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Review Article

"Organs on a Chip": Revolutionization in personalized treatment

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Abstract

"*Organs-on-a-chip*" (OOAC), involves microfluidics based biomaterial sciences, bio-engineering and cell biology majorly cell isolation and cell culturing aspects. This technology claims to develop 3-dimensional tissues structurally and physiologically in simulation to *in vivo* providing relevant results in terms of physiological and genetic aspects with virtue of its origin from human systems. In recent times, experts from diversified disciplines have developed and established many OOAC systems with an assertion of being perfect for drug research replacing convectional cell cultures and animal testing due to the technical limitations in the applicability of the same *in vivo* for systemic complexities and genetic variances. "*Organs-on-a-chip*" has attracted substantial interest for its wide range of applications in fields of drug research, regenerative medicine and personalized medicine. Successful development and establishments of different OOACs will contribute towards newer avenues in the path of precised personalized medicine.

Keywords: Organs on a chip, personalized medicine, OOAC, 3D cell culture

1. INTRODUCTION

In the constant changing world of clinical practice, one of the most challenging aspects is *personalized treatment* which leads to the development, identification and optimization of new drug candidates. [1-3] Drug development studies on a initial level use different animal models to predict responses of human pharmacokinetic. [4,5] Studies on drug development and drug testing in few instances produce non satisfactory and insufficient data as the cell lines/ animal models used are not completely suitable and fall short in prediction of pathophysiology of human disease, personalized drug sensitivities, and off-target drug toxicity. [6] There are different conventional *in-vitro* platforms developed which are being utilized for the study and identification of signal molecules (enzymes, receptors and ligands) involved in various physiological processes. However, these platforms do not mimic the extracellular mechanical environment, but the analytics performed are based on complicated cell-to-cell interactions in the body. [7] There are certain drawbacks of such conventional *in-vitro* cell culture methods, for example, the static condition with excessive amounts of nutrients, where mechanical or chemical stimuli or signaling molecules cannot be produced satisfactorily which is essential for functioning of the normal cells in a time controlled manner.

On the other hand technologies as "Microfluidics" are available that can be employed for generation of automated and time controlled different mechanical stimuli and also to study the concentration gradients of certain signaling molecules (including drugs). Natural polymers such as collagen, fibrin and agarose fabricate cell-laden microfluidic devices which provide an *in vivo*-like environment resembling living tissues. Amalgamation of microfluidic technology along with three dimensional (3D) cell cultures can be used to generate *in-vivo* like tissue analogs such as the emerging organ-on-a-chip system. These "organs-on-chip" models are cheap and offer a vast potential with highly reproducible results for cell physiology studies. It better mimics function and architecture of body tissues as compared to the 2 dimensional (2D) cell culturing models which lack the structural complexity present around cells in the natural environment. [8,9] In microfluidics, 3D cultures are advantageous than 2D cultures in drug related studies. Cells in 3D culture have gap junctions, which are crucial for cell communication processes, tissue integration and function. In case of drug diffusion, the rate of drug diffusion is much slower in 3D cultures unlike 2D cultures for the reason of multi-layer penetrability before reaching the potential target.

The "Organs-on-a-chip" models are based majorly on the knowledge advancements in tissue engineering and microfluidics enabling the precised design of customized micro-environments of the cells with better fluidic, mechanical and structural control. [10,11] "Organ-on-a-chip"

model is propitious due to the successful re-creation of the following three key aspects of human physiology,

1. Multicellular vascular or epithelial interfaces of organs [12]

2. Tissue-level organization of parenchymal cells [13,14] and

3. Systematic interaction of multiple organs. [15-17]

These attributes leads to a precised recreation up to the level of species-specific cellular structures. The precised the recreation of tissues is, the better would be the prediction power of *in- vitro* model, which is core essentiality of drug discovery.

2. *MICROFLUIDICS* AND "*ORGANS-ON-A-CHIP*" SYSTEM

Microfluidics can be defined as a science dealing with micro-scale handling of the fluid processes. The main purpose of this science is to precisely handle and control the microfluidic environment (10^{-9} to 10^{-18}L) in and around the cells by using channels that range in size from tenth to hundredth of micron. It is also termed as "lab-on-a-chip". [18-20] This field of microfluidics emerged largely in the late 1990s with introduction of poly-dimethylsiloxane (PDMS), which is an optically transparent, soft elastomer ideal for small scaled biological applications, [18,21-22] followed by the development of a range of microfluidic devices. These devices mimic all the diverse biological functions by culturing cells from blood vessels, muscles, bones, airways, liver, brain, gut and kidney. [23-27] The term "organ-on-a-chip" (OOAC) was coined by Donald Ingber, by forming a microfluidic chip to capture organ-level functions of the human lung. [12] The OOAC model is a biomimetic system having the ability to regulate key parameters including concentration gradients, shear force, cell patterning, tissue-boundaries, and integration of sample preparation to detection from the level of basic operating functions as cell culture, sorting and lysis. [28-31]

3. "ORGANS-ON-A-CHIP": A POTENTIAL RESEARCH ASSET

As a biomimetic system, there is a vast research potential of "organs-on-a-chip" system. It has been listed as one of the most emerging technologies developed with the amalgamation of multidisciplinary sciences. "Organs-on-a-chip" system is the most advanced form of 3D cell cultures with cell biology, bioengineering and material sciences as its backbone. It has been considered as tremendous ability to save the most valuable resource as "time". "Organs-on-a-chip" system also has an added benefit as it being based on microfluidic cell culture approach as compared to traditional cell culture methods. Research based benefits of "organs-on-a-chip" have been described further.

3.1. Replacement for animal disease models

"Organs-on-a-chip" system might be considered as a system with many possibilities in being a replacement of animal models for the discovery, development, and efficacy studies of drug molecules against many severe diseases. If "organs-on-a-chip" system is developed further and used for drug development, it might be able to save years of time required for the study of new therapeutic compounds' clinical trials. It might also be helpful in terms of saving a lot of valuable resources as this would replace the animal models and greatly relieve the animals from suffering which is bound to happen for the tests to assess the kinetics, efficacy and safety of drug candidates against fatal diseases as cancer, aids etc as these studies require animal disease models. [32] "Organs-on-chips" can directly culture human cells required for different research attributes, thus, gains an extra point for removal of inherent species barriers of species-to-species variations of differential gene expression profiles. [33]

3.2. Mimicking the micro-environment of tissue and organs

In comparison to traditional 2D cell culturing, organ-on-a-chip systems allows the controlled co-culture of different cells to mimic various structures and functions of tissues and organs, such as blood–brain barrier, lung and heart and other such interactions which requires more than one type of cells working in coordination in the same set-up. "Organs-on-a-chip" mimics physical and chemical signals and hence, produces a micro-environment analogous to the cell in terms of maintaining specific tissue characteristics, which might not be reproduced in 2D cell cultures. [34-35]

3.3. High level of integration and *in vivo* characterization

Due to the foresaid attributes, the "organ-on-chip" and/ or "lab-on-a-chip" systems can be utilized for the research and study of physiochemical characterization with high levels of integration of two or more cell types involved in certain physiological processes *in vivo*. This systems based on microfluidics, offers the possibilities of direct integration, hence, promoting the physiochemical characterization to utmost similarity to *in vivo*. Likewise, "organ-on-chip" can be used for different cell and organ cultures as stem cell-derived embryonic tissues, cardiac cells, connective tissues, which can be used for experiments in synchronization for research studies of the varied disease models. [36,37].

4. APPLICATIONS

The advantages of "organ-on-a-chip" endow it as a perfect model for the evolution of personalized medicine. "Organ-on-a-chip" models for individual patients can be created time and cost efficiently with a small cell sample and be harnessed to standardize the drug dosage, efficacy and safety and be tailor-made as per individualized requirements. There are a lot of possibilities for this technology in different applications areas which are yet to be explored to its complete potential.

4.1. Biological mechanism studies

"Organ-on-a-chip" technology enables studies of the structure and functions of a specific organ, along with their interactions between two or more organs efficiently. [38] For example, for confirmation of the regulating ability of Transforming Growth Factor-b1 (TGF-b1) to a four-tissue/ organ system (Liver, Lung, Kidney and Adipose tissue), an elementary "human-on-a-chip" has been reported by Zhang and colleagues, which provides a possibility to mimic the real human micro-environment *in-vitro*. [39]

4.2. Models for diseases and cancer

"Organ-on-a-chip" technology offers an excellent platform for different disease modeling, like pulmonary edema, protein-induced lung inflammation, central nervous system diseases and type 2 diabetes. [40-43] In recent past, malignant tumors in cancer disease have been reported to have the highest global fatality rate and is one amongst the top most risk factors for mortality around the world, which makes it a major subject of interest for the oncology researchers. Ling *et al.*, have utilized bio-printing technology and produced spherical cellular formations on a hydrogel array with *in situ* seeding of the cells on a chip. [41] Few more researchers too have used the same technology to develop tissue-like structures as "multi-organ-chip" with an ability to maintain the 3D structures from the organ of origin. [42]

4.3. Drug discovery and toxicity tests

"Organ-on-a-chip" systems have been reported to be more efficient in terms of results obtained from systemic substance testing and provide nearly accurate predictive cell culture models as compared to conventional cell cultures and animal testing. [43] Research studies around the world have developed many "organ-on-a-chip" systems as intestine-on-chip, liver-on-chip, kidney-on-chip and other organ-chips for systemic substance testing for drug efficacy screening and drug toxicity. [43-45] In addition, "multi-organ-chip" integrating different organs have also been buildup. Maschmeyer, *et al*., formed a multi-organ-chip platform integrating intestine, liver, skin and kidney that has a capacity to test systemic absorption and metabolism of the drugs. [44] Researchers have tried, tested and established "multi-organ-chip" system suitable for screening of drugs for its efficacy and toxicity as demonstrated by their experiments *in-vitro*, mimicking the physiological *in vivo* conditions and maintaining homeostasis with an *in-vitro* life of as long as 28 days as reported . [42-45, 49-50]

4.4. Regenerative medicine

Without the existing complications of using organ allografts, the reconstruction of organs can be of great use in regenerative medicine having a great potential to cure or replace directly damaged tissues and organs. In the context of regenerative medicine, "organ-on-a-chip" technology provides a broad platform to understand key aspects of how various fabrication strategies affect viability of cells and tissue functioning. "Organ-on-a-chip" system allows stem cell culture and its differentiation into specialized organ cells, which was further explored to fulfill the high expectations of regeneration of organ cells and/ or complete organs.[49-52] Park *et al*., explored the osteogenic differentiation potential and reported that human bone marrow-derived mesenchymal stem cells were found to be more effective when cultured in a micro-fluidic *in-vitro* environment facilitating the regenerative therapeutics. [53,54]

5. *IN-VITRO* RESEARCH MODELS

In-vitro research models of "organ-on-a-chip" technology are the need of the hour, to speed up the results of drug efficacy and toxicology studies. "Organ-on-a-chip" *in-vitro* research models are an essentiality in the fields related to clinical sciences, where time and specificity is an utmost requirement which cannot be compromised. This "organ-on-a-chip" *in-vitro* research models increases the productivity by reducing the cost and being species specific and in cases serving need of studies involving the systemic responses from multi-organs for a particular physical or chemical stimulus.

5.1. Lung-on-a-chip

The alveolar-capillary membrane, a bilayer interface constituted mainly of alveolar epithelial cells and microvascular endothelial cells which is the smallest structural and functional unit of the lungs. There are specialized epithelial cells at interface that produce mucus-containing antimicrobial and anti-inflammatory signaling molecules which are capable of triggering recruitment of immune cells to the infected areas. [54] A few interesting research studies on drug delivery have reported the use of lung tissue models generated from a single alveolar epithelial cell line with the ability to mimic the alveolar epithelium part of the lungs. [55,56] The most famous "organ-on-a-chip" model noted is the "lung-on-a-chip" also known as "breathing lung" developed by Ingber research group at Harvard University. [12] "Lung-on-a-chip" is replicated with alveolar epithelial and vascular endothelial cells being co-cultured in a microfluidic device with air-liquid interface.

5.2. Heart-on-a-chip

Cardiovascular diseases are amongst the top most risk factors for mortality in non-communicable diseases. The emergence of microfluidics has enabled *in-vitro* bionic studies of involving the myocardium, which enables direct access to cardiac tissues and study drug effects on beating cardiomyocytes and correlate the results on pumping of the heart. [57] Functional measurements of *in vitro* cardiovascular studies is important to study drug efficacy and toxicity. Grosberg and colleagues developed a system that was amenable to both striated and smooth muscle types with a feature of analyzing contractility concurrently on a single chip for both the muscle types. [58] An optimized 3D environment has been reported to yield relatively alienated functional cardiac tissues from human embryonic stem cell-derived cardiomyocytes which were used to study the effects of various cardiovascular drug effects in a PDMS model. [59] Zhang *et al.*, introduced the heart-on-a-chip device that used high-speed impedance detection that has assessed cardiac drug efficacy and cardiotoxicity as recorded by the electrophysiological methods. [60] Similarly, many researchers have also replicated the formation of Heart-on-chip which imitated native myocardium in functional and biochemical aspects with many refinements and user friendly aspects. [61,62]

5.3. Liver-on-a-chip

There were many post approval drug withdrawals (~30%), listing liver toxicity as the major cause as reported in studies in late 19th century. [63,64] Liver is the main organ to metabolize drugs entering the blood stream. The functional unit of liver, hepatocytes can be easily obtained from human biopsies and proliferated unlike cardiomyocytes. Heterotypic (crosstalk between different cell types) and homotypic (crosstalk between the same cell types) interactions between hepatocytes and stromal cells are crucial for maintaining hepatocyte functions *in-vitro*. [65-68] In earlier 21st century researchers have developed a model of functional units using hepatocytes to replicate the physiological mechanisms of the liver. [64] However, the mechanisms of action of many drugs are modulated by inflammatory pathways. Macrophage-like primary human Kupffer cells and hepatic stellate cells are co-cultured to improve accuracy of toxicity screening, into the system for the assessment of effects of pro-inflammatory cytokines on liver toxicity. [69]

5.4. Tumor-on-a-chip

The tumor microenvironment is a heterogenic and dynamically evolving molecular system in which cancer cells interact with each other by physical and chemical interactions. [70] Due to recent advances in microfluidic cell-based biochips that has led to the development of a physiologically relevant tumor microenvironment, which is one of the major factors that affects efficacy of anti-cancer drugs. [71,72] Moreover, to generate different gradients of drug concentration and to study personalized drug treatments, numerous chips have been developed aiming to reproduce the complex tumor microenvironment. [73] Recently, Kim *et al*., developed a fully automated and programmable microfluidic system for drug candidate screening applications which integrates '*on-chip*' generation of different drug concentrations with parallel culture of cells. [74] By exploiting the unique high throughput properties of microfluidic devices, the authors were able to study multiple drugs and concentrations at the same time, obtaining a more

physiological environment over conventional static culture platforms. [75]

5.5. Body-on-a-chip

Humans are composed of organs and tissues that possess multiple physiological roles and can be assumed to represent a kind of complex system. Single "*organ-on-a-chip*" models reflect the complexity, functional changes, and integrity of organ function but it fails to report the results where multiple organs are involved. [76] Hence to overcome this limitation of single "*organ-on-a-chip*" model, the "*multi-organ-on-a-chip*" models have been proposed and also developed successfully and attracted obvious research attention. [45-48,77] Body-on-a-chip devices coupled with pharmacokinetics models aim to mimic the physiological complexity of inter-organ interactions that might be used to observe continuous or linked pharmacokinetic processes such as ADME (absorption, distribution, excretion, metabolism) of various drug administration routes, and the data obtained may be applied to construct mathematical models for prediction of drug efficacy. [78-80] Although the "*multi-organ-on-a-chip*" concept remains in its infancy, major breakthroughs have been made which includes the design of two-organs [81,82], three-organs [83,84], four-organs [46,85], and ten-organs on the chip. [86]

6. CONCLUSION

"*Organ-on-a-chip*" systems have recently developed an interest from researchers all around the world due to well mimicked physical and chemical micro-environment of human cells which further aids drug efficacy and drug toxicity replacing animal models alleviating the ethical concern related to the same. "*Organ-on-a-chip*" systems also raise the bar for drug research providing species specific results with resource efficacy. Till date many "*organ-on-a-chip*" systems have developed representing different organs, some are also based on multiple organ systems which otherwise function together in human body, viz. heart-lungs. The establishment of multiple organ constructs on a chip creates physiologically relevant *in-vitro* models as compared to the animal models and 2D cell culture systems. Thus, the "*organ-on-a-chip*" system can be used to derive more pertinent information for drug research with genetic and physiological reliability. These systems are custom-built using cells of human origin with the use of tissue engineering and microfluidics which endows them has high throughput capacity and controllability. Different chips for different organs have been established and have produced a niche platform for the study of species specific systemic drug effects that may have its potential application in the development of personalized clinical therapies. Before completely replacing animal testing and convectional cell cultures, "*organ-on-a-chip*" systems has to fulfill the technical limits, which are largely compensated by accuracy of the results it provides.

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