polyclonal immunoglobulin raised in rabbits or horses against the Jurkat T cell line. These antibodies are directed against lymphocyte surface molecules such as CD2, CD4, CD8, CD5, CD3, CD18, CD11a, CD45, and HLA-DR and are effective in destroying human leukocytes. For the First time, it was reported that rabbit ATG resulted in a significant reduction of acute and chronic GvHD but with an increase in serious infection. Afterward, many studies have investigated the precise effect of ATG on the incidence of GvHD, relapse and infection. It has been shown that the Addition of ATG to the conditioning regimen, could reduce long-term immunosuppressive use. Also, the decline of chronic GvHD has been reported in many studies. While reducing the risk of developing GvHD, administration of ATG preserves the anti-leukemia effect of the URD HCT However, a very recent study demonstrated the increased use of blood products in patients received ATG-conditioning regimen, compared to alemtuzumab- conditioning regimen. Another disadvantage of ATG administration mentioned in a study, is Epstein-Barr virus reactivation. But this problem can be solved by pre-emptive or prophylactic treatment with rituximab. Overall, considering the result of all studies in this field, ATG administration in conditioning regimen, associated with favorable outcome.

Keywords: ATG; Graft-versus-host disease; Prophylaxis; Conditioning

IS-060. Allogeneic Hematopoietic Cell Transplantation for Chronic Lymphocytic Leukemia

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Background and Aim: In the recent years, the treatment options of chronic lymphocytic leukemia (CLL) have literally been revolutionized with the invention and use of pathway inhibitors (PWIs), including ibrutinib, idelalisib, and venetoclax. These treatment modalities have altered the standard treatment of CLL, which have also challenged the role of allogeneic hematopoietic cell transplantation (allo-HCT). Nevertheless, the optimum dose, duration, long-term efficacy and toxicity as well as of these agents, are not yet fully defined. A significant proportion of patients discontinue their treatment over time because of intolerance. Moreover, the disease is either refractory or progresses after a short period of time in a proportion of patients. Thus, in such cases, the prognosis of the disease might be dismal while allo-HCT could represent the treatment of choice. The allo-HSCT modality can impose a long-term control of disease with curative potential in the case of CLL, particularly with reduced-intensity conditioning (RIC) and overcomes the poor prognostic impact of 17p- and fludarabine-refractoriness. In this study, the current applications of allo-HCT as a treatment option for the CLL is presented.

Results: In the case of CLL, the disease might be considered as the high-risk issue if one/more of the following conditions are met: (i) disease refractory to purine analogues; (ii) disease relapsing within 2 years after the chemoimmunotherapy (CIT); and (iii) disease with deletion and/or mutation of the TP53 gene. The CIT-resistant patients outlooks seem to be markedly improved by the use of PWIs. In fact, the current findings support the notion of a combined approach with PWI and allo-HCT, which can be used either before the transplantation to reduce tumor burden, or after the transplantation to treat the relapse of the disease. Altogether, several studies on the RIC allo-HCT in CLL have highlighted the progression-free

survival (PFS) and overall survival (OS) rates of 50%-60% and 60%-75%, respectively, at 2 years. At 5 years, the rates were 35%-45% and 45%-65%, respectively. After allo-HCT, the long-term follow-up studies report 10-year PFS of approximately 30%. In a large registry study conducted by the European Society for Blood and Marrow Transplantation (EBMT) as well as in the prospective CLL3X trial, the PFS rate at 10 years post-allo-HCT was about 79% for the patients who passed the 5-/ 6-year landmark event-free. Approximately, 30% of all transplanted patients might durably benefit from a "targeted" GVL effect, while the TP53 abnormalities have not been associated with an inferior outcome after RIC-allo-HCT in most of the studies. A refractory disease at allo-HCT seems to be the most important risk factor for an adverse transplant outlook. As a result, patients, who have been transplanted in remission might have a better outcome with PFS rates of 55%-65% at 2 years. In addition to disease-related risk factors, the patient- and procedure-related variables (e.g., sex mismatch, age, donor type, T cell depletion and performance status) and the center experience might determine the outcome of the allo-HCT. The early-death rate of CLL allotransplants appeared to be less than 5% by means of the modern transplantation approaches. The good tolerability of RIC-allo-HCT might allow application of the procedure in older cases and patients with comorbidity. However, the non-relapse mortality (NRM) may increase to over 40% at 2 years post-transplant in patients with adverse patient-, donor- and procedure-related risk factors, in large part because of the graft versus host disease (GvHD)-related problems. Nevertheless, in patients with a combination of favorable risk factors at allo-HCT, the 2-year risk of NRM reduces to 12%. Apart from its impact on NRM, chronic GvHD is the major determinant affecting the quality of life after allo-HCT. About 25% of survivors will experience impaired quality of life during the first post-transplant years because of chronic GVHD. A recent study by the EBMT has confirmed the significant impact of using ibrutinib as a bridge to transplant strategy indicated the principle of association between PWI and allo-HCT. Besides, in the untransplanted patients, the prognosis of CLL relapse post-transplant seemed not to be inferior to that of high-risk CLL. The advent of PWIs (e.g., ibrutinib) has considerably improved the treatment options of the high-risk CLL progressing after the allo-HCT. Accordingly, some recent data propose that the safety and efficacy of ibrutinib given for CLL relapse after allotransplant are as good as in the untransplanted patients. Furthermore, Th1-mediated GVL effects may be enhanced by ibrutinib if given on a donor chimerism scenario. Accordingly, the outcome of post-transplant relapse in CLL patients has significantly improved. Open issues of allo-HCT in the PWI era are largely related to the interactions of PWI and transplantation. Some emerging evidence has accentuated that PWI can safely bridge the CIT-refractory patients to transplant. In addition, the use of PWI for the prevention of early disease recurrence post-transplant warrants thorough studies.

Conclusion: In comparison with what can be achieved using other treatment approaches, the use of an allogeneic SCT in CLL must weigh up both the risks of the morbidity of the transplant and its outcome. As the currently available novel agents become increasingly used in combination and earlier in the disease course, the allo-HCT might be beneficial for patients who fail or are intolerant or even do not have access to these novel agents. Taken all, the optimal timing of the allo-HCT continues to be of the focus of some ongoing clinical investigations, which seems to be the basis of randomized prospective clinical trials.

Keywords: Allogeneic hematopoietic cell transplantation; Chronic lymphocytic leukemia

IS-061. Diagnosis, Incidence and Treatment of Chronic Graft-Versus-Host Disease

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After an allogeneic hematopoietic stem cell transplantation (SCT), the leading cause of non-relapse mortality and morbidity is the emergence of the chronic graft-versus-host disease (cGvHD). Further, the incidence of cGvHD has markedly dramatically increased by an increased use of peripheral blood as a graft source. At the moment, over 50% of SCT recipients have been shown to develop this multisystem inflammatory disease, which happens late after the bone marrow transplantation. the predominant and diagnostic organ pathologies that develop are usually cutaneous and/or pulmonary despite the fact that cGvHD can affect almost any target tissue. As for most diseases with fibrotic manifestations, unfortunately, no satisfactory therapy is available for the cGvHD currently. Standard primary treatment is glucocorticoids with or without other immunosuppressive agents. Nevertheless, almost nearly 50% of patients continue to have inadequate control of their cGvHD and require second-line systemic treatment. Besides, systemic glucocorticoids are wrought with long term-complications and undesired issues, and hence, increasing morbidity and mortality in this patient population that are otherwise cured of their original malignancy.

Our understanding of the pathophysiology of cGvHD has substantially been advanced by the recent surge of preclinical and clinical studies. As a result, we now identify cGvHD as a complex immunologic process that associate with multiple facets of adaptive and innate immunity such as B cells, T cells, and macrophages and their interactions with the target tissue. Prominently, these studies have resulted in the recognition and classification of targetable cellular and molecular mediators of cGvHD. Such findings might broaden our choice of potential new therapeutics to manage these patients.

Recent advances in the immunobiology of cGvHD provides a great possibility based on the strategically targeted therapeutics that grants us with much more effective prevention and treatment options for controlling the cGvHD. While a number of the newly identified targetable pathways are only in the conceptual stage, repurposing of existing reagents should allow the rapid assessment of efficacy in well-designed clinical trials. Such endeavor might hopefully build effective medical armamentariums to be use alone or in combination with trivial toxicity and side effects, which should hopefully be truly steroid-sparing or steroid-free therapies for cGvHD. We review here the incidence of cGvHD in patients receiving allogeneic SCT, its causes and risk factors as well as available therapies including those recently introduced in clinical trials phases.

Keywords: Graft-versus-host disease; Clinical trials; Allogeneic hematopoietic cell transplantation

IS-062. Formative Biofabrication of Tissue and Organ Constructs Using Magnetic, Acoustic and Magneto-Acoustic Levitational Bioassembly

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Background and Aim: Since demonstration levitation of frogs in the high magnetic field the magnetic levitational bio-assembly started to attract the growing interest of several research groups around the world. Magnetic as well as acoustic fields are working in this case as a temporal and removable support or some sort of scaffold which we suggested it to be called "scaffield". The formative bio-fabrication is a new emerging research field based on using magnetic and acoustic fields. Our aim was to advance formative bio-fabrication using scaffields.

Methods: Chondrospheres bio-fabricated from chondrocytes have been used for magnetic and acoustic levitational bio-assembly experiments. Custom-designed magnetic and acoustic devices have been employed. Experiments with high magnetic field have been performed in The European High Magnet Field Laboratory (HMFL) at Nijmegen, The Netherland. The paramagnetic medium Gadolinium has been used in the magnetic levitation experiments. Mathematical modeling has been used to design the shape of magnetic and acoustic fields. The viability of tissue constructs was estimated using standard cytotoxicity tests. Levitational bio-assembly registered with the camera.

Results: Four groups of experiments have been performed, including (i) magnetic levitation in the low magnetic field, (ii) magnetic levitation in the

high magnetic field, (iii) acoustic levitation and (iv) combined magneto-acoustic levitation at the low magnetic field. Chondrospheres have been used for magnetic and acoustic levitation. It has been demonstrated that in all four cases the employed physical fields both magnetic and acoustic fields were not toxic for living cells, tissue spheroids and resulted in tissue constructs. Reproducible magnetic and acoustic levitational bio-assembly of 3D tissue constructs from tissue spheroids have been achieved. Moreover, in all experiments tissue spheroids in the construct manifested tissue fusion. Employment of high magnetic field allowed to reduce in 100 times concentration of paramagnetic media enabling magnetic levitation and containing relatively toxic Gadolinium. Combination of magnetic and acoustic fields allowed the first time to assemble from tissue spheroids the 3D tissue construct of more complex geometry in the form of a ring.

Conclusion: Our data demonstrate a principal feasibility of magnetic, acoustic and magneto-acoustic levitational bio-assembly of tissue engineered constructs from tissue spheroids. The bio-assembled constructs were not only viable but also manifested tissue fusion process. It is logical to speculate that using formative bio-fabrication it will be possible to very rapidly assemble tissue engineered tubes, branched tubes and, eventually, even the complex vascularized 3D tissue and organ constructs. Formative bio-fabrication is a very promising technology and opens new frontiers in bio-fabrication research.

Keywords: magnetic levitation, acoustic levitation, magneto-acoustic levitation, tissue spheroids, formative biofabrication

IS-063. Development of Technological Platform Enabling 3D Bioprinting of Functional and Vascularized Endocrine Human Organs

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Background and Aim: Organ printing could be defined as an automated robotic layer by layer additive bio-fabrication of 3D tissue and organ constructs using living cells and biomaterials (bioink) according to a digital model. The endocrine organs do not have ductal systems and anatomically they are much more simple then complex human organs such as lung, liver or kidney. The aim of the study was the development of a technological platform enabling 3D bioprinting of human endocrine organs such as ovary, thyroid gland and pancreatic islets.

Methods: The developed bioprinting platform technology includes several key components. At first, we have developed biocompatible bioink ("Viscoll") from viscous porcine collagen with a high level of printability of bioprinted scaffold. Secondly, we developed original multifunctional 3D bioprinter "Fabion" with capacity for continuous bioprinting of viscous collagen and digital bioprinting of one tissue spheroid a time. Organ-specific tissue spheroids have been bio-fabricated either by using cell suspension of insulin-producing cells generated from human IPS cells (in case of endocrine part of the pancreas) or by rounding microdissected explants of human organs (in case of the ovary and thyroid gland). Endothelial tissue spheroids have been bio-fabricated from human umbilical vein endothelial cells. Organ constructs have been bio-fabricated using original multifunctional 3D bioprinter "Fabion" and they have been additionally coated by fibrin spraying. Bioprinted organ constructs have been transplanted on chick and quail chorioallantoic membranes for sequential vascularization and testing their organ-specific functionality.

Results: The microdissected explants of human ovary and thyroid gland have been rounded into tissue spheroids and demonstrated a high level of viability. Insulin-producing tissue spheroids have been fabricated from human cells generated from human IPS cells. Printed scaffolds from viscous porcine collagen "Viscoll" have shown a high level of printability and fidelity. Tissue spheroids have been precisely placed into pores of printed scaffolds according to digital model using specially designed microfluidic device which was an integral part of multifunctional 3D bioprinter "Fabion". Bioprinted organ constructs have been additionally coated with sprayed fibrin hydrogel. For *in vivo* testing bioprinted constructs have been transplanted into chick and quail chorioallantoic membranes. Organ constructs maintained their viability and have been successfully vascularized by host vasculature. Pancreatic, thyroid and ovarian bioprinted organ constructs demonstrated their functionality by producing correspondent hormones such as insulin, thyroxine and estrogens. The fertile function of the ovarian construct has been tested *in vitro*.

Conclusion: Our data demonstrate the principal feasibility of bioprinting of functional and vascularized human endocrine organs such as ovary, thyroid gland and endocrine part of the pancreas using developed platform technology. Although testing of bioprinted organ constructs on birds is very cost effective and justifiable, the clinical translation of technological platform will require additional pre-clinical testing on mammalian animals such as nude mice or rats. The ethical issues related to testing of the fertile function of bioprinted human ovary must be also addressed.

Keywords: Bioprinting; Tissue spheroids; Bioink; Ovary; Thyroid gland; Pancreas

IS-064. Immune Cell Composition and Graft-Versus-Host Disease

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Allogeneic hematopoietic stem cell transplantation (AHSCT) has revolutionized the curative approaches of malignant and non-malignant hematological disorders. The grafted cells are an amalgam of stem cells, T cells, B cells, natural killer (NK) cells, and other immune and stromal cells that can be beneficial or injurious based on the purpose of treatment. One of the most serious and pernicious complications of AHSCT is unwanted priming of grafted allogeneic-T cells against the host's tissues leading to graft-versus-host disease (GvHD) and has a high rate of incidence, morbidity, and mortality. A wide range of approaches to control the GvHD are currently under research among which the preventive approaches is the most desirable to fulfill. Graft manipulation before transplantation is one of the interesting areas to decrease the probability of GvHD. Whole T cell depletion was the first choice led to a dramatic decrease of GvHD incidence but the curative effects of graft-versus-leukemia (GvL) were also abolished. Recent studies have focused on manipulation or mitigation of some harmful alloreactive-T cell or other immune cell subsets such as ab T Cells, CD45RA-expressing naive T cells, B cells, and dendritic cells while preserving the functionality of tumor-reactive NK cells and gd T cells. Another approach is to use the immune-modulating cells such as regulatory T cells, mesenchymal stromal cells, and tolerogenic dendritic cells as well as cytokines like IL-22 to ameliorate the severity of GvHD. The diversity of methods and targets and their pros and cons for preventing GvHD and preserving GvL make an interesting and controversial era of study.

Keywords: Graft-versus-host disease; Immune cell subsets; Graft manipulation

IS-065. Regenerative Medicine in Urology: Urethral Reconstruction

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Organ regeneration has different aspects including tissue engineering, stem cells and colonizing. Tissue engineering is the main part of regenerative medicine, in which cell transplantation rules, material sciences and tissue engineering are applied to develop biologic substitutes to reproduce and maintain natural organ functions. Tissue

engineering strategies can be categorized in 2 main parts: cellular and acellular matrix. Acellular tissue matrix has been reproduced by scaffold production or by eliminating cell content of tissue by mechanical or chemical manipulation in order to produce collagen-rich matrix. The cells then will be implanted on matrix and gradually reproduce new matrix. This new matrix tends to replace the extracellular matrix (ECM). The other ways to use cells for therapeutic goals are injection, hydrogels transporting or even solely. These cells can directly or after proliferation on a scaffold be implanted into recipient. This will result in least complications of functional and structural nature of cells. The best autologous cells are the cells obtained by a biopsy and then isolation and proliferation of cells in a medium and finally implanting them in recipient. However, this process can result in inflammatory responses but the lethal side effects of immunosuppressant drugs is prevented. In the majority of patients with significant organ failure it is impossible to obtain sufficient healthy cells through a biopsy, in these cases stem cells which are purified from other sources as embryonic, bone marrow and adipose tissue cells are a valuable alternative. Nowadays, a significant advance in tissue engineering in the field of genitourinary (GU) system has been achieved. The most significant advancements are obtained in the Urethral and Bladder regeneration. Various strategies have been projected over the years for the regeneration of urethral tissue. Woven meshes of PGA without cells and with cells have been utilized to regenerate urethras in various animal models. Naturally derived collagen-based materials (e.g., bladder-derived acellular submucosa, acellular urethral submucosa, and collagen gels) have also been examined experimentally in various animal models in terms of urethral reconstruction. The bladder submucosa matrix proved to be an appropriate graft for the repair of urethral defects in rabbits. The neourethras showed a normal urothelial luminal lining and organized muscle bundles. These findings were clinically validated in a series of patients with a history of failed hypospadias reconstruction wherein the urethral defects were repaired with human bladder acellular collagen matrices.

Keywords: Regenerative medicine; Urology; Urethral reconstruction

IS-066. Efficacy and Safety of Chitosan-Based Bio-Compatible Dressing Versus Nanosilver (Acticoat) Dressing in the Treatment of Recalcitrant Diabetic Wounds

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Background and Aim: Chitosan has a biocompatible, biodegradable, and nontoxic nature. The effectiveness of nano-chitosan films in the field of wound healing has been confirmed previously. Nanosilver (acticoat) dressing has also been used for the treatment of different wounds for many years. To compare the clinical efficacy and safety of two dressings (chitosan and nanosilver dressings) in the treatment of refractory diabetic wounds.

Methods: A total of 25 eligible patients with chronic non-healing diabetic wound were included and randomly assigned to receive chitosan (13 patients) or nanosilver (12 cases) dressing. The dressings were applied on the wounds based on their protocols and patients were visited and examined by an experienced dermatologist every week. The clinical assessments and healing rates were recorded using diabetic foot infection (DFI) score at the 2nd, 4th, 6th weeks during treatment. The study endpoint (the time needed for complete improvement), safety and tolerability profile were also documented.

Results: The patterns of change in total 10-item DFI wound scores didn't differ significantly over time between the two groups. In both groups, the total 10-item DFI wound score reduced continuously through the course of study. The mean percentage reduction of the total 10-item DFI wound score from baseline was 78.1% and 74.1% in the chitosan and nanosilver dressing groups, respectively. Both dressings were well tolerated and there were no product-related adverse events such as allergic reaction or infection.

Conclusion: Our findings confirmed that chitosan can be safely and effectively used for the treatment of diabetic wounds just like the nanosilver (acticoat) dressing. Further studies are recommended to design a randomized clinical trial with more volunteers.

Keywords: Chitosan; Diabetic wounds; Nanosilver

IS-067. A Novel Regulatory Mechanism to Fine-Tune OCT4 Gene Expression in Pluripotent and Cancer Cells

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POU5F1 (OCT4) is a master regulator of self-renewal in embryonic stem cells and can potentially encode several spliced variants, including OCT4A, OCT4B, OCT4B1, and OCT4C/C1. The long non-coding RNA (lncRNA) *PSORS1C3* is located upstream of *OCT4* gene. It is already reported that lncRNAs have an enhancing effect on the expression of their downstream genes. As we have shown previously, PSORS1C3 is vigorously spliced to generate 24 novel spliced variants, and that a positive correlation exits between its expression and that of OCT4. Herein, we used a CRISPR-Cas9 approach to remove the promoter of PSORS1C3, and then evaluated the transcriptome alterations in the edited cells. Our data approved a regulatory effect of PSORS1C3 on OCT4 expression. Moreover, our data revealed new targets of PSORS1C3. Altogether, our data has clarified a new aspect of OCT4 regulation by different variants of PSORS1C3 in stem and cancer cells.

Keywords: OCT4; Embryonic stem cells; Gene expression

IS-068. Oral Manifestation and *Dental Management:* A case with Chronic Graft Versus Host Disease

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Background and Aim: Chronic graft versus host disease is a potentially therapeutic procedure for patients with relapsed or refractory hematological malignancies. This case is the first to report oral manifestation and dental management in a patient with chronic graft versus host disease after allogeneic hematopoietic stem cell transplantation with a long-term follow-up.

Method: A 40-year-old man referred to the department with complaints of tongue burning, mouth dryness, and ulcers was studied.

Results: The patient had chronic myelogenous leukemia and had undergone allogeneic hematopoietic stem cell transplantation. The patient had undergone treatment with several sessions of extracorporeal photopheresis. The graft versus host disease complications had started about nine months after hematopoietic stem cell transplantation.

Conclusion: The dentist should play a role in the local therapy of cases with oral manifestation and dental management of chronic graft versus host disease. Controlling the graft versus host disease can reduce oral and dental alterations, increase the improvement of the quality of life,

and reduce the need for more intensive immunosuppressive systemic therapies.
Keywords: Hematopoietic stem cell transplantation; Graft versus host disease; Oral manifestation; Dental management

IS-069. Challenges in Regenerative Endodontics?

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Various methods and different materials have been suggested to deal with teeth having immature apices and necrotic pulp. In most of these treatment modalities root length and root dentin thickness remain unchanged and consequently, such teeth are susceptible to root fracture. The aim of regenerative endodontics procedures (REPs) in the treatment of teeth with incomplete root formation and necrotic pulp is to replace the irreversibly inflamed pulp tissue with newly regenerated tissue in an attempt to stimulate root maturation. The principle of REPs is based on the tissue engineering triad, including (i) stem cell, (ii) scaffold, and (iii) signal molecules. The prerequisite of REPs is chemical disinfection of the root canal system that achieves through profuse irrigation using sodium hypochlorite and the placement of an antibacterial paste inside the root canal system without mechanical instrumentation to avoid smear layer formation that may block the release of growth factors from root dentin. In addition, it has been suggested to use the triantibiotic paste as an intracanal medication between the first and the second appointment. However, due to the unique anatomy of the root canal system, the disinfection of the entire root canal system without injuring the surrounding stem cells is almost impossible. During the second appointment, a blood clot is induced inside the canal to act as a scaffold for regeneration and then the coronal access cavity is sealed with a bioactive calcium silicate cement to prevent bacterial penetration and to allow regeneration in a bacteria-free environment. The material used to provide the bacteria-tight seal in this context is important as it should ideally have the ability to upregulate signaling molecules and provoke regeneration. In addition, since it is not practical to circumvent the blood contamination, the sealing capability and basic physical properties of the material should not be jeopardized by moisture and/or blood exposure. More recently, the use of platelet-rich plasma and/or platelet-rich fibrin rather than blood clot has been suggested. In this presentation, the clinical consideration of REPs by emphasizing on the clinical outcome are reviewed and the recently published evidence about REPs will be critically appraised.
Keywords: Regenerative endodontics; Stem cells

IS-070. Development of a Cord Blood Stem Cell-Derived Dendritic Cell Vaccine to Prevent Relapse After Cord Blood Transplantation in AML

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Background and Aim: Dendritic cells (DC) are excellent candidates for vaccination strategies as they have the capacity to activate and prime naïve T-cells for the eradication of pathogens and tumor cells. In the setting of cord blood (CB) transplantation, DC vaccines should be generated from the donor CB. We studied the feasibility to develop a GMP-grade DC vaccine from CB-derived $CD34^+$-stem cells for pediatric AML.
Methods: Cord blood units are frozen in 80/20% fractions of which the 20% fraction (5 mL) was used to isolate C34+ cells using CliniMACS. The cells were expanded for two weeks in culture bags containing medium supplemented with FLT3L, SCF, IL-3 and IL-6. Differentiation into DCs was induced in FLT3L-, SCF-, GM-CSF- and IL-4-containing culture conditions and the CBDCs were matured using proinflammatory cytokines. For loading with the tumor-associated antigen (TAA) we compared Wilms' Tumor 1 (WT1) antigen loading by electroporation with WT1-encoding mRNA and loading by pulsing with a WT1 15-mer-peptide pool. After maturation and loading, cells were cryopreserved until use. CBDC were thawed and washed before being analyzed phenotypically by flow cytometry and functional assays were performed to analyze transmembrane migration, cytokine production, mixed lymphocyte reactions (MLR), and WT-specific T-cell activation using antigen-specific T-cell clones as well as CB-derived T-cells expanded using WT1-peptivator and sorted using WT1-tetramers.
Results: We were able to generate sufficient DCs from 5 mL CB for at least three rounds of vaccination for each patient. The CBDCs showed upregulated co-stimulatory molecules and enhanced CCR7-dependent migration towards CCL19 in a trans-well migration assay. CD83 expression was used to assess the amount of mature CBDC and CBDC produced IL-12 upon stimulation with cytomix and CD40L. In addition, CBDCs expressed WT1 protein after electroporation with WT1-mRNA. The WT1-loaded CBDCs stimulated T-cells both in an MLR and in an antigen-specific setting.
Using flow sorting of DC progenitors and RNA-sequencing we found that CBDCs exclusively originate from $CD115^+$-expressing progenitors. Functional assays demonstrate that these DC mature and migrate upon GMP-grade stimulation and possess a high capacity to activate tumor-antigen-specific T-cells. Gene set enrichment analysis displayed an enriched conventional primary DC profile within the CD115-derived DC compared to CB monocyte-derived DC.
Conclusion: We developed a GMP production process to generate sufficient numbers of WT1-loaded CBDC as a vaccine to stimulate anti-tumor reactivity of the newly developing immune system in AML patients after CB-HCT. The discovery of a committed DC precursor in CB-derived stem cell cultures further enables optimized utilization of DC-based vaccines to provide powerful anti-tumor activity and long-term memory-immunity.
Keywords: Dendritic cell; Cord blood; Vaccination; Acute myeloid leukemia

IS-071. Importance of Individualized Conditioning in Allogeneic Hematopoietic Cell Transplantation

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Background and Aim: Allogeneic hematopoietic (stem) cell transplantation (HCT) is a last-resort treatment for patients with primary immune deficiencies (PIDs), metabolic disorders, or refractory hematological malignancies. T-cell immune reconstitution (IR) after HCT is pivotal for disease control and reduces the probability of transplantation-related mortality. However, adequate immune reconstitution is critically dependent on chemo- and serotherapy (anti-thymocyte globulin (ATG)) used in the conditioning phase. We studied the relationship between exposure to ATG and clinical outcomes in adult and pediatric HCT patients.
Methods: We performed a retrospective, pharmacokinetic-pharmacodynamic analysis of data from patients undergoing an allogeneic-HCT with ATG (thymoglobulin) as part of non-myeloablative conditioning (2004-2016). Active ATG concentrations were measured using a validated bioassay and pharmacokinetic exposure measures were

calculated with a population pharmacokinetic model. We compared the probability of CD4 T-cell reconstitution, previously defined as having >50×10^6 cells/L in 2 consecutive measurements within 100 days after HCT, according to cell source (BM vs CB) and ATG exposures. Cox proportional hazard models were used to identify covariates affecting CD4 IR, including age, sex, diagnosis (malignancy, PID, BM failure syndromes, or benign non-PID), treatment period steroid- treated (grade II-IV) aGVHD, and ATG exposure after HCT. To address why IR after CB is more affected by residual ATG exposure than IR after bone marrow (BM) transplantation, we performed complement- and cell-dependent cytotoxicity assays (NK and neutrophils) in addition to phagocytosis assays using patient's neutrophils.

Results: For adult patients, ATG exposure post-HCT was the best predictor for 5-year overall survival. Optimum exposure post-transplantation was 60–95 AU per day/mL. Estimated 5-year overall survival in the optimum-exposure group was significantly higher than under- or overexposed patients, resulting in a greater event-free survival chance. In children, the ATG exposure post-CBT predicted successful CD4 T-cell IR and event-free survival chances were significantly higher in patients with successful IR and lower ATG exposure. In both populations modeled-dosing based on absolute lymphocyte counts led to higher optimum target attainment than did weight-based dosing. Multivariate log-rank tests (with correction for covariates) revealed that IR was faster after CBT than after BMT in patients with low levels (<10 AU/day) residual ATG exposure, but vice versa in patients with higher active-ATG exposures. We found no differences in ATG-binding and ATG-cytotoxicity but identified that Filgrastim treatment (only given after CBT) increased neutrophil cytotoxicity by 40-fold.

Conclusion: We show that exposure to residual-ATG affects survival after HCT in pediatric and adult patients, stressing the importance of optimum ATG-dosing. Individualized ATG dosing, based on lymphocyte counts rather than bodyweight, might improve survival chances after HCT. Filgrastim (G-CSF) exposure explained the enhanced T-cell clearance after CBT. These findings imply a revision of the use (and/or timing) of ATG and G-CSF.

Keywords: Hematopoietic cell transplantation; Individualized; Conditioning; ATG; Busulfan; Fludarabine

IS-072. NK-Cell Therapy in the Setting of Haploidentical Hematopoietic Stem Cell Transplantation

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The use of cellular immunotherapy has increased significantly over the past decade. Growing understanding of NK cell biology and their potent anti-cancer and infectious activity sustained the development of NK cell therapy for patients with hematologic disorders. In the setting of haploidentical hematopoietic stem cell transplantation (HSCT), while NK cells are the first subset to recover, we and others reported that NK cells generated after selected CD34$^+$ HSCT exhibit an immature phenotype in association with impaired functions. In the same way, after haploidentical HSCT with posttransplant cyclophosphamide (PT-Cy), the majority of mature NK cells infused with unmanipulated grafts are lost upon PT-Cy administration. Thus, blunting of Nk cell alloréactivity in this transplantation setting support the adoptive transfer of alloreactive haploidentical donor NK cells. A major challenge to the broader application of adoptive NK cell therapy is to improve methods for *in vitro* expansion of low-frequency NK cells. Large-scale expansions have been obtained with various feeder cell-based systems but these methods have major drawbacks including complicated procedures, safety issues and/or the expansion of exhausted NK cells. Developing innovative strategies to generate clinically efficient NK cells with minimal *in vitro* manipulation, in large numbers, would provide an important breakthrough in NK cell-based immunotherapy. We will discuss major clinical trial providing a solid basis for the development of NK cell therapies and report our own experience.

Keywords: Immunotherapy; Haploidentical hematopoietic stem cell transplantation; NK cells

IS-073. Haploidentical Hematopoietic Stem Cell Transplantation and Immunotherapy: The Experience of AP-HP Pitié Salpétrière hospital

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Allogeneic hematopoietic stem cell transplant (HSCT) remains the only potentially curative option for a variety of hematologic disorders. Haploidentical BMT has been associated with significant risks of graft rejection and severe graft-versus-host disease (GvHD) but over the past decade, haploidentical donors have emerged as a viable alternate graft source for patients without an HLA-matched donor. Initially, the risk of severe GvHD has been reduced in intensively conditioned recipients by grafts that have been rigorously depleted of mature T cells, but the risks of relapse, serious infection and death in these severely immune-compromised patients was high. In our experience of CD34$^+$ selected and CD3/CD19 depleted haploidentical HSCT, in high-risk acute leukemia patients, 45%, 24% and 22% of patients respectively died from relapse, infection and GVHD. In the last 10 years, different methods to selectively inhibit alloreactivity while preserving immunity to infection and malignancy have been proposed. Among them, post-transplantation immunosuppression with high-dose Cyclophosphamide (PT-Cy), was associated with an acceptably low incidence of graft rejection, severe aGvHD, and extensive cGvHD, while allowing prompt engraftment. In more of 100 haploidentical hematopoietic stem cell transplantation using high-dose cyclophosphamide, we observed an incidence of fetal GvHD less than 20%, similar to that reported in others studies; in this setting, the major causes of death were a high incidence of severe opportunistic infections (53%) and relapse (29%). Thus, it remains to boost both graft anti-leukemia and anti-pathogens effects to improve the outcomes of haploidentical hematopoietic stem cell transplant and we will report major clinical trials and our own experience of T and NK cells manipulation strategies.

Keywords: Allogeneic hematopoietic stem cell transplant; Graft-versus-host disease; NK cells manipulation

IS-074. Comparing the Clinical, Radiographical and Histological Effects of Pulpotomy with Different Materials in Dogs' Deciduous Teeth

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Background and Aim: The aim of this study was to compare the effects of different pulpotomy materials in dogs' deciduous teeth.

Methods: In this experimental study, 56 premolar teeth in 5 mixed-breed Iranian dogs of 6-8 weeks, were randomly divided into 10 groups. In the treatment groups, formocresol, Gutta-percha, mineral trioxide aggregate (MTA), collagen, SHED, BMP2 and SHED + BMP2 were placed on the canal orifice and restored by a zinc oxide eugenol (ZOE) and restorative glass ionomer. After observing mobility in most teeth, periapical radiography of the teeth was conducted under general anesthesia and all the teeth were extracted. The histologic samples were studied blindly by the pathologist in terms of their inflammatory reaction and hard tissue formation. Finally, the data were analyzed by one-way ANOVA, Kruskal-Wallis, chi-square and least significant differences analysis with SPSS Ver.

20 (α = 0.05).
Results: The clinical symptoms and the radiographic symptoms showed no significant difference between the groups (P chi-square >0.05). There was a significant difference between the mean of total inflammation index (P one-way ANOVA=0.019), and the types of hard tissue formed (P-value = 0.001) between the studied groups.
Conclusion: SHED+BMP2+Collagen showed the highest hard tissue formation and the least inflammation.
Keywords: Pulpotomy; Histology; Radiography

IS-075. Short-Term Stimulation of Stem Cells with Various Osteogenic Factors for Enhanced Differentiation Into Bone

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Background and Aim: A one-step concept for bone regeneration has been postulated in which human adipose stem cells (hASCs) are harvested, triggered to differentiate, seeded on carriers, and implanted in the same operative procedure. Toward this goal it was investigated whether short (minutes) incubation with osteogenic factors like BMP-2 and polyamines suffices to "kick-start" differentiation of hASCs seeded on calcium phosphate carriers towards the bone cell lineage.
Methods: hASCs were treated with or without physiological dosages of the various factors for 10-30 minutes, and seeded on calcium phosphate bone substitutes. The attachment was determined after 30 minutes. Proliferation (DNA content) and osteogenic differentiation (alkaline phosphatase activity, gene expression) were analyzed for up to 3 weeks of culture.
Results: We found that markers for early and late bone cell differentiation, e.g. gene expression of runx-2, collagen-I, osteonectin, osteocalcin, and osteopontin, as well as alkaline phosphatase activity were increased by short osteogenic factor pre-incubation.
Conclusion: It can be concluded that short osteogenic factor preincubation of hASCs seeded on bone substitutes had a long-lasting stimulating effect on osteogenic differentiation in vitro. These results strongly support a one-step clinical concept for bone regeneration.
Keywords: Adipose stem cells; Osteogenic factors; Osteogenic differentiation; Bone regeneration

IS-076. Blood Vessel Formation and Bone Regeneration Potential of Human Adipose Stem Cells for Jaw Bone Augmentation

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Background and Aim: Bone substitutes have been exploited as an alternative treatment modality for the autologous bone grafts in patients undergoing maxillary sinus floor elevation (MSFE) for the dental implant placement. Nevertheless, the bone substitutes lack the osteoinductive and angiogenic potential. Addition of adipose stem cells (ASCs) has been shown to possibly stimulate the osteogenesis, osteoinduction, and angiogenesis. In this investigation, in a phase I study, we aimed at studying the vascularization in terms of the bone formation potential of the ASCs containing stromal vascular fraction (SVF) of adipose tissue, which were seeded on two types of calcium phosphate carriers within the human MSFE model.
Methods: Autologous SVF was obtained from ten patients. Then, the autologous SVF was seeded on either β-tricalcium phosphate (n = 5) or biphasic calcium phosphate carriers (n = 5), and used for MSFE through a one-step surgical procedure. After six months, biopsies were obtained during dental implant placement. Then, the quantification of the number of blood vessels was performed using histomorphometric analysis and immunohistochemical stainings for blood vessel markers, including CD34 and alpha-smooth muscle actin markers.
Results: Based on our findings, the bone percentages appeared to correlate with the formation of blood vessels, which were also found to be markedly higher in the study versus the control biopsies in the cranial area, particularly in the case of β-tricalcium phosphate-treated patients.
Conclusion: Taken all, this investigation confirms the safety, feasibility, and efficiency of the application of ASCs in the human MSFE, which also implies a pro-angiogenic effect of SVF.
Keywords: Blood vessel; Bone regeneration; Human adipose stem cells

IS-077. HLA Haplotype Frequency in Iranian Patients and Their Families

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HLA haplotype frequency data is useful in anthropology studies and prediction of finding matched unrelated donors. In this study, low-resolution A, B, and DRB1 HLA haplotypes were extracted by analysis of HLA pedigree of transplant patients. From the extracted haplotypes, 696 out of 5494 (12.66%) haplotypes were unique and the frequency of 240 haplotypes were more than 0.001 (totally 3300 haplotypes). The 9 most frequent haplotypes were A*24, B*35, DRB1*11 (0.0158), A*02, B*51, DRB1*11 (0.0132), A*11, B*52, DRB1*15 (0.0131), A*02, B*50, DRB1*07 (0.0114), A*24, B*18, DRB1*11 (0.0105), A*24, B*51, DRB1*11 (0.0105), A*33, B*14, DRB1*01 (0.0103), A*03, B*35, DRB1*11 (0.0096), and A*30, B*13, DRB1*07 (0.0096). In conclusion, at least 12% of Iranian patients wouldn't have matched unrelated donors in local (Iranian) registries. Considering different B-C and DR-DQ linkages as well as different HLA alleles at high-resolution level increases this estimation significantly.
Keywords: HLA alleles; Haplotype

IS-078. Erythropoietin: A Step Forward in Optic Nerve Regeneration and Treatment of Optic Neuropathies

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Background and Aim: Methanol poisoning is able to result in an optic neuropathy which is often severe and irreversible and might occurs after the ingestion of illegal/homemade alcoholic beverages. We already published the significant effect of this cytokine in patients with traumatic optic neuropathy. In this study, we evaluated the potential neuroprotective effect of erythropoietin (EPO) on visual acuity (VA) in patients with methanol optic neuropathy. We will briefly review the role of EPO in optic nerve regeneration.
Methods: In a forthcoming, noncomparative interventional case series, patients with methanol optic neuropathy after the ingestion of alcoholic beverage were consecutively examined. All these patients were initially administered systemic therapy, including metabolic stabilization and

detoxification. Treatment with intravenous recombinant human EPO consisted of 20000 units/day for 3 successive days. Depending on the clinical responses and outcome, some patients were given a second course of EPO. VA, fundoscopy, and spectral domain optical coherence tomography were assessed during the study. The main outcome measure was the VA.
Results: Thirty-two eyes of 16 patients with methanol optic neuropathy were included. In this study, the mean age was 34.2 years (±13.3 years), and the mean time interval between the methanol ingestion and the treatment with intravenous EPO was 9.1 days (±5.56 days). The mean follow-up after the treatment was 7.5 months (±5.88 months). The median VA in the better eye of each patient before the treatment was a light perception (range: 3.90-0.60 logMAR). The last acuity median after the treatment in the best eye was about 1.00 logMAR (range: 3.90-0.00 logMAR). The VA was markedly increased in the last follow-up examination ($P < 0.0001$). Age and time to EPO treatment after methanol ingestion were not significantly related to final VA. In this patient cohort, no ocular or systemic complications occurred.
Conclusion: Intravenous EPO appears to improve the VA in patients with methanol optic neuropathy and may represent a promising treatment for this disorder. Considering our studies in EPO in optic nerve rehabilitation/regeneration it can be considered in optic nerve regeneration strategies.
Keywords: Erythropoietin; Optic nerve regeneration; Optic Neuropathies

IS-079. Direct Transdifferentiation as a Tool for Functional Studies and Drug Developing

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Direct transdifferentiation is differentiation of one cell type into another, without going through the induced pluripotent stem cell (iPSC) stage. We have developed protocols to create different cell types from fibroblasts cultured from the skin. Avoiding the genetic alterations necessary for iPSC's is essential for the functional study of mutations that affect cell differentiation, because the necessary genetic changes change the differentiation potential of the cells. The transdifferentiated cells can be used for functional studies, drug developing and patient-specific drug testing. The differentiation of fibroblasts to osteoblasts takes 3 weeks. Differentiation to smooth muscle cells takes 8 days. For patient-specific analyses this saves the time necessary for creating iPSC's.
Keywords: Drug developing; Induced pluripotent stem cell; Fibroblasts, Osteoblasts

IS-080. Autologous Transplantation with Genetically Edited Cells: Pitfalls and Possibilities

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Two developments in stem cell culture and genetic editing have to lead to high hopes of successful options for gene therapy. Creating induced pluripotent stem cells (iPSC's) from a patient, followed by CRISPR-Cas9 editing of the causative mutation for the disease was theoretical an elegant combination that could lead to gene therapy with autologous cells. The pitfalls, however were not trivial. There were more off-target effects of the gene editing than was anticipated and the modified iPSC's were also potentially dangerous by integration of the genes for alteration of the differentiation potential. Direct transdifferentiation is difficult to use in this context, because the cells need to be expanded after the gene editing and before transdifferentiation. Non-integrative induction may solve this problem, while the recently developed Base Editor technique, an improvement of the CRISPR/Cas9 technique, may avoid off-target effects of the genetic editing.
Keywords: Induced Pluripotent Stem Cells, Autologous transplantation, Genetically Edited Cells

IS-081. Biomarkers of Acute Graft-Versus-Host Disease

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Acute Graft Versus Host Disease (aGVHD) is an important complication after allogeneic hematopoietic cell transplantation (Allo-HSCT). Despite worthwhile investigation for finding biomarkers of aGvHD, the diagnosis is currently mainly based on clinical findings. An ideal biomarker is noninvasive, rapid, simple, accurate, inexpensive, and standardized and can demonstrate the state of the disease. The functions and numbers of several immune cell populations are altered in aGVHD and cGVHD disease states. Regulatory T cells, invariant natural killer T cells (iNKTs) are potential cellular biomarkers of aGVHD. In cGVHD, B cells and their modulators such as B cell-activating factor are important biomarkers. TIM-3 (T-cell Ig and mucin domain 3), ST2, REG3α (regenerating islet-derived 3-α) can be mentioned as the most valuable markers related to the T-cell-mediated tissue damage of SCT. At day + 30 post-allo-HCT a panel of inflammatory cytokines and receptors including IL-2Rα, TNFR1, HGF, and IL-8 could predict aGvHD. Studies suggest that miRNAs, a post-transcriptional regulator of gene expressions, such as miR-155 and miR-100 could not only be promising biomarkers, but also the potential new target for therapeutic agents of GVHD. Several single nucleotide polymorphisms (SNPs) have been identified as risk factors for GVHD including tumor necrosis factor (TNFα), interleukin-6 (IL-6), interferon (IFNγ), IL-10, UDP-glucuronosyltransferase 2B17. But donor selection according to SNP genotyping is still not performed clinically, although it may be available in the near future. Overall, a scoring predictive system including the most important member of these mentioned categories as an algorithm may be the best choice for utilizing in the clinical setting. GVHD biomarkers have predictive and prognostic value and the approaches to distinguish them in therapeutic clinical trials are crucial to improve outcomes post-allo-HCT.
Keywords: Allogeneic hematopoietic cell transplantation, GvHD, Biomarker

IS-082. Characterization of Late Acute and Chronic Graft-Versus-Host Disease According to the 2014 NIH Consensus Criteria

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In the current investigation, the late acute and chronic graft-versus-host disease (GvHD) were characterized per the 2014 NIH criteria among Iranian patients. Based on the manifestations at the onset, approximately 60% displayed late acute GvHD and 40% had chronic GvHD. The cumulative incidences of the late acute and chronic GvHD were about 20% and 17%, respectively. Gastrointestinal involvement with the late acute GvHD appeared to be infrequent among Iranian patients. A direct comparison of data between Iranian and other ethnicities is needed to examine ethnic differences. To characterize the incidences and outcomes of late acute (LA) and chronic GvHD in East Asians according to the 2014 NIH criteria, we retrospectively analyzed 30 consecutive Iranian patients who had a first allogeneic hematopoietic cell transplantation (HCT) at our center between 2014 and 2018. According to manifestations at onset, 18 patients (60%) had LA GvHD and 12 (40%) had chronic GvHD. The cumulative incidences of LA and chronic GvHD were 20% and 17%, respectively, at 48 months after HCT. The involved sites at the onset of LA GvHD included the skin (71%), gut (13%), and liver (8%). Our findings revealed that, 48 months after the onset of LA GvHD, the cumulative incidences of relapse, non-relapse mortality, transition to

chronic GvHD, and discontinued systemic treatment were 11%, 6%, 22%, and 46%, respectively. Cox models showed that the prior acute GvHD was associated with the non-relapse mortality. Further, the HCT from a female donor to a male patient, myeloablative conditioning, and low Karnofsky Performance Status were associated with a longer duration of systemic treatment after LA GvHD. The most frequently involved sites at the onset of chronic GvHD included the mouth, liver, skin, and eyes. Cox models demonstrated that the use of anti-thymocyte globulin in conditioning regimens was associated with a higher risk of discontinued systemic treatment after the onset of chronic GvHD. Our data showed that, 48 months after the onset of chronic GvHD, the cumulative incidences of relapse, non-relapse mortality, and discontinued systemic treatment were 16%, 11%, and 41%, respectively. These findings propose several potential differences between Iranian patients and those of other ethnicities. It seems that a direct comparison is needed to formally investigate the ethnic differences.

Keywords: Chronic graft-versus-host disease; National Institutes of Health criteria; Iranian

IS-083. Activated Platelets, a Miracle Cure?

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Background and Aim: In this modern era, regenerative medicine is considered as a popular and of great interest in the western countries, because of the increasing aging population. There is a great hope on the regenerative medicine, which is believed to be a robust strategy in improving organ dysfunction and delaying tissue degeneration. In this field, the early studies have mainly focused on the production of stem cells and mesenchymal stem cells (MSCs) in order to recreate compatible tissues in vitro to be used as "replacing patches" in the human body in vivo. Although some studies have been promising, they remain associated with several problems, including (i) high production cost, (ii) poor clinical results, (iii) necessity of repeated infusions of undifferentiated cells.

Methods: Of note, some recent studies have concentrated on the shedding and culture medium of stem cells (so-called secretome), which have the potential of stimulating mesenchymal cells of our bodies. Havin used a simple comparison, it can be suggested that the secretome could be considered a "good-stock" that could cure a designated patient much better than the "beef-piece" (the cultured stem cells). Basically, the haemo-component include platelet-rich plasma (PRP), platelet-poor plasma (PPP), platelet gel (PG), platelet-rich fibrin (PRF), serum eye drops (E-S) and PRP eye drops (E- PRP), are rich in growth factors (VEGF, PDGF, TGF beta 1. It can be used not only as transfusional system but also to promote soft/hard tissue regeneration in vivo by potentiating existing mesenchymal stem cells, without requiring the use of artificial tissues. In addition to regenerative properties, some of these elements (e.g., PG) has also anti-inflammatory and analgesic effects, and anti-bacterial impacts against some microorganisms.

Results: We report on a series of patients treated at Bolznao hospital, Italy. Three dramatic cases of wounds in oncohaematological patients were identified and treated using PG. An exhaustive documentation of all cases is reported, including Case#1 deals with a case of skin damage post for a breast cancer; Case #2 deals with a laterocervicalis abscess and jaw osteonecrosis; and Case # 3 deals with severe oral mucositis post ASCT. Furthermore, we discussed additional cases (e.g., decubitus with infected wounds).

Conclusion: Randomized studies are needed to establish the difference and the best efficacy of PG and CBPG. Furthermore, early studies by Calabrese et al (personal communication), have shown that PG could facilitate bone grafting in maxillofacial surgery. This would further support the overall idea that secretome and secretome-like substances may be "the holy water" of Regenerative Medicine.

Keywords: Cord blood platelet gel; Platelet gel; Chronic wounds; Aplasia; Mesenchymal stem cells; Growth factor

IS-084. Regenerative Medicine for Oral Mucositis

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Background and Aim: Oral mucositis represents a severe clinical issue for all patients undergoing chemotherapy, and radiotherapy. During the cytopenic phase, serious infections and organ toxicities can occur. Oral mucositis leads to a high risk of oral per-infections. Symptoms of mucositis vary from pain and oesophagitis to complete interruption of food intake. Parenteral nutrition and opiates administration are often required. Recently, platelet gel (PG) has been shown to have a strong capacity for tissue regeneration thanks to the release of growth factors. These promote the reepithelisation and angiogenesis through the recruitment of mesenchymal stem cells (MSCs).

Methods: A 68-year-old woman was admitted in December 2015 because of a mantle cell LNH. In September 2016, she underwent autologous hematopoietic stem cell transplantation (HSCT) with FEAM (fotemustine plus etoposide, cytarabine, and melphalan). During aplasia, she developed grade IV oropharyngeal mucositis, septic shock and cytomegalovirus infection. Cord blood blatelet gel (CBPG) was administered twice a day.

Results: The patient was asked to place the CBPG in her mouth cavity and to distribute it with her tongue on the inside mucosa. After only 3 days of consecutive application, a significant improvement in mucositis and pain was observed. On day 8, restituto ad integrum of her mucosa was noted. Furthermore, in association with the mucosal healing, the trend of lymphocytes showed a peak, which was not observed for the total white cell count or for neutrophils. The patient's general condition slowly improved. After 3 weeks, the patient was discharged from the hospital.

Conclusion: Our findings are in keeping with previous studies on PG. The trend of lymphocytes showed a peak immediately after CBPG application, which might have helped to clear up the CMV infection.
The positive outcome of our patient supports the need for controlled studies on the efficacy of CBPG for the treatment of severe oral mucositis in patients who receive treatment with high-dose chemotherapy.

Keywords: Cord blood platelet gel; Platelet gel; Oral mucositis; Aplasia; Neutropenia; Growth factor

IS-085. Modeling Neurological Disorders using Isogenic Human Stem Cells and Organoids

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Much attention has been given to understand how cellular processes are changed in the neurological diseases, which have long been hampered by the inaccessibility of the brain. Nevertheless, some recent technological advancements and progresses appear to be in favor of overcoming this hurdle. Of these, one is the ability to direct the differentiation of patient-specific human pluripotent stem cells (hPSCs) into the neuronal and glial cells/tissue. Editing specific loci in the genome using programmable sequence-specific nucleases provides another possibility, which might allow the precise removal or addition of mutations of interest. In this talk, I will discuss the progress made in establishing isogenic hPSC-based models of neurological disorders. I will also articulate their utility in understanding neuronal dysfunction during development and disease.

Keywords: Human pluripotent stem cells; Neurological disorders; Modeling

IS-086. Noninvasive Imaging of Immune Responses to Gauge Efficacy of Immunotherapy

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Background and Aim: Immunotherapy using checkpoint-blocking antibodies against targets such as CTLA-4 and PD-1 can cure melanoma and non-small cell lung cancer in a subset of patients. The presence of $CD8^+$ T cells in the tumor correlates with improved survival. We aimed to develop a method to monitor the dynamics of $CD8^+$ T cells in the tumor microenvironment in response to treatment to identify patterns of response in responders and nonresponders.

Methods: In this study, we report on how immuno-positron emission tomography (immuno-PET) can visualize tumors by the detection of infiltrating lymphocytes. We also show how the longitudinal observation of individual animals distinguish responding tumors from those that do not respond to therapy. In this line, we capitalized on the use of ^{89}Zr-labeled PEGylated single-domain antibody fragments (VHHs) specific for CD8 to track the presence of intratumoral $CD8^+$ T cells in the immunotherapy-susceptible B16 melanoma model in response to checkpoint blockade.

Results: The ^{89}Zr-labeled PEGylated anti-CD8 VHH was found to be able to detect the thymus and the secondary lymphoid structures as well as the intratumoral $CD8^+$ T cells. Our data revealed that the animals responded to CTLA-4 therapy displayed a homogeneous distribution of the anti-$CD8^+$ PET signal throughout the tumor. However, more heterogeneous infiltration of $CD8^+$ T cells were found to correlate with faster tumor growth and worse responses. To support the validity of these observations, we exploited two different transplantable breast cancer models, in which the yielding results conformed with predictions based on the anti-melanoma response. We also explored the dynamics and distribution of intratumoral $CD8^+$ T cells and $CD11b^+$ myeloid cells in response to anti-PD-1 treatment in the anti-PD-1-responsive MC38 colorectal mouse adenocarcinoma model. Responders and nonresponders showed consistent differences in the distribution of $CD8^+$ and $CD11b^+$ cells. PD-1 treatment mobilized $CD8^+$ T cells from the tumor periphery to a more central location. Only those tumors fully infiltrated by $CD8^+$ T cells went on to show a complete response. All tumors contained $CD11b^+$ myeloid cells from the outset, followed by recruitment of additional $CD11b^+$ cells. As tumors grew, the distribution of intratumoral $CD11b^+$ cells became more heterogeneous. Shrinkage of tumors in responders correlated with an increase in the $CD11b^+$ population in the center of the tumor.

Conclusion: The changes in the distribution of $CD8^+$ and $CD11b^+$ cells as assessed by PET served as biomarkers to gauge the efficacy of checkpoint blockade treatments. It may thus be possible to use immuno-PET and monitor antitumor immune responses as a prognostic tool to predict patient responses to checkpoint therapies.

Keywords: Immunotherapy, Checkpoint blockade, Immuno-PET, single domain antibodies

IS-087. Combined Immunotherapy and Conventional Chemotherapy in Cancer Patients

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Despite promising success in immunotherapy of cancer patients, a considerable part of these patients does not reach to optimum response to these agents such as PD-1 and PD-L1 inhibitors. Recent interest is to achieve the additive efficacy of immunotherapy in combination with conventional chemotherapy agents. The schedule and dosage of chemotherapy agents like metronomic treatment can play as a game changer in this strategy. This integration of immunotherapy with standard cancer therapy can promote immunogenic cell death with a various mechanism such as modulate the immune phenotype of residual tumor cells, minimize of the negative impact of tumor burden and up-regulation the expression of tumor antigen

Keyword: Immunotherapy metronomic treatment; Chemotherapy

IS-088. Decidua Stromal Cells for Treatment of Steroid-Refractory Graft-Versus-Host Disease

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Graft-versus-host disease (GvHD) is a life-threatening complication and a major cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HSCT). Steroid refractory aGvHD is associated with a high mortality rate (up to 80%) despite intensified treatment with other immunosuppressive agents. Placenta-derived decidua stromal cells (DSCs) are a novel therapy for acute GvHD after allogeneic HSCT. DSCs are different from bone marrow and adipose-derived mesenchymal stromal cells in terms of immunosuppressive capacity and some other characteristics. To investigate the therapeutic potential of DSCs, these cells isolated from the term placenta. Nine patients with steroid-refractory GvHD (2 patients SOS and GvHD) and 1 patient with chronic GvHD received DSCs. The dose of infused cells for all of the patients was 1×10^6 DSCs/kg. The patients were given 3 (1-8) doses, with a total of 34 infusions. GvHD response in patients as no, partial and complete was 3/2/5 respectively. No adverse events related to the treatment were observed in patients. About 1 year after DSC therapy 4 patients were alive. Causes of death in patients were an infection (despite complete response in GI, skin and liver) due to treatment with monoclonal antibodies, sinusoidal obstruction syndrome (SOS) and liver dysfunction. In conclusion, DSCs can be an acceptable treatment for patients with steroid-refractory acute GvHD and administered safely in these complicated patients but more prospective clinical trials are needed.

Keywords: Decidua stromal cells; Graft-versus-host disease; Hematopoietic stem cell transplantation

IS-089. Recent Progress in Cell Therapy in Solid Organ Transplantation

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There has been plethora of preclinical and animal experiments showing efficacy and safety of the use of various cell-based modalities, including the use of stem cells or T regulatory cells, after transplantation for tissue repair, immunosuppression or tolerance induction. Nevertheless, there has recently been a substantial progress in terms of the utilization of cell therapy in solid organ transplantation in small clinical trials. The reported findings of recent studies appear to be very promising and the use of cell therapy in solid organ transplantation seems to be plausible and safe. However, there exist some issues to overcome, including the dose and timing of the infusions. As a matter of fact, the current investigations have mainly focused on the live donor kidney transplantation. It seems that the expansion of current regimes to other organs and deceased donor transplantation might be crucial though such concept is yet to be fully addressed.

Keywords: Cell therapy; Organ transplantation

IS-090. Effect of Elaeagnus angustifolia Extract in Bone Tissue Engineering Nanofiber Technology

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Modern societies tend to use medicinal plants for their basic health care needs. *Elaeagnus angustifolia* is one of the medicinal herbs with antinociceptive, anti-inflammatory, antibacterial and antioxidant properties widely used in the treatment of patients with rheumatoid arthritis and osteoarthritis symptoms. In this work, for the first time, E.A extract was loaded on PCL-PEG-PCL nanofibers and studied their potential application in bone tissue engineering. Fourier transform infrared spectroscopy (FT-IR), field emission scanning electron microscope (FE-SEM), contact angle and mechanical test were applied to evaluate the morphology and chemical properties of nanofibers. FE-SEM images showed that all samples had bead-free morphologies with average diameters ranging from 100-200 nm. The cell response to *E. angustifolia* extract-loaded PCL-PEG-PCL nanofibers was evaluated by means of human dental pulp stem cells (hDPSCs). hDPSCs showed improved adhesion and proliferation capacity on *E. angustifolia* extract loaded nanofibers compare to pristine PCL-PEG-PCL nanofiber. Alizarin red S assay and alkaline phosphatase activity confirmed the nanofibrous scaffolds could induce the osteoblastic performance of hDPSCs (P<0.05). QRT-PCR results confirmed that *E. angustifolia* extract-loaded nanofibrous scaffolds significantly upregulated the gene expression correlated with osteogenic differentiation (P <0.05). The results suggested that *E. angustifolia* extract-loaded with PCL-PEG-PCL nanofibers might have potential applications for bone tissue engineering.

Keywords: *Elaeagnus angustifolia*; Scaffold; Electrospinning; Human dental pulp stem cell; Tissue engineering

IS-091. Graphene, Butterfly and Stem Cells Is Set to Revolutionise Medical Devices

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The world advancing rapidly in the field of technology, a simple example is our mobile phone. However, when compared to healthcare, the diagnostic and treatment of diseases are still very poor and surgery has not changed significantly compared with 50 years ago. There is plenty of news in academia/media that everything could be diagnosed and cured, but in reality, the invention has been tested in rodents and has not moved to human. This is due to; the complexity of the medical devices build in university research environment, the lack of difficulty taking devices to the clinical setting, as well as the positive outcome obtained from in vitro and rodents may not transferable to human. Therefore, needs going back to the drawing table and rethink to build medical devices that; commercially feasible, reliable, sensitive, repeatable and non-toxic and biocompatible. The potential for using smart nanomaterial and consequent research to replace damaged tissues has also seen a quantum leap in the last decade. In 2010, two scientists in the UK realized they had isolated a single layer of carbon atoms on a scotch tape. Since then, graphene has captured the imagination of researchers due to its fascinating properties. Graphene considers as a wonder material, it is the strongest material on the planet, an order of 200 times stronger than steel, super-elastic and conductive. Graphene's carbon atoms are arranged into hexagons, forming a honeycomb-like lattice. The functionalized graphene oxide (FGO) with polyhedral oligomeric silsesquioxane (POSS) from butterfly wing are nontoxic and antibacterial. FGO has been used for drug and gene delivery, development of biosensor or in nanocomposite materials development of human organs. In my talk, I present and discuss our work on the application of FGO-POSS in development of medical sensors, drug, gene and stem cells delivery, as well as the development of human organs with stem cells technology. The materials can be fabricated to human organs with the 3D printer or other fabrication methodologies. The scaffold from these materials is functionalized with bioactive molecules and stem cells technology, for the development of human organs. The data for the development of organs using these materials will be presented. In conclusion, the graphene, POSS bring new hope for gene, drug and stem cells delivery for repair and replacement of organs.

Keywords: Graphene nanomaterial; Medical devices; Stem cells; Gene delivery; Human organs; 3D printing

IS-092. Can Academic Be a Successful Entrepreneur?

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Many academic especially in the medical field, from postgraduate student to professor, may think working life is hard, with the today research moving so fast and with digital technology in the global world. To be on top of the field requires constant reading and thinking, especially if one wants to stay on the top. The delivery from an academic generally includes publication in high impact journal, obtaining funding for research and to carry out the research with positive or negative results, which both are valid. However, when these compared with the commercialization of the idea, especially in the medical field, the task is much harder. The university education is a tremendous help when entrepreneurs need to understand different cultural and social environments. But it does require learning new sets of skills and rules. Even the way of writing for funding and report change significantly, sometimes much more difficult compared writing high impact research paper. In my talk, I will highlight the difficulty and benefit of an entrepreneurship. I also highlight the route requires taking from an idea to commercialization and successful product in the global market.

Keyword: Academic; Entrepreneurship; Commercialization

IS-093. Integrative Utilization of Microfluidic Devices and Biomaterials for Advanced Regenerative Medicine

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Microfluidics systems have received much attention and shown excessive capacity in the field of tissue engineering and regenerative medicine. Studying cellular responses by microfluidic devices provide much more accurate and comprehensive outcomes. In this work, we report on a microfluidic-based device, which was used for analysis of the matrix density as a mechanical property and the concentration profile of a biochemical factor as a chemical property. The microfluidic device used in this investigation has a cell tank and a cell culture chamber to mimic both 2D to 3D and 3D to the 3D migration of three types of cells. Fluid shear stress was found to be trivial on the cells, in which a stable concentration gradient can be obtained by diffusion. The device was designed by a numerical simulation, and thus, the uniformity of the concentration gradients was obtained throughout the cell culture chamber. Adult neural cells were cultured in this device. The cultivated cells displayed different branching and axonal navigation phenotypes within varying nerve growth factor (NGF) concentration profiles. In addition, neural stem cells (NSCs) were cultivated within varying collagen matrix densities while exposed to NGF concentrations and they experienced 2D to 3D collective migration. By the generating vascular endothelial growth factor (VEGF) concentration gradients, the adult human dermal microvascular endothelial cells (HDMVECs) migrated (in a 2D to a 3D manner) and formed a stable lumen within a specific collagen matrix density. Altogether, a minimum absolute concentration and concentration gradient was found to be required to stimulate the migration of all types of cells. Taken all, we believe that his device has the advantage of changing multiple parameters concurrently and is

envisioned to have wide applicability in cell studies.

Keywords: Microfluidic devices; Cell migration; Nerve growth factor; Vascular endothelial growth factor

IS-094. Allogeneic Mesenchymal Stem Cell Therapy for Refractory Lupus Nephritis: Preliminary Results of a Phase I-II Non-Randomized Clinical Trial

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Background and Aim: Renal involvement in patients with systemic lupus erythematosus (SLE), known as lupus nephritis (LN), is associated with poor prognosis. This is mainly due to the fact that a fraction of LN patients is vulnerable to become resistant to conventional treatments. Pathogenesis of SLE is attributed to autoimmunity and dysregulation of the immune system. Hence, immunomodulation is at the top of therapeutic strategies for such patients. Mesenchymal stem cells (MSCs) are best known for their immunomodulatory effects. This study was designed to evaluate the effects of allogeneic MSC transplantation on treatment-resistant LN.

Methods: In this phase I-II non-controlled clinical trial with before-after design, a series of LN patients (class III and IV) resistant to standard treatments and having 24 h urine protein >1 g or urine RBC >10 or cellular casts in urine were included. MSCs were extracted from an adipose tissue of patients' siblings. The patients underwent systemic infusion of $1x10^6$ MSCs/kg body weight. After the intervention, the patients were followed up 3 months post-infusion (after), and their disease activity (using SLEDAI score) and 24-hour urine protein level were compared with those of 3 months pre-infusion (before).

Results: Nine patients (88.9% female) enrolled in this study with a median age of 29 (range, 19-37) years. Mean SLEDAI score of patients was 15.4 ± 5.8 in 3 months pre-treatment which reduced to 6.0 ± 4.8 in 3 months post-treatment showing a significant improvement in disease activity (P = 0.014). Nonetheless, the mean values of 24-hour urine protein excretion, despite a slight reduction after MSC therapy, were not significantly different between pre- and post-intervention (2.1 vs. 1.5 g/24 h urine, P = 0.308). MSC therapy was found to be safe as except transient hypertension in one patient after the infusion, no other acute or chronic adverse effect was observed.

Conclusion: Systemic infusion of allogeneic adipose-derived MSCs was safe and also effective to reduce the disease activity in patients with LN refractory to conventional treatments. Albeit, this was a preliminary report and patients should undergo a longitudinal evaluation. Moreover, renal function of patients in view of proteinuria did not change significantly, and so, further follow-ups are needed.

Keywords: Systemic Lupus Erythematosus; Mesenchymal stem cells; Lupus nephritis

IS-095. Local and Systemic Cell Non-autonomous Mechanisms Uniquely Drive Intestinal Stem Cell Aging

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Background: Ageing occurs as a complex interaction of cell autonomous and non-cell autonomous mechanisms. Functional decline is a hallmark of aging in multiple tissues, including the intestine, and this process is thought to be driven in part by deterioration in resident stem cell function. The intestine has a rapid turnover of every 3-5 days, owing this to the presence of Lgr5+ intestinal stem cells (ISCs), that resides at the bottom of the crypt along with their niche providing Paneth cells. Therefore, we investigated how this compartment is affected with aging.

Methods: Four and 22 months old C57BL/6 male mice were used for aging phenotype studies. Young and old mice received rapamycin (4 mg/kg) or salicylate (2 mg/mL) for 28 days. Neutralizing antibodies to IL-1β, TNF-α, IFN-γ or IgG1 were i.p. injected at a 300 µg dose for 3 weeks. Isolated crypts from Lgr5-EGFP mice were re-suspended in TrypLE Express with Rock inhibitor and DNAse I, filtered and centrifuged. Cells were then re-suspended in FACS buffer containing PE-conjugated anti-CD24 antibody and APC-conjugated anti-Epcam antibody for 15 min at 4°C, and analyzed by MoFlo. ISC proliferation was assessed using an *ex vivo* organoid assay. Briefly, isolated crypts were re-suspended in matrigel, transferred to a 48-well plate to solidify at 37°C, and 250 µL crypt culture medium (ADF, Pen/Strep, HEPES, Glutamax, N2, B27, N-acetyl-L-cysteine, Noggin, EGF and R-Spondin) was added to each well and maintained at 37°C. The organoid formation was quantified on day 9. Parabiosis surgery was carried out by the Einstein Chronobiosis core.

Results: As compared to young mice, old mice harbor declines in ISC proliferation, impaired mucosal barrier integrity, and shifts in the gut immune cell composition, including increased CD8+ T cells and dendritic cells ($P<0.05$). We next generated isochronic (Y-Y, O-O) and heterochronic (Y-O) C57BL/6 male parabionts and observed impaired ISC function in young parabionts exposed to old blood ($P<0.05$). Rapamycin or salicylate treatment restored ISC function in old mice ($P<0.05$), without further suppressing mTOR signaling. However, rapamycin reduced plasma cytokines to more youthful levels ($P<0.05$), suggesting a potential role for inflammation in mediating the stem cell dysfunction with aging. *Ex vivo* screening assays confirmed that TNFα and IFNγ potently disrupt ISC proliferation ($P<0.05$), while *in vivo* treatment with a TNFα- or IFNγ-neutralizing-antibody restored ISC function in old mice ($P<0.05$). Upon single cell ablation in the crypts, the motion of neighboring cells was dramatically impaired, and cell debris 'lingered' in the crypts of old animals ($P<0.05$).

Conclusion: We propose that intestinal aging happens as a complex interplay of cell autonomous and non-cell autonomous pathways. Moreover, IFN-γ and TNF-α can act as lead progeronic factors to drive a decline in intestinal stem cell function with aging.

Keywords: Ageing; Intestinal stem cells; Lgr5+; Inflammation; Parabiosis

IS-096. Insights from Embryology to Inform on Disease

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Background and Aim: In vertebrates, head and axial muscles arise from different mesodermal origins, and their development depends on distinct gene regulatory pathways. Tbx1, Islet1 and Pitx2 govern cranial-derived muscle fates whereas Pax3 and Pax7 are critical for somite-derived muscles. It is unclear how the distinct genetic hierarchies have led to this regional specification. Observations in the clinic have identified a wide variety of myopathies that affect subsets of skeletal muscles resulting in debilitating diseases. It is unclear why some muscles are affected while others escape the disease.

Methods: We have used an extensive array of genetically modified mice, including knockouts and transgenics with lineage derived reporter constructs to mark, perturb, and follow the fate of specific muscle populations in the mouse. Multi-color and imaging strategies including microCT scanning of 3D generated structures provide whole mount imaging views of the developmental trajectories that muscle stem and progenitor cells use to effect morphogenesis and subsequent muscle patterning.
Results: We have investigated the patterning of the extraocular (EOM), esophagus and neck muscles and provide evidence for a surprising diversity even among muscles of the same cranial embryological origin. Using a combination of genetic lineage drivers in the mouse and a variety of imaging approaches, we provided a link between key transcription factors, cell fates, and patterning. The complementary analysis of other vertebrate organisms including chick, fish has shed new light on the evolutionary relationships and functions of the neck and the associated pectoral girdle apparatus.
Conclusion: The use of mouse genetic strategies and imaging approaches have provided important insights into tissue genesis as well as an understanding of the developmental origin of some human congenital diseases such as DiGeorge syndrome where subsets of cranial muscles are affected.
Keywords: Skeletal muscle development; Genetic modification; Lineage tracing; Whole mount imaging; Muscle stem cells

IS-097. Regulation of Stem Cell Properties in the Niche

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Background and Aim: The regulation of skeletal muscle stem cells during homeostasis and regeneration involves the interplay of multiple mechanisms. The mechanisms by which niche molecules and intrinsic factors regulate muscle stem cell quiescence and properties remain largely unknown. In a series of studies, we investigated Notch as a key mediator of muscle satellite cell stability and fate. Specifically, Notch mediates extrinsic (extracellular matrix) and intrinsic (microRNA) mechanisms to stabilize satellite cells within their niche.
Methods: We used ChIP-sequencing, small RNAseq and genetically modified mice to investigate muscle stem cell properties. These studies were complemented by live imaging of explants of skeletal muscles where the consequences of perturbations in vivo were followed by monitoring by 2-photon microscopy the fate of muscle stem cells subjected to miR knockdown. Regeneration studies involve damage of the Tibialis anterior muscle with snake venom and follow up of the regeneration process over several weeks.
Results: Interestingly, we found that Notch/RBPJ-bound regulatory elements are located adjacent to specific collagen genes in adult muscle stem cells. These molecules are linked to the ECM and constitute putative niche components. Notably, we show that satellite cell-produced collagen V (COLV) is a critical component of the quiescent niche, as conditional deletion of Col5a1 leads to anomalous cell cycle entry and differentiation of muscle stem cells. Strikingly, COLV specifically regulates quiescence through calcitonin receptor-mediated activity. In other studies, we have identified a microRNA pathway that is modulated by Notch, and it is required for stabilizing muscle stem cells in their niche by regulating the migration status of the muscle stem cell. These observations lead us to propose a two-step mechanism for niche occupancy.
Conclusion: Notch is a critical regulator of the muscle stem cell niche. Through extrinsic and intrinsic mechanisms that act through collagen V and miR-708 respectively, Notch stabilizes muscle stem cells in their niche.
Keywords: Skeletal muscle; Stem cell; Niche; Notch; Extracellular matrix; Collagens

IS-098. Functional Hepatocyte-Like Cells from Human Pluripotent Stem Cells in a Scalable Suspension Culture: A Success Story 2011-2018

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Background and Aim: Biomedical application of human pluripotent stem cell-derived hepatocyte-like cells relies on efficient large-scale differentiation, which performed by a suspension culture of three-dimensional multicellular spheroids in bioreactors.
Methods: In this wok, we report on a scalable stirred-suspension bioreactor culture of functional hepatocyte-like cells from the human pluripotent stem cells. Three PhD projects designed to address the potential challenges in this subject. This method needs large amounts of growth factors and must overcome the limited diffusional transport posed by the inherent 3D structure of hPSC spheroids.
Results: Currently used protocols for the scalable and integrated differentiation of hPSCs limited due to the high cost of growth factors and technical challenges. Priming hPSCs aggregates with different small molecules in combination with different concentrations of growth factors resulted in an effective protocol for differentiation. Hepatocyte-like cells characterized and then purified based on their physiological function. The differentiated HLCs possessed multiple characteristics of the primary hepatocytes, including the expression patterns of liver-specific marker genes, albumin secretion, urea production, collagen synthesis, indocyanine green and LDL uptake, glycogen storage, and inducible cytochrome P450 activity.
Conclusion: These findings imply that sustained and localized delivery of GFs using MPs might provide a de novo method towards scalable technologies for the hepatocytic differentiation and engineer a better 3D microenvironment for cells.
Keywords: Hepatocyte-like cells; Pluripotent stem cells; Scalable suspension culture

IS-099. Chemistry and Biology Connected for the Emergence of Individual Cognition

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Background and Aim: A brain is an intricate, complex, and beautiful organ that underlies our cognition and behavior. Although we are living in an era of privilege that we can not only read our own genetic makeups, we are also becoming capable of editing them at will, we remain woefully ignorant of what all this means for the brain to become capable of constructing individual cognition.
Methods: To solve this mystery, our group focus on the post-transcriptional regulation mechanisms of RNA molecules and take three approaches to understand these cellular processes: to see, to analyze, and to mimic. To see the RNA molecules in living cells, we develop functional chemical probes for fluorescent labeling of the RNA. To analyze RNA in a genome-wide manner, we apply deep sequencing techniques and bioinformatics analysis. To mimic RNA regulation, we build artificial magnetic nanoparticles to mimic naturally occurring cellular RNA granules. In this talk, I will highlight our recent study how experience-driven activities in the brain is integrated into protein synthesis, at the close proximity to synapses of neurons. These proteins form multicomponent complexes and make up the microscopic machinery for neuronal plasticity and behavior.
Results: Recently, our group established a low-input sequencing method to study the minute amount of synaptic RNA and reported the initial draft of epitranscriptome at synapses. About 4469 methylation sites enriched in 2,921 genes as the synaptic m^6A epitranscriptome (SME) were identified in biochemically enriched neuronal synapses. SME was found to be functionally enriched in the synthesis and modulation of tripartite synapses as well as some pathways implicated in neurodevelopmental

and neuropsychiatric diseases. Interrupting m^{6}A-mediated regulation via knockdown of readers in hippocampal neurons was found to change the expression of SME member *Apc*, resulting in synaptic dysfunction such as immature spine morphology and dampened excitatory synaptic transmission concomitant with decreased PSD-95 clustering and GluA1 surface expression.

Conclusion: Our findings indicate that extremely simple chemical modifications, such as the addition of a methyl group (-CH_3) to the RNA molecules, can make critical contributions to building neuronal circuits in the brain and thus the emergence of individual cognitive abilities. This study warrants further investigations on the diverse chemical modifications at the synaptic RNA and their contribution to individual cognition. Currently 171 RNA modifications are known in nature, indicating our genetic programs can be expanded by orders of magnitude, which might be essential for creating diversity and complexity of neuronal connections.

Keywords: Synapse; RNA modification; Epitranscriptomics; Neuronal circuitry; Genetic information

IS-100. Rise of an Epitranscriptomic Era in Understanding Neurodevelopment and Neuropsychiatric Diseases

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Background and Aim: A broad spectrum of developmental and psychiatric disorders, ranging from autism and intellectual disability to schizophrenia, can be connected by commonly underlying structural and genetic causes. In the past 30 years, evidence provided by the neuroepigenetics field has made an immense impact on understanding neurodevelopmental and psychiatric disorders that were previously assumed to be irreversible. Recently a new field of epitranscriptomics has risen with new promises in connecting the dysfunction of neuronal circuits and cognitive disorders. In this talk, I will review recent evidence suggesting a potential link between epitranscriptomic regulation and neurodevelopmental and psychiatric diseases.

Methods: To understand how epitranscriptome affects synaptic function and contribute to human diseases, our group focus on N6-methyl-adenosine (m^{6}A), one of the most abundant internal messenger RNA modifications, and study the function of this cellular pathway in developing neurons. Using an in vitro neuronal culture system, we introduced genetic manipulations using short-hairpin RNAs and CRISPR/Cas9 system, to specifically reduce expression levels of m^{6}A reader proteins, or the proteins that recognize m^{6}A RNA species and direct them into distinct metabolic pathways. Furthermore, enhanced expression of m^{6}A reader proteins was induced in neurons using strong promoters. The effect of such genetic manipulations was examined using cytohistochemistry, dynamic fluorescent imaging, and electrophysiology. Key neuronal developmental steps such as polarization, axon specification, dendritic development, and synapse maturation were examined. Sholl analysis and morphological classifications were conducted to evaluate neuron development. Furthermore, immunocytochemistry under cell-membrane impermeable conditions was carried out to assess glutamate receptors trafficking to the cell membrane. All these experiments were carried out in a blind manner where experimenters were blinded with the genetic manipulations.

Results: Structural dendritic and synaptic deficits were detected in association with the interruption of m^{6}A recognition pathways. Multiple structural deficits at extending axons and growth cones, dendritic atrophy, and immature spine phenotypes were observed. Lack of post-synaptic density scaffold protein clusters at the immature spines was observed in the combination of reduced spontaneous synaptic transmission. The structural and functional deficits are consistent with RNA deep sequencing results in adult healthy mouse forebrain, as m^{6}A-modified RNA as a cohort was enriched in the human phenotype of "intellectual disability" and in categories of genes regulating synapse assembly, organization, maturation, and modulation. Furthermore, the spine immaturity deficits (elongated spine neck and smaller spine head) were reminiscent to the previously reported spine phenotypes in human infants with mental retardation. A microtubule plus-end binding protein APC has been shown to associate with spine morphology, growth cone dynamics, and axon extension. In neurons where m^{6}A-mediated regulation was interrupted via knockdown of readers in hippocampal neurons, APC protein level decreased in addition to other microtubule regulatory proteins. Altered APC protein expression may partially explain the altered phenotypes of developing neurons and deficits in molecular trafficking over a long distance in affected neurons.

Conclusion: Our findings indicate a structural and functional link between RNA methylation and neurodevelopmental and neuropsychiatric diseases. Failed recognition of this RNA modification affects neuron development at different stages and in all neuronal compartments such as axons, dendrites, and dendritic spines. We speculate that activity-dependent structural maturation of neurons requires spatiotemporally orchestrated gene expression, partially through regulating neuronal cytoskeleton development.

Keywords: Microtubule; Spine; Epitranscriptomics; Neuroepigenetics; Neuron development; Intellectual disability

IS-101. Stem Cell Application in Liver and Gastrointestinal Diseases

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Stem cells are known as cells with self-renovation and regeneration of various tissues, and they are classified according to their ability to differentiation. In recent years, stem cell research has become much broader, and a promising prospect for refractory diseases has been outlined. The current abstract is about the potential for stem cell use in liver and gastrointestinal disorders, as digestive diseases have a significant impact on the general health of the community. Although the recent studies encourage the use of stem cell therapy in some gastrointestinal diseases, especially on Crohn's disease, it seems that full clinical application of these cells in other gastrointestinal diseases requires a more comprehensive elaboration.

Keywords: Stem cells; Liver and gastrointestinal diseases; Crohn's disease

IS-102. Different Cytokines Differentially Affect Osteogenic Differentiation of Human Adipose Stem Cells

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Background and Aim: During the initial stages of bone repair, some biological phenomena emerge. Of these, proinflammatory cytokines are released in the injury site, which quickly followed by a shift to anti-inflammatory cytokines. However, the biological impacts of pro- and anti-inflammatory cytokines on osteogenic differentiation of mesenchymal stem cells seems to be controversial. In this current investigation, we studied the effect of the proinflammatory cytokines (i.e., TNF-α, IL-6, IL-8, and IL-17F and the anti-inflammatory cytokine IL-4) on the proliferation and osteogenic differentiation of the human adipose stem cells (hASCs).

Methods: In practice, the hASCs were treated with TNF-α, IL-6, IL-8, IL-

17F, or IL-4 (10 ng/mL) for 72 hours mimicking bone repair.
Results: Our findings revealed that the TNF-α could reduce the gene expression of collagen type I, while it increased the proliferation of hASC and the activity of ALP. IL-6 was found to significantly enhance the activity of ALP (18-fold), while the bone nodule formation was also increased by the hASCs. IL-8 did not affect the proliferation or osteogenic gene expression, however it lessened the bone nodule formation. IL-17F was found to decrease the proliferation of hASC, while it increased the activity of ALP. IL-4 appeared to enhance the osteocalcin gene expression and the activity of ALP; nonetheless, it decreased the gene expression of RUNX2 and the bone nodule formation.
Conclusion: Take all, our results showed that all cytokines studied displayed both inreasing and decreasing impacts on the osteogenic differentiation of hASCs, even when applied for 72 hours only. Some cytokines (i.e., IL-6) might be appropriate agents for the induction of the osteogenic differentiation of mesenchymal stem cells as a suitable strategy for enhancing bone repair.
Keywords: Cytokines Osteogenic; Differentiation; Human adipose stem cells; Bone repair

IS-103. Endometrial Stem Cells: Cinderella of Endometrial Immune Network

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Almost five hundred million years ago, the modern immune system was developed. This system had to be finely tuned and controlled so that it did not react with self-antigens leading to autoimmunity and at the same time was to be able to recognize and take action against non-self-antigens. The self/non-self (SNS) discrimination capacity of the immune system was a big challenge during development in millions of years which finally was solved in a way that self-reactive B and T cell clones are eliminated or remained in an anergic state during lymphocyte development and those reacting only with non-self molecules are developed. With SNS discrimination capacity of the immune system, most mammals encountered with another big problem: how they should manage pregnancy in which a non-self embryo is implanted in the uterus? This evolutionary challenge was also solved during the development of endometrial immune regulatory network through extensive modification of immune system at feto-maternal interface. There are a plenty of orchestrated mechanisms working in concert leading to maternal immune tolerance against developing an embryo. During the past couple of years, we have extensively studied and characterized menstrual blood stem cells (MenSCs), as a potential surrogate of eMSCs, in term of their immunomodulatory capacity. Based on our results, these stem cells profoundly modulate functional features of endometrial cells including dendritic cells, NK cells, Th17 and regulatory T cells and induce a pregnancy-friendly phenotype. Our data clearly imply that endometrial stromal cells may contribute to the induction of tolerogenic microenvironment during early pregnancy. In this regard, impairment of the function of endometrial stromal cells as the instructor of endometrial immune cells may lead to pregnancy-related disorders.
Keywords: Endometrial stem cells, Immune regulation, NK cells, Regulatory T cells

Oral Section

OS-001. Improvement of Retinal Injury After Subretinal Transplantation of Neurosphere-Derived Retinal Pigment Epithelium in the Age-Related Macular Degeneration Model

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Background and Aim: Age-related macular degeneration (AMD) is the main cause of irreversible blindness. Stem cells therapy is a promising tool in such diseases. The present study aims to improve the retinal injury following subretinal transplantation of Neurosphere-derived Retinal pigment epithelium cells (NRPELC) for replacement therapies in cases of retinal degenerative diseases.

Methods: Bone marrow stromal cells (BMSCs) were isolated from pigmented hooded male rats. These cells were transformed into neurospheres and differentiated into pigmented spheres (PS) with small molecule induction. Finally, NRPELC were immunostained with RPE65, CRALBP, and OTX2 markers. Consequently, their expression was supported by RT-PCR and RT-qPCR. Moreover, the functionality of NRPELC was evaluated by phagocytosis of fluorescein-labeled photoreceptor outer segment (POS). Accordingly, 5 µL of BrdU-labeled NRPELC cells suspension transplanted into subretinal space through scleral approach. The morphological and electroretinography (ERG) changes in RPE layer and neurosensory retina investigated in time curse from seven days to 90 days post-injection.

Results: In vitro results showed that the cultured BMSCs differentiated into neurospheres. The neurosphere at the seven days was to express the neural markers (Sox2, Oct4, Nanog, and Nestin, $P < 0.05$). The pigmented spheres obtained from neurosphere expressed the RPE markers (RPE65 (100%±1), CRALBP (100%±2), and OTX2 (92%±2), $P < 0.05$) quite well. In addition, RT-qPCR supported the gene expression after 14 days in vitro. Cultivation of PS showed expression of markers from 7 days to 90 days after induction. Furthermore, NRPELC internalized the POS after seven days in culture. In vivo results showed that BrdU-labeled NRPELC survived, migrated, and integrated 90 days post-transplantation into RPE layer. Consequently, Changes in a-wave and b-wave of ERG confirmed the improvement of neurosensory retina function and morphology after cell therapy.

Conclusion: Mesenchymal stem cells therapies are rising trends in the treatment of AMD, and future pre-clinical and clinical studies are required for standardization of the therapy and obtaining the long-time data about the efficacy and safety.

Keywords: Bone marrow stem cells; Neurosphere; Retinal pigmented epithelium; Age-related macular degeneration; Sodium iodate

OS-002. Human Chorionic Mesenchymal Stromal Cells (hCMSCs) Transplantation Ameliorates Motor Function and Prevents Cerebellar Atrophy in Rat Model of Cerebellar Ataxia

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Background and Aim: Cerebellar ataxias include a range of neurodegenerative disorders hallmarked by deterioration of cerebellum. Cell replacement therapy (CRT) offers a potential remedy for the diseases associated with the central nervous system (CNS). This study was designed to assess the neuro-restorative/protective effects of human chorionic mesenchymal stromal cells (hCMSCs) implantation on a rat model of cerebellar ataxia induced by 3-acetylpyridine (3-AP) as a neurotoxin.

Methods: To begin, hCMSCs were extracted, cultured and their mesenchymal abilities proofed. Then, experimental ataxia was induced in twenty-four male adult rats by single injection of 3-AP, and bilateral hCMSCs transplantation in the cerebellum was performed 7 days after 3-AP administration in seven of them. After an assessment of their motor coordination by Rotarod test and electromyography(EMG) changes by its device their cerebellum was sampled. Their Purkinje neuron degeneration was analyzed by Fluoro-Jade staining, then the transcription status of inflammatory, neurotrophic and apoptotic genes assessed by RT-PCR test. We also analyzed the expression level of VEGF and immunohistochemically changes of calbindin.

Results: The findings showed that transplantation of hCMSCs in a 3-AP model of ataxia ameliorated motor coordination and muscle activity, increased cerebellar volumes of molecular and granular layers plus white matter, decrease degeneration of Purkinje cells, increase transcription of neurotrophic factors and decrease the inflammatory changes and apoptosis. We also observed the increase in expression of VEGF and calbindin.

Conclusion: Taken together, human CMSCs could be considered as a suitable candidate for CRT-based therapies with a specific focus on cerebellar ataxia.

Keywords: Mesenchymal stem cells; Cerebellar ataxia; 3-acetylpyridine (3-AP); Neurotrophic factors; Motor coordination; Neurodegeneration; Inflammation; Apoptosis

OS-003. Gene Expression Pattern of CCL2 in Patients with Refractory Rheumatoid Arthritis Who Received Autologous Bone Marrow-Derived Mesenchymal Stem Cell Therapy

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Background and Aim: Mesenchymal stem cells (MSCs) differentiate into tissue types with mesodermal lineage such as chondrocyte, adipocyte, and osteocyte. Immunomodulatory and low immunogenicity of MSCs are unique properties which can be used for the treatment of many diseases like rheumatoid arthritis (RA). RA is the autoimmune inflammatory disease which various inflammatory mediators such as chemokines have a key role in its pathogenesis. Our aim was an evaluation of CCL2 gene expression in RA patients before and six months after MSCs therapy.

Methods: MSCs were derived from bone marrow of 13 refractory RA patients. These cells were cultivated and purified and then 1×10^6 cells per kg of body weight were injected intravenously to the patients. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient centrifugation and then, total RNA was extracted from PBMCs and cDNA synthesized. Gene expression of CCL2 was determined by employing the SYBER Green Real-Time RT-PCR technique.

Results: Relative gene expression of CCL2 was decreased after 6 months compared to the base line, however, this reduction was not significant ($P=0.156$).

Conclusion: Our results provided evidence that CCL2 gene expression can be down-regulated but not significant, following MSC therapy in RA patients. However, for a better conclusion, we suggest to increase the sample size and also follow-ups.

Keywords: Rheumatoid arthritis; CCL2; Mesenchymal stem cell

OS-004. Functional Critical-Size Bone Tissue-Engineered Constructs by Integration of Treated Human Adipose Stem Cells with Osteoinductive, Seeded on 3D Printed Scaffold, Cultured in a Perfusion Bioreactor

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Background and Aim: Bone tissue engineering aims to regenerate new functional bone using cells, scaffolds, bioreactors, osteostimulatory, osteoinductive, and pro-angiogenic factors. In this study, we delineate, from an engineering perspective, the progress that has been made to overcome the main challenges in the field of critical-size bone tissue-engineered constructs using human adipose stem cells (hASCs), 3D printed scaffold, modified perfusion bioreactor, osteoinductive and pro-angiogenic factors.

Methods: Modeling was performed to optimize the parameter of bioreactor and foresight what will happen in vivo. hASCs were extracted from adipose tissue and treated with osteoinductive and pro-angiogenic factors to induce osteogenic and angiogenic differentiation. Treated cells were seeded on 3D printed scaffold which was made based on the defect size and cultured in the modified perfusion bioreactor. After in-vitro culture, surgery was performed to implant bone tissue-engineered constructs. Cell proliferation and osteogenic differentiation were analyzed up to 3 weeks.

Results: Osteostimulatory and pro-angiogenic factors stimulated hASCs toward osteogenic and angiogenic differentiation. Dynamic flow using modified perfusion bioreactors enhanced osteogenic differentiation of hASCs via increasing alkaline phosphatase activity and upregulating osteogenic and angiogenic markers.

Conclusion: Treatment of hASCs with osteostimulatory, osteoinductive, and pro-angiogenic factors enhanced angiogenic and osteogenic differentiation of hASCs. Incorporation of treated hASCs into the 3D printed scaffolds was cultured within the modified perfusion bioreactor formed thicker and more uniform bone tissue-engineered constructs for implantation which is a promising approach to treat patients with large bone defects.

Keywords: Bone; Osteogenic differentiation; Angiogenesis; Human adipose stem cells; 3D printed scaffold; Modified perfusion bioreactor

OS-005. Integrated Automatic Bioreactor of Human-Sized Lungs: Achievement of One Decade's Efforts to Engineering Whole Lung Organs

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Background and Aim: Engineered lungs are an excellent solution for dealing with the increasing demand for transplantation. There is a wide range of hurdles which must be overcome to advance the field and facilitate moving toward the clinic. To do so, we tried these steps: i) engineering rodent lungs using a custom-designed bioreactor as a necessary element to guide the development of tissues, ii) establishing a non-invasive online method to monitor cell viability within lung scaffolds as an essential step toward a functional tissue, iii) building an integrated bioreactor to support large-scale tissue engineering.

Methods: In the step (i), lungs were harvested from 2-month-old male rats. The lungs were decellularized with liquid detergent. Hematoxylin and eosin staining were used to verify efficient removal of cell components of acelluar tissues. Furthermore, scanning electron microscopy proved the maintenance of the extracellular matrix architecture. Human umbilical cord vein endothelial cells (HUVECs) were cultured on acellular tissue using a perfusion-based bioreactor. In the step (ii), an online monitoring system based on respiration activity was developed to monitor cell viability within acellular lung scaffolds. Acellular lung scaffolds were recellularized with HUVECs, and then, cell viability was monitored during a 5-day period. In the step (iii), sheep lungs were decellularized using a 20-L chamber with an SDS-based protocol. The chamber with a custom-designed cap was modified to contain a human-sized lung, providing necessary connections to trachea and pulmonary artery via embedded ports. The decellularization was performed up to 24 hours at the perfusion rate of 1.1-1.4 L/min for different stages.

Results: In this study, (i) H&E staining and SEM analysis indicated that the entire pulmonary airway architecture and alveolar structure in the acellular scaffolds were intact. DNA assay indicated that the removal of the DNA content of the decellularized scaffold was approximately 94.9% (compared to the native lung), and the ultimate tensile strength values of decellularized and native samples were very similar; there was no decrease in UTS for the acellular lung scaffold. (ii) The real-time monitoring system produced a cell growth profile representing invaluable information on cell viability during the culture period. The cell growth profile obtained by the monitoring system was consistent with MTT analysis and glucose consumption measurement. (iii) The architecture of native and decellularized lungs was analyzed with H&E staining and SEM. Removal of nuclear materials was proved by H&E staining; also, SEM imaging demonstrated a total removal of cells and preserved structural architecture. Furthermore, the MTT assay showed the biocompatibility of the decellularized matrix.

Conclusion: To move faster toward the clinic, the integration of engineering and biological parameters in bioreactors is needed. This can lead to more consistent products, reduce the process time and labor intensity, maintain sterility and necessary information on tissue growth and maturity, which is required to have a functional tissue needed for commercialization.

Keywords: Bioreactor; Monitoring system; Human-sized lungs; Tissue engineering; Recellularization

OS-006. Differences Between Neural Genes Expression in Cortex and Striatum After Intra-Arterial Injection of Neural-like Cells Derived from Menstrual Blood Stem Cells in a Rat MCAO Model

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Background and Aim: Stroke is the third leading cause of death. Stem cell therapy has been recognized as an effective method in reducing brain injury. Nowadays, many studies indicated that one of the important sources of stem cells is in menstrual blood. However, lack of researches about the effects of these stem cells in each part of the brain in neurological diseases such as stroke can be observed. In the present study, we assessed the effects of neural-like cells derived from menstrual blood stem cells on neural genes expression in cortex and striatum separately.
Methods: Isolation of human menstrual blood stem cells (MenSCs) from 5 healthy women and neural cells from Wistar adult rats (270-310 g) were done. Neural differentiation by co-culture method using trans-well for one week was performed. Evaluation of neural differentiation by immunocytochemistry and ELISA for neural factors such as BDNF, NT-3, GDNF, and VEGF was done. For making middle cerebral artery occlusion (MCAO) model in Wistar rats (270-310 g, n=6), occlusion of the artery by a microfilament for 30 minutes and inserting a catheter for injection in internal carotid artery was done. Validation of the MCAO model was performed by applying Magnetic Resonance Imaging (MRI). After that intra-arterial injection of neural-like cells (105 cells) was done. Sham and vehicle group (saline) were considered as control groups. After 1 and 7 days, part of penumbra areas in cortex and striatum was determined by MTT assay. Immunohistochemistry, behavioral and molecular test (real-Time PCR) for neural genes including BDNF, MAP2, GFAP, VEGF, and Hsp70 were done.
Results: After neural differentiation, neural-like cells derived from MenSCs display changing in morphology to neural-like the shape and could produce high levels of BDNF compared to undifferentiated cells ($P<0.05$). We could see significant improvement of behavioral tests such as elevated body swing and cylinder test in stem cell group ($P<0.05$). For neural genes expression, after intra-arterial delivery of neural-like cells, MAP2 increased in day 7 in both cortex and striatum, however, increasing of this gene was higher in the striatum. We could observe this condition similarly to BDNF ($P<0.001$). Furthermore, we could observe a high expression of GFAP in the cortex on day 7 but not in the striatum. In the striatum, GFAP could decrease in day 1 and 7 significantly ($P<0.05$). HSP70 gene had a significant decrease in both cortex and striatum ($P<0.05$).
Conclusion: Our major finding is that the intra-arterial injection of neural-like cells derived from MenSCs significantly reduced HSP70 as an important heat shock protein in rats with MCAO (both in cortex and striatum). Regarding neural genes expression, it seems that there are important differences among expression of neural genes during brain repair after stroke in cortex and striatum. More studies about the role of stem cell therapy in changing neural genes expression during brain repair after stroke are required.
Keywords: Menstrual blood stem cells; Neural differentiation; Stroke; Intra-arterial injection; Gene expression

OS-007. Therapeutic Potential of Human-Induced Pluripotent Stem Cell-Derived Endothelial Cells in a Bleomycin-Induced Scleroderma Mouse Model

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Background and Aim: Vascular injury and destruction of endothelial cells (ECs) are the early events in scleroderma (SSc) patients. This study aims to investigate the therapeutic potential of human-induced pluripotent stem cell-derived ECs (hiPSC-ECs) to treat SSc. We have assessed the functional differentiation of hiPSC-ECs and compared them with human embryonic stem cell-derived ECs (hESC-ECs) by a variety of in vitro experimental approaches. Additionally, we evaluated the therapeutic potential of hiPSC-ECs in a bleomycin-induced SSc mouse model.
Methods: The hESCs and hiPSCs culture and EC differentiation Human umbilical vein endothelial cell isolation and culture Flow cytometry and cell sorting Immunostaining Colony-forming assay Uptake of acetylated low-density lipoprotein (DiI-ac-LDL)Tube formation by ECs in vitro Scleroderma mouse model and hiPSC-EC transplantation Histopathology and immunohistochemistry Mast cell number Enzyme-linked immunosorbent assay of serum cytokine
Results: Our results demonstrated that hiPSC-ECs and hESC-ECs showed similar maximum expressions of FLK1 (early EC marker) at day five during differentiation. After sorting and culturing, the FLK1-positive cells exhibited spindle and subsequent endothelial cobblestone morphology in the EGM2 medium. The hESC-ECs and hiPSC-ECs also expressed late EC markers CD31 (68% and 75%), CD144 (50% and 61%), CD146 (46% and 61%), and DiI-labeled acetylated low-density lipoprotein (DiI-ac-LDL) uptake (55% and 63%), respectively. They additionally formed capillary-like structures on Matrigel. Analyses of the transplantation of sorted CD31-positive hiPSC-ECs into the bleomycin-induced SSc mouse model demonstrated that these cells participate in the recovery of the damaged vessels. There was a reduction in collagen content; the number of total and degranulated mast cells returned to their normal state, and bleomycin-induced wounds, as well as skin fibrosis, improved four weeks after transplantation of hiPSC-ECs. Our findings have shown that the differentiation process fromhESCs and hiPSCs to vascular cell components is similar. Besides, this is the first study to determine the therapeutic potential of vascular cells from hiPSCs in the treatment of an SSc model. In the future, with further validation, these may be used as an appropriate source for the treatment of SSc patients.
Conclusion: These early data have shown the proof of concept that the transplantation of ECs derived from hiPSCs, similar to hESC-ECs, can provide some benefits in the setting for SSc regeneration. With further validation, the ECs that are isolated from hESCs and hiPSCs, as described here, can provide a continual endothelial cell source of therapy for vascular regeneration in SSc in addition to other diseases with endothelial abnormalities
Keywords: hiPSCs; Endothelial cells; Differentiation; Scleroderma disease; Bleomycin animal model cell therapy

OS-008. Conductive Drug-Loaded Electrospun Scaffold Based on Polyaniline for Nerve Tissue Engineering

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Background and Aim: Bioelectricity, along with other vital signals, plays an important role in tissue engineering and regenerative medicine. The crucial step of entering this area is to design a suitable substrate for transmitting necessary signals to the cells. Conductive nanofibrous scaffolds are an appropriate tool for this purpose and have been highly regarded due to their resemblance to the native extracellular matrix (ECM) and their ability to regulate cell behaviors. Herein, we aimed to produce a drug-loaded conductive nanofibrous scaffold for nerve tissue engineering.
Methods: Polyethersulfone (PES) nanofibers were prepared using electrospinning technique. Surface activation and enhancement of surface hydrophilicity were achieved by using oxygen plasma treatment. Freshly distilled aniline and ammonium persulfate (APS) were dissolved separately in an aqueous solution of the drug. Afterward, the PES electrospun scaffold was fixed to plexiglass frame for the retention of its original form and was immersed in the drug/aniline solution at 40°C for

a specified time. Chemical polymerization was then initiated by adding oxidant solution containing APS and conductive layer was polymerized at around 5°C for 48 hours. In this way, the core/shell nanofibers were synthesized via in situ chemical oxidative polymerization of aniline and the molecules of the drug were incorporated in polyaniline during polymerization. By adjusting the electrospinning parameters and the polymerization conditions the desirable nanofiber structure was fabricated. The PES/PANI/drug nanofibrous scaffold was characterized using scanning electron microscopy (SEM), attenuated total internal reflectance infrared spectroscopy (ATR-FTIR), electrical conductivity and cell culture study.

Results: Based on SEM images, PES nanofibers prepared here were homogenous and defect-free. As well as this, the conductive core/shell scaffold was coated uniformly. It was shown that plasma treatment had a significant effect on nanofibers coating process by increasing hydrophilicity and functionality of nanofibers surface. ATR-FTIR spectra for PES and PANI/PES/drug nanofibers and FTIR spectra for powder material of PANI confirmed the presence of PANI and drug on the surface of nanofibres. Additionally, the conductive scaffold offered acceptable value of conductivity. It was found that the conductivity and morphology of the final nanofibers were strongly influenced by the dopant properties, the concentration of aniline and dopant, and time of polymerization. In addition, the viability of adipose-derived stem cells cultured on the conductive nanofibrous scaffold was found to be excellent and cells had good adhesion to the scaffold.

Conclusion: Herein, we developed a drug-loaded conductive nanofibrous scaffold with a proper cell compatibility. By the combination of nanofibrous topography, electrical activity, and biochemical functionality new multi-functional material with unique physicochemical properties was obtained. The natural conductivity of these composites offers exciting opportunities for electrical stimulation of cells, especially those are sensitive to electrical signals such as nerve cells. So, our scaffold can be an appropriate tool for regulating stem cells behaviors such as proliferation and differentiation.

Keywords: Conductive nanofibers; Electrospinning; Polyaniline; Nerve tissue engineering; Drug delivery; Stem cells

OS-009. Effect of Amount of Tetraethylorthosilicate (TEOS) and Thermal Treatment Temperature on Mechanical and Biological Properties of Gelatin-Calcium Phosphate Scaffolds

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Background and Aim: Clinical demands for bone regeneration continues to rise due to trauma; cancer; infection; and arthritis. Nowadays, the most important topic is developing of complete bioactive three- dimensional (3D) composite scaffold in bone tissue engineering (BTE) which can regenerate bone damages successfully. Variety of materials have been utilized to create novel alternatives instead of conventional ones which had some disadvantages.

Methods: In the present work, gelatin-calcium phosphate composite scaffolds were produced via solvent casting and freeze-drying methods. Amount of tetraethylorthosilicate (TEOS) and temperature of thermal treatment were two factors in which considered as the variations. Synthesizing reinforcing particles were decorated with SiO44- to increase bioactivity and osteoconductivity and then were surface modified by Gelatin solution with the aim of forming a good interface between two phases. Finally, gelatin-glutaraldehyde solution and TEOS sol were used as two different coatings on scaffolds.

Results: Scanning electron microscopy (SEM), three-point bending test, simulated body fluid (SBF), and differential thermal analysis (DTA) coupled with energy dispersive spectroscopy (SEM/EDS), allowed us to detect the structure and behavior of the scaffold. According to SEM analyze, there is an interconnected network of pores in the range size of some microns to 200 micrometers. These scaffolds also have considerable mechanical properties from 2 to 5 MPa. Bioactivity analysis illustrated that the interactions of the materials support the apatite formation in SBF. Decoration and surface modification of glass-ceramic particles were totally effective in increasing bioactivity and final strength respectively. Scaffolds which were coated with TEOS sol had more final strength while, the other ones had more toughness According to DTA results, thermal treatment of glass particles in 1100-degree centigrade cause the crystallization of which, enhance mechanical properties, and decrease the osteoconductivity in SBF.

Conclusion: Although all of the scaffolds have been had good behavior in different tests, the scaffold which was produced with a lower temperature of thermal treatment and lower amount of TEOS in particles, is the best choice for bone regeneration in bone tissue engineering

Keywords: Bone tissue engineering; Polymer ceramic scaffold; Thermal treatment

OS-010. Developing a Cost-Effective and Scalable Production of Human Hepatic Competent Endoderm from Size-Controlled Pluripotent Stem Cell Aggregates

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Background and Aim: Dynamic suspension culture of human pluripotent stem cells (hPSCs) in stirred bioreactors provides a valuable scalable culture platform for integrated differentiation toward different lineages for potential research and therapeutic applications. However, current protocols for scalable and integrated differentiation of hPSCs limited due to the high cost of growth factors and technical challenges.

Methods: hPSCs aggregates primed with 6 and 12 µM of CHIR99021 (CHIR), a Wnt agonist, in combination with different concentrations of high-cost Activin A (10, 25, 50, 100 ng/mL). We sought to determine the appropriate treatment duration for efficient and cost-effective differentiation protocol for foregut definitive endoderm production in a dynamic suspension culture. Afterward, we evaluated the impact of the initial hPSC aggregate sizes (small: 86±18 µm; medium: 142±32 µm; large: 214±34 µm) as critical bioprocess parameter on differentiation efficacy at the beginning of induction

Results: One-day priming of hPSCs as 3D aggregates (hPSpheres) with 6 µM CHIR followed by treatment with a low concentration of Activin (10 ng/mL) for 2 days resulted in efficient differentiation to definitive endoderm that highly expressed the anterior endodermal marker HEX. These endodermal cells differentiated efficiently into mature functional hepatocytes. The medium-sized hPSpheres resulted in higher productivity and differentiation efficiency for scalable hepatocytes production, whereas small aggregates resulted in significant cell-loss after CHIR treatment and large aggregates had less efficacious endodermal differentiation. Differentiated cells exhibited multiple characteristics of primary hepatocytes as evidenced by expressions of liver-specific markers, indocyanine green (ICG) and low-density lipoprotein (LDL) uptake, and glycogen storage

Conclusion: This platform could be employed for the scalable production of hPSC-derived hepatocytes for clinical and drug discovery applications.

Keywords: Hepatocyte; Definitive endoderm; Aggregate; Scale-up differentiation; Size-controlled differentiation

OS-011. Autologous Human Stromal Vascular Fraction Injection in Post-Burn Hypertrophic Scar: A Double-Blinded Placebo-Controlled Clinical Trial

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Background and Aim: Post-burn hypertrophic scar (HTS) is a common complication of burn injury which results from the prolonged inflammatory response, and leads to cosmetic, functional, and psychological complications. Adipose-derived stromal vascular fraction (SVF) contains heterogenous cells with regenerative, anti-inflammatory and anti-fibrotic properties.

Methods: In this phase I randomized placebo-controlled clinical trial, we aimed to evaluate the safety and potential efficacy of intra-dermal injection of autologous SVF in HTS. We enrolled 20 patients who had HTS for more than 1 year with Vancouver scar scale (VSS) of ≥4. In each patient, two 6 cm^2 scar sites were randomly received 2 mL of SVF (1×10^6 cells/cm^2) or normal saline. Patients were followed till 4 months post-transplantation. Our primary endpoint was the number and severity of adverse events. Our secondary endpoints were alterations in VSS score, skin thickness (dermal and epidermal layers) measured using high resonance ultrasound, patient self-assessment of HTS improvement based on visual analog scale (VAS), and level of expression of TGF-β assessed by immunohistochemical assay. This study has been registered with IRCT.ir (identifier: IRCT2012070710201N1).

Results: Safety evaluations showed no major and serious adverse events in treated patients. The mean VSS scores of the treatment and placebo sites before the injection were 8.0±1.2 and 7.3 ±1.6 which respectively decreased to 6.4±1.4 and 6.7±1.7 at 4 months following cell transplantation. The mean VAS at 4 months after cell transplantation in SVF and control groups was respectively 3.2±2.2 and 0.7±1.8. The mean skin thickness in SVF group decreased from 2.9±0.7 mm to 2.7±0.6 mm, while in the control group was respectively 2.8 ±0.7 mm and 2.8±0.8 mm, before and 4 months after the treatment. The expression level of TGF-β3 increased in SVF group skin biopsy specimens compared to placebo group, 4 months after cell injection.

Conclusion: In this study, we demonstrated safety and tolerability of autologous SVF intra-dermal injection in HTS. Besides, our study results indicated the potential efficacy of SVF injection in HTS probably via the anti-inflammatory and regenerative properties of SVF cells. However, this needs to be confirmed performing further investigations.

Keywords: Stromal cells Burns Cicatrix; Hypertrophic Regenerative medicine

OS-012. Effect of 810, 940 nm Laser Irradiation with Different Energy Densities on Periodontal Ligament Stem Cells

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Background and Aim: At low intensities, laser therapy has shown biostimulatory effects at cellular levels. This effects may be used in tissue engineering treatments using stem cells. However, the application of appropriate dosimetric parameters is of great importance in achieving favorable results. The purpose of this study was to determine the effect of different fluences (energy densities) of near infra-red diodes lasers with wavelengths of 810, 940 nm typically used in dentistry on the proliferation and survival of Periodontal ligament derived stem cells.

Methods: In this study, periodontal tissue remnants of human teeth extracted for orthodontic purposes were used for isolation of periodontal ligament stem cells. The mesenchymal nature of the isolated cells was determined using CD45, 90, 105 surface markers. Third passage cells were then irradiated by laser radiation of 810 (L1), 940 (L2) nm, with energy densities of 0.5, 1.5 and 2.5 J/cm^2, 100 mw output powers. A non-irradiated group was considered as control. Cellular viability was measured after 24 hours of exposure to a single session of laser irradiation and proliferation was measured 24, 48, 72 hours after exposure. Propidium iodide (PI) staining was used to identify any pyknotic nuclei or nuclear fragmentation 72 hours after irradiation

Results: The isolated cells were positive for CD105 and CD90 and Negative for CD45. An increase in cellular viability 24 hours after laser irradiation was only observed with the 940 nm laser and 2.5 J/cm^2 energy density. This increase was statistically significant compared to the other settings and control group ($P< 0.001$). The proliferation of cells also showed a statistically significant increase in the 940 nm, 2.5 J/cm^2 irradiated group at all time points in comparison to other groups and control($P<0.001$). PI staining showed no adverse effect in cell nuclei in any of the groups.

Conclusion: In this study, 940 nm diode laser with an energy density of 2.5 J/cm^2 significantly increased PDLSC viability and proliferation compared to the other settings and control group.

Keywords: Photobiomodulation; Periodontal ligament; Stem cell

OS-013. Regeneration of Intervertebral Disc by Stem Cell and Injectable Hydrogel in Animal Model

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Background and Aim: Intervertebral disc degeneration is recognized to be the leading cause for chronic low-back pain which occur because of structural damage of disc and is caused by herniation. Current methods for treatment of degeneration are artificial disc and prostheses but there are caused by the limitation of movement and are invasive. Advance method for regeneration is cell and hydrogel In this study injectable hydrogel prepared that was similar to the extracellular matrix of the disc. Finally, hydrogel with nucleus pulposus (NP) cells and BMSCs injected to degenerated disc model and survey effects of its regeneration

Methods: Injectable hydrogel synthesis with chitosan, B glycerol Phosphate, collagen, gelatin, hyaluronic acid, chondroitin 6 sulfate, and fibroin silk. Bone marrow mesenchymal stem cells extracted from rabbit and cultured and differentiated to NP cells and another group undifferentiated. Rheology, MTT, trypan blue, SEM used for evaluation of hydrogel. Discs 3-6 in animal model degenerated using by needle and injection of papain. The degenerated discs divided into 4 groups: group 1 was disc 3(L3-L4) that used as control group 2 was disc 4(L4-L5) that was degenerated but without cell and hydrogel injection. Group 3 was disc 5(L5-L6) that encapsulated BMSc in hydrogel injected. Group 4 was disc 6(L6-L7) that encapsulated NP cells in hydrogel injected. Finally, effects regeneration of cells and hydrogel in animal model evaluated by MRI, histology, immunofluorescent and RT-PCR.

Results: Results of rheology showed that gelation of hydrogel started at 37°C after about 30 min. Rheological properties showed suitable mechanical properties of hydrogel and too. MTT and trypan blue tests indicated hydrogel was cytocompatible. Results of histology and immune fluorescent of rabbit IVD post injection of hydrogel-NP cells and hydrogel-BMSc determined high expression and production of extracellular matrix post injection of NP cell-hydrogel in degenerated IVD. MRI indicated

signal intensity and high of disc increased in the hydrogel-NP group.
Conclusion: Our study suggests using of compound hydrogel-NP cell is excellent for regeneration of IVD and it could use for regeneration of degenerated IVD in the human. It needs that perform our results in the big animal before clinical trial.
Keywords: Intervertebral Disc; Low back pain, Nucleus pulposus cells; Injectable; Chitosan; Collagen; Chondroitin sulfate; Fibroin silk; Hyaluronic acid; Gelatin

OS-014. Gene Expression Analysis in AHR (Aryl Hydrocarbon Receptor)-Active Fibroblast Cells to Measure Factors for Dedifferentiation of Fibroblast to Stem Cells

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Background and Aim: Aryl hydrocarbon receptor (AHR) transcription factor play key roles in cell differentiation, apoptosis, response to toxic substances, and cell growth. It effects on the transcription of many genes in the nucleus (more than 200 genes). For example, NFKB, RelB and p300 genes that play an important role in the development of organisms. The activation of this transcription factor has a role in cell fate in B cells and promoting epithelial to mesenchymal cells and it also has roles in many stem cells.
Methods: Human fibroblasts were cultured in a specific culture medium. Three samples were treated with DMSO-soluble TCDD and three samples were treated with DMSO as controls. After 24 hours, the extraction of RNA was carried out and gene expression analysis was performed using a microarray device. Data obtained by GEO 2R software were compared and the expression of genes was analyzed. Then, the results were analyzed in Reactome and Gorilla software, and molecular pathways towards the creation of stem cells were analyzed.
Results: After analyzes carried out by Reactome and Gorilla software, we found that expression of hypoxia-inducible factor 2A (HIF2A) gene, pluripotency regulator gene under decreased oxygen conditions, increase. In another molecular network, after transcription, OCT4 (octamer-binding transcription factor 4), a marker for undifferentiated cells, attach to the LN28. Sox2 and Nanog transcribe (Nanog is a factor for maintaining the ES cell self-renewal). Then, in on one of the pathways, Oct4, Sox2, and Nanog link to the Sox2 promoter and cause more transcription of this gene. In another pathway, Oct4, Sox2, Nanog, ZscAN10, PRDM14, SMAD2, FOXP1-ES bind to the Oct4 promoter and cause more transcription of this gene. Oct4, Sox2, NANOG, KLF4, PBX1, and SMAD2 bind to the Nanog promoter, and enhance the expression of this gene. Nanog, Sox2, and Oct4 are markers for embryonic stem cells.
Conclusion: Stem cells are biological cells that have the ability to convert to different cells. One of the best ways to obtain stem cells is the transform of somatic cells into stem cells. In this study, with the bioinformatic analysis of the fibroblast cells that their AHR transcription factor was activated, it was found that the expression of Nanog, LN28M, and Sox2 factors increased, which have a great effect on pluripotency.
Keywords: Aryl hydrocarbon receptor; Stem cell; Epigenetic; Development

OS-015. Effects of Astaxanthin on the Expression of PPARα and PPARγ in LPS-Stimulated Neuroblastoma Cells

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Background and Aim: Peroxisome proliferator-activated receptors (PPARα and PPARγ) activation can result in the transcription of proteins involved in oxidative stress defense and mitochondrial biogenesis which could rescue mitochondrial dysfunction in Parkinson's disease (PD). Astaxanthin (ASTA) is a red-orange carotenoid with powerful antioxidant that occurs naturally in a wide variety of living organisms. There is evidence demonstrating that ASTA confers neuroprotective effects in experimental models of chronic neurodegenerative disorders and neurological diseases.
Methods: In this study, Groups included the cell control group (SH-SY5Y cell line) that did not contain ASTA and/or LPS, the group that received the ASTA (10 mM) alone, the group that received the LPS (10 mg/mL) alone and cell group was pre-treated with ASTA (10 mM) for 1 hour, washed, and then treated with LPS for an additional 24 h (LPS+ASTA group). ASTA was treated with, SH-SY5Y cell line at 48 hours. To measure the expression of peroxisome proliferator-activated receptors (PPARα and PPARγ) and one of the genes involved in inflammation (IL-6). Using the real-time PCR technique, the gene expression of PPARα and PPARγ were measured. Also, IL-6 protein expression was assessed by ELISA test. The results were analyzed by the one-way analysis of variance (ANOVA) using Prism version 6.0 software.
Results: Our results show that treatment with ASTA (10 mM) for 1 hour attenuates the LPS-induced inflammation. The beneficial effect of ASTA is associated with the reduction inflammation by restoring the PPAR proteins expression. ASTA has a greater effect on PPARγ expression changes than the PPARα.
Conclusion: Consequently, accordingly hypothesize that ASTA has therapeutic properties protecting SH-SY5Y cells from LPS-induced inflammatory. PPAR proteins are very important in neuronal survival and inflammation. The results suggest that ASTA treatment may be beneficial for enhancing oxidative stress defense.
Keywords: Astaxanthin; Neuroblastoma cells; Gene expression; PPARα; PPARγ

OS-016. Donor-Patient Age and Gender as Influential Factors for Graft Versus Host Disease Incidence and Survival After Allogeneic Hematopoietic Stem Cell Transplantation

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Background and Aim: Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) has become routine for the treatment of many hematological malignancies, while graft-versus-host Disease (GvHD) is still an inevitable undesirable and serious consequence. A Variety of donor and patient factors including donor-recipient HLA matching, ABO compatibility, race, and cytomegalovirus serologic state can affect the GvHD incidence and survival. In this study, we examined the effect of donor-patient gender and age on GvHD incidence and survival in patients underwent allo-HSCT in Taleghani bone marrow transplantation center.
Methods: This study was carried on 196 leukemic patients who received allo-HSCT between 2008 and 2017 in the bone marrow transplantation center of Taleghani hospital, Tehran. 34.5% were male recipients with female donors, 30% male recipients with male donors, 15.5% female recipients with female donors, and 20% female recipients with male donors. Factor analyzed were donor-patient gender and age, and accordingly, patient survival was calculated by using the Kaplan–Meier method. The GvHD incidence was estimated using the cumulative incidence curves and proportional sub-distribution hazard regression model.
Results: The main findings were that HSCT with female donors resulted in significantly higher rate of GVHD (57% versus 52% , P value ≤0.0001) and lower survival (50% versus 60%, P value = 0.001) compared to HSCT with male donors. In addition, male recipients with female donors

showed an increase in the risk of GvHD incidence. (HR,1.16, 95% CI,1.04-1.25, P=0.006). younger donors (aged less than 30 years) were associated with better survival (48% versus 40%, P value = 0.009) and a lower rate of GvHD (53% versus 62%, P value = 0.04). therefore, older patient and older donor regardless of donor sex were associated with a higher rate of GvHD (P-value= 0.003 and P-value= 0.03 respectively).
Conclusion: Donor and patient characteristics such as age and gender have been demonstrated to influence the allo-HSCT outcome including GvHD incidence and survival. Overall, recipients of grafts from female donors had a significantly higher rate of GvHD and worst survival than recipients with male donors. Moreover, younger donors may result in a lower rate of GvHD and survival improvement after transplantation. Therefore, donor-patient age and sex are important risk factors for predicting the allo-HSCT outcome.
Keywords: Graft versus host disease; Hematopoietic stem cell transplantation

OS-017. The Effect of Amniotic Membrane-Derived Mesenchymal Cells Transplantation on Cardiac Function Improvement in a Model of Heart Failure in Male Rats Through the Effect on The Expression of bcl2, and bax

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Background and Aim: Heart failure is an increasing and debilitating problem throughout the world. Currently, cell therapy is a beneficial strategy compared to previous treatments with the potential to replace injured myocardial tissue. It seems that using mesenchymal stem cells derived from the amniotic membrane is an appropriate therapeutic method for heart failure.
Methods: Thirty-five male Wistar rats were randomly assigned into 5 groups (7 in each group). Study groups were: 1. Control group, 2. Heart failure group (HF), 3. Sham group, 4. Culture media group, 5. Stem cells transplantation group (SCT). Heart failure model was induced by subcutaneous injection of 170 mg/kg/d isoproterenol during 4 consecutive days and the induction was confirmed by echocardiography 28 days later. In stem cells transplantation group, 3×10^6 cells were transplanted into myocardial tissue in a 150 µL culture media. By use of echocardiography, cardiac function was examined. Western blot method was used to determine the expression of bcl2, bax and p53 proteins.
Results: Echocardiography parameters showed that ejection fraction in the HF group increased from 58.73± 0.9 to 81.25± 0.5 in the SCT group which indicates 22.52 change in this parameter. Fractional shortening increased from 27.8±53.58 in HF group to 45.6±55.91 in the SCT group indicating a change of 22.52. Protein level measurements showed increased levels of p53 and bax proteins in the HF group.
Conclusion: It seems that amniotic membrane-derived mesenchymal stem cells transplantation significantly improves the cardiac function in heart failure through effects on the expression of bcl2, bax and p53 proteins.
Keywords: Heart failure; Amniotic membrane-derived mesenchymal stem cells; Ejection fraction; Fractional shortening; p53; bcl2; bax

OS-018. Nanofibrous Three Dimensional Scaffold Produced by Freeze-Drying from Ternary Composition: Polyvinyl Alcohol, Vinyl-Based Polymer, and Nano-Graphene Oxide; for Bone Tissue Engineering

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Background and Aim: The present work aims to obtain new nanofibrous biocomposites based on polyvinyl alcohol (PVA) reinforced with vinyl based polymer (VBP) and nano-graphene oxide (nGO) by freeze-drying as a candidate for bone tissue engineering. On the one hand, the scaffolds are designed with consideration of the high implementation of the aforementioned biopolymers as biomaterials; on the other hand, the two component of the system, GO and VBP, is expected to improve porosity, biocompatibility structural stability and boost mechanical strength. Moreover, a 3D porous structure made out of nanofibers fabricated through the freeze-drying process.
Methods: We prepare two solutions of polymers:1) Polyvinyl alcohol, Vinyl based polymer, and Nano-graphene oxide (NP^+), 2) Polyvinyl alcohol, Vinyl based polymer (NP^-). Then fabricate 3D hydrogel composites through the controlled freeze-drying process. The scaffolds were characterized by Fourier-transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). Furthermore, the mechanical properties, biocompatibility, and cytocompatibility of PVA-VBP-nGO (NP^+) and PVA-VBP (NP^-) scaffold were explored.
Results: FT-IR showed the absorption regions of the specific chemical groups associated with PVA, VBP, GO in NP^+ hydrogel group and PVA, VBP in NP^- hydrogel group. Incorporation of nGO greatly improved the thermal resistance, mechanical stability, and cytocompatibility of the NP^+ nanocomposite scaffold as compared to NP- composite scaffold. SEM analysis showed that the presence of nanofibers in the structure of the above mention groups. It is also found that using of nGO resulted in more uniformly nanofibers in NP^+ 3-dimensional group.
Conclusion: In this study, for the first time, we introduced the fabrication of new PVA-VBP-nGO nanofibers 3D scaffolds through the freeze-drying process which could serve as an efficient construct for bone tissue engineering.
Keywords: Nanofibrous three-dimensional scaffold; Polyvinyl alcohol; Nano-graphene oxide; Bone tissue engineering

OS-019. Targeted Deletion of BCL11A Gene by CRISPR/Cas9 System for Fetal Hemoglobin Reactivation

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Background and Aim: Beta-thalassemia is caused by unusual structure or decreased production of β-chains. On the other hands, HbF induction would be a promising strategy to attenuate the symptoms of thalassemia. Using the CRISPR/Cas9 technology which is a versatile and powerful editing tool we proposed to reactivate HbF in the adult stage. B cell lymphoma 11A (BCL11A) is a key regulatory agent in HbF silencing, so suppression of the BCL11A protein could represent a therapeutic target for hemoglobinopathies disease.
Methods: We proposed that knockdown of BCL11A expression by erythroid enhancer disruption would result in the induction of gamma-globin expression. We used the CRISPR/Cas9 system to generate clones of HEK293 cells with a deletion of the 200bp BCL11A enhancer. The cleavage activity of each sgRNA was examined by T7EI assay. We introduced the SpCas9 and a pair of sgRNA expressing vectors into HEK293 cells by Amaxa 2b-Nucleofector kit. Clonal isolation was performed in order to isolate clonal cells containing homozygote deletions through serial dilution. Targeted genomic deletion via CRISPR/Cas9 using pairs of sgRNAs was evaluated and recognized by genomic PCR and Sanger sequencing. To assess the expression reduction of BCL11A gene, BCL11A mRNA levels were checked by RT-qPCR.

Results: Genomic PCR and Sanger sequencing results validated targeted deletion in transfected cells and RT-qPCR results showed down to a two-fold reduction in BCL11A expression in HEK293 cells.
Conclusion: This study showed that these CRISPR/cas9 constructs bring about targeted deletion and reduction in BCL11A mRNA level. The next step of this study is to examine these constructs in other erythroleukemia cell lines in order to the elimination of the inhibitory effect of BCL11A and induction of fetal hemoglobin production.
Keywords: Beta-thalassemia; Fetal hemoglobin; γ-globin; CRISPR/Cas9; Genome editing

OS-020. The Serum Levels of Uric Acid Might Be a Predictive Biomarker for Acute GvHD

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Background and Aim: Acute graft-versus-host disease (aGvHD) continues to be the major complication of allogeneic hematopoietic stem-cell transplantation (AHSCT). The diagnosis of aGvHD is dependent on clinical manifestations which mostly occur after organs damages. So, identification of predictive biomarkers can be useful to prevent and manage aGvHD. Following conditioning for AHSCT, danger signals such as uric acid (UA) are released by the affected tissues but the association of UA levels with aGvHD is still controversial. This study investigated the association of serum levels of UA with the incidence of aGvHD.
Methods: Altogether, 196 patients with hematological malignancies, who were candidates for AHSCT, were randomly chosen and 2 mL peripheral blood samples were taken with signed consent form at the day of the transplantation (day 0). The serum samples were separated from whole blood and the UA serum levels were quantified using enzyme-linked immunosorbent assay (ELISA). The occurrence of aGvHD in 100 days post-transplantation was evaluated using clinical manifestation in accordance with NIH criteria. The results were analyzed using SPSS. Patient survival was calculated by using the Kaplan–Meier method.
Results: Overall, 47 patients out of 196 recipients (24%) developed aGvHD. The UA serum levels of aGvHD patients was significantly lower than those of recipients without aGvHD. (P value = 0.0051, risk ratio=0.713). There was no significant association between UA levels and survival.
Conclusion: Our results suggest that the UA serum levels of patients at the day of transplantation might be a predictive biomarker for occurring aGvHD so that patients with lower UA serum levels are probably more susceptible for aGvHD. Determination a cut-off for this parameter can help for better prediction.
Keywords: Acute GvHD; Serum uric acid; Predictive biomarker

OS-021. Effect of Different Molecular Weight Fractions Derived From Probiotic Cell-Free Supernatant on Colon Cancer Stem-Like Cell Invasion and Apoptosis

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Background and Aim: Cancer stem cells play an essential role in tumor metastasis. Decreased expression of E-cadherin and dysregulated expression and activity of matrix metalloproteinases (MMPs), especially MMP-9 can lead to tumor invasion and metastasis. Many studies have reported anti-cancer effects of *Lactobacillus reuteri* as a probiotic bacterium. In this study, we investigated the effect of different molecular weight fractions derived from the *L. reuteri* supernatant and its heat-killed sonicated fraction (HK-SON) on invasion factors and apoptosis in previously established human colon cancer stem-like cells derived from HT-29 cell line engineered by E-cadherin shRNA (HT29-CSC like).
Methods: Cell-free supernatant (CFS) of *L. reuteri* was fractionated into defined molecular weight ranges (<3 kD, 3-10 kD, 10-50 kD, 50-100 kD, >100 kD) using ultra-filtration tubes. Also, 109 CFU/mL of *L. reuteri* was heat-killed at 100°C in DMEM medium, and then sonicated and centrifuged. HT29-CSCs were treated with different molecular weight fractions of CFS, HK-SON, and uninoculated bacterial growth media (MRS) as a control. Subsequently, cell invasion assessed using matrigel-coated insert plates. The expression of MMP-9 and TIMP-1 mRNAs were determined using real-time PCR. The gelatinolytic activity of MMP-9 was evaluated by Zymography and the apoptosis and necrosis percentage of cells were determined using Annexin V-PE and 7AAD by flow cytometry.
Results: Cell invasion was significantly decreased following treatment with HK-SON and different molecular weight fractions of CFS in comparison with an MRS-treated group ($P<0.05$). Also, HK-SON, 10-50 kD, 50-100 kD, and >100 kD fractions significantly decreased activity and expression level of MMP-9 ($P<0.05$). Furthermore, the expression level of TIMP-1 was significantly up-regulated by crude CFS, and >100 kD fraction ($P<0.05$). Apoptosis was significantly decreased following treatment with HK-SON and >100 kD fractions.
Conclusion: Our results indicate that CFS of *L. reuteri* possesses anti-metastatic properties. Since fractions containing high molecular weight secretory factors decreased cell invasion, it suggests that the inhibitory compound(s) may fall into a macromolecule range such as a protein, nucleic acid, or a polysaccharide.
Keywords: Apoptosis; Invasion; *Lactobacillus reuteri*; Matrix metalloproteinase; Probiotic

OS-023. Exosomes Isolated from Menstrual Blood-Derived Mesenchymal Stem Cells Regenerate the Beta Cell Mass in Diabetic Type 1 Animal Model

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Background and Aim: Diabetes type 1 is characterized by the lack of insulin production as a result of degeneration of insulin-producing beta cells in the pancreas. The autoimmune response against beta cells is the main reason for this disease; therefore, any strategies that help immune response regulation can be beneficial. Studies have shown the effectiveness of mesenchymal stem cells (MSCs) in the regulation of T cell response and pancreatic islet repair. However, the application of MSCs accompanies the cell therapy safety issue. The unknown fate of injected stem cells is one of the major safety concerns regarding stem cell therapies; therefore, in this study, we have used the exosomal secretome of MSCs to regenerate insulin-producing cells
Methods: MSCs were isolated from menstrual blood as a rich and noninvasive source of MSCs. Exosomes were isolated and characterized using western blot and AFM, SEM techniques. Exosomes were injected intravenously at different time points after induction of diabetes using STZ. Blood glucose and insulin levels were measured at pre-determined time points and animals were sacrificed at day 60 and regeneration of beta cells and insulin production at pancreas were analyzed using immunohistochemistry.
Results: Flow cytometric and differentiation assays confirmed the characters of MSCs derived from menstrual blood. The presence of CD81, CD63, Tsg-101, Calnexin markers on exosomes was confirmed using western blotting and AFM and SEM analysis verified the presence of purified exosomes. Altogether, the blood levels of glucose and insulin

and the histochemistry analyses represented the regenerative potential of exosomes isolated from menstrual blood-derived MSCs in the restoration of insulin-producing cells. The in vivo tracking of exosomes further confirmed the involvement of exosomes in beta cell regeneration
Conclusion: Although very successful in preclinical studies, mesenchymal stem cells have still very limited therapeutic applications in clinics mainly because of its safety concerns. Secreted exosome from these cells exerts the most beneficial properties of stem cells; however, they follow fewer safety issues as they are not active agents as cells are. This work represents the effectiveness of MSC-derived exosomes in the regeneration of pancreatic beta cells
Keywords: Stem cell; Exosome; Diabetes type regeneration beta cell

OS-024. Anti-fibrosis Effects of Extracellular Vesicles Generated From Human Embryonic Stem Cell-Derived Mesenchymal Stromal Cells in Chronic Liver Injury

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Background and Aim: Mesenchymal stromal cells (MSCs) exert their hepato-protective effects more probably through the secretion of functional extracellular vesicles (EVs) in liver injuries. Human embryonic stem cell-derived MSCs (ES-MSCs) are increasingly applied both in vitro and in vivo due to the high proliferation rate and immunomodulatory potential. The aim of this study is to investigate the therapeutic potential of ES-MSC derived EVs (ES-MSC EVs) in thioacetamide-induced chronic liver injury (CLI).
Methods: Inhibitory effects of ES-MSC EVs were determined on peripheral blood mononuclear cells (PBMCs) proliferation using mixed lymphocyte reaction (MLR) assay in vitro. To induce CLI model, 200 mg/kg TAA toxin were injected twice weekly for 16 weeks. After cirrhosis induction, ES-MSC EVs were injected intrasplenically under the guidance of ultrasound.
Results: Human ES-MSCs EVs could elicit potent immunosuppression potential compared to human bone marrow (BM)-MSCs and human adipose (AD)-MSCs in vitro. Moreover, ES-MSC EVs demonstrated immunosuppression in TAA-induced CLI. ES-MSC EVs are involved in reduction of fibrosis, collagen density and portal vein diameter as well. Downregulation of fibrosis-related genes (Col1α, αSMA, and TIMP1) and upregulation in collagenases (MMP9 and MMP13) and anti-inflammatory cytokine (IL-10) were detected following administration of ES-MSC EVs in CLI rat model.
Conclusion: The results of this study provided a new approach that ES-MSC EVs could be considered as allogenic cell-free products in liver diseases.
Keywords: Mesenchymal Stromal Cells; Extracellular vesicles; Chronic liver injury

OS-025. Association Between Body Mass Index and Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation

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Background and Aim: Hematopoietic stem cell transplantation (HSCT) is an effective treatment for hematological malignancies. A variety of risk factors such as disease stage, stem cell source, and HLA mismatch have been shown to affect the outcome of HSCT. Pre-transplantation, body mass index (BMI) has also been considered as a risk-factor for allogeneic-HSCT and may influence stem cell mobilization and outcomes after transplantation such as graft-versus-host disease (GvHD) incidence and survival. The purpose of this study was to assess the association of BMI with allo-HSCT outcomes and stem cell mobilization.
Methods: In this retrospective study, since 2008, 196 patients with hematologic malignancies including 102 acute myeloid leukemia, 53 acute lymphocytic leukemia, 10 non-Hodgkin lymphoma, 12 Hodgkin lymphoma, and 7 aplastic anemia, were treated with allo-HSCT in Taleghani hematopoietic stem cell transplantation center. The correlation between BMI and GvHD incidence, survival, CD34 and mononuclear cells (MNCs) of patients was evaluated. Student T-test and linear regression tests, Kaplan-Meier and Cox Regression tests and Spearman's Rho test were used to assess the correlation of BMI with GvHD incidence, survival, and mobilization variables respectively.
Results: From a total of 196 patients, 11.2% were underweight, 48.3% were normal weight and 42.3% were overweight. There was a statically significant correlation between BMI in underweight patients and GvHD incidence (P value = 0.001) and between BMI in overweight patients and survival (P value = 0.01) and MNCs (P value = 0.009) in allogeneic transplanted patients. But there was not a significant difference between BMI in underweight and normal weight patients and survival (P value = 0.06). Moreover, there was no significant difference between BMI in overweight, underweight and normal patients and CD34 (P value = 0.8).
Conclusion: Our data suggest Lower BMI is associated with higher GVHD incidence, lower MNCs and mobilization, and lower survival rate. Therefore, higher BMI is associated with better allogeneic transplantation outcome and stem cell mobilization in leukemic patients. It can be concluded that BMI is a useful prognostic factor in patients before and after transplantation. But further studies are required to confirm the impact of BMI on CD34 stem cells.
Keywords: GVHD; Body Mass Index; Hematopoietic stem cell transplantation; Survival

OS-026. Calreticulin: A Player in Metastatic Lung Cancer

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Background and Aim: Lung cancer is considered as one of most frequent cancers in the world causing high morbidity and mortality. Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) compromise, 80% and 20% of lung carcinoma, respectively. Although many factors such as Tobacco, asbestos, and genetic factors have been associated with lung cancer, the mechanism leading to the development of metastatic adenocarcinoma is not clear. Our recent data on genetically modified mouse model, overexpressing calreticulin under Tie2 promoter showed development of metastatic lung adenocarcinoma in these mice. Calreticulin (CRT) is a ubiquitously expressed protein in mammalian cells, with both calcium binding and chaperone activity. CRT is involved in quality control process during the folding and maturation of protein in endoplasmic reticulum.
Methods: Total RNA were isolated from lungs of transgenic mouse line (*Tie2-CRT*) that develop cancer and wild type mice using Trizol reagent. Gene expression was examined using microarray technique. Lentiviral delivery for shRNA was used to silence the long-non-coding RNA in lung tumor cell lines isolated from the *Tie2-CRT* mice. To identify down stream targets of Malat-1, cells infected with shRNA of Malat-1 or scrambled control were used to isolate total RNA. RNA sequencing were performed and genes affected were identified.
Results: Microarray analysis of lung tissue from *Tie2-CRT* mice as compared to the wild type mice, showed a significant increase in a number of long

non-coding RNAs. One of these long non coding RNA (LncRNA) known as Metastasis Associated Lung Adenocarcinoma Transcript-1 (Malat-1) has been implicated in the development of metastatic lung cancer. Malat-1 is a novel Lnc RNA that is localized to nucleus. Furthermore, its 3′ end can be spliced to form tRNA like structure called mascRNA that translocate to cytoplasm. Our data illustrate the involvement of this Lnc RNA in the increased rate of tumor cell migration and altered cell cycle. The exact targets of this Lnc RNA are not known. Using RNA-Seq. analysis of tumor cells after knockdown of Malat-1, we illustrate changes in cluster of genes involved in cell proliferation and migration.

Conclusion: Our data identifies Malat-1 as one of LncRNA upregulated in mouse lung cancer like Human lung adenocarcinoma. We also identify a number of genes which are targets of this LncRNA and assist in its metastatic effect.

Keywords: Long non-coding RNA; Lung Cancer; Cell proliferation; Cell migration; Metastasis

OS-027. MicroRNAs 200/205 as Gene Therapy Candidates in Inhibiting the Production of Gastric Cancer Stem Cells Through EMT Pathway

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Background and Aim: Epithelial-Mesenchymal Transition (EMT) could be described in two different contexts of normal and tumor cells. EMT transforms cancer cells to cancerous stem cells which result in metastases and drug resistance. Preventing the EMT phase or reversing the EMT phase to MET can be effective in controlling cancer disease. This study deliberates the potential of miR-200/205 in controlling the EMT process in gastric cancer cells and its application as a candidate for gene therapy in the metastatic cancer field.

Methods: To induce EMT pathway, Gastric adenocarcinoma cell line was treated with serum-free RPMI 1640 containing 10 ng/mL TGF-β. After 48 hours treatment, total RNA (including miRNA) of treated and untreated AGS were extracted. To confirm the induction of EMT process, RNA expression level of Mesenchymal markers (Vimentin), marker involves in migration-invasion (β-catenin), E-cadherin a key marker of epithelial phenotype, ZEB1/ZEB2 and Snail were analyzed by Rotor Genq 5plex HRM. Western blot technique used to consider protein level expression change of EMT markers. Specific monoclonal antibodies for E-cadherin, Vimentin, and β-catenin in wet blotting system were applied. To characterize miR-200a expression, specific primers were designed and synthesized. The changes in the expression level of miR-200a were studied by using the real-time PCR technique. We evaluated comparative analysis results according to GAPDH and U6 as housekeeping genes for EMT markers and miRNA respectively.

Results: In this study, we stabilized the in vitro model of TGF-β-induced EMT process in AGS cell line. The results of our Real-Time PCR in confirming the EMT model showed that the expression level of E-cadherin as a marker in the cell surface binding molecule was significantly decreased ($P<0.05$), and other mesenchymal and cancer stem cells markers including Vimentin, B-catenin, Snail, ZEB1/2 were significantly increased ($P<0.05$). The western blot results also confirmed the changes in E-cadherin, Vimentin, and B-catenin at protein levels. The results of the western blot were in line with the comparative analysis of real-time PCR. After the induction of EMT in AGS cells and its approval, the expression level of miR-200 and 205 was studied. Based on the results obtained from real-time PCR, there was a significant decrease ($P<0.05$) in the level expression of those miRs after the transformation of AGS from cancer cell to cancer stem cells.

Conclusion: Current study demonstrated that in the in vitro model of EMT, this process in gastric cancer cells decreases transcription level of miR200/205. There are some studies on non-GI cancers which identified the role of miR200/205 on the repression of EMT and inducing MET by affecting on ZEB1/2. Based on our results, miR200/205 as suppressors of EMT could be used as a candidate for gene therapy research purposes on preventing a metastatic form of Gastric cancer, which is one of the most aggressive cancers.

Keywords: Epithelial-mesenchymal transition; Cancer stem cell; Gastric cancer; miR-200; miR-205

OS-028. The Stability of Noncryopreserved Mesenchymal Stem Cells

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Background and Aim: Mesenchymal stem cells (MSCs) use, due to their multipotency, low immunogenicity, and tissue repair ability is growing rapidly. A challenge in MSCs therapy is that the bench to the bedtime of freshly cultured MSCs cannot exceed 24 hours. For use, after 24 hours, we need to cryopreserve MSCs. Cryopreservation can preserve the cells for years, but it is a costly and damaging process. Here we introduce a method to extend the bench to bed lifetime of MSCs up to 3 to 5 days without the high cost and cell-damaging effects of cryopreservation. Our method is based on preserving the MSCs in human AB+ plasma.

Methods: MSCs of 10 tissue samples - 3 adipose, 3 bone marrow, and 4 Wharton's jelly - were cultured and expanded in standard conditions. After the cell harvest, 25 million MSCs of each sample were suspended in human AB+ plasma at 2×10^6 cell/mL density. Afterward, the cell suspension was equally divided into 12 sterile capped tubes. Six tubes were put in a fridge at 5±3°C and the other 6 were left in room temperature (22±3°C). During the next 6 days, each day, two tubes (one from each group) were picked to assess the viability and ability to the growth of the MSCs. For the tubes picked on day 3, we also assessed the differentiation potential to the adipocyte and osteocyte of the MSCs. The results were analyzed by computing the means over 10 samples and applying the independent-samples *t* test to those means.

Results: The sample means for both cell expansion ability and cell viability for 1 to 6 days storage length were compared between the two "fridge" and "room temperature" groups. No significant differences between the two groups for any of the storage lengths were observed (independent *t* test). We found that more than 80% of the cells remained alive up to 6 days of storage in both groups. We observed a gradual decrease in cell growth potential from 1 day stored samples to 6 days stored samples in both groups. However, the cell confluency percentage reached 80% under 20 days for all samples except the 6 days stored ones. No significant differences were observed between the two groups. The differentiation potential to adipocyte and osteocyte which were tested in the 3 days stored tubes were positive in all samples.

Conclusion: This study showed that we can store the MSCs in human AB+ plasma at least for 3 days (probably up to 5 days) in both room and refrigerator temperatures without losing their stemness characteristic. Based on the available equipment (coolers and refrigerators), we suggest 5±3°C as a storage temperature for management reasons. Moreover, assessing the differentiation potential of the MSCs in 5 days stored samples may prove that this method can be used for storing the cells up to 5 days.

Keywords: Mesenchymal stem cells; Storage; AB plasma

OS-029. High-Resolution Typing of HLA-DRB1 Is Necessary to Prevent GvHD

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Background and Aim: Allogeneic hematopoietic cell transplantation (aHCT) is a curative solution for patients with malignant and other blood diseases. Human leukocyte antigen (HLA) and specifically HLA-DRB1 is one the most important antigens that should be taken into account in aHCT. Although the donor-recipient matching of HLA-DRB1 is considered in aHCT, the probability of life-threatening aHCT consequences such as graft-versus-host disease (GvHD) is inevitable which might be due to the low-resolution typing and matching of HLA between donor and recipient. In this study, we investigated the GvHD rate in the HLA-DRB1 donor-matched patients.

Methods: Retrospective clinical records (HLA-DRB1 type, GvHD status, and mortality rate) were collected from 190 patients who underwent aHCT with fully donor-recipient HLA-A, -B, -C, and DR-B1 matched during 2008-2017 at hematopoietic stem cell transplantation center of Taleghani hospital, Tehran, Iran. The HLA typing method was low-resolution polymerase chain reaction with single specific primer (PCR-SSP) and GvHD status was evaluated according to clinical manifestation and national institute of Health (NIH) criteria. The association of the types of HLA-DRB1 in donor-recipient fully matched patients with GvHD and mortality rate were analyzed using multivariate analysis.

Results: Despite the HLA matching between donor and recipient, patients with HLA-DRB1*11 have significant higher rate of acute GvHD (aGvHD) (P value= 0.04, RR= 1.31), chronic GvHD (cGvHD) (P value= 0.02, RR=1.32), and also significant higher mortality rate (P value= 0.007, RR= 1.04).

Conclusion: Our results illustrated that in spite of the HLA matching, the patients with HLA-DRB1*11 are more susceptible to both types of GvHD and have more mortality rate. This suggests that the HLA-DRB1 should be typed and matched with high-resolution methods or at least these susceptible patients should be managed and supported more than the others.

Keywords: Allogeneic hematopoietic cell transplantation; Graft-versus-host disease; Human leukocyte antigen; HLA-DRB1

OS-030. Plasma Levels of Epinephrine and Norepinephrine Along with Expression Levels of 2-Adrenergic Receptor Gene as a Probable Prognostic Factor of Acute GvHD

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Background and Aim: Acute graft-versus-host disease (aGvHD) is the major hindrance of allogeneic hematopoietic stem-cell transplantation (AHSCT). Epinephrine (E) and norepinephrine (NE) are adrenal glands stress hormones which affect many cells include immune cells through interaction with adrenergic receptors mainly B2-adrenergic receptor (B2-AR). The immunomodulatory effects of E, NE and AR-signaling have shown to decrease the probability of GvHD. The plasma levels of E and NE and the gene expression of B2-AR in leukocytes of patients who develop aGvHD with recipients without aGvHD have been compared in this study.

Method: The plasma samples were taken from 15 AHSCT candidates At days -7, 0, +7, and + 21 (day of transplantation was considered as day 0) and the levels of E and NE were assayed by Enzyme-linked immunosorbent assay (ELISA). cDNA was reverse transcribed from buffy coats-extracted total RNA and the expression of B2-AR was measured using real-time PCR. The incidence of aGvHD was measured according to the patient's clinical manifestations and International criteria. Mann-Whitney U test was used to analyze data.

Results: aGvHD were presented in 5 patients out of 15 patients during 100 days post-AHSCT. The mean plasma levels of E and NE and also the expression of B2-AR in leukocytes of aGvHD patients were significantly lower than non-aGvHD recipients. (P value <0.05).

Conclusion: Regarding the lower levels of plasma E and NE and 2-AR expression in aGvHD patients compared with recipients without aGvHD, we can conclude that the stress through hormones and their receptor might have a role in preventing aGvHD and could be a predictive marker for aGvHD.

Keywords: Epinephrine; Norepinephrine; 2-adrenergic receptor; aGvHD

OS-031. Protective Effect of Hematopoietic Stem Cell Transplantation Against the Progression of Liver Fibrosis in β-Thalassemia Major Patients

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Background and Aim: Patients with β-thalassemia major (TM) are vulnerable to liver fibrosis, which is mainly due to iron overload. After successful allogeneic hematopoietic stem cell transplantation (HSCT) for such patients, it is reasonable to assume that iron overload decreases because of the reduction in hemolysis and cessation of therapeutic transfusions. Transient elastography (TE) is a valuable non-invasive method to assess liver fibrosis. In this study, using TE we sought to assess the levels of hepatic fibrosis in TM patients before and after allo-HSCT.

Methods: In this prospective observational study, 68 TM patients (57.7% male) with a median age of 7.7 (2-15) years who were a candidate to undergo allo-HSCT were included. The level of hepatic fibrosis in MT patients was measured with TE using Fibroscan 502 (EchoSens, Paris, France) and with liver biopsy according to the METAVIR scoring system. TE, measured in kilopascals (kPa), was performed both in pre-HSCT and 1-year post-HSCT, while liver biopsy was only performed before transplantation.

Results: Comparing laboratory findings in 1 year after HSCT with those in pre-HSCT, a significant increase in ferritin (P= 0.001) and significant increments in liver enzymes (P < 0.001) were observed. However, no significant change in liver fibrosis occurred comparing pre-HSCT and post-HSCT TE measurements of the liver (P = 0.211). Age, gender and ferritin values did not have any impact on the level of liver fibrosis measured by TE both before and after HSCT. Moreover, pre-HSCT METAVIR score did not show a significant impact on the change in the levels of liver fibrosis (as assessed by TE before and after HSCT).

Conclusion: Transfusion-dependent TM patients experience progressive liver fibrosis over time. The progression of liver fibrosis arrests after HSCT as we found no significant change in TE measurements of the liver before and after the procedure. Hence, it can be stated that allo-HSCT is able to alleviate the hepatic fibrosis progression in TM patients.

Keywords: Beta-thalassemia; Hematopoietic stem cell transplantation; Hepatic fibrosis; Transient elastography

OS-032. Apelin-13 Protects Inner Ear Hair Cells From Oxidative Stress-Induced Apoptosis In Vitro and In Vivo

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Background and Aim: Several studies have shown that exposure to loud sounds leads to the formation of oxidative stress and free radicals in particular hydrogen peroxide (H2O2) in tissue. Apelin-13 is an adipocytokine that was first isolated from the bovine stomach extract. Recent studies have shown that apelin-13 has protective effects on the brain's ischemia-induced cell death and injury. Apelin-13 also has protective effects on the hippocampal cells and cortical neurons against oxidative stress. However, the protective effects of apelin-13 on the inner ear hair cells are not known. Therefore, in this study, we evaluated the protective effects of apelin-13 on the cochlear hair cell damage induced by oxidative stress in a culture medium and in vivo.

Methods: Stem cells were differentiated into hair cell-like cell by B27, FGF, EGF, and IGF-1. Expression of neuron-specific markers including β tubulin III, Nestin, MAP2, Neurofilament, and GFAP was tested by flow cytometry assay. As well, inner ear hair cell marker such as Myosin7, Sox 2, TrkB and AchR α expression was assayed with immunocytochemistry (ICC). Protective effect of apelin-13 was assayed in H2O2 - treated hair cells in vitro by acridine-orange staining. For in vivo assay, rats were assigned into control, noise exposure groups without any treatment or receiving apelin-13. Following chronic exposure to 110 dB white noise, apelin-13 was injected i.p, then histological, molecular and tunnel assay was done.

Results: Our findings show that apelin -13 protect hair cell-like cell damage against oxidative stress in vitro condition. As well, apelin-13 reduced cochlear hair cell death in a rat model of noise-induced hearing loss (NIHL) by inhibition of apoptosis.

Conclusion: Our study suggested that apelin-13 could be a promising target to treat noise-induced hearing loss (NIHL) through reducing apoptosis. Although more research is needed to investigate the potential therapeutic effects of apelin-13 in NIHL patients.

Keywords: Apelin-13; Stress Oxidative; Hair Cell; Apoptosis

OS-033. Whether Serum Ferritin Level Impact on GvHD and Survival in the Patients Undergoing Hematopoietic Stem Cell Transplantation?

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Background and Aim: Hematopoietic stem cell transplantation (HSCT) and graft-versus-host disease (GvHD) have a complicated biological network which can be related to many biofactors. Serum ferritin is an acute phase protein and an indicator for iron storage. Iron overload can lead to liver damage, hepatic sinusoidal obstruction syndrome, infection and other complications which substantially influence HSCT outcomes. Thus, we performed this study to investigate the prognostic significance of pre-transplantation serum levels of ferritin on the incidence of GvHD and overall survival in patients undergoing allogeneic HSCT.

Methods: In this study, we retrospectively evaluated the data (serum levels of ferritin, GvHD status, and survival) from 196 patients with hematological malignancies who underwent allogeneic HSCT in Taleghani bone marrow hematopoietic stem cell transplantation center, Tehran, Iran. We examined the association and correlation of pre-transplantation serum levels of ferritin with GvHD and overall survival of patients. The data were analyzed using Kaplan-Meier and Cox regression tests.

Results: Our results showed that 5-year overall survival (OS) is significantly higher in the patients with pre-transplantation serum ferritin levels under 800 µg/L (68% versus 62%, P value= 0.001) than the patients with serum ferritin more than 800 µg/L and there was a negative correlation between serum ferritin level and OS. In addition, it was shown that the pre-transplantation serum ferritin level is higher in the patients with GvHD but there was no significant correlation between serum ferritin level and GvHD (P>0.05).

Conclusion: This study confirmed that high serum level of ferritin before HSCT might be associated with survival rate after allogeneic HSCT and increased pre-transplantation serum levels of ferritin can be a predictor of HSCT outcomes.

Keywords: Serum ferritin level; Allogeneic hematopoietic stem cell transplantation; Graft-versus-host disease

OS-034. Optimization of a Fibroin-Based Substrate for Delivering L-MSCs and Epithelial Cells to the Ocular Surface

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Background and Aim: Silk fibroin membranes support the growth of limbal mesenchymal stromal cells (L-MSCs) and limbal epithelial cells (L-ECs). Nevertheless, this biomaterial requires improvement in cell adhesion, permeability (for optimal communication between the cells seeded on opposing side of the membrane) and strength for suturing. Thus, we evaluated the properties of an advanced formulation of fibroin using a genetically modified fibroin containing RGD (cell adhesion motifs). It is hypothesized that this modified fibroin could be a potential vehicle for co-application of L-ECs and L-MSCs to the ocular surface.

Methods: Genetically modified RGD-fibroin (RGD-F) was obtained from the National Agriculture and Food Research Organization (NARO, Tsukuba, Japan). L-MSCs and L-ECs were separately applied to either culture surfaces (tissue culture plastic [TCP], TCP coated with standard fibroin [TCP-F], and TCP coated with RGD-F [TCP-RGD-F]) for 90 minutes. The subsequent morphology and number of attached cells were examined under both serum-free and serum-supplemented growth conditions. The number of attached cells was quantified using a Picogreen assay (estimating dsDNA). Morphology and proliferation of L-MSCs were assessed in long-term (6 and 10 days) cultures on TCP, TCP-F, and TCP-RGD-F. A comparison was made between the co-cultures of L-MSCs and L-ECs established on RGD-F free-standing membranes fabricated using the regular formulation versus RGD-F prepared with poly(ethylene) Glycol (PEG as a porogen) and horseradish peroxidase (HRP as a cross-linking agent). Finally, the feasibility of applying co-cultures of L-ECs/L-MSCs growing on RGD/PEG/HRP fibroin membrane to the ocular surface was examined in the rabbit model.

Results: In 90 minutes cell attachment assay for both L-MSCs and L-ECs, no significant difference was detected between culture conditions. Morphological examination of long-term (6 and 10 days) L-MSC cultures revealed that while L-MSCs cultured on TCP-F generally formed sparsely scattered clusters, those cultured on TCP-RGD-F were more uniform in adherence. Moreover, at 6 days L-MSCs displayed evidence of significantly ($P < 0.05$; $n = 4$) superior attachment and proliferation on TCP-RGD-F compared with TCP-F as indicated by a Picogreen assay. Confocal microscopy of the L-MSC/L-ECs co-cultures grown on the opposing surface of the RGD/HRP/PEG-fibroin membrane revealed that L-MSCs enhanced stratification of L-ECs (2-4 fold more stratified than those grown in the absence of L-MSCs). The RGD/HRP/PEG-fibroin membrane was successfully sutured to the ocular surface without tearing. Pilot treatment of L-MSCs/L-ECs implantation using RGD/HRP/PEG-fibroin membrane (n=1) initially produced promising results (nearly complete re-epithelialization in 14 days), but subsequently led to a prominent epithelial defect and conjunctivalization of the ocular surface.

Conclusion: Results of the current study suggest that the benefits associated with the inclusion of RGD in silk fibroin are most effective in the long-term (6-10 day) cultures of L-MSCs. Moreover, the feasibility of using RGD/HRP/PEG-fibroin as a vehicle for delivering limbal stem cells to the ocular surface was preliminarily confirmed. The outcomes of this study provide a foundation for the use of the L-MSCs, L-ECs and the new RGD silk fibroin scaffold. This offers a stepping stone for future studies in cellular therapy for limbal stem cell diseases.
Keywords: Limbal; MSCs; Epithelial; Silk fibroin; RGD

OS-035. The Impact of ABO Incompatibility on aGVHD Incidence and Overall Survival in HSCT Patients

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Background and Aim: Allogeneic hematopoietic stem cell transplantation (aHSCT) is a proper option for treatment of patients with a variety of malignant hematological. In previous studies, have been reported that ABO incompatibility between donor and recipient is seen in approximately 25%-50% of patients who undergo acute graft-versus-host disease (aGVHD). Also, it has been observed that transplantations with ABO incompatibility enhance the chance of (aGVHD) incidence and mortality rate in allo-HCT patients. Therefore, this study aimed to investigate the role of ABO incompatibility on aGVHD incidence and overall survival (OS) in allo-HCT patients.
Methods: We retrospectively assessed 149 patients who underwent allo-HSCT at Taleghani hospital, Tehran, between 2008 and 2017. Patients received peripheral blood stem cells (PBSCs) after myeloablative (MA) conditioning regimens and nonmyeloablative (NMA) regimens. We analyzed the impact of major, minor, and bidirectional ABO incompatibility on the incidence of aGVHD and overall survival rate in allo-HCT patients.
Results: Analysis of our data showed that the incidence of aGVHD was higher in bidirectional ABO incompatibility patients compared to ABO-matched patients (hazard ratio: 1.86, P value: 0.006 and confidence interval:1.19 – 2.93). Also, we found a significantly lower overall survival in ABO-mismatched HSCT compared to ABO-matched allo-HSCT (hazard ratio: 2.3, P value: 0.0008 and confidence interval: 1.5–5.1). However, major and minor ABO-incompatibility did not have an impact on aGVHD incidence and overall survival of allo-HCT patients (P value: 0.1).
Conclusion: There are different reports from the correlation between ABO-incompatibility and GVHD incidence in allo-HSCT patients. Our data revealed that only bidirectional ABO incompatibility has a significant impact on aGVHD incidence and survival outcomes in patients who undergo allo-HSCT; So, these results are agreement with some of the previous reports. But, in another study has been reported in addition to the bidirectional ABO-incompatibility, major and minor ABO incompatibility influence aGVHD. Moreover, other factors that influence aGVHD incidences such as human leukocyte antigen (HLA) disparity, donor and recipient age etc should be considered.
Keywords: Allogeneic hematopoietic stem cell transplantation; Acute graft-versus-host disease; HSCT patients

OS-036. Alginate-Gelatin Encapsulation of Endothelial Cells Induces Angiogenesis in In Vivo and In Vitro Milieu

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Background and Aim: Up to present, numerous attempts have been collected in the favor of cell-based therapies by using sophisticated methodologies and delivery approaches. Micro-capsules have the potential to provide safe micro-environment for cells delivery during transplantation in a physiological 3D milieu.
Methods: Here, we investigate the impact of alginate gelatin encapsulation on angiogenic potential of human endothelial cells after 5 days. Human umbilical vein endothelial cells were encapsulated by the alginate-gelatin substrate and kept in vitro for 5 days. Cell survival assay (MTT) and autophagy PCR array analysis were used to study HUVECs survival rate. To monitor angiogenesis capacity, cell distribution of Tie-1, VEGFR-1, and VEGFR-2 and Tie-2, were measured by ELISA. In addition to in vitro tubulogenesis assay, we measured the protein level of VE-cadherin by Western blotting. The migration of encapsulated HUVECs was confirmed by measuring MMP-2 and MMP-9 activity via gelatin zymography. The in vivo angiogenic potential of encapsulated HUVECs was analyzed in immune-compromised mouse implant model during 7 days post-transplantation.
Results: Encapsulation increased HUVECs cell survival and proliferation rate. Compared to the control, no significant differences were observed in the autophagic response of encapsulated cells ($P > 0.05$). The cell distribution of Tie-1, Tie-2, VEGFR-1, and VEGFR-2 were induced but did not reach significant levels. Encapsulation suppressed MMP-2, -9 activities while increased the VE-cadherin peptide in enclosed cells ($P < 0.05$). Moreover, an enhanced in vivo angiogenic response of encapsulated HUVECs was evident as compared to non-capsulated cells ($P < 0.05$).
Conclusion: Data suggest that alginate-gelatin encapsulation induces the angiogenic response of endothelial lineage in the in vivo and in vitro conditions.
Keywords: Alginate-gelatin encapsulation; Angiogenesis; Human endothelial cells; Migration; Receptor tyrosine kinases; VE-cadherin

OS-038. Fecal Calprotectin: A Predictive Factor in Response to Corticosteroids in Patients with Gastrointestinal Graft-Versus-Host-Disease

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Background and Aim: Acute graft-versus-host disease (aGvHD) is the primary limitation of allogeneic hematopoietic cell transplantation, and once it develops, there is no reliable test to monitor the responses to treatment. Calprotectin was reported to be significantly associated with the response to treatments in gastrointestinal inflammation. In this study, we aimed to evaluate the amount of fecal calprotectin pre-transplant, before the initiation of treatment and during the course of treatment of GvHD.
Methods: In this study 25 patients (13 patients with GI-GvHD and 12 patients without it) which underwent allogeneic HSCT were evaluated in Taleghani hospital, Tehran, Iran. Gluckberg criteria were used for diagnosis of aGvHD independently from the date of transplantation, and

general symptoms were considered to approve the GI-GVHD, Then in order to assess the FC, stool samples were taken (early or onset) and general symptoms such as diarrhea, anorexia, nausea, and vomiting were considered to approve the GI-GvHD. Moreover, In order to assess the FC, stool samples were taken before the transplantation and the onset of GvHD symptoms and at intervals of 7 days to 3 weeks after starting therapeutic approach before the transplantation and the onset of GVHD symptoms and at intervals of 7 days to 3 weeks after starting therapeutic approach. We monitored the fecal calprotectin in all patients.

Results: Calprotectin levels decreased in both groups during follow-up from diagnosis to 3 weeks post-transplant. But these changes were more severe in patients with hypersensitivity to corticosteroids.

Conclusion: Our results suggest that the reduction of calprotectin level in patients, which underwent allogeneic HSCT might have a correlation with acute GI-GVHD incidence. Therefore, the assessment of fecal calprotectin level before and within the treatment could be considered as a reliable prognostic biomarker for GI-GVHD.

Keywords: GVHD; Calprotectin; Hematopoietic stem cell transplantation; Corticosteroid, Biomarkers

OS-039. Macrophage Repolarization Using Chrysin-Based PCL-PEG Electrospun Nanofibrous Scaffold: Possible Application in Regenerative Medicine

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Background and Aim: In the regenerative medicine therapies, the availability of engineered scaffolds that modulate inflammatory states is highly required. The aim of this study was to evaluate the efficiency of electrospun nanofibrous scaffolds containing natural substances with anti-inflammatory properties such as chrysin (Chr) to control inflammation and re-polarization of macrophages toward M2 anti-inflammatory phenotype.

Methods: For this purpose, Chr-blended PCL/PEG electrospun nanofibrous mats were successfully fabricated and characterized using field emission scanning electron microscopy (FE-SEM) and Fourier-transform infrared spectroscopy (FT-IR). MTT assay and FE-SEM were used to assess the possible effect of EO-loaded PCL/PEG nanofibrous mat on the morphology and viability of polarized RAW264.7 macrophages after 72 hours incubation. Also, the in vitro efficiency of Chr-loaded PCL/PEG nanofibrous mat to modulate macrophage polarization from M1 to M2 phenotype through alteration in the expression levels of M1 marker (iNOS2) and M2 marker (Arg1) as well as the expression levels of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 were measured using real-time PCR and ELISA.

Results: MTT results showed that macrophage viability on bead free and smooth Chr-PCL/PEG electrospun nanofibers was higher than on PCL/PEG nanofibers and control ($P < 0.05$). Additionally, stimulated macrophages on Chr-PCL/PEG nanofibers were observed to be round, pancake-like shape with few cytoplasmic projections and low spreading within in 3-day culturing indicating an M2 phenotype of macrophages. qPCR results showed a reduction in iNOS-2 and an increase in Arg-1 levels of macrophages seeded on Chr-PCL/PEG nanofibers, representing the successful polarization of the macrophages to M2 phenotype. The change in macrophage phenotype on Chr-based nanofibers could suppress the inflammation in LPS/ IFN-γ stimulated macrophages as evidenced by a major reduction in pro-inflammatory cytokine levels TNF-α, IL-1β, and IL-6.

Conclusion: Conclusively, the results demonstrated that Chr-based nanofibers efficiently modulated RAW264.7 macrophage polarity toward an anti-inflammatory M2 phenotype.

Keywords: Chrysin; Nanofiber; Macrophage polarity; Regenerative medicine

OS-040. Analytical Study on Acute and Chronic GvHD Occurrence in a Series of Pediatric Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation

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Background and Aim: Graft-versus-host disease (GvHD) is one of the main complications following allogeneic hematopoietic stem cell transplantation (allo-HSCT). In this study, we reported the epidemiologic profile and outcomes of pediatric patients undergoing allo-HSCT at a reference pediatric HSCT center in Iran. We also analyzed the impact of demographic, clinical and treatment-related variables on the occurrence of acute and chronic GvHD (aGvHD and cGvHD).

Methods: In this retrospective cross-sectional study, one-hundred pediatric patients (57% male) who underwent allo-HSCT between September 2016 and February 2018 at the department of pediatric stem cell transplantation in Children's Medical Center, Tehran, Iran were included. Data were extracted from patients' records and were entered into a predesigned checklist.

Results: Thirty-eight patients developed aGvHD, with the highest percentage having grade I (16%), followed by grade II (9%), grade III (7%) and grade IV (6%) aGvHD. Twelve patients developed cGvHD with the majority having limited (8%) followed by extensive (4%) cGvHD. The source of HSCs was peripheral blood in the majority of cases (88%). The disease class of the patients was hematologic malignancies (33%), primary immunodeficiencies (23%), inborn errors of metabolism (12%) and non-malignant hematologic abnormalities (32%). The occurrence of aGvHD was significantly more common in patients with primary immunodeficiencies and non-malignant hematologic abnormalities compared with the other two classes of diseases (P=0.038). Other variables such as gender, age, donor-recipient relationship, donor-recipient matching, the source of HSC, etc did not have any impact on the occurrence of aGvHD and cGvHD. aGvHD was successfully managed with standard care. Nine patients died during the study period and only one of them was related to GvHD (acute). Patients with cGvHD are still under observation in follow-up visits.

Conclusion: The occurrence of aGvHD and cGvHD in allo-HSCT treated pediatric patients was less than two-fifths of patients and a little greater than one-tenths of patients, respectively. The underlying disease may have an impact on the occurrence of aGvHD.

Keywords: Graft-versus-host disease; Hematopoietic stem cell transplantation; Immunologic deficiency syndromes; Hematologic diseases

OS-041. HLA-A Needs More Attention in Graft-Versus-Host Disease

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Background and Aim: Class I human leukocyte antigens (HLAs) have an important role in many kinds of transplantation specially allogeneic

hematopoietic stem cell transplantation (aHSCT) in which all major HLA-I members (HLA-A, -B, -C) must be matched between the donor and recipients. Due to the cost, routine HLA-typing is low resolution and despite the donor-recipient fully matched HLA-I, some post-aHSCT complication such as graft-versus-host disease might occur. In this study, we investigated the question that whether any matched HLA-I members has an association with GvHD.

Methods: In this retrospective study, the data of low-resolution HLA-I typing of 190 patients who underwent aHSCT as well as their GvHD status were obtained from their clinical records. All patients received HLA-A, -B, -C fully matched hematopoietic stem cells. The association between the types of HLA-I with GvHD status of patients were analyzed using multivariate analysis.

Results: Among the patients, only the patients with donor-recipient matched HLA-A*01 have significantly high rate of acute GvHD (aGvHD) (P value= 0.048, RR=1.31) and significantly mortality rate (P value= 0.04, RR= 1.21). Other donor-recipient matched class-I HLAs have no significant association with aGvHD and mortality. None of HLA-I have a significant association with chronic GvHD.

Conclusion: In accordance with the results, It seems that the HLA-A might be more important in post-aHSCT complications especially life-threatening aGvHD and should be typed with high-resolution methods.

Keywords: Human leukocyte antigen; HLA-I; GvHD; Mortality

OS-042. Combination of Sorafenib with Mesenchymal Stem Cell Can Be a Novel Therapeutic Strategy for Hepatocellular Carcinoma in Xenograft Model

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Background and Aim: Sorafenib is the first-line treatment for hepatocellular carcinoma (HCC). although the positive effect of sorafenib on the survival of patients, novel therapeutic strategies are needed to extend survival and improve the efficacy of sorafenib. In this study has been used combining sorafenib with MSC as a new approach to optimize the beneficial effects of sorafenib.

Methods: Subcutaneous xenograft model of HCC established by Human HepG2 cell lines were implanted into the flank of nude mice was used to evaluate tumor growth after treatment with sorafenib alone or in combination with MSC. AST, ALT, BUN, and creatinine levels were measured for safety assessment. Histopathological studies were performed using H&E staining, and IHC tests were performed to evaluate proliferation (Ki67) and angiogenesis (CD34). TUNEL assay was used to detect apoptosis and measure the expression of major inflammatory cytokines (IL-1a, IL-10, TNF) with real-time PCR.

Results: Sorafenib in combination with MSC, strongly inhibited tumor growth in a xenograft model. Furthermore, inhibited HCC cell proliferation, decreased tumor angiogenesis and induced apoptosis and maintains antitumor -associated anti-inflammatory effects of MSC.

Conclusion: This combination therapy strategy could be a new therapeutic approach to the treatment of HCC that significantly improved the results achieved using sorafenib as monotherapy.

Keywords: Hepatocellular carcinoma; Sorafenib; Human placenta; Mesenchymal stem cells

OS-043. Safety and Immunogenicity Evaluation of Allogeneic Mesenchymal Stem Cell Transplantation (Core Matrix Extracted)

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Background and Aim: Among the stem cells, mesenchymal stem cells (MSCs) have garnered significant attention in the regenerative medicine, largely due to their anti-inflammatory and immunomodulatory properties. They can be easily isolated and cultured from many tissues, such as bone marrow, adipose tissue, and umbilical cord. MSCs exhibit low expression of MHC I but do not express MHC II or CO-stimulatory molecules such as CD80 and CD81. So, they do not cause an immunological reaction after they are infused into an allogeneic body. In this study, we evaluate the safety and efficacy of allogeneic MSCs in patients with late-onset neurometabolic disorders.

Methods: This study is a community based and pragmatic, non-randomized single group, clinical trial without blinded postoperative care and outcome assessment. Wharton jelly-derived allogenic mesenchymal stem cells in suitable albumin solution were prepared in a clean room. After patient selection, one or much more injections planned by intrathecal and/or intravenous rout based on their organs involvements. 3-4 million cell per kilogram more than 90% viability in 20-50 mL injectable human albumin as a preservative in each intravenous injection and one million per kilogram in 5-10 mL in each intrathecal injection. Interval of these injections was between 2 days to two weeks. Patients underwent a clinical and laboratory evaluation for a period of 18 months at defined intervals.

Results: Historically, panel reactive antibody (PRA) analysis to detect HLA antibodies has been performed using cell-based complement-dependent cytotoxicity (CDC) techniques. Recently, a flow cytometric procedure (FlowPRA) was introduced as an alternative approach to detect HLA antibodies. Monitoring of anti-HLA class I and II antibodies was negative in 6 to 12 months after the first injection. In phase 1 of clinical trial no. 30396 submitted in IRCT website, 3 patients (100%) had post-transplant anti-HLA class 1 and 2 antibodies detectable by FlowPRA lesser than 4 that suggest negative immunogenicity in allogenic Wharton jelly-derived mesenchymal stem cell transplantation.

Conclusion: In this study, the immunogenicity of stem cells in the clinical phase was investigated, which measured the HLA class 1 and 2 antibodies by flow PRA method, indicating a negative immunogenicity of these stem cells

Keywords: HLA antibody; Warthon jelly; Mesenchymal stem cell transplantation; Allogenic

OS-044. Production of GBM-Induced Rat Model: A Gateway to Preclinical Studies and Personalized Medicine for Human Glioblastoma Multiforme

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Background and Aim: The glioblastoma multiforme (GBM) is an aggressive tumor in adults. It mostly develops in the brain, whereas, rarely spreads out of the central nervous system. It is the second most common CNS malignancy in humans and is associated with high mortality rates.

To establish a platform for drug discovery and preclinical studies, animal models of tumors is one of the most beneficial approaches. In this paper, we report our experience of developing a GBM-induced rat model.
Methods: Using C6 cell line (malignant glial cell lines of Rattusnorvegicus), the cells were injected into the brain of Wistar rats. The rats started having hemorrhagic discharge from their eyes and paraplegia on the median of 6 days post-injection. Brain magnetic resonance imaging (MRI) was performed on the 8th-day post-injection to confirm the development of the tumor.
Results: The model was established by employing the C6 cell line, which is immunologically compatible with its receptor. By exploiting bioinformatic tools, the similarities between aberrant gene expression and pathways have been predicted. In this regard, 337 commensurable genes and a number of pathways have been detected. We found genomic similarities between human- and rat-origin GBM. Moreover, comparing film outputs of MRIs from GBM-induced rats and 3 humans with GBM, we found close similarities. The pathologic analyses also showed comparable results between human GBM samples and GBM samples from sacrificed rats.
Conclusion: Provision of cancer-induced models is a prerequisite to investigating not only the pathogenesis and pathology of tumors but also to improve drug discovery studies, preclinical research, and personalized medicine. Although several methods and various species have been employed for this approach, the real recapitulation of the human tumor has been left under discussion. In this study, a GBM-induced rat model was established with comparable pathologic, genomic and radiologic features to human-origin GBM.
Keywords: Animal Models; Glioblastoma multiforme; Rats

OS-045. Adipose Tissue-Derived Mesenchymal Stem Cells in Peritoneal Dialysis Patients

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Background and Aim: Following promising results of mesenchymal stem cell (MSC) transplantation in PD models in the recent decade, this method is taken into consideration in clinical trials. Experiments reported that MSC in PD models attenuated submesothelial thickness, inflammation, angiogenesis, and fibrosis as well as improved ultra-filtration volume, glucose uptake, glucose mass transfer, and solute transport. Taken together we hypothesized that MSC could induce anti-fibrosis, immunomodulation and anti-apoptosis effects in these settings. Therefore, we designed the first in the human clinical trial of MSC in PD.
Methods: In this open-label, non-randomized, phase I clinical trial, we infused autologous adipose-derived MSC in 9 eligible patients and followed them for 6 months. Ten patients were regarded as the control group. The primary endpoint was the safety and the secondary endpoint was the change in membrane function.
Results: Fourteen minor adverse events (AE) were recorded in the MSC group which were subsided by itself or by supportive therapy. No serious adverse events or adverse events related to cell infusion were recorded. There was a significant reduction in Dialysate-to-plasma ratio (D/P) of creatinine in the MSC group (0.77 to 0.73, P=0.02).
Conclusion: In summary, this trial showed safety, feasibility, and tolerability of autologous MSC in ultrafiltration failure patients which could be a foundation for efficacy assessment by an RCT.
Keywords: Peritoneal dialysis; Peritoneal Fibrosis; Ultrafiltration failure; Adipose tissue mesenchymal stem/stromal cell

OS-046. Multi-Layered Cellular Biointerface for Bone Tissue Regeneration

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Background and Aim: Orthopedic surgery is one of the key applications of tissue engineering. To date, numerous and various materials (synthetic and natural) are used. However, the optimization of their properties in order to improve bone tissue restoration is still challenging. This study aims to produce a tissue-engineered construct with tissue-like biointerface using multi-layered cell sheets for bone regeneration.
Methods: Scaffolds were fabricated via surface selective laser sintering of polylactide particles. Before structuring, polylactide particles were treated with hyaluronic acid. After sintering, structures were reinforced with Irgacure 2959-assisted photo-crosslinking. Mesenchymal stromal cells (MSCs) phenotype was determined using flow cytometry. Osteogenesis was evaluated by measurement of calcium accumulation.
Results: The scaffolds ensured successful adhesion and proliferation of human bone marrow MSCs and induced calcium deposition which was detected in 7-21 days of culturing in standard medium. The novel approach to graft the scaffolds with a functional bio-interface was proposed using surface functionalization with cell MSC sheets aiming to facilitate host tissue-implant integration. The sheets exhibited contiguous morphology and high viability.
Conclusion: In this study, we presented a new approach to produce osteo-inducing constructs for bone-tissue regeneration grafted with multi-layered cellular bio-interface. The combination of cell sheet technology and scaffolding can ensure the development of novel medical devices, which improve tissue reparation and regeneration. The achieved results form the base of new tissue engineering approach to fabricate osteo-substitutes. The study is supported by the Russian Science Foundation under grant 18-15-00407.
Keywords: Tissue Engineering, Osteogenesis, Scaffolds

OS-047. One Step to Defeat the Acute Myeloid Leukemia (AML) by Ultrasensitive Detection of Single Cancer Stem Cell

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Background and Aim: One of the most common types of blood cancer is acute myeloid leukemia (AML), which highly affects the blood cells, in the context of bone marrow and other tissues. The leukemia stem cells (LSCs) have a very important role in the refractory and relapse of AML. So monitoring of LSCs is so critical during the diagnosis, and therapeutic period of AML suffering patients.
Methods: In this regard, we developed an electrochemical-based cytosensor for detection and quantification of LSCs. For this aim, we designed an immunosensor for detection of cell surface marker of CD123, which were overexpressed in the surface of KG1a cell line as a model of LSCs. It is important to declare that, approximately more than 75% of the CD123 protein is accessible and located outside of LSCs membrane.

Graphene quantum dots (GQDs) for their specific properties like high surface area, electrical conductivity, and optical properties were used as electrode platform. The glassy carbon electrode (GCE) was used as the working electrode and stepwise modified by GQDs, Au nanoparticles (AuNPs), streptavidin coated AuNPs (star-shaped), biotinylated CD123 antibody and target cells. The biotinylated antibody was used as a cell capturing element.
Results: All of the electrode preparation steps were confirmed by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. After passing the optimization of all experimental parameters, the detection limit (DL) and linear dynamic range (LDR) of the designed cytosensor were obtained as 1 cell/mL and 1 to 50 cells/mL, respectively.
Conclusion: The constructed nanomaterial-based cytosensor has top features like ultra-sensitivity, easy fabrication process and more precise results, which make it as a useful analytical device for detection and quantification of LSCs in clinical laboratories.
Keywords: Stem cell biosensing; Electrochemical; Cytosensor; Single cell detection; CD123; AML

OS-048. Engineered Tumor-Derived Exosomes: Novel Tools for CD8+ T Cells Induction

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Background and Aim: In spite of recent advances in immunotherapy of cancer, there have been limitations in cancer treatment and patient survival due to lack of antigen recognition and immunosuppressive tumor microenvironment. Although tumor-derived exosomes (TEX) are involved in the transmission of suppressive signals between tumor and immune cells, they contain tumor-specific antigens and several heat shock proteins which are necessary for the induction of immune response. In the present study, we used modified TEX with Let-7i and miR-142 to target dendritic cells and CD8+ T cells as well as tumor microenvironment.
Methods: Mouse mammalian breast cancer cell line; 4T1, was cultured. Differential ultracentrifugation was used for isolation and purification of exosome population from exosome free medium. Morphology, size distribution and protein expression of exosomes were evaluated by scanning electron microscopy (SEM), dynamic light scattering (DLS), western blot and flow cytometry. Bone marrow-derived DC was differentiated from bone marrow progenitors and miRNAs inoculated into exosomes through electroporation. Delivery of miRNAs was confirmed by real-time PCR. Immature DC was incubated with LPS, TEX and manipulated TEX as control positive, control negative and test groups respectively. T cells were isolated from lymph node and co-cultured with mature DCs. Proliferation, phenotype and cytokine release were assessed using CFSE, flow cytometry by PE-conjugated anti- CD3 and FITC conjugated anti-CD8 and ELISA for IFNg and Granzyme-B.
Results: Morphology and size of isolated exosomes evaluated using electron microscopy and dynamic light scattering. Exosomes were positive for CD81, CD63 and TSG101 using western blotting and flow cytometry. The morphology of DCs changed and branched projections on mDCs were observed during incubation with modified TEX like LPS group but not TEX. DC-modified TEX in comparison with DC-TEX, expressed a higher percentage of CD11c, MHCII, CD80, and CD40, as activatory surface molecules. Higher proliferation index and CD8+ population with a dominant increased expression of IFNg and Granzyme B were determined in the test group.
Conclusion: We found that Let-7i could efficiently increase DC maturation also a combination of Let-7i with miR-142 have notably enhanced the effect on either DC maturation or CTL induction and cytokine release. This outcome indicates that modified TEX may act as a cell-free vaccine in the future of cancer treatment.
Keywords: Tumor-derived exosomes; miRNA; Dendritic cells; Cytotoxic T cells

OS-049. Nanoemulsion Spray Analgesic and Immediate Dressing Skin Wounds, Acceleration of Skin Wound Healing

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Background and Aim: We evaluate the molecular, cellular and clinical effects of Plant compounds on wound healing. Application of natural ingredients and herbs for treating ulcers has been in the history of human life. Effects of using plant compounds on the healing process were investigated by the microscopic method, cell counting and TGF-ß gene expression in the wound bed. Nanoemulsion encapsulation of component within the vesicles, along with the presence of membrane channels to control the entrance of substrate and subsequent exit of the enzymatic reaction product.
Methods: Sixty Wistar rats weighing 200-220 gr were placed under general anesthesia and sterile conditions. Square shape wound with 1.7×1.7 mm dimension was made on the back of the neck. Rats divided into control and experimental groups and to 3 subgroups with 4, 7, and 14 days of study. In firstst experimental group Nanoemulsion spray was used twice on the wound, once in a 2nd experimental group and for positive control group phenytoin cream 1% was applied daily from the surgery days; the control group did not receive any treatment. For histological studies, samples were taken from the wound and adjacent skin. This tissue examined for histological staining, then wound surface and wound healing were evaluated separately, Also TGF-ß gene expression by estimated by RT-PCR. Results showed that fibroblasts in both groups were significantly increased, caused to the acceleration of wound healing. It concluded that twice application of Nanoemulsion spray will increase TGF-ß gene expression, ultimately accelerate wound healing process.
Results: The macroscopic and microscopic evaluation showed that wound healing increased because the fibroblast numbers in two experimental groups improved compared with the control group. The percentage of wound healing on different days in the experimental and control groups were significant. Data were analyzed by using one-way ANOVA test and $P< 0.05$ was significant.
Conclusion: The Present study showed that the twice application of topical Nanoemulsion spray can result in rapid wound healing in rats.
Keywords: Wound healing, Nanoemulsion spray, Open skin wound, TGF-ß

OS-050. A Combination of Biomesh Processing Techniques Towards Functional Improvement: In Vitro and In Vivo Assessment

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Background and Aim: Biomeshes based on decellularized bovine pericardium are the gold standard in reconstructive surgery. They were introduced into the area in 1971 by Ionescu et al as bioprostheses of heart valves. However, their xenogeneic nature shortens their service life and reduces implant/host tissue integration efficiency. To the date, significant progress was achieved in biomaterials science concerning the immunogenicity elimination. Developed approaches involve decellularization, cross-linking, supercritical fluid extraction and gene-engineering techniques. Each of these techniques possesses their own advantages and drawbacks. Aside from immunogenicity, they influence

on functional properties of biomeshes, namely, mechanical and structural characteristics, biodegradability, cytotoxicity, in vivo calcification and vascularization that predetermine the clinical outcome. In our study we performed a complex characterization of biomeshes prepared using various combinations of decellularization, cross-linking and supercritical fluid extraction protocols.

Methods: Decellularization procedure was based on alkaline treatment followed by cross-linking with a set of chemical agents and supercritical CO_2 treatment as schematically illustrated in Fig. 1. Variously treated samples were thoroughly characterized by mechanical trials (nanoindentation, tensile testing), biocompatibility (in vitro extraction test, contact cytotoxicity study, histological evaluation of explants) and biodegradability (collagenase A digestion, subcutaneous implantation in mice) evaluation and ultrastructural studies (scanning electron microscopy, atomic force microscopy in vitro and optical coherence tomography and multiphoton laser scanning microscopy in vivo).

Results: The samples prepared according to the experimental design were analyzed in a comparative study at each stage of the preparation procedure. We demonstrated that the cross-linking chemistry strongly influences the biodegradation rate. The values reduced to a different extent following the cross-linking. Optical properties were examined both in vitro and in vivo indicating ultrastructural alterations of the samples occurring in the series of treatment. Elastic modulus, in general, reduced due to the shrinkage of the material as the result of cross-linking; as well as mechanical anisotropy lowered. In vivo studies indicated better vascularization of cross-linked biomeshes when compared to the intact decellularized pericardium. We attribute this effect to structural alterations undergoing during cross-linking (increased water swelling, porosity, and proteolytic stability). Additional treatment in supercritical CO_2 reduced acquired cytotoxicity and improved elastic properties.

Conclusion: To conclude, our study revealed significant improvement of the functional properties of biomeshes following cross-linking and supercritical CO_2 extraction. These properties are crucial for the success of implant/host tissue integration and enable to tailor the material's behavior in vivo that is demanded adequate treatment. This work is supported by the Russian Science Foundation (Grant No. 18-15-00401).

Keywords: Pericardium; Reconstructive surgery; Xenotransplantation; Tissue-engineering

OS-051. Evaluation of Cord Blood Platelet Gel for The Treatment of Skin Lesions in Children with Dystrophic Epidermolysis Bullosa: A Pilot Study

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Background and Aim: Epidermolysis bullosa (EB) consists of a number of genetically skin diseases characterized by defects in the adhesion of the epidermis to the dermis that cause mechanical fragility of the skin and abnormal wound healing. Wound care is, therefore, of critical importance in the management of EB and current cure are not always satisfactory. In this study, we performed a pilot evaluation of the effectiveness of platelet gel from cord blood (CBPG) as an allogeneic product for the treatment of skin lesions in three children with EB

Methods: Cord blood samples are collected in sterile conditions. These samples were transferred to GMP grade clean room for processing. All CB samples were negative for viral and bacterial infection. Then, bags contain CB were centrifuged and two parts (RBC and PRP) were separated. Gelation was done under sterile condition by adding thrombin within 20-30 minutes. Prepared gel changed twice per week, time and size of wounds were measured.

Results: The clinical outcomes of this small group of patients were shown this trial have high efficiency and effectiveness in comparison with the current standard of care for these patients. Time of healing and size of wounds decrease significantly. CBPG releases a number of growth factors such as PDGF, TGF-β1 and -β2, IGF, EGF, FGF and EGF which can affect wound healing.

Conclusion: In conclusion, this pilot study reveals that CPBG is a promising and safe option for the treatment of EB skin lesions but need for larger studies

Keywords: Epidermolysis Bullosa; Cord blood; Platelet gel

OS-052. Plasticity of Cell Cultures from Human Eye Tissue

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Background and Aim: Application of tissue engineering is concerned to be promising in all medical fields, including ophthalmology. The injury of anterior and posterior eye sections affects multiple tissues: cornea, photoreceptors and retinal pigment epithelium (RPE) as well as a limbal zone that is the main source of eye stem cells for regeneration. Data about cell cultures from different eye regions are sometimes contradictory. The

aim of our work was to study 2D and 3D cell cultures of RPE and limbal zone.
Methods: Primary cultures of RPE cells and multipotent mesenchymal stromal cells from the limbal zone (MMSC-L) were obtained from the postmortal human eye. The study was conducted in accordance with officially approved procedures and a special authorization existing under the laws of Russian Federation. Cells were cultured for 4 passages under standard conditions (37°C, 5% CO2) in the growth medium consisting of DMEM/F12 (1:1), 2 mM/l L-glutamine, 50 μg/mL gentamicin. For RPE growth medium was supplemented with 20 ng/mL FGF, 20 ng/mL EGF, 1% solution of N2 and B27, and 5% FBS. For MMSC-L growth medium was supplemented with 20 ng/mL bFGF, insulin-transferrin-selenite (1:100) and 10% FBS. Spheroids were formed on agarose plates with micro-wells made using 3D Petri Dishes (Microtissue, USA). Time-lapse microscopy was performed with live imaging system Cell-IQ (CM Technologies, Finland). Immunophenotyping of cell cultures was conducted for the following surface markers: CD34, CD45, CD90, CD105, CD14, CD11b, CD19, CD29. For immunocytochemical analysis cells in monolayer and 7-day spheroids were fixed in 4% paraformaldehyde and were stained with antibodies to vimentin, ZO-1, N- and E-cadherin according to recommended protocols.
Results: In 2D monolayer cultures, cells were highly adhesive to Petri dish surface. RPE cell culture had polygonal "cobblestone" morphology with tight intercellular junctional complexes that expressed ZO-1 protein characteristic of epithelial cells. By the 4th passage the phenotype of RPE cells changed: they acquired mesenchymal fibroblast-like morphology and immunophenotype (CD105+, CD90+, CD45-, CD34-, CD11b-, CD19-), the level of vimentin expression was upregulated, synthesis of melanin was downregulated. MMSC-L during all the period in monolayer culture-maintained fibroblast-like morphology, expressed mesenchymal markers vimentin, N-cadherin, CD105, CD90, and CD29 almost did not express epithelial marker E-cadherin. When placed in non-adhesive conditions both cell types rapidly formed 3D spheroids. One of the characteristics of spheroids from cells of either epithelial or mesenchymal phenotype was the formation of several outer epithelial-like layers with adhesive E-cadherin+ (in case of RPE and MMSC-L) and tight ZO-1+ (in case of RPE) intercellular junctions. Moreover, spheroids from RPE and MMSC-L fused with each other and formed microtissues with epithelial cells on the surface and mesenchymal insight. 3D cell culture promoted a spontaneous mesenchymal-epithelial transition in MMSC-L, and reverted epithelial phenotype in RPE with upregulation of melanin synthesis.
Conclusion: Our study revealed a gradual transition to mesenchymal phenotype in 2D culture and spontaneous restoration of epithelial phenotype in cell spheroids. Combination of epithelial and mesenchymal characteristics in studied cultures of RPE and MMSC-L indicates their epithelial-mesenchymal plasticity, which makes these cells a promising source for regenerative medicine in the treatment of eye disorders.
Keywords: Cell plasticity; Spheroids; Epithelial-mesenchymal transition; Retinal pigment epithelium; Mesenchymal stromal cells

OS-053. High-Resolution 3D Printing of Bone Tissue Engineering Scaffolds

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Background and Aim: Extrusion-based 3D printing has been used for fabrication of bone tissue engineering (TE) scaffolds. As for hard biomaterials like bioceramics, it has been quite challenging to 3D print uniform and accurate scaffolds as the inks experience nozzle jamming/clogging and need exceedingly high pressure to impel ink to flow through a fine nozzle. Minimum nozzle size has been used for 3D printing of bioceramics is currently limited to 200 μm. The initial goal of this project focused on 3D printing of consistent and highly uniform bioceramic scaffolds for in vitro testing. The main objective was to optimize the process in order to increase the resolution. Hydroxyapatite (HA) scaffolds could be 3D printed using a nozzle diameter of 50 μm for the first time by carefully adjusting the process parameters.
Methods: An extrusion-based 3D printer was designed and set up and series of experiments were conducted using the design of experiment (DOE) approach to analyze the effects of paste solvent content, extrudate velocity and die design on extrusion pressure using the Benbow's model. The printed samples were subjected to further analysis using a scanning electron microscope (SEM) and computed tomography (CT). Cell viability and adherence were assessed by incubation and fluorescent imaging of Cell Tracker Green and Ethidium Homodimer (Molecular Probes, Oregon, OR, USA), respectively. Chorioallantoic Membrane Model (CAM) was also used to investigate blood vessel generation.
Results: Different scaffolds with a high level of accuracy (+/-20 μm) were fabricated from HA, PLA/HA, β-TCP, alumina, and zirconia using the bespoke developed 3D printer. Fig. 1 depicts the bespoke 3D printer, the bioceramics paste characterization, and the very high-resolution 3D printed HA scaffold using 50 μm nozzle by proper adjustment of printing parameters and nozzle design. Excellent cell adhesion and viability were observed by Cell Tracker Green/Ethidium Homodimer staining. The really interesting bit of results was that cells tend to row/bridge from one portion of the filament to the adjacent filament, even after just one day (Fig. 2a). Microscopic images at day 7 proved widespread cell growth, proliferation and pores filling (Fig. 2b). The scaffolds with 250 μm pore size were implanted for 7 days into CAM (Fig. 2c). Location intimate with yolk sac indicates good CAM integration and the membrane is visible surrounding HA scaffold. Fig. 2d depicts a typical scaffold with generated blood vessels after CAM implantation. As seen, there is a good blood vessel formation and adhesion.
Conclusion: Optimized 3D printing allowed production of the highest resolution bioceramic scaffolds has been reported so far while retaining a reasonable level of detail and accuracy. In vitro tests of the 3D printed HA scaffolds proved cell attachment, proliferation, and blood vessel formation.
Keywords: 3D printing; Bioceramics; Tissue engineering scaffolds

OS-054. Novel Bioactive PEEK Composites Produced Using 3D Printing and Dry Powder Printing Technologies

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Background and Aim: Bioactive materials like calcium phosphates are incorporated into Polyether-ether-ketone (PEEK) to improve the bone-implant interface (bone apposition). The current manufacturing processes have less control over the distribution of bioactive phase within the PEEK matrix. In this research work, new techniques based on 3D printing and dry powder printing were developed for the fabrication of bioactive PEEK-based composites with the computer-controlled 3D interconnected bioactive network within the PEEK matrix.
Methods: Extrusion freeforming an extrusion-based 3D printer was designed and set up for solvent-based extrusion freeforming of high resolution bioactive ceramic scaffolds. To make PEEK/HA composite, different bioactive HA scaffolds were made and over-molded with PEEK OPTIMA®LT3 UF powder. Dry powder printing (DPP) The developed dry powder printing contains two or more micro-feeders through which particles from several microns to a few millimeters could be co-fed accurately. The use of ultrasonic vibrations aids in breaking arches of particles in the nozzle by applying a continuous force. Sub-millimeter rod-shaped PEEK-OPTIMA® granule and glass filler (porogen) were fed simultaneously into a mold with the co-feeding system in different ratios for further heat treatment to prepare a homogeneous compound. The blend could then be heated at 400ºC for 45 minutes to melt the PEEK and bond to the filler to create a PEEK compound suitable for machining into test samples.
Results: Fig. 1 depicts SEM and CT images of vertical sections from typical PEEK/HA composite produced successfully through the optimized

compression molding using static loading, and PEEK-HA interface (the magnified view). As seen, HA scaffolds are fully infiltrated by PEEK in both vertical (infiltration depth is 3 mm) and lateral directions, while maintaining the HA network structure and uniformity. Images derived from X-ray CT data (Fig. 2) were used to investigate distributions of mixed particles with different size, shape and/or density of particles at different layers. The porous PEEK sample with narrow pore size distribution was fabricated by this ultrasonic co-feeding method which has made a significant improvement compared to traditional stir mixing.
Conclusion: In this work, the use of extrusion-based 3D printing and dry powder printing technologies to make bioactive PEEK-based composites is investigated. The produced composites provide new possibilities so that biological and mechanical performance can be tailored.
Keywords: 3D printing; Bioceramics; Bioactive PEEK composites; Dry powder printing

OS-055. Ca(2+) Signaling Orchestrates Cell Fate Decision in Health and Disease

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Cell-fate decisions like cell survival, senescence and apoptosis are regulated by Ca(2+) signals arising from the endoplasmic reticulum, which is in a close proximity to the mitochondria. These organelles are linked by Ca(2+)-transport mechanisms involving the inositol 1,4,5-trisphosphate receptor (IP3R). Constitutive IP3 signaling is a common feature in lymphoma and leukemia that can result in the survival, growth and proliferation of cells with oncogenic features. Suppressing the amount of Ca(2+) transferred from the endoplasmic reticulum to the mitochondria increases the resistance to apoptotic stimuli. Interfering with Ca(2+) signaling mechanisms could be exploited to design therapeutic approach that might achieve selective elimination of senescent cells by the reactivation of apoptosis. Here in, we provide an overview of the current knowledge on IP3R-Ca(2+) signaling, how they affect cell fate decision and how they can be targeted in the context of anticancer therapeutical strategy.

OS-056. In Vivo Study of Multi Potential Antigen in Generation of Dendritic Cell Vaccines

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Background and Aim: One of the most effective approaches for cancer treating is the excitation of the immune system against tumor antigens. One of the strategies to stimulate T cell effectors and memory immune response in cancer patients is to develop dendritic cell based vaccines. Microarray study indicated, LY6E is a common over expressed biomarker in three GI cancers, colon, gastric, and pancreatic. The current research purpose is in vivo study of investigation the effectiveness of adopting dendritic cells loaded with designed LY6E peptide antigen stimulating the MHC class I and II at the same time.
Methods: LY6E was chosen according the differential gene expression analysis. In the next step, Immune Epitope Database and Analysis Resource (IEDB) was used as a prediction tool for MHC class I and class II binding site and design of epitopes. After that mononuclear cells extracted from murine bone marrow were cultured in conventional cytokines. On day 7, cells were harvested and pulsed with peptide and subsequently, they were utilized for the maturation stage with LPS. DC phenotypes and characteristic were approved by flow cytometry (MHC Class II, CD80, CD40, CD14, and CD11c). Murine CT26 cell line were injected subcutaneously into Balb/c mice to create tumor and matured DCs pulsed with peptides were injected into mice. 17 days after injection, mice were sacrificed and samples were collected. Proliferation, stimulation assays, Flow cytometry, and pathology detection were carried out to confirm the efficiency of LY6E peptide.
Results: According to cytotoxicity assay (annexin/PI) extracted spelenocytes had a necrotic effect on CT26 as a cancer cell line. Regarding to tumor size, out of five treated mice, two of them showed significant amelioration. Flow cytometry analysis as another assessment, identified the proliferation of CD8 positive cells as cytotoxic T lymphocytes in the treated group. Following this, pathology samples indicated the G2 grade in treated group where the untreated group had G3 and G4 grade according to the TNM staging system.
Conclusion: Identification and development of common tumor antigens provide the possibility of immediate availability of DC vaccine. These anti-genes can provide more populations of cancer patients with available treatments. Finding a key player among the gastrointestinal cancers was the main concern of this study, therefore, by immunoinformatic approach; LY6E was detected as a core actor in three lethal GI malignancies (colon, Gastric, and pancreatic cancers). Our in vivo study characterized LY6E as a promising therapeutic candidate in GI cancers.
Keywords: Dendritic Cell; LY6E; GI cancer; Multi potential antigen

Poster Section

PS-001. Beta Thalassemia Gene Therapy by Lentiviral Transfer of Beta-Globin Gene

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Background and Aim: Beta thalassemia is a common monogenic disorder caused by a partial or complete reduction of beta globin chains synthesis. It is estimated that 1.5% of the global population (80 to 90 million people) are carriers worldwide. Common treatments have their complication. So, a molecular approach, such as gene therapy for direct healthy beta-globin gene transmission, seems quite promising to cure thalassemia. The goal of this study to design lentiviral vector carrying the healthy beta-globin gene and study beta glob gene expression in goal cell line

Methods: For our purpose, we designed the DEST Lentiviral vector carried the normal beta-globin gene and its promoter and packaged lentivirus in LentiX-293 T cell line. Then targeted cells (K562 cells) was transduced by packaged lentivirus containing β-globin cassette. After transduction, β-globin protein expression level was determined by flow cytometry

Results: Our results showed that we have successfully packaged and generated lentivirus in LentiX -293 T cell line and flow cytometry analysis showed that beta globin protein expression in a treated cell was increased. Beta-globin gene expression in control and a treated cell was achieved 23% and 85%, respectively

Conclusion: These data indicated that the vector used in this study can be useful in gene therapy in the patient's hematopoietic stem cell. The final goal of this study is to examine designed vector in hematopoietic stem cells promising therapeutic strategy for genetic diseases like beta thalassemia.

Keywords: Beta-thalassemia; Gene therapy; Lentiviral vector

PS-002. miR-195-5p Tough Decoy Alleviates Hypertrophic Markers in Micromass Model

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Background and Aim: Chondrocyte hypertrophy is a multi-stage evolutional process containing mineralization and differentiation. With regard to our knowledge of signaling pathways and, in particular, the determination of the important regulatory role of miRNAs in signaling pathways, one of the goals that can be studied in helping to achieve a method of a cop with cartilage hypertrophy is to study the effect of miRNA on the cartilage differentiation pathways. In this study, a miRNA195-5p tough decoy expresses an artificial transcript with a competitive inhibitory effect so, by absorbing the desired miRNA.

Methods: miR-195-5p tough decoy designed base on seed sequences with a bulge, In order to prevent the target from being captured by RNAi. Since we are working in the non-coding region, so firstly we put TAA as stop codon after emGFP then our construct cloned, and to make desire space, sequentially ACGCGU sequence inserted to make desired distance, Then the sequence put into a 'Tough Decoy' frame and The DNA synthesized and cloned in the PLVX vector, after analyzing by miRNAsong, plasmid gets ready to transfect by lipofectamin 2000 into c28/i2 cell line. C28/i2 cell line was followed by a relatively different transfection protocol due to its hard transfection and low rate of transfection and for the first time on the research, c28/i2 gets stabled. On 80% density, cells trypsinized and 1.7×10^4 cells seeded into six-well plates, after overnight incubation cells transfected by lipofectamine 2000 (Invitrogen, Life Technologies, NY), then followed by Micromass pellet culture with StemPro® chondrogenesis complete medium

Results: The miR-195 has a confirmed hypertrophy signaling pathway that interferes with the pathway of Col2a1, Col10a1, MMP13, DLK1, Aggrecan, VEGF. In fact, the miR-195 has direct targets on the 3'UTR of the targets that bind them and results in reduced expression, In normal mode. Also, the expression level of miR-195 is inversely related to the expression level of CCND1 and in case of high expression of miR-195, this gene expression reduced and vice versa. Another important target of miR-195, which has a very important function of differentiation and evolution, is the WNT3a gene that has some seed for miR-195 in the 3'UTR region. The high level of miR-195 reduces the expression of the WNT3a gene that leads to cell cycles stopping at G1 steps. Real-time PCR analyses showed Col2a1, CCND1, DLK1, Aggrecan, and ALP genes were up-regulated in Tough decoy stabled cell line compared to control cells during micromass pellet culture, while Col10a1, MMP13, Runx2 were downregulated.

Conclusion: In this study, we used a TUD containing cassette for miR195-5p, which is expressed by the CMV promoter. The expression of this construct leads to cytoplasm occupation with artificial miR-195-5p targets, this will result in all of the miR-195-5p absorbed by construct and main targets remain clear. By comparing the results of real-time PCR and also, Alician blue and Alirazin red tissue stain, showed that the chondrocytes cells retained their cartilage shape in TUD stabled cells more than the control cells.

Keywords: Hypertrophy; miR-195-5p; Chondrogenesis; miRNA Inhibitor; Tough Decoy

PS-003. Surface Modification of Nanofibrous PCL Scaffold Followed by Collagen Immobilization, Enhanced Endothelial Cell Attachment and Stability Under Shear Stress

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Background and Aim: Endothelial cell seeding of synthetic vascular grafts is a promising method to improve the graft function. Surface modification by functional groups followed by collagen immobilization improves endothelial cell attachment and stability under fluid flow shear stress. We aimed to study whether surface modified PCL nanofibrous scaffolds containing carboxylic groups followed by collagen immobilization, improve endothelial cell attachment and stability.

Methods: Nanofibrous PCL scaffolds were prepared by electrospinning method. Carboxylic groups were created into the scaffold surface using NaOH solution (3M, 30 minutes). Type one collagen was immobilized on the nanofibers after surface modification by NaOH treatment. HUVECs were seeded on the luminal surface of the untreated, NaOH treated and collagen immobilized nanofibrous tubular scaffolds. All three groups of scaffolds were placed in the perfusion chamber and exposed to fluid flow (35mL/min, 1h) using a peristaltic pump. The number of detached cells was assessed by the trypan blue assay. Scanning electron microscopy was used to visualize the morphology of endothelial cells attached to unmodified or surface modified nanofibrous tubs after 7 days of culture.

Results: The number of detached cells under fluid flow was significantly

decreased by surface modification and collagen immobilization. SEM images showed that collagen immobilization provides a compatible surface for endothelial cell attachment.
Conclusion: In conclusion, PCL/Collagen nanofibrous scaffold has a promising surface for endothelial cell attachment and stability under fluid flow shear stress.
Keywords: Nanofibrous PCL scaffold; NaOH treatment; Fluid flow; Collagen immobilization; Endothelial attachment

PS-004. Evaluation of the Effect of BMSCs Condition Media and Methylprednisolone in TGF-β Expression and Functional Recovery After Acute Spinal Cord Injury

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Background and Aim: Inflammation is one of the most important contributing factors to the spinal cord after injury. It makes causing Macrophages and other inflammatory agents to attack the site of the lesion. One of the most important ways to control inflammation is using Methylprednisolone (MN) and Bone Marrow Stromal Cells Condition Media (BMSC-CM). They can reduce inflammation and improve functional recovery after spinal cord injury.
Methods: BMSCs were analyzed by flow cytometry for mesenchymal markers. After acute spinal cord injury, MN and BMSC-CM were injected intraperitoneally and the recovery rate was evaluated by BBB and narrow beam test.
Results: There were significant differences in functional recovery between MN+CM group Relative to other groups. TGF-β1 expression decrease in CM+MP group Compared to the control group.
Conclusion: Simultaneous use of MN and BMSC-CM will improve recovery from spinal cord injury, reduce inflammation and improve functional recovery.
Keywords: Spinal cord injury; TGF-β1; Inflammation; BMSC; Methylprednisolone

PS-005.The Combined Effects of Mesenchymal Stem Cell Condition Media and Low-Level Laser on Stereological and Biomechanical Parameter in Hypothyroidism Rat Model

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Background and Aim: Many studies have shown the positive effect of laser radiation and usage of the Mesenchymal Stem Cells (MSC) and their secretion in stimulating bone regeneration. The aim of the study was determining the effects of MSC condition Media and low-level laser (LLL) on healing bone defects in the hypothyroid male rat.
Methods: We assigned 30 male Wistar rats randomly to three groups: control, hypothyroidism, CM+LLL. Four weeks after surgery, the right tibia was removed. Immediately biomechanical examination and histological examinations were performed
Results: Our results showed significant increase of bending stiffness (116.09±18.49), maximum force (65.41±8.16), high-stress load (23.30±7.14) and energy absorption (34.57±4.10) and trabecular bone volume (1.34±0.38) and number of osteocytes, osteoblast, and osteoclast (12.77±0.54,6.19±0.80,1.12±0.16, respectively) in osteotomy site in LLL+CM compared to hypothyroidism groups ($P<0.05$).
Conclusion: The result indicated that using the LLL+CM may improve fracture regeneration and it may accelerate bone healing in the hypothyroid rat.
Keywords: Hypothyroidism; Low-level laser; Condition media

PS-006. Neurosphere-free Trans-differentiation of Rat Bone Marrow Stromal Stem Cells Into Retinal Cells and Retinal Pigment Epithelium

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Background and Aim: Neurosphere-free (NF) trans-differentiation of bone marrow stem cells (BMSCs) into retinal progenitor and retinal cells in vitro could offer a unique opportunity for the study of regeneration of degenerative retinal diseases. In this respect, a simple and efficient protocol for getting retinal cells from trans-differentiation of rat BMSCs in the NF state is reported.
Methods: BMSCs extracted from femur hooded pigment rats and passaged to P4 were exposed to single-step protocol, including neurosphere-free, which induced trans-differentiation into retinal pigment epithelium (RPE) and different retinal cells (RC). The cells immunostained with Rhodopsin, OTX2, NF200, GFAP, RPE65, VEGF, and CRALBP markers.
Results: Cultured rat BMSCs at the fourth passage was shown to express the surface markers CD90 (95±2%), CD44 (91±2%), CD166 (98±2%), CD 106 (99±2%) and Fibronectin (96±2%), $P < 0.05$. The RC and RPE cells obtained from rat samples expressed the markers. 60 days after differentiation, the previously rat BMSCs expressed rhodopsin (58±2%), OTX2 (24±2%), NF200 (25±2%), GFAP (28±2%), RPE65 (100±1%), CRALBP (96±1%), and VEGF (97±2%), $P < 0.05$ and were thus significantly similar to the RC and RPE cells obtained from control rat samples. Morphologically, differentiated cells appeared to have similarity to RC and epithelial and cytoplasmic pigment granules.
Conclusion: Overall, these findings show a protocol, mimicking developmental stages which make it possible to derive retinal and retinal pigment epithelium cells in vitro. This is a great finding for transplantation in retinal diseases.
Keywords: Neurosphere-Free; Rat Bone Marrow Stem Cells; Retinal Cells; Retinal Pigment Epithelium, Trans-differentiation

PS-007. Effects of Bovine Serum Albumin and Heparin on the Basic Fibroblast Growth Factor Release from Smart Poly (N-isopropylacrylamide) Nanoparticles for Induction of Angiogenesis in Adult Stem Cells

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Background and Aim: Growth factor (GFs) delivery with a certain concentration and release kinetic is one of the main challenges in tissue engineering. Using controlled spatiotemporal release of the GFs can be an approach to achieve this aim. Poly(N-isopropylacrylamide) (PNIPAM) is the most prominent candidate thermally responsive polymer to deliver the drug due to its sharp transition which is around physiological temperature. The aim of this research was to investigate the basic fibroblast growth factor (bFGF) release from PNIPAM nanoparticles in different bFGF loading conditions.

Methods: In this study, temperature-sensitive PNIPAM nanoparticles were first synthesized by free radical polymerization technique. Nanoprecipitation and diffusion methods were studied to load the basic fibroblast growth factor (bFGF) in PNIPAM nanoparticles. According to the results, the diffusion method is more suitable to load the GFs. Two different formulations of bFGF with and without Bovine Serum Albumin (BSA) and Heparin (Hep) were used to load the bFGF in PNIPAM nanoparticles by the diffusion method. The effects of BSA and Hep on the bFGF release kinetic profile were carefully investigated. BSA and Hep were used to prevent adsorption to microtube walls and to stabilize the GFs, respectively. The biological evaluation of PNIPAM nanoparticles on human bone marrow stem cells (hBMSCs) was also studied via an indirect method, according to the ISO 10993-5 cytotoxicity standard.

Results: Morphology of the PNIPAM nanoparticles before and after the nanoprecipitation process and diffusion method was studied by scanning electron microscope. The results showed that the nanoprecipitation process caused polymer degradation due to using the organic N,N-Dimethylacetamide solvent, as a result, the diffusion method is more suitable to load the GFs. The PNIPAM nanoparticles had spherical grains in the range of 50–150 nm. The cumulative percentage bFGF release after 72 h for pure bFGF loaded PNIPAM (bFGF-PNIPAM) and bFGF-BSA-Hep added loaded PNIPAM (bFBH-PNIPAM) were 100 and 80%, respectively. The presence of BSA and heparin decreased the bFGF burst release from bFBH-PNIPAM compare to bFGF-PNIPAM. The biological evaluation revealed the particles up to a concentration of 3 mg/mL did not show any toxicity on hBMSCs.

Conclusion: According to the given data the GFs loaded PNIPAM nanoparticles is promising nanosystem that can be applied to deliver the bioactive GFs for tissue engineering applications. By using heparin and BSA, the bFGF release was better controlled as it had diminished burst release and more slowly drug release kinetic. Our data showed the PNIPAM NPs have the potential to load and release the angiogenic GFs for stimulation of angiogenesis in bone tissue engineering.

Keywords: Temperature-sensitive PNIPAM nanoparticles; Growth factor delivery; Angiogenesis; Human bone marrow stem cells (hBMSCs)

PS-008. Platelet-Rich Plasma and its Restorative Effect on Fertility for Rat Model of Menopause; A Pre-cinical Study

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Background and Aim: Due to natural physiological aging, women at age 50-52 years old, face menopause and its related obstacle including infertility. Early menopause occurs in about 1% of women under 40 and 0.1% under 30 years in general population. Several causes have been identified for early menopause such as iatrogenic causes (mostly because of oncology treatment), idiopathic, genetic and autoimmune diseases. Several therapies have been proposed in this regard, however, the effectiveness of them remains to be further illustrated.

Methods: In this study, we examined the restorative effect of Platelet-rich plasma (PRP) as a natural blood product in the rat model of menopause. The animal models were made within 15 days, using a daily injection of the gonadotoxic agent, 4-Vinylcyclohexene Diepoxide. The control group received saline in the whole period of animal modeling. After confirmation of menopause by hormonal (E2 and FSH) assessment and histological studies, a group of rats was injected (intra-ovarian injection) by freshly prepared rat PRP in two concentrations of 1 and 4X (15 rat/group). Other two groups were either received saline or did not get any injection. Two months were assigned for evaluation of the treatment, in which every two weeks, 3 rats from each group randomly were allocated for hormonal and histological assessment of ovarian tissue.

Results: Results indicate that the groups of rats who have received PRP in both concentrations restored somehow their ovarian function. Histological studies revealed that PRP treatment improved the follicle numbers of treated rats compared to the non-treated ones. Hormonal assessment as well showed a refinement pattern as the level of FSH has declined and the level of E2 has elevated significantly in the treated compared to non-treated rats.

Conclusion: Our results illustrate that PRP could be applied as an alternative therapeutic agent for treatment of patients who are at early stages of menopause or about to enter into this complication such as cancer patients who are undergoing through gonadotoxic chemotherapy and radiotherapy. Quite optimistic, but further experimentations should be performed to approve the safety and applicability of the technique in the human setup.

Keywords: Menopause; Infertility; Platelet-Rich Plasma; Animal Model; Ovarian rejuvenation

PS-010. The Protective Effect of Melatonin Against Induced Oxidative Stress on Adipose-Derived Stem Cells (ASCS)

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Background and Aim: Adipose-derived stem cells (ASCs) are one of the cellular populations in adipose tissue with a high potency of differentiation to specific lineages. These stem cells, based on their therapeutic potential to repair and regenerate damaged tissue, have applications in regenerative medicine. Melatonin is a natural compound that secret by the pineal gland and several studies investigated that it has strong antioxidant effect against free radicals. In this study, we aimed to determine the cytoprotective effects of melatonin on ASCs that induced by H_2O_2.

Methods: We isolate human adipose-derived stem cells (hASCs) from adipose tissue. Then we cultured them with different dosages of hydrogen peroxide and melatonin for a period of 3 hours. MTT assay was used to assess the IC_{50} of melatonin and H_2O_2. We also measured the H_2O_2

-induced cytotoxicity in cultured ASCs with LDH assay. Apoptosis analysis was investigated by using the flowcytometric annexin V/propidium iodide (PI) assay. The intracellular level of produced reactive oxygen species (ROS) during exposure to melatonin and H_2O_2 was assessed based on the oxidation of fluorescent probe 2'7'-dichlorofluorescein (DCFH). Furthermore, the signaling pathway of Nrf2/ HO-1 in ASCs treated with melatonin and H_2O_2 was studied.

Results: Hydrogen peroxide treatment caused an incremental increase in cell death. Melatonin lonely had no damaging effects on ASCs in H_2O_2-free condition. ASCs co-treated with melatonin in the presence of hydrogen peroxide prevented ASCs from cell death in a dose-dependent manner. Co-treatment of ASCs with melatonin showed significantly fewer apoptotic cells. The release of LDH was considerably induced by H_2O_2, while melatonin pretreatment dose-dependently suppressed LDH release in ASCs. The result of this study suggests that melatonin protects the ASCs against oxidative stress induced by H_2O_2. It is proposed that melatonin involves in the control of Nrf2 in ASCs.

Conclusion: In conclusion, melatonin seems to be an endogenous antioxidant agent with proficient free radical scavenging activity and may act as an effective adjunct in fat grafting in tissues.

Keywords: Melatonin; Oxidative stress; Adipose; Derived Stem Cells (ASCs)

PS-011. Study of the Eeffects of EMT Inhibitors on Cancer Stem-Like Cell (CSC) Enriched HT29 Cell Line Order to Anti-CSCs Drug Screening

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Background and Aim: CSCs have a potential for self-renewal and differentiation and are resistant to current anticancer treatments. Since CSCs generally comprise only small minorities within cancer cell populations screening for agents that specifically kill them may help to reduce cancer mortality. Studies showed that the EMT generates cells with properties of CSCs. So, recently it has been noted to EMT inhibitors as a promising strategy for CSC targeting. The aim of our research is the study of EMT inhibitors on CSCs enriched HT29 cell line in order to anti-CSC drug screening.

Methods: Pioglitazone (Pio) was selected as an EMT inhibitor. CSCs enriched HT29 cell line by EMT induction using knockdown of E-cadherin, was cultured in DMEM media. MTT test was done in 3, 5, 7 days for IC50 value calculating. After IC50 calculating, CSCs enriched HT29 cell line was treated by the appropriate concentration of Pio. After drug treatment, properties and markers of CSCs and EMT were investigated compared to control cells using several techniques including flow cytometry, Real-time PCR, and MTT assay. During the study, morphology changes were monitored by optical and fluorescent microscope daily. The parental HT29 cell line was used as the control.

Results: Pio treatment of CSCs enriched-HT29 Cell line was caused by EMT inhibition. Cell morphology changed from mesenchymal to epithelial. Markers of CSCs and EMT were decreased compared to control cells. Properties of CSCs and EMT were changed compared to control cells.

Conclusion: Pio as an EMT inhibitor can decrease CSCs in cancer cell line population. We expect significant advances in inhibition of EMT can be useful for CSCs targeting. We suggest more study for the greater understanding of EMT and CSC and their relationship to cancer treatment.

Keywords: Colon cancer; Cancer stem cell; EMT; Anti CSCs drugs

PS-012. ROBO-4/Slit-2 Signaling in Leukemic Stem Cell after Treatment with Brivanib Alaninate

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Background and Aim: Acute Myeloid Leukemia is a clonal hematopoietic malignancy with overproduction of abnormal myeloid progenitors in bone marrow. Nowadays, common chemotherapy is not efficient because of the presence of leukemic stem cells. Hence, target therapy by inhibiting a specific signaling pathway in leukemic blasts is needed. LSCs are chemo-resistant cells which are expressing receptors like ROBO-4 as a receptor for Slit-2. ROBO-4 act as a co-receptor for CXCR-4 and inhibit VEGF/VEGFR-2 signaling by binding to Slit2. Thus, we aimed to evaluate the effects of Brivanib Alaninate as a VEGFR-2 inhibitor on Slit-2/ROBO-4.

Methods: In this study, KG1a which has been considered as a drug resistance AML cell line, was used for cell culture. Cells were treated with 5 different doses of Brivanib Alaninate (Apexbio, USA) for 48 and 72 hours and then MTT assay (Sigma-Aldrich) was performed to evaluate the viability of these cell lines. Optical densities of each well were measured at 540 nm wavelength. Finally, flow-cytometry was done to confirm the results of the MTT assay. Then, real-time PCR was conducted to evaluate the expression level of VEGF, VEGFR-2, ROBO-4, Slit-2, UNC5B, and CXCR-4.

Results: The optimum dose of Brivanib Alaninate for treatment was 100 µM. Expression of VEGFR-2 and VEGF were increased in KG1-a cell line treatment. Low expression of ROBO-4 was seen in these groups while the expression of CXCR-4 was increased a bit.

Conclusion: In this study, we showed that the expression of VEGFR-2 increased after treatment as a chemo-resistance mechanism. KG1-a cell decreased the expression of ROBO-4 to avoid inhibiting the blocking of VEGF/VEGFR-2 signaling to maintain proliferation and survival capacity.

Keywords: Leukemic stem cell; Brivanib Alaninate; ROBO-4

PS-013. The Effect of Targeting EGFR Pathway in Glioblastoma Multiform

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Background and Aim: Glioblastoma multiforme is an invasive type of brain tumor that penetrates a distinct attack from single glioblastoma cells, which causes brain parenchymal infiltration. Here, we examined the effect of on miR-491 glioma cell line.

Methods: Expression of miR-491 in glioma cells was assessed by quantitative PCR (qPCR). To assess the function of candidate microRNAs. In GBM, we transfected miR-491 to cultured-U-251 cell lines. Cellular proliferation an apoptosis was assessed by flow cytometry, and gene expression in EGFR pathway was sassed by qPCR.

Results: Our studies revealed that overexpression levels of microRNA-491 can increase apoptosis and reduce cell proliferation in u-251 cell line. In addition, suppress the expression of epidermal growth factor receptor (EGFR) pathway.

Conclusion: Taken together, these results revealed that overexpression of miR-491 in u-251 cell line might suppress the EGFR pathway.

Keywords: Glioma; miR-491; Epidermal growth factor receptor

PS-014. Neural Proliferation and Differentiation of Human Adipose-Derived Stem Cells (hADSCs) On Nanofibers PCL/lignin Scaffold.

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Background and Aim: Tissue Engineering in regenerative medicine warrants our enhanced understanding of the biomaterials and its function. The aim of this study was the evaluation of the proliferation and differentiation of human adipose-derived stem cells (hADSCs) growing on Polycaprolactone PCL/lignin scaffold that we developed an aligned (PCL)/lignin nanofiber using electrospinning technique. Stability of this scaffold is in providing a favorable matrix for cell growth.

Methods: Following the isolation of hADSCs from human adipose tissue, these cells were cultured and characterized on PCL/LIGNIN scaffold. The MTT assay, lineage-specific differentiation under two-dimensional culture conditions, scanning electron microscopy (SEM), flow cytometry, and immunocytochemistry were used for analyses.

Results: The study revealed that the PCL/lignin fibers promote cell proliferation coupled with 90% cell viability and the excellent substrate for the differentiation of hADSCs to nerve cell.

Conclusion: Our results suggest that PCL/lignin is a promising scaffold for neural proliferation and differentiation.

Keywords: Nervous system; Stem cell; Proliferation; Differentiation; Electrospinning; Nanofibers

PS-015. Effect of Rat Cardiomyoblast Encapsulation with Alginate-Gelatin Microspheres on Cell Dynamic and Viability

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Background and Aim: Despite the emergence of novel therapeutic advances regarding MI, ischemic heart disease is conceived as a major cause of global mortality and morbidity. it made investigators to develop novel re-vascularization techniques that specifically target the microvasculature in ischemic myocardium. Several studies have recently reported promising results by modifying and enhancing cell-mediated ischemic myocardial repair and regeneration. In this study, we aimed to investigate the effect of alginate-gelatin encapsulation on the viability and dynamic of rat cardiomyoblasts in vitro.

Methods: Rat cardiomyoblasts cell line H9C2 were encapsulated by alginate-gelatin solution and incubated for 7 days, and the control group was rat cardiomyoblasts from the same cell line without encapsulation while incubated for 7 days as like the encapsulated group. The level of extracellular enzymes as like SGPT, SGOT, CPK, and LDH were detected in supernatants from both groups. The level of intracellular enzymes as like SOD, GPx, and TAC was measured by biochemical analyses in cell lysates. To the examination of cell viability, we used MTT method.

Results: We found that encapsulation was able to increase the viability of rat cardiomyocytes after 7 days. The increased level of intracellular enzymes including SOD, GPx, and TAC was identified compared to the control cells ($P>0.05$). No significant differences were found regarding the level of SGPT and CPK while the level of LDH and SGOT as extracellular enzymes showed decreasing compared to the control non-treated cells ($P>0.05$).

Conclusion: The results of this investigation showed that encapsulation of rat cardiomyoblasts with alginate-gelatin microspheres improves the cell's dynamic and viability.

Keywords: Rat cardiomyoblasts; Encapsulation; Alginate-gelatin Microspheres; Oxidative stress enzymes; Cell Viability

PS-016. HLA-A Gene Knockout Using Dual gRNA by CRISPR-Cas9 System

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Background and Aim: Nowadays, the treatment of many cancers and genetic diseases is focused on cell therapy and long-term allogenic engraftment, which the most important is HLA molecules. We demonstrated that HLA-A molecule on the HEK293T cell line could be eliminated by CRISPR-Cas9, which would make the process of transplantation easier.

Methods: Two gRNAs with the highest efficiency and the lowest off-target were designed by existing databases. Then, these gRNAs were separately inserted in the px458 vector, transformed and cloned in E. coli. The insertion of gRNAs into the vector validated by both PCR and Sanger sequencing, and then transfected into the HEK293T by lipofection 2000. Green fluorescent transfected cells sorted by FACS, and single cell dilution performed. Afterward, the DNA's from every single clone extracted and then both PCR and Sanger sequencing approved excised region by CRISPR-Cas9.

Results: After selecting the HEK293T cell as a model, HLA typing was performed and the allele was determined. The design of gRNAs was carried out according to the type of cell allele and for exons 2 and 3 of HLA-A2. After co-transfection of the plasmids, doing FACS and single cell dilution, the proportion of HLA-A gene-disrupted cells was 9.09%. At 2–3 weeks after sorting, 22 clones among a total of 192 wells (11.45%) were cultured and analyzed for HLA-A gene mutations.

Conclusion: The studies showed that removing both alleles of the HLA-A gene not only protects the cell against immune responses but also significantly increases the chance of finding an unrelated donor by reducing the number of alleles required for allogeneic transplantation. It has also been shown that the CRISPR-Cas9 system is an efficient and safe technique to perform gene knockout.

Keywords: HLA-A2 antigen; Gene Knockout Techniques; Transplantation; CRISPR-CAS Systems

PS-017. Universal gRNAs Design to Increase Chance of Finding a Suitable Donor for Hematopoietic Stem Cell Transplantation Using CRISPR/Cas9 System

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Background and Aim: Human Leukocyte Antigen (HLA) molecules are a complex system that expresses as major Histocompatibility Complex in the human and it is one of the most polymorphic regions of the genome. These molecules have an important role in transplantation and adaptive immunity; therefore, mismatch of HLA alleles can lead to graft rejection and graft-versus-host disease. We developed a strategy to address this issue for the Iranian population using a CRISPR-Cas9 system.
Methods: According to previous studies, the most common HLA-A alleles in the Iranian population were collected. Based on this, 14 common alleles were selected and the sequence of each allele was extracted individually from https://www.ebi.ac.uk/ipd/imgt/hla/. Using the alignment tools (https://www.ebi.ac.uk/ipd/imgt/hla/align.html), we aligned all the alleles simultaneously. In order to design a common gRNA among the identical areas of aligned sequences, we referred to the https://crispr.mit.edu. After analyzing gRNAs, the best gRNAs were selected based on efficiency and off-target.
Results: From https://crispr.mit.edu database, two gRNAs were picked up. gRNA1 covers all HLA-A alleles except HLA-A1. To encompass all HLA-A alleles, another gRNA named gRNA2 was selected, which it covers four alleles including HLA-A1.
Conclusion: Despite the high level of polymorphisms in HLA genes, two universal gRNAs for Iranian population were designed that it can be used for approximately (99.1%) all Iranian racial populations. In this way, with the new technology for gene editing, CRISPR-Cas9 can induce indel in the HLA-A gene; consequently, the cell cannot express HLA-A molecule on its surface. Failure to express this gene will make it much easier to find a proper donor in the population.
Keywords: HLA Antigens; CRISPR-Cas systems; gRNA; Transplantation; Iran; RNA; Guide

PS-018. Effect of Continuous and Pulsed Mode of Low Power Laser Irradiation on Viability of Buccal Fat Pad Derived Adipose Stem Cells

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Background and Aim: Laser photobiomodulation can be a useful method in tissue engineering to enhance the proliferation and viability of stem cells. Laser therapy with powers less than 0.5 W can result in biostimulation of cells and tissues. However, parameters such as power, energy density, irradiation mode (continuous or pulse mode) and stem cell type play significant roles in the results. The aim of this study was to compare different laser settings of a 940nm laser on the viability of adipose stem cells in order to define the best settings for photobiostimulation.
Methods: Adipose stem cells were isolated from the buccal fat pad. Third passage the cells were seeded in 96-well plates. The cells were irradiated at the intervals of 12 hours for three consecutive days. Irradiation was performed with 6 different laser settings by two modes of continuous and pulsed (50% duty cycle) and two different energy densities of 3 and 6 J/cm2 and two different 0.1W and 0.3W output power using a 940nm laser (Epic x, Biolase, USA) a non-irradiated group served as control. The test was repeated at 3 different times. Cell viability was evaluated by MTT assay at intervals of 24, 48 and 72 hours after the final laser irradiation.
Results: After 24h the 6J/cm2, 0.3w, the pulsed group showed a 13.96% higher cell viability compared to control ($P<0.01$). This group also showed a 12.3% higher cell viability compared to control after 48h, however, none of the groups had statistically significant results at this time point. 72 h after irradiation 3 J/cm^2, 0.3 W, the pulsed group showed 26% higher and the 3 J/cm^2, 0.1 W, the pulsed group showed 22% higher viability results compared to the control which was statistically significant ($P<0.01$). The 6 J/cm^2, 0.3 W, the pulsed group also showed a 15% increase, however, this result was not statistically significant. No statistically significant difference was observed in the continues mode irradiated groups.
Conclusion: According to the results of this study a pulsed mode of irradiations showed better viability results on adipose stem cells.
Keywords: Adipose stem cells; Low-power laser irradiation; Laser

PS-019. A STAT3-Decoy Oligodeoxynucleotide Suppresses Cell Growth and Induces Apoptosis in Erlotinib Resistant SW480 Colon Cancer Cell Line by Blockage of the STAT3 Signaling Pathway

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Background and Aim: Colon cancer persists as one of the most prevalent and deadly tumor types worldwide. The emergence of resistance to chemotherapy is a major clinical problem. STAT3 (signal transducer and activator of transcription 3) is frequently activated in tumor cells and plays a prominent role in proliferation, chemotherapy-resistance. The present study was to test the hypothesis that inhibition of STAT3 decrease growth and induce apoptosis in Erlotinib resistant SW480 colon cancer cell line.
Methods: First, sense and antisense sequence of decoy and scramble oligodeoxynucleotide for STAT3 transcription factor designed based on STAT3 elements in the promoter region of the MYCT1 gene. To confirm specific binding of the STAT3 nuclear proteins with STAT3 decoy ODNs EMSA dual protein-nucleic acid staining Kit was used. Fluorescent microscopy was done to determine the subcellular localization of labeled decoys. Investigation of cell viability, apoptosis and expression level of downstream genes in SW480 cells carried out by MTT, Annexin v/pi test and Real-time PCR assay, respectively.
Results: EMSA indicate that specific binding between STAT3 decoy to STAT3 protein in nuclear extract of SW480 cells. Transfection of STAT3 ODNs potently represses erlotinib-resistant colon cancer cell line in association with reducing cell viability reduction, apoptosis promotion and downstream gene expression modulation in SW480 cells compared to control groups.
Conclusion: These findings showed that STAT3-decoy ODNs is an efficient inducer of cell death and apoptosis in the Erlotinib resistant SW480 colon cancer cell line. Inhibition of STAT3 by cis-element double-stranded oligodeoxynucleotide (ODNs) strategy holds great promise for the effective treatment of colon cancer.
Keywords: Colon cancer; Decoy oligodeoxynucleotide; STAT3 transcription factor; Erlotinib; Drug resistance

PS-020. Linc-ROR Expression in Acute Lymphoblastic Leukemia

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Background and Aim: Acute lymphoblastic leukemia (ALL) is common in children and characterized by the overproduction of immature lymphocytes in bone marrow (BM). Emerging data demonstrate that long non-coding RNAs (lncRNA) are involved in pathological processes like cancer. LINC-ROR plays a role in the endogenous maintenance of

stem cells and participated in tumorigenesis by acting as p53 repressor, hypoxia-responsive lncRNA. Also, Leukemic cells in BM have shown an overexpression of hypoxia-inducible factor (HIF-1α). The aim of this study was to examine Linc-ROR expression in ALL cell lines, patients and Hypoxia condition.
Methods: We cultured T-ALL including RPMI-8402 and Jurkat in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) at 37°C in a humidified incubator containing 5% CO2. Cell lines were purchased from the National Cell Bank of Iran (NCBI) (Pasteur Institute of Iran, Tehran). For hypoxia condition cell lines were cultured in 10-cm dishes for 24 hours at 37°C in an incubator; gassed with a pre-analyzed gas mixture containing 5% CO2 and 95% N2. BM Mononuclear cells of patients were isolated from ficoll-opaque. As control T lymphocyte was isolated by nylon wool from healthy BM -nylon fibers were obtained from the Iranian Blood Transfusion Organization (IBTO, Tehran, Iran). The purity of isolated T lymphocytes was evaluated using flow cytometry, Cells were incubated with PE-conjugated Anti-human CD3 for 45 minutes at 4°C in dark. Expression of LINC-ROR, P53, and HIF-1α in cell lines, hypoxia, and Patients by quantitative real-time PCR. β-Actin was used for normalization. Relative gene expression was calculated according to the 2-ΔΔCT/ΔCT method.
Results: Our results revealed that expression of LINC-ROR was lower in Jurkat and RPMI-8402 compared to T cell Also, P53 expression was increased significantly in Jurkat and RPMI-8402 compared to control. Patient samples evaluation showed that expression of LINC-ROR was decreased in contrast to p53, which its expression pattern is the same as cell lines. Expression of LINC-ROR was significantly increased in both cell lines and P53 expression was decreased under hypoxia condition compared to normoxia condition. We evaluated the expression of HIF-1α and we observed expression of HIF-1α was higher in the patient samples compared to healthy patients. In addition, HIF-1α expression was considerably elevated under hypoxia condition compared to control.
Conclusion: Our data demonstrated, have a tight correlation between LINC-ROR and p53. linc-ROR Expression under hypoxia was increased that lead to inhibit important tumor suppressor, p53 that leads to leukemic cell growth. In addition, LINC-ROR can induce HIF-1α expression under hypoxia and it contributes to chemotherapy resistance of leukemic cells. Our findings accompanied by the previous study reveal that LINC-ROR may have a role in ALL progression especially, during hypoxia. We hypothesize that LINCROR may have a key function in a T-ALL pathogenesis and it would be a new biomarker for prognostic factor.
Keywords: Acute lymphoblastic leukemia; Long noncoding RNA; LINC-ROR

PS-021. Ferulic Acid-Loaded Polyurethane for Vascular Tissue Engineering

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Background and Aim: Autologous vessels have limited graft resources. Artificial vascular grafts failed in small diameter vascular graft (6 <mm inner diameter) applications due to thrombosis, infection, lower stability, compliance mismatch, and premature occlusion. The aim of this study was to develop scaffolds with enhanced blood compatibility and vascular cell-compatibility.
Methods: In this study, polyurethane was synthesized. The synthesized polyurethane was blended with ferulic acid (FA) to improve hemocompatibility. Scanning electron microscopy (SEM), water contact angle (CA) measurement, and tensile test were utilized to characterize the scaffolds. The polyurethane and polyurethane-FA scaffolds showed suitable tensile strengths and strains.
Results: The ferulic acid from the scaffolds released in a sustained manner. The polyurethane-FA scaffolds induced lower platelet adhesion with a reduction in hemolysis and fibrinogen compared to the polyurethane scaffold. The endothelial cells proliferated on the polyurethane-FA samples. The cells expressed endothelial cell marker (CD31).
Conclusion: Therefore, the new polyurethane modified with ferulic acid can be considered as a promising candidate for the vascular application.
Keywords: Polyurethane; Ferulic acid; Hemocompatibility

PS-022. The Odontogenic Differentiation of Human Dental Pulp Stem Cells on Hydroxyapatite-Coated Biodegradable Nanofibrous Scaffolds

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Background and Aim: The authors aimed to design nanofibrous (NF) scaffolds that facilitate odontogenic and osteogenic differentiation of human dental pulp-derived mesenchymal stem cells (DPSCs) in vitro.
Methods: For this purpose, hydroxyapatite (HA)-loaded poly (L-lactic acid)/poly (ε-caprolactone) (PLLA: PCL 2; 1) blend NFs were prepared using the electrospinning method. Alizarin red activity and cell viability were evaluated by MTT assay, and SEM revealed the proliferation properties of NF scaffolds.
Results: Optimized nanofibers PCL/PLA/HA (PCL/PLA,2:1 w:w) of various HA contents with average diameters of 100-300 nm were obtained using the electrospinning method. QRT-PCR results demonstrated that HA-loaded PLLA/PCL can lead to osteoblast/odontoblast differentiation in DPSCs through the up-regulation of related genes, thus indicating that electrospun biodegradable PCL/PLA/HA has remarkable prospects as scaffolds for bone and tooth tissue engineering.
Conclusion: The attachment, proliferation, and differentiation behaviors of the osteoblast-like cells on the PCL/PLA and PCL/PLA/HA nanofibrous mats were fully investigated to assess their potential application as scaffolds for bone/dentin tissue engineering. HA-coated nanofiber scaffolds clearly displayed a positive effect on the differentiation of DPSCs into osteogenic/odontogenic cells and moderate effect on cell proliferation.
Keywords: Dental pulp stem cells; Differentiation; Nanofibers; Odontogenic; Regenerative medicine; Scaffold; Tissue engineering

PS-023. Fibrin Scaffold as Substrate for Culture of Bone Marrow Mesenchymal Stem Cells

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Background and Aim: Mesenchymal Stem Cells (MSCs) are known as appropriate cells in regenerative medicine. Applications of MSCs in clinical practice and regenerative medicine need to keep MSCs as pluripotency stem cells. In this study, the effect of fibrin scaffold on stemness gene expression of MSCs was examined.
Methods: Bone marrow-derived MSCs were cultured in tissue culture plates (2D) and 3-dimensional (3D) fibrin scaffolds. The effect of fibrin scaffold on the proliferation of MSCs was evaluated by MTT assay. Stemness state was evaluated by qRT-PCR for OCT4, SOX2 genes, and flow cytometry for Nanog protein.
Results: Cultured MSCs on fibrin scaffold were able to proliferate according to data obtained by MTT assay. Gene expression of OCT4 and SOX2 had a significant increase in cells were cultured in the 3D condition in compared to 2D condition ($P<0.05$). Also, the higher expression of Nanog protein in 3D culture was observed ($P<0.05$).
Conclusion: Thorough evaluation of important pluripotency regulators such as OCT4, SOX2, and Nanog; we demonstrated that fibrin scaffold

could maintain MSCs in a stemness state.
Keywords: Mesenchymal stem cells; Stemness; Fibrin scaffold; OCT4; SOX2; Nanog

PS-024. Preparation, Characterization, and Evaluation of Biological Properties of Silk Fibroin-Laponite Fibrous Membranes for Guided Bone Regeneration Applications

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Background and Aim: Loss alveolar bone is a common phenomenon after extraction of teeth. One of the most effective methods to regenerate damaged tissue is guided bone regeneration (GBR) application. GBR membrane allows spaces maintained by a barrier in order to regenerate new bone tissue. Due to the functions of the guided bone regeneration, these membranes should have physical and mechanical properties similar to the extracellular matrix (ECM) of natural bone and ability of osseointegration. The aim of this study was to develop nanocomposite fibrous membranes of silk-laponite using electrospinning technique.
Methods: Silk is one of the natural polymers which have many applications for tissue engineering. Some features like appropriate mechanical strength and toughness have made this polymer a good choice for this application. However, the inability to osseointegration leads to the development of nanocomposite membranes, based on silk. Laponite with chemical composition $[(Mg, Li)_3Si_4O_{10}(OH)_2Na_3]$ is one of the nanoclay which recently has been greatly considered for tissue engineering and biomedical applications. One of the best ways to manufacture GBR membrane with interconnected porous and fibrous properties is electrospinning. Electrospinning by applying electrical power can produce micrometers size to nanometers fibers from composite solution. It could mimic the architectural of bone ECM and improve the mechanical properties compared to previous fibrous membranes.
Results: Results showed that manufactured fibrous membrane of silk fibroin-laponite through electrospinning has uniforms distribution and suitable size of fibers. Silk fibroin-5 wt% laponite revealed the best strength (1.63±0.61 MPa) and its strength decreased when laponite content of nanocomposite increased. Moreover, immersion in SBF solution for 21 days demonstrated that increasing the laponite content resulted in the improved bioactivity of nanocomposite membrane.
Conclusion: In the present study, novel nanofibrous composite membranes of silk fibroin and laponite nanopowder were successfully developed through electrospinning technique. Addition of laponite nanopowder increased the bioactivity ability and mechanical properties of pure silk fibroin fibrous membranes. Results showed that fibrous membranes consisting of 5 wt% laponite nanopowder exhibited the best morphology of fibers and significantly increased the mechanical and bioactivity properties.
Keywords: Guided Bone Regeneration; Fibrous Scaffold; Nanocomposite membrane; Electrospinning

PS-025. Using Plant-Derived Small Molecules for Safer Differentiation of Mesenchymal Stem Cells Into the Mesodermal and Cardiac Cells

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Background and Aim: Cardiovascular diseases and subsequent heart failures are the leading causes of mortality and morbidity in adults worldwide. Considering the Increasing number of heart problems versus deficiency of donors, mesenchymal stem cells has recently introduced as a new tool for cell therapies in cardiology practices. However, most of the approaches still suffer from low efficiency, high cost and the lack of safety due to using growth factors and animal-derived materials. Therefore, using plant-derived chemical compounds to control mesenchymal cell fate and differentiating them into desired lineages would tackle these concerns. Here, we aimed to develop our strategy to differentiate adipose-derived mesenchymal stem cells into definitive mesoderm and finally cardiomyocytes using plant deprived small molecules such as Rutin, resveratrol and quercetin.
Methods: Extraction of stem cells from the abdominal adipose tissue performed by collagenase enzymatic method. Following characterization using flow cytometry, cells cultured in 24 well plates and treated with a various combination of growth factors such as Activin A, bFgF, AlK5 inhibitor, rutin, resveratrol and quercetin. Moreover, crude extract of *Salix aegyptiaca* also assessed in comparison with pure synthetic small molecules.
Results: The current study showed that the addition of some small molecules could increase the efficiency of maintenance, propagation as well as directing differentiation of the mesenchymal stem cell into mesodermal cells followed by cardiac cells.
Conclusion: The finding showed pharmacological activation of the WNT signaling pathway and inhibition of the BMP signaling pathway using small molecules have a crucial role in the differentiation of mesodermal lineage. The data also showed that natural compounds or a crude extract of Salix have the same effect as synthetic chemicals. Our differentiation strategy may help to overcome the current limitations to mesenchymal stem cell therapy that are faced in the field of cardiovascular disease.
Keywords: Regenerative medicine; Mesenchymal stem cells; Small molecules; Differentiation; Definitive mesoderm; Cardiomyocyte

PS-026. ANRIL Gene Polymorphism Association with Acute Myeloid Leukemia (AML) in Iranian Population

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Background and Aim: Recently, in an effort to fully illustrate the underlying genetic causes of the acute myeloid leukemia (AML), attention has been devoted to the newest aspect of gene expression regulations which inferred to the regulatory lncRNAs. ANRIL is one of the disease-associated lncRNAs which is transcribed from the pivotal genomic region that has an important role in the expression regulation of its neighbor genes CDKN2A and CDKN2B encoding 3 main tumor suppressor genes p14ARF, p15INK4b and p16INK4a.
Methods: Since the identified variants in the CDKN2A and CDKN2B genes or ANRIL locus are indicated to be associated with pathogenicity in different diseases, we investigate ANRIL (rs10757278) single nucleotide polymorphism in Iranian AML patients in comparison to control individuals.
Results: The results showed significant association neither for allelic and genotypic frequencies nor for haplotype blocks with AML patients against healthy control.
Conclusion: Considering the indicated roles of ANRIL in epigenetic gene expression regulation, seeking its AML-associated genetic defects or its abnormal expression in patients is still a growing area of research and further investigations may illustrate its potential to serve as a diagnostic biomarker for AML.
Keywords: ANRIL; lncRNA; AML

PS-027. Gene Expression Analysis of TNF and HNRNPL-Related Immunoregulatory Long Non-coding RNA (THRIL) in Iranian Patients with Acute Myeloid Leukemia

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Background and Aim: Recently, lncRNAs have been noticed as regulatory factors for several biological mechanisms through modulation of gene expression. Among them, the TNF and HNRNPL related immunoregulatory (THRIL) lncRNA may be implicated in the pathogenesis of immune-related and inflammatory disease through regulating the expression of TNF-α.
Methods: In this study, we assessed the expression of THRIL in the blood of 25 acute myeloid leukemia (AML) patients in comparison to healthy controls using real-time quantitative reverse transcription-PCR.
Results: Results showed that there was no significant difference in the expression level of THRIL lncRNA between AML patients and control subjects. Additionally, we did not find a significant association between male subgroup and THRIL expression as well as females.
Conclusion: In conclusion, our study demonstrated that THRIL cannot be used as a reliable biomarker for AML diagnosis, although, the study should be confirmed by other studies.
Keywords: Acute myeloid leukemia, Expression analysis, Long non-coding RNA

PS-028. Mesenchymal Stromal Cell Labeling by NH2- Super Paramagnetic Iron Oxide Nanoparticles

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Background and Aim: Bone marrow mesenchymal stromal cells (MSC) are multipotent cells and their ability to circulate and homing in different tissue types as well as the lack of HLAII makes them a hot topic in cell therapies and regenerative medicine. However, it is necessary to make an optimized in vivo MSC tracking method. Superparamagnetic iron oxides (SPIOs) are a good candidate for a non-invasive efficient labeling method. The aim of this study is the development and evaluation of an NH2- dextran-coated SPIO (NSPIOs) and optimization an efficient protocol to label human MSCs (hMSCs).
Methods: NH2-Dextran SPIOs were synthesized; Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), dynamic light scattering and Zeta potential were used to evaluate the properties of the dedicated nanoparticle. Then, a new protocol optimized by using heparin and protamine sulfate. The efficiency of iron content per hMSCs and viability of treated hMSCs compared to non-treated hMSCs were measured by inductively coupled plasma (ICP) and MTT assay respectively.
Results: Synthetized NSPIOs size constantly ranges around 70-90 nm based on AFM, TEM and dynamic light scattering which increased to 680 nm after heparin- protamine sulfate coating. After NSPIO-heparin-protamine sulfate treatment of hMSCs, 17± 0.39 pg per cell iron content was measured by ICP in compare to non-treated hMS cells and MTT assay did not show any effect of cells after NSPIO-heparin-protamine sulfate treatment.
Conclusion: Our results show an efficient established protocol for tracking hMSCs. This tool can be used in tracing cells after transplantation
Keywords: Human bone marrow cells; SPIO cell tracking

PS-029. Isolation of MSC-Derived Exosome for Encapsulation of Doxorubicin

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Background and Aim: Exosomes as natural nanoparticles are extracellular membrane-derived vesicles with a 30-120 nm in diameter. Exosomes can be derived from any different cells type. Among these different cells type, MSCs have several features such as the large ex vivo expansion capacity that make them perfect candidates as a manufacturer of exosomes for drug delivery. In this regard, it was demonstrated that MSC-derived exosome can be loaded with anticancer drugs in order to increase their therapeutic index.
Methods: In the current study, mouse MSCs was isolated from the adult mice bone marrow tissue. MSCs was cultured in DMEM supplemented with 10% FBS and penicillin/streptomycin. MSCs were used at passage 3-4 for exosome preparation. The proliferated MSCs (with 7% confluency) were cultured in DMEM/F12 supplemented with 10% exosome-depleted FBS and 1% penicillin-streptomycin for 72 h. Then, the exosomes from exosome-depleted culture medium were isolated using ExoQuick-TC kit. The exosome pellets were re-suspended in phosphate buffered saline (PBS) and quantification of exosome was performed through Bradford method. For drug loading, DOX-exosome mixture was sonicated using a Model 505 Sonic Dismembrator with .25″ tip with the following settings: 20% amplitude, 6 cycles of 30 s on/off for three minutes with a 2-minute cooling period between each cycle. After sonication, the exo-DOX solution was incubated at 37°C for 60 min. At the final stage, we used dynamic light scattering (DLS) and atomic force microscopy (AFM) for characterization of exosomes.
Results: Obtained data indicated that the MSC-derived exosomes are 70–90 nm in diameter using DLS and AFM analysis. It was demonstrated that MSC-derived exosomes were successfully purified. Exosome suspensions were kept at -20°C temperature for one year. Based on the data from the Bradford method, the Quantification of exosome was about 200 µg/mL. We applied UV-spectroscopy to determine the loading capacity of DOX. The amount of DOX-loaded into exosomes was 90% approximately. DLS studies revealed that the size of exo-DOX nanoformulations increased after drug loading and became 140 nm.
Conclusion: MSC-derived exosomes are the best choice as the nanovehicle for drug delivery by overcoming various biological barriers. In order to use in the clinic, there were several limitations. Including the efficient loading exosomes with a therapeutic agent without primary changes in the content and structure of exosomal membranes and the size of these. While the use of exosomes as vehicle therapeutic drugs is still in its early stages, the potential for targeted drug delivery has been clearly confirmed.
Keywords: Exosome; Doxorubicin; Anticancer; Mesenchymal stem cell

PS-030. Comparison of the Induction Effect of 5-Azacytidine and Trichostatin A on the Cardiac Differentiation of Human Adipose-Derived Stem Cells Encapsulated in Fibrin Scaffolds

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Background and Aim: Human adipose-derived stem cells (hADSCs) are capable of differentiating into many cells including cardiac cells. Several chemical agents and different types of scaffolds are used for cell differentiation, but the best is yet to be determined. The main purpose of this study focused on the possibility of better differentiation of hADSCs towards to the cardiac-like cells in fabricated fibrin scaffold (3D) in comparison with culture plates (2D) in the presence of 5- Azacytidine (5-Aza) and trichostatin A (TSA).

Methods: After approving the characteristics of hADSCs by flow-cytometry and differentiation of hADSCs into adipocytes, osteocytes, and chondrocytes, cells were cultured in fibrin scaffold (3D) that fabricated using human plasma fibrinogen and culture plate (2D) and treated with 10 µM of 5-Aza and TSA, then followed weekly up to 4 weeks. The morphology of the scaffold was characterized by Scanning Electron Microscopy (SEM). In 2D culture, the differentiated cells were examined for cell growth and morphological changes every day by using an inverted phase contrast microscope. Immunochemistry assays and qRT-PCR were used to evaluate the expression of special cardiac genes such as NKX2.5, Cx43, and cTnI in the treated hADSCs with 5-Aza and TSA in the 3D and 2D groups. In addition, induced cells in each of the groups were examined by Transmission Electron Microscopy (TEM).

Results: The SEM images of scaffolds showed that a uniform structure of the scaffold with a mean pore size of 113.14 ± 26 µm. The fibroblast-like morphology was the dominant form in the negative control group. Gradual morphological changes of the induced hADSCs using TSA in the 2D group were multinuclear, elongated, ball-like myotube-like structures and fork like and star-like during the 4th week. Immunochemistry assay and qRT-PCR showed the significantly higher expression of special cardiac genes such as NKX2.5, Cx43 and cTnI in the treated hADSCs with TSA in the 3D and 2D groups in comparison with 5-Aza groups ($P < 0.05$). Also, treated hADSCs with TSA, within 4 weeks, showed the hADSCs could differentiate to cardiomyocyte-like cells. The TEM images showed that hADSCs in the treatment groups using TSA all had myofilaments in the cytoplasm when compared with the 5-Aza groups.

Conclusion: Taken together, these results indicated that the TSA allows a higher and sooner differentiation of hADSCs into cardiomyocyte-like cells treated alone or with fibrin scaffold and it can lead to generating of cardiac-like cells in a shorter period of time in comparison to 5-Aza. In addition to the combination of TSA and fibrin scaffold effectively promotes the cardiomyogenic differentiation of hADSCs in vitro their application may represent a therapeutic strategy for the treatment of ischemic heart disease.

Keywords: Human ADSCs; Cardiomyocyte-like cells; Fibrin scaffold; TSA, 5-Aza

PS-031. Evaluation of the Ccapability of the Wharton's Jelly Mesenchymal Stem Cell Aggregates to Express the Markers of 3 Germ Cell Lineages

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Background and Aim: The ability of stem cells to differentiate into different cell types makes them as a key component of healing damage in regenerative medicine. As human umbilical cord Wharton's jelly (HUCWJ) are available non-invasively, do not raise any ethical issues with higher differentiation potential compared to adult stem cells and the ability to express embryonic stem cell markers, they can be considered as a good candidate in regenerative medicine applications. The objective of this study was to find if these cells form cell aggregates with the same features with that formed by embryonic stem cells (embryoid body) and could form three germ layers.

Methods: In this study, the umbilical cords were cut into small pieces, and then the explants were cultivated. Once reached the passage 3rd, about 1000, 5000 and 10.000 cells/ 20µL were cultured as hanging drops for 3 days, after which the cells were incubated for an additional 3 days in non-adhesive dishes. As the center of cell aggregates formed from 5000 and 10 000 cells was darker than those formed from 1000 cells, this study focused on the aggregates formed by 1000 cells for further assessments. The immunocytochemistry and flow cytometry were performed using 3 color antibodies to detect the markers of three germ cell lineages

Results: The data showed that the embryoid-body-like aggregates expressed a low amount of ectodermal and endodermal markers and most of the cells expressed mesodermal markers.

Conclusion: These aggregates mainly kept their mesenchymal state and showed a poor differentiation potential toward the ectoderm and endoderm

Keywords: Wharton's jelly; Embryoid-body; Cell aggregates; Brachyury; HAND1; GATA4

PS-032. Infected Carrier Cells: A New Strategy for Systemic Delivery of Oncolytic Reovirus in Cancer Cell

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Background and Aim: Cervical cancer accounts for the most common cancers in women, underlining the need for the development of new therapies. Reovirus is a naturally oncolytic virus, selectively kills tumor cells. Studies have shown limited antitumor efficacy due to insufficient viral delivery. Mesenchymal stem cells (MSCs) possess the ability to protect from circulating neutralizing antibody and specifically home into tumor sites. This property of MSCs could be exploited for the delivery of various anti-tumor agents directly into tumors. We explored the effects of reovirus loaded into MSCs on TC-1 tumor model cells.

Methods: In this experimental study, MSCs derived from adipose tissue were infected with reovirus T3D. After co-culturing of MSCs-loaded with reovirus in TC-1 cell line, we assessed the ability of these cells to deliver the virus to target cell line and the effect on cell death was measured by real-time PCR.

Results: In this study, Adipose-derived MSCs (AD-MSCs) that were infected with oncolytic reovirus, delivered the virus in co-cultured TC-1 cells. Reovirus-infected MSCs induced a higher level of apoptosis in TC-1 cells compared with the apoptosis induced by their direct infection with similar virus titer.

Conclusion: AD-MSCs-loaded with oncolytic reovirus as a carrier cell may provide a novel efficient therapeutic approach for targeting the TC-1 tumor model cell.

Keywords: Reovirus T3D; Mesenchymal stem cells (MSCs); Carrier cell, TC-1 cell; Apoptosis

PS-033. Comparison of the Efficacy of Mesenchymal Stem Cells Derived from Total Human Umbilical Cord and Wharton's Jelly Explants

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Background and Aim: An increased demand for MSCs in regenerative medicine has made scientists prioritize the development of MSC isolation methods. The umbilical cord (UC) has become one of the major sources of mesenchymal stem cells (MSCs). Several techniques have

been devised for the dissociation of tissues for a primary culture that can affect the quantity and quality of the isolated cells. Wharton's jelly is one of the most promising tissue sources for mesenchymal stem cells, but not the only source. The aim of our study was to develop one of the most appropriate methods for the isolation of mesenchymal stem cells from the entire umbilical cord.

Methods: UC was divided into 10-cm pieces. After isolation vessels, both ends of each vessel were closed, to form a loop. The loops were mildly digested by collagenase type I, Enzyme activity was neutralized. Then the Wharton jelly was further cut into tiny pieces and digested with collagenase type I for 16 h. After mild digestion, vessel loops were placed in T75 flasks containing DMEM-LG. After partial digestion, the digested Wharton jelly was centrifuged, after removing the supernatant, the tiny partially digested tissues were transferred into the same T75 flasks containing the vascular loops. The UC pieces and the vascular loops were cultured in DMEM-LG. On the other hand, to compare the methods, MSCs were isolated from Wharton's jelly by explanted method too.

Results: We could retrieve 6 to 10 million MSCs for 8 to 10 days of primary culture. Average time until desired cell confluency attained was 9.2 days for our method, 20.7 days for explant method. The average of harvested MSCs per 10-cm piece of UC was estimated to be 550,000 and 150,000 in this method and explant method respectively. Also, Inverted microscopy revealed fibroblast-like morphology after 16 h for this method and 72 h for the explant method. MSCs isolated by our method express CD73, CD90, CD105, and CD44, but not CD34 and CD45 (hematopoietic markers) and CD31. The genes SOX2, OCT4, and NANOG are expressed in isolated MSCs. The capacity of these MSCs to differentiate into adipocytes and osteocytes highlights their application in regenerative medicine.

Conclusion: This method is simple, reproducible, and cost-efficient. Moreover, this method is suitable for the production of a large number of high-quality MSCs from just one UC in less than a month, to be used for cell therapy in an 80-kg person.

Keywords: Umbilical cord (UC); Mesenchymal stem cells (MSCs); Regenerative medicine

PS-034. The Protective Effect of Crocin Against Induced Oxidative Stress on Adipose-Derived Stem Cells (ADSC)

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Background and Aim: Adipose-derived stem cell (ADSC) is broadly used in cell transplantation. It is easily obtained by a minimally invasive procedure, genetically stable in long-term culture and capable of evading the host's immune system. As survival and longevity of fat grafts along with ADSCs in various tissues remain poor post-engraftment, using practical methods such as natural anti-oxidants to enhance their survival has a crucial role to improve the efficiency of fat grafting. Therefore, we conducted this study to evaluate the protective effect of crocin against oxidative stress induced by H_2O_2 on ADSCs in vitro.

Methods: ADSCs were treated with different concentrations of crocin and H_2O_2, separately. Then the MTT assay was used to determine the IC_{50} of crocin and H_2O_2. LDH assay was carried out to evaluate the H_2O_2-induced cytotoxicity in cultured ADSCs. After treatment of these cells with specific concentrations of crocin and H_2O_2, intracellular level of ROS was measured based on the oxidation of non-fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (H2DCF-diacetate) to yield the highly fluorescent DCF. Also, cell survival was measured by the trypan blue assay and DAPI (4′,6-diamidino-2-phenylindole) staining. Then the signaling pathway of Nrf2/ HO1 in ADSCs treated with crocin and $H_2O_2$was studied by real-time PCR.

Results: We investigated ADSCs survival with and without crocin pretreatment under oxidative conditions. Crocin prevented cell death in a dose-dependent manner with the concentration of 10 μM exhibited a maximal protective effect against H_2O_2-induced cytotoxicity ($P < 0.05$ vs. H_2O_2 group). Additionally, cell viability did not change by incubation with crocin (1–10 μM) in H_2O_2-free condition. H_2O_2 markedly induced the release of LDH, while crocin pretreatment dose-dependently suppressed LDH release in ADSCs. According to trypan blue assay and DAPI staining, crocin increased the cell survival dose-dependently. It has been shown that crocin is involved in Nrf2 signaling pathway in ADSCs. This study showed crocin protects the ADSCs against oxidative stress induced by H_2O_2.

Conclusion: In this study, the pivotal role of crocin as a powerful and natural anti-oxidant against oxidative stress on Adipose-Derived Stem Cells (ADSCs) has been shown. Our findings provide a novel method for increasing the survival of ADSCs used in fat-grafting in tissues.

Keywords: Adipose-derived stem cell (ADSC); Oxidative stress; Crocin

PS-036. Induction of Mesenchymal-Epithelial Transition (MET) in HT29 Cell Line Containing Cancer Stem-Like Cell (HT29-shE) with Differentiation Therapy Purpose for Cancer Treatment

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Background and Aim: Radiation therapy and chemotherapy for cancer treatment can be highly toxic and nonspecific sometimes. Newer therapeutic approaches recommend the use of chemotherapy in combination with other treatment modalities. Sox2 and Oct4 are transcriptional master regulators which express in various human tumors, so that can give rise to tumor growth promotion through interfering with progenitor cell differentiation. In the present study, inhibition of Sox2 and Oct4 transcription factors using oligodeoxynucleotide decoy strategy lead to mesenchymal-epithelial transition (MET) in HT29-shE cell line.

Methods: E-cadherin knockdown in HT29-shE cell line lead to the enrichment of cancer stem-like cells. Sox2 and Oct4 decoy and its scramble ODNs designed and synthesized. Also, EMSA assay was used to confirm the specificity interaction of ODNs decoy with their proteins. Then, ODNs transfected into cells with Lipofectamine and its subcellular localization determined by fluorescence and confocal microscopy. Cell viability, apoptosis and downstream genes expression in HT29-shE cells were analyzed by MTT, Annexin V/ PI test, and Real-time assays, respectively.

Results: EMSA data showed that Sox2-Oct4 decoy ODNs bound specifically to their proteins in nuclear extract of HT29-shE cells. The results revealed that the designed complex decoy can concomitantly target Sox2 and Oct4, which subsequently induction of MET in HT29 -shE cells compared to controls through decreasing cell viability, arresting the cell cycle in G0/G1 phases, inducing apoptosis, and modulating differentiation process.

Conclusion: Use of Sox2-Oct4 TFD strategy in HT29-shE cells can lead inhibiting cell growth and triggering differentiation. Hence, this strategy can be used in combination with conventional treatment modalities as a promising tool in cancer therapy.

Keywords: Cancer stem-like cell; Oligodeoxynucleotide decoy; Oct4 and Sox2 transcription factors; Antitumor effects; Differentiation therapy

PS-037. Inhibition of GATA3 Expression Upon miR-29b1 Overexpression in CD4+ Cells

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Background and Aim: MicroRNAs (miRNAs) are about 18-24

endogenous nucleotides non-coding RNAs. These short RNAs regulate gene expression by binding to 3'-untranslated region of mRNA that led to mRNA degradation or translation inhibition. Transcription factors are one of the most important genes that their expression is controlled by miRNAs. Several studies showed that GATA3 is the master regulator of Th2 differentiation. Th2 have a key role in allergy and hypersensitivity. The aim of this study was to determine whether miR-29b overexpression could modulate the expression of the GATA3 transcription factor in CD4+ cells.

Methods: Spleens were harvested from 6-8 weeks old C57Bl/6 female mice and single cells suspension was prepared. CD4+ cells were then purified by negative selection on MACS separator (Miltenyi Biotec). The purity of cells was confirmed by flow cytometry. Then, CD4+ cells were cultured at a density of 106 cells/well in 24-well culture plates and stimulated with anti-CD3/CD28 mAbs. After 4 hours, the cells were transfected with miR-29b1 mimic using Lipofectamine 2000. 48 hours after transfection, total RNA was extracted and Quantitative real-time PCR was performed. Expression of mature miR-29b1 and GATA3 were normalized to U47 and β-actin, respectively, as the endogenous reference gene, and relative expression was calculated using the 2-ΔΔct method.

Results: To investigate the effect of miR-29b1 overexpression on GATA3 expression, miR-29b1 mimic was transfected into the purified mouse CD4+ cells. RNA extraction and Quantitative real-time PCR revealed that miR-29b1 mimic was successfully transferred to CD4+ cells. Furthermore, GATA3 expression was significantly decreased in miR-29b1 transfected cells compared to control cells.

Conclusion: Here, we showed that overexpression of miR-29b1 decreased GATA3 expression in CD4+ cells. Since this transcription factor has a key function in the differentiation of T naive to T cell subsets, miR-29b1 overexpression could inhibit Th2 differentiation in allergic diseases.

Keywords: miRNA; Lymphocytes; Th2; GATA3; Differentiation

PS-038. Comparison of 2 Methods of Cell Culture on the Structure and Differentiation of Quail Bone Marrow Stem Cells

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Background and Aim: Investigating, isolating and cultivating stem cells and studying animal models is one of the strategies for accurately understanding the functioning of these cells in the context of stem cell research. This study was conducted to determine the effect of isolation ways and cell culture on differentiation and morphology of stem cells in mesenchymal cells of bone marrow in quails.

Methods: For this purpose, 8 Japanese quails (free of the pathogen) were raised. 1 mL of bone marrow at the age of six months was obtained from a large bone marrow area and the fluid was transferred to DMEM medium. Two groups were used for this study first group as direct with RBC and the second group RBC defected by Ficoll loading method. The culture medium contained 10% bovine embryo serum with penicillin and streptomycin antibiotics. Fourth passage cells were studied structurally and differentiated into fat cells, bone marrow cells, and cartilage cells. The phenotypic measurement criterion in this study is the effect of adipose, osteogenesis, and chondrogenesis of stem cell populations.

Results: Based on the results, cloning in direct culture medium was created more than the loading medium on the Ficoll. In a direct culture medium, more cells received the ability to differentiate into cartilage, bone and fat cells. In a direct culture, most of the cells had fibroblastic morphology.

Conclusion: In conclusion, direct culture media had better performance for bone marrow mesenchymal cells.

Keywords: Quail; Bone marrow stem cell; Mesenchymal cells; DMEM

PS-039. Increased Level of Fetal Hemoglobin in Umbilical Cord Blood CD34+ Hematopoietic Stem Cell by New Drug GSK-LSD1 for Treatment of Thalassemia

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Background and Aim: Switch from fetal γ-globin to β-globin gene expression occurs at birth. The genetic regulation of this switch has been studied for decades, and the molecular basis for γ-globin silencing and change in molecular mechanisms in the expressed genes have been elucidated. Elevated γ-globin synthesis can significantly alleviate the symptoms of β-globin disorders. Several adult-stage γ-globin repressors, such as BCL11A, Ikaros, GATA1, and SOX6 have been identified that interact with each other to repress the γ globin genes. In addition, Lysine-specific demethylase-1 (LSD1) was recently shown to associate with co-repressors, including DNA methyltransferase I (DNMT1) and TR2 and TR4, as components of a core heterotetrameric complex. An activating epigenetic signature. Recent data suggest that CD34+ is involved in the maintenance of the progenitor cells in a phenotypically undifferentiated state. Primary human CD34+ cells isolated from umbilical cord blood mononuclear cells (MNCs) include hematopoietic stem and progenitor cells. Recently, it was shown that lysine-specific demethylase-1 (LSD1) that remove monomethyl and dimethyl residues from the lysine 4 can repress γ- globin gene expression. In this report, we investigated the inhibition of LSD1 by the GSK-LSD1 inhibitor in human erythroid CD34+ cells to increase γ-globin gene expression.

Methods: We grew and differentiated the cells ex vivo into the erythroid lineage in 14 d by a two-phase culture method described previously. We examined the effects of the GSK-LSD1 inhibitor on CD34+ cells are isolated from cord blood using positive immunomagnetic separation techniques, cells ex vivo. Cell number and viability were determined by trypan blue staining. Cell morphology was examined by Wright-Giemsa staining (Sigma-Aldrich). Flow-cytometric analysis showed that HbF was induced in all of the cells in a dose-dependent manner. We treated the cells with 0, 0.5, 1.5, and 5 μM of the GSK-LSD1 inhibitor on days 4 to 14 of the differentiation culture. Then we performed an analysis of the expression of LSD1 and γ-globin genes comparable levels throughout differentiation with real-time PCR using the IQ SYBR Green Master mix.

Results: After treatment GSK-LSD1 inhibitor at 0.5, 1.5, and 5 μM did not alter cell proliferation or viability, but 5 μM GSK-LSD1 reduced cell proliferation and delayed differentiation without affecting cell viability. In 1.5-μM concentration of the GSK-LSD1 inhibitor, the mean of γ-globin mRNA expression was induced up to 33-fold. We observed a decrease in the LSD1 mRNA expression in a 5-μM concentration of the GSK-LSD1 inhibitor.

Conclusion: Our results indicated that LSD1 played an important role in γ-globin silencing in adult erythroid cells. Further, the GSK- LSD1 inhibitor increase concentration of HbF induction within the therapeutic plasma concentration. Finally, LSD1 is thus a promising therapeutic target for γ-globin induction, and GSK-LSD1 inhibitor leads compound for the development of a new γ-globin inducer.

Keywords: Umbilical Cord Blood; CD34+ cell; GSK-LSD1; γ- globin; Lysine-specific demethylase-1

PS-040. Analysis of a Mutant form of Dystrophin

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Background and Aim: Duchenne muscular dystrophy (DMD) is severe X-linked neuromuscular disease, caused by a mutation in *dmd* gene. DMD has a prevalence of 1/5000 boy. Affected boys have muscle weakness and progressive movement disorder. Finally, death occurs at the age of 30. *dmd* gene is the largest known gene in human, many different types of mutation described in this gene such as large and small deletion and duplications, point mutation and small rearrangement. The aim of this study is an analysis of different type of mutations in *dmd* gene according to the http://edystrophin.org data.

Methods: According to the database, we collect 30 common mutations that described in *dmd* gene and analyze the morphology of dystrophin after mutation in this region.

Results: Morphology of all 30 dystrophin mutants analyzed. Based on our data, we found a mutation in critical regions of dystrophin such as N and C terminal. Although it may be repaired by a novel technique like exon skipping or CRISPR/Cas9, the repaired dystrophin is not functional. In hot spot region of dystrophin, exon 45-55, which has repetitive morphology, is the best sit for repair to produce truncated functional dystrophin.

Conclusion: Many different types of mutation described in this gene such as large and small deletion and duplications, point mutation and small rearrangement. The selection of the best sit for repair to produce truncated functional dystrophin is so important. Before *dmd* gene repair, the mutated region should be analyzed in database and bioinformatics software.

Keywords: Duchenne muscular dystrophy; Dystrophin mutants; DMD analysis

PS-041. Gene Editing by CRISPR-Cas9 System

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Background and Aim: CRISPR-Cas9 is an acquired immune system in prokaryotes that help them for resistance against foreign genetic element such as phages. Today researchers are using CRISPR-Cas9 for in vivo and in vitro gene editing. Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by progressive muscle degeneration and weakness. It is one of nine types of muscular dystrophy. DMD is caused by an absence of dystrophin. The aim of our study was in vitro *dmd* gene editing

Methods: HEK-293 cell was cultured in DMED high glucose supplemented with 10% FBS (Fetal bovine serum), 500 µL Pen/Strep. gRNAs were designed to target Exons 48 to 53. This gRNAs were inserted in a vector that carrying Cas9 and GFP. After cloning, this vector transected to the cells by lipofectamine 2000. According to the gRNAs that we designed, we expected a500 bp band after deletion in dmd gene.

Results: Cells that were transfected with the vector were isolated. After DNA extraction and PCR, we demonstrated 500 bp band in edited cells. To confirmation of edition, Sanger sequencing was done.

Conclusion: In conclusion, CRISPR-Cas9 can be used as a powerful tool for gene editing and helpful for a genetic disorder and cancer treatment.

Keywords: Gene editing; dmd; CRISPR/Cas9

PS-042. Evaluation of Electrospun Polycaprolactone/Gelatin/Polydimethylsiloxane Nanofiber Scaffold for Endometrial Cell Growth and Proliferation as Part of Uterine Tissue Engineering

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Background and Aim: In this study a PCL- G- PDMS copolymer was further engineered into porous fiber scaffolds by electrospinning. The biocompatibility was evaluated, using fibroblasts and endometrial cells and assessing cell metabolic activity by MTT assay. the aim of this study was to develop and evaluate electrospun nano-fibrous scaffolds to support the growth of endometrial cells. The aim of this project is to solve the problems similar to hysterectomized uterus, rennet uterus (surrogacy), and the lack or defective womb using an engineered uterine tissue.

Methods: PCL- G- PDMS segments with different ratios were synthesized. Cell interaction and growth of endometrial cells and endometrial cell viability were investigated by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assay on PCL- G- PDMS fibers. With an informed written consent, endometrial cells were extracted from normal human uterine tissue samples. The surface markers (CD90+, CD105+, CD146+, and CD45) were distinguished them from mesenchymal stem cells (MSCs). After sterilization under UV light for 20 minutes, the electrospun fibers were placed as a disk at the bottom of every well in a 24-well cell culture plate and were seeded with endometrial cells at a density of 10,000 cells per well. Cells were harvested at days 1, 3, and 7 for growth and phenotypical evaluation and MTT test. By triplicate assay, the activity and survival of the cells on nanofibers were calculated as a percentage of samples to controls.

Results: The electrospinning technique has been used to tailor microstructures including fiber diameter and pore size of nano-fibrous scaffolds for tissue engineering applications. Electrospinning allows for the generation of fibers with nanoscale diameters. Small fiber diameter combined with the high specific surface area, together with porous structures of nano-fibrous scaffolds, are essential for tissue engineering applications. There are several parameters that can be manipulated to tailor fiber diameter and pore size. These factors affect cell adhesion on the scaffold. So the random, aligned scaffolds were developed by the electrospinning technique, and their morphological microstructures were investigated by SEM. SEM micrographs of the scaffolds were obtained before seeding of the cells. The endometrial cells on all six types of scaffolds were found to attach and spread well. There is a difference in cell growth due to differences in fiber diameter and porosity per percent. Also, a significant increase in cell growth and replication on the scaffold compared to control samples indicates the lack of toxicity of the wizard for the scaffold adaptation.

Conclusion: In this study, we synthesized a series of novel biodegradable scaffolds comprising different ratios of PCL- G- PDMS. These PCL- G- PDMS copolymers were further engineered via electrospinning. The PCL- G- PDMS fibers showed decent mechanical properties and hold great potential for various biomedical applications. The advanced mechanical properties and biocompatibility of these PCL- G- PDMS fibers would allow them to become promising candidates for tissue engineering. Evaluation of endometrial cells on this scaffold could be the first step for uterine tissue engineering. We planned to evaluate a fertilized egg's reaction to this novel scaffold.

Keywords: Endometrium; Nano-fiber; Polycaprolactone; Gelatin; Polydimethylsiloxane; Tissue engineering

PS-043. 9-tBAP From Spiroaminopyrimidones Family Decreases cell Proliferation and Down-Regulation of Survivin Concomitant with Induction of Apoptosis in NB4 Leukemia Cells

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Background and Aim: It has been recently reported the activity of aminopyrimidone family to induce apoptosis in human cancer cells. In this study, we reported an active compound from spiroaminopyrimidone family with apoptotic activity against NB4 acute promyelocytic leukemia cells.

Methods: The cells were seeded in 96-well plates at 1×10^5 cells/well and treated with 10-150 µM of the 2, 4-Diamino-1, 3-diazaspiro [5.5]-9-tert-butyl-2, 4-diene- 5- carbonitrile (9-tBAP). This compound was found to be highly active cell growth inhibitor with IC50 of 30±3.5 µM as determined by MTT assay. Evaluation of survivin expression in NB4 cells treated with 9-tBAP was performed by real-time PCR.

Results: 9-tBAP decreases cell proliferation of the NB4 cells in a dose- and time-dependent manner. The IC50 value following 72 h exposure was found to be 30 µM for the cells. Furthermore, real-time PCR analysis revealed that the treatment with the compound down-regulated the expression of survivin in a time-dependent manner.

Conclusion: These data further suggest that 9-tBAP may provide a novel therapeutic approach for the treatment of leukemia.

Keywords: Survivin; 9-tBAP; NB4 cells; Apoptosis

PS-044. Development of an Alginate-Based Microcarrier for the Expansion of Anchorage Dependent Cells

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Background and Aim: Clinical implementation of regenerative medicine in the treatment of various diseases necessitates the development of the cell culture methods which provide sufficient cell number of the specified tissues. Microcarrier-Based cell culture overcomes the limitations of the conventional 2D cultivation methods by supplying high surface to volume ratio. Alginate hydrogels are great of an interest in tissue engineering due to their advantages such as biocompatibility, none toxicity and mimicking several extracellular matrix characteristics. We hypothesized that treatment of Ca-alginate hydrogels with either an ECM protein or a polycation would promote cell attachment.

Methods: The calcium-alginate microbeads were prepared by dripping sodium alginate solution into an aqueous solution of calcium chloride under high voltage electrostatic field. Gelatin and chitosan solution of different concentrations were applied for the surface treatment of the hydrogels. Chitosan was coated on the hydrogel surfaces by two different methods. In the first method, the hydrogels were immersed into the chitosan solution under gentle agitation, while in the second strategy alginate solution directly dropped into the chitosan solution containing calcium chloride. Gelatin coated alginate microbeads were prepared via incubation of hydrogels in gelatin solution under continuous agitation at 37˚C. To promote covalent crosslinking of gelatin, the beads were further crosslinked by glutaraldehyde. The mouse preosteoblast (MC3T3-E1) cell line was cultured on the modified microbeads in stationary as the model system and its proliferation was assessed by MTT method.

Results: MC3T3-E1 cells attached and expanded rapidly on the gelatin-coated hydrogels, whereas no significant cell growth was observed on the chitosan coated ones at all tested concentrations. Glutaraldehyde crosslinking significantly improved the stabilization of the covalent gelatin as the cells could not attach and proliferate on the beads coated with gelatin solely. Among examined gelatin concentrations (1, 1.25 & 1.5% (w/v)), treatment of the microbeads with gelatin solution of 1.25% had the best results of the cell proliferation after 48 h.

Conclusion: Our results demonstrated that gelatin coated Ca-alginate hydrogels can be applied as biocompatible microcarriers in tissue engineering applications. Moreover, it was revealed that chitosan would not be an appropriate coating for microcarriers to promote cell attachment and proliferation.

Keywords: Microcarrier; Ca-alginate; Tissue engineering; Gelatin; Chitosan

PS-045. A Combination of Gelatin Addition as the Sacrificial Fiber and Ultrasonication, to Increase the Pore Size of PCL-nHA Electrospun Scaffolds

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Background and Aim: Electrospinning is one of the techniques in preparation of the scaffolds for bone tissue engineering. However, due to the small pore size of electrospun scaffolds, cellular infiltration and tissue ingrowth into the scaffold are not possible. The use of sacrificial fibers (such as polyethylene oxide (PEO)) in a combination with ultrasonication has been shown as an appropriate way to increase the pore size of the electrospun scaffolds in our previous work. But, due to the high cost of PEO, it is not economical.

Methods: In this study, gelatin (a water-soluble polymer) was chosen as a novel sacrificial agent and co-electrospun with polycaprolactone-nanohydroxyapatite (PCL-nHA). Gelatin and PCL-nHA were co-electrospun on a rotating collector from two separated spinners. After electrospinning, gelatin was washed with water for three days and then, the prepared scaffold was ultrasonicated. In order to find the optimized ratio of PCL-nHA and gelatin, the samples were weighed before and after the washing and the amount of lost weight was calculated. Morphology and structural properties of the prepared scaffolds were studied by SEM. The mean pore size and fiber diameter were measured by ImageJ software. Fourier transform infrared (FTIR) spectroscopy and water contact angle tests were used to evaluate the removal of gelatin.

Results: According to the SEM results, the pore size of the scaffolds, after gelatin removal and ultrasonication, was increased 3-folds compared to the control sample (PCL-nHA without the addition of gelatin as the sacrificial agent and ultrasonication), at PCL-nHA: gelatin ratio of 70:30. Our results showed that an increase in the pore size, caused by the removal of gelatin, was approximately equal to that of PEO, in our previous work. FTIR results indicated incomplete gelatin removal. According to the contact angle results, due to incomplete gelatin removal, the samples were almost hydrophilic, although, after the removal of gelatin, the hydrophilicity was less. Also, the contact angle after plasma treatment showed that in both of the cases, they were hydrophilic.

Conclusion: Our finding suggested that using gelatin as the sacrificial agent, is more appropriate for increasing the pore size of electrospun scaffolds.

Keywords: PCL; Gelatin, Pore size; Co-electrospinning; Ultrasonication

PS-046. On-Chip Synthesis of Chitosan-Alendronate Nanoparticles for Bone Tissue Engineering

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Background and Aim: In this study, chitosan (CHI)-alendronate nanoparticles were produced by microfluidic technology and ion gelation techniques. In microfluidic devices, there is a powerful control over the production of uniformly sized nanoparticles due to the laminar flow. The innovation of this project is in the removal of additional crosslinkers. Eliminating additional factors creates many benefits in introducing the drug to the body.

Methods: In this study, we developed two T-shape microfluidic devices to create a hydrodynamically focused flow, one device has two inlets and one outlet. The central inlet for CHI and lateral flow for AS (Chip1). Another device consists of three inlets, one for AS at basic pH (pH>10), one for CHI, another for acidic water (pH<7) and one outlet for the fabricated nanoparticles (Chip2). Microfluidic devices were fabricated with poly(dimethylsiloxane) (PDMS) using a standard micro molding process. The mixing channel was 150μm wide, 40 μm high and 3 cm long. CHI has the ability of gelation; spontaneously, in contact with multivalent polyanions due to the formation of inter- and intramolecular cross-linkage mediated by the polyanions. We have described the ionic crosslinking of CHI-drug using AS molecules in microfluidic chip for the first time.

Results: From DLS measurements, Chip2 configuration enabled the production of nanoparticles with 140 ± 15 nm of particle size, 0.10 ± 0.02 of PDI. In this study, two types of microfluidic chips were designed. In chip1, the ratio of lateral to central flow was 1-30 and various pH of AS was tested. In which all of the cases the gelation phenomenon occurred. One reason for that is that fast encountering of CHI and AS. So, the chip 2 was designed. In this chip, first, AS molecules penetrate into the water and after that to CHI, and then the nanoparticles are formed. In this case, the mixing time becomes smaller. Different flow ratio has been tested, under critical flow ratio with the gelation phenomenon. After particle synthesis, samples were tested with DLC measurement.

Conclusion: In this study, we developed a new method for the development of a continuous and homogeneous production CHI-AS nanoparticles. We have demonstrated the possibility of using a central water stream in order to slow down molecules' diffusion, favoring the synthesis of CHI/AS nanoparticles in a simple and reproducible microfluidic method.

Keywords: Alendronate; Microfluidics; Ionic gelation; Bone tissue engineering

PS-047. Effect of Gelatin Modification on Enzymatically-Gellable Pectin-Gelatin Hydrogel Scaffold for Soft Tissue Engineering Application

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Background and Aim: In recent year, modification of natural and available materials is one of the solutions for the production of biodegradable hydrogel scaffolds

Methods: In this study, therefore, the modifications of pectin and gelatin were accomplished through cross-linking with EDC and NHS, and the phenolic group was placed on the carboxyl –COOH groups of the materials for hydrogenation in the presence of the horseradish peroxidase enzyme and hydrogen peroxide. Pectin modified (2%)-gelatin (1%) (P.ph-G) and pectin modified (2%)-gelatin modified (1%) (P.ph-G.ph) were prepared as two types of hydrogel scaffolds by peroxidase enzyme and their properties were investigated

Results: The P.ph-G.ph scaffold was mechanically more resistant than P.ph-G as the P.ph-G scaffold was broken by 54.7 kPa (73% strain) while P.ph-G.ph at 155 kPa remained intact. More resistance means less degrades, as the degradation rate of P.ph-G and P.ph-G.ph hydrogels were observed at 50.6% and 25.3%, respectively. By considering SEM images, P.ph-G pore sizes were two times more than P.ph-G. As the larger pores can absorb more water, the swelling ratios of P.ph-G and P.ph-G.ph were obtained 41.4% and 10.6%, respectively. Gelation time showed a faster gelation of the P.ph-G compared to P.ph-G.ph hydrogels. The results of cell test after 14 days using chondrocyte cells showed that the cells proliferated 2.29-fold in the P.ph-G hydrogels and 8.81-fold in the P.ph-G.ph hydrogels, respectively.

Conclusion: The results of this study demonstrate that P.ph-G.ph hydrogel has a valuable potential for use in the fabrication of soft tissues, especially cartilage.

Keywords: Enzymatically-gellable hydrogels; Pectin, Gelatin; Soft tissue engineering

PS-048. Biophysical Manipulation of Olfactory Ensheathing Cells by Static Magnetic Field

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Background and Aim: Olfactory ensheathing cells (OEC) have been successfully used in transplantation experiments, however, their biophysical properties have remained unknown. Their motility and migratory properties root into their cytoskeleton activities that lead to filopodia formation, elongation, and orientation and so on, playing important role in regenerative medicine. In this study, OEC were isolated from mouse olfactory bulbs and exposed to 10mT SMF. The motility characteristics (velocity and directness) in the presence and absence of SMF were analyzed to address possible application of the approach in the treatment of spinal cord injury (SCI).

Methods: The olfactory bulbs were isolated and digested using collagenase type I and trypsin 0.1%. The sediment was cultured in DMEM: F12 15% FBS and 2 mM L-Glutamine for 18 hours, followed by 48-hour culture in the replaced supernatant, and eventual culture in new poly L-lysine treated plates for 96 hours. Characterization of cells and induction means of sphere formation was carried out in the presence of BFGF and EGF but the absence of FBS, and analyzed by immunocytochemistry (ICC) technique using P75 and GFAP antibodies. Then, the positive cells were cultured and their migration was studied by time-lapse experiments recording their activities and morphology at 5-minute intervals in the presence and absence of 10mT SMF for 48 hours. The characteristics of the membrane and cytosolic organelles recorded in high-resolution photos were assessed by ImageJ and Gradientech software and the biophysical effects of SMF were investigated at cellular and molecular levels.

Results: About 95% of cells attached to the poly L-lysine coated plates were found to be OEC which used for further characterizations. The ICC positive cells were used for scratch assay test and their migratory properties were studied. The velocity of the cells in the presence and absence of the 10 mT SMF was about 10.48 μm/hour and 6.97 μm/h, respectively. The directness of the cell in the presence and absence of the 10 mT SMF with respect to the N pole was 0.76 and 0.65, respectively. The exposure of the OEC to SMF increased the rate of velocity and orientated the directness of the cells towards the N pole of the magnet in comparison to control group which showed less velocity and a random directness. Furthermore, the microtubule arrangement in the presence of SMF showed a long stretch in comparison to control group.

Conclusion: Along with all the efforts of science, the lack of a biophysical perspective for stem cell therapy is palpable. The promising results obtained here prove the potential effect of the magnetic field and represent it as a biophysical modality to manipulate stem cells with possible application towards the treatment of spinal cord injury and application in the regenerative medicine in the near future. This is why further studies should be conducted to investigate the matter at various intensities of SMF in order to obtain comprehensive information to fabricate an optimum non-invasive treatment regime.

Keywords: Static Magnetic Field; Olfactory ensheathing cells; Biophysical treatment

PS-049. Silk Nanofibrous Electrospun Scaffold Enhances Differentiation of Induced Pluripotent Stem Cells Into Insulin-Producing Cells

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Background and Aim: The scaffolds accompanied with stem cells have great potential for applications in pancreatic tissue engineering. Fabrication of nanofibrous scaffold similar to extracellular matrix is one of the applicable methods in pancreatic tissue regeneration. The aim of this study was the fabrication of a silk nanofibrous scaffold as a microenvironment for pancreatic guiding differentiation of induced pluripotent stem cells (iPSCs) toward beta-like cells.
Methods: IPSCs were seeded on silk scaffolds and differentiated to insulin-producing cells (IPC) using pancreatic medium supplemented with growth factors for 21 days. The generation of IPC was confirmed via the evaluation of specific pancreatic markers; insulin and pdx1 using immunocytochemistry and real-time PCR.
Results: Our results in the MTT assay showed that silk scaffold supports the attachment and proliferation of iPSCs. The expression of pancreatic markers was higher in cells grown on silk scaffold in compare to monolayer group.
Conclusion: This study suggests electrospun silk nanofibrous scaffold as an appropriate substrate for pancreatic induction of stem cells that is applicable for islet transplantation.
Keywords: Induced pluripotent stem cells, Silk nanofibrous scaffold, Insulin-producing cells, Tissue engineering

PS-050. High Yield Differentiation of Mesenchymal Stem Cells to Neural-Like Cells Using Magnetoelectric Nanocomposite Scaffold

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Background and Aim: Advanced polymers have versatile applications in diversified fields, such as drug delivery, tissue engineering, energy harvesting, sensors, and actuators. One of the most interesting polymers is polyvinylidene difluoride (PVDF) with a broad application in tissue engineering due to its biocompatibility, flexibility, and facile processing techniques The magnetoelectric (ME) effect is the interrelation between magnetic and electric properties of a material and is defined as the variation of the electric polarization in response to a magnetic field (ΔH). In addition, it is a variant of the magnetization under an applied electrical field.
Methods: Given the fact that PVDF shows one of the most piezoelectric properties among polymers, it was selected as the piezoelectric material in this research. Moreover, $CoFe_2O_4$ nanoparticles were used as the magnetostrictive phase due to their large magnetostrictive coefficients and high Curie temperatures. We electrospun the piezoelectric PVDF polymer containing the magnetic nanoparticles to form magnetoelectric nanofibers. In the case of ME composites, a motion, which is induced by the magnetic field in the magnetostrictive component, is transferred to the piezoelectric component that converts the mechanical movement to polarization changes. The piezoelectric and magnetoelectric properties of the composite are becoming of interest for neural cell differentiation.
Results: While the differentiation factors have been widely used to differentiate mesenchymal stem cells (MSCs) into various cell types, they can cause harm at the same time. Therefore, it is beneficial to propose methods to differentiate MSCs without factors. Herein, magnetoelectric nanofibers were synthesized as the scaffold for the growth of MSCs and their differentiation into neural cells without factors. According to the results of the current research, MTT and H&E assays confirmed the biocompatibility of the composite nanofiber. In addition, RT-PCR and ICC assays confirmed that the good potential of the obtained magnetoelectric nanofiber for neural differentiation.
Conclusion: Our findings can be applied to fabricate high-performance magnetoelectric scaffolds to be used for neural cell differentiation with no need for chemical differentiation media, which leads to avoiding their unwanted effects. The achievements of this study can provide a new way to fabricate magnetoelectric scaffolds of high performance to be used for neural cell differentiation with no need for chemical differentiation media and consequently avoiding their unwanted effects.
Keywords: Scaffold; Magnetoelectric; Electrospinning; Mesenchymal stem cells; Neural differentiation

PS-051. Dendrimer Modified Magnetic Nanoparticles as a Promising Platform for Cancer Theranostics

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Background and Aim: Over the past decades, increasing interests have been devoted to the synthesis of functionalized magnetic nanoparticles (MNPs) due to their biocompatibility, biological degradability, targeting ability, and remarkable magnetic properties. Nanoparticles have high surface energy, so tend to agglomerate resulting in a loss of magnetism and dispersibility. To overcome this obstacle, it is necessary to protect nanoparticles by polymers or other agents such as dendrimers, taking into account their biocompatibility, to chemically stabilize the magnetic nanoparticles against aggregation.
Methods: In this study, a precipitation method had been used for the synthesis of iron oxide nanoparticles. At first, the MNPs had been modified with (3-aminopropyl) triethoxysilane, and then PAMAM functionalized MNPs had been synthesized cycling. These MNPs had been characterized by SEM, TEM, XRD, and VSM. The toxicity of these MNPs had been evaluated by MTT. Thereafter their inductive heat property by an ac magnetic field generator and the MRI properties were investigated.
Results: In this paper, various characterization techniques were used to investigate the properties of the synthesized nanocomposites. Cytotoxicity assay confirmed the biocompatibility of the nanocomposites. A superior heat generation was achieved for the given concentration according to the hyperthermia results. MRI results show that the synthesized nanocomposites are a favorable option for MRI contrast agent.
Conclusion: We believe that these dendrimer functionalized MNPs have the potential of integrating therapeutic and diagnostic functions in a single carrier.
Keywords: Magnetic nanoparticles; Dendrimer; Hyperthermia; Magnetic resonance imaging

PS-052. Dopaminergic Induction of Human Adipose Tissue-Derived Stem Cells Under Serum-Free and Low-Serum Conditions

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Background and Aim: Dopaminergic (DA) neurons regulate locomotion and emotion. Degeneration of DA neurons is involved in different neurodegenerative disorders including Parkinson's disease (PD). Cell replacement therapy has been regarded as a promising strategy for the treatment of PD. Human DA neurons have been generated from multiple types of stem cell in vitro. We aimed to study the efficiency of generating dopaminergic neurons from ADSCs under low-serum and serum-free culture conditions.

Methods: Human adipose tissue samples were collected by abdominoplasty and ADSCs were isolated by collagenase treatment. For dopaminergic differentiation, third to fifth-passaged ADSCs were induced by a dopaminergic cocktail containing SHH, bFGF, FGF8, B27, and BDNF in serum-free (Neurobasal medium) or low-serum (DMEM+1% FBS) condition for 12 days. The expression of several neuronal and dopaminergic markers was compared by RT-PCR, qPCR, immunocytochemistry and flow cytometry analyses. Also, secretion of dopamine was measured by high-performance liquid chromatography (HPLC).
Results: RT-PCR results showed that NSE, NEFL, NURR1, EN1, GLI1, PTX3, TH, VMAT2 and GIRK2 mRNAs were expressed in both serum-free and low-serum conditions. Based on the qPCR analysis, the expression of NEFL and NURR1 was not different between two protocols, while NSE, EN1, GLI1 and TH expression in serum-free condition was significantly higher than the low-serum condition. A significant proportion of differentiated cells were positively immunostained for TH and MAP2 proteins. Flow cytometry analysis revealed that TH protein was expressed in about 41% and 13% of the cells in the serum-free and low-serum conditions, respectively. Moreover, the cells differentiated under serum-free condition released a higher amount of dopamine in response to KCl-induced depolarization.
Conclusion: The results of the current study indicated that human ADSCs can be induced by a growth factor cocktail to produce dopamine-secreting cells. Dopaminergic differentiation in serum-free (neurobasal medium) condition was more effective than low-serum (DMEM+1% FBS) condition. Therefore, the serum-free condition may be used to produce dopaminergic cells for the future cell replacement therapy in Parkinson's disease.
Keywords: ADSC; Differentiation; Dopaminergic neuron; Serum-free; Low-serum

PS-054. ANRIL rs4977574 Polymorphism and Acute Myeloid Leukemia Risk

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Background and Aim: Acute myeloid leukemia (AML) is a type of blood cancer which normally begins in cells that turn into white blood cells. Genetic variants like polymorphism have been shown to implicate in pathogenicity of AML. Evidence indicates that variation in ANRIL gene at any step of expression can develop the AML. The aim of the present case-control study was to investigate the association of ANRIL rs4977574 Polymorphism in Iranian AML patients.
Methods: According to the identified variants in the ANRIL locus and its tumorigenicity in a variety of cancers, we assessed the rs4977574 of ANRIL gene in the Iranian AML patient in comparison to the controls.
Results: The results showed no significant association of alleles and genotype frequency of ANRIL (rs4977574) polymorphism between AML patients and control subjects.
Conclusion: Since the ANRIL is juxtaposed to the INK4b-ARF-INK4a gene cluster and may have a role in epigenetic gene expression regulation, hence further investigation on the other ANRIL gene polymorphism must be done to determine the role of this gene in pathogenicity of AML.
Keywords: ANRIL; rs4977574; Acute myeloid leukemia

PS-055. Association of ANRIL (rs1333045) Gene Polymorphism with the Risk of Acute Myeloid Leukemia

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Background and Aim: Dysregulation of cell cycle genes can lead to different kind of cancers. It has become apparent that lncRNAs are an important regulator of the gene expression. Promotor polymorphisms are one of the main reasons for gene expression dysregulation. ANRIL which also known as CDKN2B-AS is a lncRNA that located within the p15/CDKN2B-P16/CDKN2A-p14/ARF gene cluster. Single nucleotide polymorphisms of this gene are associated with many diseases, particularly cancers. The aim of this study was to evaluate the association of ANRIL polymorphism with acute myeloid leukemia in Iranian patients
Methods: In the present study, AML patient and age- and sex-matched control were included.
Results: The results showed no significant association of alleles and genotype frequency of ANRIL (rs1333045) polymorphism between AML patients and control subjects.
Conclusion: We didn't find a significant association between ANRIL (rs1333045) polymorphism and the risk of acute myeloid leukemia.
Keywords: Association; Polymorphism; ANRIL, Acute myeloid leukemia

PS-056. Expression Analysis of HOTAIR lncRNA in Iranian Patients with Acute Myeloid Leukemia

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Background and Aim: A lot of studies show that lncRNAs may have potential as predictive biomarkers in different cancers. HOTAIR is a lncRNA that has been known to have an oncogenic role and its Over-expression has been reported in several types of cancer.
Methods: According to the major role of the gene in developing of different cancer, we investigated the expression level of HOTAIR lncRNA in the blood of AML patients and control subjects using qRT-PCR.
Results: Our results showed no significant difference in HOTAIR gene expression level between patients and healthy individuals.
Conclusion: We conclude that HOTAIR lncRNA is not a reliable biomarker for the diagnosis of AML.
Keywords: HOTAIR; Expression Analysis; Acute myeloid leukemia

PS-057. Telomerase Activity of Myeloid Leukemic Cells Was Suppressed by Rat Mesenchymal Stem Cells

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Background and Aim: Chronic myeloid leukemia (CML) is a myeloproliferative disorder that may arise from a clinically manageable chronic phase (CP) to an incurable blastic phase. As is the case in all cancers, telomerase activity plays an important role in the progression of CML. A significant increase in the telomerase activity has been reported in each of the three phases of CML, and this enhancement is accentuated during the progression of the disease. The multi-lineage nature of CML suggests that stem cell transplantation are important targets of this disease. Therefore, the aim of this study is to investigate the effect of bone marrow-derived mesenchymal stem cells on the telomerase activity of the K562 cell line.
Methods: In this experimental study, the bone marrow of the adult Rattus was flushed, mononuclear cells were separated by Ficoll-Paque and bone marrow-derived mesenchymal stem cells (BMSCs) were isolated. In the following, the flow cytometry method and multi-lineage differentiation were performed to investigate the MSCs-surface markers and multi-potency differentiation, respectively. On the other word, K562 as chronic myeloid leukemia cell line were cultured in RPMI/1640. In the following, BMSCs co-cultured with K562 cell line for 7 days (1:10). At the end of co-culture time, K562 cell line was collected, the protein was extracted and subjected to PCR-ELISA TRAP assay for investigating the telomerase activity.
Results: It was found that BMSCs had high levels of expression of CD44 (94.5%) and CD90 (87.1%) and hematopoietic cell lineage-specific

antigens, such as CD31 (0.07%), and CD56 (0.9%) were not expressed in these cells. In addition, PCR-ELISA TRAP assay showed that BMSCs cause to significantly decrease the telomerase activity.
Conclusion: Taken together, the data showed that bone marrow-derived mesenchymal stem cells cause to decrease the telomerase activity of the K562 cell line as a therapeutic approach for regenerative medicine.
Keywords: Telomerase activity; PCR-ELISA TRAP assay; Bone marrow-derived mesenchymal stem cell

PS-058. Using Retro Viral Vector for HAX1 Gene Delivery in Severe Congenital Neutropenia

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Background and Aim: Neutrophils are considered as one abundant innate immune cells in peripheral blood that quickly act in bacterial and fungal infections. Decrease and malfunction of these cells cause severe infectious diseases. Severe neutropenia is recognized with the Absolute neutrophil count less than 500 per microliter that many of the patients have a mutation in ELA2, HAX1, G6PC3, GFI1, VPS45, and JAGN1. In 2007 HAX1 gene is defined as one of the genes cause severe neutropenia with autosomal recessive inheritance and the affected patients have repeated infections.
Methods: In the past years, gene therapy is introduced as a therapeutic approach for some of the monogenic disorders that some of the clinical trials have confirmed that. In this current project, the retroviral vector contains HAX1 gene isotype A was produced and using Plat-A cells as packaging cell, viral particles were produced and HEK293T as targeted cells were transduced. To confirm HAX1 protein expression, indirect intracellular staining and flow cytometry was applied and double positive cells – GFP and HAX1 protein – confirmed HAX1 protein expression.
Results: Retroviral vector contains HAX1 was confirmed by EcoRI and BgLII double digest and sequencing. Using TurboFect transfection reagent, Plat-A cell as retroviral packaging cell was infected and fluorescent microscopy confirmed the infection. To quantify infected cell, flow cytometry for GFP was done and it was about 20 %. HEK293T cell was chosen as a target cell to analysis transduction efficiency. This cell was transduced with 70 % confluency with help of polybrene. To analysis transduction efficiency, indirect intracellular staining flow cytometry for HAX1 protein was done. Double positive cells for HAX1 protein and GFP were about 40 %.
Conclusion: In the next step, we hope by transduction hematopoietic stem cell of HAX1 deficient patient; we want to analysis in vitro resolving maturation arrest and myeloid differentiation series.
Keywords: HAX1; Gene therapy; Severe neutropenia

PS-059. Choosing the Right Source for Induction of Pluripotent Stem Cells for Cell Therapy Proposes

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Background and Aim: IPSCs are the cells with the potential to differentiate to all of the three embryonic layers. Although many attempts have been made to differentiate these cells to a specific type, many of them may be hindered by many factors such as apoptosis, partial reprogramming, cancer consideration, and etc. One of the best ways to overcome this problem is finding a new source of somatic cells which have enough potential to differentiate with optimal conduction to needed cells.
Methods: We analyze the NGS FASTA files of 27 samples from IPSC with fibroblast, hepatocytes, melanoma, mesenchymal source and embryonic cell line and embryonic body with gene pattern and GEO software package. Their differential signature has been identified and heat maps have been established for a comprehensive and comparable search. After this stage, we have picked the subsets of genes with a p-value under 0.05 and their networks have been established by gorilla and Reactome package. We compared the results to selecting the right source for cell therapy proposes.
Results: The results show us the IPSC with fibroblast cells have been the lowest amount of catabolic and metabolic in lipids, amino acids, and glycolysis pathways differences with normal embryonic stem cells. It is noteworthy that changing in this pathway is the main step in the formation of cancer stem cells. This data shows us that fibroblast cells are the safest source for using for cell therapy and clinical usages. Our data indicate IPS cells with hepatocytes source are prone to differentiate to beta cells, these cells have an upregulation in secretion and insulin-related pathway and they preserve their protein localization and structural pathway which are an important condition for tissue engineering and engrafting concerns.
Conclusion: Based on these finding we can conclude that fibroblast cells generally can be considered as the safest source for induction toward pluripotency, this is an important consideration as it has been seen in teratoma tests. Another finding of ours is, IPSC with a hepatocyte source show similar pattern to stem cell progenitor of the pancreas and they are a great source in regenerative medicine for diabetic and pancreatic related diseases.
Keywords: Pluripotent stem cells; cancer cell therapy

PS-060. Effect of Thiol Compound Cysteamine and β-Mercaptoethanol on In Vitro Maturation and Embryo Development Oocytes with/without Cumulus NMRI Mouse

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Background and Aim: The present study examined the relationship between cysteamine and β-mercaptoethanol on in vitro maturation and embryonic development in oocytes with-cumulus and without cumulus NMRI mouse. Unfavorable conditions in cell culture medium and high levels of free radicals and eggs low quality seem to be the cause of failure of this method and may cause low fertility rate. The purpose of this study was to compare maturation and embryo development germinal vesicle with and without cumulus in NMRI mice in the presence and absence of cysteamine and β-mercaptoethanol.
Methods: In this study, 4-6-week immature NMARI mice were used. Animals were handled according to the guidelines for the care and use of laboratory animals and maintained in a 12 D photoperiod under constant temperature and relative humidity. Food and water were provided suitable, and 7.5 unites PMSG (pregnant mare serum gonadotropin) was used for ovaries stimulation. After 48h, mice were killed with dislocation of cervical vertebrae. GV oocytes with and without cumulus cells were isolated from ovaries and cultured in TCM199 media in addition 100 µm BME and CYS for 24 hours. Then mature oocyte (MII) were fertilized with sperm in BSA+T6 medium (IVF). Then, they were analyzed using SPSS software and evaluated for development egg to blastocyst under an inverted microscope. Blastocyst formation in a defined IVM medium for mouse immature oocytes increased. The effect of supplementation with Cys or β-ME on IVM rate and fertilization and developmental competence following intracytoplasmic sperm injection (ICSI) were investigated. The ICSI procedure was performed according to the method of Yong et al.
Results: In the present study, our findings indicated that 100 µm BME & CYS in medium culture can improve in-vitro maturation ($P<0.05$). Also, oocytes with cumulus increased in vitro maturation in medium culture ($P<0.05$). The addition of cysteamine and β- mercaptoethanol to defined maturation medium enhanced the blastocyst formation. There is a positive and meaningful relation between BME & CYS and in-vitro maturation oocytes in stages 2-cell, 4-cell, 8-cell, and blastocyst. Existence cumulus-oocytes complexes (COCs) increased the maturation in-vitro.

Conclusion: The oocytes with-cumulus may play an important role in the oocyte maturation by regulating the meiotic progression and supporting the cytoplasmic maturation. High efficiency in the mouse by further increasing the GSH reservation created by oocytes complexes was matured in vitro in TCM199 media. In all supplemented groups, the intracytoplasmic GSH concentration was significantly higher than the control medium for the improvement in embryo production. There are a positive and meaningful relation between BME&CYS and in-vitro maturation oocytes in the stages 2-cell, 4-cell, 8-cell, and blastocyst. The existence of cumulus-oocytes complexes (COCs) could increase the in-vitro maturation.

Keywords: Cysteamine; β-mercaptoethanol; Cumulus oocytes; Embryonic development

PS-061. Zebrafish Liver Tissue-Derived Mesenchymal Stem Cells Expressed the CD44 and CD90 Epitopes as Mesenchymal Cell Surface Markers

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Background and Aim: Effectiveness of MSCs in the treatment of wide ranges of disease (e.g., hematopoietic recovery, inflammatory diseases, bone regeneration, infarcted myocardium, joint diseases etc) has been well documented. As a result, isolation and characterization of MSCs from different species for extensive preclinical studies are required. Zebrafish liver tissue-derived MSCs described here fulfill the basic MSC characteristics and these criteria. The aim of this study was to investigate the immunophenotypical characterization of liver-derived MSCs for the first time for using zebrafish as a suitable animal model for regenerative medicine in cell therapy.

Methods: In this experimental study, zebrafish (*Danio rerio*) was purchased from a local fish dealer. Liver of zebrafish was obtained and carefully minced under sterile conditions. In the following, MSCs were isolated enzymatically from liver tissue of Zebrafish as previously reported in another study by Fathi et al. Subsequently, the RT-PCR, as well as flow-cytometry, were used to analyze the expression of a panel of immunological cell surface markers CD44, CD90, CD31, and CD34. Also, alizarin red, oil Red-O, and toluidine blue staining were carried out to evaluate the multipotency of zebrafish liver tissue-derived MSCs.

Results: In the immunophenotyping analysis, the liver-derived MSCs was found to be immunopositive for CD44 and CD90 epitopes as mesenchymal stem cell markers, and immunonegative for CD31 and CD34 epitopes as hematopoietic stem cell markers. These results are, in combination with the RT-PCR results, were also positive and negative for gene expression of CD44 and CD90 and CD31 and CD34, respectively. These findings are in line with the results of other MSCs characterization; these reactions are generally characteristic of MSCs.

Conclusion: Our results show that liver tissue-derived MSCs exhibited expression of a typical set of classic MSC surface markers, which will contribute to a more extensive characterization of zebrafish MSCs prior to their use in cell therapy and regenerative medicine.

Keywords: Zebrafish (*Danio rerio*); Mesenchymal cell surface markers; CD44 and CD90 epitopes

PS-062. Cytokine Antibody Array of Rattus Norvegicus Bone Marrow-Derived Mesenchymal Stem Cells

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Background and Aim: Growth factors and cytokines secreted from mesenchymal stem cells (MSCs) as well as other types of cells relate to cell fates toward survival versus death and interaction versus protection, etc. It is now well documented that the main stem cell properties, including self-renewal, multi-lineage differentiation, and tissue engraftment, are principally influenced by the local microenvironment and soluble molecular signals. Therefore, the identification of these factors is very important for further consideration. The aim of this study is the identification of secreted cytokines from rat bone marrow-derived MSCs.

Methods: In this study, 6- to 8-week-old male Rattus rats were euthanized, bone marrow from tibia and femur was obtained by flushing and MSCs was isolated by the Ficoll-Paque method. MSCs was cultured until reach confluency. For antibody array, Rat Cytokine Antibody Array–Membrane (ab133992, Abcam) consisting of a total 34 different cytokine antibodies spotted in duplicate onto two membranes were used. For this purpose, supernatants from cells of the passage 3-6 were collected at culture days 7. Array membranes, each in separate wells of provided 8-well plate, were incubated for 30 minutes in 2 mL of blocking buffer, further incubated for 2 hours in a shaker at room temperature with 2 mL of fresh culture medium. After the membranes were thoroughly washed with wash buffer I and II, 2 µL of biotin-conjugated was added to each membrane, and the mixture was incubated on a shaker 4°C overnight. Following the wash, the membranes were incubated with HRP-conjugated streptavidin for 2 hours at room temperature. Proteins were detected by detection buffer C and D provided in kit and signals were captured by CCD camera. The exposure time was 5 and completed within 20 minutes as chemiluminescence signals will fade over time. Arrays images were processed with Image J software.

Results: The cytokine antibody array membrane incubated for 2 hours with a fresh culture medium as suggested in the manufacturer's protocol yielded a number of spots whose intensities were significantly stronger than the background level, indicating that culture medium of bone marrow-derived MSCs featured a single predominant hybridization signal for TIMP-1 (tissue inhibitor of metalloproteinases-1).

Conclusion: The significant expression of TIMP-1 suggests that this cytokine as well other secreted cytokines could be involved in the cell interactions. The identity of another molecule involved in the anti-proliferative effect of bone marrow-derived MSCs requires further investigation.

Keywords: Cytokine secretion profile; Rat bone marrow-derived MSCs; TIMP-1

PS-063. Early-Stage Expression of VE-Cadherin During Endothelial Differentiation of Mesenchymal Stem Cells Using SPR Biosensor

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Background and Aim: The mesenchymal-to-endothelial transition is an essential phenomenon during tissue regeneration, leading to an increased vascular density in target tissues. Thus, the development of highly sensitive and accurate biosensor approaches for detecting stem cells differentiation, particularly in the earliest steps, is more crucial than ever. In the current study, a label-free SPR (surface plasmon resonance) method was developed for the monitoring of the early-stage differentiation of mesenchymal stem cells into endothelial-like phenotype in term of VE-cadherin over a period of 14 days.

Methods: Human amniotic mesenchymal stem cells (hAMSCs) line (Cat No: C10680) was obtained from Iranian Biological Resource Center. Low glucose-DMEM (DMEM/LG, Gibco) cell media were used for the cultivation of hAMSCs. To induce endothelial differentiation, hAMSCs were maintained in endothelial cell growth media, M-199, supplemented with an EGM-2 cocktail (Cat No: C-22010, Promocell) and 2% fetal calf serum (FCS, Promocell) for 14 days. The medium was replenished

every 2 to 3 days. The differentiation of hAMSCs into endothelial-like phenotype was investigated by SPR biosensor method and also with flow cytometry analysis and immunofluorescence microscopy on days 1, 2, 3, 5, 7 and 14. The morphological changes in relation to the endothelial acquisition were monitored through the experiment. A multi-parameter SPR device (MP-SPR Navi 210A, BioNavis Ltd, Tampere, Finland) with gold chips (BioNavis Ltd, Finland) was used to examine antibody-cell affinity interactions.

Results: The combination of SPR method and Ab immobilization on-chip resulted in early detection of VE-cadherin as a cell surface marker in the endothelial differentiation of hAMSCs. Consistent with endothelial differentiation of SCs, a high level of VE-cadherin was encoded on the cell surface resulting in an increased attachment of the cells to the immobilized antibody on the chip surface. This attachment, in turn, caused profound changes in the refractive index values in the detection region which were reflected as increased RU intensities during differentiation stages. After subtracting the nonspecific binding responses, we recorded 0, 80, 120, 360, 510 and 610 RUs×10^{-4} for differentiating hMSCs following 1, 2, 3, 5, 7 and 14 days, respectively. The flow cytometric method was unable to discriminate the hAMSCs expressing VE-cadherin during the first 4 days of the differentiation, especially on day 0, 1 and 2, toward endothelial lineage.

Conclusion: In the present study, SPR technique could sense the early stage differentiation of hAMSCs on day 3 in real-time and label-free form without affecting cell viability, but flow cytometry and fluorescent microscopy methods were not able to detect the cell differentiation at the same time. This sensitive method presents hopeful views for monitoring and identification of rare and specific cell populations like tumor cells, cancer stem cells and etc.

Keywords: Human amniotic mesenchymal stem cells; SPR Biosensor; VE-cadherin

PS-064. Evidence of The Correlation Between Wnt Signaling and The Expression of Telomerase Components in the Human Mesenchymal Stem Cells Derived from Peripheral Blood

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Background and Aim: Telomerase activity has been known to play a critical role in the remaining proliferation potential of stem cells including mesenchymal stem cells (MSCs). The low number of these cells limited the application of these cells in the regenerative medicine and treatment of diseases. To more understand the molecular mechanisms involving in the replicative potential of MSCs, the expression of telomerase components and the Wnt signaling pathway was evaluated in the early culture as well as the late culture of MSCs.

Methods: The mononuclear cells containing MSCs were separated from peripheral blood using a Ficoll density gradient. After reaching to 70%-80% confluence, the expression of hTERT, hTERC, TCF4, CTNNB1(β-catenin) and GSK were evaluated by Real-time PCR in early culture (8-9th days) and late culture (14th-17th days) of MSCs as compared with the control group (6th days of MSCs culture).

Results: Our data indicated that there was a negative correlation between GSK and telomerase RNA component (TERC) in the early culture of MSCs (t-test; -2.29, $P < 0.05$). Furthermore, the decrease of the mean expression of TERT and TERC in late culture was accompanied by the diminished expression of TCF4 and the enhanced expression of GSK as compared with the early culture of MSCs.

Conclusion: The diminished expression of TCF4, as well as the enhanced expression of GSK, suggested that the inhibition of Wnt signaling pathway plays a role in the decreased expression of human telomerase reverse transcriptase (hTERT) and TERC in the late culture of MSCs. The obtained results could be contributed to the identification of mechanisms involving in the maintenance of telomere length and the proliferation potential of MSCs.

Keywords: Telomerase; Wnt signaling pathway; Mesenchymal stem cells; Cellular senescence

PS-065. Optimization of Best Model for TanCAR T Cell Targeting CD123 and Folate Receptor Beta

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Background and Aim: Acute myeloid leukemia (AML) is considered as a type of cancer with abnormal myeloblasts in the bone marrow. To tackle this disease, we capitalized on the chimeric antigen receptors (CARs), which are artificial molecules that change the specificity of T cells to particular antigens. A CAR was consisted of two different antigens (CD123 and FRβ) recognition domains presented in tandem on a single transgenic receptor that mediates bispecific activation and targeting of T cells. We assumed that this tandem CAR (TanCAR) can exclusively recognize each target molecule and facilitate a synergistic activation and functionality when both are used concurrently. While there exist some possibilities in modeling the CAR, we capitalized on various methods in order to optimize the physical properties of the final CAR protein.

Methods: Different computational modeling tools were exploited to predict the functionality of CAR molecules that can mediate the bispecific activation and targeting of T cells. To uncover whether the aforementioned molecular arrangements were possible, we used TanCAR structural models predicted by modeling web server, ModWeb. Patchdock and FireDock, software tools for docking and refining two structures. These analyses were carried out based on the shape complementarity and docking of FRβ- and CD123- scFvs to their respective target molecules. We also looked into some other important parameters in computational designing of the chimeric protein, including stability, proper energy level and etc. Finally, the best model was selected through comparison between the predicted structures and physical properties, which might simulate the function of CAR molecule in the in vivo condition.

Results: It was found that the potential interactions of the best TanCAR model could accommodate the planned bispecificity, and as such, an initial model to explore the ability of TanCAR to interface with the target molecules individually predicted. The designed chimeric antigen receptor demonstrated stability and same docking as the original scFvs. The modeled construct displayed a good energy level to making the proper conformation based on its sequences. Both paratope domains possessed free physical configuration and were able to recognize each target molecule solely.

Conclusion: Various de novo CAR T cell therapy modalities have recently been articulated. Among them, bi-specific CAR constructs are the most attractive methods. They are able to recognize and bind to two different antigens separately, and thus, result in an enhanced specific function of recombinant T cells. Likewise, the TanCAR molecules provided both recognition domains in tandem, imposing T cells to facilitate a synergistic activation and functionality. This study confirmed the potency of the best TanCAR based on CD123 and FRβ as a new candidate for the treatment of AML, which might simulate the function of CAR molecule *in vivo*.

Keywords: Acute myeloid leukemia; CD123; FRβ; TanCAR; Modeling

PS-066. Evaluating the Expression of Bone Gamma-Carboxyglutamate Protein in Differentiated Cells Subjected to Iron Nanoparticles

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Background and Aim: Iron nanoparticles (NPs) in medical fields has been utilized broadly in magnetic resonance imaging. It is crucial to formulate NPs containing appropriate characteristics for therapeutic approaches. The broad application of Mesenchymal stem cells (MSCs) in regenerative medicine attracted the attention of scientists. The expression of the bone gamma-carboxyglutamate protein (OCN) as one of the marker genes of osteoblasts can be considered in the accuracy of induction of the osteogenic differentiation. In the current study, we assessed the adverse effect of Zn-Mn-iron NPs on the expression of OCN gene in the osteocytes.
Methods: In the current study, we exposed umbilical cord MSCs to osteogenic differentiation media. At day 21 the morphology of cells has been altered to osteoblasts. The changes of OCN gene expression at the mRNA level was evaluated by SYBR Green and real-time quantitative PCR.
Results: The expression of OCN gene of treated cells with Zn-Mn Ferrite NPs showed no significant changes in comparison with untreated cells.
Conclusion: The results of our study showed that ferrite NPs do not have any adverse effects on the expression of OCN gene at the mRNA level. In order to utilize ferrite NPs in therapeutic aspects, yet more investigations are required to ensure the safe function of this approach.
Keywords: Iron nanoparticle, Mesenchymal stem cells, Bone gamma-carboxyglutamate protein

PS-067. Potential of Tumor Suppressor miR-145 to Reduce the Expression of KLF4 in Order to Control the Progression of MCF7 Breast Cancer Cell Line

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Background and Aim: Recent studies indicate that miR-145 plays an important role as a tumor suppressor and metastasis in a variety of tumor. This gene can also induce the differentiation of embryonic stem cells. KLF4 is a necessary transcription factor for maintaining the pluripotency of embryonic stem cells. KLF4 is considered as an oncogene. Reduction of the KLF4 self-renewal property cause suppresses the cell migration and invasion of breast cancer cells. The aim of this study is the investigation of the effect of miR-145 overexpression on KLF4 gene expression in mcf7 cell line.
Methods: The gene construct of miR-145 expression vector was designed; then, it was transduced to mcf7 cell line. Total RNA extraction and cDNA synthesized. Gene expression of KLF4 measured by real-time PCR and flow cytometry technique and the result were analyzed.
Results: Preliminary results indicate the KLF4 downregulation following the overexpression of miR-145 in mcf7 cancer cells, and induces differentiation resulting in cancer cells reduction.
Conclusion: The results of this study for the first-time show, the effect of miR-145 on the expression of KLF4 in a breast cancer cell line. This study, according to the theory of cancer stem cells may be effective in the control of the progression of cancer through molecular intervention.
Keywords: Breast cancer; KLF4; miR-145

PS-068. Assessment of CD44, SYNDECAN and Integrin Gene Expression in Mesenchymal Stem Cells (MSCs) After Priming by Poly (I:C)) as a Synthetic Analog of Double-Stranded RNA (dsRNA) in Order to Effective Type 2 Interaction

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Background and Aim: The use of MSCs for cell therapy relies on the capacity of these cells to recruit, homing and engraft into the target tissue. Homing of MSCs can occur but does so with only poor efficiency. Thus, the efficiency of MSCs transplantation is limited by lower homing of MSCs. MSCs possess immunoregulatory properties due to the expression of the key components of innate immunity such as TLRs. The aim of the present study was to evaluate the role of TLR3-primed MSCs on mRNA gene expressions of several CAMs that involved in stem cell homing.
Methods: At first, to quantify the impact of the TLR3 agonist poly (I:C) on the mRNA expression levels of TLR3 in hMSCs, we performed RT-PCR and real-time RT-PCR assays. RT-PCR analysis revealed that 4 h exposure to poly (I:C) elevated TLR3 mRNA expression in hMSCs in a concentration and time-dependent manner. Real-time RT-PCR showed that TLR3 mRNA levels reached the highest amount in MSCs exposed to 5 μg/mL poly (I:C) for 4 h during different exposure times. Also, the incubation with 5 μg/mL poly (I:C) for 4 hours preferably elevated proinflammatory cytokines mRNA levels.
Results: Here, we show that exposure to the TLR3 agonist poly ((I:C)) increased the mRNA expression levels of TLR3 and cell adhesion molecules such as CD44, Syndecan and integrin isoforms such as α1β1, α2β1, α3β1, α5β1 and αvβ5. Poly(I:C) exposure elevated intracellular signaling pathway that associated with TLR3 signaling including TRIF, TRAF-3, NF-kB and IκB and decreases proinflammatory cytokines such as IL-6. On the other hand, TLR3 agonist prompted an expression of integrin isoforms and integrin-mediated adhesion molecules that involved in cell-cell interaction and homing. In addition, we observed that other cell adhesion molecules such as CD44 and Syndecan were significantly up-regulated in response to TLR3 priming of BM-MSCs
Conclusion: TLR3 priming may enhance MSCs to the expression of several cell adhesion molecules that involved in MSCs recruitment, mobilization, homing and retention. Our findings not only clarify the novel signaling cascade from TLR3-priming to immunoproperty process but also implicate potential targets for genetic and pharmaceutical manipulation in MSCs-based therapy for increasing efficiency of recruitment and homing of MSCs for future clinical applications.
Keywords: TLR3; Innate immunity; MSCs; Homing

PS-069. Liposomal miRNAs Modulate Expression of Parp1 in Human Mesenchymal Stem Cells

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Background and Aim: Human mesenchymal stem cells (MSCs) have been of recent particular interest due to their unique features of self-renewal and potency for differentiation to many somatic cell types which is the basis for regenerative medicine. The parp1 plays an indispensable role in the preservation of this process because it can act together with

other pluripotency factors. RNAi strategy including siRNA and miRNA represents a post-translational gene expression modification. miRNA-based therapies face with some obstacles such as difficulty in passing the cell membrane barrier and also passage within the cells.
Methods: In the present study, a new liposome-based formulation incorporating two miRNAs was synthesized and characterized by precise techniques in order to confirm its optimization in terms of stability and efficient transfection. Then free miRNA and lipoplexes containing miRNA were used for transfection into MSCs, and their effect on the expression level of parp1 was evaluated by qPCR.
Results: Parp1 gene expression was over-expressed in the presence of miR-302a, however, miR-34a had the opposite effect on Parp1 mRNA levels and down-regulated the level of Parp1 mRNA significantly.
Conclusion: Our findings suggest that parp1 is a key component in the regulation of pluripotent cells and this can be regulated by the use of miRNAs. The high efficiency of the novel formulation of the liposome is promising.
Keywords: Human mesenchymal stem cells (hMSCs); Liposome; miRNA; Potency; Regenerative medicine; Transfection

PS-070. A Computational Model for Investigating the Effects of Strain on Axonal Injuries in a Microfluidic Cell Culture Platform

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Background and Aim: Traumatic brain injury and spinal cord injury are debilitating causes of traumatic death and disability worldwide. Inertial forces that occur during acceleration/deceleration of the brain can lead to the generation of forces exerted on axons causing axons to be injured. Reliable and accurate models that can simulate an applied injury need to be developed. These models must be capable of applying physiological levels of injury and assessing the extent of the injury. Here, we focus on models that include stretch injuries. A finite element model was used to provide quantitative measures for mechanical changes and their effects on the function of the axon.
Methods: Our study models an axon in cell culture platform using a three-dimensional geometry (based on experimental works) for axon and platform, a uniaxial strain device as an injury platform in a finite element analysis. By using a microfluidic device like a cell culture environment, we can distinguish and observe a single axon. In order to accurately predict the strain applied to an axon, a 2nd order Ogden hyperelastic model is used for describing PDMS. A pneumatic pressure is applied to the cavity under the microchannel layer to produce a uniaxial strain in the axon. Different injected volumes and rate of injection produce magnitudes of strain (11%, 25%, and 42%) and strain rate (260 ms^{-1}, 50 ms^{-1}, and 22 ms^{-1}). To assume proper mechanical properties of the axon, we use data from in vivo experiments and results from mathematical models. In order to observe the effects of the fluid in microchannel on the axon, an FSI model was used. We describe the whole axon as a solid hyperelastic cylinder with an approximate radius of 1.5 µm. The commercial finite element software, COMSOL Multiphysics 5.2, is used for the simulation.
Results: The results from the FSI models showed no significant effect from fluid on the mechanical response of the axon (strain value was equal to 9.33e-3%), thus we did not include this in our future considerations. It was shown that the role of flow on the microchannels is just to provide a proper environment for the axon to grow. Results have shown that in high strain and strain rate values as to be equal to 42% and 22 ms^{-1}, we see that injury occurs. The model showed a limit for the strain that will cause injuries in the axon. It is also showed that the rate of strain has also a great role in causing injuries. Breaking microtubule of axon has the most important role in injuries, due to this reason that mechanical integrity of axon comes from microtubule bundles
Conclusion: This study aimed to show a basic model for axon and inspect its mechanicals behavior as a basis for future works in regenerative medicine. There are several important factors (e.g., strain, the rate of strain, axon structure and mechanical properties and the effect of cell culture fluid in the microchannel) that have been discussed. This mechanical model can be coupled with a chemical or electrochemical model in order to obtain greater clinical relevance.
Keywords: Biomechanics; Cell mechanics; Traumatic brain injury; Axon; Strain

PS-071. Isolation of Colorectal Cancer Stem Cell Exosomes in Order to Use in Tumor Cell Invasion Studies

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Background and Aim: Colorectal cancer (CRC) is the second most common cancer in the world. Cancer stem cells (CSCs) have been attributed to mediate chemo-resistance, recurrence, invasion, and metastasis in cancer. Tumor-derived exosomes are nanometer-sized vesicles (30-130 nm) which proposed to have a role in invasion and drug resistance by transferring proteins and RNA cargo (mRNAs and microRNAs). Therefore, a better understanding of exosomes function will propose new opportunities in diagnostic or therapeutic strategies against cancer. This study designed to evaluate colorectal CSCs derived exosomes role on cancer invasion and drug resistance.
Methods: In this study, HT-29 cell line colonspheres were cultured by hanging drop method in the serum-free media under non-adherent conditions (poly-HEMA coated plates). Putative colorectal CSC markers including CD166, CD133, CD44, and DCKL-1 were evaluated using flow cytometry analysis. In addition, the expression of well-known stemness genes (OCT-4, SOX2, KLF-4) were assessed by real-time PCR. Exosome Purification Kit was used for isolation and purification of intact exosomes from CSCs culture medium. CSCs derived exosomes were characterized and confirmed by scanning electron microscopy (SEM) analysis and dynamic light scattering (DLS) measurement.
Results: HT29 derived spheroids made by hanging drop method after 10 days culture. Flow cytometry analysis showed higher expression of CD166, CD133, CD44 and DCKL-1 markers in spheroid cells than parental cells. Moreover, relative to control parental cells, the expression of stemness genes including OCT4, SOX2 and KLF-4 were increased in the spheroid cells. Scanning electron microscopic examination of the purified exosomes indicated that they have a spherical shape with a diameter of ~30– 150 nm. Further exosome size evaluation by dynamic light scattering showed a single bell-shaped size distribution with a peak at ~135 nm.
Conclusion: This purified and confirmed exosomes could be facilitated subsequent studies in cancer and CSCs fields such as drug resistance, invasion, and metastasis. Moreover, exosomal contents assessment may be useful for detection of promising CRC biomarkers involved in invasion and drug resistance.
Keywords: Colorectal cancer; Cancer stem cells; Exosome

PS-072. Adenosine Prevents Apoptosis in Neural Stem Cells by Down-Regulating Mst1 Expression

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Background and Aim: Overproduction of free radicals during oxidative stress induces damage to key biomolecules and activates programmed cell death pathways. Apoptosis is regulated by the expression or activation of proapoptotic genes and proteins including mammalian sterile 20 like kinase 1 (Mst1). Neuronal cell death in the nervous system leads to a number of neurodegenerative diseases. The aim of the present study was to evaluate the neuroprotective effect of adenosine on inhibition of apoptosis induced by hydrogen peroxide (H_2O_2) in bone marrow-derived neural stem cells (B dNSCs), with focus on its regulatory effect on the expression of Mst1, as a novel proapoptotic kinase.

Methods: Bone marrow-derived stromal cells (BMSCs) isolated from Wistar rats and evaluated using CD90. To form B dNSCs, isolated BMSCs cultured with NSC expansion medium containing DMEM/F12 supplemented with B27, bFGF, and EGF. Immunocytochemical evaluation performed for Nestin as an NSC/progenitor cell marker. B dNSCs were exposed to adenosine at different doses (2, 4, 6, 8 and 10 µM) for 48 hours followed by 125 µM H2O2 for 30 minutes. The studied groups included: N (untreated NSCs), NA (NSCs treated with 6 µM adenosine), NH (NSCs treated with 125 µM H2O2), and NAH (NSCs treated with 6 µM adenosine and 125 µM H2O2). MTT assay was used to determine an optimal concentration of adenosine. Using TUNEL assay, immunocytochemistry and Real-Time qPCR, the effect of adenosine on apoptosis and expression of Mst1 gene at the protein and mRNA level were evaluated in pretreated B dNSCs compared with control groups.

Results: The B dNSCs from neurospheres expressed a high level of nestin. Results of the MTT assay indicated 6 µM adenosine to be the most protective dose in terms of promotion of cell viability. Subsequent assays using this dosage indicated that apoptosis rate and Mst1 expression in B dNSCs pretreated by 6 µM adenosine were significantly decreased compared with the control groups.

Conclusion: These findings suggest that adenosine protects B dNSCs against oxidative stress-induced cell death via down-regulating Mst1 expression. Adenosine can cross the blood-brain barrier and therefore may be considered as a suitable drug for the treatment of diseases of the nervous system caused by oxidative stress.

Keywords: Neural stem cells; Adenosine; Mst1; Apoptosis; Oxidative stress

PS-073. Role of Natural Biopolymers in Behavior NP Cells of Human Intervertebral Disc

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Background and Aim: The low back pain is one of the major economical and social issues nowadays. The herniation in the intervertebral disc and central degeneration of disc are two main causes of low back pain, which happen due to the structural defects of disc. The intravertebral disc contains three parts (i.e., annulus fibrosus, transitional region, and nucleus polpusus), which form the central nucleus of the disc. Reduction of cell count and extracellular matrix, in particular in the nucleus pulposus (NP) cells, can result in the degeneration of disc. To tackle such an issue, various natural and synthetic scaffolds have been exploited for tissue repairing and regeneration of intravertebral disc in tissue engineering. Most scaffolds used possess some degrees of biodegradability and biocompatibility, at which they provide a fine condition for proliferation and migration of cells. In this work, we studied the proliferation of NP cells of human intravertebral disc compromised in chitosan-gelatin and alginate scaffolds.

Methods: NP cells were isolated from nucleus pulposus through an enzymatic hydrolysis using collagenase. The cells were isolated from patients who underwent open surgery for the discectomy in Alzahra Hospital (Isfahan, Iran). Chitosan polymer was blended with gelatin polymer while glutaraldehyde was used to cross-link these polymers (chitosan-gelatin scaffold). Then, alginate scaffold was prepared. Cellular suspension with a density of 1×10^5 cells transferred to each scaffold and cultured for 21 days. The viability and proliferation of cells were investigated using trypan blue and MTT assay. The SEM imaging was used to study the morphology and porosity of the scaffolds.

Results: The MTT assay revealed that the cell viability of the third day culture displayed a marked difference in contrast by the first day in both scaffolds. Accordingly, there was a significant decreased in cellular viability from day 3 to day 21. The cell count analysis demonstrated a prompt elevation cell numbers for the alginate scaffold but there was no similar result for the chitosan-gelatin scaffold.

Conclusion: Alginate scaffold provided a better condition for the proliferation of NP cells as compared to the chitosan-gelatin scaffold. Results of this study suggest that alginate scaffold could be useful for the in-vivo studies and clinical applications.

Keywords: Intervertebral disc; Alginate; Chitosan; Gelatin; Intervertebral disc

PS-074. Development of Injectable Hydrogel Based on Nanocomposite of Nanocrystalline Cellulose/Chitosan/Pectin for Cartilage Tissue Engineering

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Background and Aim: Cartilage tissue engineering (CTTE) is proposed as an emerging promising therapeutic strategy that offers advantages over the current treatment approaches, which seeks to overcome the cartilage self-repair limitation through the development of cellular scaffolds that closely mimic the complex structure of cartilage tissue. Among a variety of biomaterial scaffolds used in CCTE, in situ injectable hydrogel systems are highly desirable for the clinical applications due to the biocompatibility, highly hydrated 3-D environment similar to the cartilaginous ECM structure, ability to effectively deliver cells and/or bioactive molecules to targeted sites.

Methods: About 1.5 g aliquot of pectin was dissolved in 150 mL of purified water in a 500 mL flask. A total of 0.8 g of NaIO4 was dissolved in 10 mL of purified water and added dropwise to the pectin solution. The mixture was allowed to stir for 2 hours at room temperature before 0.4 mL of ethylene glycol was added. The solution was dialyzed for 2 days. The same procedure was used for the oxidation of cellulose CNCs. Finally, a double-barrel syringe was used to make injectable hydrogels of chemically cross-linked pectin and CNCs. Barrel A contained a 4 wt % chitosan solution in purified water and barrel B contained a 4 wt % pectin-CHO and CHO-CNC solution. Then, all materials were sterilized and the polymer solutions at different weight ratios were prepared in the presence of chondrocytes in a specific medium. Subsequently, chondrocytes were incorporated into the hydrogels and the cell viability and proliferation of cells were assessed.

Results: In this study, a biomimetic injectable chitosan/pectin hydrogel was produced with excellent properties for CTTE scaffold. The hydrogel was optimized and showed suitable physicochemical properties, including thermal stability, compressive strength, viscoelastic behavior, swelling ratio, and degradation rate. Having such characteristics, the hydrogel meet the requirements for the cartilage repair. The hydrogel precursors and cross-linked hydrogels were thoroughly characterized regarding their chemical, morphological, microstructural and mechanical properties, as well as their swelling and degradation profiles. The hydrogels provided a host tissue-mimetic microenvironment for maintaining chondrocyte phenotype.

Conclusion: Altogether, we successfully prepared the biomimetic injectable hydrogel without incorporating any extraneous cross-linking agents under physiological conditions. The properties of the hydrogel such as gelation time, mechanical properties and degradation behavior, were easily adjusted. When chondrocytes were encapsulated into hydrogels, it was found that cell behavior was remarkably affected by CS/pectin composition. The in-vitro study showed that the incorporation of chondrocytes to the hydrogel was able to maintain long-term chondrocytes survivability and improve cartilaginous ECM deposition. We believe the biomimetic injectable hydrogel based on polysaccharides is very promising scaffolds for CTTE application.

Keywords: Injectable hydrogels; Adipose-derived stem cells; Cartilage tissue engineering

PS-075. Study Genetics of Lip Prints Pattern in Iranian Population

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Background and Aim: In the last few years, the study of lip prints become an aid in individual identification in the legal investigation and forensic medicine. The present study aimed to ascertain whether the lip patterns are inheritable from parents to children.

Methods: This study was conducted on 49 males and females belonged to several relatives of Iranian population in Khorasan Razavi province in which families are 3-generation, in other words, families were considered from grandparent to grandchild. Lips of individuals were covered by a red or brown lipstick gently. The lip prints were recorded by sticking a piece of cellophane tape on lips, then moving on A4-sized white paper. Also, by using a digital camera (16 MP) some pictures of lips were taken. Lip prints and pictures were studied by utilizing a magnifying lens and classification of Suzuki and Tsuchihashi.

Results: The result of the study showed that no two people have similar patterns and only 10.20% of females and males had the same pattern in the same site identically as they mothers had, but in fathers and children no similarities appeared in patterns. Moreover, no relation was seen between patterns of grandparents and grandchildren. To some extent, resemblances were observed between siblings and also between aunts and nieces or nephews.

Conclusion: As the low level of resemblances was observed (10.20%) in individuals lip prints pattern from 2 generations, it is concluded that even though mother has a scant role in heredity of patterns, lip prints pattern is unique and do not follow a direct inheritance system and every individual inherits their pattern and grooves separately from their parent or grandparent.

Keywords: Cheiloscopy; Genetics; Iranian population; Lip print; Physical anthropology

PS-076. Mimicking Radial Porosity Gradient of Bone Structure Using Bioprinting and Controlled Release of Dexamethasone

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Background and Aim: The number of bone disorders has grown dramatically due to numerous reasons including aging and obesity. Bone tissue engineering has been considered as a promising method to overcome limitations of conventional treatment procedures of bone damages.

Methods: Here, by means of PioneerX4 bioprinter, a bone scaffold has been fabricated with radial porosity gradient. PioneerX4 is a home-made and the first commercial Iranian 3D bioprinter that builds up 3D constructs by coordinating the motion of a print module. This device is able to print up to four different bio-inks including in each station. The aforementioned radial gradient porosity not only mimics the bone structure but also leads to cell differentiation. In this study, a custom-built PCL bio-ink is coupled with alginate hydrogel as inks for the printing process. The PCL bio-ink is used as structural material and as a bioactive bio-ink, alginate is printed between them. Furthermore, dexamethasone mixed with alginate hydrogel to both induce cell differentiation and suppressing inflammatory responses.

Results: SEM images show radial porosity gradient design and prove printing accuracy. The diameter of PCL and alginate strands varies in the range of 285 µm and 432 µm. The scaffold consists of three regional parts distinguished by different porosities including 66.8%, 58.8%, and 25.9% from the inner part to the outer, respectively. The compressive modulus of printed PCL bio-ink scaffold is about 19.2±2.2 MPa. Hydroxyapatites nanoparticles (HA NPs) were prepared using the chemical wet method and used as carriers for sustained releasing of dexamethasone. Dexamethasone loaded HA NPs were mixed with alginate hydrogel to both induce cell differentiation and suppressing inflammatory responses after surgery. The sustained release of dexamethasone lasts more than 3 weeks. Finally, after 1 month most of the alginate degraded and the scaffold degradation reached 40.8%.

Conclusion: Bioprinting is not only a novel method for scaffold fabrication but also it is more alluring for bone tissue engineering due to its control of pore interconnectivity and porosity gradient. Both dexamethasone and hydroxyapatite stimulate bone regeneration leading to accelerating bone formation.

Keywords: Bioprinting; Bone Tissue Engineering; Radial Porosity Gradient; Controlled Release

PS-077. Bioinformatics Prediction of miRNAs Regulating the Differentiation of Pluripotent Stem Cells Into Insulin-Producing Cells

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Background and Aim: Nowadays, the cell type conversion is a new method for the generation of insulin-producing cells (IPCs) from other differentiated or stem cells to treat diabetic patients. Identification of the molecular mechanisms involved in the differentiation and development of IPCs is critical in the cell type conversion assays. In this regard, microRNAs (miRNAs) are important molecular tools, which post-transcriptionally regulate the expression of genes involved in IPCs differentiation and development. The present in silico study was designed to analyze and identify the effective miRNAs that control the differentiation of pluripotent stem cells (PSCs) into IPCs.

Methods: To do this, a list of 8 effective genes (*Sox17, FoxA2, Pdx1, Nkx6.1, Ptf1a, Sox9, Ngn3*, and *Neurod1*) that promote the differentiation of PSCs into IPCs was obtained from previous studies. All possible miRNAs, which target 3′-UTR of the selected genes, were separately predicted by using miRWalk and miRmap tools and then sorted based on the miRNAs which target common sequences. Finally, the miRNAs which target the greatest number of genes selected from the list.

Results: The results of this in-silico analysis presented 3 possible miRNAs. These 3 miRNAs inhibited the expression of *Sox17, FoxA2, Ptf1A, Sox9,* and *Neurod1* genes, which are important for the differentiation of PSCs into IPCs. In this regard, it found that miR-3529 targeted Sox17, FoxA2, *Ptf1A,* and *Sox9*. Moreover, results indicated that miR-5011 inhibited the expression of *Ptf1A, Sox9,* and *Neurod1*. Our results also showed that miR-4775 targeted *Sox9, Pdx1,* and *Neurod1*.

Conclusion: In conclusion, the results of this in silico study provide a prospect to design experimental studies for examination of the genuine activity of the identified miRNAs and their effects on the expression of genes that control the differentiation of PSCs into IPCs

Keywords: Insulin-producing cell, Pluripotent stem cell, Differentiation, miRNA, Bioinformatics

PS-078. Investigation of the Relationship Between Mir129a and Mir149 with the Ability to Differentiate Umbilical Stem Cells Towards

T Lymph Nodes

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Background and Aim: Micro-RNAs are small non-coding RNAs that are about 19 to 24 nucleotides long and have an effect on RNA inhibition, post-transcriptional expression, and gene expression. Micro-RNAs count for about 3 percent of the total human genome and RNAs play an important role. In the regulation of immunological functions, including acquired immune responses, the development, and differentiation of immune cells and self-healing. These small regulators of expression of genes have specific patterns for expression, such as a specific pattern of expression in particular tissues, as well as in terms of differentiation, illness.

Methods: Separation of CD133+ stem cells by MACS was performed. After 9 days, the tendency towards T lymphocytes with CD4 and CD8 markers was performed by flow cytometry. Real-time PCR and RT-PCR were used for the analysis of microRNAs expression. Finally, the data were analyzed by SPSSV.24 software.

Results: The results showed that expression of Mir129a with a mean of 5823 with a standard deviation of 395 and Mir149 with a mean of 5769 with a standard deviation of 162 in treated cells was higher than that of non-transfected cells. Also, the expression level of CD4 markers and CD8 in hematopoietic stem cells that are adjacent to the virus lacking the desired fragment is much less than cells treated with these two markers.

Conclusion: Based on these results, with the increased binding of Mir129a and Mir149, the ability to differentiate to T lymphocytes in the CD133 hematopoietic stem cells isolated from the umbilical cord blood is greater.

Keywords: Mir129a; Mir149; T-lymphocyte; Stem Cell; Umbilical cord

PS-079. Proliferation of Human Mesenchymal Stem Cells on PLLA-Nisin

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Background and Aim: Tissue engineering is of growing interest in biomedical research. Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into the osteocyte, adipocyte, chondrocyte cell type. Recently Scientists have been attracted by stem cells. Three types of stem cells include totipotent, pluripotent, multipotent that have a capacity of division, self-renewal, and proliferation and also these cells can differentiation to some cell lines, such as hepatic, kidney and etc. In addition, there have been significant advances in the development of bone scaffolds with various compositions and three-dimensional configurations using a variety of techniques.

Methods: The biodegradable poly-L-lactic-acid (PLLA) nanofibers have been widely studied for its applications in tissue engineering and controlled release systems because of its good processability, good biocompatibility, and suitable mechanical properties. Probiotics as a kind of alive microorganisms that have different types. In the health of human, Bifidobacterium and Lactobacillus have a more effective role than others. Of course, the underlying mechanisms probiotic properties normally depend on the relationship of probiotics with other microorganisms or to the cross-talk of probiotics with host cells. In the present study, poly-l-lactic-acid (PLLA) was fabricated by electrospinning and treated with an O_2 plasma to enhance surface hydrophilicity, cell attachment and growth potential.

Results: Scaffold characterization was done by scanning electron microscope (SEM). Then, human bone marrow mesenchymal stem cells were pre-condition with nisin and cultured on PLLA nanofibers. Cytotoxicity assay and proliferation of MSCs-nisin were determined by MTT assay and acridine orange staining. MTT assay showed that the proliferation of MSCs-nisin increased on PLLA nanofibers and acridine orange staining confirmed the biocompatibility of PLLA-nisin.

Conclusion: In conclusion, it was demonstrated that pre-conditioned MSCs with nisin on PLLA scaffolds could serve as a suitable condition to support the proliferation of MSCs in vitro for use in bone tissue engineering.

Keywords: PLLA, Nanofiber; Nisn; Pre-conditioned MSCs; Proliferation

PS-080. Graphene Oxide Dysregulates MiR-21 Expression in Breast Cancer MCF-7 Cell Line

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Background and Aim: Inhibition of microRNA-21 (miR-21) has emerged as a promising therapeutic strategy for many malignancies including breast cancer. Graphene oxide (GO) has been used as a suitable carrier for drug delivery due to its' unique properties which include large surface area and capability of chemical modifications. However, the molecular basis for in vivo and in vitro GO toxicity has been remained unclear till now. Recent studies have detected changes in miRNAs expression after exposure to GO. In this study, the cytotoxicity effect of GO on the expression of miR-21 in MCF-7 cells has been investigated.

Methods: The cytotoxicity of GO was quantitatively evaluated by MTT assay. MCF- 7 Cells were seeded in 96-well plates and were treated with different concentrations (5,10, 20, 40, 80, 160 µg/mL) of GO for 24 h. Then cells were incubated with GO for 24 hours and were stained with PI and Hoechst 33324 and analyzed by fluorescence microscopy. Furthermore, the expression of miR-21 was analyzed in incubated MCF-7 Cells with GO by Real-Time PCR technique.

Results: Our results indicated that GO has no cytotoxicity in MCF-7 cells up to a concentration of 20 µg/mL. Fluorescence imaging analysis of Hoechst and PI staining revealed that GO did not induce cell apoptosis in the concentration of 15 µg/mL. Real-time PCR result indicated that GO increased the expression of miR-21 in MCF-7 cells.

Conclusion: Although our results showed too little apoptosis in breast cancer MCF-7 cell line exposed to GO at a non-toxic concentration of 15µg/mL, we found induction of miR-21 expression after GO treatment. Considering that inhibition of miR-21 is the therapeutic goal, new strategies such as chemical modification are needed to eliminate the positive effect of GO on miR-21 expression.

Keywords: Graphene oxide; MCF-7 cells; miR-21; Apoptosis

PS-081. Functional Convergence of Akt Protein with VEGFR-1 in Human Endothelial Progenitor Cells Exposed to Sera from Patient with Type 2 Diabetes Mellitus

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Background and Aim: Diabetes mellitus type 2 predisposes patients to various microvascular complications

Methods: In the current experiment, the potent role of diabetes mellitus

was investigated on the content of VEGFR-1, -2, Tie-1 and -2, and Akt in human endothelial progenitor cells. The gene expression profile of mTOR and Hedgehog signaling pathways were measured by PCR array. The possible crosstalk between RTKs, mTOR and Hedgehog signaling was also studied by bioinformatic analysis. Endothelial progenitor cells were incubated with serum from normal and diabetic for 7 days.

Results: Compared to non-treated cells, diabetic serum-induced cell apoptosis (~2-fold) and prohibited cell migration toward bFGF ($P < 0.001$). ELISA analysis showed that diabetes exposed cells had increased abundance of Tie-1, -2 and VEGFR-2 and a reduced amount of VEGFR-1 ($P < 0.0001$) in diabetic cells. Western blotting showed a marked reduction in the protein level of Akt after cells exposure to serum from diabetic subjects ($P < 0.0001$). PCR array revealed a significant stimulation of both mTOR and Hedgehog signaling pathways in diabetic cells ($P < 0.05$). According to data from bioinformatic datasets, we showed VEGFR-1, -2 and Tie-2, but not Tie-1, are master regulators of angiogenesis.

Conclusion: There is a crosstalk between RTKs and mTOR signaling by involving P62, GABARAPL1, and HTT genes. It seems that physical interaction and co-expression of Akt decreased the level of VEGFR-1 in diabetic cells. Regarding data from the present experiment, diabetic serum contributed to uncontrolled induction of both mTOR and Hedgehog signaling in endothelial progenitor cells. Diabetes mellitus induces mTOR pathway by involving receptor tyrosine kinases while Hedgehog stimulation is independent of these receptors

Keywords: Human endothelial progenitor cells, Diabetes mellitus, Receptor tyrosine kinases, mTOR and Hedgehog signaling pathways

PS-082. Nanoencapsulation of Rosa Damascena Mill L. Extract Enhanced Its Local Analgesic Effects on Cornea of Rats.

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Background and Aim: *Rosa damascena* mill L. extract (RDE) is commonly known as "Gole Mohammadi" in Iran is widely used as cosmetic and pharmaceutical adjuvant because of its analgesic, antibacterial, antioxidant and anti-inflammatory properties. In order to enhance the bioavailability of RDE, niosomes encapsulating RDE were prepared and examined for topical analgesic properties on the cornea.

Methods: RDE-loaded liposomes were formulated and characterized in terms of their morphology zeta potential and entrapment efficiency. The niosomes were a diameter of less than 150 nm and remained well dispersed for at least 2 weeks. The obtained RDE niosomes were examined for analgesic effects on rat cornea before application of hypertonic saline. Corneal and retinal tissues were evaluated under a light microscope after H&E staining.

Results: Niosomal RDE significantly showed more analgesic potential than RDE alone. In addition, compared to the free niosome, RDE niosomes significantly increased the analgesic efficacy. Accordingly, the histological results coreanal care properties of RDE will be significantly enhanced by niosome encapsulation and also no retinal side effects were observed.

Conclusion: The present niosomal RDE should have a great potential as an effective natural formulation for induction of corneal local analgesia.

Keywords: *Rosa damascena* extract; Nanostructures; Analgesia; Cornea; Topical; Rats

PS-083. The In Vivo Effects of Ischemic Renal Tissue Conditioned Medium Mesenchymal Stem Cell Therapy in Acute Ischemic Kidney Injury

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Background and Aim: Renal diseases are a common condition in the general population and resulted in significant morbidity and mortality. Acute kidney injury (AKI) occurred in 8-16% of hospital admissions. The results of recent studies showed mesenchymal stem cells (MSCs) can improve renal ischemia-reperfusion injury (IRI), glycerol and cisplatinum model of AKI, glomerulonephritis model, Alport's syndrome, and diabetic nephropathy. The aim of this study was to test the therapeutic potential effects of administration of conditioned medium (CM) treated MSCs, immediately after reflow in a rat model of IRI/AKI.

Methods: MSCs were generated from the femur and tibia of 6 weeks old male Wistar rats. MSCs morphology and phenotype were confirmed by differentiation and FACS analysis. Passage 3-4 was used in all experiments. CM was prepared from the supernatant of rat kidney that undergoes 40 minutes of ischemia. MSCs were treated by 50 µg/mL CM for 24 hours. Adult male Wistar rats weighing 250–300 g were used. The rats were randomly assigned to the following groups: IRI, IRI with MSC treated, and IRI with CM-MSC treated. After laparotomy, renal pedicles were clamped for 40 minutes, and after reflow, ~2×10^6 MSCs were injected systemically via the supra-renal aorta. Blood samples were collected at baseline and day 1 post-IRI.

Results: MSCs were confirmed by morphology, differentiation and FACS analysis. 40 min of clamping in animals led to severe renal insufficiency, as evidenced by a rise in serum Cr to 3.41±0.22 mg/dL and serum BUN to 86.8±6.65 mg/dL in IRI rats at 24 h post-ischemia. Animals infused with MSC and CM-MSCs, had significantly lower serum Cr, 1.04±0.08 and 0.98±0.09 mg/dL and BUN levels, 58.9±3.96 and 55.2±6.65 mg/dL respectively at 24 hours after cell injection compared with IRI animals. However, the serum Cr and BUN levels were slightly decreased in CM-MSCs treated animals compared with MSC treated; the differences were not significant.

Conclusion: This work provides further evidence that MSCs might have substantial protective effects in IRI/AKI rat model. Accordingly, with our results, there might not be any transdifferentiation events of MSCs within the first day and the protective effects occur via differentiation-independent mechanisms. The CM contains substances that affect the migration of MSCs toward the injured tissue. Take all, our findings showed the kidney-protective effect of MSCs in IRI/AKI experimental model. We also witnessed the effects of ischemic renal tissue on in vitro MSCs properties. We believe more studies are required to clarify the mechanism responsible for this recovery.

Keywords: Mesenchymal stem cell; Acute kidney injury; Conditioned-medium; Cell therapy

PS-084. Overexpression of Stem Cell Protein, PIWIL2, Promotes the Invasive Characteristics of DU145 Prostate Cancer Cell Line

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Background and Aim: Prostate cancer is the second cause of cancer death in the world. Invasion of malignant tumor cells via epithelial-

to-mesenchymal transition (EMT) is the main cause of death in cancer patients. The stem cell protein, PIWIL2, is a member of the Argonaute family, plays an important role in the EMT process by controlling and enhancing the invasive properties of cells. In this study, we aimed to examine the effect of overexpression of piwil2 gene and its effects on EMT process in the prostate cancer cell line, DU145.

Methods: DU145 cell line was cultured in the RPMI media containing 10% fetal bovine serum (FBS) with 1% penicillin-streptomycin (Pen-Strep) suggested by ATCC. In order to provide DU145-PIWIL2 cell line, these cells were transfected by PCDNA3 vector expressing human piwil2 gene driven by CMV promoter via electroporation method. To provide DU145-mock cells, PCDNA3 empty vector was transferred to the cells via electroporation. G418 was then applied to the transfected cells for 4 weeks to establish stable cell lines before further cell and molecular analysis. To investigate the effect of *Piwil2* gene overexpression on cell growth, the doubling time method was performed to compare the cells growth rate between DU145-PIWIL2 and DU145-mock cells. Moreover, the expression of EMT biomarkers was investigated in the two cell lines using RT-PCR and Real-Time PCR.

Results: The doubling time analysis profiled a significant increase in DU145-PIWIL2 cells growth rate compared to DU145-mock cells, exhibiting 2.31 times higher growth in the cells overexpressing PIWIL2. Quantitative real-time PCR indicated changes in some canonical EMT biomarkers including E-cadherin, Vimentin, and Snail, with 0.77 decreases in E-cadherin expression, as well as 1.36 and 2.01 increase in Vimentin and Snail expressions respectively.

Conclusion: In this study our data showed that overexpression of *Piwil2* gene promoted the growth rate of DU145 cells and induced the EMT process in these cells, hence enhancing the invasive potential of cancer cells. Therefore, these data profiled piwil2 as an important biomarker in assessing the malignant state of prostate cancer with application in procedures related to cancer diagnostics and targeted therapies.

Keywords: Piwil2; DU145; Prostate cancer; Epithelial-to-mesenchymal transition

PS-085. Neuroprotective and Neurodifferentiative Properties of Ferulic Acid

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Background and Aim: Ferulic acid (FA) is a phenolic compound with known anti-apoptotic and anti-oxidative properties. Effect of FA on the induction of neurogenesis and neuro-differentiation beside its effect on neuroprotection makes this compound very interesting for stem cell researches. We designed this set of experiments to evaluate the effect of FA on neuronal differentiation and neuroprotection.

Methods: ROS-mediated apoptosis was induced on PC12 cells by using hydrogen peroxide then with the treatment of different concentrations of FA was started. To evaluate the differentiation-inducing the effect of FA, PC12 cells and mouse neural stem cells (mNSCs) were treated with different concentrations of FA. MTT, quantitative real-time RT-PCR and immunostaining assays were performed on cells.

Results: FA treatment at low concentrations significantly reduced the apoptosis rate in treated PC12 cells. Real-time RT-PCR and western blot assays confirmed that FA revealed this effect through stabilization and degradation of P53 by increasing the expression rate of SIRT1, SIRT7 and MDM2 and down-regulation of USP7. Beside this anti-apoptotic effect, FA treatments on PC12 cells and mNSCs at higher concentrations on PC12 cells and mNSCs increased their differentiation toward mature neurons. Immunocytochemical staining against beta-tubulin III and Map2 verified the presence of mature neurons, and western blot assay on FA treated PC12 cells showed a stepwise rise of phosphorylated-ERK1/2 as the concentration of FA was increased.

Conclusion: our findings showed that FA at low concentrations has a neuroprotective effect through up-regulation of SIRT1, SIRT7, and MDM2, and in higher concentrations can promote neural differentiation and neurite outgrowth.

Keywords: Ferulic Acid, PC12 Cells, Apoptosis, Survival, Neuronal differentiation

PS-086. Investigating Cytotoxic and Anticancer Properties of Coumarin Derivatives Contain Geranyl Groups at Different Position on Cervical Cancer

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Background and Aim: Coumarin compounds have been known to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antimicrobial, anti-arrhythmic, anti-osteoporosis, antiviral, and anticarcinogenic activities. Due to this wide variety of pharmacological values, coumarins and its derivatives have received more attention in synthesis and production.

Methods: In the present study, we aimed to investigate the effect of geranyl position on coumarin backbone on the cytotoxicity of these synthetic compounds. To do so, first, the cytotoxic effect of 3-geranyloxycoumarin (3-GC), 4-geranyloxycoumarin (4-GC), 5-geranyloxycoumarin (5-GC), 6-geranyloxycoumarin (6-GC), 7-geranyloxycoumarin (7-GC) and 8-geranyloxycoumarin (8-GC) were assessed by MTT assay on HeLa (cervical cancer) and HDF (human dermal fibroblast) cells. Furthermore, the apoptosis-inducing potential of these coumarin compounds was determined by flow cytometry.

Results: The results of the MTT assay revealed that 3-GC, 4-GC, 5-GC, 6-GC, and 8-GC had significant cytotoxic effects on HeLa cells. While they did not show any significant cytotoxicity on normal HDF cells. Moreover, the flow cytometric analysis, using PI/ FITC-Annexin V, showed that 3-GC, 4-GC, 5-GC, 6-GC and 8-GC induced apoptosis in HeLa cancer cells. Thus, they can be used as potent anticancer compounds.

Conclusion: Altogether, our results indicate that changes in the level of cellular toxicity on HeLa cells are associated with the position of the geranyl group; which puts an emphasis on the importance of the relationship between structure and activity in coumarin compounds. Our results showed that position 6 was the best site for the geranyl group. Further studies on various cancer cell lines are required to confirm these findings and future studies need to be focused on the molecular mechanisms of toxicity inducing potential of these compounds both in vitro and in vivo.

Keywords: Cervical cancer; Anticancer; Geranyloxycoumarin

PS-087. Quantitative Efficiency Comparison of Mouse Embryonic Fibroblast Isolation Methods

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Background and Aim: Fibroblasts are spindle-shaped cells that derived from mesenchymal stem cells. Because of their ability to produce nutrients and growth factors such as LIF and b-FGF, these cells are used as a feeder layer for maintaining stem cell pluripotency and self-renewal. In this study, we compared the cell yield of two enzymatic and mechanical methods for mouse embryonic fibroblast isolation (MEF).
Methods: All animal experiments of this study were approved by the ethics community of the university. A pregnant BALB/c mouse was sacrificed and the embryos were isolated following uterine horn removing. After removal of head and red organs, the embryos were divided into two groups of equal numbers (n=5). The first embryo group was minced by razor, incubated with trypsin 0.05% for 10 min and then centrifuged at 12 000 g for 5 minutes. Finally, the pellet was re-suspended in MEF medium, and transferred to T25 culture flask. The second embryos group was minced and passed 10times through a syringe with needle 22G. The obtained cells were washed by PBS, and then cultured as above. After 24 hours, the cultured cells were trypsinized and counted using Neobar lam.
Results: Viscous and clumpy materials were observed in the enzymatic method, but not in the mechanical method. In comparison to the enzymatic method, the mechanical method using needle 22G yielded a significantly higher number of cells. The obtained cell number after 24hours culture were 5×10^4 and 1.5×10^6 cells/flask for enzymatic and mechanical methods, respectively.
Conclusion: The mechanical method provided higher numbers of cells than the enzymatic method. Probably, MEFs are trapped in the viscous material of the mechanical method, due to nucleic acid-triggered clotting, making it difficult to isolate these cells.
Keywords: Pluripotent stem cells; Feeder cells; Cell separation

PS-088. Molecular Signatures of iPSC Derived Cardiomyocytes Highlight Sex-Specific Differences

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Background and Aim: Men have a higher risk of cardiovascular disease than women, but the molecular and physiological mechanisms remain unclear. We have previously reported the differentially expressed genes and pathways between male and female heart tissue using donor and postmortem adult human heart samples. Here, using RNA-Seq results, we compared the transcriptome expression profile of male and female iPSC-derived cardiomyocytes (iPSC-CM).
Methods: We compared 5 female iPSC-CM versus 11 male iPSC-CM. All iPSCs generated using Sendai virus technique and differentiated into cardiomyocytes using a small-molecule-based monolayer method. cDNA libraries for 16 samples were generated and sequenced. We used these RNA-Seq data that were publicly available in the NCBI GEO database. RNA-Seq analysis was performed using tuxedo pipeline.
Results: RNA-Seq results indicated that 1811 genes differentially expressed between male and female iPSC-CMs. We found 1114 genes upregulated in male and 700 genes upregulated in the female. From 87 differentially expressed X- linked genes, 44 genes overexpressed in female and 43 overexpressed in the male. Fourteen Y-linked genes expressed in all-male iPSC-CMs. RNA-Seq results indicated very low expression of pluripotency markers (Nanog, POU5F1, etc) and high expression of cardiac marker genes (TBX5, TTN, etc) in all iPSC-CMs. Gene ontology analysis indicated the overexpression of cardiac contractile genes (ventricular myocyte phenotype genes and atrial myocyte phenotype genes), adhesion molecules and calcium signaling pathway genes in male iPSC-CMs and overexpression of Pluripotency markers and Wnt/-catenin pathway genes have been observed in female iPSC-CMs.
Conclusion: The gene expression profile of iPSC-CMs reveals gender-specific differences. RNA expression results showed that male iPSC-CMs are more similar to differentiated cardiomyocytes and female iPSC-CMs are more similar to neonate cardiomyocytes. Due to sex differences in gene expression profile of iPSC-CMs, we recommend using both male and female cells in all biological studies.
Keywords: iPSC derived cardiomyocytes; RNA-Seq; Differentially expressed genes; Pluripotency

PS-089. Construction of Validating Vectors for Investigation of MSI2-miRNA Direct Interactions

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Background and Aim: MSI2 is one of the members of Musashi family, considered as key regulators in the maintenance and self-renewal of stem cells. MSI2 higher expression level is associated with poor prognosis of leukemia. It has been shown that myeloid leukemias are very diverse diseases that are associated with expression aberrations of some microRNAs. According to previous studies, knocking down of MSI2 sensitizes cancer cells to treatments. Therefore, MSI2 can be considered as a promising target for cancer treatment. In this study, several targeting miRNAs for MSI2 were predicted by bioinformatic studies.
Methods: Prediction of MSI2-targeting miRNAs was performed by several online bioinformatic software such as TargetScan, miRWalk, PicTar, and miRDB. 3′UTR of MSI2 (5.1 kb) and precursors of predicted miRNAs were amplified by specific primers. Amplified fragments were subcloned into pTZ57R/T vector. After sequencing analysis, 3′UTR of MSI2 and miRNA precursors were subcloned into psiCHECK-2 and pBud-EGFP, respectively. Also, the 3′UTR fragments containing mutations in miRNA-binding sites for each miRNA were amplified using SOEing PCR and subcloned into psiCHECK-2 vector separately. Last constructs are being used as the negative controls. The accuracy of constructed vectors was confirmed with restriction digestion.
Results: According to bioinformatic studies, the highly conserved miRNAs were confirmed. The miRNAs were selected according to the results of different software as the candidates for further studies. Precursor structure of each selected miRNA was subcloned successfully in the pBud-EGFP vector. Also, the 3′UTR of MSI2 gene was subcloned into psiCHECK-2 downstream of Renilla luciferase in order to apply luciferase reporter assay. These constructions will be used to explore the direct interactions between selected miRNAs and 3′UTR of MSI2 in HEK293T cells.
Conclusion: Some of the predicted miRNAs were previously reported as the tumor suppressors in hematopoietic malignancies. Based on bioinformatic data, we predict that the selected miRNAs have the potential to directly target MSI2 gene. For verifying this capability, the aforementioned vectors should be employed to transfect both HEK293T and hematopoietic cells.
Keywords: MSI2; miRNA; Myeloid leukemia

PS-090. Imaging of Lung in Following Stem Cell Therapy in The Preclinical Study

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Background and Aim: Acute respiratory distress syndrome has a poor prognosis. ARDS is a non-homogeneous combination of different

disorders sharing some clinical and radiological abnormalities and requiring the development of novel therapies. Radiographic findings of ARDS are non-specific and cannot identify it from typical pulmonary edema or hemorrhage. Chest x-ray features usually develop 24 hours after initial lung damage. In contrast to cardiogenic pulmonary edema, ARDS persists for days to weeks so that ct-scan plays key role in the findings of ARDS depend on the phase of the disease and can help us in diagnosis and treatments.

Methods: Adult Sall sheep (10 male) were selected and divided into two groups. In the treatment group, bone marrow stem cell (BM-MSC) was isolated and expanded. An experimental model of ARDS was induced by endotracheal endotoxin of E. coli strains-O55: B5 infusion and confirmed after 24 hours of ARDS-induce, 5×10^7 cells/sheep were aoutologically engrafted with the tracheal catheter. For evaluate effects of cell therapy, the thoracic computerized tomography (CT-scan) from the lung of all anesthetized sheep was taken by the aid of Somaris Spirit class II (Siemens) before and at 12, 24, 48, 72 and 168 hours after MSCs transplantation or PBS infusion. Quantitative estimation was done for different adjacent CT sections with Siemens Leonardo workstation and software tools. Lung parenchymal margin was manually demarcated and then average Hounsfield unit and volume obtained for each section.

Results: Hounsfield unit and volumes of the aerated and non-aerated area of right and left lungs were measurements in CT-scan images. The measure demonstrated the Hounsfield unit had increased one day after ARDS inducing that the present's replacement air with mucus and inflammatory cells. A significant decrease in the Hounsfield unit found at hours of 168 (P=0.028) post-transplantation in the treatment group compared with inflammation time and in 72 (P=0.012) and 168 (P=0.036) times compared with the control group. Also, variation volumes were compared and showed that total lung volume in both groups increased after inflammation, but transplantation of BM-MSCs had decreased the process at 168 (P=0.013) time in compared with inflammation time and at 72 (P=.030) and 168h (P=0.011) times compared with control group. In the acute phase of ARDS, CT scans typically show a non-homogeneous distribution and a ventrodorsal gradient of density.

Conclusion: CT scans were provided additional information that not obvious on bedside chest radiographs and led to a change in management in animal modeling. Moreover, CT was helped us to understand the pathophysiology of ARDS and the complex interplay between lung parenchyma and mechanical ventilation. According to our findings, a CT scan will be able to follow and integrate clinical evolution of cell treatment in ARDS, and it is certainly a worthwhile data.

Keywords: ARDS; CT-scan; Stem cell therapy

PS-091. Analysis of the Blood Differential Test for the Prognosis of Cell-Based Therapy in Acute lung Inflammatory Phenotypes

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Background and Aim: Acute respiratory distress syndrome is a heterogeneous syndrome that produces an inflammatory response, such as elevated WBC and this can lead to acute organ dysfunction. Until now any drug has proved beneficial in the prevention of ARDS. Mesenchyme stem cells are the best-described cells and the most used as a cell therapy. Of the various cell-based therapy options, BM-MSCs having the most efficacy for lung injury. Analysis of blood samples is typically one of the first steps in diagnosing and is an important part of the research to provide useful information to in diagnosis and management.

Methods: This study was excluded from ten healthy Shall Sheep in two groups of control and treatment. Bone marrow samples were collected from the treated group, and MSCs were isolated and cultured. Then an experimental model of ARDS was induced by endotracheal administration of E.coli-lipopolysaccharides strains O55: B5 and inflammation confirmed. After 24 hours of ARDS, 50×10^6 BM-MSCs were autografted in the treatment group, and PBS was injected in the control group intrapulmonary. Venous blood samples were collected in EDTA anticoagulated tubes before and after therapeutic induction. Hematological parameters were assessed with an automated analyzer include the hematocrit, erythrocytes, hemoglobin, platelets, white blood cells (WBC), heterophile, eosinophils, monocytes, basophils, and lymphocytes immediately. The CBC results were available from the animal model at the time of the 3, 6, 12, 24, 48 and 168th hours after cell-based therapy. The results are expressed as mean±SD for continuous variables. Data analysis was performed using SPSS software.

Results: Factors affecting levels during treatment were a determined, and inflammatory response such as elevated white-cell count, a segmented neutrophil count showed a significant increase in the two groups after inducing an experimental model of ARDS. In the treatment group, reduction of WBC and segmented neutrophil count were significant at times 24, 48, 72 and 168 hours in compared with baseline and also, in compared with the control group at times 24, 48, 72 and 168 hours. Reduction of band cells was significant at 24, 48, 168 hours compared with the control group. Also, the Lymphocyte counts at 24 hours and monocyte counts at 72 and 168 hours were significant. Statistical analysis in the hematocrit, platelets, eosinophil, basophile showed no important difference between the two groups at different times.

Conclusion: Analysis of clinical blood samples is typically one of the first steps in diagnosing disease. It has the potential when interpreted carefully and about the clinical history, to provide very useful information to assist in diagnosis and management. In this research abnormalities in the complete blood count were confirmed in the inflammation time so that the microscopic review of blood smears from sheep to detect band cells or segmented neutrophil change is valuable and could help in assessing disease severity and prognosis in cell therapy approach.

Keywords: BM-MSCs; ARDS; Blood differential test

PS-092. A New Strategy for Cancer Therapy by Conditioned Medium Derived From Human Amniotic Epithelial Stem Cells

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Background and Aim: Human amniotic membrane (hAM) is the innermost layer of fetal membranes, which surrounds the developing fetus and forms the amniotic cavity. hAM and hAM-derived cells possess unique properties that make them excellent candidates for use in regenerative medicine, such as low immunogenicity, promotion of epithelization, anti-inflammatory and antimicrobial properties as well as angio-modulatory, anti-fibrosis and anticancer properties. We examined the effect of human amniotic epithelial cells- conditioned media (hAECs-CM) on cancerous cell lines proliferation.

Methods: Human placentas were obtained at term pregnancy during Caesarean sections from women with negative for HIV-I, and hepatitis B and C. The amnion was manually separated from the chorion and washed extensively in PBS. The epithelial cells were isolated enzymatically. Briefly, amnion was treated with 0.15% trypsin-EDTA for 15 and 40 min

at 37°C. Tissues were then filtered through a 70µm cell strainer to collect single cell suspension and centrifuged at 1200 rpm for 10 min. The cell pellet was resuspended and cultured in flask 25 in complete culture media. The medium was changed once after the density of cells reached 85% confluency, and hAEC-CM was collected 48 h later while cells were growing exponentially. The hAEC-CM were then collected, 0.22 mm filtered, and used in subsequent experiments. Four different cancer cell were seeded in 96-well tissue culture plates. After 24h the culture medium was exchanged with hAEC-CM. After 48h of the incubation, cell viability was determined by the MTT assay.

Results: Percentage cell viability of breast cancer cell lines includes MDA-MB-231, MCF-7, and BT-474 recorded by 67%, 73%, and 56%, respectively. Amongst four cell lines, Hela (cervical cancer cells) showed the least viability by 38%.

Conclusion: Our results indicated that hAECs-CM able to inhibit the proliferation of cancer cells. Taken together, these results provide strong evidence that hAECs-CM can be used as a safe and effective cancer-targeting cytotherapy for treating breast and cervical cancer.

Keywords: Human amniotic membrane; Cancer; Stem cell; Viability

PS-093. The Effects of Chitosan-Gelatin Scaffold in Differentiation of Wharton's Jelly-Derived Stem Cells to Chondrocyte

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Background and Aim: Osteoarthritis is the most common type of arthritis affected millions of people known as a second debilitating disease. Cartilage tissue engineering composed of different elements as cells, scaffold and chemical factors. Recently Wharton's jelly mesenchymal stem cells (WJSCs) has been introduced as a suitable source for repair of cartilage lesions. These cells have similarities to the cartilage extracellular matrix. Among different scaffolds, chitosan-gelatin scaffolds seem to be a good candidate because of their similarities to cartilage ECM and anti-bacterial properties. So, the aim of the present study is to investigate whether WJSCs can differentiate into chondrocyte on chitosan-gelatin scaffolds.

Methods: Chitosan-gelatin scaffolds fabricated by freeze-drying technique. WJSCs at fourth passage were delivered on to chitosan-gelatin scaffolds and then treated with chondrocyte differentiating medium for 10 days. Then, after samples were prepared for evaluating gene expression profile using real-time RT-PCR. The viability of WJSCs on this scaffold also was evaluated using MTT technique. Attachment of cells to the scaffolds was discerned by SEM. Fourier transform sub-spectrometry (FTR-IR) for qualitative and quantitative identification of compounds and determination of the functional group and the links in the sample is being used.

Results: MTT assay technique revealed the viability of WJSCs on chitosan-gelatin scaffolds. The high porosity of scaffolds was detected using scanning electron microscopy. Real-time RT-PCR showed that the gene expression profile of collagen type II, Sox-9 and comp was significantly higher compared to the monolayer culture. Gene expression profile of aggrecan was higher in monolayer culture. The position of absorption bands for functional groups is in very good agreement with the FT-IR data of chitosan-gelatin scaffolds, which has been reported in previous studies.

Conclusion: Chitosan-gelatin scaffolds can be introduced as a good candidate for supporting the differentiation of WJSCs into chondrocytes.

Keywords: Osteoarthritis; Chondrocyte; Scaffold; Wharton's jelly; Stem cells

PS-094. FOXO3 Expression as a Predictive Factor in Developing Chronic Myeloid Leukemia Imatinib Resistance

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Background and Aim: At now the major obstacle on the way of CML treatment is TKIs resistance patients. So many mechanisms have been proposed. One of the major targets of ABL kinase is FOXO3 that introduced as a mechanistically driver of CML initiating leukemia cells maintaining. We hypothesized that TKI resistance can be predicted by evaluating the FOXO3 expression level.

Methods: In this study, we have explored the expression of FOXO3 and its activator, Sirt-1, transcripts in CML responder to Imatinib in compared with non-responder samples in GEO datasets. Moreover, we have studied the correlation between FOXO3 and Sirt1 expression.

Results: The expression FOXO3 was significantly ($P<0.007$) lower in CML nonresponders compared with Imatinib responders (7.9±0.4 vs 8.6±0.49, respectively) but did not show any significant correlation with Sirt1. Moreover, the expression of Sirt-1 has no significant differences between the samples.

Conclusion: Our findings in line with previous studies confirmed that response to TKIs down-regulated the FOXO3 expression. Moreover, we have found that monitoring of FOXO3 expression could predict TKIs resistance development.

Keywords: Leukemia; Myelogenous; Chronic; BCR-ABL positive

PS-095. Fibroblast-Myofibroblast Crosstalk after Exposure to Mesenchymal Stem Cell Secretome

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Background and Aim: Mesenchymal stem cells have a long-term clinical application and widely used in autoimmune disease and regenerative medicine. However, some MSCs derived cytokines such as TGF-β could have a dual role in suppression or progression of the disease. Fibroblast activation and extracellular matrix production are two key features of wound healing and contrivers in fibrogenesis. In this study we consider the role of MSC derived condition media as a source of MSc derived secretome on human fibroblast activation.

Methods: To isolate human stem cells, bone marrow was collected from a healthy donor. After separating cells and cultured on DMEM/ FBS10%, cells with fibroblast shape were isolated as mesenchymal stem cells. To characterize the MSC, flow cytometric assays for surface receptor molecule expression and differentiation were performed. Human foreskin fibroblast (HFF) was cultured under standard condition. To collect MSC-CM, cells were cultured in the serum-free media for 72 hours. HFF cells were plated at a seeding density of 2×10^5 cells/well in a 6-well plate and after 24 hours they were treated with MSC-CM and exogenous TGF beta was applied as a positive control. Total RNA from samples was isolated and purified using the RNeasy mini kit (Qiagen). Collagen and alpha-SMA expression as a fibrotic marker was determined by RT-PCR. Expression levels of all genes were analyzed according to comparative

analysis method by Rest and GraphPad software.
Results: Flow cytometry and differentiation methods identified cells as MSc with purity of 98%. MSc markers including CD73, CD90, and CD105 were positive and CD14, CD34 and CD 45 reported as negative. After 7 2h treatment of HFF by MSc conditioned media, alpha-SMA showed 2-fold upregulation in comparison with control. Collagen III as a second fibrotic marker also showed a significant increase of mRNA ($P<0.05$). TGF-β also showed similar results compared to TGF-B and condition media, it was shown; MSC-CM unregulated the expression of collagen III and alpha-SMA target genes which are involved in fibrosis and known as a myofibroblast marker.
Conclusion: MSCs assumed to be a reliable source of stem cells in clinical application. Our data demonstrate that MSC produces cytokines that stimulate matrix production. It will be essential to determine whether these factors can play a role in attempts to use MSC for therapeutic approaches. These data suggest that MSC can significantly stimulate the differentiation of fibroblast cells to myofibroblasts which has a pivotal role in the onset of a Fibrogenesis. However, there is a deal to be learned about the mechanism through which MSC play a role in the development of the disease.
Keywords: MSC-secretome; Fibroblast; TGF-B; αSMA; Collagen III; Myofibroblast

PS-096. Niosomal Nano-Curcumin-Induced Cell Cycle Arrest and Apoptosis in Brain Cancer Stem Cells

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Background and Aim: Glioblastoma multiforme (GBM) is the most malignant tumor with a high mortality rate. An important reason for the high recurrence of the tumor is the presence of cancer stem cells (CSCs), which is thought to be the ''root'' of growing tumors. Targeting these cells may provide a useful therapeutic approach. Niosomal nano-curcumin is synthesized from curcumin, an anti-tumor compound, assumed to inhibit cancer stem cells survival. The current study was aimed to investigate the apoptotic role of niosomal nano-curcumin in stem cells isolated from the brain tumors of patients with glioblastoma.
Methods: The cancer stem cells were isolated from the tumor tissue sample. The MTT cell viability assay was used to examine IC50 in 24, 48 and 72 hours. The Real-Time PCR assay was also utilized to determine the mRNA expression of BAX-BCL2. Flow cytometric studies using the propidium iodide showed accumulation of cells in the sub-G1 phase.
Results: In this study, IC50 of the nano-curcumin was determined to be 137 μM, 101.4 μM and 32.8 μM in 24, 48 and 72 hours, respectively. Also, we demonstrated that the treatment of GBM stem cells with niosomal nano-curcumin increased the Bax/ Bcl-2 ratio significantly. Additionally, the number of cells in the sub-G1 phase was elevated at 24 ($P<0.05$) hours after treatment.
Conclusion: Based on our data, it is anticipated that niosomal nano-curcumin will perform as a potential therapy owing to improved curcumin delivery and therapeutic efficacy on GBM stem cells. Niozomal nano-curcumin may protect against CSCs by causing cell cycle arrest and inducing apoptosis.
Keywords: Stem Cell, Glioblastoma, Niosomal nano-curcumin, Apoptosis, Cell cycle arrest

PS-097. Bioinformatics Design of the CRISPR/Cas9P300 System for the Purpose of Creating Epigenetic Changes on the Aryl Hydrocarbon Receptor (AHR) Transcription Factor Gene

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Background and Aim: AhR(aryl hydrocarbon receptor) is one of the cellular transcription factors that is inactive in many somatic cells. The chemicals used to activate this transcription factor are largely non-specific. One of the new and practical methods for activating specific genes is the use of the CRISPR/dCas9 system. Scientists add specific components specifically designed for this system to produce epigenetic changes for specific regions that confer epigenetic modifications, and by activating and deactivating genes.
Methods: The AhR promoter region was studied and the areas that have been acetylated were identified with use of UCSC browser software. Then using CRISPR, CHOPCHOP, and E-CRISP software, sgRNA especially was designed for areas close to the sites of the acetylated. In the next step, the measurements were needed to have the lowest off-target and the maximum efficiency. In addition, the CRISPR/Cas9P300 system with maximum efficiency was used to target an area of interest on acetylated DNA and to activate the gene.
Results: One of the newest systems used to create epigenetic changes for gene expression changes is CRISPR/Cas9P300. In this study, new designing of the system proposed to create targeted epigenetic changes on the Ahr gene. With designed sgRNAs that showed maximum efficiency and minimum off-target to create a specific deacetylation on the AhR gene, expression of the gene increased thousands of folds. Designing this system is the most important part of creating changes in the Ahr gene. Designing highly efficient sgRNAs with a very low off-target, fully specific for sites that are acetylated is important in deacetylation and gene-specific activation. For best function, this design must be analyzed by several software. In this study, CRISPR, E-CRISP, and CHOPCHOP software were used, and with this highly specialized design, the goal has been achieved.
Conclusion: The P300 with acetylation, control of transcription machine, and acting as an adapter molecule, acts on target locations. With the precise design of the sgRNAs for the closest locations to the acetylated area, the CRISPR/dCas9P300 system with a high efficiency leads to precise activation of the gene. The most important advantage of this design system is the precision and high specificity of the target area, which can indicate the specific function of the system.
Keywords: Epigenetic; CRISPR/Cas9P300; Acetylation; sgRNA; Aryl hydrocarbon receptor

PS-098. Alteration of Epigenetic Factors in Human Fibroblasts with Aryl Hydrocarbon Receptor (AHR) Activation

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Background and Aim: Epigenetic, alters the gene expression that occurs by making certain chemical changes on the DNA packing proteins (histones) and the DNA itself. These changes play a major role in creating vast differences in gene expression, differentiation of somatic cells, and stem cells properties. Knowing the signaling pathways leading to these changes greatly helps to recognize the mechanism of the effect of

epigenetic changes in AHR activated fibroblastic cells.
Methods: Human fibroblast cell cultured in DMEM/Ham's F-12, Fetal Bovine Serum (FBS) 10%, 2 mmol/L L-glutamine and 100 U/mL penicillin/ streptomycin media and transfer to 5% Co2 incubator with temperature 37°C. Cells were treated either with TCDD (2,3,7,8-tetrachlorodibenzo-P-dioxin) or DMSO. RNA extraction and RNA labeling using Cy3 and cRNA proliferation were performed with Agilent Technologies protocol. Then, using the Microarray system, the transcriptome of the cell groups was investigated. The results were compared using GEO2R software and the upregulation/downregulation of the selected genes was evaluated. Finally, using Reactome software, signaling pathways leading to epigenetic changes were evaluated.
Results: AHR transcription factor activation in human fibroblast cells by TCDD, leads to activation of several pathways involving in epigenetic changes. Studies conducted by Reactome software show that Polycomb Repressive Complex 2 (PRC2) is used in chromatin and lysine 27 methylates histone H3 and uses DNA methyltransferase. TET1,2,3 oxidizes 5-methylcytosine to 5-carboxyl cytosine with oxidation and splits 5 Formyl cytosine and 5-carboxylic cytosine splits. DNA is demethylated and DNA methylation is performed by DNMT3A, DNMT3B, and DNMT3L. Positive epigenetic regulation of rRNA expression is also accomplished by the B-WICH complex and histone acetyltransferase, ERCC6 (CSB) and EHMT2 (G9a). Negative regulation of epigenetic expression of rRNA is also mediated by SIRT1 and NoRC signaling.
Conclusion: Inducing epigenetic alterations in somatic cells causes changes in the transcriptomic level. Activating certain transcription factors leads such epigenetic alterations. By activating AHR transcription factor, large gene expression changes occur, many of which are due to epigenetic changes that the cell goes through the transcription of the totipotency factors and with demethylation, acetylation, and other epigenetic alterations increase the likelihood of stem cell transplantation.
Keywords: Epigenetic factors; Aryl hydrocarbon receptor; Transcription factors; Human fibroblast cell

PS-099. Evaluation of Electrospun Biodegradable Polycaprolactone and Hyaluronic Acid Nanofibers Containing Vitamin C for Tissue Engineering

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Background and Aim: Electrospinning technique is one of the most promising methods for scaffold fabrication to micro and nanoscale in tissue engineering. Hyaluronic acid is a biocompatible polysaccharide which is the main component of the extracellular matrix. Vitamin C is a principal antioxidant in human blood which plays a crucial role in the synthesis of collagen. In this study, polycaprolactone and polycaprolactone/ hyaluronic acid/ vitamin C nanofiber scaffolds were fabricated through electrospinning technique and L929 fibroblast cells seeded on nanofibers scaffolds.
Methods: Polycaprolactone (PCL) and polycaprolactone (PCL)/ hyaluronic acid (HA)/ vitamin C with weight ratios of (100:0), (90:10), (85:15), (80:20) and 40 mg vitamin C were dissolved in formic acid and acetic acid (70:30). The solution was electrospun with the flow rate of 0.1 mL/h, with an applied voltage of 15 KV and 10 cm spinning distance. L929 fibroblast cells were seeded on nanofiber scaffolds to evaluate the proliferation ability with an MTT assay. SEM was performed on L929 fibroblast cells cultured on PCL and PCL/ HA/ vitamin C.
Results: The SEM images showed higher cell growth, proliferation and adhesion of L929 fibroblast cells on the PCL/ HA/ vitamin C scaffolds compared to the PCL scaffold. SEM results showed that adhesion of fibroblast cells increased with increasing the concentration of hyaluronic acid. The results of the MTT assay showed PCL and PCL/ HA/ vitamin C nanofiber scaffolds were non-toxic.
Conclusion: The results showed that PCL/ HA/ vitamin C nanofiber scaffolds have a great potential for using in tissue engineering. In-vitro tests showed high cell adhesion and viability on the electrospun scaffolds.
Keywords: Polycaprolactone; Hyaluronic acid; Nanofibers; Tissue engineering

PS-100. Dissecting IL-2 Signaling Pathway in Non-Small-Cell Lung Cancer Stem Cells

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Background and Aim: Lung cancer is one of the most common cancers worldwide. Cancer stem cells (CSC) are known to initiate lung cancer. CSC are capable to differentiate into many cells and driving tumor growth by attenuating immune surveillance through secretion or expression of immune-suppressive factors or by the recruitment of accessory cells that locally suppress the immune response. Therefore, recognizing the signaling pathways by which CSC manipulates the immune system to escape recognition is of utmost importance.
Methods: Cancer stem cells were isolated from normal lung epithelial cells and lung cancer cell lines (A549 and NCI-H 2170, respectively). Using Affymetrix microarray, the transcriptome of the normal and cancerous stem cells was compared in GEO2R software and the results were analyzed in Reactome software. Eventually, signaling pathways ending in IL-2 in stem cells were evaluated.
Results: To activate interleukin 2 cancer stem cells, beta-interleukin 2 receptor binds to JAK1 and IL-2RG binds to JAK3. The IL-2 alpha receptor is attached to IL-2 and these two are linked to IL-2 beta receptor and the IL-2 beta receptor binds to the gamma subunit of the IL-2 alpha receptor. The complex JAK1 and JAK3 phosphorylate IL2R and JAK1, Y392, Y338, Y510 phosphorylate IL-2 beta receptor. Y338 phosphorylation of Interleukin-2 beta receptor enables SHC and Y392, Y338, or Y510 phosphorylation of interleukin-2 beta receptor enables STAT. SH1 attached to the interleukin 2 and STAT5 receptors are phosphorylated. Phosphorylated STAT5 dimerizes and goes to the nucleus. SYK is bound to Interleukin 2 beta receptor and is a substrate for JAK1. PTK2B is connected to JAK3 and is phosphorylated.
Conclusion: CSC can affect other cells through signaling pathways leading to the production of interleukins. This effect can be one of the escaping mechanisms from the immune system. Currently, Interleukin 2 is used to treat cancers, but the same interleukin can also convert adult T cell into Treg cells. Therefore, recognizing the signaling pathway leading to the production of interleukin 2 in cancer stem cells can provide a precise vision for new therapeutic processes.
Keywords: Cancer stem cell; Interleukin 2; Signaling; Immune system

PS-101. Evaluate the Possibility of Induction of Neurogenic Differentiation of Mesenchymal Stem Cells Using Curcumin

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Background and Aim: Neurodegenerative diseases are defined as diseases that cause impaired nervous system function and neurons death. Today, Scientists are seeking to use more effective, less costly and less harmful substances for the treatment of neurodegenerative diseases. The purpose of this study was to examine neurogenic induction effects of curcumin on

the bone marrow –mesenchymal stem cells (BM-MSCs).
Methods: The BM-MSCs were extracted from rats. MSCs were cultured in a 96-parts plate to determine the curcumin toxicity. For evaluation of neuron differentiation, at the first, BM-MSCs were cultured in 24 well plates with DMEM medium containing 10% FBS. In the second day, pre-induction medium, including DMEM, BME, and FBS, was added into the cells. In the third day, plates were divided into two groups: the experimental group and positive control group. The positive control group was induced by two induction mediums separately: induction medium containing BME or BHA and DMSO. The experimental group was also induced by two inductive groups: curcumin induction group and curcumin induction group with BHA and DMSO. After cells induction, every hour, each plate was photographed for six hours. In the final stage, after removing the medium from plates, both positive and experimental control groups were placed in the DMEM medium containing N2-supplement, butyl hydroxyanisole, potassium chloride, valproic acid, and forskolin.
Results: The results of MTT showed that curcumin 1 to 10 μM did not have any significant toxicity effects on BM-MSCs. The BM-MSCs were successfully induced in two positive control groups that were treated by BHA and BME, although the differentiation rate was higher by BHA induction medium and the toxicity of this substance was lower than BME. Curcumin remarkably was able to induce neurogenic differentiation of BM-MSCs, although this effect was noticeable increase when the cells were treated by the combination of curcumin and BHA.
Conclusion: Curcumin supposed to be a good drug for neurodegenerative diseases based on the induction of neurogenic differentiation
Keywords: Curcumin; Neurogenic differentiation; Neurodegenerative diseases; Stem cells

PS-102. The Effect of ROCK Inhibitor on Recovery and Proliferation of Thawed Pluripotent Stem Cells

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Background and Aim: Both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have significant obstacles for culture with inactivated feeder layer when defrosted from cryopreserved stocks. ROCK inhibitor treatment enhances stem cell survival efficiency and poor recovery after thawing. In this study, we investigated the effect of different incubation times of a specific pyridine-based Rho kinase (ROCK) inhibitor on recovery enhancement and proliferation of thawed hESCs and iPSCs.
Methods: The protocol of this study was approved by the local ethics committee (code IR.TBZMED.REC.1396.1031). Mouse embryonic fibroblasts (MEFs) were cultured in DMEM supplemented with 10% FBS. Before use as a feeder layer, their growth was arrested with Mitomycin C (2 mg/mL) for 3 hours in 37°C incubator. Inactivated cells were seeded at 600 000 cell density on 6 cm culture plates. Pluripotent stem cell medium containing DMEM/F-12 and Knockout serum replacement was conditioned for 4 hours on inactivated MEFs. Cryovials of Royan H6 hESC and R1 hiPSC4 lines were thawed immediately into conditioned media with Y-27632 ROCK inhibitor at 10 μM concentration. To investigate the effect of Y-27632, the medium was refreshed with basic medium without inhibitor at different time points after 8-24 hours. The number of colonies and cell proliferation was assessed microscopically.
Results: We observed that the number of adherent colonies was significantly higher when the medium supplemented with Y-27632 removed after 8-12 h from culture plates (≥2-fold versus later time points). In addition, the rate of reaching confluency after 10 days was higher in the mentioned culture condition (80% versus <60% at later time points), which was more prominent in iPSCs. But when we removed ROCK inhibitor after 18 or 24 hours, both hESCs and iPSCs reached to confluency later, after 12 days.
Conclusion: We demonstrated that a short time treatment with ROCK inhibitor only for 8-12 hours is more appropriate for recovery and proliferation of thawed pluripotent stem cells, maybe due to the inhibitory effect of ROCK inhibitor on cell proliferation in long-term incubation. According to our results optimizing of pluripotent stem cell culture condition with respect to ROCK inhibitor treatment time is essential.
Keywords: Pyridines, Embryonic stem cells, Induced pluripotent stem cells, Cryopreservation

PS-103. Evaluation of Different Culture Media to Differentiate Human Embryonic Stem Cells Toward Eye Field Specification

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Background and Aim: Degeneration of retinal pigment epithelium (RPE) or photoreceptors is one of the common causes of blindness around the world. Most of recently replacement therapies are focused on human embryonic stem cells (hESCs) derived RPE or photoreceptor. Several differentiation protocols are under consideration for the generation of highly efficient retinal cells. During differentiation of hPSCs to rostral cell fate, one of the most important components is the cell culture media.
Methods: In this study, we compared two widely used basic media, GMEM, and DMEM/F12, in order to assess the effects of culture media inducing the efficient generation of rostral differentiation. The hESCs were cultured on Matrigel-coated dishes in the presence of two different media which were supplemented with the same components including 10% KSR, 2 mM L-glutamine and 0.1 mM NEAA. The morphological changes were followed for one month. Also, the expression of specific eye filed markers at the RNA level was characterized.
Results: Routinely, the hESCs were maintained in DMEM/F12 supplemented by 20% KSR. To whether GMEM medium could induce rostral differentiation rather than DMEM/F12, in addition to declining KSR concentration, in some groups the medium was changed to GMEM. The results showed that the morphology of hESCs was changed significantly in the presence of GMEM. Quantification of the pigmented area as a rostral differentiation criterion as well as neural tube-like structures was effectively higher in GMEM medium when the results were compared to DMEM/F12 as the control group. The assessment of eye field markers revealed the efficiently inductive effect of GMEM medium to rostral differentiation of hESCs in compare to DMEM/F12.
Conclusion: In conclusion, although DMEM/F12 medium is widely used in many in vitro research studies such as proliferation and differentiation of hESCs, our results demonstrated GMEM would be a better replacement medium for rostral differentiation especially in vitro eye field specification studies.
Keywords: Culture medium; Human embryonic stem cell; Cell differentiation; Eye field

PS-104. Neural Differentiation of Human Induced Pluripotent Stem Cells on Polycaprolacton/Gelatin/Polyaniline Bi-electrospun Nanofibers

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Background and Aim: In recent years, tissue engineering has gained much attention because of its application in tissue and cell regeneration.

Thus, in the past years, a lot of efforts have been given to improve tissue regeneration approaches. Recent advances in cell culture have resulted in the discovery of induced pluripotent stem cells. These cells appear to provide great potential in comparison with any other types of stem cells, and thus, are more achievable and usable in terms of tissue regeneration. Nanofibrous scaffolds are considered as useful tools, in part because of their exceptional structure and potentials. Despite such efforts and achievements, the establishment of scaffolds with great features seem to need greater efforts. Many polymers have been used for such purposes as a single form or in combination with other polymers.

Methods: In this study, polycaprolactone (PCL) and gelatin (Gel) and polyaniline (PANi) were exploited to engineer a composite for the differentiation of human induced pluripotent stem cells (hiPSCs) to neural cells. The composite was fabricated by electrospinning. MTT assay was used to evaluate the toxicity on the composite. The hiPSCs were seeded on the fibers and after 14 days of the differentiation, real-time PCR and immunocytochemistry (ICC) tests were performed to validate the differentiation of the cells. For the analysis of morphological changes, scanning electron microscopy (SEM) was used.

Results: Our findings showed a total differentiated of hiPSCs to neural cells. This study highlights that PCL-GEL-PANi composite not only has the capability to support the differentiation of hiPSCs to the neural cells but also can improve the differentiation process. Taken all, PCL-GEL-PANi appears to be a feasible, reliable and simply accessed composite for the tissue engineering approaches.

Conclusion: Our results strongly confirm the potential of composites in tissue engineering as they are shown and proven to support and enhance cell attachment, proliferation, and differentiation. It should be noted that the effect of PCL-GEL-PANi composite on the differentiation and cell support was significant.

Keywords: Human induced pluripotent stem cells; Neural cells; Tissue engineering; Scaffold; Polycaprolactone; Gelatin; Polyaniline

PS-105. Change in the AQP1 Gene Expression After Ischemia-Reperfusion in the Liver of Mice

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Background and Aim: Ischemia-reperfusion (IR) injury usually occurs during organ transplantation. Aquaporins (AQPs) are transmembrane channels that facilitate water permeability through cell membranes and are essential for the regulation of water homeostasis. Changes in the AQPs expression have been correlated with several inflammatory diseases. Less is known about the effect of ischemia-reperfusion on the AQPs gene expression. To clarify the role of AQPs in IR injury, in this study we investigated the gene expression pattern of AQP1 in the liver after IR injury.

Methods: Male bulb/c mice were exposed to partial (70%) hepatic ischemia for 65 minutes and then randomized into five groups of reperfusions [0 h (A), 8 h (B), 1 day (C), 3 days (D), and 7 days (E)]. A surgical group was also selected as the sham group. Serum and liver tissue samples were collected for evaluation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and liver histopathology. Real-time PCR was performed to evaluate the AQP1 gene expression.

Results: We observed a significant increase in ALT and AST ($P<0.05$) compared to sham mice in each group. The gene expression of AQP1 was significantly increased in the IR group compared with the sham group ($P<0.05$). After 8 hours (group B) showed the highest gene expression in comparison with other groups. Pathologic changes in the liver after reperfusion were confirmed the IR. In the IR group cytoplasmic vacuolization, inflammatory cell infiltration and focal necrosis were detected.

Conclusion: In conclusion, our findings indicated that the ischemia-reperfusion injury in the liver can change the expression of AQP1 gene, which can interfere with hepatocellular homeostasis and their function. Upregulation of AQP1 could contribute to the development of hepatocellular swelling after hepatic IR injury.

Keywords: Ischemia; Reperfusion; AQP; Liver; Hepatocyte

PS-106. Role of Mesenchymal Stem Cells in Delivering Newcastle Disease Virus to Non-Small Cell Lung Cancer

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Background and Aim: Lung cancer represents one of the major challenges in oncology. About 85% of lung cancers are non-small cell lung carcinoma (NSCLC). Despite progress in chemo-radiotherapy and targeted therapy, its survival rate is still low. Hence, using oncolytic viruses such as Newcastle disease virus (NDV) can be effective. NDV significantly cause tumor regression in intratumoral injection but the absence of an effective delivery system and immune clearance system cause non-significant in the systemic injection. Mesenchymal stem cells (MSC) have a tropism to the tumor and they can be used as a carrier of NDV.

Methods: MSCs were isolated from bone marrow and adipose tissue. The evaluation of a characteristic of MSCs by biomarkers (CD90, CD73, and CD105) was performed using flow cytometry. B1, V4 and La-Sota strains of NDV were propagated in MDBK cell line and allantoic cavity of 7- to 9- day-old embryonated chicken eggs, purified using ultracentrifugation and titrated using HA assay. Infection of MSCs with a different strain of NDV evaluated using flow cytometry by staining with anti-virus conjugated antibodies. The effect of infected MSC with the best strain of NDV on A549 cells (NSCLC cell line) was evaluated using the XTT assay. This evaluation was performed with co-culture and using the supernatant medium of infected MSC with appropriate controls including MSC alone, NDV alone at different concentrations and PBS.

Results: MSCs were isolated and confirmed by biomarkers. The titer of B1, V4 and La-Sota strains of NDV, propagating in MDBK cell line, were 1 HU, 8 HU, and 4 HU respectively. The titer of them reached to at least 36 HU after propagation in embryonated chicken egg and 65536 HU after purification. The different strains of NDV could infect MSCs and the best stain was V4 with 1000HU/mL. The evaluation of the effects of NDV alone, MSC alone and infected MSC with NDV on A549 cells demonstrate that NDV induced a dose-dependent cell death in A549 cells and MSCs are more resistant to NDV than A549 cells. In groups existing MSCs, A549 cells highly proliferate and formed multi-layer colonies. Nonetheless, the proliferation in infected MSC with NDV group was less than MSC group.

Conclusion: The proliferation of A549 cells was increased in co-cultured MSCs and using the supernatant medium of MSCs, probably due to the release of various growth stimulants. However, it was less in NDV-infected MSCs which may due to the effects of NDV releasing from infected MSCs. The results showed that MSCs can be employed to deliver NDV to NSCLC cell line. Considering the limitation of chemotherapy and radiation therapy of NSCLC, treatment with MSC-mediated targeted

oncolytic NDV may provide a novel effective therapeutic approach for the treatment of advanced NSCLC.

Keywords: Mesenchymal stem cells; Newcastle disease virus; non-small cell lung cancer

PS-107. Mesenchymal Stem Cell and Electromagnetic Field

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Background and Aim: Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) are a population of stem cells in umbilical cords Wharton's jelly that can be a notable therapy option for tissue engineering and regenerative medicine due to lack of tumorigenicity and much more immunosuppressive than MSCs isolated from adult tissue such as the bone marrow and adipose. Electromagnetic field (EMF) seems to be able to change many biological functions such as proliferation, cell cycle, differentiation, DNA replication, cytokine expression, apoptosis, and etc. The aim of this study is to investigate the effects of electromagnetic fields on the proliferation of WJ-MSCs.

Methods: After isolating WJ-MSCs from umbilical cords Wharton's jelly, the treatment, and control cell groups were cultured in DMEM medium. The treated cells were exposed to electromagnetic fields with a square wave and 1mT intensity for 60 min exposure/15 min rest for 3 cycles at 47 Hz for 3 days. The effects of EMF on cell viability and proliferation of WJ-MSCs were investigated using MTT assay and trypan blue staining.

Results: WJ-MSCs were significantly dead in the treatment group, while in the control group, the cells proliferated and doubled after 3 days.

Conclusion: While several studies have reported the effect of EMF on MSCs proliferation, but many of them have not mentioned to kind of waves they have applied. In our study, we used a square wave with 3 cycles of 60 minutes, and we observed that this condition not only did not affect the proliferation and survival of the MSCs but also leads to the death of cells. It seems that this EMF condition may be effective in destroying and killing cancerous cells and be a good option for cancer therapy.

Keywords: Wharton's jelly-derived mesenchymal stem cells; Electromagnetic field; Proliferation

PS-108. The Effects of Fluoxetine on the Human Mesenchymal Stem Cell Proliferation and Differentiation

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Background and Aim: Fluoxetine is one of the most commonly used antidepressants. Fluoxetine could prevent the mesenchymal stem cells differentiation in lung fetus of the rat. Moreover, the mesenchymal stem cells are also present in adult tissues. Therefore, in the current study, we aimed to investigate the effects of fluoxetine on both proliferation and adipogenic/osteogenic differentiation of human adipose tissue-derived adult mesenchymal stem cells (ADSCs).

Methods: After culturing of human ADSCs, these cells were treated with two concentrations of fluoxetine (10 and 20 µM). Then, cells were differentiated by adding osteogenic and adipogenic media. The effect of fluoxetine on human ADSCs proliferation was evaluated by MTT assay. Fluoxetine role on the adipogenic and osteogenic differentiation of human ADSCs was analyzed by oil red and alizarin red staining and RT-PCR reaction.

Results: According to MTT assay, fluoxetine showed a time and concentration-dependent proliferation response and eventually decreased human ADSCs proliferation. RT-PCR analysis indicated that fluoxetine significantly diminished the expression of osteogenesis-related genes such as RUNX2 and ALP. Data also revealed a significant reduction in the expression of PPARγ and FABP (specific genes of adipogenic lineage). In addition, fluoxetine decreased mineralized matrix and the number of lipid droplets in human ADSCs by staining methods.

Conclusion: Our observation demonstrated that the effects of fluoxetine may be time dependent. This drug possesses an increasing phase in the proliferation and survival of human ADSCs (first 24 h) following a decreasing phase (after 48 h). Moreover, fluoxetine could attenuate both osteogenic and adipogenic differentiation of human ADSCs.

Keywords: Fluoxetine; Human adipose tissue-derived adult mesenchymal stem cells (ADSCs); Proliferation; Osteogenic differentiation; Adipogenic differentiation

PS-109. New Strategy of Decellularization and Production of Human Kidney Scaffolds

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Background and Aim: Incidence of end-stage renal disease (ESRD) is greatly increasing. Renal transplantation is one of the goal treatments for ESRD. The development of tissue engineering and regenerative medicine have catalyzed due to a gap between limited organ supply and increasing demands. Natural scaffolds prepared from an extracellular matrix (ECM), have emerged as an ideal tissue microenvironment for this goal. An important technique in regenerative medicine to prepare an acellular ECM is the decellularization of native tissues. Therefore, the aim of this study was to determine the effective method for decellularization of human kidney and producing the natural human kidney scaffold.

Methods: After Nephrectomy, human kidneys that could not be transplanted were used in this study. Adipose tissue and capsule around the kidney were removed. We cut kidneys into transverse sections (approximately 10 × 10 × 2 mm^3 pieces) using a scalpel. Then Cortex-medulla kidney sections were washed twice with phosphate buffered saline (PBS), followed by decellularization in a solution of either 1% Triton X-100 or sodium dodecyl sulfate 1% (SDS). The sample was decellularized at 4℃ using shaker (200 rpm). Decellularization solution was changed 4 hours after initial tissue harvesting and then every 24 hours until tissues were transparent (for 14 days). In order to confirmation of decellularization, hematoxylin-eosin (H&E) staining was performed on

days 2, 5, 10, and 14.
Results: Comparison of H&E staining of the decellularized and native kidney tissue revealed successfully elimination of cell nucleus in SDS and Triton-treated sections. Also, H&E staining revealed that in the Triton-treated sections the native ECM architecture, integration of renal vascular and glomerular structures was more preserved than the SDS-treated sections.
Conclusion: We have developed an effective decellularization method for the preparation of human renal ECM scaffold. Additionally, it may be possible to use the scaffolds that prepared with 1% Triton X-100 for kidney regeneration. These results also indicate that discarded human kidneys are a suitable source of renal scaffolds and their use for tissue engineering applications may be more clinically applicable and beneficial than kidneys derived from animals.
Keywords: Human kidney; Regenerative medicine; Scaffold; Decellularization; 1% Triton X-100

PS-110. Safety and Efficacy of Allogenic Placental Mesenchymal Stem Cells for Treating Knee Osteoarthritis: A Pilot Study

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Background and Aim: Knee osteoarthritis (OA) is a common skeletal impairment which can cause many limitations in normal life activities. Stem cell therapy has been studied for decades for their regenerative potency. Different sources and doses of mesenchymal stem cells (MSCs) in varying delivery systems have been investigated for treating OA. We investigated the safety and efficacy of intra-articular placental MSCs in knee OA healing.
Methods: In this double-blind, placebo-controlled clinical trial, twenty patients with symptomatic knee OA were randomly divided into two groups to receive an intra-articular injection of either MSCs or saline. The visual analog scale, Knee OA Outcome Score (KOOS) questionnaire, knee flexion range of motion (ROM), and magnetic resonance arthrography were evaluated for 24 weeks post-treatment. Blood laboratory tests were performed before and 2 weeks after treatment.
Results: There were no serious adverse effects except self-limited acute post-injection events such as mild effusion and increased local pain which improved in 48 hours. There was a significant knee ROM improvement and pain reduction started early after treatment. Improved quality of life, the activity of daily living, sport/recreational activity, and decreased OA symptoms were noted post-treatment which were clinically and statically significant until 8 weeks but were only clinically significant in 24 weeks post-treatment. Chondral thickness was improved in about 10% of the total knee joint area in the intervention group in 24 weeks. There was no significant healing in the medial/lateral meniscus or anterior cruciate ligament.
Conclusion: Single intra-articular allogenic placental MSC injection can alleviate joint pain and increase ROM in knee OA safely and effectively.
Keywords: Allogen; Placenta; Mesenchymal stem cell; Osteoarthritis

PS-111. Comparative LncRNA and miRNA as Biomarker in Early Detection of Cholangiocarcinoma Based on Bioinformatics Methods

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Background and Aim: Cholangiocarcinoma (CCA) as the second most prevalent primary hepatobiliary cancer and aggressive malignancy which arise from bile duct's epithelial cells have an incremental incidence in the last 30 years. Since gene expression pattern is greatly changed in CCA background, it is of urgency to identify new genomic biomarkers such as miRNA and lncRNA for CCA patients. Tumor-suppressing and oncogenic functions of lncRNAs and miRNAs lead them to play the crucial role in tumor growth by affecting on matrix invasion and might cause CCA by disrupting key signaling pathways.
Methods: In this bioinformatic study, all of the lncRNAs and miRNAs were collected from the literature studies that they were screened based on high throughput technics like, RNAseq and microarray screening of normal and cancer sample of patients with CCA. The gene expression profiles of CCA were obtained from GEO (available at http://www.ncbi.nlm.nih.gov/geo/) database. The following keywords and their combinations were used: homo sapiens and cholangiocarcinoma. The original studies that compared gene expression profiling between CCA and normal control (NC) biopsy tissues or cultured cells were included in this study. Nonhuman studies and reviews were deleted. The GEO IDs of the eligible dataset were GSE26566, GSE32225and GSE45001. Totally, 553 cases and 52 controls were included for integrated analysis. The NC samples were coming from normal intrahepatic bile ducts (n = 7), paired non-cancerous liver tissues from intrahepatic cholangiocarcinoma (ICC) patients (n = 7), cultured normal biliary epithelial (NBE) cells (n = 4) and nontumor areas from 20 patients with ICC.
Results: Analysis of lncRNAs and miRNAs database: After completing the final analysis, we collected 10 valuable lncRNA such as CPS1, GAS5 and HOTAIR and also 20 valuable miRNA such as mir-200a, mir-320b, mir-191as a novel panel of biomarkers to detect CCA at the early stage. All these advice panels of biomarkers were valid in a database like "LncRNAs Disease databases", "miRBase" and "miRTarBase". differentially expressed genes (DEGs) in the integrated analysis of microarray datasets: After an electronic search, there were 26 microarray studies obtained according to the inclusion criteria. By integrated analysis, 12 274 genes were obtained and a set of 712 DEGs were identified in the CCA compared with non-CCA, including 306 upregulated and 406 downregulated DEGs.
Conclusion: In recent years, many studies have discovered the link between miRNA/lncRNA and cholangiocarcinoma in the process of tumorigenic and tumor progression, some of which suggest that miRNAs and lncRNAs may serve as new markers for CCA patients. The integrated analysis appears to be a useful approach to identify DEGs between CCA and normal tissues. The most consistently overexpressed genes of PKM, COL1A1, and COL1A2 might participate in the pathology of CCA, and they will be the important biomarkers for the diagnosis of CCA and in future studies, this panel will be generalizable.
Keywords: Homo sapiens; Cholangiocarcinoma; Biomarker; miRNA and (or) lncRNA

PS-112. cfDNA Promoter Methylation Quantification of DNMT1 and MGMT Genes in Papillary Thyroid Cancer Tissues

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Background and Aim: Endocrine tumors are endocrine system related malignancies like thyroid that is the most common type with mounting trends over the last three decades worldwide. Papillary thyroid cancer (PTC) is making four-fifths of all thyroid cancers. Finding some detection markers in order to discriminate malignant from the benign one before metastasis could be really important for thyroid cancer patients and clinicians. Epigenetic silencing through aberrant DNA methylation of tumor suppressor genes linked to a variety of devastating consequences of human cancer. Two DNA methyltransferases DNMT1 and MGMT were selected as the key epigenome writers. We determined the quantity of methylation in twelve candidate promoter regions of four tumor suppressor genes using the Methylation-sensitive high-resolution melting (MS-HRM) assay.
Methods: Fresh frozen tissues and blood of 35 PTC patients and 34 goiter patients were collected after surgery. DNA was extracted from plasma and tissue using QIAGEN Kit according to the manufacturer's protocol. DNA purity and quantity were determined using a Thermo Scientific™ NanoDrop™ spectrophotometers 2000c spectrophotometer and then stored at -80°C. For bisulfite treatment DNA from each sample were treated with sodium bisulfite conversion kit. The MS-HRM analyses were run based on the three main steps of Holding, Cycling, and Melt curve. Statistical analysis was done by the Statistical Package for the Social Sciences (SPSS) version 16.0 and P <0.05 was considered statistically significant.
Results: Promoter methylation of MGMT and DNMT1genes has meaningful differences between PTC cases and goiter controls.
Conclusion: Among the different selected promoter region of two selected genes the, MGMT (a), MGMT (c), MGMT (d), and DNMT1 (b) methylation status were meaningfully different between PTC cases and controls. Our result suggested that as epigenome writer, MGMT plays a more important role in papillary thyroid carcinogenesis than DNMT1.
Keywords: DNA methyltransferase; Goiter papillary thyroid cancer

PS-113. Alginate Based Microcapsules Containing Galactosylated Chitosan as a Synthetic Matrix in 3D Culture of Hepatocytes

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Background and Aim: Microencapsulation provides a suitable 3D platform for tissue engineering and plays an important role in developing bioartificial liver devices. Alginate-based microcapsules have been tested for the treatment of liver failure. The hepatocytes in pure alginate microcapsules exhibit low viability and poor liver-specific functions. The employment of galactosylated chitosan (GC) is one effective method for improvement of alginate-based microcapsules. In this work, an alginate-galactosylated chitosan/chitosan (AGC/C) microcapsule has been presented for the 3D culture of HepG2 cells and the microcapsule characteristics and liver-specific functions of cells have been evaluated.
Methods: For investigation of the mechanical property of microcapsules, hydrogels were placed in a material testing machine (Zwick/Roell Z010, Germany) and compacted with a crosshead speed at 2.0 mm/min. For swelling test, the microcapsules were put in PBS solution at 37℃ for 7 days and the diameter of microcapsules was measured using an optical microscope (IX71 Olympus, Japan). For permeability of the microcapsules, the release of bovine serum albumin (BSA) was investigated. Finally, for cell functionalities of HepG2 cells were suspended in an alginate-based solution with a density of 2×10^6 cells/mL. For the preparation of Alg/GC solutions with the proportion of 1%/0.5%, the GC solution was dropped into Alg solution and mixed at a high rate. The microcapsules were prepared by means of an electrostatic microencapsulation method (9 kv), and eventually, the AGC microcapsules were coated with 0.3% chitosan solution. MTT assay was used to measure the viability of cells. The concentration of glucose and lactate in the supernatant was determined using a kit (GOD PAD).
Results: The microcapsules of AGC/C showed the faster release of BSA than alginate/chitosan (AC) microcapsules, indicating the presence of GC in the AGC/C microcapsule improve the permeability of microcapsules. This improvement is related to the interaction of carboxyl groups of alginates with amino acid groups of GC. In addition, the large volume and spatial hindrance of GC in the core lead to decrease the interactions between alginate and external chitosan, causing structural loosing of the membrane. Analysis of compression assay showed that stiffness of AGA/C and AC microcapsules were 6.75 and 7.62 kPa, respectively. MTT assay for cell proliferation of AGC/C microcapsules revealed about 20% increase in the 5th day compared to the AC microcapsules. During the five days of cell culture, the secretion of albumin was increased.
Conclusion: The solubility of the chitosan derivatives increases by introducing galactose groups. The increase of galactose ligands enhances the viability and formation of multicellular spheroids of HepG2 cells cultured in AGC/C microcapsules and improves their liver-specific functions than in traditional AC microcapsules. Therefore, GC because of excellent adhesion and spheroid formation of hepatocytes has potency as one of synthetic ECMs for liver tissue engineering.
Keywords: HepG2 cell line; AC microcapsule; Galactosylated chitosan; Mechanical stability

PS-114. The Use of 3D Culture of Hanging Drop Mesenchymal Stem Cells in Collagen Scaffolds for Tissue-Engineered Repair of Cartilage

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Background and Aim: The aim of regenerative medicine is to revive injured tissues by developing efficient cell, tissue, and organ replacements to repair or increase biological function in damaged tissues. We hypothesized that the 3D culture of mesenchymal stem cells (MSCs) in collagen scaffold (Collagen rat tail, Type I) can improve stem cells differentiation into cartilage. With regard to these considerations, the current study aimed to reconstruct a culture environment for cartilage tissue engineering.
Methods: The human MSCs derived from Wharton's jelly were cultured in completed Dulbecco's Modified Eagle's medium (DMEM) in primary culture. Then, the cells were characterized as MSCs by flow-cytometry. The cells were cultured in hanging drop. The collagen scaffold was made and lyophilized. Then, the 3D culture hanging drop cell put on collagen scaffolds for 2 weeks. Glycosaminoglycans (GAGs) was identified in the extracellular matrix of MSCs by Safarin-O and Alcian blue staining. All the scaffolds were evaluated by scanning electron microscope (SEM). Scaffold porosity was also determined.
Results: FTIR and SEM analysis identified that this scaffold is highly porous with interconnection. This can provide an ideal matrix which is suitable for cell growth and chondrocyte differentiation. The cells were differentiated to chondrocyte in this 3D porous scaffolds that can be useful in tissue engineering.
Conclusion: The cell cultured in a new method showed that a different physical and biological property is useful in cartilage tissue engineering applications.

Keywords: Scaffold; Mesenchymal stem cells; 3D culture; Hanging drop

PS-115. Investigation of In-vitro Osteogenic Potential of Human Adipose-Derived Stem Cells Cultured on Hydrogel Based 3D Nanocomposite Scaffold Made of PVA - VBP – nGO

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Background and Aim: The purpose of the present study was to investigate the osteogenic differentiation of human adipose-derived stem cells (hASCs) in three-dimensional (3D) constructs in vitro. Previously, we create two groups of 3D composite scaffolds made of polyvinyl alcohol (PVA), vinyl based polymer (VBP), and nano graphene oxide (nGO) (NP+ group), and PVA and VBP (NP- group) through controlled freeze-drying technique. In this study, we assessed the potential of these scaffolds combined with hASCs for bone tissue application.

Methods: human adipose-derived stem cells (hASCs) were isolated from subcutaneous adipose tissue of 7 healthy female donors (30-40 years old) that had undergone liposuction through the enzymatic procedure. In passages 3, cell surface markers (CD73, CD90, CD105, CD34, CD45) were identified by flow cytometry. After sterilization of the two groups of the scaffold, 5×10^6 hASCs were seeded on each scaffold and cultured in osteogenic media (OM). The presence of the hASCs on the scaffold were emphasized by SEM, DAPI staining, and hematoxylin-eosin (H&E) staining on the 1st, 7th,14th, 21th day of culture. Then, osteogenic differentiation of seeded cells was evaluated by alkaline phosphatase activity and Alizarin Reds in 14th, 21th day of culture. Moreover, real-time PCR, Western blot, and immunocytochemistry (ICC) were done for specific osteogenic markers including Runx2, col Iα, Osteocalcin, and Osteonectin.

Results: The positive expression of CD73, CD90, and CD105 and negative expression of CD34, CD45 of hASCs were identified by flow cytometry. Results from SEM, DAPI staining, and H&E staining showed that the presence of most of the cells on the two groups of scaffolds (NP+,NP-) after the 1st, 7th, 14th, 21th day of culture. In vitro osteogenic induction of the 3D construct showed that the expression levels of Runx2, col I, Osteocalcin and Osteonectin in NP+ scaffolds group were significantly higher than NP- scaffolds group on days 7 and 14 after cultured in osteogenic medium (OM). Western blotting and ICC showed that the expression of osteogenesis-related proteins was significantly higher in cells seeded on NP+ scaffolds group than NP- scaffolds group.

Conclusion: These results indicated that 3D nanocomposite constructs (NP+) consisting of hASCs had the great ability to promote mineralized matrix formation and could be used for the repair of bone tissue defects.

Keywords: Bone tissue engineering; Human adipose-derived stem cells; Polyvinyl alcohol; Nano-graphene oxide; 3D Nanocomposite scaffold

PS-116. In Vivo Osteogenic Potential of hADSCs-PVA-VBP -nGO 3D Constructs for Bone Regeneration in a Rat Critical Sized Calvarial Defect

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Methods: Thirty-five rats were randomized into 5 groups (n = 7): 1) Defect alone (control), 2) PVA/ VBP scaffolds (NP-), 3) PVA/ VBP /nGO scaffolds (NP+), 4) PVA/ VBP scaffolds with hASCs cultured 7 days in osteogenic media (NP-osteoblast-like cells), and 5) PVA/ VBP /nGO scaffolds with hASCs cultured 7 days in osteogenic media (NP+ osteoblast-like cells). An 8 mm critical-size circular calvarial defect was made in each rat. The scaffolds belong to each group implanted in the defects and follow up for 6 to 12 weeks. The animals were sacrificed and bone repair was evaluated using multiple computerized tomography (CT) and hematoxylin-eosin (H&E) staining at 6 and12 weeks in all experimental groups.

Results: These data provide evidence that constructs made of NP+ and NP- along with osteoblast-like cells transplantation have great bone regeneration capability in critical-sized calvarial defects than constructs without osteoblast-like cells. NP+ osteoblast-like cells-treated animals showed significantly improved regeneration of new bone formation compared to those in the other groups ($P<0.05$). Histological analysis showed that cells migration and healing process in rat calvarial defect treated by NP+ osteoblast-like cells and NP- osteoblast-like cells were higher than in the other groups.

Conclusion: PVA/ VBP /nGO scaffolds with osteoblast-like cells may have wide applicability for bone repair as an alternative bone graft material alone.

Keywords: Bone tissue engineering; hADSCs; PVA; nGO; Calvarial defect model

PS-117. Effect of Gelatin on Characteristics of an Enzymatically-Gellable Alginate Hydrogel Scaffold for Cartilage Tissue Engineering

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Background and Aim: Alginate as a natural polysaccharide is widely used in biomedical applications due to its excellent biocompatibility and biodegradability. However, this material lacks cellular interactions and suffers from poor cell adhesion. In this work, the phenolic moieties were introduced into alginate to form enzymatically-gellable alginate (Alg-ph) for mechanical strength improvement in culture medium and gelatin (Gel) was also used to enhance the cellular adhesion properties. In order to prevent gelatin from moving the scaffold, this compound was also phenolized (Gel-ph).

Methods: Tyramine and EDC were used to add phenolic groups of the alginate and gelatin. Hydrogels of Alg-ph, Alg-ph + Gel and Alg-ph + Gel-ph were prepared using Horseradish peroxidase (HRP) and H_2O_2 and analyzed by UV, microstructure (SEM), mechanical strength, degradation by lysozyme enzyme, swelling ratio, gelation time and cell culture test. Chondrocyte cells were expanded in low glucose Modified Eagle maintained at 37°C with 5% CO2 in the incubator. Media containing Chondrocyte cells at 1×10^6 cells/mL were mixed with all hydrogels at final concentrations of 1.5% (w/v).

Results: Alg-ph and Gel-ph showed absorption at 275 nm wavelengths, indicating that phenolic groups were successfully introduced on the compounds. By adding gelatin to Alg-ph, the swelling and strains of hydrogel increased 3.5 and 5-fold, respectively. Also, with the addition of Gel-ph compared to gelatin, swelling increased about 5.5 times and the breaking strain was reduced 0.8 times due to the porosity created in the hydrogel structure. In the analysis of degradation, Alg-ph + Gel and Alg-ph + Gel-ph hydrogels were 1.6 and 2.1 times higher than Alg-ph, respectively. SEM images showed uniform pore sizes in Alg-ph +Gel-ph samples. Evaluation of chondrocyte cell proliferation within the hydrogels for all three types of hydrogels at the same seeding density revealed that the cells proliferated approximately 4 times in the Alg-ph + Gel-ph hydrogels, the value of which was 3.7 times for the Alg-ph + Gel hydrogels, after 21 days.

Conclusion: Characteristics of hydrogels such as swelling, mechanical strength and degradation could be controlled by introducing the ph groups to alginate and gelatin. According to the observed results, Alg-ph

+ Gel-ph revealed an appropriate scaffold property for use in soft tissue engineering, especially for cartilage tissue.
Keywords: Scaffold; Alginate; Gelatin; Cartilage tissue engineering

PS-118. Synthesize of Carbon Quantum Dot Scaffold for the Use in Tissue Engineering

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Background and Aim: Carbon quantum dots (CQDs), also called carbon dots (CDs or C-dots) or carbon nano-dots (CNDs), are a novel class of carbon nanomaterials and quasi-spherical nanoparticles with ultrafine sizes below 10 nm. CDs have slowly become a valuable structure in the nano-carbon family, because of being non-toxic, abundant and low-cost nature. Tissue engineering includes strategies to facilitate the replacement or repair of organs and restore their functions. In this regard, selection of appropriate stimulating cues and synthesis of three-dimensional scaffolds will provide a suitable microenvironment which leads to unlocking the innate powers of differentiation and regeneration of cells. This motivated us to study whether a nanocomposite based on alginate and carbon quantum dot would support an adequate microenvironment or not.
Methods: In this study, we used alginate in order to synthesize a hydrogel, which contains CDs. We aim to use this hydrogel to encapsulate mesenchymal stem cells (MSCs) to investigate the role of carbon dots as inducer factor. We used the micro-droplet technique to make microspheres which could be used to encapsulating MSCs.
Results: Hydrogels are composed of crosslinked hydrophilic polymers that absorb large amounts of water without dissolving and are commonly used in tissue engineering applications due to their low cytotoxicity and structural similarity to native ECM. They generally exhibit high biocompatibility and non-immunogenicity. On its own, alginate does not provide mammalian cell-adhesive ligands, however, cell adhesion and differentiation can be facilitated through the addition of gelatin. The main method of crosslinking alginate hydrogels is through ionic crosslinking with Ca^{+2} ions and the gelation rate can be modified by changing the temperature and concentration of the crosslinking agent. One of the most frequently used agents to do this is $CaCl_2$. Here we used DMEM as a solvent for CDs, alginate and gelatin and $CaCl_2$ as a crosslinking agent. For solving alginate and gelatin no heat should be used. Next, we examined Cds and hydrogel by FTIR test and determined degradation rate of the hydrogel by incubating samples of microspheres in PBS solution at 37°C.
Conclusion: In this study, a novel carbon dots alginate nanocomposite was developed by utilizing interdisciplinary knowledge of material science, bioscience, and nanotechnology. Biodegradation of alginate/CDs nanocomposites were examined. Based on its great potential in biodegradation, biocompatibility, and safety, this nanocomposite might be a candidate for tissue engineering and bioprinting applications
Keywords: Tissue Engineering; Carbon Quantum Dot; Alginate; Gelatin

PS-119. Local Injection of Autologous Bone Marrow-Derived Mononuclear Cells as a Treatment Method for Idiopathic Lower Limb Lymphoedema: Clinical Trial Phase I

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Background and Aim: lymphedema is lymphatic system draining dysfunction. Currently, conventional treatments for primary lower limb lymphoedema (LLL) are preserving treatment and are not curative. Stem cells have been considered in the treatment of lymphatic drainage disorder over the last few years, and some success in vivo studies have been published. Therefore, due to the inadequacy of studies and evidence in this field, this study aimed to investigate the effect of subfascial injection of autologous bone marrow-derived mononuclear cells in patients with idiopathic LLL by Royan Institute and Shohada-e Tajrish Hospital.
Methods: This open-labeled, non-randomized clinical trial phase I which has registered on IRCT website with IRCT201611241031N19 code, enrolled 6 idiopathic LLL patients aged 10 to 35 years who referred to Shahdai Tajrish Hospital's vascular surgery clinic. After the eligible criteria assessment, informed consent was obtained. Patients with secondary causes of Lymphoedema are excluded. All patients underwent bone marrow aspiration equals 2 cc/kg. Bone marrow-derived mononuclear cells (MNCs) isolation was done under GMP standards. The cell suspension was transferred to the hospital on the same day and transplanted sub-facially in 3 cm distance points of the involved leg. These patients are followed at 1, 2, 3, 6 and 12 months after transplantation by safety assessment, water displacement leg volumetry, circumference measurement, weight, and SF-36 Health Status Questionnaire.
Results: Totally, 6 patients, 4 females and 2 males with grade II and III of disease were enrolled. Two patients had bilateral involvement, one right leg, and 3 left legs. At least 11 years and at most 32 years. The mean age of non-congenital patients was 22.25 ± 4.50 years. The primary outcome was safety evaluation. No complications were observed after injection during follow up time. Besides, data showed some evidence of symptom relief, volume reduction and quality of life improvement in patients.
Conclusion: In a 12-month period, local transplantation of the autologous MNCs-derived bone marrow had no complications for idiopathic LLL patients. Also, there is little evidence of the therapeutic effectiveness of these cells in which needs to be reviewed and confirmed by further studies.
Keywords: Idiopathic lymphoedema; Autologous bone marrow-derived mononuclear cells; Cell therapy; Stem cell

PS-120. Intracoronary Transplantation of Autologous Bone Marrow-Derived Mononuclear Cells for Idiopathic Dilated Cardiomyopathy in Pediatric: A Phase I/II, Randomized, Single Blind Clinical Trial

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Background and Aim: Dilated cardiomyopathy as the most common form of cardiomyopathy is a rare but life-threatening disorder in children. Despite the developing in the medical and surgical treatment, standard treatments may stabilize the condition, but will not restore heart function to its previous condition. Therapy remains complex and expensive. For some not all children the heart transplantation is the only option. Cell-based therapies offer an innovative approach to reverse cardiac function towards normal, possibly reducing the need for aggressive therapies and cardiac transplantation.

Methods: In this study children with idiopathic dilated cardiomyopathy were enrolled at Shahid Rajaie Cardiovascular, Medical & Research Center in collaboration with Royan Institute. According to the inclusion and exclusion criteria of the trial, 24 patients with left ventricular ejection fraction less than 45% who resistance to the standard medical therapy signed a consent form and was randomly allocated in 2 groups including BM-derived mononuclear (BMMNC) (n=12) and control (n=12). Only the MNC group underwent the bone marrow aspiration and intracoronary injection. We followed all of the patients at 2 weeks, 1, 2, 4 and 6 months after transplantation for cell therapy group or registration for placebo by physical examination, laboratory tests and imaging such as echocardiography, CXR, and CMR. (ClinicalTrials.gov Identifier: NCT02256501)
Results: The mean (SD) age of 24 patients was 8.0 (5.2) years in control and 9.4 (2.9) in MNC. 28.6% and 62.5% of participants were female in control and MNC group, respectively. We have no significant differences in baseline characteristics between groups. Almost 583×10^6 mononuclear cells were injected to the coronary artery during less than 6 hours of autologous bone marrow aspiration for MNC group. The primary outcome was safety assessment. No procedure-related unexpected adverse events occurred. The MNC group patients showed an improvement with respect to New York Heart Association classification (greater than or equal to 1) and reducing in brain natriuretic peptide serum levels. Although LVEF improvement, relative to baseline was significant in the BMMNC group.
Conclusion: In this clinical trial, intracoronary injection of BMMNC was feasible and safe. Additionally, transplantation of BM-derived mononuclear cells compared to the control group can improve the imaging, laboratory and clinical cardiac function.
Keywords: Idiopathic dilated cardiomyopathy; Bone marrow-derived mononuclear cells; Cell therapy

PS-121. The Novel Cryopreservation Technique for Preparation of Decellularized Human Amniotic Membrane as an Appropriate Extracellular Matrix for the Regenerative Medical Purposes

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Background and Aim: The human amniotic membrane (HAM) is applied for various regenerative purposes in the reconstruction of damaged epithelial skin and/or ocular surface as an appropriate biological extracellular matrix (ECM). The fresh HAM also called fetus layer, is widely used by surgeons as a surgical hand-friendly scaffold for the renovation of the cellular expansion. Decellularization of HAM is currently performed by an enzymatic procedure (Trypsin-EDTA) that affects the cellular expansion. In the represented study, a novel biomaterial free procedure for the preparation of denuded HAM is clearly expressed.
Methods: The whole placenta was obtained from HCV, HIV, and syphilis seronegative cesarean delivery from the maternity ward of Imam Reza Hospital, Mashhad. Then the placenta was washed three times by balance salt solution BSS) in order to remove the blood residues. The thick chorion was separated completely from transparent amnion. The separated amniotic layer was kept in cryopreservation media for 48 hours at -80°C. Then, the transparent amniotic membrane was vigorously washed with sterile injectable NaCl 0.9% three times, consecutively. At the final step, the denuded HAM was expanded on the sterile nitrocellulose paper, epithelial side up, sealed in the appropriate cover and stored at -80°C. All procedures performed under sterile cabinet laminar flow class II.
Results: The obtained Denuded HAM was used for explant culture of limbal stem cells. The data illustrate the amount of P 63 positive limbal stem cells have raised on our cryopreserved denuded HAM compare to the commercial enzymatic denuded HAM (P=0.005).
Conclusion: Based on our results and data obtained from other studies, the novel cryopreservation method applied for decellularization of the human amniotic membrane support the cellular proliferation while it retains and supports the limbal cell stemness which plays the key role in regenerative medicine compared to the commercial enzymatic denuded HAM. To sum up, the grafts of cryopreserved human amniotic membrane seems to be the appropriate extracellular matrix biological membrane for epithelial layer reconstruction.
Keywords: Human amniotic membrane; Cryopreservation; Cell expansion; Limbal stem cells; Decellularization

PS-122. Novel Engineering-Based Methods for Tissue Growth Assessment in Lung Regeneration

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Background and Aim: Lung tissue engineering is a promising field with the goal of developing functional substitutable tissues for transplantation or in vitro monitoring of drug toxicity in model disease tissues. To obtain efficient engineered tissues, monitoring cell viability is one of the significant difficulties that should be overcome. This study aimed to establish monitoring strategies which were required to assess the effect of different stimuli and feeding and aeration strategies on cell survival and growth.
Methods: In this research, two different monitoring systems were developed based on respiration activity. Human umbilical cord vein endothelial cells (HUVECs) were seeded into rat acellular lung scaffolds, and then, cell viability was monitored during the culture period (3-day and 10-day periods).
Results: The monitoring systems provided cell growth profiles representing essential information on cell viability and growth states during the culture period. MTT analysis, H&E staining, SEM imaging, and glucose consumption measurement proved the consistency of the results obtained by the monitoring systems.
Conclusion: The monitoring strategies provided useful information which helps control, optimize, and modify the lung regeneration process and eventually produces a functional implantable lung.
Keywords: Acellular lungs; Monitoring strategy; Tissue engineering; Regenerative medicine; Lung regeneration

PS-123. Synthesis and Evaluation of Nanocomposite Scaffolds Based on Polyurethane and its Effect on Mesenchymal Stem Cells Proliferation

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Background and Aim: Polyurethane (PU) is a biodegradable and biocompatible polymer with various medical applications which is proper for use in the proliferation of stem cells. Different nanoparticles can improve the structural characteristics of scaffolds for adhesive, migration and proliferation of cell lines such as Au, Fe, Zn and cobalt

nanoparticles (CoNPs). The aim of this study is designing a new and proper scaffold to evaluate the stem cells adherence and proliferation
Methods: PU scaffolds and CoNPs were characterized by following methods and techniques: the size of CoNPs was reported by Zeta Sizer (DLS), and scaffold properties were evaluated by Fourier transform infrared spectroscopy (FTIR), contact angle, scanning electron microscopy (SEM) and differential scanning colorimetric (DSC). MTT assay and Acridine Orange/Ethidium Bromide (AO/EB) staining were used to assess the biocompatibility of CoNPs and PU scaffolds regarding cell attachment and proliferation support.
Results: DLS technique showed the average range of 30 nm for CoNPs. FTIR results showed the presence of CoNPs inside the PU nanocomposite scaffolds. The contact angle results indicated that the surface hydrophilicity of the PU-CoNPs was increased after adding CoNPs to PUs. SEM images showed that the cells were adhered, migrated and proliferated well on the scaffolds. Acridine Orange staining and MTT assay showed that 1 mg/mL of CoNPs and 1% PU-CoNPs are not only toxic to the cells but also increase their proliferation. Our results showed that CoNPs/PU is highly effective, biodegradable and biocompatible enough to apply in MSCs proliferation.
Conclusion: According to the results, PU-CoNPs could be an appropriate scaffold for supporting cell adherence and proliferation as well as osteogenic differentiation of stem cells.
Keywords: Nanocomposite Scaffold; Cobalt nanoparticles; Polyurethane; Stem Cells Proliferation

PS-124. The Panel of Circular RNAs and Target Gene Expression as a Biomarker to Early Detection of Gastric Cancer Based on Bioinformatics Methods

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Background and Aim: Gastric cancer (GC) is one of the most common cancers worldwide Gastric cancer is a multi-factorial and multi-step process that involves significant molecular participation and complex network regulations. Advancements in molecular medicine help predict the possibility of the recurrence of late-stage gastric cancer with molecular markers effectively. In a series of molecular markers, circular RNAs (circRNA) provides several advantages. Tumor-suppressing and oncogenic functions of circRNA plays a strong regulatory function in carcinoma. In this study, we aimed to identify target genes and specific biomarkers for the identification and treatment of GC.
Methods: In this bioinformatic study, all of the circRNAs were collected from the literature studies that they were screened based on high throughput technics like RNAseq and microarray screening of normal and cancer sample of patients with GC. The gene expression profiles of GC were obtained from Gene Expression Omnibus (GEO) (available at http://www.ncbi.nlm.nih.gov/geo/) database, which included 20 gastric cancer tissue samples from previously untreated patients and 20 normal gastric tissue samples. The following keywords and their combinations were used: homo sapiens and Gastric cancer. The original studies that compared gene expression profiling between GC and normal control (NC) biopsy tissues or cultured cells were included in this study. Nonhuman studies and reviews were deleted. Gastric cancer gene expression profiles of the GSE27342 and GSE22804 datasets were downloaded from the GEO.
Results: Analysis of circRNA database: After completing the final analysis, we collected 30 valuable circRNA includes the 15 upregulated and 15 downregulated such as Has-circ-0080517, Has-circ-0000278, Has-circ-0026674 and Has-circ-0054220 as a novel panel of biomarkers to detect GC at the early stage. All these advice panels of biomarkers were evaluated in the "Cancer Specific CircRNA Databases". According to the limited number of studies regarding the role of CircRNA in GC more studies will be needed in the future to demonstrate the importance of CircRNA in tumor initiation, invasion, and metastasis, as well as their utility as therapeutic targets. Differentially expressed genes (DEGs) and circRNAs (DECs) in the integrated analysis of microarray datasets: After the electronic search, there were 42 microarray studies obtained according to the inclusion criteria. A total of 3327 DEGs; 1330 of which were downregulated and 1997 of which were upregulated, and 50 DECs, including 5 down and 45 upregulated, were identified in GC samples compared with healthy gastric tissues.
Conclusion: Gastric cancer was the leading cause of cancer-related mortality worldwide. In recent years, many studies have discovered the link between biomarkers and gastric cancer in the process of tumorigenic and tumor progression, some of which suggest that circRNA may serve as new biomarkers for GC patients. The most consistently overexpressed genes of CXXC5, NOTCH1, MALAT1, MYH9, and other genes might participate in the pathology of GC, and they will be the important biomarkers for the diagnosis of GC and in future studies, this panel will be generalizable.
Keywords: Gastric cancer; Homo sapiens; Bioinformatic and CircRNAs

PS-125. Multipotent Stem Cells Derived from the Adult Mouse Ovary

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Background and Aim: The type(s) and differentiation potential of stem cells present in the mammalian ovary are largely unknown; while oogonial stem cells have been reported, we explored the possibility that multipotent stem cells may reside in the ovary and have wide differentiation potential.
Methods: Homogenates of whole mouse ovaries were sorted using the stem cell surface markers Sca-1 and SSEA-1/CD15. Viable double-positive cells (3-10 μm in diameter) were evaluated immediately after sorting and after culture using differentiation conditions. Ovarian derived stem cells were differentiated into the three mesenchymal cell type's adipocytes, chondrocytes, or osteocytes. The subsequent culture was performed in media containing BMP-4 and/or retinoic acid (RA). RA, BMP-4 or the two agents in combination, consistently stimulated germ cell gene expression.
Results: RA treatment strongly stimulated germline gene expression and also the development of cells that were morphologically reminiscent of oocytes. The germ cell genes Dazl, Ddx4, Figla, Gdf-9, Nobox, Prdm9, and Sycp-1 were all detected at low levels. Remarkably, treatment with BMP-4 alone significantly increased protein expression of the granulosa cell product Anti-müllerian hormone (AMH).
Conclusion: We have shown that an inclusive isolation protocol results in the consistent derivation of multipotent stem cells from the adult ovary; these cells can be differentiated towards the germ cell fate (RA alone), somatic ovarian cell fate as indicated by AMH production (BMP-4 alone),

or classical mesenchymal cell types. Taken together these data suggest the presence of multipotent mesenchymal stem cells in the murine ovary
Keywords: Stem Cells; Ovary; BMP-4; Retinoic Acid; Multipotent

PS-126. Menstrual Blood Stem Cells Show Higher Efficiency in Comparison with Their Conditioned Media in Repair of Cardiac Function in Rat Model of Myocardial Infarction

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Background and Aim: Myocardial infarction (MI) assumes to be a major public health problem in the world. The aim of the present study was to compare the effects of administration of menstrual blood stem cells conditioned media (CM) with menstrual blood stem cells (Mens SCs) on cardiac function in myocardial infarction in rats as an animal model.
Methods: For this purpose, forty male Wistar rats were randomly divided into 4 groups: 1) sham, 2) myocardial ischemia (MI), 3) Mens SCs received, and 4) CM received. The animals were anesthetized with 80 mg/kg of ketamine and 20 mg/kg of xylazine intraperitoneally and placed under positive pressure ventilation. After thoracotomy, the heart was exposed and the left anterior descending (LAD) coronary artery was permanently ligated by 0-6 prolene suture. 30 minutes after ligation and induction of MI, the animals belonged to the MI and CM groups received an intramyocardial injection of 120 µL phosphate-buffered saline (PBS) and CM into the peri-infarct zone at 3 points, respectively. The animals of group 3 received 1.5×10^6 Mens SCs suspended in 120 µL Mens-CM at 4 sites (30 µL per each site) in the borders of MI region. Sham-operated rats were submitted to an identical procedure except for LAD ligation. Echocardiography and histopathological evaluation were performed at day 7 and 28.
Results: The results demonstrated significant scar size reduction and increased ejection fraction (EF) and fractional shortening (FS) after Mens SCs therapy ($P<0.05$) however, the group received CM, showed no significant improvement in myocardial infarction ($P>0.05$). Histopathologic studies in the group received Mens SCs showed a decrease in the amount of fibrosis and also relatively preservation of the cardiomyocytes in the ischemic region. In the CM –received group, in the infarct area, most cardiomyocytes were lost and lysed. Scar size was significantly smaller in Mens SCs received group compared to the CM-received group ($P < 0.05$). Interestingly, cartilage metaplasia was observed in MI and the CM-received groups, considered as a negative consequence of tissue regeneration in severe cardiac ischemia. However, Mens SCs therapy sounds to protect from this change.
Conclusion: Our findings indicated that application of Mens SCs could improve cardiac structure and function in animal models of myocardial infarction
Keywords: Menstrual blood stem cells; Conditioned media; Rat; Histopathology; Echocardiography

PS-127. MicroRNA-221: A Promising Tool for Acute Myocardial Infarction

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Background and Aim: Cardiovascular disease is a polygenic disorder that is caused by several genetic and non-genetic risk factors. The identification of genes that are involved in its pathogenesis has greatly increased our knowledge of the mechanisms that underlie this condition. Recently, the general principles of microRNAs biomarkers and stem cell therapy have provided new modalities for effective treatment and early detections.
Methods: Fifty patients with acute myocardial infarction under percutaneous coronary intervention (PCI) without requiring open-heart surgery and one-hundred healthy controls in this study were selected. Expression levels of and microRNA-221 in blood using real-time PCR and other cardiac chemistries such as CTNI, CK-MB, Creatinine were evaluated.
Results: Our data analysis revealed that microRNA-221 levels were significantly increased in primary PCI myocardial infarction patients compared to the healthy group. Expression levels of microRNA-221 were 210-fold compared to the healthy group. Receiver operating characteristic analysis was shown that the area under the curve (AUC) of miR-221 was 0.945($P<0.001$) in primary patients
Conclusion: The appropriateness of early detection and medical therapy for acute myocardial infarction depends on many factors. In this way, microRNAs can be appropriate biomarkers for patients with myocardial infarction.
Keywords: MicroRNA-221; Acute Myocardial Infarction; Early detection

PS-128. 3D Bioactive Alkyl Peptides Scaffold to Conduct Neural Differentiation

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Background and Aim: Biocompatible and non-immunogenic materials like peptides are a great candidate for 3D scaffolds construction in tissue engineering. Self-assembling alkyl peptides which can construct nanofibers in at this category of materials. There is the possibility of functionalizing of the scaffolds by bioactive peptides for various properties like differentiation. Here, a functionalized alkyl peptide with neural differentiating properties has been taken to investigate its fiber formation and differentiation activity. The FAQRVPPEEEGGGAAAAK(C16) is the alkyl peptide sequence which FAQRVPP plays neural differentiation role and the rest of the sequence applied for different properties.
Methods: we considered two different systems composing of just all functionalized alkyl peptides and a combination of functionalized and non-functionalized (EEEGGGAAAAK(C16)) system with 1:2 ratios. The later one could help to have a less condensation of epitopes of the fiber surface and more stability of fiber and accessibility of epitopes. So by consideration of previously computational study of fiber formation from alkyl peptides they were synthesized and analyzed by CD, FTIR spectroscopy, and TEM imaging.
Results: The CD and FTIR results showed that in the combined system more beta structure has formatted which can verify more prepotency of fiber construction of this system than the all functionalized system. Beside TEM image visualized the nanofibers with 8 and 10 nm in diameters for all functionalize and combined system respectively. This fibrous structure also formed a 3D scaffold in hydrogel networks to be used for cell culture.
Conclusion: To summarize two different types of nanofiber have been formed. The combined system showed a more cylindrical fiber type with more width and less length and the all-functionalized system have a lesser width and higher length. these two different types of fiber can affect the differentiation efficacy and mechanical interaction of cells those surfaces.
Keywords: Bioactive scaffolds; Alkyl peptides; Self-assembling nanofiber; Amphiphilic peptides

PS-129. Characterization of Adipocytes Differentiated from Primary Human Dermal Fibroblasts of Normal and Diabetic Patients

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Background and Aim: The sharp rise in the incidence of diabetes and obesity has reached an epidemic state worldwide. Obesity is one of the risk factors for onset of type 2 diabetes, it also can affect the cardiovascular health leading to the onset of cardiometabolic disease. The expansion of white adipose tissue in obese individuals can affect both lipid and glucose metabolism. Diabetic patients often suffer from increase adipose tissue that is defective in glucose metabolism. Therefore, it is essential to examine the cellular and molecular changes in adipocyte of human diabetic patients. Since obtaining human adipocytes from patients are challenging, we aimed to establish protocols for the differentiation of human skin fibroblast cells from normal and diabetic patients to adipocytes. Our main objective was to characterize these adipocytes and determine whether they maintain the diabetic phenotypes after differentiation.

Methods: In our current study, we differentiated primary human skin fibroblast cells from normal and diabetic patients into adipocyte after 10 days culture in differentiation media. Oil Red O staining was utilized to assess differentiation to adipocytes. Real-time PCR was used to assess changes in gene expression.

Results: Oil red O staining showed a significant accumulation of lipid droplets in the differentiated cells. Real-time PCR of non-differentiated skin fibroblast showed a significantly higher mRNA level of Adiponectin, PPAR gamma and CEPB beta of diabetic patients compared to normal. After differentiation the level of Adiponectin mRNA in diabetic cells further increased however, there was no difference in CEBP beta mRNA level in diabetic as compared to normal human differentiated adipocytes. Furthermore, we observed a significant increase In FABP mRNA level in diabetic patient accompanied by a significant decrease in GLUT-4, insulin receptor and insulin receptor substrate 1 mRNA.

Conclusions: Our data illustrates that adipocytes differentiated from skin fibroblasts of diabetic patients maintain the characteristics of the disease state. These adipocytes could be used for further biochemical studies.

Keywords: Dermal fibroblast differentiation; Adipocytes; Diabetes; Gene expression

PS-130. Gene Expression Profile of Immunoregulatory Cytokines Secreted by Mesenchymal Stem Cells Obtained from Different Tissues

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Background and Aim: Mesenchymal stem cells have a long-term clinical application and are well-known due to their immunomodulatory characteristics. Many clinical applications have been proposed in arrays of studies, therefore, we attempt to investigate which mesenchymal stem cells source and passage can provide the best possible immunoregulatory responses in patients.

Methods: We gathered samples of adipose tissue and bone marrow from volunteer donors. Bone marrow mononuclear cells were isolated by Ficoll, the cultivation of cells carried out standard conditions (5% CO2, 37°C). MSCs isolated were identified according to phenotype, physical properties, and cell surface markers. MSCs were attached to the plastic surface of the flask and getting fibroblast shapes. To characterize them as a Mesenchymal stem cell CD73, CD90 and CD105 as appositive markers and CD14, CD34 and CD45 were considered by flow cytometry. Four different passages of stem cells contain 3, 5, 7 and 9 selected as different groups to study the expression level of cytokines. All were utilized for RNA extraction and cDNA synthesis. Expression of the main immunomodulatory cytokines ICAM, IDO, VCAM, TGF-beta, IL-6, IL-10, PGE2 was measured by real-time PCR. Expression levels of all cytokines were analyzed according to comparative analysis method by Rest software.

Results: Totally MSCs were isolated from 8 donors. They were equally obtained from bone marrow and adipose tissue. Expression level Variation of cytokines in different passages was observed. In addition, cytokine panel of Mesenchymal stem cells with different source was not identical. According to the bone marrow-derived MSCs Real-Time PCR results; IDO, VCAM expression in passage 3, PGE2, ICAM, IL-6 gene expression in passage 5, and TGF-beta in passage 9 were more pronounced. On the other hand, in adipose tissue sources, the expression of ICAM, IDO, VCAM, IL-6 genes in passage 7 was up-regulated.

Conclusion: MSCs are the reliable source of stem cells in clinical application. However, some MSCs derived cytokines stimulate disease progression. IL-6 could be nominated as a stimulating factor in Liver Fibrogenesis. So, investigating in the immunoregulatory mechanism of MSCs is demanded. According to our results, passage 7 in Adipose tissue MSCs and passage 3 and 5 of the Bone marrow MSCs would be better in clinical applications of autoimmune disorders. In other side Passage number 5 of BM-MSCs seems not to be a suitable candidate for fibrotic diseases.

Keywords: MSC; Immunoregulatory; Adipose tissue; Bone marrow; Cytokine

PS-131. Zeolite Based Composites for Bone Tissue Engineering

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Background and Aim: A wide variety of injuries and orthopedic problems such as trauma, tumor, deformity and etc caused bone regeneration and repair faced problems. Tissue engineering offers the possibility to create completely natural tissue and replace failing or malfunctioning organs. The aim of this study was to design the nanofiber scaffolds containing Poly Caprolactone (PCL), Poly Ethylene Glycol (PEG) and Different Percent of Zeolite to investigate adhesion and proliferation of Human Amniotic Mesenchymal Stem Cells (HA-MSCs) and cell viability. Scaffolds were produced by Electrospinning method.

Methods: PCL and PEG were synthesized via Ring-Opening method. PCL-PEG co-polymer provides good mechanical properties for bone tissue engineering applications. PCL-PEG co-polymer provides Initial expectations of suitable scaffold for bone regeneration. Zeolite powder was made by the hydrothermal method. X-ray Powder Diffraction (XRD) of Zeolite was obtained and compared with Powder Pattern Identification Table. Scaffolds were prepared by electrospinning method (Fanavaran Nano-Meghyas, Iran) in 3 categories include PCL-PEG, PCL-PEG-Zeolite 0.05 g, and PCL-PEG-Zeolite 0.1 g. The production of nanofibers via electrospinning is affected by many operating parameters include electrospinning parameters, solution properties, and environmental parameters. Fourier Transform Infra- Red (FTIR) analysis of nanofibers was obtained. HA-MSCs were seeded on fabricated scaffolds and viability of them on scaffolds investigated by MTT assay on the 3rd, 7th and 14th days. MTT assay is a cytotoxicity test for Cell viability measurement. Cells were fixed via Glutaraldehyde on nanofibers on the 14th day and coat with gold nanoparticles for SEM studies.

Results: The result of XRD compared with Powder Pattern Identification Table and specific Zeolite peaks were identified. FTIR showed functional groups of scaffolds elements. MTT assay results showed that HA-MSCs have the maximum growth and cell viability on PCL-PEG nanofibers on the 3rd day and on PCL- PEG-Zeolite 0.1 g nanofibers on 7th and 14th days. SEM showed excellent adhesion and growth of HA-MSCs

on PCL- PEG-Zeolite 0.1 g scaffold. The presence of Zeolite in scaffold structure increased cell attachment and improve cell properties so that cell viability on PCL-PEG-Zeolite 0.1 g nanofibers is more than PCL-PEG-Zeolite 0.05 g nanofibers and cell attachment on PCL- PEG-Zeolite 0.1 g scaffold is better than PCL-PEG-Zeolite 0.05 g nanofibers. SEM images showed excellent cell adhesion of HA-MSCs on PCL- PEG-Zeolite 0.1 g nanofibers. This scaffold is recommended for bone and dental therapeutic applications because of it's an ideal property in regeneration.

Conclusion: PCL-PEG co-polymer improves scaffold properties. Investigation of MTT results showed that amount of Zeolite effect on growth, cell viability, and adhesion. Increasing percent of zeolite in electrospun scaffold improves cell adhesion on designed scaffolds and cell viability. A scaffold composed of PCL-PEG-Zeolite 0.1 g could be a good candidate for bone development, bone repair, skeletal regeneration therapy, and bone tissue engineering applications. Scaffold composed of PCL-PEG-Zeolite 0.1 g could be a good candidate for bone development, bone repair, skeletal regeneration therapy and bone tissue engineering applications. Zeolite presence in scaffolds improved osteoconductivity, osteogenesis and cell behavior. Increasing the percentage of Zeolite in scaffold's structure improved cell attachments and cell behavior on designed scaffold.

Keywords: Tissue engineering; Bone regeneration; Zeolite

PS-132. Microencapsulated Hematopoietic Stem Cell Model Proliferation in a Small Scale Stirred Tank Bioreactor: Influence of Mixing Rate

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Background and Aim: Stem cells are known for their self- renewal properties. Due to the importance of stem cells in the treatment of diseases, their use for medical applications is very important. Static systems are not responsive and it is necessary for the development of dynamic systems (bioreactor). Among the bioreactors, stirred bioreactors are simply used for design and construction for dynamic growth.

Methods: In this study, a bioreactor with optimal hydrodynamic conditions (a height of 6 cm, diameter of 6.5 cm and impeller diameter of 3.25 cm) was designed and built. Hydrodynamic parameters (integrated shear factor (ISF), the maximum shear rate (τmax) and etc) were calculated. The working volume of bioreactor was 100 mL and the initial cell density was 1×10^5 cells/mL, respectively. The proliferation of hematopoietic stem cell model (U937) in suspension and microencapsulated in Alginate- Gelatin microcapsules (1%- 1.25%) was compared at different impeller speeds (50 and 100 rpm) with static T- flask. Microcapsules with an average diameter of 500 µm (±50 µm) were produced through electrostatic methods. The amount of substrate consumed and produced metabolites were also measured during the process.

Results: The results showed that at 50 rpm, compared to the static culture, the final number of suspended cells increased by 10%, and the final number of cells in the bioreactor after 7 days reached 274 million cells, while the cells at 100 rpm in the bioreactor showed statistically decrease in proliferation as compared to the static cultures. This can be due to the high shear stress at 100 rpm as ISF was calculated 5.46 1/s and 10.46 1/s at 50 rpm and 100 rpm, respectively. To reduce shear stress exerted on the cells at 100 rpm, the cells were microencapsulated in alginate-gelatin microcapsules. The number of microencapsulated cells reached 11 million after the end of the 7 day period at 100 rpm. The size of the microcapsules containing the cell was larger than eddies created at 100 rpm. Therefore, the collision of eddies and microcapsules reduced growth and proliferation of microencapsulated cells.

Conclusion: Given the need for at least 2×10^6 cells per kilogram for treatment of stem cell-based blood diseases, the use of this bioreactor can have a good potential for the growth and proliferation of suspension and microencapsulated stem cells in the treatment of blood patients. However, the size of microcapsules and mixing rate in the bioreactor are important issues that should be considered.

Keywords: Hematopoietic stem cell; proliferation; Microcapsule; Small-scale stirred tank bioreactor

PS-133. Injectable Kappa-Carrageenan-Graphene Hydrogel: Properties and Biomedical Applications for Cartilage Tissue Engineering

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Background and Aim: Injectable hydrogels are examined for cell encapsulation and delivery as they can shield cells from high shear forces. Kappa-carrageenan is a natural polysaccharide closely resembling the native glycosaminoglycan structure, one of the most important constituents of cartilage tissues extracellular matrix. The aim of this study is to modify photo-crosslinkable Kappa-carrageenan-graphene based for cartilage tissue engineering. By creating the injectable hydrogel, injectable therapy will be achieved, which has an unparalleled advantage in which intricate therapy sites like cartilage, can be easily targeted with minimally invasive procedures.

Methods: In this respect, methacrylate Kappa-carrageenan (KaMA) was primarily synthesized to create chemically crosslinked hydrogels emphasizing their use in the context of tissue engineering. Consequently, KaMA-Graphene based nanocomposite was synthesized using graphene oxide (GO) and by increasing interaction with polymers resulting in the formation of shear-thinning hydrogels. Here in parallel, we develop another versatile 2D nanomaterial, GO functionalized with poly(dopamine) (GOPD).

Results: Results showed that incorporation of GOPD significantly improved the mechanical properties of KaMA hydrogel and also it did not have bad properties of graphene oxide usage. The combination of chemical and physical crosslinking procedures enables the formation of hydrogels with highly versatile physical and chemical properties, in order to mention the viability of encapsulated cells.

Conclusion: Ultimately, Kappa-Carrageenan-Graphene engineered systems such as these shows the potential for use as 3D cell culture scaffolds in cartilage tissue engineering, owing to their high-water content, similarity to the natural extracellular matrix (ECM), minimal invasive properties, to match irregular defects.

Keywords: Carrageenan; Hydrogels; Injectable; Shear-thinning; Cell delivery

PS-134. Short Osteostimulation Enhances Osteogenic Differentiation of Human Adipose Stem Cells Seeded on Biphasic Calcium Phosphate Substrate

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Background and Aim: Bone tissue engineering aims to regenerate new functional bone using cells, scaffolds, and clinically applicable osteostimulative factors. Lack of efficient short-term treatment strategies to stimulate osteogenic differentiation of precursor cells has restricted the development of a one-step surgical procedure for bone tissue

engineering. Earlier, we developed a cost-effective, patient-friendly one-step surgical procedure for bone augmentation in patients with bone defects using human adipose stem cells (hASCs). Here we studied whether short treatment of hASCs with an osteostimulative factor, fitting within the procedural time frame, suffices to induce osteogenesis.
Methods: hASCs were shortly (minutes) pre-treated with/without osteostimulative factor, seeded on biphasic calcium phosphate, and cultured for 2 weeks in the absence or presence of the osteostimulative factor. Cell attachment was assessed 30 min after cell seeding. Cell proliferation and osteogenic differentiation were analyzed up to 2 weeks.
Results: Our data showed that short osteostimulation enhanced cell attachment to biphasic calcium phosphate compared to non-treated cells. Compared to untreated controls, short osteostimulation of hASCs increased proliferation and alkaline phosphatase activity of hASCs. Short osteostimulation of hASCs upregulated the osteogenic markers RUNX2 and SPARC, while this was not observed upon continuous osteostimulation. Short osteostimulation of hASCs also upregulated VEGF189 expression which may contribute to the promotion of angiogenesis via stimulation of endothelial cell proliferation.
Conclusion: In conclusion, short osteostimulation of hASCs not only enhances cell attachment to the scaffold at the time of cell seeding but also promotes proliferation and osteogenic differentiation of hASCs. This indicates that short osteostimulation for bone tissue regeneration is highly promising for the treatment of patients with bone defects in a one-step surgical procedure.
Keywords: Bone, osteogenic differentiation; Osteostimulation; Human adipose stem cells; One-step surgical procedure

PS-135. Evaluation of Cord Blood Platelet Gel for The Treatment of Second Degree Burn Wound: A Pilot Study

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Background and Aim: Burn injuries are the most traumatic injuries affecting every organ system and leading to significant morbidity and mortality. Slow wound healing, infection, pain, and hypertrophic scarring continue to remain a major challenge in burn research and management. This study aims to provide an option to an emergency burn patient management that have required an integrated approach. We performed a pilot evaluation of the effectiveness of platelet gel from cord blood (CBPG) as an allogeneic source of different growth factors which play important role in the treatment of second-degree burn wound.
Methods: Cord blood samples are collected in sterile conditions. These samples were transferred to GMP grade clean room for processing. All CB samples were negative for viral and bacterial infection. Then, bags contain CB were centrifuged and two parts (RBC and PRP) were separated. Gelation was done under sterile condition by adding thrombin within 20-30 minutes. Prepared gel changed twice per week, time and size of wounds were measured.
Results: The clinical outcomes of this small group of patients were shown this trial have high efficiency and effectiveness in comparison with the current standard of care for these patients. Time of healing and size of wounds decrease significantly. CBPG releases a number of growth factors such as PDGF, TGF-β1 and -β2, IGF, EGF, FGF and EGF which can affect wound healing.
Conclusion: In conclusion, this pilot study reveals that CPBG is a promising and safe option for the treatment of burn wounds but need for larger studies.
Keywords: Platelet gel; Cord blood; Burn; Wound healing

PS-136. Two-Year Report on Hematopoietic Stem Cell Transplantation for Patients with Wilms' Tumor in the Pediatric Center of Excellence of Iran

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Background and Aim: Wilms' Tumor (WT) is known as the most common pediatric malignancy of the kidney. The majority of patients respond to tumor resection and standard chemotherapy. Relapse, however, is associated with poor prognosis leading to the 4-year survival of approximately 50%. For patients who experience relapse, high dose chemotherapy (myeloablative) followed by autologous hematopoietic stem cell transplantation (auto-HSCT) is a recommended clinical option. Here, we report the outcome of a series of relapsed WT patients treated with auto-HSCT.
Methods: The HSCT department of Children's Medical Center, known as the largest children's hospital in Iran and the country's pediatric center of excellence, has been established since September 2016. In this retrospective study, epidemiologic profile of relapsed WT patients undergoing myeloablative conditioning regimen (busulfan plus cyclophosphamide) followed by auto-HSCT, from Sep 2016 to Sep 2018, is reported. In addition, treatment details and outcomes are presented.
Results: During the two-year period, 178 patients with various diseases underwent HSCT (both allogeneic and autologous) in the department, of whom, four relapsed WT patients (2.2% of all patients) with a median age of 5.5 (range, 3-7) years were treated with auto-HSCT. Three patients were female. HSCT graft was obtained from peripheral blood employing G-CSF mobilization. The median number of cells infused in HSCT was 15 × 10^8/kg WBCs, 7.5 × 10^8/kg mononuclear cells, and 3.3 × 10^6/kg $CD34^+$ cells. After the procedure, all patients achieved hematologic engraftment as well as full chimerism according to short tandem repeat analysis. The median times to granulocyte engraftment and platelet recovery were 10 days and 11 days, respectively. No cytomegalovirus infection and graft failure occurred. The median length of hospital stay was 25 days. Up to the end of the study period, all patients were alive and relapse occurred in only 1 patient.
Conclusion: Despite the remarkable improvement in the overall survival of WT patients, the prognosis of relapsed WT patients has remained disappointing. In such patients, the long-term outcomes have improved dramatically with the advent of adjusted myeloablative conditioning regimens followed by auto-HSCT. Our study provides further evidence for favorable outcomes following myeloablative auto-HSCT in relapsed WT patients.
Keywords: Hematopoietic Stem Cell Transplantation; Wilms' Tumor; MAC Conditioning regimen

PS-137. hsa-miR-140 as a Potent Cell Cycle Regulator in Glioblastoma by Inhibiting CDKs

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Background and Aim: Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor among adults and one of the most lethal cancers. The median survival of patients is 12–15 months after initial diagnosis due to radiation therapy and chemotherapy resistance. Hence new therapeutic approaches are required. GBM is first cancer, which studied by The Cancer Genome Atlas Research (TCGA). According to this study, 78.9% of tumors had one or more alteration affecting Rb function: 7.6% by direct RB1 mutation/deletion, 15.5% by amplification of CDK4/6, and the remainder via CDKN2A deletion.
Methods: miRNAs play a role of gene's regulators by affecting the 3'UTR region of its mRNA. We have chosen CDK4 and CDK6, upregulated genes in GBM and used TargetScan and miRWalk databases to reach a list of miRNAs, which can exert cell cycle arrest via inhibiting CDK4/6 transcription/translation. The hsa-miR-140-5p was the most probable miRNA having wanted function. hsa-miR-140 was cloned in the pLenti-III-eGFP vector. HEK 293T cells were used to package virus particles by using lentiviral packaging plasmids, psPAX, pMD2.G and desirable vector. U-251 cell line is our model for this study. Cells were transduced by lentiviruses carrying hsa-miRNA-140 and after 72 hours cell was trypsinized for RNA extraction and cDNA synthesis. Real-Time PCR method was used to find out the alteration of gene expression's level in treated cells versus the control group. Internal control is beta-2-microglobulin (B2M) gene.
Results: Over 80% of U-251 cells have been transduced successfully after 48 and emitting green fluorescent light under a fluorescence microscope. REST software is used for data analysis and results are shown below. Expression of CDK6 is reduced significantly ($P<0.001$) to 0.158-fold. Although the expression of CDK4 is reduced to 0.201-fold, it is not significant. (N=2)
Conclusion: Needless cell proliferation caused by uncontrolled cell cycle and eventually cancer appeared. CDK4 and CDK6 are genes that overexpressed in glioma tissue, pair with Cyclin D and let cells pass G1 checkpoint and enter to S phase. In this study, we have shown has-miR-140 can be a good suppressor of CDK6, which is a promising approach to stop cancer proliferation. This study should examine in other glioma cell lines such as U87 and A172. In addition, more assays like cell migration and luciferase should be done to approve our claim.
Keywords: Glioblastoma; Cyclin-dependent kinase 4; Cyclin-dependent kinase 6; MicroRNAs; miR-140

PS-138. Evaluation of PCL-PANi-GEL Nanofibrous Scaffolds for Tissue Engineering Purposes

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Background and Aim: The structure of biocompatible scaffolds is one of the biggest concerns of tissue engineers. Ideally, a scaffold should mimic structural and biological functions of the extracellular matrix (ECM); both in terms of physical structure and chemical component. Finding a feasible combination of polymers to fabricate a feasible scaffold is a big concern of scientists, therefore, we aim for finding a proper setup for a useful scaffold for tissue engineering purposes.
Methods: Nanofibers were fabricated using electrospinning and to define their physical structure SEM imaging was used to evaluate the fibers ability and potential in tissue engineering MTT assay was used furthermore the fibers ability to help stem cell differentiation was evaluated by osteogenic differentiation of hMSCs.
Results: Our results showed that there can be a fine composition to help stem cell differentiation using PCL PANi GEL not only we observed that this nanofiber is able to support stem cell differentiation but it can also help stem cell proliferation and cell attachment.
Conclusion: we concluded that PCL PANi GEL is a feasible setup for supporting stem cell differentiation and proliferation also it can be concluded that this combination of polymers is able to help support stem cells for different purposes of tissue engineering.
Keywords: Nanofibers; Nano-scaffold; Stem cell differentiation

PS-139. Influence of Gelatin on Characteristics of Enzymatically Cross-Linkable Alginate as a Promising Injectable Hydrogel for Tissue Engineering Applications

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Background and Aim: Alginate, a natural polysaccharide, is one of the most common biomaterials due to its abundance, biocompatibility, and gentle cross-linking procedure. However, the stability of alginate capsules generally weakens over time due to exposure to certain species in the cell culture medium. Modification of alginate with phenol moieties (Alg-Ph), which can be gel enzymatically with horseradish peroxidase (HRP) in the presence of peroxide hydrogen, can increase alginate hydrogel stability in the cell culture medium.
Methods: In this work, alginate was modified by tyramine hydrochloride in the presence of EDC/NHS for 1 day at 25°C. To provide adhesive motifs, gelatin was added to the enzymatically-gellable alginate and the obtained hydrogel (Alg-Ph-Gel) was characterized by SEM, gelation time, enzymatic degradation, mechanical and swelling properties as well as water vapor loss for use in cartilage tissue engineering applications. Chondrocytes (at X0 = 2×10^6 cells/mL gel) were cultured for 14 days and MTT assay was used for cell activity measurements. Direct subcutaneous implantation of the cell-laden hydrogels, as well as the hydrogels without cells, was carried out in rats and H & E staining was performed after 1 month.
Results: SEM images showed the addition of gelatin could increase the uniformity of the pore sizes inside the Alg-Ph-Gel hydrogels. Alg-Ph-Gel hydrogels also showed higher gelation time, degradation rate, as well as swelling properties as the hydrogels, swelled 1.6- fold more than the Alg-Ph hydrogels after 72 h in PBS. However, mechanical strength (1.7-fold), as well as water vapor loss, decreased for the Alg-Ph-Gel hydrogels. Chondrocyte cells cultured in the Alg-Ph-Gel hydrogels could maintain their original phenotype and proliferate more than 1.4-fold higher than the cells cultured in the Alg-Ph hydrogels after 14 days ($P < 0.05$). The subcutaneous hydrogels could be identified readily without complete absorption and signs of toxicity or any untoward reactions after 1 month. Viable chondrocyte cells inside globular aggregates were seen as red-colored areas in the cell-laden hydrogels.
Conclusion: The study demonstrates that gelatin can improve the enzymatically-gellable alginate hydrogels for cartilage tissue engineering application.
Keywords: Tissue engineering; Alginate; Gelatin; Stem cells; Hydrogel

PS-140. Legal and Social-Ethical Issues of Stem Cells

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Background and Aim: The human embryonic stem cells have great

promise in the treatment of many serious illnesses, due to their high ability to differentiate and produce various types of body cells. In our country, in recent years, the activities have led to success in the formation of stem cells, while the processing and use of this technology have been accompanied by a wide range of ethical and legal issues in the world.
Methods: Includes the collection of related articles that were conducted through e-learning from academic and online colleges.
Results: Research on human stem cells has been of public interest. Pluripotent stem cells (cells that can become many different cells in the body) are separated from human embryos that are multi-day. The pluripotent stem cell also evolved from the embryo tissue (which has grown for more than 8 weeks). As science and technology are progressing, there are ethical perspectives on these developments. It is important to study scientific issues and ethical issues.
Conclusion: By studying similar studies in this field, one can conclude that in some countries of the world ethical foundations and legal regulations in this field have been developed and in many cases the void and the need for it are fully perceptible, and given the fact that in Iran an archetype In this regard, which is consistent with our national, religious and legal culture, it has not yet been designed and developed, therefore, it is necessary to formulate a comprehensive code in this regard.
Keywords: Stem cells; Ethical foundations; Embryo

PS-141. Curcumin Decreases H2O2-Induced Oxidative Stress and PDGF Gene Expression in Cultured Endothelial Cells

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Background and Aim: Oxidative stress increases platelet-derived growth factor (PDGF) gene expression in endothelial cells which contributes to vascular dysfunction and atherosclerosis. The dysregulated redox balance between ROS and antioxidant producing systems results in oxidative stress. Further, Curcumin as a main antioxidant part of turmeric has anti-inflammatory, antioxidant, anticancer and antitumor effects. The aim of this study was to assess the property of Curcumin on reducing the risk of atherosclerosis by a decrease in H_2O_2-induced oxidative stress and PDGF gene expression in endothelial cells.
Methods: The cultured Bovine aortic endothelial cell (BAEC) model was used for evaluating the stimulatory effect of H_2O_2 on increasing oxidative stress and PDGF gene expression, and also the inhibitory effect of Curcumin on decreasing oxidative stress and PDGF gene expression in cultured endothelial cells. There were 3 groups as; Group 1 (Control): untreated group, Group 2: treated with 8 µL of 5 µM H_2O_2 for 24 h, Group 3: treated with 8 µL of 5 µM H_2O_2 and 2 µL of 10 µM Curcumin for 24 h. Real-Time PCR was used to analyze PDGF-β gene expression in treated and untreated Bovine aortic endothelial cells.
Results: There was a significant increase in the level of PDGF-β mRNA expression in group 2 (treated with 20 µL H_2O_2) compared with control and group 3 (treated with 20 µM H2O2-10 µM Curcumin) ($P < 0.05$). Also, group 3 as a treated group with H_2O_2-Curcumin showed a significant decrease in the level of PDGF- β gene expression compared with the H_2O_2 treated group.
Conclusion: H_2O_2 as a potent oxidant can promote atherosclerosis by increase on atherosclerosis-induced genes expression including PDGF-β. Then, PDGF-β causes vascular smooth muscle cells (VSMCs) proliferation and migration, as a critical factor for promoting atherosclerosis. Curcumin co-treatment in H_2O_2 incubated cells showed a significant decrease in the expression of PDGF-β mRNA versus H_2O_2 treated cells. Curcumin reduces the risk of VSMC proliferation and atherosclerosis in oxidative stress-exposed endothelial cells. Thus, our results support the protective effect of Curcumin against atherosclerosis.
Keywords: Atherosclerosis; Bovine aortic endothelial cells; Curcumin; H_2O_2; PDGF; Oxidative stress

PS-142. Electrospun Poly-L-lactic acid/poly Vinyl Alcohol Nanofibers Improved Insulin-Producing Cells Differentiation Potential of Human Adipose Derived-Mesenchymal Stem Cells

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Background and Aim: Pancreatic differentiation of stem cells will aid treatment of patients with type I diabetes mellitus (T1DM). Synthetic biopolymers utilization provided extracellular matrix (ECM) and desired attributes in vitro to enhance conditions for stem cells proliferation, attachment, and differentiation. A mixture of polycaprolactone and polyvinyl alcohol (PCL/PVA)-based scaffold, could establish an in vitro three-dimensional (3D) culture model.
Methods: The objective of this study was investigation of the human induced pluripotent stem cells (hiPSCs) differentiation capacity to insulin-producing cells (IPCs) in 3D culture were compared with conventional culture (2D) groups evaluated at the mRNA and protein levels by RT-qPCR and immunofluorescence assay, respectively. The functionality of differentiated IPCs was evaluated by C-peptide and insulin release in response to glucose stimulation test.
Results: Real-time RT-PCR results showed that iPSCs-IPCs expressed pancreas-specific transcription factors (Insulin, Pdx1, Glucagon, Glut2, and Ngn3). The expressions of these transcription factors in PCL/PVA scaffold were higher than 2D groups. In addition to IPCs specific markers were detected by immunochemistry. These cells in both groups secreted insulin and C-peptide in a glucose challenge test by ELISA showing in vitro maturation.
Results: Real-time RT-PCR results showed that iPSCs-IPCs expressed pancreas-specific transcription factors (Insulin, Pdx1, Glucagon, Glut2, and Ngn3). The expressions of these transcription factors in PCL/PVA scaffold were higher than 2D groups. In addition to IPCs specific markers were detected by immunochemistry. These cells in both groups secreted insulin and C-peptide in a glucose challenge test by ELISA showing in vitro maturation.
Conclusion: The results of the current study demonstrated that the PCL/PVA nanofibrous scaffolds can enhance the differentiation of IPCs from hiPSCs. In conclusion, this research could provide a new approach for beta cell replacement therapies and pancreatic tissue engineering for T1DM in the future.
Keywords: Adipose-derived mesenchymal stem cells; Poly-L-lactic acid/polyvinyl alcohol; Scaffold; Insulin-producing cells; Differentiation

PS-143. Adipose Tissue-Derived Mesenchymal Stem Cells Promote Angiogenesis and Prolong Graft Function in Pancreatic Islet Transplantation

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Background and Aim: Although pancreatic islet transplantation has been suggested as an alternative therapy for type 1 diabetes mellitus (T1DM), there are efficiency concerns that are attributed to poor engraftment of transplanted islets. Avascular phase and the poor formation of blood vessels in the late period lead to islet allograft loss which contributed

to inefficiency and short-acting of islet transplantation. To overcome this limitation, in this study, we attempted to determine whether the inclusion of adipose tissue-derived mesenchymal stem cells (AT-MSCs) with islet transplantation could improve the graft function and neovascularization.
Methods: Islets were transplanted, either alone or with an in vitro-expanded AT-MSCs, into an omental pouch in a rat model of streptozotocin (STZ)-induced diabetes. After transplantation, the grafted animals were monitored every 5 days for their non-fasting blood glucose levels and changes in body weight. In addition, at the end of the specified treatment period (day 75), blood and omentum-bearing islet graft were collected from different groups for biochemical and histological analysis in serum and tissue. Moreover, to test and evaluate the impact of transplantation of AT-MSCs on the in vivo vascularization of the islet grafts, we also assessed CD31 staining, a marker of endothelial cells.
Results: Our results indicated that transplantation of 2000 islets only achieved normoglycemia with graft survival of >50.5±14.15 days (mean±standard error of the mean), whereas that of 1000 allogeneic islets associated with the shorter islet graft survival (16.75±2.18 days). Interestingly, co-transplantation of 1000 islets with 6×10^6 AT-MSCs, with half of the required number of islets for successful islet transplantation alone, reversed diabetes and significantly prolonged graft survival (>47.25±16.03 days) and resulted in an improvement of islet allograft outcome similar to that of sole islet transplantation. Although, obtained results indicated the positive expression of CD31 in the grafts of both transplanted groups, a more pronounced CD31 staining was evident in the rats transplanted with MSCs+islets (1000) as compared to the islets alone (2000) transplanted rats, suggesting a potential role of AT-MSCs in stimulating neovascularization.
Conclusion: Therefore, our findings provide the evidence for the protective action of AT-MSCs on islet survival and function through the improvement of revascularization suggesting that AT-MSCs-assisted islet transplantation might be a better strategy for the treatment of T1DM, compared to the conventional method of islet transplantation.
Keywords: Co-transplantation; Mesenchymal stem cells; Pancreatic islets; Type 1 diabetes mellitus; Vascularization

PS-144. Stem Cells Injected Into the Deaf Rat Cochlea via Round Window Could Survive for at Least 1 Week in the Endolymph

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Background and Aim: Noise-Induced Hearing Loss (NIHL) occurs due to prolonged exposure to high-intensity sound. Although NIHL has been widely studied over the past decades, hearing aids and cochlear implant are only clinically effective in the treatment of hearing loss. In recent years, stem cell transplantation has been investigated to repair damaged tissues of the inner ear (hair cells and auditory neurons). Because of small size and the complex structure of the cochlea, stem cell implantation is difficult. It is necessary, stem cell injection procedure has a minimal adverse effect on the target tissue. Cochleostomy was currently used for stem cell injection which can damage to the hearing. In addition, the cell survival rate is an essential factor for damaged hair cells regeneration. Therefore, we investigated the survival rate of injected bone marrow-derived mesenchymal stem cells (BMSCs) through a round window in a rat model of NIHL.
Methods: For the hearing loss induction, adult male rats were subjected to white noise (110 dB) paradigm for 6 hours in 5 days. Distortion-product otoacoustic emission (DPOAE) were recorded before and after noise exposure. Hoechst labeled - MSCs were implanted into the rat cochlea via the round window. Number of Hoechst- labeled cells were assayed in the endolymph for 24 h, 72 h, 7 and 10 days after injection.
Results: Our data show that stem cell transplantation into the cochlea through round window niche is a safe surgical approach. As well, implanted cells survived in the endolymph for a postoperative period of at least 1 week.
Conclusion: BMSCs can survive in the adult rat cochlea for at least 1 week after injection through the round window niche.
Keywords: BMSCs; Round Window; Survival Rate; Endolymph

PS-145. Osteogenic Differentiation of Mesenchymal Stem Cells on Polyurethane Nanocomposite Scaffold Containing Modified Clay Nanoplates

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Background and Aim: In regenerative medicine, a combination of biocompatible scaffolds, growth factors, and the cells are often used for the recovery of damaged tissues. Adult mesenchymal stem cells (MSCs) are attractive cell sources for new treatment strategies in regenerative medicine due to their multipotency and ease of harvest. Three-dimensional scaffolds could be used for localization, attachment, proliferation, and differentiation of stem cells. In the present study, modified clay nanoplate-polyurethane (MCNPs-PU) nanocomposite scaffolds with high porosity and bimodal architectures were prepared and used to induction of osteogenic differentiation of the human adipose tissue mesenchymal stem cells (hADSCs).
Methods: Gene expression in differentiated cells on MCNPs-PU and pure PU scaffolds, as well as MCNPs, were analyzed by real-time RT-PCR. In addition, the expression of two protein markers including osteocalcin and osteopontin was investigated by immunocytochemistry (ICC) during osteogenic differentiation of hADSCs on day 14 after induction.
Results: The relative expression of five important bone-related genes including ALP, collagen 1, osteonectin, osteocalcin, and RUNX2 was significantly enhanced by MCNPs-PU scaffolds. In addition, the expression of osteopontin and osteocalcin protein markers were qualitatively better on PU-MCNPs scaffolds compared to other groups. It seems that the PU polymers have been able to provide a suitable porous and biocompatible scaffold for attachment, proliferation and proper cell differentiation. It also seems that the presence of MCNPs inside the PU scaffolds has been increased the efficiency of osteogenic differentiation.
Conclusion: According to the results, the PU-MCNPs scaffolds by providing 3-D space could be a candidate substrate for bone tissue engineering and tissue repair. In addition, our findings show that the surface modification of clay nanoplates, as well as their presence into the PU scaffolds, mediates osteoinductive and osteoconductive responses of hADSCs. Therefore, these biocompatible nanocomposite porous scaffolds with suitable cell adhesion and proliferation as well as the ability to induction of high-performance osteogenic differentiation could be used for bone tissue engineering purposes in the future.
Keywords: Osteogenic differentiation; Mesenchymal stem cells; Polyurethane scaffold; Nanocomposite; Modified clay nanoplates

PS-146. Study of the Association Between Polymorphisms of *Nrf2* and *HIF1a* Genes with the Growth Rate Of Umbilical Mesenchymal Stem Cells

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Background and Aim: Mesenchymal stem cells have the ability to differentiate into different cell types and have immunosuppressive

properties and the ability to migrate and replace in the affected area. The Nrf2 and HIF1a genes are the main regulators of oxidative stress and inflammation. The aim of this study was to determine the association between the polymorphisms of *Nrf2* and *HIF1a* genes with the growth rate of umbilical mesenchymal stem cells.

Methods: In this study, we used three recombinant plasmids, including pcdna3.1-Nrf2 and three recombinant plasmid pcdna3.1-HIF-1α for the transfection. The extracted mesenchymal cells from the umbilical cord were transfected for the expression of *Nrf2* and *HIF1a* genes in cells that were genetically modified and tested by real-time PCR. The cells were then examined using the WST-1 method and cell survival after exposure to stress was investigated in terms of against oxidative stress and oxygen deficit and serum poverty.

Results: The survival rate of umbilical mesenchymal stem cells with Nof2 and HIF1a genes (89.3% ± 2.9%) were more than the control group (49.7 ± 28%). Also, the cells with Nosf2 and HIF1a genes encountered the stresses (6.9 ± 21%) were more than the control group (51.9 ± 6.2%). The expression of *Nrf2* and *HIF1a* genes in comparison with the oxidative groups with the oxidative stress groups was markedly higher in the S-HIF-*NFR2* gene than other groups (82% ± 7.7%). The survival rates of NSC-HIF genes were the lowest as compared to the other genes (65.2% ± 6.7%) in the MSCHIF-NRF2 oxygen deficiency group. The genes had a high survival (86.5% ± 8.9%) and in serum (P> 0.05). The frequency and survival of the genes were higher than the other genes (28.7 ± 7.9%).

Conclusion: Based on the results, the survival rate of the umbilical mesenchymal stem cells with the *Nrf2* genes and *HIF1a* was increased against oxidative stress and oxygen deficit and serum poverty.

Keywords: Stem Cells; *Nrf2*; *HIF1*; Umbilical cord

PS-147. Evaluation of Gene Expression of LncRNA BC012900 in Patients with Inflammatory Bowel Disease

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Background and Aim: Inflammatory bowel disease (IBD) is a multifactorial inflammatory disease that contains Crohn's disease (CD) and ulcerative colitis (UC). Incidence and prevalence of IBD are increasing geographically around the world. Long noncoding RNAs (lncRNAs) were recently identified to have a key role in regulating biological functions in human disease. Although the exact role of lncRNAs in IBD remains unknown, it is determined that the expression of some lncRNAs is changeable in IBD. We aimed to evaluate the expression of BC012900 lncRNA in patients with IBD CD and UC.

Methods: In this study, BC012900 lncRNA in intestinal blood and tissue samples were detected by quantitative real-time PCR. We performed this test in 53 patients including 20 people with ulcerative colitis (UC), 13 people with Crohn's disease (CD) and 20 normal people as the control.

Results: In this study, we identified a widespread dysregulation of lncRNA in both CD and UC patients compared to the normal controls. We found that lncRNA BC012900 expression was elevated in IBD patients (P < 0.05). This upregulated of BC012900 lncRNA was different in UC and CD patients, UC patients showed significantly a higher expression of BC012900 (P< 0.0001) in compared CD patients (P=0.0078).

Conclusion: Our data present that lncRNA BC012900 is involved in inflammatory bowel disease patients, which provide a potential prognostic biomarker and therapeutic targets.

Keywords: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; BC012900

PS-148. A Biodegradable Triboelectric Nanogenerator for Temporary Biomedical Applications

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Background and Aim: The outstanding development of biomedical implantable devices for wireless healthcare, monitoring, and diagnostic systems, has pointed up the application of harvesting based on biomechanical energy, such as contraction /expansion of the muscles, heart beating, blood pressure, breathing and continuous vibration of the lung. This technology could overcome most of the issues such as the requirement of external power sources like batteries, the low capacity of the batteries and psychological annoyance to the body.

Methods: In this way, the use of triboelectric nanogenerators technology was selected because of the wide range of material selection, easy manufacturing process, and desirable output power. In order to make dielectric layers, the polycaprolactone- graphene oxide (PCL/GO) and silk fibroin fibrous layers were used and Ag particle was sputtered on both of substrate as an electrode. The nanogenerator was encapsulated by a protective layer of the poly(lactic-co-glycolic acid) (PLGA) as a biodegradable polymer against moisture and the body's electrolyte environment. By applying a constant force (about 10N) and a frequency of 2 Hz to the TENG, the short-circuit current and the open circuit voltage were measured with the micro-ammeter and the oscilloscope.

Results: Our triboelectric nanogenerators could even have a reasonable output by applying the encapsulated layer. Nanofibrous structure of layers was also able to produce more efficiencies and power output relative to flat substrates. The friction layers possessed not only high strength and toughness but also were susceptible to electrostatic acceptance.

Conclusion: This work demonstrates the potential of biodegradable triboelectric nanogenerators as a self-powered system for transient medical devices

Keywords: Self-powered system; Triboelectric nanogenerator; Biodegradability

PS-149. Engraftment in Early and Late Autologous Stem Cells Transplantation in Multiple Myeloma

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Background and Aim: Autologous stem cell transplantation (ASCT) is considered the standard care for multiple myeloma (MM). According to the results of a meta-analysis, early autologous hematopoietic stem cell transplantation prolongs PFS and OS in patients with newly diagnosed multiple myeloma compared with delayed transplantation. The aim of this study was to compare the differences of platelet and WBC engraftment and hospitalization duration in Multiple Myeloma patients who received the first ASCT within 12 months of diagnosis (early ASCT) and at a later date (late ASCT).

Methods: We reviewed the results of 186 multiple myeloma ASCTs in Taleghani Hospital (Tehran, Iran) between 2010 and 2017 years. Data from 94 and 92 early and late auto-transplantation patients respectively analyzed using. All Data were analyzed by parametric test (T-Test) and expressed as means ± SD. Analyses were performed using the SPSS Statistical 22/0 program.

Results: Result of 186 patients included in this study (50.54% early HSCT, 49.46% late HSCT) showed no significant difference in WBC engraftment in both groups of patients (P: 0.07). The means time of platelet engraftment was 14.01±6.09 days in early ASCT and 16.04±4.65

days in late ASCT. The mean duration of hospitalization was 18.47± 8.9 in early ASCT and 19.53± 7.93 in late ASCT. Therefore, the time of platelet engraftment and hospitalization duration showed a significant increase in late ASCT compared to early ASCT (p-value: 0.009, 0.02 respectively).
Conclusion: This study demonstrated that the time of transplantation in newly diagnosed MM patients can affect platelet engraftment and hospitalization while there was no difference in WBC engraftment in early and late ASCT. Therefore, Autologous Stem Cells Transplantation within 12 months after diagnosis can improve the quality of engraftment and reduce the time and cost in Multiple Myeloma patient and these differences could be taken into account when designing strategies to treat MM patients.
Keywords: Engraftment; Early ASCT; Late ASCT; Multiple Myeloma

PS-150. Human Placenta Mesenchymal Stem Cell Accelerates Wound Healing in Mice

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Background and Aim: Skin is one of the large human body organs that approximately consists 5.5% of total body weight in an average 70 kg healthy adult human male. The function of the skin is to maintain hemostasis of the internal environment and protects the body against the external environment. Any injury or illness that causes disruption in skin integrity may result in significant disability or even death. The purpose of this study was to investigate the effect of transplanted human placenta mesenchymal stem cell (hPMSCs) on wound healing.
Methods: The full-thickness cutaneous wounds were created by an incision in the dorsal skin of BALB/c mice and treated with hPMSCs around the wound intradermally. The rate of wound healing was evaluated by histologic analysis. A total of 20 BALB/c mice, 2 wounds per animal, which were divided into 4 groups were examined in this study. The skin tissues were alternately harvested after wound creation. The harvested tissue sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT).
Results: The histomorphometric analysis revealed re-epithelialization was minimum in the negative control group and the defect area was mostly filled with immature granulation tissue ($P < 0.05$). The best re-epithelialization was detected in the MSCs incorporated Matrigel group. MT staining showed that among the experimental groups, MSCs incorporated matrigel induced the greatest collagen synthesis. In contrast, the rate of collagen fiber synthesis and deposition in the wound area were the lowest in the negative control group followed by the Matrigel group.
Conclusion: Our results demonstrated that hPMSCs can increase wound healing rate compared to the control group. Our data showed that hPMSCs are a promising cell source for the treatment of skin wounds. hPMSCs suppress inflammation in the wound and prepare the wound environment to heal and accelerate collagen and skin layers formation without any immunologic response induction in the genetically unrelated donor.
Keywords: Human placenta mesenchymal stem cell, Matrigel, wound, Skin, Cell therapy

PS-151. The Effect of Mesenchymal Stem Cell-Derived Microvesicles on Erythroid Differentiation of Umbilical Cord Blood-Derived CD34+ Cells

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Background and Aim: Mesenchymal stem cells (MSCs) play an important role in the proliferation, apoptosis, and differentiation of hematopoietic stem cells (HSCs) in the bone marrow via cell-to-cell contact, as well as secretion of cytokines and microvesicles (MVs). In this study, we investigated the effect of mesenchymal stem cell-derived microvesicles (MSC-MVs) on the erythroid differentiation of umbilical cord blood-derived CD34+ cells
Methods: In this descriptive study, CD34+ cells were cultured with a mixture of SCF (10 ng/mL) and rhEPO (5 U/mL) cytokines in the complete IMDM medium as a positive control group. Then, in MV1- and MV2-groups, MSC-MVs at 10 and 20 µg/mL concentrations were added. After 72 hours, erythroid-specific markers (CD71 and CD235a) and genes (*HBG1, GATA1, FOG1,* and *NFE2*) were assessed by flow cytometry and qRT-PCR, respectively
Results: The expression of specific markers of the erythroid lineages (CD71 and GPA) in the presence of different concentration of MSC-MVs were lower than that of the control group ($P<0.001$). Also, the expression of specific genes of the erythroid lineages (*NFE2, FOG1, GATA1,* and *HBG1*) was investigated in comparison to the internal control (GAPDH). Among all of them, *HBG1* and *FOG1* genes were significantly decreased to the control group ($P<0.0001$) but *GATA1* and *NFE2* gene expressions were not significant
Conclusion: The results of this study showed that MSC-MVs decrease the erythroid differentiation of umbilical cord blood-derived CD34+ cells. Therefore, MSC-MVs play a key role in the regulation of normal erythropoiesis
Keywords: CD34+ cells; Mesenchymal stem cells; Extracellular vesicles; Microvesicles; Erythroid differentiation

PS-152. Antioxidant Activity of Chrysin-Loaded Electrospun Nanofibrous Mats Increases Stemness Preservation of Human Adipose-Derived Stem Cells

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Background and Aim: An ideal biomaterial in regenerative medicine should be able to regulate stem cell proliferation without the loss of its pluripotency. Chrysin (Chr) is a naturally occurring flavone with a wide spectrum of biological functions including anti-inflammatory and anti-oxidant properties. The present study describes the influence of Chr-loaded nanofibrous mats on the regulation of proliferation and stemness preservation of adipose-derived stem cells (ADSCs).
Methods: Chr-loaded PCL/PEG nanofibrous mats were produced via electrospinning process and the successful fabrication of these bioactive mats was confirmed by FE-SEM and FTIR. ADSCs were seeded on the nanofibers and their morphology, proliferation, and stemness expression was analyzed using FE-SEM, MTT, and qPCR assays after 2 weeks of incubation, respectively.
Results: The results display that ADSCs exhibit better adhesion and significantly increased proliferation on the Chr-loaded PCL/PEG nanofibrous mats in relative to the PCL/PEG nanofibers and TCPs. The greater proliferation ability of ADSCs on Chr based nanofibers was

further confirmed by higher expression levels of stemness markers Sox-2, Nanog, Oct-4, and Rex-1.
Conclusion: These findings demonstrate that Chr-loaded PCL/PEG electrospun nanofibrous mats can be applied to improve cell adhesion and proliferation while concurrently preserving the stemness of ADSCs, thus representing a hopeful potential for application in stem cell therapy strategies.
Keywords: Chrysin; Nanofiber; Stem cell; Pluripotency

PS-153. Potential of Chrysin-Loaded Nanofibrous Mat for Wound Healing Applications Through its Antioxidant, Cytoprotective and Anti-Inflammatory Effects

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Background and Aim: Chrysin (Chr), a naturally occurring flavone, with anti-inflammatory and anti-oxidant properties has been recently investigated for wound healing applications. However, some challenges such as poor water solubility, low in vivo stability, rapid intestinal and hepatic metabolism, and low cellular uptake might limit its clinical applications. Considering to the high level of oxidative stress and persistent inflammation related to delayed healing in chronic wounds, the present study was conducted to assess the bioactivity and potential of encapsulated Chr into PCL/PEG nanofibrous mats as an antioxidant and anti-inflammatory agent.
Methods: For this purpose, Chr-loaded PCL/PEG nanofibrous mats were successfully fabricated by optimizing the electrospinning parameters and characterized using FE-SEM, FTIR, and UTM. MTT assay and FE-SEM were used to evaluate the viability and attachment of Human foreskin fibroblast cells (HFF-1) cultured on the fibers for 1, 3, and 5 days, respectively. To measure the antioxidant activity of Chr-loaded nanofibrous mats, An Oxygen Radical Absorbance Capacity Assay (ORAC) was applied. Also, the cytoprotective potential of Chr-loaded PCL/PEG nanofibrous mats compared to PCL/PEG nanofibers in a situation of oxidative stress was determined using MTT assay. The induction (relative mRNA levels) and release (protein expression levels) of three pro-inflammatory cytokines, IL-6 and IL-1β and TNF-α, were investigated in LPS-induced J774A1 macrophages seeded on the nanofibers. Furthermore, the Griess reaction assay was used to measure nitrite production.
Results: The randomly oriented structures of Chr-PCL/PEG nanofibers revealed smooth surface and bead-free structures with a broad diameter distribution (250-650 nm). Viability assay showed that HFF-1 have more than 80% viability on Chr-loaded nanofibers. Also, the good biocompatibility of Chr-loaded PCL/PEG nanofibers was confirmed through cell adhesion assay. The capability of Chr-loaded nanofibers to maintain the viability of HFF-1 cells under an oxidative stress condition demonstrated the antioxidant activities of the fibers. Also, Chr-blended nanofibrous mats reduced overexpression of IL-6, IL-1β, TNF-α and excessive production of NO in J774A1 macrophages following stimulation by LPS.
Conclusion: These results suggest that the proposed natural substance based nanofibrous mats can accelerate the wound healing process with cell proliferation, antioxidative and anti-inflammatory activities.
Keywords: Chrysin; Nanofiber; Wound healing

PS154. Platelet Rich Plasma Improves Experimental Colitis in Rat Model

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Background and Aim: Nowadays, platelet-rich plasma (PRP) has opened a new window for inflammatory disease such as osteoarthritis. PRP has shown significant anti-inflammatory, therapeutic and trophic potential. In this study, we have designed an experiment to assess PRP therapy potential on experimental colitis model.
Methods: Acute colitis in rat induced by intracolonic administration of 50 mg/kg trinitrobenzene sulfonic acid (TNBS). Rats were divided into five groups: control group, TNBS group, sulfasalazine group, PRP group. Groups with PRP and sulfasalazine received a daily dose of intraperitoneal injection of PRP and sulfasalazine, respectively. Body weight, disease activity index (DAI), colon length, colon weight to length ratio were measured
Results: PRP significantly suppressed TNBS induced body weight loss, colon length shortening and decreased the colon weight to length ratio.
Conclusion: The results of this study suggested that PRP as a new promising therapeutic option for inflammatory diseases are able to show anti-inflammatory effects on colitis.
Keywords: Cell therapy; Disease activity index; Platelet-rich plasma; Regeneration, Anti-inflammatory effects

PS-155. Study of Expression of OAZ3 and CLGN Genes as Haploid Markers in Sperm Differentiation Pathway in Induced Human Hair Follicle Stem Cells and Spermatogonial Stem Cells

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Background and Aim: Men account for about 45-50% of infertility. Infertility in men has several causes that azoospermia is one of these causes. For treatment of azoospermia patients, stem cell therapy is a new candidate method of treatment. Follicular stem cells and spermatogonial stem cells can be induced to differentiate into sperm cells. In this pathway, the genes of the haploid-specific phase should be expressed. The purpose of this study is to evaluate of expression of OAZ3, CLGN genes differentiated stem cells in sperm production pathway.
Methods: In this study, follicular stem cells and spermatogonial were used. Hair follicle cells were obtained by written consent from fertilized men who naturally had a child, and spermatogonial cells were obtained by satisfying the testicles of biopsy patients. Samples were cultured in a laboratory environment. The expression of these genes was investigated using real-time PCR.
Results: Stem cells grow in the culture medium. These cells were differentiated and altered by induction agents at specific time intervals and ultimately resembled sperm cells, and these cells express the genes.
Conclusion: Stem cells exhibit the ability to convert into sperm-like cells and can express some genes related to the haploid index.
Keywords: Adult Germline Stem Cells; Spermatogonial; Hair follicle; Infertility male; Azoospermia

PS-156. Cytotoxic Effect of ZnO on the Human Lung Cancer (A549) and HUVEC as a Normal Cell Line

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Background and Aim: Lung cancer is one of the most common cancers in both men and women and it is growing worldwide. Previous studies have reported that using nano zinc oxide (ZnO) can be a promising agent in cancer treatment because of their unique properties. Our purpose in this study was investigating the cytotoxic effect of ZnO on the human lung cancer cells (A549) and HUVEC as a normal cell line.
Methods: A549 and HUVEC cells were seeded at a density of 1×10^4 in 96-well plates and were treated with different concentrations of ZnO for 1, 3, 5 and 7 days. Cell viability was evaluated by trypan blue staining and MTT assay. In addition, Giemsa and AO/EB staining were used to investigate morphological changes of cells and detect apoptosis.
Results: In this study, treatment of A549 cells with ZnO at a concentration of 100 µg/mL resulted in a decrease in cell viability to 49%, 22.1%, 8.65%, and 6.28% respectively after 1, 3, 5 and 7 days. Moreover, treatment of

HUVEC cells with ZnO at a concentration of 40 µg/mL decreased the cell viability to 51%, 10.19%, 4.58% and 3.19% correspondingly after 1, 3, 5 and 7 days. Indeed, the Trypan blue and AO/EB results were consistent with the MTT results.
Conclusion: In conclusion, our results suggested that ZnO was able to induce apoptosis dependent on the proliferation rate of cells. So that, HUVEC cells as the normal cells were more sensitive than A549 as the cancer cells to ZnO cytotoxicity because of high proliferation rate of the cells.
Keywords: Apoptosis; A549 cells; Zinc oxide; Human umbilical vein endothelial cells; Cell survival

PS-157. The Use of Liposomes as a Vector and a Member of Nanocarriers Group in Order to Transfer the HGF Gent to Stem Cells for the Treatment of Keratoconus

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Background and Aim: Today, eye cornea is one of the most common diseases in the eye. It usually occurs in the age group of 10 to 25 years. The glasses or soft lenses that can be used in mild forms of the disease. However, with thinning and deformation of the cornea, this treatment is replaced by hard lens next treatment is corneal transplantation, which allows rejection of the transplant to the patient's life. Due to the presence of hereditary. We present the method of research and treatment of the stratum (Stroma). In this method, we tried to use the liposome to transfer the hgf gene to the nucleus Fat-derived stem cells that are compatible with the body and have no side effects. Diagnostic techniques A device called slit lamp is detectable by the keratoconus ophthalmologist in the medium to advanced stages, but in the early stages of the disease with tools such as keratotomy and topography Determining the corneal plane (purity of the cornea) can be identified
Methods: The use of liposomes as a vector and a member of the nanocarrier group is used to transfer the *hgf* gene to the nucleus of fetal-derived stem cells in the treatment of keratoconus. Hgf is used as a growth factor for growth and debridement of cells. Mesenchymal stem cells due to their ability Differentiating into different cell types is a good option for cell therapy and gene transfer in the disease. In this method, we first introduce the *hgf* gene through the transfection process into liposomes and then transfer these nanocarriers to the mesenchymal stem cell culture medium Derived from fat are added as a vector of the gene to stem cells They import. Subsequently, by injecting these cells into the ophthalmic layer, the cells express the *hgf* gene and secrete a protein that destroys the opaque cells and restores the tissue
Results: Nowadays, Most Researches and New Therapeutic Methods Use Nanotechnology and Stem Cells. In this research, liposomes were used to transfer the *hgf* gene to the stem cells nucleus. The stem cells are then inserted into the eye tissue through the drop. Next, stem cells enter the eye tissue through the droplet and the stem cells treat keratoconus. Keratoconus is a disease that causes the cornea to thin out. Usually, surgery, laser, and lens are used to treat the disease, while they have complications. But the non-invasive drops of cells do not have complications.
Conclusion: Nowadays, research on nanotechnology and stem cells and regenerative medicine is very important. We envision that this approach might be an effective treatment modality.
Keywords: Keratoconus; Liposome; *hgf* gene; Stem cells

PS-158. Gene Co-Expression Network Analysis for Identifying Modules and Functionally Enriched Pathways in Leukemia Stem Cells

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Background and Aim: Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells, leukemia stem cells (LSCs) in AML played important roles in leukemia initiation, progression, and were considered to be the root of chemotherapeutic drug resistance and disease relapse, in this study, we focus on the applications of differential regulatory analysis (DRA) based on gene coexpression network (GCN) in Leukemia stem cells research.
Methods: Gene expression datasets from NCBI Gene Expression Omnibus (GEO) database was collected from normal hematopoietic stem and progenitor cells and acute myeloid leukemia sub-populations, Normalization was performed with rma function from the affy package in R, Gene co-expression network and modules were constructed with the weighted gene co-expression network analysis (WGCNA) package in R, performed pathway enrichment analysis of selected modules by using two network-based gene set enrichment analysis tools, KOBAS 3.0 and Enrichr. This includes enrichment in predefined pathways by, for example, KEGG, and in the end Module visualization and further analysis was performed with VisANT software.
Results: In this study, we analyzed gene expression datasets of acute myeloid leukemia patients and healthy controls by applying a systems biology approach that combines the WGCNA with functional enrichment analysis. Novel co-expression gene network modules associated with AML cancer stem cell were elucidated, which in turn provided a basis for the identification of potential pathways and biomarker genes that may be involved in the development of normal hematopoietic stem cell to Leukemia stem cells.
Conclusion: LSCs in AML were considered to be the root of chemotherapeutic drug resistance and disease relapse, Identification of the specific features of LSCs such as potential pathways and biomarker genes for purpose of expanding innovative strategies in the diagnosis and treatment of Leukemia stem cells.
Keywords: Acute myeloid leukemia; Acute myeloid leukemia; Weighted gene co-expression network analysis

PS-159. Induction of Autophagy by GW9508 and Implications for Cancer Therapy

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Background and Aim: Colorectal cancer (CRC) constitutes a significant portion of mortality and morbidity due to cancer around the world. The regulation of autophagy has emerged as a promising new target in cancer treatment. Previous studies have reported that long unsaturated fatty acids exhibit antiproliferative effects through induction of oxidative stress and possess the potential to cure cancers. Similarly, the GW9508 small molecule is an unsaturated free fatty acid receptor agonist. In this study, we investigated the autophagic and anticancer effects of GW9508 on the HT29 tumor cells cultured in the 3D fibrin scaffold.
Methods: Fibrin hydrogel was prepared by polymerization of fibrinogen (3 mg/mL) by thrombin (120 U/mL). HT29 cells were seeded at a density of 50×10^3 into 24-well plate in 3D fibrin hydrogel and then were exposed to various concentrations of GW9508. MTT assay was used to measure cell viability. Also, Acridine Orange/Ethidium Bromide (AO/EB), DAPI, and monodansylcadvarine (MDC) stainings were used to detect autophagy. Moreover, the expression levels of LC3-II, AKT and mTOR genes were examined by qRT-PCR.
Results: Our results revealed a decrease in cell viability of treated cells with IC50 concentration (500 µM) of GW9508 to the extent of 52%, 10.5% and 6% after 1, 3, and 5 days respectively, in a dose and time-dependent manner. Morphological observation, AO/EB and DAPI staining confirmed autophagic morphology in treated cells. Also, the activation of autophagy was determined through changes in the number of autophagy vesicles (AVs) infected with MDC. Furthermore, the expression levels of AKT and mTOR were down-regulated whereas, LC3-II expression was

increased after GW9508 treatment compared with control samples.
Conclusion: Therefore, we suggest that GW9508 exerts anticancer actions, cytotoxic effects and promoting autophagy and can be used as a new therapeutic target for colorectal cancer treatment.
Keywords: Autophagy; Colorectal cancer; Fibrin hydrogel scaffold; GW9508; 3D culture

PS-160. An Efficient Method for Decellularization of Human Prepuce as a Biologic Scaffold for Skin Regeneration

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Background and Aim: The use of ECM derived from decellularized tissues and PAM (preputial acellular matrix) are widely used in both preclinical animal studies and clinical applications to repair tissues in regenerative medicine. Depending on a number of factors including the efficacy of decellularization, the use of chemical crosslinking agents, and the age of the source tissue from which ECM scaffolds are harvested, the host response to implanted ECM-derived biomaterials may vary from unacceptable to excellent. In this research, using ionic detergent, Triton X-100and Hank's buffer, we optimized the preparation procedure for the decellularized Prepuce.
Methods: Prepuce tissues were prepared from circumcision of children (an informed consent was obtained from parents) under the sterile condition. The outer-layer fat was removed mechanically by microdissection. Then, cellular components of prepuces were removed by using 5% (SDS) at room temperature for 4 h, by shaking. In the next step, the treated prepuces were washed with distilled water. Afterward, trypsin (0.05 %) (EDTA) (0.01%) was added and incubated at 4°C for 4 h. Fragments were rinsed with Hank's balanced salt solution (HBSS), digested with 1% Triton X-100 for 1 h, and then washed in HBSS at 4°C for 48 h. In order to evaluate the efficacy of our acellularization procedure, acellular samples were fixed in 10% formalin, the samples were paraffin embedded and sectioned to 5 µm thickness. Hematoxylin and eosin (H&E) were applied to visualize extracellular matrices and fibers. The DNA content in tissue samples was assessed by Hoechst staining and then observed under a light microscope.
Results: H&E and Hoechst staining of PAM showed an acellular collagen-based matrix with fibers orientation similar to the natural prepuces tissue. No cellular or nuclear remnants were preserved in scaffolds, while ECM was satisfactorily preserved
Conclusion: Many variables including cell and matrix density, thickness, and morphology can alter the efficacy of tissue and organ decellularization as well as the integrity and physical properties of the resulting ECM scaffold. The current method can be used efficiently for the decellularization of the prepuces biologic scaffold with satisfying biocompatibility for both in vivo and in vitro applications
Keywords: Decellularization; Human prepuce; Skin regeneration

PS-161. Elimination of Beta 2 Microglobulin Expression in HEK293 Cell Line Toward Producing HLA Class 1 Deficient Universal Cells

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Background and Aim: In cell therapy, transplantation of allogeneic hematopoietic stem cell (HSC) seems to be a beneficial and high throughput method for not only hematologic but also non-hematologic and immunologic disorders. However, the use of HSC has some limitations like; transplant rejection resulting from HLA mismatching between donor and recipient and identification of a suitable donor due to the HLA polymorphism. Elimination of HLA class 1 from surfaces of HSCs by CRISPR/Cas9 system, that is easy to use the method with high efficiency compared to TALEN and ZFN, can be a way to reduce transplant rejection.
Methods: In this study, we transfected dual gRNAs into HEK293T cell line using lipofectamine 2000 in order to a knockout B2M gene, which is non-polymorphic and codes B2M protein which is essential for surface expression of HLA class 1. Forty-eight hours after transfection, transfected cells were separated by FACS. Due to the diversity of cell population, clonal single cell lines were isolated. DNA extraction followed by PCR and Sanger sequencing to detect the deletion in the targeted locus.
Results: Two gRNAs were designed in this study, one for exon 1 and the other one for intron 1 to target the B2M gene. Using PCR, we observed deletion of both alleles in B2M gene in 11.11% of sorted clonal cell lines, and also only one allele of B2M gene was deleted in 22.22% of cell lines. Sanger sequencing confirmed elimination of alleles.
Conclusion: We revealed that a dual gRNAs can be a suitable approach to directly predicted deletions at any targeted loci and therefore can lead to a knockout gene. This process can also lead to generate a universal cell line for therapeutic aims in different patients regardless of HLA nature
Keywords: Transplant Rejection; B2M, HLA; CRISPR/Cas9; Universal Cell

PS-162. Investigating the Antioxidant Effects of Quercetin in Freezing-Thawing Process of Mouse Spermatogonial Stem Cells

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Background and Aim: Current cancer therapy approaches can be harmful to stem cells, in particular for spermatogonial stem cells (SSCs) which can lead to infertility problems. SSCs preservation techniques can be helpful and applied to pre-pubertal boys before starting their treatment. In other hand, cryopreservation protocols are associated with increased reactive oxygen species (ROS) production which subsequently leads to cellular damages.
Methods: In this study, we treated mouse SSCs by Quercetin before cryopreservation procedure and then evaluated its antioxidant effects on cell viability, ROS contents and apoptosis in SSCs after thawing. SSCs were isolated from 3-6-day-old neonate mice and were cultivated in a culture medium containing 40 µM Quercetin for 48 hours and then frozen for 2 weeks. Cell viability was evaluated by methylthiazoltetrazolium (MTT) test and ROS content was determined using the dichlorofluorescein diacetate (DCFDA) assay. Apoptosis was analyzed by detection of Phosphatidylserine externalization using flow-cytometry and also by gene expression evaluation of Bax and Bcl-2 using Real-time PCR.
Results: Our result indicated that pre-treatment of SSCs by Quercetin significantly decreased apoptotic cell numbers ($P<0.001$) and ROS content ($p<0.05$) after the freezing-thawing process in comparing to the control group. MTT assay confirmed that Quercetin decreased the mortality of SSCs from damage induced by vitrification. The results showed that the survival rates of the frozen-thawed SSCs in the Quercetin-pretreated group were significantly increased compared with the control group (OD: 0.5396 ± 0.006638 vs 0.486 ± 0.01047, $P<0.001$). Phosphatidylserine (PS) externalization was detected using Annexin V. A significant difference between the mean percentage of vital SSCs (AnV-/PI-) in the Quercetin-pretreated group and the mean percentage of these cells in control group was observed (61.9 ± 1.0 vs 28.6 ± 5.4, $P< 0.01$). The mean percentage of apoptotic SSCs (AnV+/PI-) significantly decreased in the Quercetin-pretreated group (9.345 ± 0.75 vs 29.65 ± 1.65, $P<0.001$). Also, the mean percentage of necrotic SSCs (AnV+/PI+) in Quercetin-pretreated group decreased in comparison with the control group (19.29 ± 1.11 vs 35.3 ± 4.3, $P<0.001$). Gene expression level of Bcl2 was increased ($P<0.05$) and Bax expression was decreased ($P<0.01$) by Quercetin.

Conclusion: Our results suggest that Quercetin can increase antioxidant potential of the mSSCs for high production of oxidative species during cryopreservation, and so, our approach can be a promising strategy to improve the fertility preservation techniques.
Keywords: Spermatogonial stem cell; Cryopreservation; Oxidative stress; Quercetin

PS-163. Microfluidic Preparation Routes for Organic Nanoparticles and Vesicular Systems for Nanomedicine Applications

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Background and Aim: Microfluidic systems, with their capability for precise handling and transport of small liquid quantities, have emerged as a promising platform for designing advanced drug delivery systems. We report the development of a microfluidic-based process for the production of polymeric micelles in continuous-flow microreactors where Pluronic tri-block copolymer is used as model polymeric biomaterial relating to drug delivery applications. We investigate the effect of polymer concentration, flow rate ratio and the microreactor dimension on the PMs size characteristic and drug encapsulation efficiency.
Methods: In this study, We developed a T-shape microfluidic device to create hydrodynamically focused flow consists of three inlets, one for the polymeric solution, two for deionized water, and one outlet for the fabricated nanoparticles. Microfluidic devices were fabricated with poly(dimethylsiloxane) (PDMS) using a standard soft lithography process. To make the master molds, silicon wafers were spin-coated with SU-8 2050 photocurable epoxy to a thickness of 60 µm. The mixing channel was 150 µm wide, 60 µm high and 1 cm long.
Results: Pluronic nanoparticle morphology trends found through dynamic light scattering analysis were confirmed with Transmission electron microscopy (TEM). DLS measurement revealed that synthesized nanoparticles had the size between 250-400 nanometers.
Conclusion: The diameter of the nanoparticles increases with an increase in flow ratio and polymer concentration. Moreover, the polydispersity index (PI) for the microfluidic nanoparticles was found to be remarkably lower (PI<0.2) than bulk synthesized particles (PI > 0.6) which is an important factor for drug delivery systems.
Keywords: Polymeric micelles; Microfluidic; Drug delivery; Hydrodynamic flow focusing

PS-164. Triiodothyronine-Loaded Nanofiber Biomaterial Fabrication for Accelerating Wound Healing

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Background and Aim: Skin is the largest organ and plays important roles in human bodies. Skin can be self-regenerated due to the presence of stem cells. Notwithstanding the presence of stem cells in the skin, the ability of skin to regenerate is limited to deep injuries, which leads to chronic wounds. In recent years, the attempts to find a way to treat chronic non-healing wounds attract a lot of attention. The use of synthetic bioactive substrates which deliver growth factors, hormones, and drugs at the site of injuries is an effective way to treat the wounds. Hormones are a class of active molecules that regulate physiological and behavioral functions of different organs. Triiodothyronine (T3), the active form of thyroid hormone, stimulates growth in wound healing via thyroid hormone receptor. It was shown that topical delivery of T3 on the site of injury in mice promoted wound healing process through the increase in hair follicular keratinocyte protein and improvement in organizing collagen bundles. Due to the toxic effect of intravenous administration of T3 caused by excess levels in the blood, controlled release of T3 is recommended. Our aim in this study was to create a bioactive biocompatible substrate releasing T3 in a sustained manner. In this study, T3-loaded chitosan nanoparticles were added to polycaprolactone (PCL) solution, and electrospinning technique was selected to produce nanofiber substrate.
Methods: To produce T3-loaded nanoparticles, ionic gelation method was employed. 1.7 mg/mL chitosan solution was mixed with 12 mL of tripolyphosphate solution containing T3 as a crosslinker. The mass ratio of chitosan to T3 was 100:1, respectively. Electrospinning technique was used to produce a nanofiber substrate. PCL solution contained 3%w/v of T3 loaded-chitosan nanoparticles. The flow rate of the PCL solution was fixed at 0.5 mL/h. The high voltage applied and the distance between needle and collector were 16 Kv and 15 cm, respectively.
Results: The characterization of the size and distribution of T3-loaded chitosan nanoparticles was carried out using dynamic light scattering (DLS) method. The results showed a mean diameter of 91 nm for the T3-loaded chitosan nanoparticles. The loading capacity and encapsulation efficiency of T3 were reported 87% and 8.3 by using ELIZA technique. In addition, the release profile of T3 from nanofiber substrate was monitored by using ELIZA technique. The profile showed that the release of T3 from substrate lasted for 14 days, and the concentration of T3 was reached to 133 ng/mL within first 4 days. It continued to increase to 165 ng/mL at the end of the 14th day and remained at non-toxic level. SEM micrographs of PCL embedded chitosan nanoparticles were used to measure fiber diameter. The average size of nanofibers was 738 nm.
Conclusion: The dose-dependent effect of drugs, hormones and growth factors in physiological condition highlights the use of biomaterials releasing bioactive molecules in a sustained manner in biomedical application. In this study, composite nanofiber substrate produced by the electrospinning technique showed a controlled release of T3 in the therapeutic window which prevents thyrotoxicity caused by high concentration of T3 in the circulation system.
Keywords: Triiodothyronine; Polycaprolactone nanofiber; Chitosan nanoparticle; Wound healing; Controlled release

PS-165. Evaluation of Dose-Dependent Cytotoxic Effects of Graphene Oxide-Iron Oxide Nanocomposite on Caprine Wharton's Jelly-Derived Mesenchymal Stem Cells

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Background and Aim: The experiment was aimed to study the cytotoxic effects of Graphene oxide-iron oxide nanocomposite on caprine Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs).
Methods: Ex vivo caprine WJ-MSCs were isolated and cultured. Cytotoxic effects of different concentrations of GO-Fe2O3 nanocomposite (10 µg/mL, 50 µg/mL and 100 µg/mL) were analyzed by observing cell morphology, cell viability, growth kinetics, population doubling time and colony forming unit (CFU) assay in caprine WJ-MSCs.
Results: Morphological alterations in nanocomposite-treated cells (50 µg/mL and 100 µg/mL GO-Fe2O3) were distinct as compared to lower dose (10 µg/mL GO- Fe2O3) and control group. Cell viability assay indicated a highly significant ($P<0.01$) decrease in live cell number when they were exposed to 100 µg/mL and 50 µg/mL GO- Fe2O3 nanocomposite and these effects were intensified with time (24 h & 48 h post-exposure). Retarded growth rate and significant ($P<0.01$) increase in population doubling time (PDT) of exposed cells (50 µg/mL and 100 µg/mL) were observed as compared to control group and low dose treatment group (10 µg/mL). Colony forming unit (CFU) assay indicated that cells treated with 50 µg/mL and 100 µg/mL nanocomposite formed a smaller number of clones than the control group and 10 µg/mL treatment group.
Conclusion: On the basis of results, we conclude that lower doses (10 µg/mL) of the nanocomposite are safer in caprine WJ-MSCs however

with increasing doses of nanocomposite (50 µg/mL & 100 µg/mL) the potential toxicity increases. The present study reports the tolerable doses of GO-Fe2O3 nanocomposite which will help in future applications like tracking, imaging, and differentiation of caprine WJ-MSCs.
Keywords: Stem cells; Nanocomposite; Graphene oxide; Iron oxide; Nanotoxicity

PS-166. Diabetic Human Mesenchymal Stem Cells Release Extracellular Vesicles with Distinct Size and Zeta Potential Properties

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Background and Aim: Extracellular vesicles (EVs) are nano-sized vesicles released by many cells as intercellular communication tools. Recent studies have shown that diabetes mellitus (DM) affects the biology of stem cells especially mesenchymal stem cells (hMSCs) through changes in their secretome. We aimed to study the effect of serum from type 2 diabetic mellitus patients on the size and Zeta potential (ZP) of hMSCs-derived EVs in vitro.
Methods: hMSCs were allocated into two groups as follows; Control (cells received DMEM/LG and 10% sera from healthy subjects) and Diabetic (cells received DMEM/LG and 10% diabetic sera). Treatment was carried out for a period of 7 days. EVs were then isolated according to the isolation kit (Publication No: MAN0006949, Invitrogen) instructions. Flow cytometry and scanning electron microscopy were used to characterize the EVs. To analyze size distribution and ZP, EVs were subjected to the Zetasizer Nano Z system (Nano ZS ZEN 3600, Malvern). Furthermore, to determine whether the DM sera could affect the dynamic of the fatty acids profile, we used Gas Chromatography (GC). Data are expressed as means ± SD. Student's t-test was used to calculate the significance of differences between the groups. Values of $P<0.05$ were considered statistically significant.
Results: The size of the EVs derived from the diabetic group significantly increased compared to the control group (p control versus diabetic <0.05). Furthermore, in comparison with the control group, the ZP of diabetic hMSCs-derived EVs significantly decreased (p control versus diabetic <0.05). Data from GC showed that treatment with DM sera resulted in a highly significant increase in the content of Palmitate (16:0) (p control versus diabetic <0.01) and a significant decrease in the content of Myristate (14:0) and Linoleate (18:2) compared to the control group (p control versus diabetic <0.05).No significant differences in the content of other fatty acids between the control and diabetic groups were observed (p control versus diabetic >0.05).
Conclusion: We conclude that diabetic mellitus alters the properties of EVs and the fatty acid profile leading to modifications in the cell biology of hMSCs.
Keywords: Extracellular vesicles; Mesenchymal stem cell; Diabetes mellitus; Zeta potential

PS-167. Endothelial and Pericyte Differentiation of Human Mesenchymal Stem Cells Were Inhibited in Diabetic Condition

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Background and Aim: Human mesenchymal stem cells (hMSCs) with self-renewal activity, trans-differentiation into different lineages contribute to the reconstitution of injured tissues by augmentation of angiogenesis. It was reported that in patients with diabetes type 2 DM2 deficient and abnormal angiogenesis are very common. hMSCs are more sensitive to the persistent diabetic condition. In the present study, we aimed to study the effect of sera from DM2 patients on the differentiation capacity of hMSCs into endothelial and pericyte cells.
Methods: For in vitro assays, hMSCs were classified into three groups as follows; Control: cells received FBS; Non-diabetic: cells received sera from healthy subjects and Diabetic groups treated with diabetic sera. hMSCs were exposed to DMEM/LG containing 10% FBS, healthy and diabetic sera over a period of 7 days. To measure the differentiation capacity of hMSCs, cells were incubated with endothelial and pericytes differentiation media after 7-day exposure to FBS and sera from healthy and diabetic subjects. To monitor the endothelial- and pericyte-like differentiation, the expression of VE-cadherin (CD144) (Cat no 53-1449-41, ebioscience) and NG2 (Cat no: 53-6504-82, ebioscience) were monitored by a flow cytometry and low-density lipoprotein (LDL) uptake assays. Data are shown as mean SD. One-way analysis of variance (ANOVA) and Tukey post hoc test was used in experiments between groups. Values of $P < 0.05$ were considered statistically significant.
Results: Diabetic serum inhibited hMSCs differentiation capacity by down-regulating VE-cadherin as compared to cells from FBS and non-diabetic groups (P diabetic sera vs. FBS <0.000001; P diabetic sera vs. non-diabetic <0.01). The maximum level of VE-cadherin revealed in FBS group which was more than non-diabetic counterpart ($P < 0.0001$). In the absence of differentiation factors, 14-day incubation of hMSCs with FBS, non-diabetic and diabetic sera showed a similar pattern in the dynamic of VE-cadherin. Data from fluoresce microscopy confirmed that the potential of hMSCs in FBS and non-diabetic groups to uptake Dil-Ac-LDL after being exposed to endothelial induction medium. While the ability for uptake of Ac-LDL in differentiated cells and lipoprotein lipase activity was profoundly abolished in diabetic condition. There was a significant inhibitory effect of diabetic sera on pericyte differentiation capacity in hMSCs (P diabetic sera vs. FBS <0.001). Compared to FBS group, we also found a significant reduction in pericyte differentiation of hMSCs ($P < 0.01$).
Conclusion: Our data revealed that DM2 could potentially decrease both endothelial and pericyte differentiation of hMSCs.
Keywords: Endothelial; Pericyte; Human mesenchymal stem cells; Diabetic Mellitus

PS-168. The Investigation of Chondrogenic Differentiation of Adipose-Derived Stem Cells Co-Cultured with Mature Chondrocytes

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Background and Aim: The restricted regenerative ability of human cartilage imposed a drastic need for the applying stem cell related regenerative treatment. Adipose-tissue derived stem cells (ADSC) have provided promising potentials for supporting new clinical concepts in cellular therapy. While it is still unclear if they can be implied for repairing impaired cartilage cells. This study aimed to decipher the cellular and molecular differentiation of ADSCs co-cultured with mature chondrocytes.
Methods: The adult ADSCs for chondrocyte differentiation were obtained from the human intra-patellar fat pad and were seeded into polycaprolactone scaffold following a co-culture system with mature chondrocytes. The control sample was prepared using ADSC cultured on scaffolds. After twenty-one days, differentiation of co-cultured samples was investigated and compared with control samples according to chondrogenic gene expression and microscopic features using Real-time RT-PCR and transmission electron microscopy (TEM).
Results: Based on the Real-time RT-PCR results, the co-cultured ASCs expressed significantly lower chondrogenic differentiation marker of

Indian hedgehog (IHH) compared with the control group (P = 0.004). However, the expression of other chondrogenic markers such as aggrecan (ACAN) and collagen type 2 didn't differ significantly between the two samples. The images of TEM revealed stretched shaped co-cultured cells as opposed to round formation of chondrocytes and control cells. The nucleus of co-cultured cells occupied the majority of cell volume, while the nucleus proportion to cytoplasm was smaller in both control cells and chondrocytes. There were also some multi-laminar particles containing autophagic vacuole within the cytoplasm of both study samples, which didn't exist in normal chondrocytes.

Conclusion: These study findings indicated that mature chondrocytes didn't induce chondrogenic differentiation in human ADSCs.

Keywords: Adipose-Derived Stem Cell; Chondrogenesis; Chondrocyte characteristics; Co-culture

PS-169. Preparation of Smart pH-Responsive Cell Laden Hybrid Hydrogel Microsphere Using Carbon Dots

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Background and Aim: Cell culture in 3D microenvironments mimicking physiological conditions. One of the suitable models for solid tumor is microsphere due to the formation of the poor perfusion condition which causes similar hypoxic and acidic region in tumor mass. Accordingly, measuring the amount of acidity inside these types of cancer models can help to understand the status of the cells. To achieve this goal, we proposed a facile method for recording the interior pH of cell-laden hydrogel microsphere without sectioning and staining.

Methods: In the first step, the carbon dots (CDs) were synthesized using a hydrothermal method. The prepared CDs were characterized by dynamic light scattering and fluorescent spectrofluorimetric techniques. Then the CDs solution was lyophilized and CDs dry powder was added to the mixture of alginate hydrogel and cell culture medium with a cell concentration of 103 cells/mL. In the next step, the CDs-hydrogel particles were prepared trough automatic pipetting the prepared mixture to the CaCl2 solution. The size of hydrogels was adjusted by pumping flow rate. After bead formation, the hydrogel particles removed and washed with PBS buffer. The prepared cell-laden hydrogels were transferred to the 6-well plate contacting culture medium and incubated at 37 o C. The fluorescent intensity of incubated hydrogels was measured through lab made smart-phone spectrofluorometer over a period of 5 days.

Results: Excitation and emission wavelengths of prepared CDs were independent which is suitable for pH measurement. The MTT assay showed low toxicity effect of CDs on MCF7. The shape and the diameter size (500 ± 45 μM) of prepared hydrogels were uniforms. The fluorescence intensity of the cell-laden hydrogel particles increased over time and peaked on the 5th day. This fluorescence emission increment indicates an increase in the acidity of the microsphere interior.

Conclusion: The proposed method for preparing hybrid hydrogel cell-laden microsphere provide appropriate tumor model for imaging and anticancer drug investigation.

Keywords: Cell-laden microsphere; Hydrogel; Carbon dots; pH-responsive

PS-170. Engineered Channels Throughout a Silk Cylindrical 3D-Porous Scaffold Enhance G292 Pre-Osteoblast Proliferation and Distribution in a Static Cell Culture

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Background and Aim: Cell density and oxygen distribution in 3D-porous scaffolds are important issues for the creation of large engineered constructs, which can be used in large bone defects. We assumed development of channeled, 3D-porous scaffolds might enhance the uniformity of oxygen within 3D-porous scaffolds and increase cell density through 3D-porous scaffold in a static culture. One of the critical shortcomings for current tissue engineering approaches is to create densely populated constructs. In this study, we aimed to employ silk cylindrical 3D-porous scaffolds with engineered channels to assess cell proliferation and oxygen distribution in a static culture.

Methods: Silk cylindrical 3D-porous scaffolds (radius 1.1 cm, height 1 cm) were used without channels and with 15 channels (1-millimeter diameter each channel) throughout the 3D-porous scaffold in static culture for cell culturing of G292 pre-osteoblast for 14 days. Cell viability on the 3D-porous scaffolds was determined using the Alamar-Blue assay after 7, 14 days. The results of cell viability for scaffolds were normalized compared to day zero. The oxygen concentration of culture medium measured in center scaffolds using needle-type oxygen microsensors with fixed sensor tip (NFSx; PreSens).

Results: In a static cell culture, the cellular activity of cell/polymer constructs increased from channeled to non-channeled scaffolds after 7 days (4%, it was not significant) and after 14 days (34%, it was significant) ($P < 0.05$). The oxygen concentration of culture medium in center scaffolds increased from channeled to non-channeled scaffolds after 3 days (70%, it was significant) and after 7 days (133%, it was significant) ($P < 0.05$).

Conclusion: Our data show that using channels in 3D-porous scaffolds enhanced G292 pre-osteoblast cell proliferation and spatial distribution of cells within the scaffolds, which could significantly improve cell viability and distribution within the 3D-porous scaffold in the static cell culture. We have shown the channeled scaffolds are a promising approach toward creating thick tissue-engineered constructs.

Keywords: Bone tissue engineering; 3D-porous scaffold; Channeled scaffold; Oxygen transport; Cell proliferation; Static culture

PS-171. Synthesis and Characterization of Magnetic Nanoparticles with Mesoporous Bioactive Glass Coatings for Osteoporotic Cancerous Tissue Therapy

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Background and Aim: Cancer is now becoming prevalent worldwide with a great number of researches focusing on the treatment of this disease. Nowadays, with the field of nanotechnology great advancements in cancer therapy have been achieved. Here, we propose a new and promising approach for bone-related cancer treatments with the aid of a core-shell system composed of magnetic nanoparticles (MNP, core) encapsulated in bioactive glass nanoparticles (BGN, shell).

Methods: Initially MNPs were synthesized using the co-precipitation method and fully characterized. Optimized structure of MNP was then coated using BGNs. then using a sol-gel technique by a cationic surfactant (CTAB) for encapsulating the Fe3O4 nanoparticles in a Bioglass Matrix as a shell.

Results: These particles were evaluated using SEM, FE-SEM, TEM, XRD, VSM, FTIR, DLS/Zeta potential and VSM. Results confirmed the formation of MNPs with a spherical and uniform structure with high crystallinity and purity. BGNs showed to be bioactive by forming a hydroxyapatite layer on their surface. In addition, the hyperthermia application of MNP-BGN core-shell system was also studied. It was found that the particles reach a temperature of 42°C in an alternating magnetic field.
Conclusion: Overall, it is suggested that the particles have a great dual application in magnetic hyperthermia and bone regeneration.
Keywords: Bioglass; Hyperthermia; Magnetic; Nanoparticles

PS-172. Mesenchymal Stem Cells Therapy in the Lesioned-Rat Barrel Cortex for Recovery of Tactile Dysfunctions

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Background and Aim: The vibrissal sensory system of rodents is functional to explore the levels of objects and textures by tactile discrimination. Whiskers deflections produce sensory stimuli for the Somatosensory cortical region which known as whisker barrel field cortex. A mesenchymal stem cell can improve the functional deficits in many central nervous system diseases including traumatic brain injury. The aim of the present study is dental pulp stem cells transplantation in the lesioned-rat barrel cortex to evaluate of tactile dysfunctions.
Methods: In this experimental study, 42 Wistar male rats (200-230 gr) have been used. Subjects were divided into 4 groups, randomly (n=7). In the differentiated/ undifferentiated dental pulp stem cell therapy groups (DCT/Un-DCT), stem cells transplanted directly into barrel cortex following removal of part of right barrel cortex by using a skin punch (diameter, 3 mm). In the lesion group, only barrel cortex was eliminated. Rats in the control group were intact. After one week of recovery, the ability of novel texture discrimination of each subject was investigated for 4 consecutively weeks.
Results: The lesion group had a significant decrease in time of exploration of novel texture compared to controls during 4 weeks behavioral tests ($P<0.05$). In the first week, there is no significant statistical difference among the DCT and Un-DCT groups compared to lesion group, while the performance of DCT and Un-DCT rats were significantly improved during weeks 2-4 compared to lesion group ($P<0.05$).
Conclusion: The lesioned-barrel cortex rats cannot discriminate the novel object from the old object during 4 weeks of behavioral tests. Transplantation of dental pulp mesenchymal stem cells can improve tactile discrimination of rat. Therefore, stem cell therapy can provide a promising regenerative medicine for traumatic brain injury.
Keywords: Barrel cortex; Lesion; Dental pulp stem cell; Rat

PS-173. Metagenomics Using Next-Generation Sequencing for Biological Products

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Background and Aim: Metagenomics technology enables sequencing of biological material with a flow much higher than before. Thus, the arrival of this technology allows microbiomes of very diverse habitats to be described. The fetal bovine serum is a potential source of viral contamination for cell cultures used in the production of biological products for human or animal use and is therefore routinely subjected to a range of virus-specific tests to ensure an absence of viral contaminations.
Methods: Using viral metagenomics we describe here the virome in serum from calves. For this purpose, collected samples are to be classified, homogenized, filtered, and then treated with nucleases prior to nucleic acid extraction. Following random RT-PCR and use of Nextera reagents (Illumina), we will generate doubly tagged DNA from each supernatant for sequencing. Sequence reads will be assembled de novo by use of the Ensemble program, and computational analysis will be performed using a designed pipeline.
Results: The nearly complete genomes of different virus species including some from potentially novel RNA and DNA viral families are characterized and phylogenetically analyzed, significantly expanding the known serum-associated virome.
Conclusion: This study further characterizes the diversity of viruses in calf serum with the potential to infect fetuses and through fetal bovine serum contaminate cell cultures.
Keywords: Next-generation sequencing; Cell culture; Virus

PS-174. Study of Expression of AKAP 4 and Catsper Genes as Motility Markers in Sperm Differentiation Pathway in Human Induced Hair Follicle Stem Cells and Human Spermatogonial Stem Cells

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Background and Aim: Nearly 72.4 million couples have infertility problems in the world. Men account for about 45-50% of infertility. Infertility in men has several causes that azoospermia is one of them. Nowadays, for the treatment of azoospermic patients, cell therapy is a new way. Infertile men can be used to induce follicular stem cells and spermatogonia to produce sperm. If the distinction is made into sperm, the genes of the mobility index should be expressed. The purpose of this study is to evaluate the genes of Catsper, AKAP4, that contribute to sperm motility.
Methods: In this study, follicular stem cells and spermatogonial were used. Hair follicle cells were obtained by written consent from fertilized men who naturally had a child, and spermatogonial cells were obtained by satisfying the testicles of biopsy patients. Samples were cultured in a laboratory environment. The expression of these genes was investigated using real-time PCR.
Results: Stem cells grow in the culture medium. These cells were differentiated and altered by induction agents at specific time intervals and ultimately resembled sperm cells, and these cells express the genes. These cells have tail and spindle shape head. Eventually, we prove the expression of these genes by a real-time technique in different weeks.
Conclusion: Stem cells exhibit the ability to convert into sperm-like cells and can express some genes related to the mobility index. These genes expressed in different Sperm production of stages and expression of them increases gradually. Expression of these genes can be a reason for sperm maturation but it is better to study other sperm motility genes and haploid dependent genes.
Keywords: Azoospermia; Infertility; Real-time PCR; Stem cell

PS-175. Mechanical and Biological Properties of Mg-Al-Graphene Nanocomposite for Bone Regeneration Application

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Background and Aim: Millions of people suffer from bone diseases in the world. In recent years, implants have been developed to repair bone fractures. While the primary implants used in the human body were non-degradable, the secondary surgery led to research on implants with good mechanical and biological properties. The aim of this study is magnesium and its alloys be applied as degradable metallic materials in orthopedic implants due to their degradability and resemblance to human cortical bone.
Methods: Magnesium (Mg) is naturally present in bone composition, and it is one of the required metals for metabolism. However the fundamental problem of Mg-based implants is their low corrosion resistance and weak

mechanical properties. In this study, we introduced a new approach to control the corrosion rate of magnesium. So in this method, Mg–1Al-0.5Cu-xGraphene (x=0.18, 0.5) nanocomposites were synthesized using the powder metallurgy method. Also this novel nano processing route is better than other methods that produces heat which is a big problem when dealing with magnesium.

Results: The best content of Graphene was 0.18 wt.% for this nanocomosite. Compression properties of Mg-1Al-0.5Cu-0.18 Geraphen increased compare with alloy of magnesium. Corrosion rate of synthesized nanocomposites proved to be better than pure magnesium and the rate of degration same to repair of human cortical bone.

Conclusion: In this paper the novel nanocomposite of magnesium was fabricated via powder metallurgy. Addition of geraphene improved the mechanical properties and corrosion rate of Mg-1Al-0.5Cu-0.18Geraphene nanocomposite. Also aluminium and copper prevented to agglomeration of geraphene. So Mg-1.0Al-0.5Geraphen nanocomposies with a controllable biological, mechanical properties and corrosion rate is suitable for damaged bone tissue and leads to stimulation, repair and reconstruction of bone tissue.

Keywords: Bone biodegradable implants; Nanocomposites; Corrosion; Biocompatibility

PS-176. Combination of EPI-NCSCs and DMF Improves Their Potential Therapeutic Functions by Upregulation of Expression of Neurotrophic Factors; In vivo Study

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Background and Aim: Stem cell therapy has been considered as a new modulation in the treatment of neurologic diseases. Epidermal neural crest stem cells (EPI-NCSCs) are multipotent stem cells which are present in the bulge of the adult hair follicle and their therapeutic functions have been reported. Dimethyl fumarate (DMF), which has been used in the treatment of multiple sclerosis is newly introduced as effective neuroprotective therapy in stroke models. In the current study, we evaluated the effect of DMF on the relative expression of trophic factors in EPI-NCSCs and their potential therapeutic benefits.

Methods: The bulge of adult rat hair follicles were placed onto the collagen coated cell culture plate and after EPI-NCSCs migration, cells were detached. Verification of expanded EPI-NCSCs was performed using immunofluorescent staining. To determine the appropriate concentration of DMF for EPI-NCSCs treatment, the MTT assay was employed and based on acquired data, EPI-NCSCs treated with 10µM DMF for 6, 24, 72 or 168 hours. In each time point, the quantitative RT-PCR technique was used to evaluate transcripts of NGF and VEGF.

Results: The acquired data showed that 10µM DMF significantly increased the mRNA expression of NGF 72 hours following treatment; however the inhibitory effect of DMF on GDNF mRNA expression was observed in various time points.

Conclusion: Our findings revealed that DMF up-regulated one of the major neurotrophic factors in the epidermal neural crest stem cells. Therefore, the combination of EPI-NCSCs therapy with DMF treatment might be a valuable strategy to improve their potential therapeutic functions in vivo.

Keywords: Dimethyl fumarate; Epidermal neural crest stem cell; Trophic factors; Gene expression

PS-177. Low Magnitude Electromagnetic Field Enhances Bone Cell Proliferation: Implications for 3D Bone Tissue Engineering?

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Background and Aim: Tissue engineering of three-dimensional (3D) bone constructs is a promising strategy for large bone defect repair. A challenge is to obtain 3D cell-seeded scaffolds with a uniform cell distribution and high cell density. Physical stimulation such as low magnitude electromagnetic field (LMEMF) is crucial, that is known to affect cell proliferation in a specific range (magnitude, frequency). The effect of LMEMF on bone cell proliferation is dependent on cell density is unknown. We compared the effect of LMEMF on G292 bone cell proliferation in low and high cell density.

Methods: The G292 osteosarcoma cell line from the Pasteur Cell Bank of Iran (NCBI code: C116, species: human) was used. Cells were cultured in RPMI 1640 medium with 10% fetal bovine serum and antibiotics. Cells were cultured at 37ºC in a humidified atmosphere with 5% CO2. The cells were seeded at 1.5×10^4 cells/cm^2 (low cell density) and 3×10^4 cells/cm^2 (high cell density), and cultured for 3 days. Cells were exposed daily to 8 mT LMEMF for 20 min/d by using a magnetic device manufactured by the Research Center for New Technologies in Life Science Engineering, University of Tehran.

Results: In low cell density cultures, LMEMF did not affect cell number at day 1, but significantly increased G292 bone cell number at day 2 by 2.3-fold, and at day 3 by 1.2-fold. In high cell density cultures, LMEMF slightly, but not significantly, increased G292 bone cell number at day 1 by 1.1-fold, at day 2 by 1.2-fold, and at day 3 by 1.1-fold.

Conclusion: In conclusion, LMEMF more profoundly affects bone cell proliferation in low cell density cultures than in high cell density cultures. These findings could help to better understand the effect of LMEMF on bone growth in vivo and to develop new therapies using LMEMF for treatment of large bone defects.

Keywords: Low magnitude electromagnetic force; Bone cell proliferation; Bone; Cell density; Bone tissue engineering

PS-178. Fabrication of Titania Nanotubes-Graphene Hybrid for Using in Photothermal Therapy of Breast Cancer

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Background and Aim: Breast cancer is a serious health concern for women. There are several ways for the treatment of breast cancer. Nevertheless, the lack of selectivity in these methods leads to undesirable adverse effects. Photothermal therapy is a noninvasive cancer therapy technique in which tumor tissues are exposed to light and received light is converted to heat to promote tumor destruction. Photothermal therapy contains raising the temperature of tumor cells to the range of 42-46 °C, leading to cell death. The aim of this study is the fabrication of TiO_2 nanotubes-graphene hybrid for using in photothermal therapy.

Methods: TiO_2 NTs layer was formed by electrochemical anodization of

Ti thin foils. Then the anodized samples were sonicated in ethanol for 5h to separate the TiO_2 nanotubes from the substrates. The TiO_2 nanotubes-graphene hybrid was made as follows: The GO was dispersed in ethanol by ultrasonic treatment for 30 min. Subsequently, this suspension was mixed with the TiO_2 solution and the mixture was stirred for another 20 min. The reduced form of the GO, as well as the TiO_2 nanotubes attachment, was obtained by UV irradiation for 3h. Meanwhile, the UV irradiation led to photoreduction of the GO to the graphene (RGO) and the suspension color changed from gray to black.

Results: In order to TiO_2 nanotubes formation the electrochemical anodizing process was performed. The SEM image of the anodized substrate showed that the TiO_2 nanotubes were orderly formed throughout the substrate. The estimated inner diameter of nanotubes is 90 nm and the outer diameter of nanotubes is 160 nm. The XRD pattern of TNTs-graphene hybrid showed that the photocatalytic reduction of graphene oxide occurred by ultraviolet irradiation. Also, there is no graphene oxide peak in this pattern, which indicates that all of the graphene oxides have been reduced and converted into the graphene (rGO). The graphene (rGO) sheets can be observed in the SEM image of TNTs-graphene hybrid. Moreover, it can be seen that TiO_2 nanotubes were distributed on the graphene sheets.

Conclusion: In this research, we investigated the fabrication of TiO_2 -graphene hybrid, which could be used for photothermal therapy of breast cancer. At first, the TiO2 nanotubes were formed by electrochemical anodizing process and results showed that the TiO_2 nanotubes were completely formed on the Ti substrate. Then the hybrid of TiO_2 nanotubes and graphene was synthesized. The photoreduction of graphene oxide was completely occurred by UV radiation and the TNTs-graphene hybrid was obtained.

Keywords: Photothermal therapy; Breast cancer; Titania nanotubes

PS-180. Improvement of Diabetic Open Skin Wound Healing by Human Bone Marrow-Mesenchymal Stem Cells Conditioned Media: A Preclinical Study

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Background and Aim: Wound healing is a complex process that is impaired in diabetic patients due to several factors. So far, the positive effects of mesenchymal stem cells secretome in the wound healing process have been reported. In this study, we investigated the effect of human bone marrow mesenchymal stem cells conditioned media (hBM-MSC-CM) on gene expression of some effective factors involved in wound healing and the strength of healed wound in diabetic rats.

Methods: The animals were divided into the normal wound (NW), diabetic wound (DW) and diabetic wound treated by the condition media (DW-CM) groups. Diabetes was induced by Alloxan. A full skin thickness wound was created on the back of the rats. Then, the conditioned medium was prepared from hBM-MSC and DW-CM rats received 200 microliters of the CM intravenously. Then, the wounds were sampled and expression of KGF and TGF-β1 genes was examined by RT-PCR on days 4 and 7 after wounding. Also, on the 15th day, a standard sample from each healing wound was submitted for the biomechanical examination using tensiometric study.

Results: There were no significant differences between DW-CM and DW groups in the term of KGF gene expression at 4th and 7th days. While expression of the TGF-β1 gene in DW-CM group decreased significantly ($P<0.05$) compared to DW group at 4th day. Furthermore, biomechanical parameters of the healing wounds in the DW-CM group significantly ($P<0.05$) increased in comparison to the DW group.

Conclusion: It seems that using the conditioned medium derived from human bone marrow mesenchymal stem cells positively affects the expression of inflammatory factors involved in wound healing, and leads to improvement of biomechanical parameters of the healed wound in the diabetic condition.

Keywords: Mesenchymal stem cells; Conditioned medium; Diabetic wound healing; Gene expression; Tensiometry

PS-181. Menstrual Blood Stromal Stem Cells: An Optimized Protocol for Isolation and Proliferation

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Background and Aim: stem cells are frequently used in various medical therapies and have also been proposed as promising candidates for future therapies. Recently, numerous studies have been demonstrated that stem cell-based therapy has a great potential in a wide range of diseases such as fertility. However, Cell therapy is found in the early stages for treatment of female infertility. Stromal stem cells can be considered as one of the commonly used stem cells in cell therapy researches, which have prominent characteristics, including highly proliferative and differentiated powers, along with immunosuppressive properties. Menstrual blood stromal stem cells (MBSCs) are considered as a novel source of endometrial stem cells (EnSC) which their therapeutic potential was verified in several studies. The choice of a suitable cell source and develop and optimize its own isolation and expansion protocol, are the first step in the initiation of cell therapy.

Methods: A human EnSC line was derived from the menstrual blood of 30-40 years old infertility women such as endometriosis, polycystic ovary (PCO), premenopausal menopausal syndrome (POF) and idiopathic were selected as four experimental groups. Menstrual blood was collected during the second or third days of menstruation with IUI catheter. Mononuclear cells were separated by Ficoll-Paque density-gradient centrifugation. Cells were then expanded in DMEM medium containing 10% FBS, 1% penicillin/streptomycin.

Results: EnSCs isolated from the women showed fibroblast-like cells morphology. The isolated EnSCs were propagated in vitro and passages with excellent cell viability.

Conclusion: In general, our findings suggest that the present protocol can be used to isolate stem cells derived from the menstrual blood of infertile patients, in order to of differentiating into oocyte-like cells, and eventually introducing an innovative cell-based therapy for infertile women. In addition, this protocol is a very simple, non-invasive, low cost, short period, and high-efficiency method, which can be used for clinical and in vitro research. However, part of this research is still underway and will continue to differentiate to oocyte-like cells.

Keywords: Stromal stem cells; Optimization; Infertility women; Menstrual blood

PS-182. Effect of Elaeagnus Angustifolia Extract in Bone Tissue Engineering Nanofiber Technology

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Background and Aim: Modern societies tend to use medicinal plants

for their basic health care needs. *Elaeagnus angustifolia* is one of the medicinal herbs with antinociceptive, anti-inflammatory, antibacterial and antioxidant properties widely used in the treatment of patients with rheumatoid arthritis and osteoarthritis symptoms.
Methods: In this work, for the first time, *E. angustifolia* extract was loaded on PCL-PEG-PCL nanofibers and studied their potential application in bone tissue engineering. Fourier transform infrared spectroscopy (FT-IR), field emission scanning electron microscope (FE-SEM), contact angle and mechanical test were applied to evaluate the morphology and chemical properties of nanofibers. FE-SEM images showed that all samples had bead-free morphologies with average diameters ranging from 100-200 nm. The cell response to the *E. angustifolia* extract loaded PCL-PEG-PCL nanofibers was evaluated by means of human dental pulp stem cells (hDPSCs).
Results: hDPSCs showed improved adhesion and proliferation capacity on the *E. angustifolia* loaded nanofibers compare to pristine PCL-PEG-PCL nanofiber. Alizarin red S assay and alkaline phosphatase activity confirmed the nanofibrous scaffolds could induce the osteoblastic performance of hDPSCs ($P<0.05$). QRT-PCR results confirmed that *E. angustifolia* loaded nanofibrous scaffolds significantly upregulated the gene expression correlated to osteogenic differentiation ($P<0.05$).
Conclusion: The results suggested that *E. angustifolia* extract loaded with PCL-PEG-PCL nanofibers might have potential applications for bone tissue engineering.
Keywords: *Elaeagnus angustifolia*; Scaffold; Electrospinning; Human dental pulp stem cell; Tissue engineering

PS-183. The Effects of Mouse Embryonic Stem Cells Injection into the Morula on Pluripotency and Trophectoderm Genes Expression in Mice Chimeric Blastocysts

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Background and Aim: Chimera is an animal with two or more populations of genetically distinct cells. Chimera has a significant impact on biological and developmental researches. Nevertheless, chimeric animal exhibits less viability and more fetal and placental abnormalities than a normal animal. The aim of this study was to determine the effects of mouse embryonic stem cells (mESCs) injection into the mouse embryos on some cell lineages genes expression in chimeric blastocysts
Methods: In the first part of our experiment, the GFP-mESCs, 129/Sv, were injected into the in vivo-derived pre-compacted and compacted mouse embryos, C57BL/6, and the incorporation rate of the injected cells was determined. In the second part, the expressions of inner cell mass (ICM) (Oct4 and Nanog) and trophectoderm (TE) (Tead4 andCdx2) lineage-specific genes in the blastocysts derived from different approaches were evaluated by real-time PCR and compared. The approaches were as follows: i) in vivo-derived blastocysts (control); ii) injection of mESCs into the subzonal space of in vivo-derived morula (chimeric blastocysts); iii) blastocysts derived from in vivo morula, and iv) blastocysts derived from in vivo morula which received a sham injection.
Results: The expressions of Oct4, Nanog and Tead4 in chimeric blastocysts were lower than those in blastocysts of other groups ($P<0.05$). A significantly increased expression of Cdx2 was observed in the chimeric blastocysts compared to sham ($P>0.05$), while there was no significant difference in Cdx2 expression of chimeric blastocysts with other groups. There was a significant difference in the production of chimeric blastocyst using two different stages of embryos so that subzonal injection of GFP-mESCs at the pre-compacted embryos had greater advantages than a compacted stage in the production of chimeric blastocysts ($P<0.05$)
Conclusion: Our data suggest that the formation of chemric blastocysts may be negatively affected by alteration in the expression of genes in ICM and TE. Furthermore, mESCs injection before embryo compaction increase incorporation rate of the injected mESCs into the ICM and subsequently the chance of mouse chimeric blastocyst formation.
Keywords: Genes Expression; Mice Chimeric blastocyst; Mouse Embryonic Stem Cells; Morula; Pluripotency; Trophectoderm

PS-184. Thymoquinone-induced Antitumor and Apoptosis in Human Lung Adenocarcinoma Cells

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Background and Aim: Lung cancer has the highest mortality rate in the world. Cancer chemotherapy can manage the progression of lung cancer and plants may have large contents of new antineoplastic agents. Thymoquinone (TQ) found in the plant Nigella has a potent anti-oxidant and anti-inflammatory activity. Objective: The purpose of the current research was to evaluate the chemo-preventive action and related mechanisms of TQ against human lung cancer.
Methods: The A549 cells were treated with the different concentrations of TQ for the three following days. Cytotoxicity was tested through MTT evaluation, production of necrosis and apoptosis by fluorescence-activated cell sorter analysis with propidium iodide (PI) and Annexin V, activation of caspase-3 and -9 by cleavage of specific substrates. DNA fragmentation through gel electrophoresis.
Results: TQ inhibited proliferation in human tumor cell line and death in the cells induced through apoptosis pathways. TQ also inhibited the apoptosis induced by the DNA fragmentation and activation of caspase-3 and caspase-9. Treatment with TQ on lung cancer induced a significant elevation in Bax/Bcl2 ratios. Up-regulation of P53 by TQ showed the other pathway for inducing apoptosis in A549 cells. TQ caused apoptosis which is accompanied by the effect of caspases-3 and -9, acting as an upstream activator. Only recently patents of TQ derivatives for the treatment of cancer are developed. This article also discusses our finding with the patents for this agent.
Conclusion: the current result may propose new pathways for the prevention and treatment of lung cancer through TQ for apoptosis induction in A546 cells.
Keywords: Anti-proliferative; A549 cells; Bax; Bcl2; Caspases; MTT; P53; Thymoquinone

PS-185. Scaffolds of Pectin-Graft-Polycaprolactone/Polyaniline Blend Nanofibers for Bone Tissue Engineering

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Background and Aim: Electrically conducting polymers (CPs), discovered in the late 1970s, have received tremendous attention because they combine the attractive properties of traditional polymers with those of metals and other inorganic conductors, including optical and electrical activity. One reason CPs have attracted the attention of biomedical

engineers is the discovery that many cell types (e.g., neurons, osteoblasts, fibroblasts) respond to electrical currents in vitro and in vivo. This led to the consideration that rationally designed conducting scaffolds could play a role in tissue engineering. On the other hand, the scaffolds should be biodegradable with appropriate mechanical properties that do not require surgery to remove scaffolds again. The scaffolds must have the mechanical properties appropriate to the target tissue.

Methods: Pectin-polycaprolactone (PEC-g-PCL) grafted copolymer and homo-polyaniline were synthesized via the ring opening polymerization of caprolactone and the chemical oxidation polymerization of aniline, respectively. Biocompatibility of the electrospun nanofiber was evaluated by assessing the adhesion and proliferation of mouse osteoblast MC3T3-E1 cells line and in vitro degradability.

Results: We successfully fabricated the PEC-PCL/PANI scaffold by the improved electrospinning process. Electroactivity of nanofibers, which here was for the presence of PANI, is an essential factor in its performance; because the electrical signals are pivotal physiological stimuli that control the adhesion and differentiation of various cell types. After twelve weeks, the mass loss for PEC-g-PCL/PANI sample was 55%. Scaffolds based on PEC-g-PCL/PANI nanofibers having an average diameter of 90–130 nm and electrical conductivity of 0.035 S cm^{-1} imitated the natural microenvironment of extracellular matrix (ECM) to regulate cell attachment, proliferation, and differentiation.

Conclusion: This work demonstrated that scaffold properties such as porosity, mechanical characteristics, degradation, and electrical properties can be tailor-made through the composition of nanofibers

Keywords: Polyaniline; Pectin; Nanofiber; Scaffold; Electrospinning; Tissue engineering

PS-188. Effects of Lignin on Proliferation and Differentiation of PC12 Cells in Electrospun Poly(Glycerol Sebacate)/Poly(vinyl Alcohol) Fibers

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Background and Aim: Peripheral nervous tissue injury is relatively common which could result from tumor resection, trauma, systemic diseases (e.g., diabetes), and infections (e.g., Lyme disease). Electrospinning of natural and synthetic polymers is one of the most practical approaches for using in tissue engineering. Lignin is considering as an antioxidant and antiviral material promoting neural cell differentiation. Poly(glycerol sebacate) (PGS) and poly(vinyl alcohol) (PVA) has also introduced as a promising biomaterial for nerve tissue engineering.

Methods: In the current study, aligned PGS/PVA/lignin fibers were fabricate using electrospinning technique to assess proliferation and differentiation of PC12 cell culture. MTT assay, scanning electron microscopy (SEM), DAPI was utilized to the evaluation of cell proliferation, adhesion, and viability. To study the study differentiation, SEM was used to measure the length of neurites.

Results: The results show the effective role of lignin on the proliferation and differentiation of PC12 cells.

Conclusion: Our results suggest that PVA/PGS/lignin is a promising nanocomposite scaffold for peripheral nerve regeneration.

Keywords: Poly(glycerol sebacate); Nerve regeneration; Poly(vinyl alcohol); Lignin; Electrospinning

PS-189. Finite Element Modeling of Fluid Dynamics, Oxygen Transport, and Cell Proliferation Inside 3D-Printed Scaffolds in a Perfusion Bioreactor

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Background and Aim: A major bottleneck in the 3D-bone tissue engineering field is the lack of efficient vascularization strategies since a constant flow of oxygen and nutrients is needed to maintain viability and functionality of bone tissue constructs. Fluid dynamics and oxygen concentration in 3D-printed scaffolds within perfusion bioreactors are crucial for bone cell proliferation and distribution. We aimed to determine the fluid dynamics, oxygen transfer, cell proliferation and distribution in 3D-printed scaffolds inside perfusion bioreactors by finite-element modeling.

Methods: M3T3-E1 osteoblasts were treated with pulsating fluid flow (PFF, frequency 1 Hz) for 1 h with low (0.8 Pa; low-PFF) or high peak shear stress (6.5 Pa; high-PFF), and nitric oxide production was measured to validate the sensitivity of the cells to fluid shear stress. Fluid flow and oxygen transfer between scaffold-strands were simulated at three inlet flow rates (0.4, 2, and 10 mL/min) for 5 days.

Results: High-PFF more strongly stimulated nitric oxide production by osteoblasts compared to low-PFF. 3D-simulation demonstrated that fluid velocity reached a maximum (100-2400 μm/s) between scaffold-strands dependent on inlet flow rate. Fluid shear stress (0.24-6 mPa) and wall shear stress (0.04-25 mPa) reached a maximum on scaffold-strand surfaces. At all inlet flow rates, cell distribution was homogeneous, while cell density increased due to sufficient oxygen transfer.

Conclusion: We conclude that fluid dynamics inside 3D-printed scaffolds can be controlled by changing the inlet flow rate of a perfusion bioreactor. The oxygen concentration and cell proliferation appear homogeneous independent of the inlet flow rate. Our findings provide a quantitative insight into the fluid dynamics, oxygen transport, and cell proliferation and distribution within a 3D-printed scaffold containing cells in a perfusion bioreactor, which will have important implications for bone tissue engineering strategies using bioreactors, scaffolds, and cells.

Keywords: Finite element modeling; Fluid dynamics; Oxygen transfer; Perfusion bioreactor; Three-dimensional scaffold

PS-190. Implication of Intraarticular Injection of Adipose-Derived Stem Cells for the Treatment of Knee Chondral Injuries: An In Vivo Study

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Background and Aim: The treatment of post-traumatic knee chondral defects is still a therapeutic challenge. The limited regenerative capacity of resident chondrocytes has led to the introduction of stem cell-based therapies as a novel therapeutic approach. Recently, adipose-derived stem cell (ADSC) may provide regenerative potentials for cartilage injuries. The aim of the study was to determine the effectiveness of isolated adipose-derived stem cells to repair knee chondral injuries through in vivo models of study.

Methods: The study population included ten sheep models of surgically imposed knee chondral injuries. The right joint of each was regarded as

a control sample and the left as the experimental joint. The ADSCs were obtained from the intra-articular fat pad of each animal before inducing injury. A mean of 105 stem cells was prepared and seeded into 4*4 mm of scaffold tissues, then were administered to the selected knees of animals. After six months, cartilage samples were extracted from both knee joints and macroscopic morphology of defect site as well as molecular and immunohistochemistry (IHC) of samples was studied.

Results: Macroscopic morphology of the experimental sample showed much improvement in chondral regeneration compared to control samples. Histological evaluation demonstrated thick, hyaline-like cartilage regeneration. Enhanced metachromatic regions stained with toluidine blue in treatment groups implied the increased level of proteoglycan molecules during the healing process. IHC results revealed a significantly increased level of collagen type 2 expressions in the extracellular matrix in treatment samples compared to controls ($P<0.001$).

Conclusion: Our study revealed that intra-articular injection of ADSC provokes regeneration of meniscal tissue, and has promising results in the treatment of knee cartilage injuries.

Keywords: Adipose-Derived Stem Cell, Knee Cartilage, Chondrocytogenesis, Injury, Regeneration

PS-191. Preconditioning of BM-MSCs by Innate Immunity Agonists Influences Mesenchymal Stem Cell Properties In vitro

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Background and Aim: Mesenchymal stem cells (MSCs) therapy has thus far been shown for inconsistent outputs, indicating an essential need to rectify therapeutic efficacy. Although administration of sufficient cells is necessary to attain maximal therapeutic benefits, documented MSC trials relied on injections of ~1×10^6 cells/kg, which appears too low to elicit a robust therapeutic response according to published animal studies. However, frequent cell passaging necessary for the scale-up expansion of MSCs causes cellular senescence, remodeling of cells and reduces stem cell potency. We hypothesized that preconditioning with TLR3 agonist significantly enhance cell proliferation via the Ras-Raf-MEK-ERK signaling pathway.

Methods: Experiments were performed using human bone marrow MSCs. The hMSCs were cultured in low-glucose DMEM supplemented with 10% fetal bovine serum and 100 U/100 µg/mL penicillin/streptomycin at 37°C in a humidified 5% CO2 incubator. The cells were fed with fresh medium every 3–4 days and used at passages 5 and 6. The hMSCs were incubated with poly (I:C) (1, 2 and 5 µM/mL, TLR3 primed) in the culture medium for 4 h. Total RNA was extracted from hMSCs and was reverse-transcribed and subsequently, the resultant cDNA was amplified using Evagreen mastermix. RT-PCR primer pairs for target genes were synthesized and quantitative real-time PCR was performed on an ABI step-one plus real-time PCR system. The amplified PCR products were detected by agarose gel electrophoresis and ethidium bromide staining.

Results: Using the poly(I:C) to engage MSC Toll-like receptor 3 (TLR3), we found that poly(I:C), signaling through multiple mitogen-activated protein kinase pathways, induced therapeutically specific trophic factors such as interleukin-1,6, TNF-α types cytokines, stromal-derived factor 1, and VEGF and slightly influence the proliferation of MSC. Poly (I:C) preconditioning enhanced proliferation of MSCs, stimulated expression of the Ras-Raf-Mek-Erk pathway. So, Poly (I:C) preconditioning provided a novel strategy in maximizing biologic and functional properties of MSCs.

Conclusion: These molecular quantifications thus establish the utility of TLR3 engagement for enabling the low-dose MSC therapy that may be translated to more efficacious clinical applications.

Keywords: TLR3; Innate immunity; BM-MSCs; Proliferation

PS-192. Chondrogenic Potential of Adipose Stem Cells and Chondrons In Vitro and Their Regenerative Capacity In Vivo

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Background and Aim: In articular cartilage regeneration, adipose tissue stem cells (ASCs) and chondrocytes have been used in several studies. Chondrocyte with peri-cellular matrix (PCM) is called chondron preserves its chondrocyte phenotype. Also, there are promising data from preclinical studies indicate the importance of using mesenchymal stem cells for clinical purposes. This study investigated chondrogenic potential and regenerative effects of ASC and chondron in vitro and in vivo.

Methods: In vitro study on human ASCs, chondrons and combination of them cultured for 4 days. The gene expressions of Sox9, runt-related transcription factor-2 (Runx2), aggrecan (AGG), cartilage oligomeric matrix protein (COMP), collagen type 10 (Col10), matrix metalloproteinase-13 (MMP13) were assessed for different stages of chondrogenesis at day 4 by real-time RT-PCR. In vivo study ASC, from the peri-renal fat of male rat and chondron from primary newborn rat hyaline cartilage were isolated. ASCs were cultured for at least three passages in vitro. Six weeks after OA induction, rats were randomly distributed in five groups of control, osteoarthritic, ASC, chondron and co-cultured. ASCs (107), chondrons (107) and the combination of chondrons and ASCs (107) were injected into intra-articular space of the rat knee. The effect of treatments was evaluated by microscopic examinations.

Results: PAS staining showed PCM of chondrons. The gene expression of Sox9, Agg, COMP and link protein results of chondrons were significantly higher than ASCs and coASCs at day 4. Histologic finding of in vivo study showed that more repair of the articular surface in ASC and coculture groups versus chondron group. H&E staining technique showed evidence of repair site of articular surface without erosion and fibrillation versus OA group which showed a thin layer of hyaline cartilage over tidemark and spontaneous fibrocartilage formation.

Conclusion: ASCs improve articular hyaline cartilage formation than chondron instead of high chondrogenic potential of chondrons in vitro. Joint microenvironment may be an effective factor on regeneration potency of different cell types.

Keywords: Adipose stem cell, Cell therapy, Chondron, Co-culture, Induced osteoarthritis

PS-193. Anti-Proliferative Effect of *Aloe vera* Gel on Fibroblast and Endothelial Cells

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Background and Aim: *Aloe vera* is used for a large variety of biological activities such as wound healing, antifungal activity, anti-inflammatory activity, hypoglycemic immunomodulatory, gastroprotective and anti-cancer effects. It has been reported that *Aloe vera* has tumor preventive effect by inhibiting angiogenesis. However, wound healing and anti-

cancer effects are a controversial issue based on the angiogenesis process. Therefore, this study evaluated *Aloe vera* gel effects on endothelial cells and fibroblasts.
Methods: The fresh white pulp of mature leaves Aloe Vera is homogenized by a mixer, centrifuged and sterilized by chloroform. Monolayer culture of human fibroblasts and endothelial cells were treated with Aloe 10% Vera gel. The toxicity and proliferation rate were evaluated by MTT assay for both types of cells.
Results: Our microscopic observation showed no morphological changes in Aloe vera-treated fibroblast and endothelial cells. However, both of cells in the first 24h were proliferated and on the second day, some cell debris were observed. MTT assays revealed that *Aloe vera* has no toxicity on cells on the first day of cultures and the growth rate of both cells in *Aloe vera*-treated cells was the same as control. *Aloe vera* significantly ($P<0.005$) decreased optical density of endothelial cells in MTT assay after 48 hours.
Conclusion: The recent study showed that the antiproliferative effect of *Aloe vera* on human fibroblasts and endothelial cells. For anti-cancer effects of the plant, the anti-angiogenic activity must be evaluated.
Keywords: *Aloe vera*, Anti-cancer, Endothelial cells, Fibroblasts, Wound healing

PS-194. Behavior of Human Umbilical Cord Wharton's Jelly Mesenchymal Stem Cells on Electrospun Poly(lactic Acid)\Wax Nanofibers

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Background and Aim: Extracellular matrix (ECM) contains extracellular molecules secreted from cells. It has important roles in biochemical and structural support of surrounding cells and regulates the critical cell behaviors. Scientists use nanotechnology, especially nanofibers to develop the tissue engineering scaffolds. In the present study, in vitro responses of Human umbilical cord, Wharton's jelly mesenchymal stem cells (WJ-MSCs), on polylactic acid/WAX (PLA/WAX) electrospun nanofibrous scaffold were reported in comparison with those of the cells on corresponding PLA scaffold.
Methods: In this study, umbilical cord (UC) was taken from cesarean delivery and transported to the laboratory in 2 hours. MSCs were isolated from umbilical cord Wharton's jelly using the explant method. After MSC characterization, cells were passaged for 4-6 times and cultured both two- and three-dimensionally (2D and 3D). Bee wax was used for the modification of PLA scaffold surface. For electrospinning, PLA and Wax were dissolved in hexafluoroisopropanol (HFIP) solvent and chloroform, respectively. Different scaffolds were fabricated by an electrospinning technique (PLA, PLA/wax 8:2) and Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscope (SEM), MTT assay and DAPI staining were used for the evaluation of cells morphology and viability.
Results: Our results demonstrated that cells are able to attach to the PLA/wax nanofibrous scaffold easier and this scaffold is a better support for the attachment and proliferation of WJ-MSCs than the corresponding PLA scaffold. In addition, PLA scaffold had the average fiber diameter of 350 nm while PLA/wax scaffold had a significantly decreased average fiber diameter (70 nm). Toxicity of the scaffolds was tested and the results indicated that PLA/wax scaffold was more biocompatible than PLA scaffold. MTT assay results also showed that nanofibrous scaffolds could significantly improve the viability of WJ-MSCs compared with 2D culture.
Conclusion: Consequently, the results of this study confirm that WJ-MSCs can sense the chemical composition of the materials and their physical properties. These components are able to regulate the behavior of these cells accordingly. We also conclude that Nanofibrous PLA/wax scaffolds can be used as a suitable broad-spectrum scaffold for tissue engineering.
Keywords: Adhesion; cell viability; WJ-MSC; Bee Wax; Electrospinning Scaffold

PS-195. Use of Natural Chemical Compounds for Differentiation of Human Adipose-Derived Mesenchymal Stem Cells Into Mesoderm Lineage

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Background and Aim: Administration of human adipose-derived mesenchymal stem cells (ADMSCs) has recently considered as a new therapeutic approach in clinical trials for widespread indications including cardiac, neurological, hematological and pulmonary disorders. However, efforts to exploit these cells have been met with only modest success mainly due to the low efficiency of the differentiation process, using animal-derived materials and inadequate expansion of the large quantities of desired cell derivatives under good manufacturing practice. In the present study, we have to access the effects of applying various natural small molecules and plant extract on efficiency of differentiate human ADMSCs into the definitive mesoderm lineage as the initial step in differentiation into other mesoderm derivatives, such a cardiomyocyte, hematopoietic, endothelial cell, smooth muscle cell, skeletal muscle cell, renal cell, adipocyte, chondrocyte and osteocytes.
Methods: For this purpose, first mesenchymal stem cells isolated from the human adult fat tissue, propagated and characterized using flow cytometry and relevant markers. In second passage cells transferred to 24 well plates and treated with various plant-derived small molecules mainly Wnt/β-catenin signaling pathway, one of the most conserved intercellular signaling activators including resveratrol, stilbene and different plants and evaluate their effects on their differentiation into the definitive mesoderm lineage.
Results: Data obtained in this study might lead to formulating a defined safer and more efficient, cost-effective culture medium for mesoderm differentiation.
Conclusion: These findings can lead to the improvement of the current approach toward achieving a reproducible and efficient differentiation, verification, and application of the ADMSCs for clinical purposes.
Keywords: Natural small molecules; Differentiation; Mesoderm; Adipose-derived mesenchymal stem cells

PS-196. A Novel Stirred Tank Bioreactor with Sequencing Batch Aeration System for Mass Production of Stem Cells: Conceptual Design, Modeling and Performance Analysis

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Background and Aim: It is necessary to proliferate stem cells from the low initial amounts to desirable values for use in regenerative medicine, cell therapies, and other medical/scientific applications. Regarding the vulnerability of these cells due to the lack of cell wall, it seems essential to design processes which could provide appropriate conditions to monitor and control the parameters influencing the growth. Hence, in this study, an innovative cell culture system was designed with the goal of reaching the maximum obtainable cell number on the base of simplicity, efficiency, and economic regards.
Methods: At first, all of the effective parameters were defined and studied. Then the best conditions with respect to existing facilities were selected for designing an upgraded stirred bioreactor with a sequencing batch

aeration system, in a way that it could attain to the final amounts needed by the patient, from small amounts of the initial biopsy in an economical manner. Finally, after writing related mass balances, modeling and simulation were done to predict the possible behaviors of the system.
Results: The overall volume of a bioreactor, required for both liquid and gas phases, were obtained equal to 400 mL. The novel bioreactor system can work with daily replacement of total gas phase (95% air/5% CO2, with 1% positive pressure), and the replacement of half of the liquid phase, three-day once with fresh medium. Ultimately according to the graphs obtained from the simulation, it is concluded that in the designed bioreactor with explained sequencing batch aeration, the final number of cells needed by the patient (about 2∈108 cells) could be achieved during the 10-day cultivation without the problems caused by a shortage or critical accumulation of any materials.
Conclusion: The novel designed economical bioreactor system demonstrated a great potential for mass production of stem cells particularly suspension stem cells, although further experiments are needed to evaluate the real performance of the system for this purpose.
Keywords: Mammalian cells; Proliferation; Stirred bioreactor; Sequencing batch process

PS-197. Comparison of Colony-Forming Efficiency Between Breast Milk of Mothers with Preterm Delivery and Full-Term Delivery

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Background and Aim: The beneficial effect of breastfeeding for the health of mothers and infants are well recognized. The human breast milk is the unique complex fluid which consists of the heterogeneous cellular populations and biological macromolecules satisfying nutritional needs with immunological properties. Breast milk is the novel source of stem cells forming during pregnancy and lactation. It has been suggested that breast milk stem cells (BMSCs) have the key role in organ development and health of infants with short and long-term effects. In the present study, the colony-forming efficiency (CFE) of breast milk-derived stem/progenitor cells in breast milk of mothers with preterm delivery (gestational age less than 37 weeks) will be assessed with mothers with full-term delivery (gestational age more than 37 weeks).
Methods: Breast milk samples were obtained from neonatal intensive care and maternity ward, Imam Reza Hospital (Mashhad, Iran). About 10 mL of fresh breast milk was mixed with an equal amount of balanced salt solution (BSS) and centrifuged at 1380 rpm for 20 min at 25°C in a clean room. Then, the sediment cell pellet was washed with BSS (×3) and cultured in the 12-well plate. The viability of cells was determined using trypan blue staining protocol, then cells were cultured at a seeding density of 4×10^4 cells/well and incubated at 37°C, 5% CO2, 80% humidity for 14 days. The colonies were counted using an inverted microscope in order to determine the efficiency of colony forming.
Results: Our results showed that the infants with the weight of 3000-4000 g have the significant correlation with the CFE day 5 (P=0.03, 130±62) and CFE day 15 (P=0.021, 105±26).
Conclusion: The results demonstrated increased numbers of stem cells colony in preterm infant mothers' breast milk. Thus, it is the source of cells than mature milk.
Keywords: Breast milk; Stem cells; Preterm delivery; full-term delivery

PS-198. Recellularization of Well-Preserved Rat Acellular Kidney Scaffold Using Mesenchymal Stem Cells

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Background and Aim: The development of tissue engineering have catalyzed due to a gap between limited organ supply and increasing requests for patients with the end-stage renal disease. The choice an appropriate scaffold for cell seeding and production of the functional organ for transplant purpose is the critical challenges in tissue engineering. An important technique in regenerative medicine to prepare an acellular ECM is the decellularization of native tissues. The purpose of this study was to acquire effective method for preparation of decellularized kidney scaffold which can proliferate and differentiate of MSCs into kidney cells.
Methods: After removing the kidney, the adipose tissue and the capsule around the kidney were removed. Kidney sections were washed twice with phosphate buffered saline (PBS), followed by decellularization in a solution of either 1% Triton X-100 or sodium dodecyl sulfate 1% (SDS). The decellularization solution was changed 4 hours after initial tissue harvest and then every 24 hours until tissues were transparent (for 14 days). In order to confirmation of decellularization, hematoxylin-eosin (H&E) and DAPI staining were performed on days 2, 5, 10, and 14. Then, we seeded 2×10^5 hAd-MSCs onto time sufficient and suitable sterile kidney scaffold to allow attachment of cells, 1 hour after seeding 1 mL DMEM was added to any of wells, and they incubated at 37°C and 5% CO2 for 3 weeks. H&E staining was performed for measure cell viability and proliferation of cells within the renal scaffold.
Results: DAPI staining approved the SDS-treated sections were more decellularized than the Triton-treated sections at all times. The results of hematoxylin and eosin staining revealed that in the SDS-treated sections the native ECM architecture, integration of renal vascular and glomerular structures was more preserved than the Triton-treated sections. Moreover, migration and establishment of a number of cells to the renal scaffold were observed after recellularization. In addition, cell accumulation on the scaffold surface as well as the migration of the cells to the depth of kidney-formed an epithelium-like structure.
Conclusion: Our methods can successfully decellularize rat kidneys to produce functional renal ECM scaffolds. These scaffolds maintain their basic components, retain intact vasculature and show promise for kidney regeneration.
Keywords: Kidney; Extracellular matrix scaffold; Decellularization; Recellularization; Mesenchymal stem cells

PS-199. Preparation of Hyaluronic Acid-Polycaprolactone Based Polymersome for Targeted Delivery of Doxorubicin to CD44+ Non-Small Cell Lung Cancer: In Vitro Evaluation

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Background and Aim: Polymersomes are polymeric vesicles possess core-shell construction which confers this flexibility to control their functions, properties, and structure. Numerous small and large molecules can be loaded into the hydrophobic bilayer and hydrophilic core. Therefore, polymersomes are increasingly being investigated as carriers for drug delivery and imaging probes. In this research, we synthesized hyaluronic acid-polycaprolactone copolymer in order to prepare self-assembled

polymersomes which are loaded with doxorubicin to target non-small cell lung cancer.
Methods: The nanoparticles were produced by the nanoprecipitation method. HA-PCL was dissolved in dimethylsulphoxide (DMSO) and doxorubicin was dissolved in distilled water. The polymer mixture was added dropwise to a stirring water. After 2h stirring in 50°C, the mixture was dialyzed by float-A-lyzer (Mw=8-10 kDa) for 6h and then freeze-dried to achieve the final powder formulation. Determination of particle sizes and polydispersities, drug content, DSC and SEM were done. The in vitro differential cytotoxicity of DOX-loaded HA-PCL nano-polymersomes (HA-PCL@DOX) in comparison with free DOX on A549 cells (CD44+ cells) was evaluated.
Results: Herein, we developed HA-PCL DOX-loaded nano-polymersomes for targeted delivery against A549 cells as models of NSCLC. Our findings demonstrated that HA-PCL@DOX NPs increased the cytotoxicity of the DOX payload as compared with free DOX ($P<0.05$) in A549 cells.
Conclusion: Overall, it was demonstrated that HA-PCL nanoparticles could be studied as a potential carrier for further research of cancer therapy.
Keywords: Hyaluronic Acid; Polycaprolactone; Polymersome; Doxorubicin; A549 cells

PS-200. Endothelial Cells Regeneration with Collagen/Hyaluronic Acid/ Bioglass Nanoparticles Scaffolds

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Background and Aim: Recently, many investigations have focused on the fabrication of scaffolds for vascular regeneration with multiple structures. Among various scaffold fabrication techniques, freeze drying, as an energy inexpensive and the most effective methods considering it provides high water removal can be used to create porous scaffolds for inducing angiogenesis and endothelial cells regeneration. During the recent decade, bioglass has been extensively studied as a potentially suitable candidate for scaffold fabrication because of its great physicochemical features and excellent biocompatibility.
Methods: In this study, collagen/2% and 2.5% hyaluronic acid incorporated with various ratios of bioglass nanoparticles (BGNPs) (0.5 wt%, 1.5 wt%, and 3 wt%) were prepared and their biomechanical and physicochemical properties were examined by scanning electron microscopy, Fourier transform infrared spectroscopy, Raman, and Rheological study. Expression of endothelial cell markers in treated cells on the collagen/hyaluronic acid/BGNPs scaffolds was assessed by immunocytochemistry and real-time PCR analysis.
Results: Bioglass nanoparticles were uniformly distributed inside the collagen/hyaluronic acid scaffolds without affecting their biomechanical and physicochemical properties. Collagen/hyaluronic acid/BGNPs scaffolds increased the endothelial differentiation of Wharton's Jelly mesenchymal stem cells without inhibiting their proliferation.
Conclusion: These results recommend that collagen/hyaluronic acid/ BGNPs scaffolds can be applied to make a broad spectrum of strategies for the fabrication of promising scaffolds to improve vascular tissue regeneration due to their potential to promote angiogenesis.
Keywords: Stem Cells; Endothelial Cells; collagen/hyaluronic acid/ BGNPs; Freeze-drying method

PS-201. The Role of TiO2 Nano-Tubes in Treatment of Neuronal Cancer

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Background and Aim: Titania (TiO_2) has been demonstrated as an excellent candidate for local and targeting drug delivery systems owing to its chemical stability, photocatalytic activity good biocompatibility and low cost. The common drawbacks of conventional cancer treatments are non- targeting, toxicity leading to limiting the amount of desired drug, poor biodistribution, and efficacy. For this purpose, we used TiO_2 biomaterial with the suitable structure as drug carriers for the effective and targeted transfer of drugs to target cells. TiO_2 nanotube surface with biocompatible materials containing a chemical group that provides a desirable platform to a conjugate of the drug molecules to substrate. In this study, we used TiO_2 nanotube for neuroblastoma cancer cell killing under UV light.
Methods: Anatase TiO_2 nanoparticles with a diameter 10-25 nm were used as starting material. Morphological studies were carried out using transmission electron microscope (TEM), x-ray diffraction (XRD) patterns of the powder samples were recorded on a Philips PW3710 diffractometer using CoKα radiation (λ= 1.79Ao) at 40kV and 30mA. Neuroblastoma cell line (C549) was purchased from Pasteur Institute Tehran, Iran. Metabolic activity of neuroblastoma cells lines cultured was assessed by the (MTT) reduction assay. The cytotoxicity assays were performed thrice. Finally, the optimum dose of nanotubes was chosen for cell studies according to the MTT results. The cells were incubated with RPMI media +15% FBS & 1% pen/strep. They were cultured for a period of 5-7 days and divided to control and treatment groups. Cells treated with TiO_2 at a range of concentration of 50-100 ng/mL and 5 µg/ mL cisplatin, an anti-cancer drug too.
Results: The morphology and structure of the nanotubes were characterized by TEM images revealed hallow and open ended tubular structures. The result of XRD pattern indicates that the TiO_2 is completely comprised of anatase phase (JCPDS 001-0562). Significant peaks were observed at 2 λ = 29.44, 44.34, 56.82, 63.49, 65.21 and 74.37 corresponding to (1 0 1), (1 0 3), (2 0 0), (1 0 5), (2 1 1) and (2 1 3), respectively. The viability of cells on control compare to experimental groups was examined with MTT assay in days of 1, 3 and 5 after incubation. The cell survival rate in 3 and 5 days was significant ($P<0.001$). Cell viability withTiO2 and cisplatin was more significant than cell group with only cisplatin.
Conclusion: Results showed the potential of using TiO_2 and cisplatin platforms as nanocarriers to treat cancer. Anatase TiO_2 nanotubes can strongly absorb the UV light, be excited and generate electron-hole pairs and react with OH– or molecular oxygen to form various reactive oxygen species (ROS) which lead to the death of the tumor cells
Keywords: Cisplatin, Cell culture; Therapy; TiO_2 nanotube; Photocatalytic activity; Cancer

PS-202. The Effect of Intraovarian Injection of Ckit Positive Cells Derived from Bone Marrow Cells in Ovarian Rejuvenation of Menopause Rats; A Pre-clinical Study

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Background and Aim: Today Cell therapy is one of the new therapeutic methods in medical sciences and fertility related complications such as menopause. Bone marrow derived c-kit positive cells, have the ability to differentiate into different cell types. In this study, intra-ovarian injection of bone marrow derived c-kit positive cells with the aim of ovarian rejuvenation was implemented in the rat model of ovarian failure.
Methods: To induce the rat model of menopause, daily injection of 4-vinylcyclohexene dioxide (VCD) was performed for 15 days. 75 Wistar female rats were randomly divided into 5 groups: Control (IP injection of saline for VCD control), VCD free (IP injection of VCD and letting them free without intervention), Ckit positive (IP injection of VCD and intra-ovarian injection of Ckit positive cells), Ckit negative (IP injection of VCD and intra-ovarian injection of Ckit negative), saline injection (IP injection of VCD and intra-ovarian injection of saline). Histological evaluation and hormonal assay (FSH and E2) continued up to 8 weeks (once every 2 weeks). At the end stage, reproduction performance of 3 rats for each group was assessed.
Results: Preliminary results from histological evaluation showed an improved follicles counts in the c-kit positive group compared to c-kit negative and saline groups, whereas more atretic follicles in different stages were noticed in c-kit negative rats and saline group. Reproductive performance assessment after mating revealed a significantly higher birth rates in c-kit positive group compared to c-kit negative, saline and VCD-free groups.
Conclusion: Intra-ovarian injection of Ckit positive derived from bone marrow cells is a useful approach for ovarian tissue rejuvenation and reproductive performance return. Future studies and clinical trials will hopefully focus on the uses of Ckit positive cells in the restoration of human ovaries.
Keywords: Infertility; Menopause; CKit positive; Bone marrow cells; Ovarian rejuvenation

PS-203. Vitamin D Receptor Knockdown Using RNAi Changes the Expression of Several Genes in Jurkat T and U87-MG Cells

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Background and Aim: Vitamin D regulates a number of important biological processes. It protects the adult stem cells or limits the expansion of cancer stem cells. Recent the experimental data have indicated the molecular function of vitamin D in vitro cell culture systems using cancer cell lines. Vitamin D signaling is through the Vitamin D receptor (VDR). VDR is expressed in the kinds of cells and involved in apoptosis, cell cycle, proliferation, immunity, and inflammation. In this study, we investigated the effects of VDR knockdown on some gene expression in Jurkat and U87-MG cell lines.
Methods: In the present study Jurkat and U87-MG cell lines were used. These cell lines were maintained in RPMI-1640 medium. In order to knock-down VDR, Jurkat T and U87-MG cells were transfected with pGFP-V-RS shRNA clones targeting human VDR transcripts and a scrambled non-targeting control. Following the transfection, the expression of GFP was observed under a fluorescence microscope. Next, Total RNA was extracted from cells and cDNA was synthesized. To analyze the expression of IL-10, NF-KB, TGF-β1, TGF-β2, TGF-β R I and TGF-β R II mRNAs and Test knockdown efficiency, real-time PCR was performed at 24 h and 48 h after transfection.
Results: Quantitative PCR showed that the VDR transcript expression was decreased in VDR shRNA-transfected groups. In Jurkat T cells the reduction of the VDR mRNA expression at 24 and 48 h after transfection was77 % and 71% down respectively and in U87-MG cells was 74% and 76% respectively. Analysis of qRT-PCR showed that the expression of IL-10, NF-KB, TGF-β1, TGF-β R I and TGF-β R II in Jurkat T cells transfected with VDR-shRNA was significantly decreased compared to Scramble-shRNA cells and the untransfected cells, but no significant difference was observed in these cells for TGF-β2. Additionally, the expression of NF-KB, TGF-β1, TGF-β2, TGF-β R I and TGF-β R II in U87-MG cells after transfection with the VDR-shRNA, was significantly increased compared with Scramble-shRNA cells and the untransfected cells, but this difference was not significant for IL-10.
Conclusion: We have shown a fundamental role of vitamin D receptor in regulating TGF-β signaling and highlighting the potential function of VDR to control some gene expression in cancer cell lines; Jurkat T and U87-MG. There are clearly still significant gaps in our understanding of the VDR roles in stem cells. In future investigations, it will be of scientific and clinical interest to investigate effects down-regulation VDR on the apoptosis, cell cycle and other biological processes in different stem cells.
Keywords: RNAi; Jurkat T cells; Stem cells; TGF-β signaling; IL-10

PS-204. Development of a Novel Rat Model of Chronic Wound Using Magnetic Feature for Regenerative Medicine Applications

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Background and Aim: Chronic wound development is a result of delayed wound healing process. Briefly, the wound progression into the chronicity is associated with the prolonged and excessive inflammatory response and the myriad of factors including poor circulation, vascular insufficiency, diabetes, aging, malnutrition and even local factors such as bacterial infection and pressure. Developing the proper animal model would be an advancement to understand how chronic wound develops in humans. Finally, this provides the therapeutic field for further investigation in chronic wound treatment.
Methods: Mature rats were purchased and the animal experiments were conducted in accordance with the guideline approved by the Institutional Animal Use and Care Committee of Shahrekord University of Medical Sciences. After anesthetizing the rats by ketamine (75-100 mg/kg) and xylazine (10 mg/kg); they were shaved and disinfected with 75% ethanol. A surgical incision was made on the back of the rat and one sterilized stainless-steel ring with a magnetic feature was implanted in the depth of the skin. Next, the wound was closed by suturing and proper wound dressing. Afterward, the wound was faced with a magnet every 8 hours and each time for 20 minutes to prevent the normal wound healing process. After 21 days, under the anesthetic condition, the ring was removed and the wound was analyzed by applying hematoxylin & eosin (H&E) staining. The non-healing status of the wound and its elevated level of inflammation should be seen. After confirming the chronic wound creation, this model can be used for regenerative medicine applications
Results: After 21 days, the wound had become chronic and the wound had produced exudates which contained biofilm. The histological examinations showed that the granulation tissue does not exist and the epidermis does not develop to close the wound. Therefore, these results indicate that chronic wound has been developed in rats.
Conclusion: Considering the psychological, physiological, financial and social effects of chronic wounds for both government and affected patients, there is a huge need to have a proper model of a chronic wound. In addition, it will contribute to the understanding of how wounds become chronic and how they can be treated efficiently. In this regard, we introduced a novel method for developing a rat model of the chronic wound within 21 days and at a lower cost for regenerative

medicine applications
Keywords: Wound healing; Chronic wound; Inflammation

PS-205. An Investigation of Promoter-Targeted Small Activating RNAs On BMP2 Up-regulation

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Background and Aim: saRNAs (Small activating RNAs) are small double-stranded RNAs (dsRNAs) that target gene promoters to induce transcriptional gene activation in a process known as RNA activation (RNAa). In this study, we are going to investigate the likelihood to enhance the BMP2 expression in human Mesenchymal Stem Cells (hMSCs) using specifically designed saRNAs. If so, we can use the designed saRNAs as affordable small osteogenic factors in synthetic bone grafts and substitute materials instead of expensive BMP2 proteins with side effects.
Methods: First, two different dsRNAs targeting BMP2 promoter are designed using short hairpin RNA target design tools including DSIR and Dharmacon. Secondly, the dsRNA inserts are separately ligated into the pCDH vector and then transformed using heat shock in home-made competent cells. In the following, target cells will be individually transfected with two kinds of constructs expressing BMP2 promoter-targeted saRNAs with the backbone construct serving as negative control. Using qRT-PCR, the relative expression of BMP2 and other marker genes in osteogenesis are measured at the mRNA level in both treated and control cells
Results: Using target design tools we chose "GAATATATTTATAGAAATATA" and "CTGCATTTGTCCTGGATTTCG" sequences as the potent saRNAs to activate BMP2 gene expression. The sequences were successfully cloned. Following target cell transfection, the results show that our designed osteogenic dsRNAs could affect osteogenic pathway through targeting the BMP2 promoter. Based on the literature survey and saRNA target design tools, we believe our designed saRNAs hold a high potential to induce osteogenesis. Such a finding may have a great impact on gene therapy of bone associated disease, which would obviate the need for cloning of the large BMP gene by using a short RNA sequence instead
Conclusion: Our finding will provide a novel approach to up-regulate the desired genes in different cells. However, the cell-type dependency of RNAa makes it tough to make a common algorithm for designing saRNAs. In addition, promoters consisting of certain elements such as the TATA box and CGIs (CpG islands) appeared to be more potent to be affected by dsRNAs
Keywords: saRNA; RNAa; BMP2

PS-206. Comparison of the Properties and Bioactivity of Glass-Ceramic Nano Powder Produced by 2 Distinct Synthesis Methods (Sol-Gel and Crushing) and Their Bioactivity Fixation with SBF Body Simulant

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Background and Aim: Biologically active glasses, including biomaterials, are used in various medical fields. In this study, two types of biologically active hydroxyapatite (basic biomaterials for bone fracture repair) were synthesized, and to investigate the phase composition of glass-ceramics and available ion bands found in the composition and morphology of each sample were used for Fourier-Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM), respectively. For the formation of apatite and study about the stability behavior of the structure in vitro conditions, we floated two of samples in a simulated body fluid (SBF) for 20 days, and with regard to created weight gain, we compared their bioactivity.
Methods: Biologically active glasses, including biomaterials, are used in various medical and medical fields. In this study, two types of biologically active hydroxyapatite (basic biomaterials for bone fracture repair) were synthesized, and to investigate the fusion of glass and transplants found in The composition and morphology of each sample were used for infrared Fourier transform and scanning electron microscopy, respectively, and for the formation of apatite and study of the behavioral stability of the structure in external conditions, in a simulated body solution for 20 days, and with regard to weight gain Created, you compared their bioactivity
Results: Biologically active glasses, including biomaterials, are used in various medical and medical fields. In this study, two types of biologically active hydroxyapatite (basic biomaterials for bone fracture repair) were synthesized, and to investigate the fusion of glass and transplants found in The composition and morphology of each sample were used for infrared Fourier transform and scanning electron microscopy, respectively, and for the formation of apatite and study of the behavioral stability of the structure in external conditions, in a simulated body solution for 20 days, and with regard to weight gain Created, you compared their bioactivity
Conclusion: Biologically active glasses, including biomaterials, are used in various medical and medical fields. In this study, two types of biologically active hydroxyapatite (basic biomaterials for bone fracture repair) were synthesized, and to investigate the fusion of glass and transplants found in The composition and morphology of each sample were used for infrared Fourier transform and scanning electron microscopy, respectively, and for the formation of apatite and study of the behavioral stability of the structure in external conditions, in a simulated body solution for 20 days, and with regard to weight gain Created, you compared their bioactivity
Keywords: Sol gel; Hydroxyapatite; simulated body solution; bioactivity; glass-ceramics

PS-207. Design and Fabrication of Injectable Hyaluronic Acid Hydrogels by Guest–Host Assembling

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Background and Aim: Injectable hydrogels afford direct injection or catheter delivery to tissues. However, many injectable hydrogels require long reassembly times or the use of triggers such as chemical initiators or heat.
Methods: We developed an injectable hyaluronic acid (HA) hydrogel based on the guest–host interactions of adamantine modified HA (guest macromer, Ad-HA) and β-cyclodextrin modified HA (host macromer, CD-HA).
Results: The ability of conjugation to HA was confirmed by 1H NMR spectroscopy. Mixing of Ad-HA and CD-HA resulted in rapid formation of a hydrogel composed of guest–host bonds.
Conclusion: The guest–host assembly mechanism allowed both shear-thinning behavior for ease of injection and near instantaneous reassembly for material retention at the target site. These hydrogels show potential as a minimally invasive injectable hydrogel for biomedical applications and tissue engineering.
Keywords: Shear thinning hydrogels; Hyaluronic acid; Graphene oxide; Cardiovascular tissue engineering

PS-208. Expression Analysis of BORIS During Retinoic Acid-Iinduced Differentiation of Human Embryonal Carcinoma NCCIT Cell Line

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Background and Aim: BORIS belongs a group of genes that are expressed normally in testis, and abnormally in various types of cancers. Recently, expression of BORIS in cancer stem cells and its role in expression induction of some CSC markers and in the regulation of CSC features has been demonstrated. Embryonic carcinoma (EC) cell line are the pluripotent cells derived from tumors known as teratocarcinomas and are generally considered to be the archetype of cancer stem cells. Here expression alternation of BORIS was analyzed during RA-induced differentiation of human EC NCCIT Cell Line
Methods: NCCIT cell line was cultured in RPMI supplemented with 10% fetal bovine serum and maintained at 37°C in humidified air with 5% CO2. To induce differentiation, NCCIT cells were treated with 10 µM RA for 21 d. Total RNA was extracted using the total RNA isolation kit. Primers for detection of all isoform of BORIS as well as the β2M gene as a reference were designed by Oligo 7 software. After cDNA synthesis, one microliter of complementary DNA products was amplified with SYBR green real-time PCR master mix in Analytik Jena real-time PCR system. Quantification of target genes was normalized using the β2M gene as a reference and subsequently expressed as relative to untreated control cells. Fold change was calculated according to the following equation: 2(-ΔΔCT).
Results: Although designed primer for BORIS can amplify all isoform for BORIS, Real-time PCR did not show expression of BORIS in NCCIT cell line. Expression of BORIS was not induced during RA-induced differentiation of NCCIT cell line
Conclusion: In fact, NCCIT cell line is a heterogeneous population of cells comprising a small population of BORIS-expressing cancer stem cells. Therefore, in order to investigate the expression of BORIS in this cell line, at first stem-like cells must be first isolated according to their markers and properties. It is probable that the heterogeneous population of NCCIT does not show the expression of this gene.
Keywords: BORIS; Human embryonal carcinoma NCCIT cell line; Retinoic acid; Differentiation

PS-209. Study of the Effect of Donepezil Hydrochloride Drug on Genes Expression During the Osteogenic Differentiation of Mesenchymal Stem Cells on PLLA Nanofibers

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Background and Aim: Human mesenchymal stem cells (hMSCs) have potential to self-renewal and differentiation to other subtype cells such as osteoblast, adipose, and cartilage. PLLA (poly L-lactic acid) is a biodegradable and biocompatible nanofibrous scaffold that mimics the physical and biological properties of the extracellular matrix. Donepezil hydrochloride is an acetylcholine esterase inhibitor that prescribes in patients who suffer from dementia. In this study, we examined the proliferation and osteogenic differentiation of hBMSCs on PLLA nanofibrous scaffold in the presence of donepezil.
Methods: PLLA nanofibrous scaffold synthesized by an electrospinning technique, and then were exposed to oxygen plasma to induce hydrophilicity and better cell adherence. hMSCs were seeded in four conditions to achieve the purpose of promoting cell proliferation and bone differentiation: 1-control group, 2-PLLA nanofiber, 3-donepezil hydrochloride and 4- PLLA- donepezil hydrochloride then, all of them were treated with osteogenic induction media. The biocompatibility of scaffolds and cytotoxicity of the drug were studied by MTT assay and Acridine orange staining. Bone-related markers were evaluated by calcium content, alkaline phosphatase (ALP) activity, von Kossa, and Alizarin Red staining. In addition, bone-related genes expression (ALP, Osteonectin, Osteocalcin, and RUNX2) was evaluated by immunofluorescence staining and quantitative real-time PCR (qPCR) analysis.
Results: MTT results showed that the fabricated PLLA scaffold did not have any cytotoxicity as well as promote the cell attachment and proliferation of hBMSCs. Under a fluorescence microscope, viable cells appear green color with the recognizable plasma membrane. MTT assay showed that donepezil hydrochloride caused toxicity in more than 50 µg/mL but in the range between 500 ng and 1 µg it acts as a co-stimulator. Our results showed higher ALP enzyme activity, bio-mineralization, von Kossa staining with more intensity in differentiated cells on PLLA-donepezil hydrochloride. The expression level of 4 genes related to osteoblasts was evaluated on four conditions. A higher level of alkaline phosphatase activity, as well as osteonectin, osteocalcin and RUNX2 genes expression on donepezil/PLLA, was detected. The level of osteocalcin and osteonectin protein markers was increased in the period of differentiation of hMSCs into osteogenic lineages.
Conclusion: A higher level of osteogenic markers such as ALP activity, calcium content and bone-related gene expression was detected on the donepezil/ PLLA scaffolds. According to the results fabricated PLLA nanofibrous scaffolds in presence of donepezil hydrochloride, revealed high capabilities in support of proliferation and osteogenic differentiation of hMSCs.
Keywords: Stem Cell; Osteogenic Differentiation; PLLA Nanofiber; Donepezil Hydrochloride

PS-210. Intramural Transplantation of Homologous Fibroblasts Promoted the Wound Healing in Rat Model of Intestinal Anastomosis

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Background and Aim: This study was aimed to evaluate the intramural transplantation of homologous fibroblast on the healing of colonic anastomosis in rats by means of histopathological parameters.
Methods: Thirty-six male mature Wistar rats were divided randomly into three groups and undergone the surgical procedure of colonic resection and anastomosis. Primary fibroblasts were isolated from the skin of rats. The abdominal cavity of rats in the sham group was approached by midline incision, the whole intestine was manipulated and then the abdominal wall was sutured. In both groups of control and treatment, ascending colon was exposed and a 0.5 cm section of colon was resected and thereafter anastomosed by simple interrupted sutures. Rats in the control group were received 0.5 mL PBS in the seromuscular layer and in the treatment group 0.5 mL PBS containing about 1×10^6 fibroblasts were injected in the same manner. On day 7, all rats were euthanized for sampling.
Results: Histopathologic findings indicated that angiogenesis and number of mature fibroblasts, collagen deposition, re-epithelialization, the thickness of granulation tissue and wound contraction were significantly increased in the treatment group and all indices were normal in the sham group.
Conclusion: Results of the present study suggested that intramural injection of fibroblasts in the anastomosis site, could improve the histopathological indices of wound healing.
Keywords: Colon; Anastomosis; Fibroblast; Healing; Histopathology

PS-211. The Effect of Clay Nanoparticles on Surface Topography of Polymeric Scaffolds and Mesenchymal Stem Cell Attachment

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Background and Aim: The unique properties of nanomaterials in tissue engineering have captured a great deal of attention as experimental tools in biomedical applications. A plenty of researches has provided a strong evidence that nanostructures not only passively interact with stem cells but also actively engage and mediate these cells functions. To address this issue, we have decorated clay nanoparticles in the electrospun nanofiber scaffolds, characterized the composite material, and investigated their effect on the surface topography of nanofiber scaffolds and mesenchymal stem cells attachment.

Methods: First of all, we used the enzymatic process for isolation of mesenchymal stem cells from human adipose tissue which characterized by flow cytometry. Then, we fabricated electrospun fiber scaffolds and evaluated the fibers by scanning electron microscope (SEM). Fiber coverage by the clay nanoparticles and the scaffold topography was also evaluated by a transmitting electron microscope (TEM) and an atomic force microscope (AFM), respectively. To assess the cytotoxic effect of scaffold for mesenchymal stem cells, MTT assay was done. The attachment of mesenchymal stem cells within the scaffolds was shown by DAPI staining on day 7. For further evaluation of the mesenchymal stem cells' interaction with the fibrous scaffolds, the cell constructs were first fixed and then imaged using a scanning electron microscope (SEM).

Results: The flow cytometric analysis demonstrated that the isolated mesenchymal stem cells expressed CD90 and CD105 while they were negative for CD34 and CD45. The fabricated electrospun fiber scaffolds diameter was evaluated near 260 ± 70 nm. SEM images showed that nanoclay-fiber scaffolds were homogeneous. Also, TEM images of the fibers revealed the ideal internal morphology of constructs. AFM results provided evidence for modification of the surface of the fibers with nanoscale roughness providing topographic cues. We have shown that the scaffolds are not toxic for mesenchymal stem cells via MTT assay. Also, mesenchymal stem cells attachment were demonstrated by DAPI staining fluorescence microscopy images. In addition, SEM images confirmed mesenchymal stem cells' attachment to the nanofiber scaffold.

Conclusion: Nowadays, biomaterials with topographical properties are well known because they can influence cell–surface interactions. Also, nanoscale features at guiding cell behavior are of particular interests because of many interactions occurring at that scale, which are keys in cell survival process. In this study, we demonstrated the ideal topography of clay decorated scaffolds and its positive effects on mesenchymal stem cell attachment. Therefore, we envision that such cellular constructs may be useful in the future as implantable cellular devices for repairing damaged tissues.

Keywords: Clay Nanoparticles; Scaffold; Topography; Mesenchymal stem cell

PS-212. Pre-Transplantation Platelet Count and Hemoglobin Level Can Predict RBC and Platelet Transfusion Requirement in Allogeneic-Hematopoietic Stem Cell Transplantation

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Background and Aim: Allogeneic-hematopoietic stem cell transplantation (Allo-HSCT) is the selected treatment for patients with a variety of malignant and nonmalignant disorders. The conditioning regimen before HSCT can affect the hematopoietic system, therefore the patients received packed RBC and platelet until engraftment occurs. The aim of this study was to determine the relationship between hemoglobin (Hb) level and platelet count before transplantation as an index for predicting the requirement of RBC and platelet unit after allogeneic-HSCT

Methods: This retrospective study includes 196 patients with hematological malignancies who underwent allogeneic HSCT at bone marrow transplantation center of Taleghani hospital between 2008 to 2017. The Hb level and platelet count of the patients were quantified before allo-HSCT and the data of the RBC/platelet requirement of patients were obtained from patients' files. The correlation between the platelet count and Hb level with received packed RBC and platelet units after allo-HSCT were assessed using the Pearson correlation test.

Results: The results indicated a significant positive correlation between the pre-transplantation Hb level with RBC transfusion requirement after allo-HSCT (P= 0.02) but the pre-transplantation platelet count did not have a correlation with the transfused platelet units after allo-HSCT (P=0.07). According to our data patients with lower Hb levels before transplantation received RBC more often after HSCT than the patients with higher pre-transplantation Hb levels.

Conclusion: Regarding the results, It seems that the Hb level before allo-HSCT can be used as a predictive marker of RBC requirement in allogeneic hematopoietic stem cell transplantation so that the patients with low levels of Hb are more susceptible for more transfusion support after HSCT. The platelet count might be lesser important to be measured before transplantation than Hb.

Keywords: Allogeneic hematopoietic stem cell transplantation; RBC transfusion; Platelet Transfusion; Hemoglobin; Platelet count

PS-213. Pretransplant Hemoglobin Level and Platelet Count as Prognostic Markers of RBC and Platelet Transfusion Requirement in Autologous Hematopoietic Stem Cell Transplantation

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Background and Aim: Hematopoietic stem cell transplantation (HSCT) is an effective treatment for hematological and non-haematologic disorders. Conditioning regimen for HSCT can partially ablate the patient's hematopoietic system; therefore, the patients post-HSCT are dependent on transfusion support and receive multiple units of RBC and platelet until engraftment occurs. Transfusion requirement for patients receiving HSCT depends on various factors. The aim of this study was to determine the relationship between hemoglobin (Hb) level and platelet count pre-transplantation as an index for predicting the requirement of RBC and platelet unit after HSCT

Methods: We studied 270 patients with hematological malignancies who underwent autologous HSCT. We measured the Hb level and platelet count of these patients before HSCT and examined the relationship between these markers with RBC and platelet units that received after HSCT.

Results: The results showed a correlation between Hb level and Plt count before HSCT with RBC and random platelet transfusion after HSCT (P= 0.01 and 0.05, respectively). Also, the patients with sub-group analysis patients categorized according to the malignancy that there was no significant correlation between Hb levels with RBC units that transfused, but the platelet count in Hodgkin's lymphoma patients had the correlation with received Platelet units after HSCT (P=0.001). According to our data patients with lower Hb levels and plt count are more susceptible to more transfusion support.

Conclusion: These results suggest that pre-transplant Hb levels and platelet count may be useful markers for predicting RBC and Platelet unit requirements after HSCT. Knowledge of cut-off for these parameters can help with the better selection of patients, thus reducing the number of

transfusions in HSCT patients.
Keywords: Bone marrow transplantation; Hematopoietic stem cell transplantation; RBC transfusion; Platelet transfusion; Hemoglobin; Platelet count

PS-214. Effect of Mummy Substance on the Proliferation of Human Adipose-Derived Stem Cells in Acellular Scaffold

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Background and Aim: Regenerative medicine is focused on wound healing and the proliferation of different human tissue. Acellular scaffold extracted from the extracellular matrix has been used for constructive and regenerative medicine. Adipose tissue is commonly used as the source of adult stem cell. These cells enhance the vascularization capacity of scaffolds. Mummy is a pitch-like substance that found naturally in the altitudes. It used to wound healing in ancient medicine. The aim of this study was estimating the effect of the mummy on the proliferation of human ASCs (adipose-derived stem cells) in the acellular scaffold.
Methods: Decellularization of sheep jejunum was carried out using chemical reagents and various physical conditions in order to determine its morphology and the degree of decellularization, H&E staining was applied. Then, the scaffolds were treated with mummy substance and for characterized using FTIR, FE-SEM, and UTM. ADCs were seeded on the scaffold and the morphology and adhesion of the cells to the scaffolds were assessed through FE-SEM after 24 and 48 h incubation. Also, the efficiency of a mummy for inducing the proliferation of ADCs on the scaffolds was evaluated using the MTT assay.
Results: Findings revealed satisfactory decellularization of jejunum which could be due to its thin thickness. H&E sections clearly showed that decellularization had removed all cell nuclei, but some cell nuclei appeared to be present in demineralized sections. Mechanical properties and structural form were preserved in both mummy-loaded and unloaded decellularized matrices. FE-SEM showed that the mummy loaded and unloaded decellularized matrix possesses similar matrix morphology with a randomly oriented fibrillar structure and interconnecting pores. No toxicity was observed in all treatments, and viability, expansion, and cell proliferation were supported in both scaffolds. Importantly, the proliferation capacity of ASCs on Mummy-loaded decellularized scaffold significantly increased after 24 and 48 h incubation time compared to the unloaded scaffold ($P<0.001$). These results clearly indicate that mummy accelerates ASCs proliferation on the mummy-loaded matrices.
Conclusion: The results of this study suggested that mummy has potentials to accelerate proliferation ASCs on the acellular scaffolds. This shows that mummy substance in agreement with public beliefs and ancient medicine probably effective in stimulating re-epithelialization phases of wound healing by in vivo application.
Keywords: Tissue engineering; Mummy substance; Acellular scaffold; Adipose-derived stem cell; Cell proliferation

PS-215. Glioma Stem Cells-Derived Microvesicles Promote Tumor Growth Through Modification of Astrocytes

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Background and Aim: Glioma stem cells (GSCs) play an important role in glioblastoma prognosis. Several studies indicated the importance of glioma stem cells-derived microvesicles (GSCs-MVs) in the communication between the tumor and its surrounding microenvironment. In this study, we addressed whether glioma stem cells use microvesicles to modulate normal astrocytes to facilitate their invasion.
Methods: Microvesicles were isolated from glioma stem cells using differential ultracentrifugation and characterized by the use of Transmission Electron Microscopy and Dynamic Light Scattering. The microvesicles were co-incubated with normal astrocytes and then cell growth and expression of genes involved in extracellular matrix remodeling were evaluated.
Results: We found that co-incubation of normal astrocytes and GSCs-MVs upregulate the Expression of glial fibrillary acidic protein (GFAP), and activate normal astrocytes. Furthermore, our data showed that GSCs-MVs affects the expression of genes involved in tumor invasion and growth in normal astrocytes.
Conclusion: Cumulatively, our findings indicate that GSC-MVs can facilitate tumor growth and invasion by the modifying of the normal astrocytes' phenotype.
Keywords: Glioma stem cells (GSCs); Extracellular vesicles; Glial fibrillary acidic protein

PS-216. Fas-AS Long Noncoding RNA and Acute Myeloid Leukemia

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Background and Aim: Recently, long noncoding RNAs (lncRNAs) have been noticed as potential predictive biomarkers for prognosis of different human cancers. One example is the FAS-AS RNA 1 located in the 10q23.31 region which is transcribed from the opposite strand of the FAS gene. FAS has a major role in the regulation of apoptotic pathways and there is a reverse correlation between FAS-AS1 expression level and production of the soluble form of Fas.
Methods: So in this study, we assessed FAS-AS1 expression in blood samples of de novo AML patients and control subjects using qRT-PCR.
Results: The result of this study showed that there is no significant difference in FAS-AS1 lncRNA expression between AML patients and healthy individuals.
Conclusion: We conclude that FAS-AS1 is not an informative biomarker for AML diagnosis.
Keywords : Acute myeloid leukemia; Long non- coding RNA, FAS- AS1

PS-217. Use of Fat-Derived Stem Cells to Treat Emphysema

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Background and Aim: We use this patient-naive pulmonary stem cell (NIPAM), an injectable hydrogel, in this project. Emphysema means the destruction of the lung parenchyma, which results in the loss of lung elasticity and the loss of the lung's alveolar wall This reduces the amount of oxygen in the absorbing respiratory tract, all smokers develop to some degrees of emphysema. In this condition, the wall of the alveoli is gradually eliminated and the individual suffering from shortness of breath, the progression of the disease depends on the amount of smoking.
Methods: In this project, we introduce the cells from the culture medium into the colloid containing the nanogel precursor and additionally add some of the culture medium, which is called the encapsulation of pulmonary stem cells in the nanogel. Recent studies have been conducted to treat this disease by lung stem cells for treating the disease on mice while using this nanogel as a scaffold to increase cellular concentration and also prevent the development of an immune response. For treatment of cardiac MI, it has been used to encapsulate stem cells. A temperature-sensitive polymer was purified before being synthesized by crystallization in a hexane and dried at room temperature. Then, the polymer is synthesized continuously for 5 hours under the protection of a nitrogen atmosphere at 70°C and used for the formulation of nanogel.
Results: These nanogels have no adverse effect on the function and

growth of stem cells, and the muscles are not repulsed after injection and do not give rise to immune responses and if practiced, cause the lost lung parenchyma tissue to improve respiration.
Conclusion: In this study, we have a new idea of temperature-sensitive polymer to design an appropriate cell culture and cell-plate separation constructed by tissue engineering without the need for a trypsin enzyme, and also the porosities of the polymeric structure can cause oxygen to enter and prevent the immune response.
Keywords: Emphysema; Lung stem cells; Encapsulation

PS-218. Application of Mesenchymal Stem Cells in the Treatment of Diabetic Wound Healing

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Background and Aim: Wound healing, as a complex process, emerges upon any skin injuries/damages. The healing a wound in a short period of time span with trivial side effects is a challenging issue. Much attention has been given to attain "supernormal" wound healing modality. In this line, the mesenchymal stem cells (MSCs) derived from bone marrow tissue has been investigated. In this work, we aimed at studying the wound healing and stem cells impacts on managing full-thickness wounds in vivo.
Methods: This work was carried out using 54 adult male diabetic Wistar rats weighing 200-250 g and ages of 3-4 months. The rats were divided into the control and two experimental groups and on the back of the neck of animals, a square 1.5×1.5 wound was made. The control and experimental groups were subdivided into three subgroups corresponding to 4, 7, and 14 days of study. MSCs isolated from bone marrow were cultured. While the control group did not receive any treatment, the MSCs were used once on the wound in the first experimental group and the second experimental group was given 1% phenytoin cream on the wound. Samples taken from the wound and adjacent skin were examined using hematoxylin and eosin histological staining. Wound surface and wound healing were evaluated and data were analyzed using one-way ANOVA followed by Tukey test and a p value less than 0.05 was considered statistically significant.
Results: The microscopic analyses revealed that the histological parameters in wounds bed (i.e., the number of fibroblasts, blood vessels, neutrophils, and macrophages) in the experimental group were significantly different than the control group. The macroscopic and microscopic evaluations demonstrated that the percentage of wound healing in the control and the experimental group significantly varied ($P< 0.05$) on different days.
Conclusion: These results highlight the beneficial potential of MSCs in the wound healing process, which might be complemented by the effects of growth factors and ECM produced by the native placenta tissue cells. The use of MSCs in an open wound may quicken the healing process.
Keywords: Diabetic wound healing; Open skin wound; Rat; Mesenchymal stem cells

PS-219. Investigation of the Relationship between Growth Factors of SCF and FLT-3 in the Proliferation of Umbilical Cord Blood CD + 8 Stem Cells

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Background and Aim: Stem cells are a cell of high-division cells that are split and multiply throughout life. Growth factors of SCF and FLT-3 are natural molecules with protein or steroid structures that are involved in the growth and differentiation of many cells. The mutation in SCF and FLT-3 disrupts the proliferation of CD8 + stem cells. The purpose of this study was to examine the relationship between S and F growth factors in the proliferation of cord blood CD8 + stem cells
Methods: Statistical population: In this study, 38 umbilical cord blood samples from newborns from Motahari Hospital of Urmia were used. Real-Time PCR technique was used to extract Total RNA to isolate and extract CD8+ stem cells using flow cytometric method by MACS system. Stemline was used for culture. After extraction, SCF and FLT-3 factors were used for the proliferation of cells on days 5, 10 and 15. After replication of growth factors for Total RNA, a real-time PCR technique was used and the data were analyzed by SPSS V.24 software was analyzed
Results: The expression of FLT-3 growth factor expression in CD8+ stem cell proliferation on day 5 (during reproduction) ($P <0.001$) decreased the growth trend. The expression of SCF growth factor on day 5 ($P = 0.014\%$, 469 ± 281). The frequency of FLT-3 was 10 (0.041 ± 0.041) on day 10 and the frequency of SCF was 10 (019.0 ± 09.29, $P = 0.019$), which increased on day 5, the incidence of SCF growth factor Day 15 (017.4 ± 4.2) and FLT-3 growth factor (15%, $P <0.01$) were observed at day 15 ($P <0.01$).
Conclusion: Based on the results, growth factors of SCF and FLT-3 in cord blood CD8+ cell proliferation have a significant relationship with cord blood, which can be used as a substitute for non-growth.
Keywords: SCF -FLT-3 Stem Cell-Cord

PS-220. Development and Characterisation of Hemostatic Polymeric Hydrogel Membrane from Blends of Oppositely Charged Biopolymers as a Potential for Wound Healing

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Background and Aim: The principal goal of wound healing is to prompt recovery of damaged tissue. However, these materials predominantly undergo degradation under physiological environment. Therefore, the crosslinking formed between two oppositely charged polymers leads to the formation of a polymeric membrane stabilized polyelectrolyte complex. The aim of this study is to develop a novel oppositely charged biopolymers composed of chitosan (CS) and κ-carrageenan (KC) with adjustable hemostatic, mechanical, and biological properties for wound healing application.
Methods: Hydrogel membranes with various volume ratios of CS:KC (10:90, 20:80, and 30:70) were prepared using a gel casting technique. Primarily, separate CS and KC aqueous solutions with the concentration of 1.5 wt.% were prepared in water+10 v/v% acetic acid and water, respectively. After stirring at 60°C for 3 h, pH of the KC and CS solution 7.5 and 5.5, respectively. Then, two solutions were mixed at the various volume ratio of at room temperature for 2 h to get a homogenous solution. Finally, after degassing, the suspensions were transferred into Petri-dish and maintained for 72 h to be completely polymerized. To evaluate kinetic clotting property, the membranes (n=3) were placed at the small vials containing diluted blood and was kept in the incubator at 37°C. Tensile properties of the samples were determined by a Hounsfield H25KS tensile tester. The cytotoxicity of membranes was considered by MTT assay using human dermal fibroblasts.
Results: Result of blood clotting test revealed that the absorption value of the hemolyzed blood solution in contact with all samples reduced with increasing time. It is due to the fact that a negatively charged surface is known to activate factor XII and platelet factor 3 initiating a series of proteolytic reactions that lead to intrinsic blood coagulation. The main disadvantage of hydrogels in the wound healing process is improper mechanical stability and weak mechanical strength. The electrostatic force formed between two oppositely charged biopolymer lead to enhance mechanical strength 3-folded for CS:KC (20:80). Afterward, the strength decreased 1.7-folded due to the high amount of KC. Moreover, the

proliferation of human dermal fibroblast cells seeded on the membrane gradually enhanced from day 1 to day 7. This observation reflected the cell-compatibility of the membranes and confirmed nontoxicity of crosslinking treatment. After 7 days of culture, the cell viability of cells on CS:KC (20:80) membrane enhance 2.5 folded more than day 1.
Conclusion: The aim of this study was to prepare a novel oppositely charged membrane of chitosan and κ-carrageenan and study the effects of KC concentration on the hemostatic, mechanical and biological properties of the membranes. Results confirmed that the blending of CS:KC (20:80) with oppositely charged network significantly enhance the hemostatic a mechanical properties of the membranes. Moreover, it was found that CS:KC (20:80) membranes are nontoxic toward human fibroblast skin cells. Our results suggest that CS:KC membrane could be an ideal biomaterial for wound healing applications at the optimal concentration of 20:80.
Keywords: Oppositely charged; κ-carrageenan; Chitosan; Wound; Hemostatic

PS-221. The Correlation of Serum C-Reactive Protein Levels and Engraftment of Autologous Hematopoietic Stem Cell

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Background and Aim: Autologous hematopoietic stem cell transplantation (AHSCT) is a curative option for many hematological disorders. Early prediction of the engraftment is valuable since patients require extensive supportive care during this period to prevent opportunistic infections. C-reactive protein (CRP) is a blood acute-phase protein which rises in response to inflammation. In this study, we evaluated the association between CRP level and WBC engraftment (WE), platelet engraftment (PE) and length of hospital stay (LOHS) in AHSCT patients.
Methods: 2 mL peripheral blood samples of 208 multiple myeloma (MM) and lymphoma patients candidate for AHSCT were gathered on the admission day and the plasma CRP levels were quantified using enzyme immunoassay. The correlation between the CRP and WE, PE and LOHS were analyzed by Pearson correlation test and linear regression model.
Results: The significant positive correlations of CRP levels were found with WE, PE and LOHS without considering the disease (P-value: 0.001, 0.007, 0.001, respectively). Depending on the disease, only in Hodgkin's lymphoma patients (n=80), we observed a significant association between CRP and WE (P: 0.001).
Conclusion: Based on our data, we conclude that the plasma CRP levels on admission day correlate with some engraftment factors of AHSCT and could reflect the inflammation influencing the post-transplantation conditions. Regarding the first report of such correlation, it could give a valuable insight to clinicians about the outcome of AHSCT.
Keywords: C-Reactive protein; Autologous hematopoietic stem cell transplantation

PS-222. Chitosan-Baghdadite Nanocomposite Coating on 316L for Implant Materials

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Background and Aim: 316L Stainless Steel has been selected as a substrate with excellent corrosion resistivity, oxidation resistance, and high deflection strength. However, when implanted in the body, various ions such as nickel, chromium, and iron may release from it in tissue that causes sensitization and tissue stimulation. Implants need antibacterial properties, but 316L does not have these properties. To overcome this challenge, polymeric, ceramic or composite coatings have been developed.
Methods: Baghdadite has been considered as a bioactive bioceramic with excellent biocompatibility, good mechanical properties, and the ability to stimulate bone growth. In another word, chitosan is a biopolymer with antibacterial activity, excellent biocompatibility, and good biodegradability.
Results: Results showed that incorporation of Baghdadite significantly improve the biological properties of the composite coating and made them promising for implant materials.
Conclusion: The purpose of this study was to fabricate chitosan/Baghdadite nanocomposite coating on 316L stainless steel using electrophoretic technique and study the role of Baghdadite on the improvement of bioactivity and biocompatibility of implantation materials used in the body.
Keywords: Chitosan, Baghdadite, Stainless steel 316L

PS-223. Immortalization of Human Dental Pulp-Derived Stromal Cells Through hTERT Overexpression

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Background and Aim: Mesenchymal stromal cells (MSCs) can be easily harvested from a large variety of human tissues. Among MSCs, human dental pulp-derived stromal cells (hDPSCs) have high proliferative and regenerative capacity and can differentiate into various cell types including osteoblasts. hDPSCs are being evaluated for regenerative medicine; however, their limited culture lifespan may represent an obstacle for both pre-clinical research and therapeutic use. To overcome this problem, hDPSCs immortalization was performed in order to obtain cells with prolonged lifespan but still maintain their mesenchymal marker expression and ability to differentiate.
Methods: hDPSCs were obtained from Human and Animal Cell Bank of Iranian Biological Resource Center (IBRC). The cells were cultured in DMEM:F12 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin and maintained at 37°C under 5% CO2 atmosphere. To induce hDPSCs immortalization, human telomerase reverse transcriptase (hTERT) was used. The hTERT gene was digested from the pBABE-hygro-hTERT vector and subcloned into the Lentiviral vector pLenti-III to construct a pLenti-III-hTERT expression vector. The pLenti-III-hTERT vector was co-transfected into the 293LTV cells using transfection reagent, together with the other two plasmids, pMD2.G and psPAX2. After three days, the supernatant was collected, centrifuged and stored at -70°C. Lentiviral transduction of hDPSCs was conducted according to the manufacturer's protocol. Overexpression of hTERT in hTERT-hDPSCs was confirmed by Real-time PCR and western blot. For immunophenotyping, hDPSCs and hTERT-hDPSCs were analyzed for CD34, CD45, CD29, CD90, and CD105 markers. The osteogenic and adipogenic differentiation assay was performed as described previously.
Results: We found that hTERT failed to immortalize hDPSCs. Overexpression of hTERT in hTERT-hDPSCs was confirmed by Real-time PCR and western blot. hTERT-transformed hDPSCs largely resembled the parental cells with a minor reduction in plastic adherence. Both hDPSCs and hTERT-hDPSCs were cultured for up to 20 passages in culture. hDPSCs and hTERT-hDPSCs showed a population doublings (PDs) of 36 and 20, respectively. Comparative differentiation studies between hDPSCs and hTERT-hDPSCs showed that the in vitro osteogenic and adipogenic differentiation properties of hTERT-transformed hDPSCs reduced significantly compared to the parental cells. Interestingly, CD29, CD90, and CD105 marker expression in hTERT-hDPSCs largely decreased compared to hDPSCs.
Conclusion: Our results demonstrated that hTERT-transformation merely is not a practical strategy to immortal DPSCs. Upon immortalization, hTERT-transformed cells maintain their morphology while fail to fulfill

the minimal criteria for defining multipotent mesenchymal stromal cells as demonstrated by the International Society for Cellular Therapy.

Keywords: Dental pulp MSCs, Lentiviral transduction, hTERT, Immortalization

PS-224. CEA- DNA Vaccination Strategy against Colorectal Cancer

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Background and Aim: Carcinoembryonic antigen (CEA), a glycosylated protein of MW 180 kDa, is overexpressed in colorectal cancer. CEA as a target for anti-cancer specific immunotherapy. We have studied the effects of CEA as a DNA-vaccine Candidate in mice bearing colorectal cancer.

Methods: The CEA cloned into the pCDNA vector. Cloning of CEA was confirmed by enzymatic digestion and sequencing. The CEA was expressed and confirmed by anti-CEA western analysis. To evaluate the ability of the CEA, spleen lymphocytes of immunized mice were analyzed with BrdU proliferation kit, Cytokine assay, and Elispot assay. Also, we explored the protective potency of CEA as a vaccine Candidate in mice bearing colorectal cancer.

Results: The finding demonstrated that injection of CEA construct could elicit strong T-helper 1 (Th1) immunity against the antigen, as indicated by the specific high-level production of IFN-γ and IL-2 using spleen lymphocytes and lymphoproliferative responses.

Conclusion: Animals with CEA expressing tumors showed diminished tumor growth and prolonged survival when immunized with CEA-plasmid DNA compared to controls.

Keywords: Carcinoembryonic antigen; Colorectal Cancer; T-helper 1; Vaccine

PS-225. Chondroitinase ABC and Chondroitin 4-Sulfate as Potent Inducers for Neural Differentiation of Human-Induced Pluripotent Stem Cells

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Background and Aim: A lot of studies focused on the human induced pluripotent stem cells (hiPSCs) for treating patients who suffered from spinal cord damage. Chondroitinase is a bacterial enzyme, which allows new nerve fibers to grow across the damaged nerve. In this study, neural differentiation of human induced pluripotent cells (hiPSCs) was investigated qualitatively in the presence of chondroitinase ABC and chondroitin 4-sulfate as its substrate.

Methods: The cytotoxicity of chondroitin 4-sulfate was evaluated by Acridine Orange (AO) staining and MTT assay at 10, 50, 100, 200 and 500 µg/mL concentrations. hiPSCs derived embryoid bodies were plated on gelatin-coated dishes and were treated with an induction medium including forskolin and IBMX. Neural differentiation of hiPSCs was done in the presence of chondroitinase ABC and chondroitin 4-sulfate on days 7 and 14. To confirm the neural differentiation, Von Kossa staining and immunocytochemistry (ICC) analysis for MAP-2 protein as the neuronal marker were performed.

Results: About 100 µg/mL was selected for neural differentiation of hiPSCs based on MTT assay and AO staining results. The ICC staining of hiPSCs showed that the expression of MAP-2 in differentiated hiPSCs in supplemented media containing forskolin, IBMX, chondroitinase ABC, and chondroitin 4-sulfate was significantly higher than induction media without enzyme and substrate. The results of von Kossa staining were more intense in wells containing enzyme and substrate, compared to other wells. In addition, neural differentiation of hiPSCs on day 14 was higher than day 7 for ICC analysis and von Kossa staining.

Conclusion: In conclusion, our results indicated high efficiency for differentiation of hiPSCs into neural lineages in the presence of chondroitinase and chondroitin 4-sulfate on day 14 after induction.

Keywords: Chondroitinase ABC; Chondroitin 4-sulfate; Neural differentiation; Human-induced Pluripotent stem cells

PS-226. Human Adipose-Derived Mesenchymal Stem Cells Attenuated Rat Model of Experimental Ulcerative Colitis

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Background and Aim: Nowadays, cell therapy has opened a new window for treating inflammatory bowel disease (IBD). Adipose-derived stem cells (ASCs) have shown significant anti-inflammatory, therapeutic and trophic potential. In this study, we have designed an experiment to assess combination therapy of ASCs and sulfasalazine (as a reference drug for IBD treatment) on experimental colitis model.

Methods: Acute colitis in rat induced by intracolonic administration of 50 mg/kg trinitrobenzene sulfonic acid (TNBS). Rats were divided into five groups: control group, TNBS group, sulfasalazine group, ASC group, ASC + sulfasalazine group. Groups with ASC received a single intraperitoneal injection of ASCs 3 hours after colitis induction and groups with Sulfasalazine received a daily dose of sulfasalazine (30 mg/kg). Body weight, disease activity index (DAI), colon length, colon weight to length ratio were measured.

Results: Coadministration of ASC + sulfasalazine significantly suppressed TNBS-induced body weight loss, colon length shortening and decreased the colon weight to length ratio.

Conclusion: The results of this study suggested that ASCs as a new promising therapeutic option for inflammatory diseases are able to show potent anti-inflammatory effects on colitis when co-administered with a low dose of sulfasalazine.

Keywords: Cell therapy; Inflammatory bowel disease; Mesenchymal stem cells; Combination therapy

PS-227. In Silico Evaluation of miR-124 and its Targets in Metastatic Tumor Samples of Lung Cancer

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Background and Aim: Lung cancer is one of the malignancies with a high mortality rate. Despite enormous developments in lung cancer treatment, metastasis still prevents a successful treatment. MicroRNAs (miRNA) are Non-coding RNAs with 18-22nt length, dysregulation of which has been shown to play a pivotal role in many pathological processes such as cancer. Recent studies indicate the outstanding role of miRNAs in the metastasis of cancer. In this study, we aim to predict the role of miR-124 which functions as a tumor suppressor and its target genes in metastasis of lung cancer.

Methods: The changes in expression levels of genes (GSE5364) in metastatic lung tumor samples versus the normal adjacent samples were analyzed using GEO2R algorithms. On the other hand, targeting genes of miR-124 were found using miRTarbase, Then the mutual genes between the two groups were found via R programming-(R project).

Results: Upregulation of some of the target genes of miR-124, for instance, two transcription factors such as KLF4 and FOXA2 observed in metastatic tumor samples in comparison to normal ones. KLF4 involves in the

differentiation of epithelial cells and contributes to the down-regulation of p53/TP53 transcription, and FOXA2 is a known transcription factor for a number of oncogenes in cancer. According to our findings, it could be interesting to follow this relationship experimentally.
Conclusion: FOXA2 and KLF4 as examples of miR-124 targets might act as oncogenic transcription factors which are frequently upregulated in cancers. Moreover, they are related with FoxO signaling pathway which has an important role in metastasis of lung cancer.
Keywords: miR-124, Lung cancer, Metastasis

PS-228. The Nanostructured PLLA Scaffold and Conditional Medium Enhance Proliferation of Human Mesenchymal Stem Cell

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Background and Aim: Human Mesenchymal stem cells (hMSCs) are of great interest due to their high potency to differentiate. The behavior of these cells depends on the paracrine and biophysical signals received from the 3D microenvironment. The simultaneous use of suitable nanofibrous scaffolds and conditional media can simulate the microenvironment needed by stem cells. The aim of this study is to fabricate and evaluate the effect of the Tissue-engineered scaffold of Poly (L-lactic acid) (PLLA) and conditional medium on mesenchymal stem cell culture.
Methods: Poly (L-lactic acid) (PLLA) nanofibrous porous scaffolds were prepared using by electrospinning method. The structure of the scaffolds was investigated using scanning electron microscopy. The identity of bone marrow-derived hMSCs was confirmed by osteogenic and chondrogenic differentiation and flow cytometric analysis and then cultured in the PLLA scaffold in the presence of MSCs conditional medium. Cellular adhesion of hMSCs was assessed using by SEM. The viability and proliferation of the cells were then determined by an MTT assay.
Results: SEM data confirmed the porosity of scaffold with suitable pore size. Histological and flow cytometric analysis confirmed bone marrow-derived hMSCs. The MTT assays revealed the biocompatibility of PLLA nano scaffold under conditional medium.
Conclusion: The 3D architecture of PLLA nanofibrous scaffolds can support the viability and proliferation of hMSCs under conditioned medium.
Keywords: Scaffold, Mesenchymal stem cells; Conditional medium; Poly-L-lactic acid

PS-229. Surface Topography-Dependent Calcium-Phosphate Coating of 3D-Printed PCL Scaffolds: Enhanced Osteogenic by Pre-osteoblasts

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Background and Aim: Poly(ε-caprolactone) (PCL; FDA approved) scaffold is often used in bone tissue engineering because of its high stiffness, long degradation time and degradation products removable by natural pathways. Since PCL is not bioactive, surface coating of PCL scaffold with calcium phosphate (CaP) is commonly used to render the surface bioactive. We aimed to test the effect of surface topography on biomimetic CaP coating on 3D-printed PCL scaffolds and osteogenic potential of CaP-coated PCL scaffolds.
Methods: PCL scaffolds were fabricated using an extrusion-based 3D-bioprinter. Scaffolds were etched by 3 M NaOH for 24 or 72 h to create topography on the surface. Biomimetic CaP coating was carried out through alternate dipping of etched PCL scaffolds in 200 mM CaCl2 and K2HPO4.3H2O aqueous solutions. Scanning electron microscopy (SEM) was used to study the surface topography. The elemental composition of the surface was studied by energy dispersive spectroscopy (EDS). MC3T3-E1 pre-osteoblasts were cultured up to 14 days on PCL scaffolds with or without CaP-coating. Alkaline phosphatase (ALP) activity was measured and normalized to protein content after 14 days of culture using p-nitrophenol liberated enzymatically from p-nitrophenyl phosphate.
Results: SEM showed that NaOH etching changed the scaffold surface topography from smooth to a honeycomb-like pattern with small (24 h NaOH-etched) and large (72 h NaOH-etched) pores. EDS showed that after CaP coating, percent Ca and P atoms on the surface of 24 h NaOH-etched scaffold was 2.5-fold higher than on 72 h-etched scaffolds. Cells showed increased ALP activity by 3.4-fold on 24 h NaOH-etched CaP-coated scaffold and 1.5-fold on 72 h NaOH-etched CaP-coated scaffold compared to the scaffold without CaP coating.
Conclusion: Our results suggest that a honeycomb-like surface topography enhances biomimetic CaP coating on 3D-printed PCL scaffolds and therefore, enhances osteogenic activity of pre-osteoblasts which might have implications for bone tissue engineering.
Keywords: Poly(ε-caprolactone), Surface topography, Calcium-phosphate coating, Osteogenic activity

PS-230. Human Unrestricted Somatic Stem Cells (USSCs) Attenuate Systemic Inflammation in the Mouse Model of ALI

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Background and Aim: Sepsis is the oldest life-threatening challenge in medicine with difficult condition to diagnose and treat. The third International Consensus Sepsis is defined as unregulated host reaction to infection, characterized by systemic inflammation. Regarding the complicated pathophysiology, the management of sepsis is a very considerable procedure in the hospital's intensive care unit (ICU). Cell therapy is a promising option that shows significant outcomes in inflammatory diseases. In the present study, we investigated the therapeutic potential of Human unrestricted somatic stem cells (USSCs) isolated from the umbilical cord in the mouse model of ALI.
Methods: ALI model was established via injection of LPS. Mice were randomized into three groups: (1) LPS group, (2) LPS+ PBS group, and (3) LPS+ USSC group. The mice were sacrificed at 24 hours after injection. Serum was collected for cytokine concentration measurements, lung and liver harvested for evaluation of injury, and histology.
Results: USSCs improved survival rate and suppressed concentrations

of pro-inflammatory mediator's TNF-α, and IL-6 and the level of anti-inflammatory cytokine IL-10. The cells showed significant improvement in lung and liver injury, edema and hepatic enzymes.
Conclusion: USSCs significantly enhanced the survival rate of mice suffering from ALI and considerably reduced systemic inflammation. USSCs can be the appropriate therapeutic approach in the management of sepsis throw the anti-inflammatory effects.
Keywords: Acute lung injury; Umbilical cord; Unrestricted Somatic Stem Cells; Inflammation; Cell therapy

PS-231. Efficacy and Safety of PRP Therapy for Patients with Knee Osteoarthritis

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Background and Aim: Platelet-rich plasma (PRP) has been frequently used to regenerate tissue damages in different organs such as skin, mucosal membranes, and joints. In this study, we sought to investigate the regenerative capacity of PRP to treat cartilage lesions of patients with knee osteoarthritis (OA).
Methods: In this phase 1 clinical trial, 14 patients with mild to moderate knee OA referred to the Rheumatology Clinic in Imam Reza Hospital were enrolled. All patients received a single intra-articular injection of 2 mL of autologous PRP. All subjects were visited serially 1, 3 and 6 months after treatment and were evaluated for the amount of pain (assessed with visual analog scale), joint stiffness, the range of motion and crepitus.
Results: The PRP treatment was found to be safe and no adverse events occurred. Significant and almost linear improvements in stiffness and range of motion were observed. In addition, pain and crepitus decreased significantly during the 6-month follow-up. Multivariate analyses showed no significant impact of age, gender, and BMI on the level of clinical response.
Conclusion: The intra-articular injection of PRP showed clinical improvement in patients with mild to moderate knee OA and produced no adverse events. This provides the evidence for efficacy and safety of PRP as an alternative regenerative treatment for knee OA.
Keywords: Intra-articular injections; Knee; Osteoarthritis; Platelet-rich plasma

PS-232. The Evaluation of Myelination and the Expression of Neurotrophic Factors in Human Adipose-Derived Stem Cells Induced into Schwann- Like Cells at the Presence of Laminin

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Background and Aim: Cell-based therapy for repairment of nerve injury is one of the important strategies in nerve regeneration. Schwann cells (SCs)play a critical role in nerve regeneration, furthermore, SCs secretes neurotrophic factors (NTFs), a main group of polypeptides contributing to the maintenance of neuronal cells and promoting development. However, there is a limitation in obtaining SCs to use. Human Adipose-Derived Stem Cells(hADSCs) are multipotent stem cells and are suitable to use. Laminin is an essential protein in ECM synthesize by SCs. Also. The objective of this study was to induced hADSCs into SC-like cells and assess the effect of laminin-coated culture on the efficiency of SC-like cells differentiation, the myelination potential and the expression of neurotrophic factors.
Methods: The human ADSCs were isolated from the adipose tissue, these cells were plated in pre-induction medium, after neurospheres formation, these cells were re-plated with complete differentiation medium in the presence and absence of laminin-coated flasks. Then, Schwann cell markers S100β, GFAP and myelin basic proteins (MBP) of SC-like cells were assessed through immunocytochemistry. The quantify genes expression for Schwann cell markers, MBP and neurotrophic factors like brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial cell-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) was analyzed by the real-time reverse transcription polymerase chain reaction (RT-PCR) technique.
Results: The results of this study indicated that the phenotype of the differentiated ADSCs was converted to bi-polar or spindle shape in the absence and presence of laminin. Immunocytochemistry analysis revealed that the mean percentage of co-markers GFAP/S100β and S100β/MBP positive cells can increase the differentiated rate and myelinogenic potential of SC-like cells, coating surface by laminin as compared to control group. The genes expression levels of SCs markers, MBP and neurotrophic factors in SC-like cells in two condition was performed by RT-PCR. The findings data demonstrated that gene expression of SCs markers and MBP, in addition, neurotrophic factors BDNF and GDNF increase significantly in the presence of laminin compared to a plastic surface ($P < 0.01$). In versus, the expression of NGF was downregulated significantly on laminin-coated plates as compared to that of the absence of laminin ($P < 0.05$). Also, the expression of CNTF was upregulated but not significant.
Conclusion: The findings of this present study suggest that the expression of myelination-related gene and neurotrophic factors genes becomes upregulated in the presence of laminin. Therefore, laminin may provide a more appropriate microenvironment for peripheral nerve regeneration and neurodegenerative diseases.
Keywords: Adipose-derived stem cells; Laminin; Myelin; Neurotrophic factors

PS-233. Design, Construction and Functional Evaluation of a Polycistronic Modified mRNA with the Aim of Human iPS Cell Induction

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Background and Aim: During the last decade, induced pluripotent stem cells (iPSCs) technology had an enormous impact on the progress of stem cell biology and personalized regenerative medicine. Several methodologies are accomplished to generate iPSCs. Among them, synthetically-modified mRNAs (mmRNA) have shown great potential to derive safe and highly efficient integration-free iPSCs. However, low stability of mRNAs has raised the necessity of daily transfection, which is time-consuming. To overcome this limitation, in the current study we have designed and constructed a polycistronic mmRNA containing WPRE element that improves the stability of the produced mRNA.
Methods: Due to the stoichiometric expression of the reprogramming factors we developed a 2A-mediated polycistronic plasmid containing a single expression cassette with several open reading frames (ORFs) of human pluripotency transcription factors (LIN28, NANOG, SOX2 and OCT4) along with an EGFP reporter gene and WPRE element. We cloned the polycistronic DNA fragment in an appropriate vector containing a T7 promoter, untranslated regions and poly (A) tail used as a template DNA for the transcription. During in vitro transcription (IVT), mmRNAs are synthesized using T7 RNA polymerase, modified nucleotides and cap analogs. Phosphatase treatment was then carried out to reduce the immune responses. To evaluate the length, integrity and quantity of the transcript, an aliquot of transcription reaction was run on a denaturing

agarose gel. Finally, synthesized polycistronic mmRNAs were transfected into HEK293T cells and EGFP expression was monitored at defined time points by fluorescent microscopy and flow cytometry in order to evaluate the functionality and stability of the mmRNAs.

Results: Primary polycistronic plasmid consisted of four human reprogramming factors and EGFP coding sequences have been constructed in Royan Institute, Previously. In this study, we successfully inserted the WPRE element into this vector. Correct orientations of cloned fragment in the vector were confirmed by PCR and sequencing analysis. Subsequently, the total ORF subcloned into another plasmid designed for IVT reaction, and its accuracy was confirmed by restriction digestion. After transcription reaction, the efficient production of mRNA transcript with expected length was confirmed by agarose gel electrophoresis. After transfection of mRNAs into HEK293T Cells, the green fluorescent signal was assessed by fluorescent microscopy and flow cytometry. The results demonstrated that synthesized polycistronic mmRNA were efficiently expressed in HEK293T cells and also the presence of WPRE in the downstream of the transgenes resulted in significantly enhanced mmRNA stability, which in turn led to a higher level of protein expression.

Conclusion: In conclusion, we have developed a stabilized polycistronic mmRNA with potential application for the generation of human iPS cells. The application of the 5′cap, modified nucleotides and 3′poly-A tail in the structure of synthesized mRNA enhanced its stability and translation efficiency. On the other hand, phosphatase treatment, which was performed after in vitro transcription removed the 5′triphosphates at the end of the uncapped mRNAs to reduce the cellular immune responses, and applied WPRE element in the expression cassette increased the mmRNA stability as well as the yield of protein expression.

Keywords: iPSCs; Modified mRNA; Polycistronic; 2A Peptide; Woodchuck Hepatitis Virus Posttranscriptional Regulatory (WPRE) element

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