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Microfluidics as a promising technology for personalized medicine

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Abstract

Introduction: Due to the recent advances in biomedicine and the increasing understanding of the molecular mechanism of diseases, healthcare approaches have tended towards preventive and personalized medicine. Consequently, in recent decades, the utilization of interdisciplinary technologies such as microfluidic systems had a significant increase to provide more accurate high throughput diagnostic/therapeutic methods.

Methods: In this article, we will review a summary of innovations in microfluidic technologies toward improving personalized biomolecular diagnostics, drug screening, and therapeutic strategies.

Results: Microfluidic systems by providing a controllable space for fluid flow, three-dimensional growth of cells, and miniaturization of molecular experiments are useful tools in the field of personalization of health and treatment. These conditions have enabled the potential to carry out studies like; disease modeling, drug screening, and improving the accuracy of diagnostic methods.

Conclusion: Microfluidic devices have become promising point-of-care (POC) and personalized medicine instruments due to their ability to perform diagnostic tests with small sample volumes, cost reduction, high resolution, and automation.
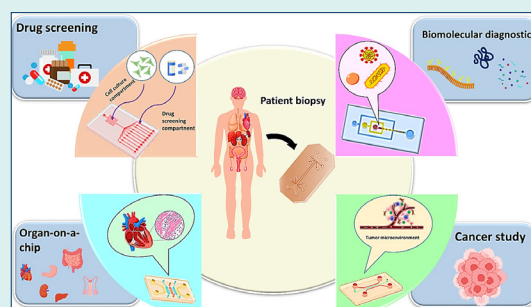
Introduction

During the recent decades, microfluidic technology has become widely used in biology research. Microfluidics is an interdisciplinary science in which various disciplines such as biotechnology, engineering, and physics are involved. The microfluidic device is made of specific structures—including very small-scale channels—that enable the manipulation of small volume of fluids at microliter or picolitre levels [1] (Fig. 1).

These attributes have several advantages: a remarkable decline in the use of resources (such as expensive/tracer reagents or samples), a more accelerated experimental process, extra precise control over the experiment. These features make this technology an ideal tool for using in biological researches. Another important feature of microfluidics is that laminar flow assists the simulation of interstitial flows within the body, the spatial orientation of cells, and the gradient of concentration of various chemical factors.[2] Hence, microfluidic technology enables the culture and study cell behavior in a controlled
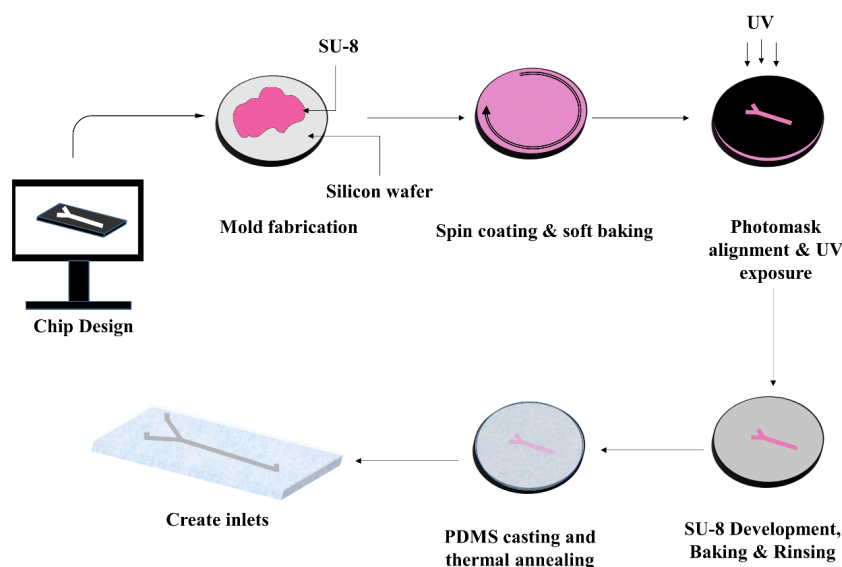
Fig. 1. Schematic steps of fabricating a typical PDMS microfluidic device via soft lithography method.

condition.[3] Surface modification and coating of the polydimethylsiloxane (PDMS) and glass, as a mostly applied materials for the fabrication of microfluidic devices,[4] by extracellular matrix (ECM) components such as fibronectin, collagen, or matrigel improves cell attachment, and then cell proliferation.[5,6]

Conventionally, research on human diseases and drug screening was performed on 2D cell culture using petri dishes that is oversimplified and not possible to mimic microenvironment complexity and physiological condition in living tissues. Therefore, results obtained from 2D models may not be completely reliable.[7,8] On the other hand, even though physiologic environments can be found in animal-based systems, but the use of animal models is sometimes problematic, and the results in this system are not always generalizable to humans. These hitches eventually led to the development of 3D cell culture based approaches that provide both the physiologic microenvironment of the cells and the ability to more accurately control the cell conditions.[9]

Nowadays, microfluidic devices providing a 3D platform for cell culture enabled the reconstruction of tissues and organs. In this regard, numerous microfluidic devices with complex microstructures and substantial biochemical/bioelectrical performance have been achieved. These microfluidics platforms have the potential to be extended for 3D biomimetic tissues and organotypic culture for a wide range of purposes.[10-13] Therefore, in this article, first, explanations about personalized medicine and microfluidic systems were presented. Then we discussed the application of microfluidic systems that have been recently developed in various fields of biomedicine, including molecular diagnosis, oncology studies, drug screening, and organ-on-a-chip with a personalized medicine approach.

Personalized medicine and microfluidic technologies

Over the past decades, increasing knowledge on the molecular mechanism of gene expression and mutation has revealed the mechanism of many diseases, and researchers have used this information to bridge the gap between experimental researches and therapeutic approaches.[14] In this regard, scientists are applying genetic-based diagnostic tests and molecular mechanisms to more accurately predict patients' response to targeted therapies. Therefore, nowadays most efforts have focused on developing new treatments and optimizing prescriptions by directing patients to the best drug, appropriate dose at the right time.[15]

Completion of the human genome project in 2003 opened new horizons for medical science. The data from this project has helped a great deal in medical science, especially in the field of oncology,[16,17] as it provided valuable information on gene sequences, polymorphisms and mutations. Presented genetic data have important applications such as prognostic value and significant therapeutic implications.[18] The analysis of these data led us to realize that genetic differences in individuals will affect almost all aspects of the physiological function of individuals. In fact, the human genome project offered new perspectives to a better understanding of the genetic origins of individual differences and their application in the medical fields.[18,19] This developing field that uses risk factors, molecular diagnostics, targeted therapies, and pharmacogenomics to improve the efficacy of treatment is called personalized medicine. Personalized medicine links one's molecular signature to its medical profile to help to make the right decisions about the patient's treatment.[15,20]

Studying the molecular markers (DNA, protein, or mRNA) has changed our view of diseases and also created molecular classification for diseases. Genetic

variations in individuals such as polymorphisms and even environmental effects can influence the uptake, excretion, distribution, and metabolism of drugs (for instance through changes in enzyme kinetics), thereby affecting the response to treatment.[21] Therefore, approaches based on the origin of the disease will replace conventional therapies. In this regard, a pharmacogenomic test will be needed to evaluate the patient's therapeutic response to the drug based on the patient's genomic profile. It can be expected that; personalized medicine can make a big breakthrough in the pharmaceutical industry and medical practices in the not-too-distant future.[22,23] Alterations in the medical system's pattern from reactive treatment to early detection or pro-active prevention, reduction of trial-and-error in the treatments and formation of an information-based treatment system for patient management are some of expected changes.[24]

Despite advances in the molecular genetics and medical fields, there are still some obstacles in providing appropriate genetic analysis methods or disease models. Hence, it is vital to produce models that are compatible with each patient's genetic system for clinical study. Personalized disease model organoids can be a powerful tool in the determination of treatment approaches. These organoids can be used as live models for investigating the effects of drugs, gene editing, surgery or even preventive studies.[25,26] In this regard, various studies have demonstrated the potential of microfluidic technique to improve the similarity of 3D cell culture models to in-vivo physiological conditions and organoid construction.[27,28]

Microfluidic can be a precise and quick tool for clinical diagnostics. The microfluidic device is capable of using whole blood and giving a genetic profile endpoint.[29] Microfluidic genetic analysis (MGA) systems can be applied for nanoliter flow control, electrophoresis, DNA purification and PCR amplification. Besides, these MGA systems have significant advantages such as reduced time and reagent consumption per trial, a reduced operator based variability or errors and providing better safety for the operator.[30,31] Microfluidic systems can also be used to extract exosome from bodily fluids as a liquid biopsy for personalized medicine. Exosomes contain valuable information such as disease status, treatment response, exposure to environmental symptoms, and numerous other health factors that have diagnostic and therapeutic applications.[32] Thus, eventually, we expect that microfluidic technology will create a leap in personalized medicine era.

Application of microfluidic in biomedicine
Microfluidic technology has become the ideal model for biomedical researches.[33] In this article, practical examples of the microfluidic system in various aspects of biomedicine will be discussed as well as diagnosis, prognosis, and treatment with regards to the personalized medicine approach.

Biomolecular diagnostics
Over the past decades, biomolecule diagnostic methods have been widely used in medicine since can provide highly valuable information for detection, characterization, and prognosis of diseases. Foreign DNAs, modified gene expression levels, specific proteins, and genetic mutations are sometimes linked to pathologic conditions such as infectious diseases, various cancers and genetic disorders.[34] Early detection, especially in the cases of high mortality diseases, infectious diseases, and cancer, has received much attention today. Early diagnosis considerably reduces the mortality rate and cost of treatment.[35] On the other hand, personalizing a molecular diagnosis based on each person's molecular pattern can provide more accurate and precise diagnosis, optimizes treatment, and helps to implement an effective medication (pharmacogenomics). Immunoassay based methods such as ELISA or blotting techniques are routinely used in biomolecular diagnosis but, these methods typically require several steps, long incubations, and more consumption of reagents and samples. PCR has improved these drawbacks but there is still a need for faster, more sensitive and affordable techniques.[36] Thus, developing methods for measuring faster, more accurately, more sensitively and in small quantities of the biomolecules have become a challenge in this field and various techniques have been proposed accordingly.[37] The microfluidic and lab on chip strategies have become a critical tool in biomolecule diagnosis by providing these features (Table 1).[38]

The miniaturization of molecular techniques such as PCR on microfluidic devices will considerably reduce the sample loss, cost and laboratory space required for the processes as the entire procedure will be performed on a single chip. Moreover, simultaneous analysis of multiple samples with precision at the single cell or single gene level is also possible.[48,49] Extracellular vesicles (EVs) are also valuable biomarkers, containing nucleic acids and proteins. investigation of EVs provides valuable data about the patient's condition. Hence, their confinement from biological liquids could be a potential diagnostic strategy. In this respect, microfluidic frameworks are utilized as a reasonable instrument for isolating EVs (Fig. 2).[50]

Several microfluidic platforms with the ability to nucleic acid extraction, purification, PCR amplification, electrophoresis, and DNA microarray have been developed lately.[48,49] In this area, a nucleic acid purification chip device based on the use of silicon beads has been developed. In this device, the mixing of the beads with the sample is regulated through the buttons installed, by controlling the flow rate and the valves. A reciprocating flow is applied in this device in order to recover the sample in the system and increase the purification efficiency.[51] In another study, a button actuation chip consists of

Table 1. Microfluidic-based studies for biomolecular detection

| Target biomolecule	Microfluidic platform	Ref
Rapid capture and fluorescent detection of rolling circle amplification products (RCPs)	Combination of silica-based microfluidic device with thin film photodiodes to capture and detect florescent labeled rolling circle amplification (RCA) product	39
Isolation of circulating tumor cells (CTCs) from prostate cancer patients	Functionalized microfluidic device with dual antibodies (anti-PCA and anti-EpCAM)	40
Tau Protein	A fiber-optic sensor containing anti-tau antibodies and a SnO2−x thin film in which the binding of serum tau protein to anti-tau can be sensed via SnO2-mediated wavelength shifts.	41
Glucose	A microfluidics containing microneedle patch for transdermal biofluid collection and an electrochemical biosensor for high sensitivity glucose detection	42
miR-21	Binding of miR-21 to the hairpin probe leads to the exposure of a single-strand fragment of the hairpin to the electrochemical probe and initiation of an electrochemical process	43
Detection and quantification of IL-6 and PSA proteins	An ECL based immuno-detection microfluidic device with IL-6 and PSA capture Ab coated channels. Secondary Abs are coated on a silica nanoparticle	44
Detection of disease by analyzing of physical features of blood	A microfluidic device consisted of capillary scanning viscometer and pendant drop tensiometer combined with system architecture for disease topography	45
miRNAs detection	Power-free microfluidic chip for miRNAs detection using a sandwich hybridization system	46
The simultaneously detection of inflammatory biomarkers	Combination of localized surface plasmon resonance (LSRP) sensor with one layer-four channel microfluidic device which utilized by automated microfluidic control	47
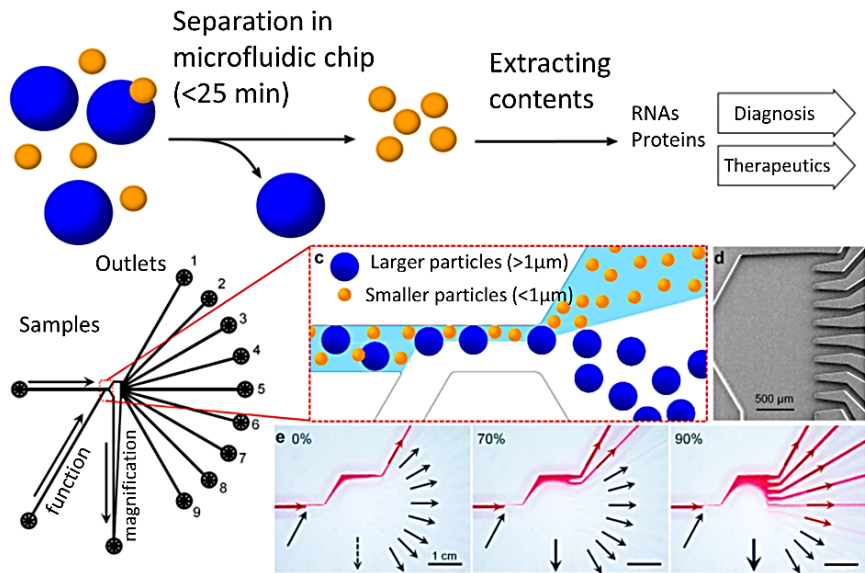
Fig. 2. A tunable microfluidic device for noninvasive size-based separation of EVs. Reprinted from Shin et al[50] under a Creative Commons Attribution 4.0 International.

two plasma separation and nucleic acid extraction/purification parts was designed to detect the nucleic acid of pathogenic bacteria in blood samples. Silica-coated magnetic nano particles (MNPs) is used to separate bacteria from plasma, and the steps of lysis and washing are carried out by the buttons installed on the device that control the pumps and valves.[52] Integrated platforms are also designed based on a variety of electrophoretic methods such as capillary electrophoresis (CE),[53] capillary gel electrophoresis (CGE),[54] isoelectric focusing (IEF) and SDS-PAGE.[55-57] Immuno-affinity miniaturization devices are also developed as another on-chip biomolecular diagnostic tools that have some advantages over conventional methods such as enhanced performance, speed up and simplify the process as well as employing lower volumes.[58] On-chip immune-affinity approaches

are based on detecting key biomarkers associated with the various diseases such as EGFR (head and neck cancer) and C-reactive protein (CRP) as a biomarker of coronary heart diseases,[59] detecting vascular-endothelial growth factor (VEGF), prostate-specific antigen (PSA), and PCa circulating tumor cells (CTC) in human serum. For fabrication of this device first, aptamer and antibody have been coated on gold nanorods (GNR) and a UV-Vis-NIR spectrophotometer have been used as the detection system.[60]

Among the various instruments designed, Lee and colleagues developed a simple and rapid colorimetric system for the simultaneous detection of multiple microRNAs. MicroRNAs (miRNAs) are small (typically 18 to 24 bases) non-coding RNAs, which regulate gene expression by binding to 3′UTR of target mRNAs and

inducing their degradation.[61,62] The recent discoveries have suggested miRNAs as promising biomarkers for various diseases,[63] drug sensitivity/resistance, and associating with personalized medicine[64] and so, several microfluidic-based methods for miRNAs detection have been invented.[65] Lee and colleagues have been used polyethylene glycol hydrogel as a substrate to facilitate the hybridization of microarrays with designed probes. Also, detection is done by colorimetric examination of gold nanoparticles attached to the target using dark field imaging.[66] A droplet microfluidic high throughput device 300-500 cells/minute with analysis efficiency provided by Gou et al. This continuous-flow microfluidic platform utilizes two hairpin DNAs structures for capturing target miRNA from the lysed cell. Conformational changes in hairpin DNAs after binding lead to the separation of fluorophore and quencher pairs and eventually forming a signal senses by a UV detector.[67] Additionally, microfluidic paper-based analytical devices (μPADs) are also designed as a biosensor for semi quantitative/quantitative detection goals. Sun, et al developed a paper-based electrochemical/visual biosensor for miRNA detection. In this platform, a biocompatible porous cellulose fiber web (μPADs) is modified with Au-NRs which assists the hairpin DNA probes to be immobilized on the surface by Au-S bonds. When the target miRNA binds to the hairpin probe, a single-strand fragment of the hairpin will be exposed and binds to the electrochemical probe (CeO2-Au@GOx).[68]

Different types of microfluidics-based biosensors are proposed such as glucose biosensors, urea biosensors, cholesterol biosensors, toxin detection biosensors.[69] Biosensors are one of the most widely used molecular diagnostic tools due to their speed and ease of performance and hence their combination with microfluidic technology will improve their usage in molecular diagnostics. For instance, optofluidic devices can be categorized based on optical detection methods such as plasmon resonance technique, fluorescence, and interferometry. The integration of microfluidics with optical detection systems will reduce hardware costs and improve their sensitivity.[70,71] The optofluidic devices are usually consist of three layers: The topmost layer consists of the microfluidic controls such as valves and pumps, the middle layer with microfluidic channels, and the third layer that contains the optical structures, sensors, and waveguides.[72] A metal film or fluids can be used for coupling optical functionalities on-chip.[73] One of the promising platforms of the optofluidic biosensor is a chip designed based on a liquid crystal system. In this device, the sample containing protein biomarkers enters the channel and is exposed to laser light which is amplified by liquid crystal and a whispering-gallery-mode (WGM) laser emission spectrum is formed. The presence of protein causes a shift in the WGM spectrum, which is detected by a spectrometer.[74]

Exosome isolation and analysis

Exosomes are 30-150 nm sized extracellular vesicles secreted by cells into bodily fluids. These intercellular communication tools contain a variety of proteins, RNAs, DNA and lipids from the original cell. Therefore, exosomes can be considered as enriched information packages, the study of which provides valuable clinical information, assisting personalized diagnosis and therapy. The performance of conventional separation techniques is not adequate in separating exosomes from other vesicles and cells in the bodily fluids. Hence, microfluidic separation systems with higher resolution than other separation techniques are proposed. So far, various types of microfluidic systems have been developed for exosome isolation as reviewed in Table S1 in supplementary data.[75] Isolation methods based on physical properties can also well be used to separate exosomes. For example, acoustic-based microfluidic systems can isolate exosomes in a blood sample based on size and shape. Porous membrane based microfluidic platforms also can simply separate microvesicles from biofluids. The diameter of these pores is optimized to only micro vesicles are allowed to pass, and the cells are trapped behind the filter. Performing an on-chip electrophoresis in the next step helps to better discretion of the microvesicles from the plasma proteins.[75-77] Isolation methods based on immuno-affinity capture are one of the most routine separation methods. In these methods, a ligand is coated into the system's micro-channels to trap exosomes with a certain extracellular marker. This method is also used in cancer diagnosis due to this fact that exosomes carry significant amounts of cancer biomarkers. Research has shown that in addition to apoptotic cells, malignant cells and cancer derived microvesicles also contain large amounts of externalized phosphatidylserine (PS). Kang, et al. designed an ExoChip to isolate these exosomes containing externalized PS. This device has a large number of circular chambers containing coated annexin V protein which has a Ca^{2+} dependent affinity for PS. The large number of these trapping chambers implanted on this device increases its separation efficiency.[78]

Magnetic based microfluidic systems are also highly efficient for on-chip exosome isolation and analysis. In this approach, a specific ligand is attached to the magnetic beads and beads are mixed with the serum sample. The serum sample containing the beads enters the microchannels of the microfluidic device and by applying magnetic force and several washing steps, only the exosomes with the target biomarker remain. On-chip exosome analysis is performed by steps of fluorescence detection and TEM imaging.[79,80]

Drug screening

A recent discovery toward the human genome has discoveries toward the human genome has revolutionized

diagnostic and therapeutic methods. The introduction of individual medicine has led to an attempt to carefully optimize therapies tailored to everyone's genetic mechanism. This has also affected the pharmaceutical industry. Genetic diversity amongst individuals causes variant response to medications. Thus, drug screening is crucial especially for life threatening diseases like cancer.[20,81] High-throughput, automation, inexpensiveness, and efficiency with low-sample volumes are important in drug screening. Drug screening microfluidic platforms, in addition to addressing these issues, allow for three-dimensional cell culture and drug screening in in-vivo-like conditions (Table 2).[82,83]

This is important in two ways; First, the results of drug screening in laboratory animals cannot always be generalized to humans[92] and second, 3D microenvironment simulation is effective in drug screening, especially in cancer. In the tumor microenvironment, various factors can impress the potency of the chemotherapy agents and for this reason microfluidic systems are advantageous options for drug screening.[93-95] Determining the effective dose of the drug is vital for positive therapeutic response and a prescription inappropriate dose of the drug both increases the probability of side effects of the drug and may lead to drug resistance. Microfluidic devices can be used to apply drug concentration gradients to determine the effective dose, cell viability, and the period of drug influx. Different doses of the chemotherapy drugs with the cell culture medium enter the central inlets of the microfluidic system and the result of their efficacy is inspected.[84,90] (Fig. 3).

These multi-drug screening platforms make it possible to investigate the synergistic effect of several antibiotics and achieve an effective inhibitory dose.[96] To prevent drug resistance, three-dimensional culture of biopsied samples on microfluidics and afterward drug screening have been performed.[97] Eduati et al developed a braille valve chip capable of single/combinatorial drug screening on very small volumes of biopsy samples, in a period of 48 hours. Performing plug technology separation of samples using different chemical phases provided different test conditions on one chip at the same time. Moreover, several computer-controlled, movable pins contrived to precisely control drug circulation inside microchannel networks.[87]

Several microfluidic platforms (bleeding chips) have been designed to simulate in-vivo thrombosis condition[98-101] for screening antithrombotic agents and determining the appropriate dose, even with a personalized treatment approach. Thrombosis is a pathophysiologic condition associated with a high rate of mortality worldwide correlated with many clinical complications including immune-mediated disease, cardiovascular disease, stroke, atherosclerosis, and malignancies.[102] However, the urgent need for anticoagulant screening became even greater when the link between thrombosis and mortality from the global COVID-19 issue was identified.[103] As the world sought to reduce the mortality rate of this pandemic, high throughput systems for drug screening came to the fore.[88] The polydimethylsiloxane-based bleed chip designed by Lakshmanan, et al. is an example of these studies that two orthogonal channels embedded on this device. The main channel as the blood vessel and side-channel called the bleeding channel and three circular pillars located at

Table 2. Microfluidic-based studies for drug screening

| Drug	Microfluidic platform	References
Doxorubicin and Cisplatin	A device providing the possibility of studying different doses of the doxorubicin and cisplatin on tumor cell lines. Different drug-medium concentrations enter each chamber.	84
carboplatin, gemcitabine, capecitabine, topotecan, and navelbine	A tow-part chip containing a CTC isolation and culture chamber, and six separate drug screen cavities. Due to the structure of the separation chamber, CTCs are separated from other blood cells based on their size and are trapped and expanded under non-adherent culture conditions. Then, by applying fluid flow, CTCs enter the drug screening chambers, and their viability is measured by luminescence analysis.	85
Doxorubicin	A droplet system to create homotypic and heterotypic spheroids and investigate the effect of chemotherapeutic agents on these spheroids. The purpose of creating spheroids is to mimic the microenvironment of a tumor and to investigate the effects of cell interactions with each other and with the surrounding environment in response to treatment.	86
Gemcitabine Oxaliplatin TNFα	A braille valve chip capable of a single/combinatorial drug screening on very small cancer biopsy samples, in 48 hours. Two chemotherapy drugs for pancreatic cancer (Gemcitabine and Oxaliplatin), specific kinase inhibitor drugs, and one cytokine (TNFα) that activates apoptosis have been tested alone and in combinatorial in this device.	87
Anticoagulant drugs	A bleeding chip with two orthogonal channels, one acts as a blood vessel, and the second is the bleeding channel. The hemostatic plug will form at the site of pillars.	88
100 compounds and amphotericin B mixtures	A chip with 20 units which each unit has micro-channels with a diameter of 3 to 6 microns, so Candida albicans are trapped individually in these channels. Fluorescent microscopy was used to determine live and dead cells.	89
Doxorubicin and Tirapazamine	A chip providing an oxygen tension gradient environment for cancer study. The MDA-MB-231cells are seeded in a fibrin gel (3D microenvironment) and gas channels apply the oxygen tension. The response of cancer cells to anti-cancer drugs in the hypoxic tumor microenvironment can be examined (Fig. 3).	90
Cisplatin	A multi-channel device for selective trapping cuboidal-shaped micro dissected tissues and exposure to different drug doses	91
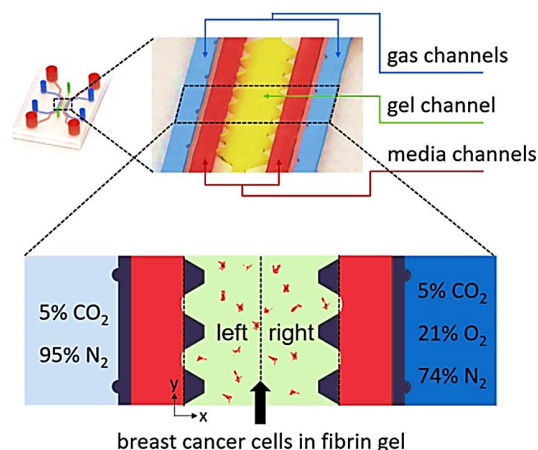
Fig. 3. Schematic of an oxygen tension gradient providing device for studying the reaction of cancer cells to doxorubicin and tirapazamine in the hypoxic tumor microenvironment. Reprinted with permission from Nam et al.[90]

Fig. 4. Schematics of microfluidic systems replicating cancers: **(A)** Ovarian adenocarcinoma,[110] **(B)** Kidney to liver metastasis (Reprinted from Wang et al[111] under the terms of the Creative Commons Attribution License; https://creativecommons.org/licenses/by/4.0/).

the junction of two channels. The influx of whole blood into the channels of the bleeding chip with a determined flow rate led to the aggregation of platelet at the site of the pillars that led to the formation of the hemostatic plug caused blockage of the bleeding channel.[88]

Cancer study

Cancer is a heterogeneous disease described by uncontrolled cell proliferation and invasion[104] that is the main health problem and a leading cause of death (millions of deaths each year) worldwide.[105] However, the cancer mortality rate has decreased since the mid-1970s,[105] but its prevalence is increasing due to changes in lifestyle such as smoking, lack of physical activity, and improper diets.[106] Since early diagnosis of cancer is very important, different methods have been developed for detection and characterization of this disease. Nowadays, strategies for tumor diagnosis and treatment are critically influenced by the viewpoint of personalized medicine. A set of alterations at transcriptome, proteome, and metabolome levels are involved in cancer incidence, therefore, a molecular panel as a recognition pattern is necessary for prediction, diagnosis, and treatment of cancer. Predictive, preventive, and personalized medicine (PPPM) is an approach based on discovering key tumor molecule-panel for individuals and providing a treatment based on each person's own panel.[107] In this regard, several microfluidic platforms have been developed for studying cancer biology, capturing cancer cells and subsequently enabling on-chip post-processing after capture , detecting cancer biomarkers, and testing drug sensitivity[108-111] (Table 3; Fig. 4).

Although biopsy of tumor tissue is a common way of cancer diagnosis, due to its invasive nature, it is an inconvenient method for patients and can cause problems such as tissue damage. Studies have shown that ind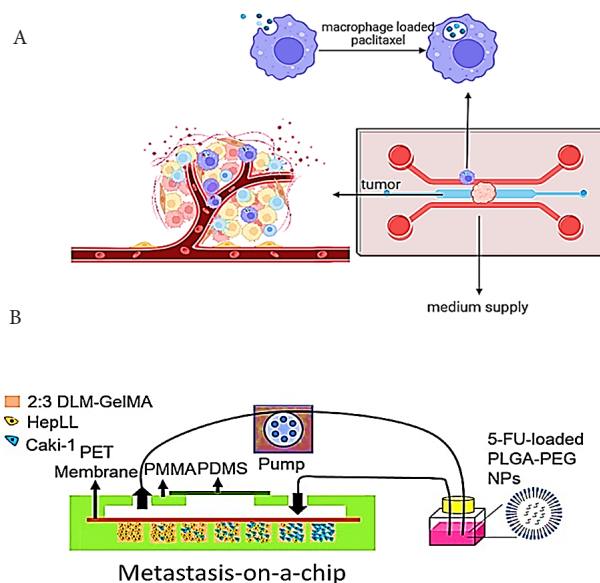ividuals' body fluids contain biomarkers (DNA, RNA, protein and circulating tumor cells (that can be used in cancer detection and treatment follow up (based on each person's specific biomarkers).[119] Microfluidic is a non-invasive, quick and suitable method for in vitro studying of individual's body fluids.[120] Paper-based systems can be mentioned among the tools provided. Paper-based microfluidics is one of the diagnostic tools that have found wide application in the field of biosensors due to their cheapness and ease of use. Wang et al developed a four-layer paper microfluidic system for the simultaneous detection of carcinoembryonic antigen (CEA) and neuron-specific enolase (NSE) cancer biomarkers. The diagnostic electrochemical system of this instrument has three electrodes and two Au-NP-based nanocomposites applied to immobilize the aptamers and intensify the electron transfer. The advantages of this system include sensitivity, selective power, and high detection speed.[121]

At a tumor site, some of the tumor cells (circulating tumor cells; CTCs) shed into the bloodstream and are responsible for the spreading of cancer to distant sites that provide personalized information about the stage of the disease and the efficacy of treatment.[122,123] Thus, CTCs isolation from patient blood offers a valuable biomarker for diagnosis, characterization, and monitoring. Because of the low circulating levels and short lifespan, CTC separation requires a rapid and sensitive method. In this regard, immune-affinity based techniques have been investigated for CTCs isolation but these methods with multiple steps are time-consuming and have a high risk of loss of target cells in the separation stages. Microfluidic technology is a promising approach for high throughput, and sensitive separation of CTCs comparing to other methods.[124] One example of a microfluidic system for CTC

Table 3. Cancer on a chip studies

Type of cancer	Microfluidic platform	Ref
ovarian adenocarcinoma	A middle micro channel surrounded by two lateral channel which is separated by array of PDMS column to loading of hydrogel for culturing of tumor spheroids and macrophage in separated channel. To create of tumor-microenvironment model, the tumor spheroid placed in collagen gel and macrophage were embedded in adjacent channel (Fig. 4A).	110
Leukemia-on-a-chip	A device simulating leukemic bone-marrow niche and B-ALL. Three region including central venous sinus (central red part), a medullary cavity (green parts), and endosteal regions (external ring) are emulated in this device. These areas are divided by trapezoidal micro-columns containing cell-hydrogel mixture, balancing surface tension and capillary forces, to imitate the leukemic BM architecture .	112
Ovarian cancer on a chip	A two-layered device with a separating thin, porous membrane imitating the microarchitecture of the tumor-vascular connection. Three ECM, tumor (in outer layer) and vascular (bottom layer) microchannels are separated by hexagonal micropillars which facilitates cell invasion. This device enables the study of intravenous perfusion of platelets, their penetration through the endothelium and cancer cells invasion.	113
Lung cancer-on-a-chip	This chip includes a central chamber where the Hepatic stellate cells (HSCs) and hepatocellular carcinoma (HCC) cells enter this chamber together with matrigel. Endothelial cells are cultured in the two side chambers and they play the role of blood vessels. Cellular crosstalk, Activation of HSCs, endothelial invasion, drug resistance and inefficiency of natural killer (NK) cells is studied on this chip.	114
Pancreatic cancer-on-a-chip	A two-part microfluidic device was used to model the heterogeneous tumor microenvironment of pancreatic ductal adenocarcinoma. Patient-derived primary cancer cells (PCCs) along with stromal cells and macrophages were cultured in the upper chamber on the matrigel matrix. The lower chamber which is separated from the upper chamber by a permeable layer, provides the culture medium required for cell growth.	115
Kidney to liver metastasis-on-a-chip	A poly (methyl methacrylate) (PMMA)/ PDMS fabricated device with 7 cell culture microwells containing different ratios of co-cultured immortalized hepatocytes HepLL and kidney cancer Caki-1 cells into an ECM of 2:3DLM/GelMA hydrogel to mimic the metastasis of kidney cancer to the liver (Fig. 4B).	111
breast-cancer-on-chip	A tow-layered device which the upper layer contained three independent capillary vessel channels comprising an endothelial monolayer, connected ECM channel and a multicellular tumor spheroids (MCTS) culture channel with 11 U-shaped microchambers. Second layer was embedded under the U-shaped microchambers creating the perfusion flow along with preventing cells evasion. This system has been used for the assessment of drug delivery systems and toxicity studies.	116
Lung cancer on a chip	A glass-based microfluidic device to investigate drug toxicity. This system is designed to provide a sensor integrated organoid for lung cancer monitoring and evaluation of the toxicity of candidate drugs. For real time monitoring the survival of cultured cells on glass-based chip, a trans-epithelial electrical (TEER) impedance sensor is embedded in this device. An optical pH sensor also is installed to check the pH of the culture medium. Therefore, this system has the ability to investigate the cell toxicity and determine the appropriate therapeutic dose	117
Tumor vessel-on-a-chip	A MF device preparing the opportunity to imitate the formation of thrombi and hemorrhage in vascular tumors in vitro. Device is made of micro-sized hollow channels coated with fibronectin and endothelial cells. A microfluidic syringe pump has been installed to perfuse the blood at physiological flow rates and shear stress.	118

separation is the three-part system designed by Wu, et al. In this chip, the sample first enters the detection section, and by applying a continuous flow (Deterministic lateral displacement structure), CTCs are separated from the rest of the peripheral blood cells and enter the second section for pure isolation by encounter with specific antibody.[125]

It is worth mentioning that one of the most important microfluidic applications in the field of oncology is providing a proper 3D microenvironment for the study of cancer biology, metastasis, and drug resistance. The interaction between malignant cells and tumor associated cells at the site of the tumor forms the tumor microenvironment (TME) which plays a crucial role in metastasis, angiogenesis, and chemo resistance. Microfluidic platforms offer highly controllable conditions through controlling the shear stress, microperfusion and cell-cell or cell-matrix communication for better mimicking the TME. Thus microfluidic propose suitable models for studying the physiological processes of cancer as well as a high throughput system for drug screening.[126,127] Moreover, microfluidic technology is a valuable tool for the analysis of cancer cell invasion and metastasis. Recently, several microfluidic devices have been offered for studies of cancer cells migration[128] such as: the Kühlbach et al chip for study of the trans-migration and invasion of tumor cells from blood vessels[129]; growth factor gradient based systems[130]; chemotaxis-based prompted migration system[131]; perfusable vascular structures as a tumor angiogenesis and drug delivery model[132]; and mechanical confinement systems for studying spontaneous migration of cancer cells.[133]

Organ on chip
In recent years, studies have shown that cell niches have a tremendous effect on cellular demeanor, consequently the behavior of cells in two-dimensional cultures is far different from three-dimensional conditions. On the other hand, in-vivo animal studies also have not been able to overcome the challenge of in-vivo modeling of diseases and therapeutic responses due to species differences. Therefore, in recent years, many researchers have focused on in-vitro regeneration of human body organs (Fig. 5).[134,135]

Organ-on-a-chip (OOC) is an approach in which cells in a microfluidic cultivation environment are cultured with controlled in-vivo like conditions. As a result, tissue or organ formed on these microfluidic devices are true simulations of human tissues/organs. OOC can be used
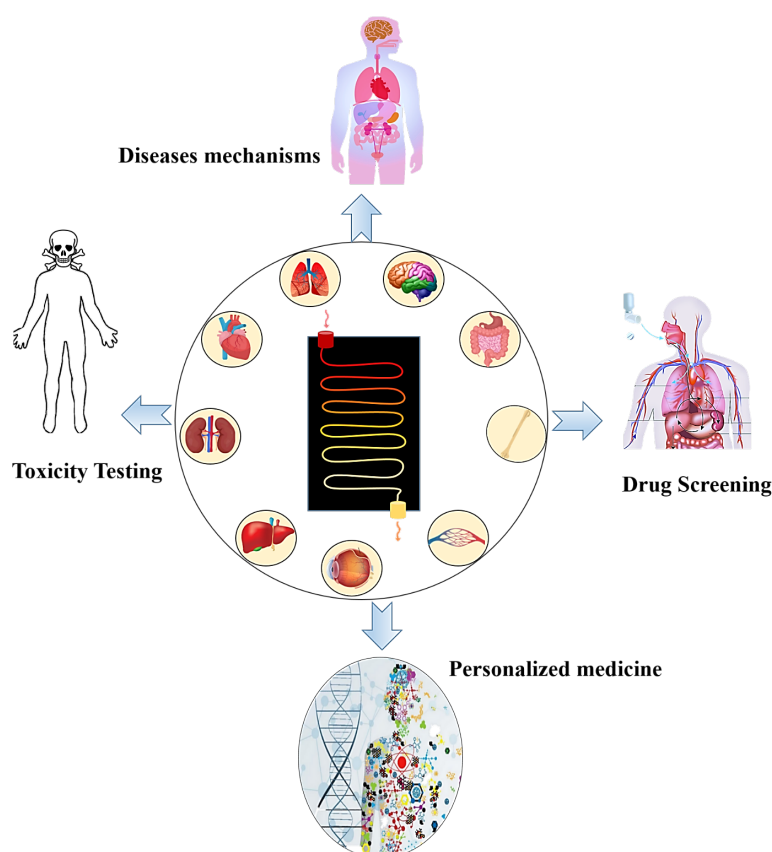
Fig. 5. Applications of organ on a chip in biomedicine.

for a variety medical purposes and personalization of treatment strategies where a specific therapy with more success probability and less side effects is required.[136,137] So far, different types of human organs have been successfully remodeled on microfluidic platforms.[135] For example, the lung on a chip is a reconstructed model of the lung that restores the function of this organ in an in-vitro condition. The physiology of this regenerated organ is similar to that of a normal organ, i.e. the epithelial and endothelial cells are arranged in a three-dimensional microenvironment similar to in-vivo conditions and perform the rhythmic function of respiration. These models can be used to study the pathological conditions of diseases, pharmacological and toxicology investigations with a personalized treatment perspective.[138,139] Various platforms have been proposed to mimic lung function on the microfluidic system[138,140,141] as shown in Table 4 and Fig. 6.

In lung organoid-based toxicological studies, a new agent/drug is exposed to remodeled organoid and the effect on tissue function is investigated. Nanoparticles (NPs) have recently been used in various industrial and clinical fields, therefore, the study of their effects on various human organs is vital. The formation of reactive oxygen species and the subsequent inflammatory responses are among the side effects of NPs. Some NPs also can cross body barriers such as the alveolar-capillary barrier and penetrate different parts of the body. Zhang et

al developed a microfluidic device simulating the alveolar-capillary barrier function for assessment of NPs toxicity.[40]

Given the vital role of the liver in metabolism and detoxification, it is valuable to study of the physiology of liver disorders and discovery of effective drugs. In recent years, advances in liver organoids have greatly assisted to provide a more informative model to upgrade therapies toward more efficient, targeted, and personalized treatments.[152] Liver disease accounts for 3.5% of global deaths and about 2 million deaths are recorded annually due to these diseases. Cirrhosis, hepatitis and hepatocellular carcinoma are among the most important liver diseases.[153] Up to now, various two-dimensional (2D) and three-dimensional (3D) liver models have been reconstructed on the microfluidic system.[154] An important challenge in liver regeneration is the reconstruction of the tubular structure and the provision of conditions for nutrient transfer between hepatocytes and vascular endothelial cells. To address this limitation, Meng et al fabricated a pluronic F127 (F127-DA) hydrogel microfluidic device, imitating the structural and functional properties of human liver. This F127-DA fabricated chip is able to afford high channel resolution along with tolerating the perfusion state. The hepatic spheroids are in the outer microchannel and the inner microchannel is covered with a single layer of endothelial cells. The culture medium at a flow rate similar to the physiological conditions is

Table 4. Organ-on-a -chip studies

Type of OOC	Cell source	Microfluidic platform	study
Alveolar-capillary barrier-on-a-chip	Alveolar epithelial cells ,and endothelial cells	This model consists of 3 parallel microchannels. The middle channel, which contains the matrigel imitating the extracellular matrix (ECM), separates the two side channels remodeling the alveolar and capillary structures. Alveolar epithelial cells are cultured on one side and endothelial cells on the other side. Nanoparticles exposed to epithelial cells and in the capillary side a fluidic flow similar to the human lung is applied.	[142]
Liver-on-a-chip	Hepatocellular carcinoma HepG2, human hepatic stellate cell LX-2 ,and human umbilical vein endothelial cells (HUVECs)	A system consists of endothelial cells and the permeable hydrogel to mimic diffusion. The microfluidic chip is consisted of two adjacent but discrete channels: inner channel which represent the capillary vessels, its surface is covered with the HUVECs and exposed with medium to show the shear stress in the physiological condition), outer channel: the outer channel is filled with the hepatic spheroid shapes which is formed by the concaved micro wells. The nutrient from inner channel could uptake through the infusion by the hydrogel.	[143]
Liver-kidney-on-a-chip	HepG2 ,and Hek293 cells	A microfluidic device consists of two connected chambers simulating kidney and liver function. Using an optimal flow velocity, the conditions for evaluating the primary and secondary toxic effects of drug candidates on liver and kidney are provided, respectively.	[144]
Kidney-liver -on-a-chip (an acute renal injury model)	Kidney tubuloid and liver biopsies	The aim of this study was to investigate the therapeutic effect of Mesenchymal stromal cell (MSC)-derived small extracellular vesicles (sEVs) on the repair of kidney damage, therefore, the modeling of kidney and liver organoids (accumulation place of sEVs) was done on a microfluidic system with two interconnected chambers. To create a functional tubular epithelium detaching the blood and urine, kidney compartment was cultured on a semipermeable membrane and kidney injury was caused by hydrogen peroxide.	[145]
Stomach-on-a-chip	Human-derived adenocarcinoma epithelial cell line MKN74, and human stomach normal fibroblast cell line NST-20	This chip is a nine-part structure mimicking three different layers of the gastric mucosa; the epithelial barrier, the basement membrane made of fibronectin thin gel, and the lamina propria layer comprising collagen type I gel and normal gastric fibroblasts. A peristalsis-like motion is also applied to the system to reconstruct the dynamic stomach microenvironment.	[146]
Lung-on-a-chip	Human alveolar epithelial cells (HPAEpiCs), Human umbilical vein endothelial cells (HUVECs)	A breathing alveolar–capillary chip consisting of the alveolar epithelial cells cultured in the upper side and pulmonary microvascular endothelial cells in the bottom side of the elastic membrane. Applying an air flow induce epithelial cells differentiation (Fig. 6A).	[140,147]
Vessel-on-a-chip	human umbilical vein endothelial cells (HUVECs)	A coaxial flow microfluidic device with a helical microfiber for fabricating different vessel structures such as multilayer, hollow. Coaxial flows are consisting of an inner alginate flow and outer $CaCl_2$ flow (outer and inner flows are shifted in hollow microfibers). Two- and three-layer structures are also made by increasing the number of infusion micro-channels. HUVECs were seeded in the inner layer of the constructed membrane forming the endothelium layer as the lumen of blood vessels.	[148]
Heart-on-a-chip	Human induced pluripotent stem (hiPS) cells derived cardiac cells	Cardiac micro-tissues derived from human iPSCs cultured on a micro-electro-mechanical microfluidic system, comprising of a microchannel, a push bar placed on the top, a chamber and diaphragm. The cardiac cells are cultured on the push bar within a fibronectin matrix. Inside the microchannel, a culture medium containing fluorescent particles flows. Instinctive pulse generated by cardiac micro tissue can be measured by fluorescent particle displacement (Fig. 6B).	[141]
Heart-on-a-chip	Ventricular cardio myocytes (CMs) were derived from the human embryonic stem cell (hESC) ,and the human induced pluripotent stem cell (hiPSC)	A rectangular micro-well interconnected by two parallel grooves containing Poly (octamethylene maleate (anhydride) citrate) (POMaC) polymer wires. POMaC wires have an inherent fluorescence leading to monitoring the passive and active tension of cardiac tissue.	[149]
Brain-on-a-chip	Human induced pluripotent stem (hiPS) cells	A microfluidic system with two parallel chambers for cell culture and three separate channels. hiPS derived EBs mixed with matrigel are cultured in two parallel channels. The lateral channels contain the culture medium. The middle micropillar-shaped channel also contains the culture medium, causes fluid flow perfusion, and facilitates the exchange of oxygen and nutrients to cell-containing channels.	[150]
Kidney-on-a-chip	Human induced pluripotent stem (hiPS) cells	A device consists of a stretchable PDMS elastomer that comprise two parallel channels with opposite directions and segregated by a porous flexible PDMS membrane that its coated by laminin 511.	[151]

pumped amid these two channels. This in vitro 3D liver model expresses the potential for a range of applications including drug discovery and cellular toxicity analysis.[143]

In addition to studies on liver regeneration, studies have also examined the interaction of the liver with other organs. Remodeling of these interactions helps to study physiologic processes and related diseases. Reconstruction of the liver-gut model has a researching value to study metabolic routes and transmission of gut-absorbed lipids to the liver. This model can also be used to study the
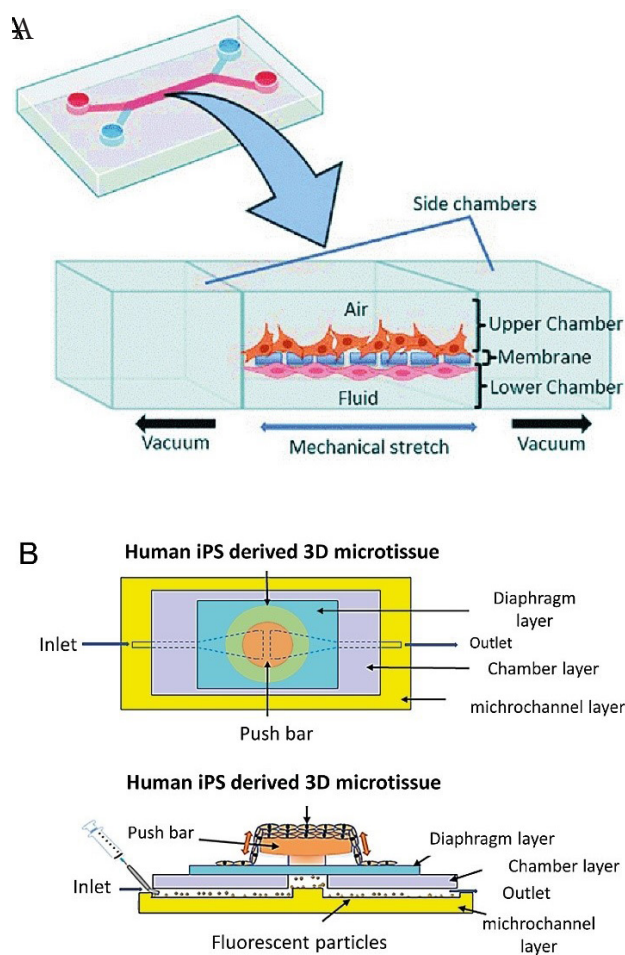
Fig. 6. (A). The design of breathing alveolar–capillary chip, the alveolar epithelial cells are cultured in the upper side and pulmonary microvascular endothelial cells in the bottom side of the elastic membrane. Applying an air flow induce epithelial cells differentiation[140] **(B).** Schematic of a cardiac micro-tissues derived from human iPSCs (Reprinted from Abulaiti et al[141] under a Creative Commons Attribution 4.0 International License).

hepatic steatosis and drug screening for this prevalent disorder. To remodel the gut-liver interaction on the chip, a microfluidic device with two anti-parallel micro channels with opposite inputs and outputs was designed. Gut cells were cultured in the upper microchannel and hepatocytes were cultured in the lower microchannel. These channels are connected in the center, in which a permeable membrane was placed to exchange lipids. Screening of anti-steatotic agents on this system showed that compounds which help strengthen the gut barrier function, have a desirable outcome in treating hepatic steatosis.[43]

In addition to liver disease, heart diseases also have a high prevalence and the lethality risk. Thus, the reconstruction of an in vitro functional model of heart has been considered in recent years. These models have applications in physiological, pathological and drug screening studies. Whereas, the physical characteristics of the culture microenvironment, such as geometry and stiffness determine the differentiation and functionality of

cardiac cells, the embedding of these conditions on a chip positively affect the outcome of the remodeling process.[155] In addition to providing a suitable microenvironment for cell differentiation, the application of an adjusted electrical force is required, to induce cardiac cell contraction. Monitoring the active force and passive tension of cardiac cells also importantly authorizes personalized drug screening and toxicology studies.[149,156] Moreover, the evaluation of the action potential presents vital information on the pathophysiologic state of cardiac cells. Liu, et al. developed a bioelectronic chip to study cardiac function under the hypoxia condition. The design of this micro-fluidic device is such that the input oxygen content is controlled by micro-channels so that embedded electrodes and platinum nanoparticles provide real-time monitoring of electrophysiological response of cardiac cells.[157]

The brain on a chip is perhaps one of the most important achievements of the microfluidic system. Although we have not been able to fully regenerate the brain due to the complex structure and technical limitations, successful regeneration of some parts and modeling of nervous system disorders has been promising for personalized treatment of neurological disorders. The human brain is a layer structure made up of different cells, including neurons, astrocytes, microglia, and oligodendrocytes. Neurons play a role in brain function through the establishment of synapses.[158] Studies have shown that the neuron's microenvironment including adjacent cells and ECM affects its function. Neurons isolated from different parts of the brain demonstrate differences in metabolism, protein expression, and electrical activity. Therefore, an important challenge in in-vitro brain reconstruction is to provide this microenvironment to mimic the function of the target region in the brain.[159] Interestingly, Park et al designed a microfluidic system for the simultaneous culture of neurons, astrocytes, and microglia cells and subsequently investigated the cellular interactions and mechanism of neuronal cell destruction during Alzheimer's disease. This 3D model is capable of recapitulating beta-amyloid aggregation, phosphorylated tau accumulation, neuroinflammatory activity, recruitment of microglia and cellular damage mediated by microglia cells.[160] The study of cell communication is also important in the incidence of secondary neuronal cell death. Released glutamate from damaged cells, as well as synaptic connections, are involved in the spread of neuronal damage at the stroke or injury region. Samson, et al designed a microfluidic device with five parallel interconnected culture chambers for investigation the expansion of neurotoxicity. These chambers are designed to study the spread of cytotoxicity to adjacent regions through synapses. Therefore, in this model, synaptic dependent hyperactivity, sudden ion influx into neurons causing depolarization and cytotoxicity due to glutamate accumulation can be

simulated. The results of this study revealed a protective mechanism to prevent the spread of cytotoxicity. In the era adjacent to the site of injury, signaling of the GluN2A receptor triggers the recruitment of GABA receptor, thereby hindering spreading cytotoxicity. These results could open new horizons for the treatment of progressive brain injuries.[161] Blood-brain barrier (BBB) modeling is another important study in the area of the brain on a chip. BBB by controlling the entry of substances into nerve cells is a competent research option for the studies of diseases mechanism as well as drug delivery. Several CNS diseases and even neurological malignancies such as glioblastoma are associated with impaired BBB permeability. Due to the complexity of this system as well as species differences, 3D modeling of BBB on a chip can be superior to animal studies and 2D culture systems.[162,163] In a recent study, Yu, et al. designed a microfluidic platform mimicking the BBB structural complexity and function. This model imitator the interactions between endothelial cells (ECs), pericytes, and surrounding astrocytes in BBB organization and the fluid flow condition in the brain microvasculature. One inlet, one outlet and a microchannel are embedded in this microfluidic device, and the inlet and outlet heights are designed in such a way that the fluid moves towards the outlet by the siphoning effect and via gravity. To slow down the flow speed as blood flow in brain microvasculature, a paper cylinder flow resistor installed in the outlet reservoir. The microchannel is also made in the form of a hollow lumen of type I collagen hydrogel as a matrix for 3D cell culture. Yu, et al. then used TNF-α to induce the inflammatory condition modifying the BBB permeability, as is the case with many CNS diseases. This device can provide a suitable drug screening platform for the treatment of neurovascular disorders.[164]

Concluding remarks

Given the valuable advances in biomedical science in recent decades, as well as the increasing knowledge of disease mechanisms, it is expected that personalized medicine will replace traditional methods of treatment in the not-too-distant future.[20] This evolution certainly requires reformation in existing tools in the field of diagnosis, disease monitoring, and especially treatment providing the ability to tracking small amounts of biomolecules or mimicking the complex conditions within the patient body.[165] Microfluidic devises, which attracted the attention of biomedical researchers due to their high accuracy and automation, were able to address many of the challenges in increasing the speed and accuracy of diagnostic methods as well as personalization of therapeutic approaches.[166] A variety of microfluidic platforms are offered in biomedical applications and due to the advantages of these devices over traditional techniques, microfluidic technology is becoming a valuable portion in the field of personalized medicine.[43] Point-of-care (POC) diagnostics,[167] body

Review Highlights

What is the current knowledge?
- Genetic differences in individuals will affect their response to drugs and treatments.
- Microfluidic instruments have the ability to accurately control the internal conditions of the system, with use in biological studies.

What is new here?
- Different types of microfluidic systems with the ability to perform molecular tests have been invented for the purpose of quick and early diagnosis of diseases.
- Microfluidic systems with 3D cell culture capability are designed to regenerate organs and diseases in laboratory conditions.
- Organoid models allow for novel drug elucidation and researches in various human disorders.

on a chip, disease modeling, and rapid drug screening systems are all valuable microfluidic achievements in biomedicine.[135] Increasing advances in the design and construction of microfluidic systems in these areas will be promising for further advances in the early diagnosis and the personalization of treatment methods.

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