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Body-on-a-chip: Microfluidic based futuristic approach for Drug Screening

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ABSTRACT

Preclinical testing using animal models has been crucial in drug development process, but issues of ethical factors and species variances still remain. However, human derived in vitro cell-based assays are being actively practiced to study drug's pharmacokinetics and pharmacodynamics. Although, current in vitro cell assays give the drug's potential therapeutic benefits, it is only specific to a tissue but not to an entire body to accurately predict the compound's efficacy, toxicity and inter-organ interactions. In recent years, ever-growing scientific research and innovative advancements in microfabrication technologies have impacted notably on bioengineering and biomedical engineering. One such approach is 'Microfluidics' that has emerged as a powerful tool to provide the opportunity to stimulate different organs. The technology of organ-on-a-chip has made it probable to physically and chemically recapitulate the in-vivo conditions by utilizing microfluidic approach. Till now, in vitro models of multi-organs including the gut, liver, kidney, lung, heart, and bone have been developed to mimic the organ physiology. Likewise, an integration of multiple organs on a microfluidic platform known as body-on-a-chip model has also been proposed to replicate organ cross talk. This review provides a overview on cell culturing within microfluidics systems, design, fabrication of biochips, organ and body on a chip models, and its applications in the coming future.

Keywords— *Microfluidics, organ-on-a-chip, body-on-a-chip, microfabrication, drug screening.*

1. INTRODUCTION

The current drug discovery route from research to development is a laborious, expensive process. Every new drug takes about 10 - 12 years to come to the market with the odds of <1 out 10 getting approved and its average capital costs about $2.5 billion during the R&D phase as estimated [1-2]. Most drugs fail due to insufficient achievement of efficacy, pharmacokinetics and in preclinical testing.

In preclinical trials, animal testing are the gold standards used to estimate the drug profiling for metabolism, toxicity, safety and investigating systemic interactions, however drug metabolic rate in animals and humans can vary extensively due to species differences. Often animal models do not foresee human response realistically [3]. In addition, issues of high handling costs, ethical considerations, and limited testing with inaccurate extrapolation of data from animal experiments to humans are associated with the use of animal models so these have also been the reason for the rejection of various drug leads earlier to clinical trials. It is likely that the trend towards cutback or ban on animal testing may perhaps be seen in drug discovery in the time ahead.

Presently, controlled culturing of cells derived from human tissues are an possible alternative for in-vivo animal assessment. The cell culture assays are an applicable means to test cytotoxicity, viability as an initial drug screening. Yet, these assays have setbacks, for example when cells are cultured in petri-plates or microtiter plates, cells can evidently lose their function, responsiveness and communications between organs which makes it difficult to directly record the responses. Consequently, there are dissimilarities between data attained from animal testing and traditional in vitro models for pharmacological forecasts [4]. Thus, an alternative, advanced model platforms are in great demand.

Microphysiological systems (MPS, commonly as organ-on-a-chip (OOC), body-on-a-chip (BOC)) have grown over the past years as an appealing system to probe the response to pharmaceutical drugs or chemicals [5]. These platforms indulge in the fields of microfluidics, tissue engineering and have significantly progressed from conceptual phase to developmental phase showing notable success in mimicking the physiological functions of in vivo tissues. The body on a chip technology purpose is to create an artificial microenvironment representing interactions among human organs with advantages such as modelling of tissue

microarchitecture, physiological flow conditions and kinetic diffusion of drugs compounds and signaling factors [6]. This review, focuses on the overview of design, working, potential applications of integrated body on a chip system.

2. CELL CULTURE WITH MICROFLUIDIC TECHNOLOGY

Development of these microfluidics approaches for cell cultures have opened entirely new promises to build in vitro models that re-comprise more intricate 3-dimensional organ structures. Microfluidics is defined as the precise control, manipulation of fluids which are confined in a microenvironment generally through microchannels. At microlevel, even though they are small, they have huge mass transfer capability and more surface area, favoring low usage of reagent, volume controllability, quick responses, high mixing rates and accurate control of physical and chemical properties [7].

Normally, cell cultured in 2D cultures lack the actual physiological functions of the human system. It becomes really difficult to sustain the cellular functions for long periods. In vivo, cells receive nutrients and oxygen through blood flow, also receive physical, chemical and electrical stimulation from the surrounding environment. For example, lung stretching movement, shear stress, chemical transmission in nerves and electrical impulses in myocardial tissues. While conventionally cells are cultured within a controlled artificial environment, where it depends only on diffusion for testing the application of candidate substances to the cells. Such dissimilarities of morphology and environment between in vivo and in vitro might be the reasons for failure in maintaining their cellular functionality in cultures. In order to overcome this, scientists have applied microfluidic technology to cell cultures to develop body-on-a-chip systems over the past decade and has been recognized as one of the most promising upcoming technology.

3. FABRICATION AND DESIGN OF BIOCHIPS

Biochips are fabricated through nano semiconductor fabrication techniques. In fabrication procedures widely used potential materials are observed to be polydimethylsiloxane (PDMS) or polystyrene (PS) or polymethylmethacrylate (PMMA) as it features biocompatibility, low cost and excellent oxygen permeability. To build microfluidic systems, numerous methodologies have been devised and used from a fabrication standpoint. The processes of (1) hot embossing and (2) injection moulding are two of the most common methods. Wherein, the first involves nano thermal imprint lithography which builds nanoimprint patterns for microfluidic-based products. The second is formed by pouring molten material into a mould where it cools and hardens according to the mould configuration [8].

The key elements of biochips comprise of (1) microfluidics; (2) live cell cultures (3) external stimuli and (4) real-time sensing. The microfluidic element is illustrated by automated, miniature structure of integrated system that consists of fluid culture input and liquid waste output flowing out throughout the culturing process, as well as the microchannels that deliver the medium for the cells in the defined area [9]. Live cell culture element refers to spatially alignment of certain cell type on biocompatible materials, like as hydrogels - which have the ability to resist mechanical damage in 3D arrangements. An external stimulus of dynamic mechanical cues and chemical signals are given to establish the actual in vivo conditions. A fixed sensor element or a optically visualized transparent chip-based visual function evaluation system is used to detect and compile data. Peel et al. [10] imaged multicellular OOCs using automated techniques, resulting in comprehensive cell types and mathematical models for estimation.

Amongst the first, organ-on-a-chip model was developed by the scientists from the Wyss Institute [11] which was made of transparent elastic polymer material in the size of a microscope glass slide. It comprised of two hollow parallel microchannels about 1mm wide and porous membrane parting the two channels. Organ-specific cell cultures were on one side of the parenchymal channel, while vascular endothelial cells mimicking blood flow vasculature were on the other. Each channel was perfused with cell type-specific media separately. The porous membrane was semi permeable to chemicals including cytokines, oxygen, nutrients, growth regulators, and drug break-down products disposed while metabolic reactions in the organs, to communicate and exchange between the two compartments. This development of miniatured organ encouraged the scientists to further research and experiment with various other organ cell types and integrating multi-organ microfluidic models.

4. ORGAN-ON A-CHIP TECHNOLOGY

So far, scientists have suggested organ-on-a-chip systems that mimic diverse organ and tissue activities. Although it is beyond the scope of this study to provide an overview of all of these devices, we do provide some of the developed in vitro models that reflect important organs such as gut, liver, and kidney in this review as they are essential in drug metabolism.

4.1 Gut on a chip model

Gut models provide a robust platform to investigate the physiology, pharmacology and etiopathology of the human gut. Gut plays an essential part in proper functioning of different organs, as its primary functions is to take up and transport electrolytes, drugs, nutrients from the digestive tract to the blood stream for dispersal throughout the body. As a result, it is important for administration of orally consumed drug metabolism. The presence of microbiota and specialized cells called villi, microvilli in the gut makes it part of both immune and endocrine system of the body [12]. As the name suggests, it aims to recapitulate the barrier of the intestinal lumen and blood vesicles. The organ specific, intestinal epithelium cells used in these models are commonly Caco-2 cell type along with microbiota and the cells to represent blood vesicles used are human intestinal microvascular endothelial cells (HIMECs) and human umbilical vascular endothelial cells (HUVECs) [13]. Kimura et al [14], devised a gut-on-a-chip (fig 1) device having a visual evaluation system for long term monitoring of cell cultures and to assess the distribution of rhodamine 123 by fluorescent based on-line assays. Also, device performance was characterized by monitoring the cells polarisation transport activities. The mechanical stimuli in presence of flow rates through the channels impose a biomimetic shear stress in the chip. Different analytical tests like photo-analysis, confocal microscopy and fluorescent tests are commonly done to

interpret cell arrangements and polarization. Released effluents and media composition are assessed for metabolites, dissolved O2, signaling molecules pH, ion and drug concentration.

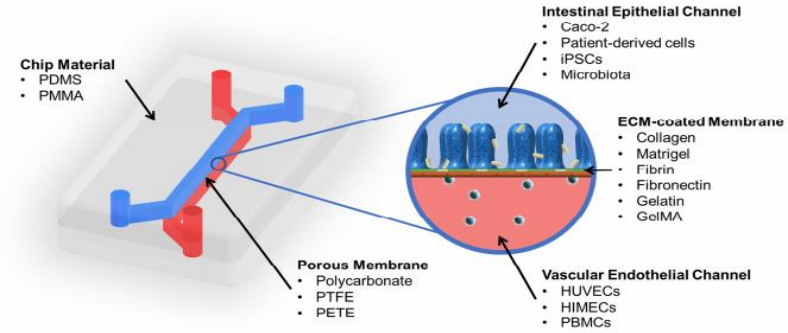

Fig 1: Potential materials and elements used in gut on a chip model. [15]

4.2 Liver on a chip model

Liver has a multifaceted physiology constituting different cell phenotypes such as hepatocytes, fibroblasts, stellate cells and kupffer cells making it a versatile organ as a whole. Each zone of the liver involves in different level of drug metabolism and clearance along the central vein to portal vein. Liver performs numerous pivotal functions to maintain proper physiological processes, including blood sugar and ammonia regulation, hormone production, endogenous and exogenous chemical detoxification, protein synthesis and glycogen storage [16]. Liver renders immense complexity for recapitulating its organ physiology, unlike liver-on-a-chip (fig 2) technology has offered models to co culture different cell architecture, hepatic zonation and tissue hierarchy within bio printed scaffold. Continuous perfused cell culture medium was proposed by Powers et al. [17], including 3D scaffolds, cell-holding filter and structural support to allow perfusion over top of the arrangement and via cell masses in each channel. Fluid shear stress was provided through flow rates according to the estimated cellular oxygen consumption within the functional range of 2 dyne/cm^2. The findings explain that the device formed hepatocellular aggregates resembling structures of hepatic acini and sustain its viability for 2 weeks. However, scaffolds impose problems too such as inherent stability and its unpredictable effects on signaling pathways, thus Weng et al. [18], designed their device by scaffold-free using hepatic stellate cells with hydrophilic flow for cell adhesion. Peristatic pump was used for medium circulation along the six inlets shaped in hexagonal arrangement and a central outlet. The flow passed through the hepatic cord radially simulating biomimetic radial flow in the liver, further scanning electron microscopy progressed in examining culture morphogenesis and measuring urea and albumin levels for liver functionality study. To evaluate the competence of drug metabolism and clearance of the device, the activity of cytochrome P450 3A4 (CYP 3A4) were quantified, as it is one of the most important enzymes involved in the metabolism of xenobiotics. It was observed that, this metabolic activity had sustained for weeks in liver-on-a-chip model.

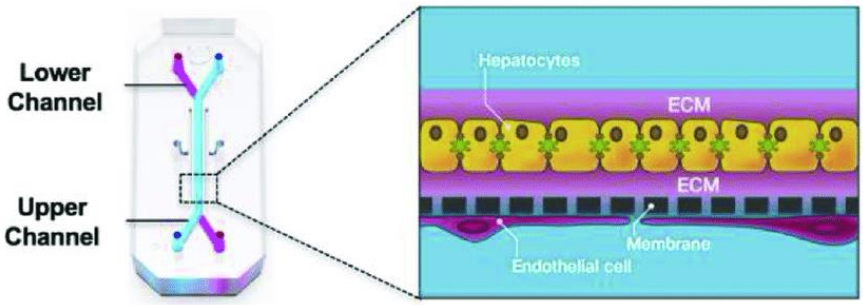

Fig 2: Liver on a chip model [19]

4.3 Kidney on a chip model

Kidney is a filtering unit of the body, made up of nephrons which controls the fluid balance in the body and eliminates waste products by performing the complex mechanisms of filtration, re-absorption, and excretion. Kidney is more susceptible to damage as it is a target of drugs, chemicals, heavy metals and toxins leading to nephrotoxicity. In kidney-on-a-chip model (fig 3), attempts have been made to develop that bio-mimic different segments of nephrons namely glomerulus, proximal tubule and distal tubule or collecting duct. As of glomerulus models, Zhou et al [20], designed the chip comprising closely opposed layers of human endothelial glomerular cells and cells of murine podocyte precursor with two compartmentalized channels. This successfully modelled hypertensive nephropathy, cytoskeletal rearrangements, damage to cells and their junctions that led to increased glomerular leakage by fluid flow. Proximal tubule models were created by bioprinting proximal tubule epithelial cells which were continuously subjected to osmotic variations and fluidic shear-stress gradients. Homan et al [21], housed actively perfusable tissue chips in which cells were fully embedded within an extracellular matrix through the open lumen. They examined for nephrotoxin and cyclosporine A and showed a response in dose dependent manner. Distal tubule models were cultured using Madin-Darby canine kidney cells (MDCKCs) in the microfluidic channels made of fibronectin-coated PDMS. The culture compartments were linked to the vascular channel by a second layer. But it was observed that when flow rates similar to those seen in vivo (50 L/min) were given to the system, it hampered cell proliferation and survival [22]. Kidney-on-a-chip models of each separate sections in a nephron could mimic the in vivo physiological activities to an extent, but until now, a complete nephron-on-a-chip system with integrated individual elements has not been realized [23].

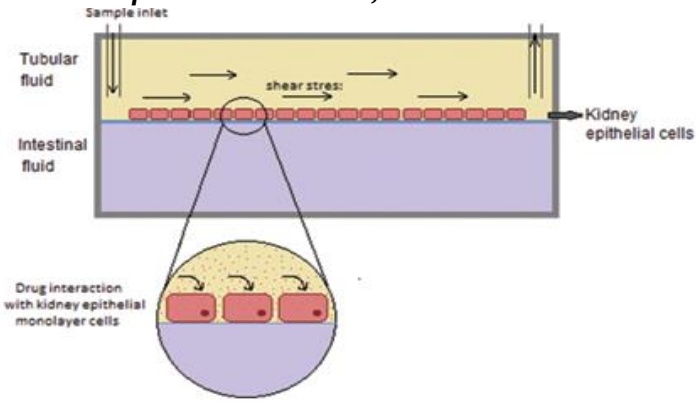

Fig 3: Kidney-on-a-chip model [24]

4.4 Body on a chip model

The human body is composed of multiple organs interacting together to perform the complex physiological processes. In recent years, organ-on-a-chip technologies have gained tremendous momentum and hence, scientists have proposed a relative technology of creating a human body chip. Body on a chip focuses on the combining different organs models into a single chip which emphasize on answering organ-organ crosstalk, pharmacokinetics and metabolic effects in pre-described organ levels.

In recent times, Novak et al [25], presented a highly modulated platform which was operated via automated fluid handling apparatus named the 'Interrogator' instrument. It has a capability of culturing ten different organ on a chip model and transfer liquids across the cell layer of vasculature to stimulate the in vivo condition of normal circulation system. This employs perfusion, media flow, liquid exchange, collection of samples, convention software, fluid-transfer automation for programmed culturing and connected microscopy for imaging the cell organization inside the organ chips. In this work, 8 organ chips i.e., heart, liver, lung, kidney, skin, intestine, brain and blood-brain barrier were serially linked along their vascular channels, that perfused channels containing organ-specific cells and a highly optimized common blood substitute. For over three weeks, the viability of all tissues and their organ-specific functions were maintained which allowed to quantitatively analyze the tissue-specific distribution of a chemical across the system. While testing the instrument, configuration of gut, kidney and liver models linked by a central arterio-venous (AV) fluid mixing reservoir (as shown in Fig 4) were used, allowing for realistic blood flow and drug exchange between the individual organs. Nicotine, a oral drug for neurodegenerative and inflammatory bowel diseases was administered using mass spectroscopy. The computational and experimental data demonstrated the capability to model drug uptake, metabolism and accurately predict changes in drug blood levels that have been previously seen in human clinical trials. These experimental advancements have led to increased research on body-on-a-chip systems.

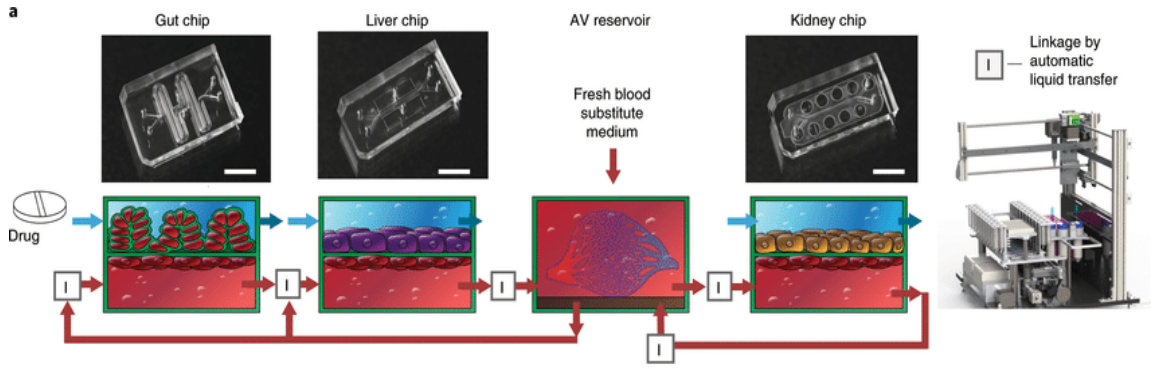

Fig 4: Integrated system of gut, liver and kidney on a chip model [26]

5. APPLICATIONS AND FUTURE PROSPECTS

Multi-organ on a chip can be encompassed into drug discovery and development workflows and increase translation of pre-clinical assays. ADME (Absorption, Distribution, Metabolism and Excretion) responses, drug-drug interactions and safety or toxicology of drug can be analyzed without the use of animal models. Fluidically linked organotypic models allow modelling of complex disease processes, biodistribution of molecules, organ-organ crosstalk and find new therapies. Ultimately, with progressive development, it might be possible to create biochips that are made solely from an individual's stem cells. That would be personalized medicine at its best, understanding your specific disease and using your cells to determine the most effective therapy for you.

6. CONCLUSION

Body on a chip technology renders a novel system which can replace the existing drug discovery process and accelerate the drug evaluation method by presenting a more definite replicate of the human physiology and various inter-organ communications using engineered biomaterials and microfluidic techniques. These platforms show promising revolution from two-dimensional cell cultures to microfluidic cell cultures. It would likely change the dependency on the need of animal models in preclinical testing phase of drug discovery. Although there are challenges to commercially implement this, it poses as a remarkable way to perform precision medicine, disease modelling, common cell culture media development, and drug development.

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