An Update to Space Biomedical Research: Tissue Engineering in Microgravity Bioreactors

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ABSTRACT

Introduction: The severe need for constructing replacement tissues in organ transplantation has necessitated the development of tissue engineering approaches and bioreactors that can bring these approaches to reality. The inherent limitations of conventional bioreactors in generating realistic tissue constructs led to the devise of the microgravity tissue engineering that uses Rotating Wall Vessel (RWV) bioreactors initially developed by NASA. Methods: In this review article, we intend to highlight some major advances and accomplishments in the rapidly-growing field of tissue engineering that could not be achieved without using microgravity. Results: Research is now focused on assembly of 3 dimensional (3D) tissue fragments from various cell types in human body such as chondrocytes, osteoblasts, embryonic and mesenchymal stem cells, hepatocytes and pancreas islet cells. Hepatocytes cultured under microgravity are now being used in extracorpororeal bioartificial liver devices. Tissue constructs can be used not only in organ replacement therapy, but also in pharmaco-toxicology and food safety assessment. 3D models of various cancers may be used in studying cancer development and biology or in high-throughput screening of anticancer drug candidates. Finally, 3D heterogeneous assemblies from cancer/immune cells provide models for immunotherapy of cancer. Conclusion: Tissue engineering in (simulated) microgravity has been one of the stunning impacts of space research on biomedical sciences and their applications on earth.

Introduction

Vacanti et al introduced tissue engineering based on synthetic biodegradable polymer scaffolds in 1988 for potential replacement of missing or defective cartilage. Tissue engineering/regenerative medicine has the ultimate goal of generating functional 3D constructs which can be utilized as replacement organs with normal function, or serve for in vitro study of drug toxicity, safety and efficacy (Unsworth and Lelkes 1998, Langer 1997). So far, three principal approaches have been followed in tissue engineering: I; direct implantation of freshly isolated or cultured cells, II; in situ tissue regeneration, and III; assembly of cells and scaffolds in vitro (Korossis et al 2005). Novel model tissue engineering systems have two features: I; a biodegradable scaffold that determines the final shape and dimension of the constructs, and II; the culture environment that provides essential nutrients and appropriate mixing which will ensure a uniform cell seeding and proliferation (Freed and Vunjak-Novakovic 1997b).

Homotypic or heterotypic 3D multicellular spheroids provide a more natural cellular differentiation than 2D monolayer cultures and show improved mimicry of the behavior and function of actual tissues (Hoffman 1993). When spheroids are cultured in conventional Petri-dishes or bioreactors, the restricted nutrient and oxygen diffusion into the spheroids results in a hypoxic, necrotic center in constructs larger than 1 mm in size (Sutherland et al 1986) which limits the functional properties of the constructs. Microgravity has advanced the field of tissue engineering by facilitating diffusion of nutrients and oxygen into these spheroids and thus creating constructs devoid of necrotic centers (Unsworth and Lelkes 1998). Under microgravity conditions, aggregation of cells is also enhanced by induction of differentiative cellular signaling. These issues have led to achieving constructs larger than those engineered in conventional bioreactors or 2D cultures (Unsworth and Lelkes 1998). In this review article, we will discuss the development of microgravity bioreactors along with cartilage and bone tissue engineering under microgravity. Advances in pancreas and liver tissue engineering, their potential applications in treatment of diabetes and acute liver failure and the important role of tissue engineering in cancer research and pharmaco-toxicology are also discussed.

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Microgravity bioreactors for tissue engineering

Bioreactors are biomechanically active simulation systems that use mechanical means to influence biological processes. Bioreactors can contribute to in vitro formation of tissues by providing and tightly controlling the biochemical and physical regulatory signals to cells. The mechanical stimulation by bioreactor can encourage cells to differentiate (Altman et al. 2002) and produce extracellular matrix more quickly (Carver and Heath 1999). Bioreactors provide tissue cultures by required nutrients and gases and facilitate nutrient transport to and waste transport from the tissue. Bioreactors and especially microgravity bioreactors can maintain a spatially-uniform cell distribution throughout the tissue engineering scaffold (Partap et al 2010, Goldstein et al 2001, Yu et al 2004).

Space research contributed to the expanding field of tissue engineering by combining cell culture and microgravity. Years ago, it was recognized that microgravity might benefit tissue engineering by promoting cell-cell association while minimizing turbulence and shear stress. Indeed, cells in suspension tend to aggregate when exposed to microgravity (Hymer et al 1996, Dintenfass 1986). Microgravity promotes co-location of cells and initiation of differentiative cellular signaling via induction of specialized cell adhesion molecules and extracellular matrix proteins. These in turn, may lead to establishment of 3D tissue constructs (Freed and Vunjak-Novakovic 1995). Microgravity has also been shown to potentiate stem cell proliferation while sustaining their capability for differentiation (Yuge et al 2006), which can be of utmost importance in tissue engineering.

First examples of microgravity devices fabricated by researchers at NASA were Slow-Turning Lateral Vessel (STLV) and the High Aspect Ratio Vessel (HARV) (EL-Haj AJ and Cartmell 2010, Martin and Vermette 2005). RWV bioreactor was introduced for studying tissue generation and cell behavior under microgravity (Schwarz et al 1992). In a RWV bioreactor, two concentric cylinders exist: an inner cylinder from silicone rubber that is stationary and is meant for gas exchange and the outer cylinder capable of rotating at a constant angular speed. Rotation of the vessel provides an upward hydrodynamic drag force against the downward gravitational force. When the gravitational forces are balanced with centrifugal forces, a microgravity-like culture condition is created within the cylinders in the annular space (Partap et al 2010). With gradual increase in the size of the tissues in bioreactor, the rotation speed must increase to balance the gravitational force and maintain the scaffold in suspension (Partap et al 2010, Kwong et al 2008). Media can be exchanged through a fluid pump. RWV bioreactor is now commercially available from Synthecon in USA (Houston, Texas) and from Cellon in Europe (Luxembourg). Fig. 1 shows a schematic representation of RWV bioreactor, which is often used in tissue engineering under, simulated microgravity.

The fluid dynamics of RWV bioreactors allows for diffusion of oxygen and nutrients to the cell aggregates and results in tissue constructs devoid of necrotic cores (Hammond and Hammond 2001, Unsworth and Lelkes 2000). Shear stress can be harmful to the engineered tissue constructs. Recently, the safe range of microcarrier radius or tissue size to avoid shear stress in RWV bioreactors has been determined (Farrag 2009). In another attempt, appropriate operating parameters for a RWV bioreactor such as oxygen transport and consumption and optimal rotation speed were determined (Kwon et al 2008). In a numerical simulation, different parameters involved in a successful simulation of microgravity such as fluid shear, mass transport, collisions between microcarriers and between the microcarrier and walls of the cylinder, and the use of adequate and appropriate controls have been discussed (Ayyaswamy and Mukundakrishnan 2007). One of the limitations of RWV bioreactors is that when tissue engineering scaffolds with more density than the culture environment is used, cell aggregates fall to the bottom of the cylinder.

As first attempts of tissue engineering in microgravity, RWV bioreactors were used for formation of cartilaginous constructs composed of round cells, collagen and glycosaminoglycan, and cardiac tissue constructs contracting spontaneously and synchronously (Freed and Vunjak-Novakovic 1997b). In this study, constructs grown in microgravity had the highest fractions of regenerated tissue and glycosaminoglycan content (the component required for cartilage to endure compressive forces) compared to constructs grown in rotating bioreactors, turbulent mixers and Petri-dishes (Freed and Vunjak-Novakovic 1997b). However, 3D aggregates did not form in every case, e.g. the insect ovary cell line SF-9 did not aggregate in RWV bioreactor (Francis et al 1997). A good list of early examples of cells and tissues cultivated in (simulated) microgravity has been presented by Unsworth et al (1998).
Cartilage tissue engineering in microgravity

Chondrocyte aggregates have been generated on beads (Duke et al. 1996), meshes (Hu and Athanasiou 2005) and novel porous biopolymers such as chitosan (Nettles et al. 2002) in RWV bioreactors. Ohyabu et al. reported the rapid regeneration of 3D large cartilaginous tissue from rabbit bone marrow cells (without a scaffold) using a RWV bioreactor (Ohyabu et al. 2006). The same group succeeded to control the cartilage tissue shape from rabbit bone marrow cells by RWV bioreactor using a collagen sponge scaffold which enhanced the glycosaminoglycan content of the generated tissues and strengthened the compression strength of the product (Ohyabu et al. 2009). The cartilaginous tissue aggregates formed without scaffold from bone marrow-derived cells using the RWV bioreactor were placed in critical osteochondral defects in rabbit femur and rapid regeneration of defects were reported (Yoshioka et al. 2007). In an attempt to engineer rat articular cartilage articular chondrocytes were cultured on 3D macroporous poly(DL-lactic-co-glycolic acid) (PLGA) sponges under microgravity with chondrogenic medium (containing TGF-β1) which led to redifferentiation of rat chondrocytes and formation of hyaline-like rat cartilage (Emin et al. 2008). In RWV bioreactor, a hyaline cartilage tissue, which possessed favorable morphological properties, was engineered from human bone marrow-derived cells (Sakai et al. 2009).

However, cartilage constructs flown in space are mechanically inferior to constructs grown on earth while those built in RWV bioreactors are quite similar in both composition and mechanical strength to natural cartilage (Freed et al. 1997a, Freed et al. 1998). This inferior quality of space-flown cartilage is in line with the widely-known adverse effect of space on bone, cartilage and even muscles (Stamenkovic et al. 2010, Rucci et al. 2007, Nabavi et al. 2011).

Bone tissue engineering in microgravity

RWV bioreactors have been used to generate microcarrier-based (Granet et al. 1998, Bothchewy et al. 2001) and porous scaffold-based (Turhan et al. 2005, Song et al. 2006, Song et al. 2008, Kyriakidou et al. 2008) osteoblastic cell culture. HARV bioreactor has been used for bone tissue engineering using poly(lactic acid glycolic acid)/nano-hydroxyapatite composite microsphere-based scaffolds (Lv et al. 2008). 3D osteoblast cell cultures on bioceramic microspheres and degradable composite microspheres were obtained in RWV bioreactor (Qiu et al. 1999, Qiu et al. 2001). Bone tissue engineering has also proved promising with mesenchymal stem cells grown on mineralized PLGA scaffolds (Koc et al. 2008). Undifferentiated embryonic stem cells were encapsulated within alginate hydrogels and cultured in a rotary cell culture microgravity bioreactor. The generated constructs displayed the morphological, phenotypic, mechanical and molecular properties of the osteogenic lineage (Hwang et al. 2009). Bone constructs engineered by culturing bone marrow mesenchymal stem cells on ceramic bovine bone scaffolds in static flasks and in rotating vessels were transplanted into Sprague-Dawley rat cranial bone defects. The engineered bone constructs under dynamic culture were found to repair the defects better than static counterparts after 24 weeks of in vivo implantation (Jin et al. 2010). 3D environments such as Rotary Cell Culture System (RCCS), enhances osteoblast cell aggregation and mineralization (Facer et al. 2005). Osseous-like tissues were also engineered in small volumes from preosteoblasts cultured in RWV bioreactors (Schneider et al. 2011). Hydrodynamic microgravity can thus modulate the composition, morphology, and function of the engineered bone (Song et al. 2006).

Improved mass transfer in the microgravity bioreactor and appropriate scaffold material have been suggested as decisive factors in bone tissue engineering (Araujo et al. 2010). Shear stress is also known to have a role in osteoblastic differentiation, mineralization and calcium deposition of stem cells and has been reviewed comprehensively along with bioreactors used in bone tissue engineering by Yeatte and Fisher (2011).

Liver tissue engineering in microgravity

3D assemblies of human liver cells (up to 3 cm long) were achieved in simulated microgravity. Bile duct-like structures, cohesive hepatocytes, complex stromal structures, reticular fibers, bile canaliculi, and tight cellular junctions were identified in the 3D assemblies by electron microscopy (Khaoystov et al. 1999). Later, simulated microgravity environment was shown to maintain key metabolic functions and promote aggregation of primary porcine hepatocytes (which are difficult to maintain in normal culture) (Dabos et al. 2001). Low-shear modeled microgravity has also been shown to maintain morphology and differentiated functionality of primary porcine hepatocyte cultures which is hard to achieve in normal culture (Nelson et al. 2010). Rat hepatocytes cultured initially as spheroids on culture plates and then transferred into HARV, retain cellular and physiological properties of the intact liver, including drug-metabolizing enzyme activities, plasma protein production, and long-term viability (Brown et al. 2003).

Entrapment of hepatocyte spheroids in a hollow fiber bioreactor was hypothesized as a potential bioartificial liver (BAL) in 1995 (Wu et al. 1995). In an attempt to design an extracorporeal BAL device (Innsbruck Bioartificial Liver or IBAL), Hochleitner et al. designed a bioreactor containing aggregates of porcine hepatocytes.
grown under simulated microgravity. Cell culture was possible for at least 10 days in the device (Hochleitner et al. 2005). IBAL was then tested in pigs through induction of fulminant hepatic failure. The survival of pigs was significantly prolonged by about 150% with IBAL treatment as compared to controls (Hochleitner et al. 2006). Small human hepatocytes in rotary culture were then utilized to construct a prototype BAL support system, in which cells demonstrated high viability (90-95%), and thus proved promising in establishment of a fully autonomous BAL as a bridge to transplantation (Wurm et al. 2009). Very recently, a simple dummy liver assist device was shown to prolong anhepatic survival in a porcine model of total hepatectomy (Thiel et al. 2011).

Microgravity tissue engineering and diabetes

Xenogeneic islets have been considered for transplantation in patients with insulin-dependent diabetes mellitus (Thompson and Mandel 1990). Allogeneic islet transplants have been successfully used in diabetic recipients, but chronic immunosuppressive agents are needed to prevent the rejection of transplanted cells (Ryan et al. 2002, Shapiro and Lakey 2000a, Shapiro et al. 2000b). Microgravity not only enhances the survival or proliferation of beta islet cells (Song et al. 2004a, Song et al. 2004b), but also reduces their immunogenicity by depleting dendritic cells which express the class II MHC (Rutzky et al. 2002). Besides, islets have a better morphological, insulin normalizing and secretory profile under microgravity (Hou et al. 2009).

Pancreatic islets from neonatal pigs, and Sertoli cells from prepubertal rats co-cultured in simulated microgravity, have been shown to form insulin-secreting, Sertoli-enriched tissue constructs (Cameron et al. 2001a), which have been suggested for long-term transplantation treatment of diabetes (Cameron et al. 2001b). Han et al transplanted islets and Sertoli cell aggregates co-cultured under microgravity to streptozotocin (STZ)-induced diabetic rats (Han et al. 2009). STZ is used to induce diabetes in rats (Ghaffari et al. 2012). During the in vivo studies, the animals remained euglycemic and the Sertoli-islets cell aggregates did not elicit allogeneic transplantation rejection, reducing the need for immunosuppressive agents (Han et al. 2009).

Microgravity tissue engineering for generation of model tissues

3D tissue models mimic specific tissue-like structures and functions better than two-dimensional (2D) cultures. 2D cultures are easy to set up, but lack tumor cell–tumor cell, tumor cell–stromal cell, and tumor cell–extracellular matrix interactions of a typical tumor (Kurioka et al. 2011). 3D tissue technology may be used to produce tissue models of cancer, which may help glean new information about cancer development and biology by recreating the in vivo tumor phenotype (Hutmacher et al. 2010, Jessup et al. 1993, Jong Bin 2005, Ingram et al. 2010). A cancer model may also be used for high-throughput pre-animal and preclinical evaluation of anticancer drug candidates because 3D tissues can mimic the tissue response and drug resistance better than 2D cultures (Burdett et al. 2010, Kunz-Schughart et al. 2004). 3D co-cultures may contribute to cancer research when heterogeneous cell populations (cancer along with cancer stem/tumor-initiating cell populations) are used to generate multicellular heterotypic spheroids (Hirschhaeuser et al. 2010, Friedrich et al. 2007). Cancer stem cells are now considered as adjunct targets that must be shut down to decrease the possibility of tumor relapse after chemotherapy or immunotherapy. Tumor-immune cell co-cultures can be considered as models for testing novel immunotherapeutic treatment strategies. 3-D model tissue constructs may as well, provide in vitro systems to improve the predictive value of cell-based assays in toxicology and food research (Mazzoleni et al. 2009)

Model endothelial cells (Sanford et al. 2002), skeletal muscle (Marquette et al. 2007), erythroid cells (Sytkowski and Davis 2001), adipose tissue (Frye and Patrick 2006), cortical-like tissues (Ma 2008), hepatic tissue (Ishikawa 2011), vaginal epithelial cells (Hjelm et al. 2009), human intestinal epithelial cells (Skardal et al. 2010), cardiac cells (Rungarunlert et al. 2011), retinal-like structures (Dutt et al. 2003) and lacrimal gland acinar cells (Schrader et al. 2009) have been constructed under microgravity conditions. 3D models of melanoma (Marrero et al. 2009, Licato et al. 2001), carcinoma (Nakamura et al. 2002), colon carcinoma (Goodwin et al. 1992), breast cancer (Vamvakidou et al. 2007), lung cancer (Vertrees et al. 2009), neuroblastoma (Redden and Doolin 2011), hepatocellular carcinoma (Tang et al. 2011) and ovarian and endometrial cancer (Grn et al. 2009, Goodwin et al 1997) have been engineered using microgravity bioreactors.

Carvalho et al have expanded the application of microgravity tissue engineering by developing a 3D tissue culture model from human intestinal epithelial HCT-8 cells using RCCS for the study of attach and efface lesion formation by enteropathogenic and enterohemorrhagic Escherichia coli (Carvalho et al 2005). A 3D Huh7 cell culture system was also engineered for the study of hepatitis C virus infection (Sainz et al. 2009). Human norovirus infection of Caco-2 cells was modeled by growing tissue in a RWV bioreactor to develop an infectivity assays (Straub et al. 2011). Researchers have simulated the HIV pathogenesis in artificial lymphoid tissue (Margolis et al 1997), Borrelia burgdorferi virulence in human tonsillar tissue (Durr et al 2005) and cryptopso-
Theheartafteraminorinfarctionseemstobe
achievable targets for regenerative medicine (Badylak
also shown promise as extracorporeal BALs. These
systems are now under study as Extracorporeal Liver Assist
Device (ELAD), HepatAssist, Bioartificial Liver Support
System (BLSS) and Extracorporeal Liver System
(MELS). Tissue engineered vascular products, neural
products (for treatment of spinal cord injury) and cellular
products for the constructive functional remodeling of
the heart after a myocardial infarction seem to be
achievable targets for regenerative medicine (Badyal
and Nerem 2010).

Current commercial tissue products
Many commercial tissue engineering products are cur-
tently available in clinic, however most are not from
microgravity origin. The presence of these products
demonstrates that tissue engineering is a viable medical
and commercial approach.

Carticel®, as the first cell therapy (cartilage) product
approved by the FDA, has proved very successful clini-
cally; no reports of serious adverse effects exist. In this
approach, autologous chondrocytes are grown in vitro
and then grafted into a cartilage defects (Gillogly and
Myers 2005). Matrix-induced Autologous Chondrocyte
Implantation (MACI), ChondroArt, co.don chondro-
transplant, co.don chondrosphere, BioSeed®-C, NOVO-
CART®, Cartilage Repair System (CaRe S), ArthroMatrix®
and ChondroCelet® are all similar commercial
products prepared in a similar approach as Carticel®
(Samadikuchaksaraei 2010).

Skin replacement therapies are intended for treatment of
acute or chronic skin disorders and cosmetic surgeries.
Integra, Epiderm®, Biobrane®, Suprathel®, Matriderm®
and Transcyte® are examples of products targeted to
burn victims (Hentze et al 2007, Dieckmann et al 2010).
Dermagraft®, EpiDex®, Epibase, Laserskin, Permacol®,
Oasis® and Apligraf® are available for patients with
chronic skin ulcers. BioSeed-M® and MelanoSeed® are the
two products being used in cosmetic surgery
(Samadikuchaksaraei 2010). For an excellent and up to
date review of the regenerative medicine in dermatology
refer to (Dieckmann et al 2010). Tissue engineered bone
products include BioSeed-Oral Bone®, co.don osteo-
transplant® and Osteocel. Hepatocyte preparations have
also shown promise as extracorporeal BALs. These sys-
tems are now under study as Extracorporeal Liver Assist
Device (ELAD), HepatAssist, Bioartificial Liver Support
System (BLSS) and Extracorporeal Liver System
(MELS). Tissue engineered vascular products, neural
products (for treatment of spinal cord injury) and cellular
products for the constructive functional remodeling of
the heart after a myocardial infarction seem to be
achievable targets for regenerative medicine (Badyal
and Nerem 2010).

Future outlook
In this review article, we focused on microgravity tissue
engineering of cartilage, bone, liver and pancreas as well
as 3D models of different organs; however, other tissues
such as epidermis, periodontal ligament and arteries have
also been constructed in microgravity (Gao et al 2012,
Lei et al 2011, Li et al 2009). The culture of whole sen-
sory organs and other high-density structures in rotating
bioreactors can provide in vitro models for physiological
and pathophysiological investigations (Arnold et al
2010, Hahn 2008). Very recently, a 3D cell biology
model of human hepatocellular carcinoma was con-
structed in vitro by culturing MHCC97H cells on mole-
cular scaffolds within a RWV bioreactor (Tang et al
2011). A modified RCCS bioreactor, Rotary Cell Culture
System! (RCCS!), was used to engineer a 3D model of
bone matrix for studying osteocytes’ differentiation and
bone matrix formation (Mazzolenia et al 2011). Some
researchers speculate that microgravity tissue engineer-
ing will allow for testing chemotherapeutics on cells
taken from an individual patient and grown in vitro.

However, there are yet some obstacles to overcome after
achieving tissue constructs of desired sizes and qualities.
One of these issues is the variability of patient response
regarding resorption, recellularisation and regeneration
of the implanted tissue (Korossis et al 2005). Spontane-
ous vascularisation of the in vitro grown tissue also re-
 mains an issue (Korossis et al 2005). One question that
remains to be answered is whether the differences in cell
physiology and gene expression in cells and tissues con-
structed under microgravity could adversely affect pa-
tients treated with these products. Bone loss in space for
example, has been attributed to changes in gene expre-
sion of osteoclasts (Sambandam 2010, Tamma et al
2009). With advances in the field and overcoming these
obstacles in near future, we may witness a golden era in
which tissue replacement therapy of defective organs
will be a viable option.

Ethical issues
None to be declared.

Conflicts of interest
The authors declare no conflict of interests.

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Tissue engineering in (simulated) microgravity


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