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Review Article

Recent Advancements In Microfluidic Organ On A Chip Technology: A Short Review

Vinita Patole[b], Sneha Mali[a], Mayuri Bhosale[b], Radhika Baheti[b], Santosh Bhujbal[a*]

[a]*Department of Pharmacognosy,* [b]*Department of Pharmaceutics, Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune 411018, India*

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An emerging idea known as "Organ-on-a-Chip" has possible applications in the investigation of the physiology and pathophysiology of tissues, including the determination of drug toxicity, and the fabrication of tissue models which could substitute animal experimentation. Organ on a Chip as a significant research platform entered in the current frontier of medical science and drug discovery due to a number of benefits, such as a reduction in sample utilization, improved separating methodology, analytical precision and high-throughput characteristics, flexibility, and reliability. This paper gives an overview on the concept of an organ on a chip and its scientific advancement in the past few years. The review also discusses the primary models developed and utilized for drug discovery and development along with their recent advancement, limitations, and future applications of organ on chip technology.

Introduction

New drug discovery attempts have increased costs and a poor chance of succeeding in clinical trials, owing to a lack of accurate preclinical in vitro models and emphasis on animals in research, which often fails to precisely replicate human reactions [1]. Consequently, critical human pharmacokinetics and pharmacodynamics properties, like elimination, tolerance limits, safety, and efficiency, are often incorrectly predicted [2]. Developing new pharmaceuticals should be examined at three stages: preclinical, clinical, and post-approval. Cell culture and animal studies are the two types of research included in preclinical investigations [3]. Techniques of cell culture consist of developing living cells in a plate; where there is no blood flow, and the conditions are different from those found in an organ [4]. Furthermore, data from animal research is not necessarily predictive of human responses. They are fundamentally limited by differences in anatomy and biological processes [5].

Drug screening is progressing towards quick, effective, and high-throughput development due to the ongoing development of screening approaches and technologies [6]. The microfluidic systems integrated with drug carrier supports dynamic control for movement of small molecule drugs [7]. In comparison to traditional approaches, the microfluidic system enables target specific and prolonged delivery, and improving safety and compliance by reducing pain [8]. Firstly, the concept of microfluidics was dedicated to considerably reducing sample usage and enhancing separation technique effectiveness [9]. As a miniaturization technology, microfluidic devices could deliver analytical accuracy and high-throughput capabilities without compromising precision and automation [10].

A microfluidic cell culture device called an "Organ on a Chip" is made using microchip manufacturing techniques and has one or more continually infused compartments packed with viable cells that mimic tissue or organ function [11]. Due to their small size, these chips have a larger surface area, resulting in high mass transfer and high analytical outputs [12]. The development of drugs and its impact on diverse organs are the primary features of Organ on Chip (OoC) [13].The first step in establishing OoC of any target organ is to reduce the organ to its fundamental anatomical parts which are essential for physiological functions particular to that organ [14].To determine fundamental cell structures with distinguishing characteristics, the morphological organization of the various cell and tissue types, as well as the biochemical and mechanical cues occurring in their local microenvironment, the functional units of the organ are extensively studied [15]. They are often composed of translucent 3D polymeric micro-channels layered with human cells which mimic three key characteristics of complete organs: 3D microarchitecture is characterized by the

* *Coresponding author*
E-mail address: santosh.bhujbal@dypvp.edu.in (**Dr. Santosh Bhujbal, Professor & HOD, Department of Pharmacognosy, Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Pimpri, Pune 411018, India**)

physical arrangement of various tissues, functional tissue interface as well as sophisticated organ-specific biological and mechanical microenvironments [16]. In order to overcome the difficulties of cell-based drug screening, microfluidic platforms were designed. These devices have advantages including reducing the volume of reagents needed and generating a 3Dcell culture that mimics both physiological and pathological micro-environment [17]. In this review, we address the significance of this emerging technique for basic and applied research.

Organ On a Chip

When compared to cells cultivated in dishes, an OoC is a microfluidic-based cell culture technology that provides a more physiologically accurate in vitro model. Constantly perfused compartments populated with living cells and configured to mimic tissue and organ-level physiology is a significant aspect **(figure 1)** [18].

In this micro-engineered 3D in vitro tissue model, numerous microfluidic channels connect micro-compartments. It facilitates the reproduction of the physiological environment of any organ [12]. It is comprised of four essential elements:microfluidics, living cells, drug delivery, and detection [19]. This component is typically distinguished by its miniaturization, integration, and automation. The living cell tissue component in 2D or 3D system refers to the factors that precisely match the cell type.

Organs on a chip - Design Concept

Micromachining and cell biology are combined in OoC which helps to control external environment and simulate physiology of cell. Cell patterning, concentration gradient, field shear force, mechanical stress are essential components on chip [98].

Field shear force: Micropump perfusion due to microfluidics maintains the dynamic culture of cells which is more predictable to in vivo conditions and also prevails organ polarity [99].

Concentration gradient: Microfluidics utilize microvalves and micropumps to alter flow velocity in important biological phenomenon like angiogenesis, invasion and migration and therefore gaining three-dimensional biochemical concentration gradient [100].

Dynamic mechanical stress: Elastic porous membranes are used in microfluidics to simulate mechanically stressed tissues such as cartilage, bone, skeletal muscles and blood vessels [101].

Cell patterning: The human body is complex system and consists of multiple cells which are to be aligned orderly. This cell patterning includes surface modifications, templates and 3D printing on the chip [102].

The cell sources used in OoCs are primary cells, stem cells, immortalized cell lines and gene edited stem cells. Potential materials used in chip production are Polydimethylsiloxane (PDMS), glass, thermoplastics, hydrogels, silicon, metals and membranes. Various fabrication techniques involved are soft lithography, hot embossing, injection molding and 3D printing [103].

Biomimetic compounds are typically used to create 3D structures. Such materials can prevent mechanical damage and form three-dimensional structures [20]. Several specific organ-on-chips have been created to date **(figure 2**), including lung on a chip [21], heart on a chip [22], liver on a chip [23], kidney on a chip [24], blood-brain on a chip [25], bone on a chip[26], bone marrow on a chip [27], gut on a chip [28], skin on a chip [29], as well as multiple organs on a chip[30].

Lung-on-a-chip

Lung on a chip is one of the first anticipated and produced OoC which offers innovative techniques to develop biomimetic respiratory system micro-environment as well as in-vitro development of respiratory disease modelling techniques [31]. The study aimed to show that multi-layered microfluidic devices can be used to create differentiated airway epithelium exhibiting morphological and secretory characteristics like those reported in vivo [32]. Indeed, research provides biomimetic microsystems that help in assessing the fundamental aspect of the lung, particularly the contact betweenalveolar and capillary membranes [33]. The most recent lungonachip design comprises two micro-channelled exterior portions, a flexible and porous Polydimethylsiloxane (PDMS) membrane to separate the air-blood chamber, and auxiliary vacuum channels that allow stretching of the PDMS membrane to replicate lung movement processes [34].Hugh and colleagues recreated the blood-air barrier to develop the first lung on a chip[35].In 2014, Sellgren et al. constructed a compatible microfluidic system that

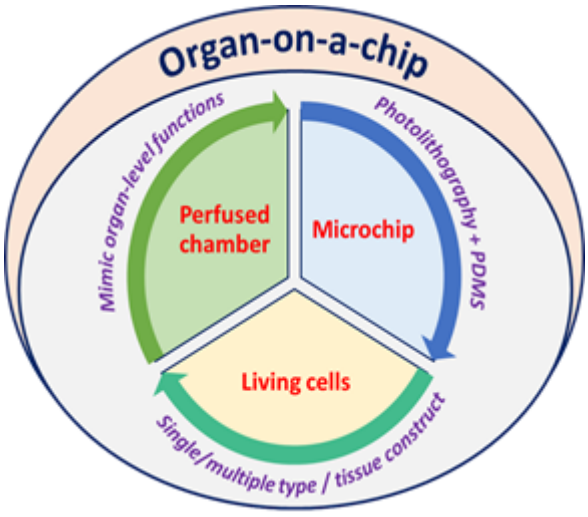

Figure 1: Composition of Organ on a Chip

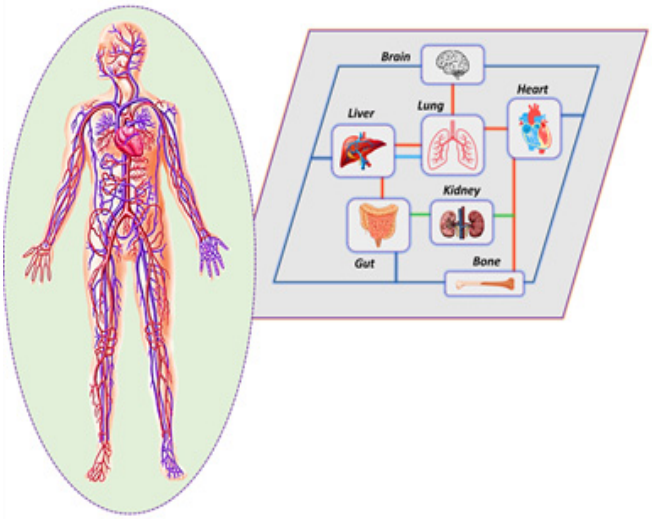

Figure 2: Integration of multiple organs on a chip

included three independently accessible channels, and vertical layered sections separated by a synthetic nanoporous membrane [36]. Furthermore, Benam and colleagues. proposed a chronic obstructive pulmonary disease model utilizing viable human epithelium tissues of bronchioles attached to a device which "breathes" cigarette smoke into and out of a small lung on a chip [37]. Biomedical experts, physicists, and engineers have recently been captivated by the confluence of diverse methods [38].

The lung-on-a-chip is applied in drug evaluation and model establishment for diseases associated with lung. Diseases are not limited to lung tumor, pulmonary thrombosis and pulmonary oedema but also helpful in lung viral infections such as Influenza virus, SARS-CoV-2, Rhino virus, Pseudo type virus [105]. Coronavirus Disease 2019 (COVID-19) is a severe pneumonia pandemic caused by SARS-CoV-2, a beta coronavirus that emerged in late 2019, leading to a global outbreak. Cao, T., et al. developed a 3D biomimetic alveolus-on-a-chip that can replicate the necessary physiological characteristics of human alveolar units. This study provided a reliable foundation for studying viral infection at organ level. Chemicals that are similar to viruses and pseudo-viruses were used to investigate the ability of antibodies to block viruses during infection and virus pathogenesis. Inflammation response and immune system was assessed by giving poly(I:C) treatment, which simulated viral infection. The efficacy of antibody inhibition was also evaluated using SARS-CoV-2_del19AA-GFP pseudovirus infection. This 3D biomimetic alveolus-on-a-chip was found to have great potential to study human lung at physiological and pathophysiological level as compare to 2D monolayer cell culture [106].

Heart-on-a-chip

Heart-on-a-chip received a lot of attention as a technique for creating in vitro heart models. Numerous fabrication techniques have been created, and various purposes, including illness modelling, physiology studies, and drug screening, have been suggested [39].The heart on a chip has four parts: microfluidic chip, cell or micro-tissues, micro-actuator to create the micro-environment, and micro-sensors for measuring outcomes [40]. Among the newest works is a platform in which micro-engineered cardiac tissue was designed to support human cardiomyocytes beating in three dimensions [41].Kamei et al. constructed the whole heart on a chip utilizing living tissue. The microfluidic device had a micropump as well as pneumatic valves to mimic how the heart works and check the anti-cancer drug doxorubicin [42].Heart on a chip was designed by Varghese et al., who studied the contractions of cardiomyocytes when provided with electric stimuli [43]. Zhao and his colleagues designed a heart on a chip with bio-wire constructions that may be utilized for testing of drugs and gene expression analysis. And could determine both contractions strength as well as the calcium content [44]. Wu et al. designed a chip to replicate the circulatory system [45].

Cardiovascular disease remains a leading cause of death worldwide. Myocardial infarction or heart attack is caused by platelet-rich thrombi that occlude the coronary arteries. Recently Berry, J. et al. developed a microfluidic device called "occlusive thrombosis-on-a-chip" to address the shortcomings of in vivo models, specifically their unpredictability and lack of relevance to human disease. In this study a device which was made up of poly(dimethyl siloxane) was able to monitor the effect of antithrombotic drugs on occlusion time in an objective manner without any bias. Eptifibatide, an approved anti-platelet drug was used to record a difference in occlusion time in developed microfluidic devices compared to control. Different results were shown by eptifibatide in quenched and unquenched devices due to downstream EDTA quenching of coagulation. Thismicrofluidic device is novel and durable, and it produced consistent results in producing occlusive thrombi at shear rate of artery. Therefore, this technology provides insights into cardiovascular health and can be valuable for drug testing and understanding the mechanisms of heart related diseases [107].

Liver on a chip

Depending on in-vitro experiments, studies of liver disease mechanisms focused on drug screening and hepatic tissue engineering techniques. Liver on a chip and micro platform-based bioreactors that can create precisely specified microenvironments have been developed in response to the limitations of conventional liver models [46]. The liver is the primary part of the human body engaged with metabolism; therefore, the potential to imitate liver functions is essential for drug development [47]. In vitro hepatic models attempt to simulate functioning and imitate both the regular and abnormal physiology of the liver [48]. Recent advances throughout the creation of 3-D hepatic modelling, as well as the comparatively simple need for producing the required, have all contributed to prevalence of liver-on-a-chip systems in use today. The 3-D liver model used in the liver-on-a-chip technology described by Bhise et al. uses bio printed HepG2/C3A spheroids. The PDMS chip is divided into three compartments: two external compartments attached to a syringe pump's nozzle, and a core bioreactor with the spheroids. Spheroids were tested for their generation of albumin, transferrin, ceruloplasmin, and alpha-1-antitrypsin and determined to be consistent throughout 30 days. The spheroid's responses towards acute acetaminophen shown to be equivalent to what was shown in animal experiments, proving that OoC can imitate drug-induced liver damage [49].

Tumor microenvironment that surrounds the cancer cells has a significant impact on the progression of disease and the effectiveness of treatment. Shen, P. et al. developed hepatocellular carcinoma (HCC)-on-a-chip according to the standard photolithography method to mimic liver cancer. This chip was composed of tri cell culture (tumor cells, endothelial cells, hepatic stellate cells (HSCs)) to evaluate the effect of HSCs on HCC. The results revealed that HSCs were responsible for drug resistance, endothelial invasion, tumor cell proliferation, natural killer(NK) cell exhaustion and infiltration. LIPOCALIN-2 (LCN-2) played a major role in remodelling tumor microenvironment and this was evaluated by RNA sequencing analysis and cytokine array. LCN-2 was targeted by various drugs such as 5-fluorouracil, sorafenib, and oxaliplatin to demonstrate anti-tumor effect in vivo mouse model and in vitro biomimetic chip. It was concluded from this study that microfluidic platform can be used to mimic tumor microenvironment which proves to be helpful in drug screening, developing personalized and targeted anti-cancer therapy [50].

Kidney on a chip

The key functions of kidney comprise the filtration of blood to eliminate toxins, regulating the body's fluid-electrolyte balance, and maintaining metabolic homeostasis [51]. The construction of kidney-on-a-chip was improved to facilitate drug discovery and to better comprehend the physiological alterations of specific proteins and associated interactions[52]. Cell lines such as Madin-Darby canine kidney and porcine Lilly Laboratories cell, pig kidney cells are currently used in kidney on a chip, which is easily accessible, create compact monolayers and are not human-derived[53]. A system of hypertensive glomerulopathy was created by Zhou et al. using the glomerulus on a chip concept. Further proving the usefulness of these devices as glomerulopathy disease models, Wang et al. further revealed employing a glomerulus on a chip microdevice to evaluate early diabetic nephropathy [54]. A design for mimicking a nephron was put forth by Weinberg et al. in 2008, and it included a system made up of the glomerulus, PT, loop of Henle, and connector [55].

Chronic kidney disease (CKD) remains a significant global health

challenge. Perin, L. et al. fabricated an innovative "glomerulus-on-a-chip" (GOAC) first by developing extracellular matrix channel followed by two step seeding process i.e., podocyte seeding and glomerular endothelial cell seeding that imitates the characteristics and functions of the glomerular filtration barrier, including its reaction to damage. This system recapitulates permselectivity which is one of the structural functions of the in vivo glomerulus. The results confirmed that this system is impermeable to physiological albumin concentrations while inulin can diffuse freely. When exposed to nephrotoxic agents such as high concentration of glucose or puromycin aminoglycoside leads to enhancement in albumin leakage and loss of albumin impermeability. When the chip is exposed to blood serum from patients who have anti-podocyte autoantibodies, it shows albumin leakage while this leakage does not occur when exposed to serum from healthy individuals. This confirms that the chip responds to injury in a functional way. Hence, GOAC platform can be employed for drug testing and disease modelling of different chronic kidney diseases [108].

Blood brain barrier-on-a-chip

The blood brain barrier (BBB) models based on microfluidic technology are of special interest because they offer a cutting-edge method for doing research on the brain, including high-throughput drug screening and modelling of neurodegenerative illnesses. Animal models, cell-based Transwell assays, and parallel artificial membrane permeability assays are the three traditional methods used to evaluate transfer across the BBB. However, the first two techniques have problems such as ease and an absence of physiologic significance, while the latter has had limited results in anticipating medical outcomes for humans due to cross-species variances. [56]. Because of advancements in microfluidic technologies, nanostructures, and embedded sensors, a newly developed in vitro organ-on-chip BBB models has emerged [57]. Microfluidic in-vitro BBB models can be used to provide more accurate dimensions and geometries for BBB modelling, as well as to expose endothelial cells to physiologic fluid flow. In "BBBs-on-chips," one may directly analyze functionality: the permeability of the cellular barriers, as well as marker expression that could provide information about the organ-level function. Transwell models, for example, are commonly used to evaluate permeability, by incorporating sensors and actual sensor data, BBBs on chips have the possibility to measure more BBB functions. The complex and different mechanism of leukocyte recruitment at the BBB as that of leukocyte extravasation in the lung-on-a-chip during bacterial infection is not studied in Transwell [58].

The choroid plexus (ChP) is a secretory tissue which is highly organized with complex vasculature present withinbrain's ventricles. It is responsible for production of cerebrospinal fluid (CSF) which acts as a barrier to keep blood and CSF separate. Thus, remodelling the unique properties of the ChP in a microenvironment that is physiologically relevant is challenging. Lim, J., et al. developed ChP-on-a-chip that mimics ChP pathophysiology and immune responses by demonstrating cytotoxic effects of macrophages which was helpful in modelling brain metastasis. The microfluidic device was engineered using 3D printer and extracellular matrix was made up of laminin for constructing epithelial and endothelial barriers of brain. The pulsatile flow of CSF was produced by using rocking system and evaluated by computer simulation and image processing. This study stated the use of Chp-on-a-chip for drug screening and immune oncology screening by spreading breast cancer cells (HER-2) on chip and targeted trastuzumab to evaluate drug response. Therefore, human brain microenvironment can be created on a chip for development of anti-cancer therapeutics to treat cancer that have spread in choroid plexus [109].

Bone on a Chip

The majority of research on bone regeneration and cancer-mediated bone destruction use traditional in-vitro 2D cell cultures or in animal models, however, these strategies are not able to simulate the 3D nature of the bone extracellular matrix or a significant number of dynamic cell-cell and cell-matrix interactions that seem to be vital for physiological bone function. Microfluidics has already been proposed as a method to imitate the physiologic microenvironment of various tissues in order to get around these challenges [59]. In 2022, Galván-Chacón et al., published a paper describing bone on a chip where advance techniques like creation of 3D model with two-photon polymerization (2PP) laser lithography by a 3D phase-contrast nano computed tomography scan of trabecular bone are involved that may be used to examine bone regeneration processes and mimic the physiochemical properties of microenvironment of bone. A direct printing process known as 2PP allows for the production of products with sub-micrometer precision for uses such as medical applications [60].

The process of bone remodeling relies on the balance between bone formation and resorption. Mesenchymal stem cells play a crucial role in tissue engineering. Alamán-Díez, P., et al. developed a 3D bone-on-a-chip to promote differentiation and to mimic bone microenvironment. Pre-differentiated adipose-derived stem cells (ADSCs) were involved to develop collagen hydrogel-based model as they have several advantages such as quick doubling time, easy of tissue harvesting and self-renewal ability. To determine the differentiation of cells and the progress of the culture, certain osteogenic markers were evaluated within the microfluidic devices. These markers include calcium mineralization, alkaline phosphatase (ALP) activity, and expression of specific proteins such as bone sialoprotein II (BSP II), Osteocalcein (OCN), and dentin matrix acidic phosphoprotein I (DMP I). ADSCs differentiated for 7 and 14 days were used. The results shown that after 14 days of incubation, differentiated cells (ADSCs) developed a complete bone model within the microfluidic platforms. Cells that were differentiated for seven days also produced an early bone model, but the expression of osteogenic markers did not indicate a fully differentiated phenotype, even though they exhibited a coordinated cellular network. This study was successful in developing 3D in vitro bone model for personalized bone tissue engineering [110].

Bone marrow-on-a-chip

The microenvironment of bone marrow includes a complex set of biological, chemical, structural, and physical factors required to maintain the hematopoietic system's viability and efficiency [61].As the bone marrow plays vitalizing role in the process of haematopoiesis and constitutes a significant portion of the lymphatic system, its introduction to the field of organs-on-chips is regarded as a milestone [62].Red bone marrow is found in the medullary cavity of flat bones wherein haematopoiesis occurs. It is a flexible compartment of bone marrow that includes hematopoietic stem cells (HSCs), multipotent cells and terminally differentiated cells capable of developing all blood cells such as platelets, T cells, B cells, macrophages and erythrocytes [63]. Torisawa et al. used modified bone marrow to mimic the in vitro bone marrow responses to drug toxicity and the effects of poisonous protective agents from gamma radiation [61]. In 2018, Sieber et al. were the first to maintain the bone marrow hematopoietic microenvironment purely in vitro for a period of four weeks [64].

B cell acute lymphoblastic leukemia (B-ALL) is characterized by production of dysfunctional B cell blasts that eventually take over the microenvironment of bone marrow leading to disease relapse and chemoresistance. The current in vivo models fail to investigate these heterogenous mechanisms among different subtypes of B-ALL and bone marrow. Ma, C., et al. demonstrated a unique in vitro 3D organotypic "leukemia-on-a-chip" that mimics in vivo pathophysiology and heterogeneity of leukemic bone marrow. The biomimetic model was able to explore interactions between B-

ALL blasts and niche cells (endosteal osteoblasts, vascular endothelial cell) and was also able to determine different roles of niche cells such as cell proliferation, NF- êB signalling, and cytokine regulation. Single cell RNA sequencing was used to investigate B-ALL subtype-specific niche signals. Therefore, development of leukemia-on-a-chip was helpful in identifying heterogeneity of chemoresistance mechanisms and also in understanding of microenvironment during leukemia pathogenesis providing a base for establishing optimized therapy for relapsed and refractory B-ALL patients. In conclusion, preclinical use of leukemia-on-a-chip can be used for dose prediction, disease management and patient specific therapy screening [111].

Gut on a chip

An innovative and effective in vitro system for investigating the physiology, pathophysiology, and pharmacology of the human gut is provided by gutonachip (GOC) technology [65]. These OoC systems will contribute to improvements in the understanding and treatment of diseases like colorectal cancer [66] and inflammatory bowel disease [67].To focus attention on the pathological conditions of intestinal diseases at initial phases, the latest developments in GOC systems mimic gut inflammation and host-microbiota interaction. In particular, GOC model offers functional readouts for detecting the biological responses and trying to recreate the key elements of intestinal physiology. Relevant replicates gut aspects include barrier function, which can be achieved through the use of 2D cell cultures and 3D microstructures,and biomechanical cues such as shear force, an oxygen gradient, and mechanical distortions, which can be achieved through the use of perfusable chambers [68-71]. Using a gut on a chip system developed by Kim and Ingber, Caco-2 cells are subjected to physiologic external signals such as shear stress and periodic mechanical strains, that mimic peristalsis-like activities in vivo [72].

The gut microbiome has an impact on the biological processes of the organism. Over the past decade's static transwell and animal models were used to mimic intestinal physiology but they have certain limitations such as expensive, legal and ethical concerns. Thus, development of gut-on-a-chip is essential to analyse intestinal microenvironment and also to study interaction between host cells and gut microbiomes. Jeon, M. et al. developed gut-on-a-chip based on microfluidics to imitate mucus secretion, epithelial cell differentiation and membrane integrity of gut. Osmotic pump was used to create fluidic flow that mimic intestinal fluid. Lipopolysaccharide induced inflammation and injury was employed as disease model. The results revealed that prebiotics recover barrier function without overgrowth of bacteria thus defining therapeutic efficacy of prebiotics. In this study, effect of microbiome to intestinal epithelial barrier function was studied by using co-culture (Caco-2 cells, HUVECs) system. Hence, gut-on-a-chip helps to find out crosstalk between commensal gut microbes and intestinal epithelia thus providing a platform for drug development and disease treatment [112].

Skin on a chip

Miniaturized technologies such as dermal studies of topical formulations, pathology testing of skin disorder and cosmetic science are gaining popularity in diagnostics, therapeutic testing, as well as biomedical fundamental science[73]. Wufuer et al. described the 'Skinonachip' technology which was developed for imitating the structural as well as functional human skin responses. The three layers of the suggested model were used to develop human epidermal, dermal, and endothelial components. Each layer of the microfluidic system was differentiated from the others by porous membranes to enable intercommunication during the co-culture of human skin cells. To show the system's effectiveness, skin irritation and oedema were generated by applying tumour necrosis factor alpha to the skin layer [74]. Kim et al. developed a single-tissue replicated skin on a chip consisting of two channels separated by a red blood cell screen to evaluate responses of neutrophils to the presence of bacteria on skin [75-76]. To imitate epidermal and dermal tissues, Mori et al. developed a skin on a chip integrating perfusable vascular canals as well as an interface layer [29, 77]. Kim K et al. used a pumpless skin on a chip to test the efficacy of a medication which is derived from the product of natural origin *Curcuma longa* leaf extract and they investigated the antiaging effect [78]. Pumpless SOC employs a microfluidic chip that operates on the concept that if the chip is slanted the medium flow down the microfluidic channel down due to gravity [79].

Skin acts as a biological barrier to protect the body from foreign agents, environmental conditions and other harmful substances. Hence there is a need to access and detect skin allergens and irritants that are responsible for various skin disorders. Skin-on-a-chip is a recently technology that employs microfluidic system to evaluate cosmetics or drug candidates. Li, Q., et al. developed a biomimetic triple-well epidermis-on-a-chip (EoC) based on microfluidics to develop melanin mimicking model that exhibits epidermis barrier and being friendly for semisolid substances as the model allows effective testing of semisolid specimens. The EoC is composed of stable and well-differentiated epidermis including spinous, basal, corneal and basal layer along with epidermis markers (keratin-10, filaggrin and loricrin). In this study four different chemicals (glycerol, 1-bromohexane, cyclamen aldehyde, isopropanol) were tested for their penetration and irritation properties. They have also tested the whitening effect of a cosmetic by monitoring melanin production. Therefore, development of automated physiological chip is an effective strategy for potential measurement of skin evaluation indices, irritation, phototoxicity, corrosiveness, and permeation detection which plays an important role in development of cosmetic and pharmaceutical products [113].

Multi-organ on a chip

Organ systems inside the body are dependent on one another. Different cell and tissue types are mutually dependent on each other for their development and growth [22, 80]. Most of the single-cell types included in the models are unable in representing the relevant function of human organs. As a result, OOC technology has advanced over the past several years in the direction of integrating multiple organ functions on a chip [81]. Integrating a human on a chip with functioning organ components are present creates an effective tool for studying the effects of different drug delivery systems (including oral, aerosol, and transdermal administration) in human subjects [82]. MOCs have been used to investigate drug absorption as well as metabolism [83]. Wagner et al. created a multi-organ chip using human liver and skin co-culture to evaluate troglitazone toxicity on day 6 after incubation. Following a 6-day treatment, a dose dependent response to troglitazone was found [84]. Miller and Shuler designed a multi-organ chip system for a system of 13-organ containing different cell lines by replicating primary parenchymal organs and tissues that act as physiological barriers inside the body to study the transfer of biological substances to different organ for drug response [85]. In order to recreate the response for entire body based on compartmental cell culture, Vozzi et al. introduced a bioreactor for multiple compartments for building in vitro tools of multiple organs and body systems. Every compartment is created specifically to replicate a certain organ characteristic and microenvironment, and they are linked together to model inter organ or tissue interaction [86]. Shuler and Esch developed the system, which includes multiple culture chambers containing multiple cell types, to anticipate responses of entire-body to drugs during the Pharmacokinetic and dynamic process. They also introduced the physiologically based concept of pharmacokinetic-pharmacodynamic modelling[87].

Long-term testing of MOC platforms has been made possible by

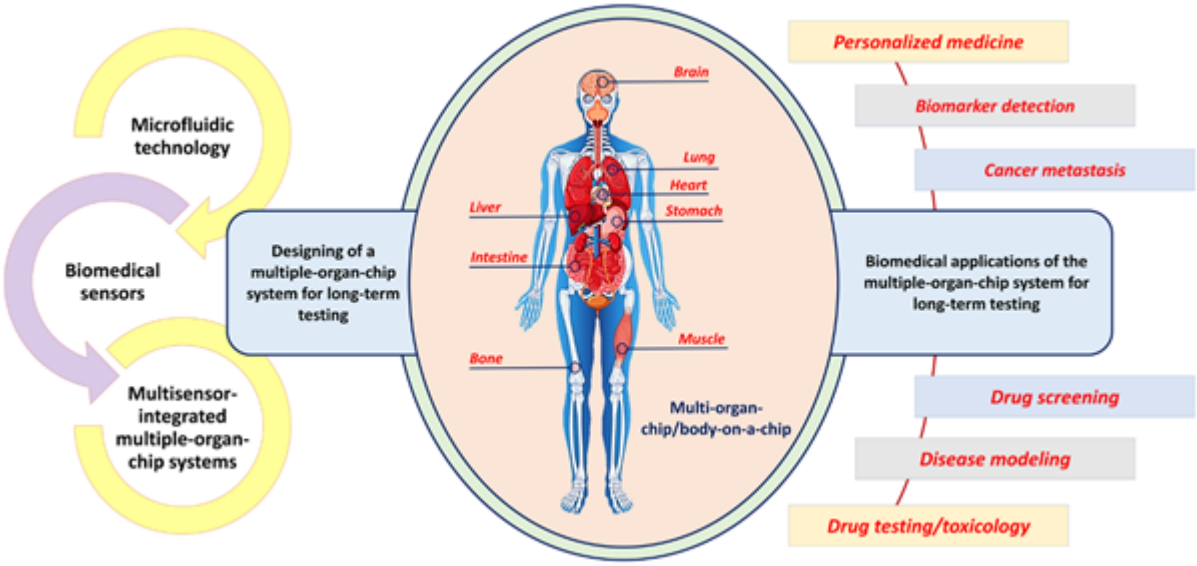

Figure 3: Designing and Biomedical application of multiple organ chip system for long term testing

the development of a wide range of MOCs that integrate microfluidic technology with biological sensors [88]. Many initiatives including tissue platforms or in vitro multi organ in the field of drug evaluation were proposed to achieve this goal [89-92]. A multi-organ disease model gives detailed information of human diseases, enabling the improvement of human physiological and pathological responses and elevates the efficiency of therapeutic strategies as compared to the traditional in vitro models and animal models [93]. Multi organ systems have gained a lot of interest and become a possible tool for screening of drugs such as anticancer. This is

Table1: Recent advancements in organ-on-a-chip with their applications

Organ-on-a-chip	Application	Ref.
Lung alveolus on a chip	To study acute radiation induced lung injury (RILI) useful for assessment of new radiation therapeutics	[114]
Lung on a chip	To mimic alveolar-blood barrier and exploring relationship between polystyrene nano plastic and COPD	[115]
Lung airway-on-a-chip	Mimics breathing cycle by incorporating bidirectional airflow and also demonstrate presence of epithelial glycocalyx and its role in different respiratory diseases and therapeutics	[116]
Lung on chip infection model	Investigate immune response of lung towards influenza A virus	[117]
Heart-on-a-chip	To determine optical and electrical dual response in testing hormone toxicity	[118]
Cardiac microtissue on chip	Human induced pluripotent stem cells (hiPSCs) were used to study the response of stimuli or drugs towards endothelial cell barrier	[119]
Heart-on-a-chip	Electrospinning and 3D-printing was employed to mimic cardiac tissues and explored it as preclinical platform for testing cardiotoxicity and drug efficacy	[120]
Liver-on-a-chip	Hepatic steatosis is modeled using HepG2 cell line to evaluate efficacy and toxicity of therapeutics	[121]
Proximal tubule-on-a-chip	3D cell printing is used to develop a model for determination of drug induced toxicity and therefore beneficial in drug screening	[122]
Gut-on-a-chip	The gut-immune-skin axis is explored to predict allergen sensitization of food (proteins)	[123]
Skin-on-a-chip	Melanoma-on-a-chip and breast cancer-on-a-chip was developed to model skin cancer, melanoma and metastases of breast cancer to the skin and also to determine effect of chemotherapy and photodynamic therapy in cancer treatment	[124]
Brain tumor-on-a-chip	Beneficial in brain cancer treatment by assessing crossing capability of blood brain barrier, side effects of nutlin-3a, an anticancer drug. This helps in selecting drugs and nanomedicines.	[125]

due to their ability of precise, comprehensive and reliable in vitro evaluation of drug potency [94, 95]. Cancer metastasis is a medical condition that requires the inclusion of multi organ model, in addition to the various uses mentioned above [96].

Applications of OoCs

Pharmacokinetics and Pharmacodynamics Analysis: During drug development OoCs play crucial role in preclinical assessment by investigating physicochemical properties and microenvironment, modelling therapeutic efficacy. OoC scan simulate real life in vivo states which helps to study drug metabolism into single and multi-organ models and provide treatment strategy along with personalized medicine.

Disease Modelling: OoCs are integration of physical, biochemical and electrochemical sensors which enables continuous dynamic monitoring of physiological parameters and microenvironment.

Safety assessment: Toxicity evaluation is possible using OoCs where the unsafe drugs are screened out. Mostly multi-organ models give predictive results for hepatotoxicity, cardiotoxicity, neurotoxicity and therefore minimizes the use of animals [104].

Future and Conclusion

Organ on a chip has been a trending technology for the last two decades as its potential to solve the critical challenges of the healthcare system is outstanding. Advancements in the organ on a chip help researcher to make real-time cellular and tissue level environments on small devices using microfluidics technology. The ethical concerns to use animal models in drug preclinical trials are getting fulfilled by the application of suitable OoC techniques. This magnificent technique will positively alter the pattern of preclinical trials in the future. Using the OoC technology and the infected cancer cells we will able to screen a variety of small and large molecules to find potential therapies for cancer. Similarly, this method will help to understand the critical pathways of various diseases in the microenvironment. New toxicological screening models using this technology will emerge to estimate the toxicity of the medicinally concerning molecules.

OoC is beneficial in drug development, disease remodeling, personalized medicine and toxicology research by employing different culture-based models. Despite these advantages OoC have few drawbacks. The surface effect is the first drawback that must be considered. Due to the minute size of the fluids, surface effects predominate over volume effects. This may be an indication of the low quality of the analysis, and some of the products of interest may have been adsorbed. A further disadvantage of these platforms is that specialized equipment is required to acquire accurate findings in certain studies [13]. Mimicking the entire complex and heterogenous organ is challenging. Current models in the market may not be sufficient enough to replicate the dynamic interactions and functions of entire organs which leads to a loss in real time monitoring of responses. Another challenge is maintaining and culturing cells on chips over an extended period of time can be difficult. This can compromise the reliability of experimental findings by affecting cell viability and functionality. Furthermore, the scalability of OoC is major problem. Integrating and modeling large and more interconnected multiple organ models on a single chip by maintaining accuracy is a major task and provides a scope for further advancement.

In the current review, we provided a comprehensive overview of current advancements in the application of OoCs. Microfluidics brought new concepts and effective approaches to drug discovery, primarily drug synthesis, delivery, and drug development. We anticipate this study will encourage scientists and the community to make crucial advancements toward moving the entire microfluidics-based drug discovery chain to a more adaptive, highly dependable, and efficient approach.Emerging OoC platforms will allow for actual, in-situ, and dynamic testing and inspection of a wide range of physiological parameters, including shear force, ionic strength, oxygen, cytokines, and chemokines, as well as off-chip assessments of molecular fingerprint, cellular physiology, and tissue pathophysiology utilizing conventional methodologies like Enzyme linked immunosorbent assay, Real time polymer chain reaction, and single-cell mRNA sequencing [97].

Future of OoC lies in refining the design of microfluidic channels, enhancing biomimicry to better replicate the complexity and heterogeneity of human body, and incorporating more advanced sensing capabilities for improved physiological relevance.

Stem cells are more likely to be the primary tissue source for Organ-on-a-Chip (OOC) models in the future. This is due to ease of extraction, higher physiological representativeness as compared to tissue biopsies many primary cell types. OOC models can be improved and stem cell methodologies can be enhanced through continued research into techniques for on-chip differentiation of stem cells into functional organ models.

Another advancement in future of OoC can be development of sensor integrated OoC by investigating different sensors such as biosensors, pressure sensors, optical sensors, microelectrodes, impedence sensors, force sensors, acoustic sensors to gather real-time data on cellular responses allowing for accurate representation of in vivo conditions.One possible use of OoCs is for preclinical testing of implantable devices. Microsensors and bioelectronics are combined to create these cells, which can simulate the tissue-implant interface. This allows for more accurate assessment of the effectiveness and safety of devices prior to implantation.

Moreover, there is a potential for tailoring OoC models to benefit patients with personalized and optimised therapy. 3D printing may be used for improving fidelity of OoC by providing an advantage in fabricating realistic tissue structures of complex organs on a chip e.g., 3D Inkjet bioprinted lung-on-chip. When designing microfluidic chips to replicate tissue function, it is important to consider biological, structural, and engineering elements. OoC can also be used to evaluate targeting efficacy and toxicity of nanoparticle-based drug delivery system. This organ-on-a-chip technology can be capable of studying pathophysiology of different diseases such as Zika virus, COVID-9, Middle East Respiratory Syndrome (MERS), microbiome related disorders, autoimmune disorders, neuropsychiatric disorders and rare genetic disorders.

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