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Original Article

Organ-on-Chip and Digital Twins: Emerging New Approach Methodologies for Advancing Animal Free Preclinical Drug Testing- Challenges and Future Perspectives.

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ABSTRACT

Preclinical drug testing plays an important role in identifying the safety and effectiveness of new medicines, but conventional models do not always reproduce the complexity of human biology. Two-dimensional cell cultures provide useful information but have limited ability to mimic tissue-level interactions, while animal models may show differences from human physiology. In this context, organ-on-chip (OoC) technology and digital twins are emerging as complementary approaches within New Approach Methodologies (NAMs). OoC platforms use human-derived cells, controlled fluid flow, mechanical stimuli and tissue-specific microenvironments to reproduce selected aspects of organ function in vitro. Digital twins, supported by approaches such as physiologically based pharmacokinetic (PBPK), pharmacodynamics (PD), quantitative systems pharmacology (QSP) and data-driven modelling, can use experimental and patient-related information to simulate biological responses and treatment scenarios. This review discusses the principles, pharmaceutical applications and current evidence supporting OoC and digital-twin technologies in preclinical drug development, including drug efficacy, toxicity, ADME and pharmacokinetic assessment. Particular attention is given to the possibility of linking organ-chip-generated data with computational models to improve interpretation and translational prediction. The review also highlights challenges related to validation, standardization, reproducibility, scalability, data integration and regulatory acceptance, while discussing future opportunities for more human-relevant and animal-sparing preclinical research.

Keywords: Organ-on-chip, Digital twins, Preclinical drug testing, New Approach Methodologies, Microphysiological systems, PBPK, QSP, Drug development, Non-animal methods

INTRODUCTION:-

Organ-on-a-chip (OoC) is a micro scale platform that can recapitulate the key structural and functional features of human organs [1, 2]. This Technology overcomes the limitations of traditional two-dimensional cell culture, better mimicking the in vivo microenvironment and providing a more reliable platform for drug development, disease modeling and toxicity testing [3, 4]. The Core components of the OoC are microfluidic channels, cells Culture chambers and biomimetic scaffolds [5, 6]. Among them, The biomimetic scaffold is a supporting structure for cell attachment, growth and differentiation, which not only provides physical support but also regulates cell behavior through its biochemical and mechanical properties, mimicking the extracellular matrix (ECM) environment in vivo [7, 8]. The choice of biocompatible scaffolds in OoC directly influences The performance and reliability of the chip [9]. An Ideal scaffold should possess good biocompatibility, appropriate degradation rate, mechanical properties similar to natural tissues, and adjustable pore structures and surface characteristics [10]. Organ-on-a-chip technology next uses microfluidics to simulate in vivo research while Maintaining the controlled parameters of in vitro research. Standard cell biological Research relies on in vitro research, i.e., research performed in a test tube, culture dish, or Elsewhere outside of the living organism. OOC technology is crafted to address challenges associated with in vivo and in vitro research [2]. Microfluidics has major advantages over working on a macro scale including Increased efficiency, lower cost, and increased control of volume and other experimental parameters [5].

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

1.1 Overview of Organ-on-Chip Technology

Organ-on-chip (OoC) technology has emerged as an advanced generation of in vitro models, following the development of conventional two-dimensional (2D) cell cultures and three-dimensional (3D) cell culture systems. It represents a multidisciplinary approach that combines recent advances from several scientific and engineering fields [11] OoC platforms are primarily based on microfluidic and cell biology principles and are designed to reproduce important structural and functional characteristics of human organs within miniature micro devices [12]. These devices are commonly fabricated using transparent polymeric materials, such as polydimethylsiloxane (PDMS) (figure 1) and contain micro scale fluidic channels and porous membranes [13,14]. Living cells can be cultured within these channels, while controlled fluid flow, mechanical forces, and biochemical gradients are applied to recreate important features of the physiological microenvironment of human tissues and organs [15]

The major objective of OoC technology is to establish physiologically relevant in vitro models that can help reduce the translational limitations associated with conventional cell culture and animal-based experiments [16,17] The development of these systems has resulted from the integration of several disciplines, including microfluidics, tissue engineering, materials science, and cell biology [18,19]. During the early 21st century, researchers began applying microfabrication and microfluidic technologies to develop miniature devices capable of supporting controlled cell culture conditions [20].A major milestone was achieved in 2010, when researchers at the Wyss Institute at Harvard University introduced the concept of a “lung-on-a-chip.” The system was designed to reproduce key features of the alveolar environment, including breathing-associated mechanical movements, within a micro engineered device. This pioneering work stimulated extensive research and accelerated the development of different types of OoC platforms worldwide [21]

Since then, researchers have developed numerous organ-specific models, including lung-, heart-, liver-, kidney-, intestine-, and brain-on-chip systems [11,21] More recently, efforts have also focused on linking multiple organ-specific chips to create interconnected “human-on-a-chip” platforms. Such integrated systems are being explored for studying the distribution and systemic effects of drugs and for providing a more representative model of human physiological responses.

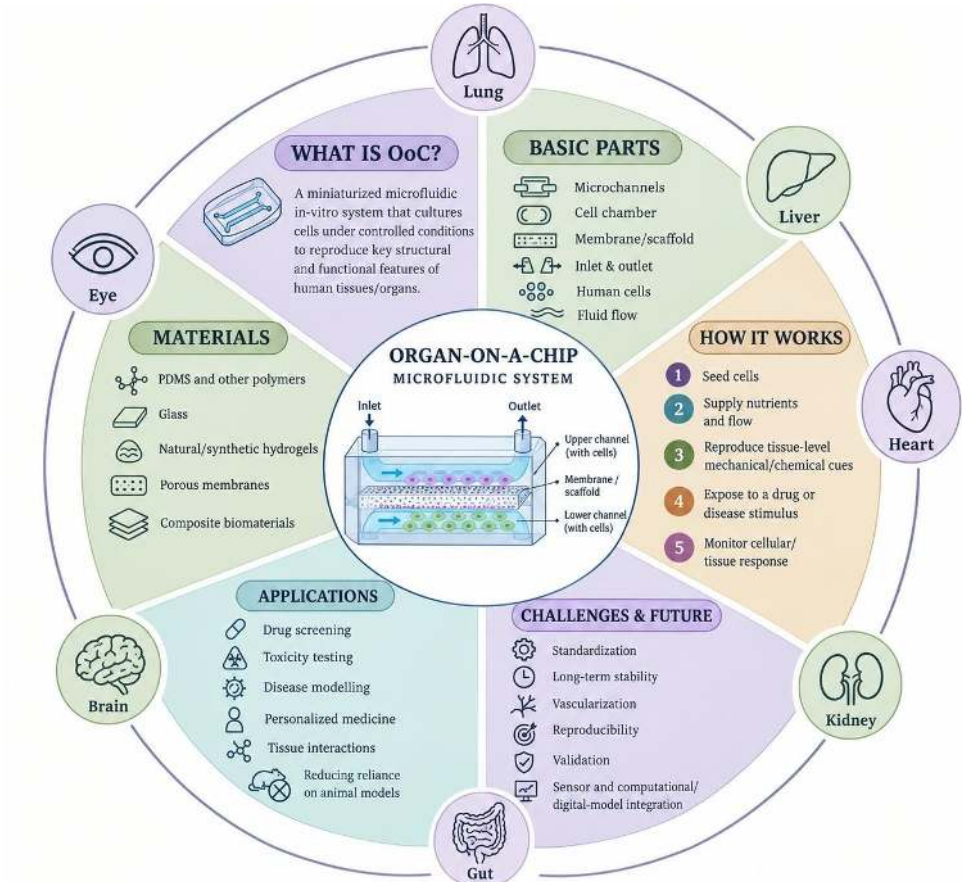


Figure 1: Overview of Organ on Chip Technology

2.Material Selection for Organ-on-Chip Fabrication :-

The choice of material used for fabricating an organ-on-chip (OoC) device is an important factor that can influence its overall performance and potential applications. An appropriate material should ideally provide good biocompatibility, optical transparency, sufficient gas permeability, ease of fabrication, and reasonable cost. Among the materials currently used for OoC fabrication, polydimethylsiloxane (PDMS) remains one of the most widely employed options. Its high optical transparency, gas permeability, flexibility, and compatibility with microfabrication techniques make it particularly suitable for developing microfluidic devices [22]

Despite these advantages, PDMS has certain limitations that need to be considered during OoC development. Its hydrophobic surface can promote the nonspecific adsorption of proteins and other biological molecules, which may affect cell culture conditions and experimental outcomes. Therefore, surface-treatment approaches such as oxygen plasma treatment or modification using hydrophilic coatings, including polyethylene glycol (PEG), are often applied to improve its surface properties [23]

Apart from PDMS, several other polymers have been investigated for OoC fabrication. Common examples include Polymethyl methacrylate (PMMA), polycarbonate (PC), and cycloolefin copolymer (COC), which offer different combinations of optical, mechanical, chemical, and fabrication-related properties. In recent years, the development of newer material platforms has further expanded the possibilities for OoC design. These include temperature-responsive polymers and hydrogel-integrated materials, which can provide additional control over the cellular microenvironment and introduce functions that may be difficult to achieve using conventional materials alone [24-27]

Table 1: Common Material Used in Microfluidic Application

Material	Property/Advantages	Disadvantage	Application	Reference
Collagen	Biocompatibility, control the structure	Lack of mechanical strength and structural stability	Bio sensing and film assembly	[28,29]
Agarose hydrogel	Biodegradable, mechanical stability	Poor cell adhesion, inconsistent properties	Cell culture, sensor	[30-33]
Teflon	Easy of fabrication	Hydrophobicity	Ultra clean tools, valves and pumps	[34]
Photo curable Resin/ polymer	High resolution	Volume shrinkage	Observation of cell growth	[35-37]]
Acrylonitrile Butadiene Styrene (ABS)	Excellent surface finishing material	Hydrophobicity	Pathogens detection, biological assay	[38-40]]
Polyethylene glycol (PEGs)	Relatively inexpensive, biocompatibility	Poor cell adhesion, limited biodegradation	Microfluidic valve, microfluidic lifetime improvement	[12,41-43]
Polyhydroxyalconate(PHAs)	Biocompatibility, biodegradables	Hydrophilic, lacking of sufficient moisture resistance	Barrier for vapor and oxygen	[44]
Gelatin Methacrylate (gelMA)	Photoplymerizable porous membrane	Week mechanical properties, fast degradation	Vascular and valvular biology cell research	[45-47]
Polylactic acid,polyglycolic acid	Biodegradable	High degradation rate	Porous scaffolding, better adhesive	[12-48]
Styrene Ethylene Butylene Styrene (SEBS)	Biocompatibility, electrical surface properties, reduced drug absorption	Poor dimensional stability	Microdivice for electrokinetic application cell culture,complex network	[49-50]]

3 | Microfabrication Techniques

Microfabrication plays a central role in the development and construction of organ-on-chip (OoC) devices. These techniques are used to create the small-scale channels, chambers, membranes, and other structural features required for reproducing the desired microenvironment within the chip. Several fabrication approaches are currently used in OoC development, with commonly applied methods including soft lithography, micro milling, hot embossing, and three-dimensional (3D) printing.

Table 2: Common Microfabrication Techniques:-

Microfabrication techniques	Main advantages	Major limitations	Common material	Reference
Soft lithography	Provide high resolution microstructure Relatively inexpensive and easy to perform Suitable for rapid fabrication of occ device	Require fabrication of a master mold Difficult to directly scale up for large scale production	PDMS	[5,51,52]
3D printing	3D printing Offer considerable freedom in design Allow rapid prototyping and fabrication of customized structure	Resolution can be limited Suitable printable a material are restricted	Photosensitive resin,secilized bioink	[6,53]

Microfabrication techniques	Main advantages	Major limitations	Common material	Reference
Hot embossing	Appropriate for producing large number of devices Relatively economical for mass fabrication	Required elevated temperature and pressure Provide less flexibility for design modification	Thermoplastic polymer	[54]
Micro milling	Directly machines the desired structure Does not required preparation of a master mold.	Produce comparatively lower resolution May result in rougher surface	PMMA,PC	[55]

4.Cell Sources

The choice of cell source is an important consideration in OoC development because it can influence the physiological relevance, functional stability, and overall research value of the model. Different types of cells can be used depending on the purpose and requirements of the organ-on-chip system. Commonly investigated sources include primary cells, cell lines, mesenchymal stem cells (MSCs), and induced pluripotent stem cells.

Primary cells are obtained directly from biological tissues and generally retain many of the physiological characteristics and functions of the cells present in the body. Therefore, they can provide a relatively realistic representation of tissue-specific functions within an OoC model. However, primary cells may show considerable donor-to-donor variation and generally have limited proliferative capacity. These factors can contribute to variability and heterogeneity between independently developed organ models [56].

Cell lines, in contrast, consist of immortalized cells that can overcome some of the limitations associated with the restricted proliferation of primary cells. Their ability to grow for extended periods makes them convenient and relatively stable for long-term OoC experiments. Nevertheless, prolonged culture and immortalization may lead to genetic changes or alterations in cellular phenotype. Despite these limitations, immortalized cell lines provide several practical benefits for OoC research, including good batch-to-batch consistency, extensive proliferative capacity that supports large-scale experiments, and a substantial body of previously reported information regarding their biological behavior, drug responses, and genetic characteristics [57].

5.Biomimetic Scaffolds in OoC

Within an OoC platform, the bio scaffold serves as a physical support for cell attachment, growth, organization, and functional activity. Consequently, the selection and structural design of the scaffold can have a direct influence on the physiological relevance and functional reliability of the resulting model. Different scaffold materials and designs have therefore been investigated to create a microenvironment that more closely resembles the characteristics of native tissues.

This section provides an overview of the material characteristics, preparation approaches, and potential applications of different bio scaffolds used in OoC systems. Particular attention is given to major scaffold categories, including hydrogel-based scaffolds, HMs, synthetic polymers, and bio ceramic materials . Their physicochemical characteristics, biocompatibility, structural tunability, and suitability for reproducing specific tissue microenvironments are considered when evaluating their potential application in OoC platforms [57].

6.Types of Organ-on-Chip Models

Organ-on-chip (OoC) technology can be adapted to reproduce important features of different organs, including the kidney, lung, heart, and liver. These platforms generally combine microfluidic systems with biocompatible materials and suitable sensing technologies. Depending on the design and purpose of the device, sensing may be achieved through automated imaging, integrated output sensors, or other types of micro sensors [58].

6.1Kidney-on-Chip

The kidneys play several essential roles in maintaining normal body function. They remove metabolic waste products and excess water from the blood to form urine, while also contributing to the regulation

of electrolytes such as sodium, potassium, and calcium. In addition, the kidneys produce hormones involved in blood-pressure regulation and stimulate the production of red blood cells in the bone marrow [59].

Jang et al. [60] were among the first researchers to develop a multilayered microfluidic system designed to reproduce renal filtration using mouse kidney cells. Subsequently, Musah et al. [61] developed a kidney-on-chip model that reproduced important structural and functional features of the renal capillary wall. In their system, mechanical pressure could be applied to the cultured cell layer through vacuum chambers positioned on both sides of the device.

Kidney-on-chip platforms have since been investigated for several applications, particularly disease modelling, drug screening, and toxicity assessment [61]. For example, Kim et al. [62] evaluated the nephrotoxicity of gentamicin using a kidney-on-chip model under two different pharmacokinetic administration profiles. Their study showed that continuous infusion resulted in lower nephrotoxic effects compared with bolus administration [62]

6.2 Lung-on-Chip

The alveoli are small air sacs in the lungs that play a central role in respiratory gas exchange by allowing oxygen to enter the blood and carbon dioxide to be removed during breathing. Reproducing this complex process under conventional in vitro conditions has been difficult because it involves both cellular interactions and mechanical changes associated with respiration. OoC technology has provided an opportunity to develop more physiologically relevant lung models that can reproduce important features of the pulmonary microenvironment and support controlled gas exchange [59].

Current research on lung-on-chip systems has focused on reproducing key physiological conditions, including mechanical changes associated with airway function and interactions occurring at the blood–air barrier [63]. Huh et al. [64] developed a lung-on-chip model consisting of two compartments separated by a permeable interface. The upper compartment contained alveolar cells, whereas the lower compartment was populated with pulmonary vascular cells, thereby providing a model of the alveolar–capillary barrier. Vacuum was applied to the device to generate cyclic mechanical deformation, mimicking the expansion and contraction of the alveolar region during breathing. The system could also incorporate inflammatory stimuli, with neutrophils introduced through the microfluidic channels to investigate inflammatory responses within the lung microenvironment [64].

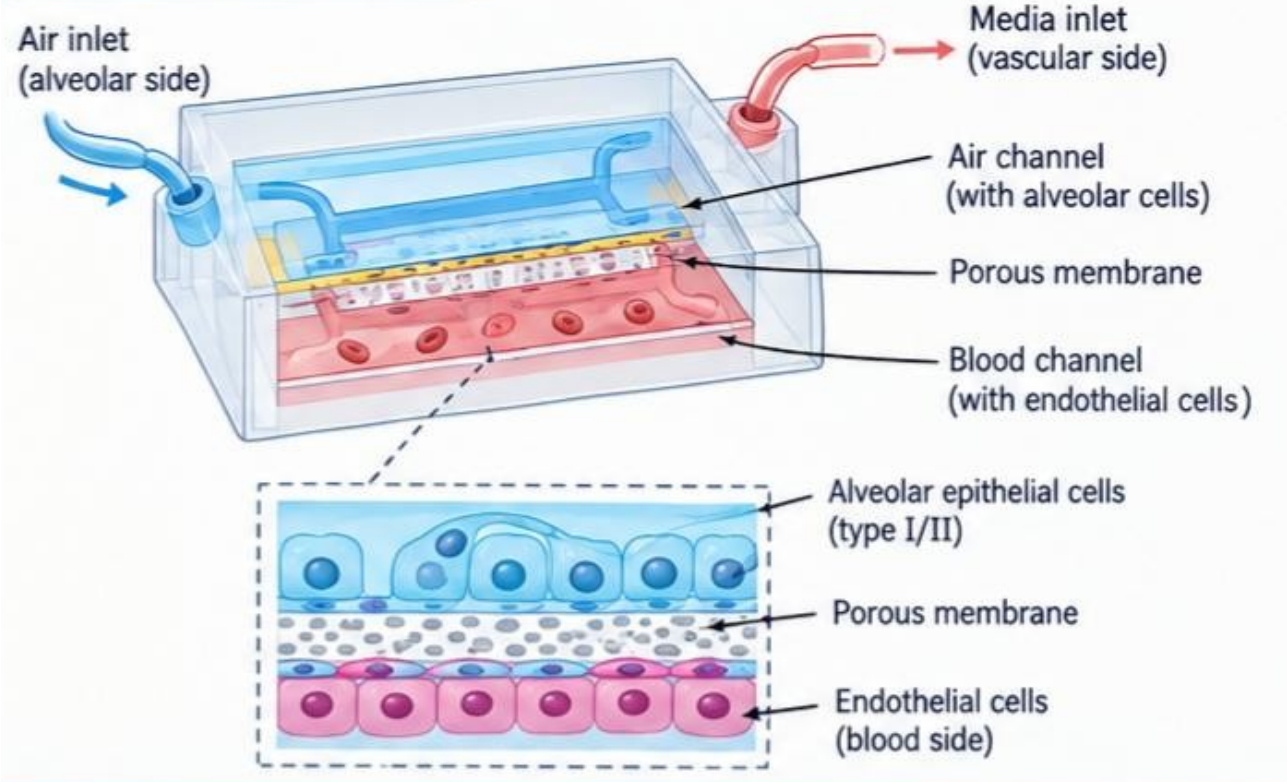


Figure 2: Lung on Chip Model

6.3 Heart-on-Chip

The myocardium forms the muscular tissue of the heart and is primarily composed of specialized cardiac muscle cells known as cardio myocytes (CMs), which are responsible for the contractile activity of the heart [59].

Grosberg et al. [65] developed a PDMS-based heart-on-chip system in which rat cardio myocytes were cultured on a flexible membrane to form a contractile muscle layer. As the cardio myocytes contracted, the membrane underwent bending or curling. The extent of this deformation was measured to assess the contractile behavior of the cardiac cells.

Marsano et al. [66] developed another heart-on-chip platform designed to reproduce important physiological and mechanical characteristics of the cardiac microenvironment. The system incorporated cardio myocytes within a three-dimensional structure, while pressure was applied to the lower compartment of the device. This pressure caused deformation of the separating membrane and compression of the 3D cardiac construct, thereby generating cyclic mechanical stimulation that reproduced aspects of the heart's pumping activity.

The heart-on-chip device developed by Marsano et al. incorporated two PDMS chambers containing cardiac cells embedded within a fibrin-based gel matrix. Side channels supplied the cellular construct and allowed flexibility in configuring the geometry of the system. By pressurizing the lower chamber, the PDMS membrane was displaced and compressed the 3D cell construct against supporting posts. Repeated pressure cycles generated mechanical stimulation intended to mimic physiological phases of cardiac activity [61]].

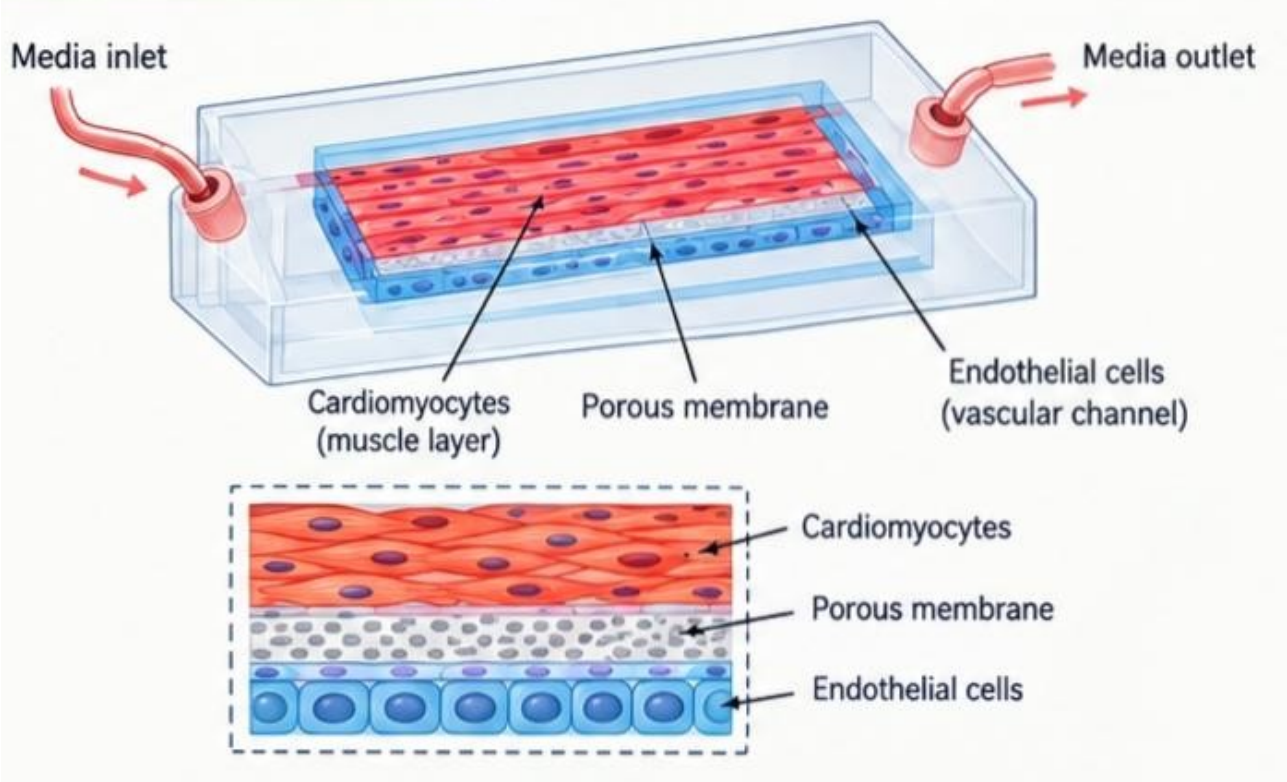


Figure 3: Heart on Chip Model

6.4 Liver-on-Chip

The liver is one of the major organs responsible for the metabolism and detoxification of drugs and other foreign substances. Its basic structural units are known as hepatic lobules, which contain hepatocytes that perform several important functions, including processing blood arriving from the digestive system, detoxifying chemicals, and metabolizing drugs [67].

Kane et al. [68] developed one of the early microfluidic systems for liver modelling. Their platform incorporated rat liver cells along with mouse fibroblasts to reproduce features of a tissue interface. Rat hepatocytes cultured within the device demonstrated stable albumin synthesis and metabolic activity, indicating the potential of the platform for maintaining important liver-specific functions.

Lee et al. [68] subsequently developed a liver-on-chip model designed to reproduce aspects of the interstitial region between liver endothelial cells and hepatocytes. This type of arrangement provided an opportunity to investigate cellular interactions and the exchange of substances within the hepatic microenvironment. Ho et al. [69] used electric-field gradients and electrophoresis-based approaches to reorganize hepatic cells into a configuration resembling the hexagonal architecture of the hepatic lobule on a circular PDMS chip.

Chong et al. [70] investigated drug metabolism using assays designed to monitor skin-related responses to drug exposure. Such liver-based platforms may contribute to drug-screening studies by providing an opportunity to identify compounds that could be associated with systemic skin reactions. Liver-on-chip systems have also been explored for studying different disease and injury conditions. For example, Kang et al. [71] investigated hepatitis B virus (HBV) replication using a liver-on-chip model, while Zhou et al. [72] developed a model for examining alcohol-induced liver injury.

Noh et al. demonstrated the induction of HBV infection on a microfluidic platform by introducing HBV-genome cDNA and subsequently exposing the cultured cells to HBV genetic material expressed using a recombinant adenovirus [73] These studies demonstrate the broader potential of liver-on-chip systems for investigating hepatic metabolism, drug-related effects, infectious diseases, and liver injury.

Table 3: Common Material, Sensor, Cell Types Used in Lung on Chip Application

Material	Sensor/reabout	Cell type	Reference
Polyethylene Methacrylate (PMMA)	Electrochemical	Hepatocytes	[74]
Polycarbonate	Optical	Liver sinusoidal endothelial cells	[75]
Glass	pH	HepG2 cells	[76]
Alginate	Not specified	Hepatocytes, hepatic stellate cells	[77]

6.5 Intestine on chip:-

The human small intestine contains numerous finger-like structures called villi that increase the surface area available for nutrient absorption. Similar to the alveolar structures of the lungs, these specialized features are important for maintaining the physiological functions of the intestine. Intestine-on-chip models are increasingly being investigated for studying the absorption of orally administered drugs, interactions between intestinal cells and microorganisms, and the complex microbial environment of the gut [59].

Kim et al. [78] developed an intestine-on-chip platform consisting of two chambers separated by a porous membrane lined with intestinal epithelial cells. Drugs or inflammatory cells could be introduced into the lower chamber to investigate absorption processes and cellular responses. Vacuum chambers positioned on both sides of the cell-containing region were used to generate cyclic mechanical strain, thereby reproducing the repeated deformation experienced by the human small intestine during normal intestinal movements.

Kasendral et al. [79] developed a microfluidic model representing the human duodenum, which is the first section of the small intestine. The model was designed to improve the understanding of intestinal morphology and the microbial environment of the gut. In another approach, Firouzinezhad et al. [80] co-cultured both aerobic and anaerobic microorganisms within an intestine-on-chip system. This setup allowed the oxygen gradient within the intestinal microenvironment to be controlled and monitored. Such platforms may provide useful experimental models for investigating the gut microbiome and exploring potential therapeutic strategies related to microbiome-associated conditions [80]..

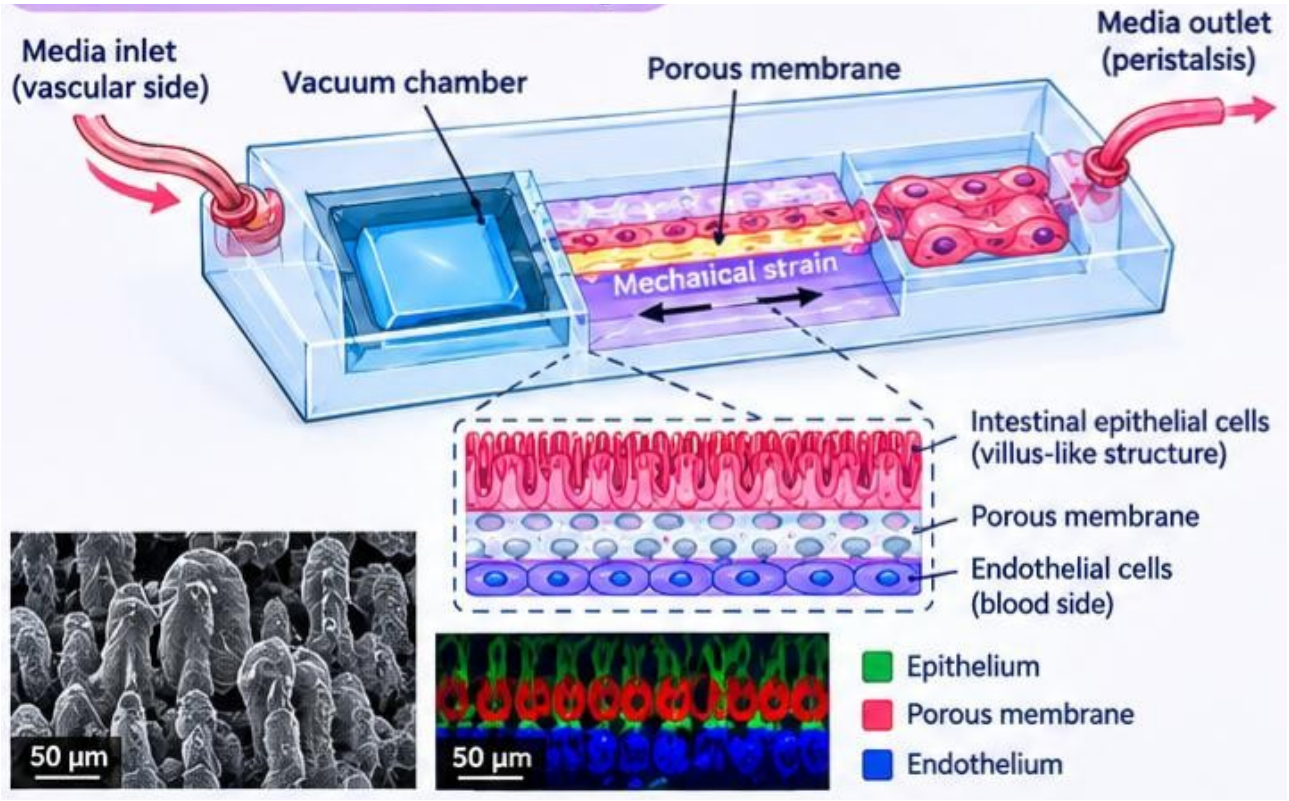


Figure 4: Intestine on Chip Model

6.6 Brain on chip:-

The brain is a highly complex organ composed of numerous specialized regions and different types of cells, each contributing to specific physiological functions. Developing brain-on-chip models can provide a controlled platform for investigating neurological processes and evaluating potential drugs for central nervous system (CNS)-related disorders, while potentially reducing the time and resources required during early-stage research.

However, reproducing the complete functionality of the human brain within a single microfluidic device remains extremely challenging. The brain contains diverse cell populations with different culture requirements, making it difficult to incorporate all of its structural and functional characteristics into one model. Consequently, brain-on-chip platforms are generally designed to represent a particular brain region, cellular interaction, or physiological process rather than attempting to reproduce the entire organ [81] As noted by Dr. Maoz, there is no single "one brain-on-a-chip fits all" platform; instead, the design of each model depends on the specific research objective and may involve different practical and technical considerations [81]. Examples of materials, sensing approaches, and cell types investigated for brain-on-chip development are summarized in the relevant table.

Tong et al. used a microfluidic platform to investigate axonal regeneration following axotomy, a process of particular relevance to neural injury. Their system was designed to reproduce selected features of the in vivo neural microenvironment. Following axotomy, the axons were exposed to an inhibitory molecule, allowing the researchers to observe and evaluate the subsequent axonal response within the microfluidic environment [82].

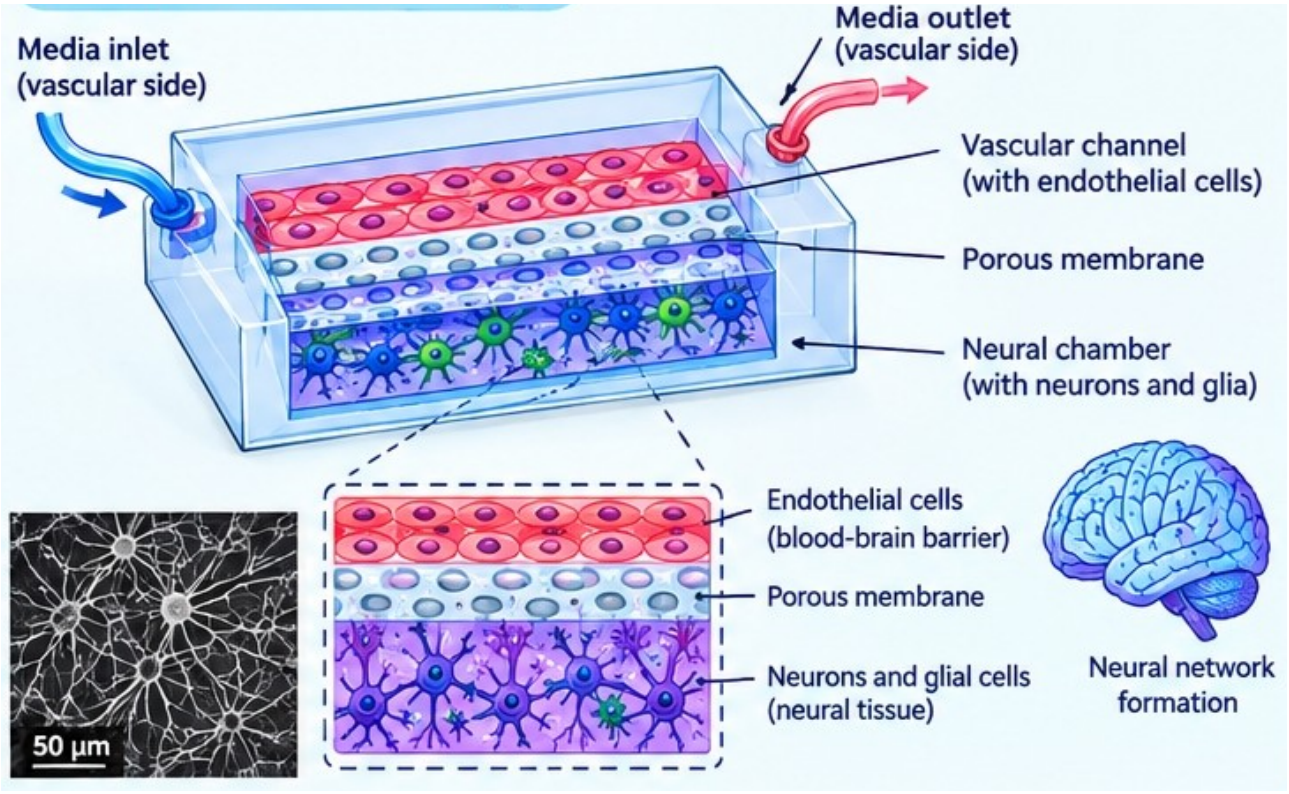


Figure 5: Brain on Chip Model

Myelination is another important process in the CNS, in which a myelin sheath forms around axons and facilitates faster and more efficient transmission of neuronal signals. A microfluidic model has been used to investigate different stages of myelination using neurons derived from embryonic stem cells, providing an experimental platform for studying this complex neural performance [83].

Table 4: Various Brain on Chip Sensor, Cell Types and Material

Material	Sensor	Cell type	Reference
PADMS	Electrical	hCMEC/D3 human brain endothelial cell	[84]
PMMS	Electrical	bEnd.3 murine brain endothelial cell	[84]
Polyethylene tetra phthalate and PDMS	Electrical	Human iPSC- derived endothelial cell, human astrocytes	[85]

6.7 Nerve on chip:-

The development of effective treatments for neurodegenerative diseases remains challenging, as many candidate therapies fail during clinical evaluation. It has been reported that fewer than 7% of neurological drugs ultimately reach commercial availability [86] .One of the major difficulties is the complexity of the nervous system, particularly the interactions between neurons and glial cells. As a result, micro physiological systems designed to predict human neurological responses have progressed more slowly than comparable models developed for several other organs. The limited availability of human-relevant in vitro models is particularly evident for peripheral nerves [87].

Nerve-on-chip platforms have therefore emerged as simplified in vitro systems for investigating neuronal and peripheral nerve functions while reproducing selected features of the complex in vivo environment. Microfabrication techniques can be used to construct controlled extracellular recording interfaces that allow action potentials to be monitored. These platforms can improve experimental reproducibility and support the analysis of statistically meaningful numbers of samples [88].

Sharma et al. [89] recently developed a three-dimensional functional model of the human peripheral nerve using a nerve-on-chip platform. The researchers generated human neurons and primary human Schwann-cell spheroids within a dual-hydrogel system. The model supported long-term cell culture and substantial neurite outgrowth. This work represented an important step toward developing a biomaterial-based micro physiological model of a myelinated human peripheral nerve that could be used to investigate electrophysiological properties that have traditionally required in vivo experimental approaches [89].

6.8 Pancreas on chip

Insulin therapy remains a standard treatment approach for patients with type 1 diabetes. However, exogenous insulin administration cannot completely reproduce the precise and dynamic regulation normally provided by pancreatic beta cells and may be associated with episodes of hypoglycemia [90] Consequently, approaches such as pancreatic beta-cell transplantation and islet replacement therapy have been investigated as potential strategies for insulin-dependent diabetes [91].

Before transplantation, determining the quantity and functional quality of pancreatic islets is an important consideration. Pancreas-on-chip (POC) technology provides a controlled platform through which researchers can assess islet quality and functional activity under standardized experimental conditions. These systems have also been investigated for studying islet organization and function within controlled microfluidic environments [92]. Several POC platforms have been developed to examine pancreatic islet responses under conditions associated with specific pancreatic disorders. For example, Shik et al. used a pancreas-on-chip platform in 2019 to investigate islet-cell function in the context of cystic fibrosis [93].

6.9 Placenta on chip:-

The placenta plays an essential role in maintaining a healthy pregnancy. It acts as an important interface between the maternal and fetal systems, regulating the exchange of oxygen, nutrients, metabolic waste products, and other endogenous or external substances while also contributing to protection against potentially harmful agents [59].

Placental transport can be influenced by several factors, including umbilical blood flow, metabolic activity, and the thickness and structural characteristics of the placental barrier. OoC technology provides an opportunity to investigate these processes under controlled conditions by combining microfluidic systems with microfabrication approaches. Placenta-on-chip models can reproduce important features of the placental barrier in vitro using human trophoblasts, umbilical vein endothelial cells, and extracellular-matrix-based membranes.

Such platforms may help overcome some limitations of existing placental models, including conventional three-dimensional organoid systems derived from human blastocysts. By providing a controllable and physiologically relevant experimental environment, placenta-on-chip technology has potential applications in reproductive biology, placental transport studies, and reproductive medicine [94].

6.10 Skin-on-a-Chip

In vitro and ex vivo studies in dermatology, pharmacology, and cosmetic research commonly use reconstructed skin tissues, artificial membranes, or excised skin samples as experimental models. These models can be used to investigate several skin-related properties and responses, including permeability, irritation, corrosion, toxicity, and different pathological conditions [59].

Dr. Risueno and colleagues classified skin-on-chip platforms according to the way in which skin tissue is incorporated or generated within the device. Two major approaches have been developed for constructing microfluidic skin models. In the first approach, a skin fragment obtained from a biopsy or a human skin equivalent (HSE) is directly transferred into the microfluidic device. This approach is commonly referred to as a transferred skin-on-a-chip model [95].

The second approach involves generating the skin tissue directly within the microfluidic platform and is therefore described as an in-situ skin-on-a-chip model. Jones et al. (2022) developed a full-thickness skin model containing human-derived primary and immortalized cells within a microfluidic device equipped with a pre-established microvascular network. The incorporation of this vascular component provided an opportunity to investigate the efficacy and safety of drugs administered through systemic routes [96].

Kim et al. (2022) demonstrated that human skin analogs could also be used to reproduce important pathological features associated with atopic dermatitis. Such models may provide a more human-relevant alternative for studying the disease, particularly because findings obtained from conventional mouse models may not always translate directly to human conditions [98].

6.11 Stomach-on-a-Chip

Conventional laboratory models and animal studies have several limitations when used to reproduce human gastrointestinal disorders. These approaches may not fully represent the complex multicellular organization and functional characteristics of human primary gastrointestinal tissues [98].

Lee et al. developed a microfluidic imaging platform incorporating peristaltic luminal flow to maintain human gastric organoids (hGOs) under in vitro conditions. The system supported stable, long-term three-dimensional development of hGOs and enabled controlled delivery of substances into the organoid lumen through a peristaltic pumping mechanism [99]

Li et al. developed another microfluidic platform to investigate mucin secretion and its protective role against acidic digestive fluids in the stomach. Their findings indicated that the mucin layer can limit the diffusion of acid toward the epithelial surface. This type of model may therefore provide a useful platform for investigating interactions between gastric mucus and drug molecules under controlled conditions [100].

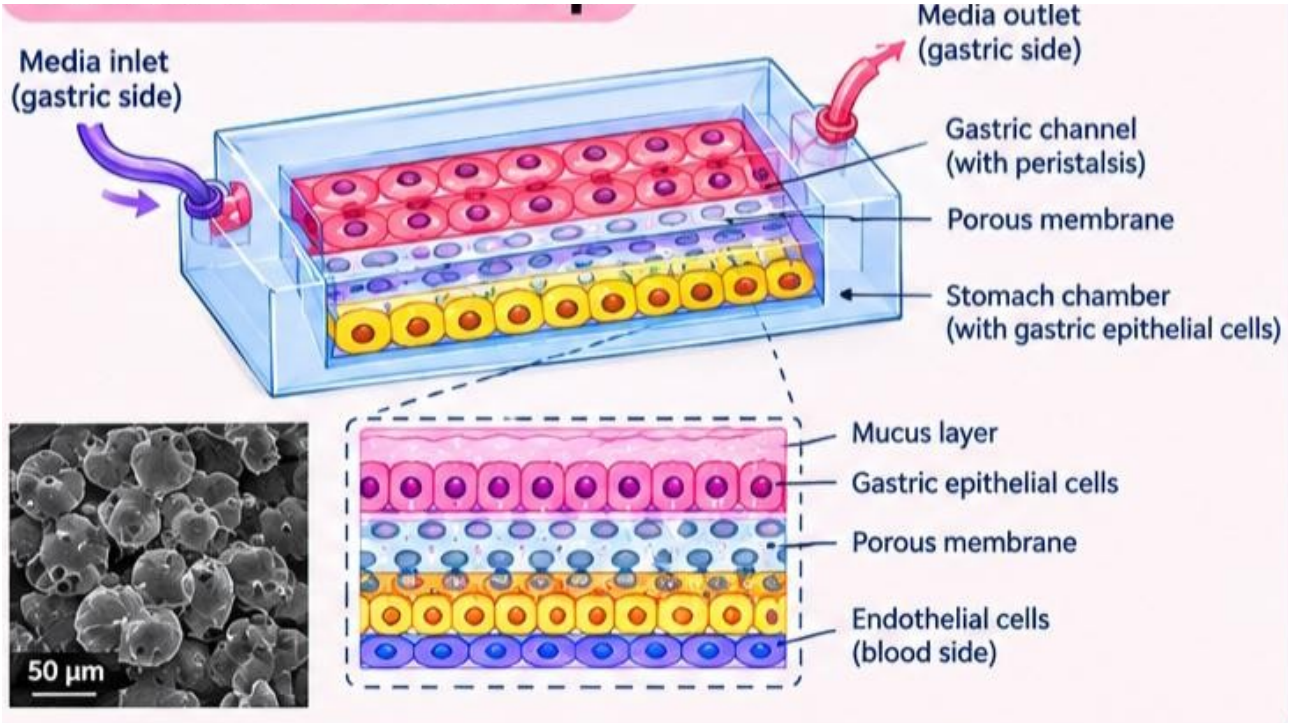


Figure 6: Stomach on Chip Model

6.12 Muscle on chip

Skeletal muscle is primarily responsible for producing contractile forces and supporting several essential physiological activities, including mastication and eye movements [101] Tissue-engineered skeletal muscle models provide a useful approach for developing physiologically relevant platforms to investigate the musculoskeletal system. Such models can be used under in vitro conditions to study how human skeletal muscle responds to injury, disease-related changes, and therapeutic interventions [102].

Micro engineered muscle models can also be designed to reproduce important three-dimensional structural and morphological features of native skeletal muscle. Agrawal et al. [103] described the development of a three-dimensional (3D) skeletal muscle tissue model within a microfluidic device, providing a controlled platform for investigating muscle structure and function [103].

6.13 Eye on chip:-

Choroidal neovascularization (CNV) involves abnormal vascular growth and disruption of the outer blood –retinal barrier and is associated with several retinal

disorders [104]]. Advances in eye-on-chip (EOC) technology have enabled researchers to reproduce important features of CNV pathology under controlled in vitro conditions [105].

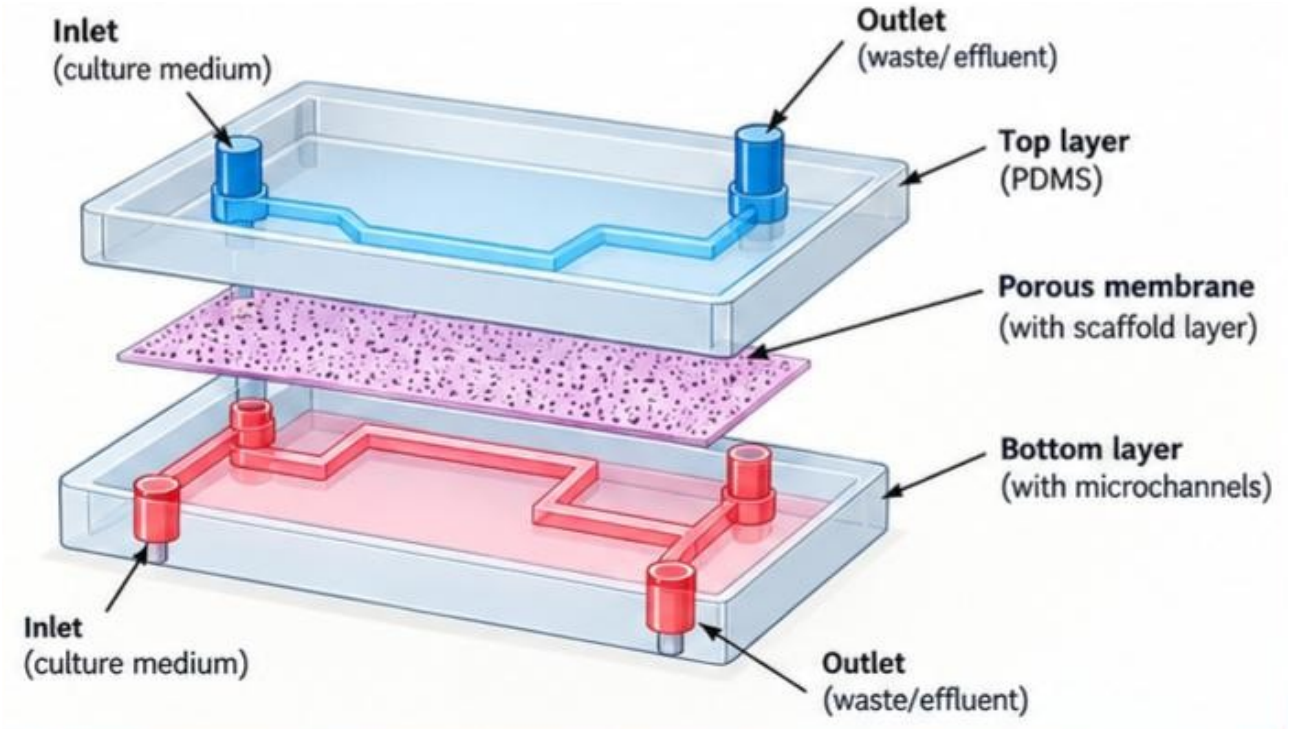
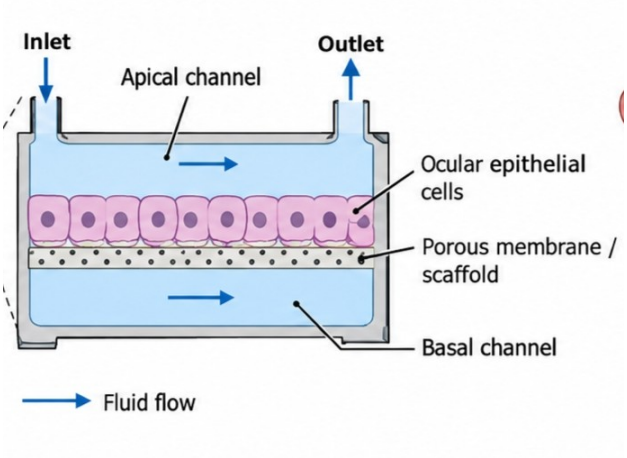


Figure 7: 3D Schematic of Eye on Chip Model

EOC platforms have also been used to investigate the effects of therapeutic agents used for CNV. For example, these models have been employed to study the dose-dependent effects of bevacizumab, a clinically used treatment for CNV. The platform enabled researchers to observe Angiogenic sprouting from pre-existing choroidal vessels and assess the subsequent effects on the retinal pigment epithelium (RPE) monolayer [106]. Such models provide an opportunity to investigate disease mechanisms and therapeutic responses within a controlled ocular microenvironment.



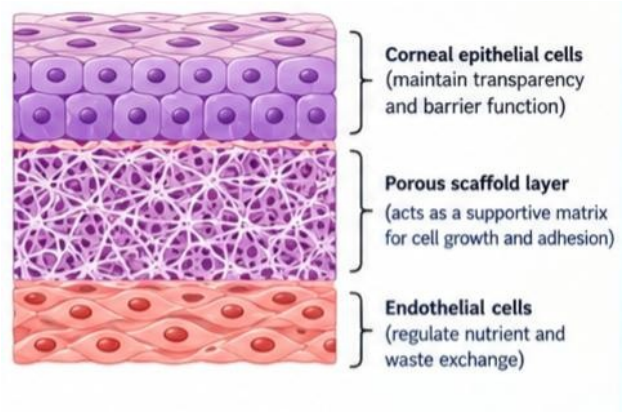


Figure 8: Eye on Chip Figure 9 :- Close -up View of Occular Cell

6.14 Vagina on chip

Mahajan et al. developed a vagina-on-chip model to investigate interactions between the vaginal host tissue and its associated microbiome [107]. More recently, a human vagina-on-chip platform was used to co-culture microbial communities from the human vaginal microbiota with primary human vaginal epithelial cells. This approach enabled investigation of physiological responses of vaginal tissue to healthy and symbiotic microbial communities [107].

The platform incorporated parallel hollow microchannel containing living cells and allowed the

cellular environment to be maintained under dynamic fluid-flow conditions [107]. Such systems may provide a useful human-relevant model for studying host–microbiome interactions and the physiological responses of vaginal tissue.

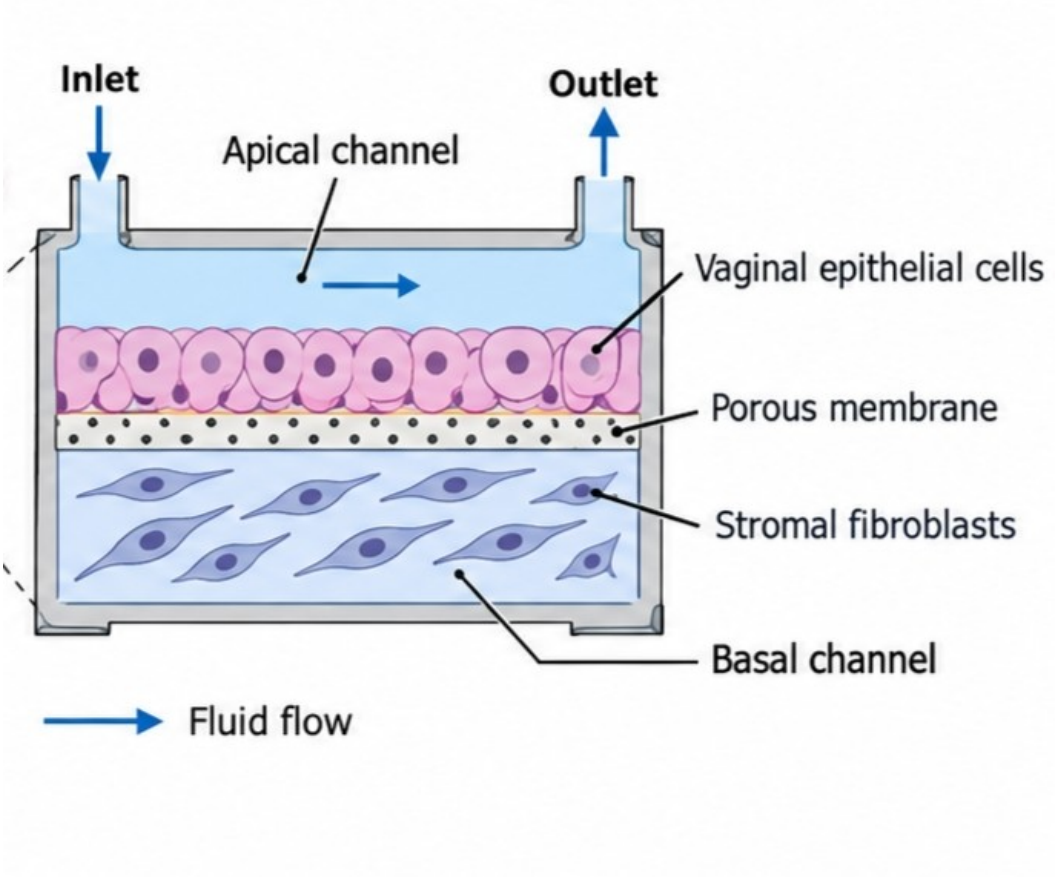


Figure 10: Vagina on Chip

7.Digital twins

7.1 Introduction

Digital twins (DTs) are virtual representations of physical entities, systems, or processes that can be used to understand, monitor, and optimize their real-world counterparts. In drug discovery and development, DTs provide an opportunity to integrate patient-derived information with computational models to simulate different scenarios, predict potential outcomes, improve processes, and support scientific decision-making [108].

The concept of digital twins has already been applied in several fields, including manufacturing, transportation, logistics, and smart-city development, and is now emerging as a promising technology in healthcare [109,110] Within healthcare, virtual patients (VPs) represent a closely related concept. Although VPs and DTs may be generated using the same underlying simulation framework, they can differ in the type of data incorporated and their primary objectives [111]. Growing interest in these approaches has encouraged researchers to explore how VPs and DTs can be incorporated into drug development and how they may contribute to more efficient and informative research strategies.

7.2 Overview of digital twins

According to the National Academies of Sciences, Engineering, and Medicine (NASEM) report, a digital twin can be described as a collection of virtual information constructs designed to represent the structure, context, and behavior of natural, engineered, or social systems, including interconnected systems [108]. In biological applications, DTs can be developed at different levels, ranging from individual cells and tissues to organs, organ systems, or an entire individual [112].

A key feature of a DT is its ability to remain dynamically connected with its real-world counterpart. Data

obtained from the physical system can be incorporated into the virtual model, allowing the DT to be updated continuously and used to generate predictions or support decision-making. Bidirectional interaction between the physical and virtual components is therefore an important characteristic of a DT framework [108]. In addition, a functional DT generally requires two major components: the collection and integration of data from relevant sources, and computational or simulation models capable of providing insights into system behavior and potential outcomes.

DTs can broadly be divided into two categories according to the type of technology used to represent the physical system [113]. One approach uses data-driven methods, including machine-learning algorithms, whereas the other relies on mechanistic models that describe interactions and processes within the system. In drug development, mechanistic models of human physiology, particularly quantitative systems pharmacology (QSP) models, are widely used to investigate and predict the safety and efficacy of investigational drugs in patients .[114,115,116].

Combining mechanistic and data-driven approaches can provide complementary advantages. Mechanistic models contribute an understanding of biological processes and relationships, while machine-learning approaches can identify patterns within complex datasets. Their integration can therefore support the development of predictive DT frameworks that retain mechanistic interpretability while adapting to newly available data [117](as shown in figure 11) Such hybrid models may assist in hypothesis generation, optimization of study designs, and more efficient decision-making during pharmaceutical research and development. By incorporating biological complexity into computational models, this approach may also help researchers use experimental resources more efficiently and improve the evaluation of potential drug candidates.

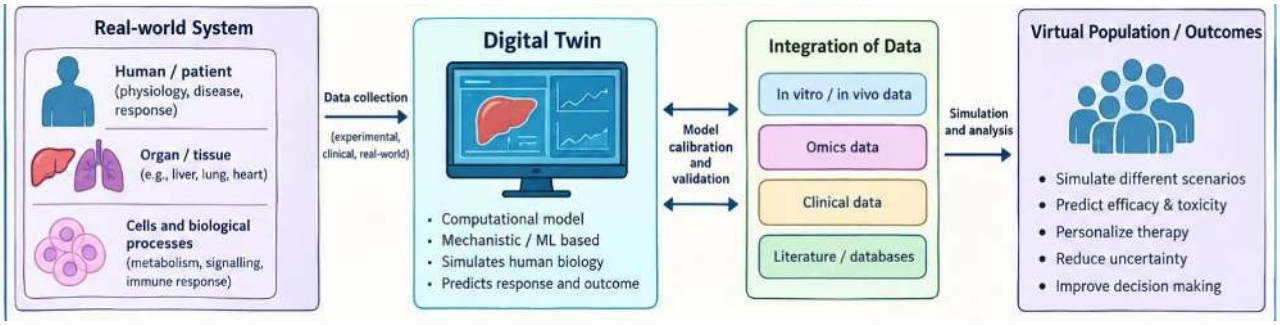


Figure 11: Overview of Digital Twins

7.3 Digital twins in Preclinical drug testing

Digital twins are increasingly being explored as computational tools for improving the relevance and efficiency of preclinical drug research. Their application can be considered across different biological levels, beginning with individual cells and progressing through cell cultures, tissues, organs, and integrated biological systems. At each level, experimental information can be combined with computational models to represent selected biological processes and predict responses to interventions.

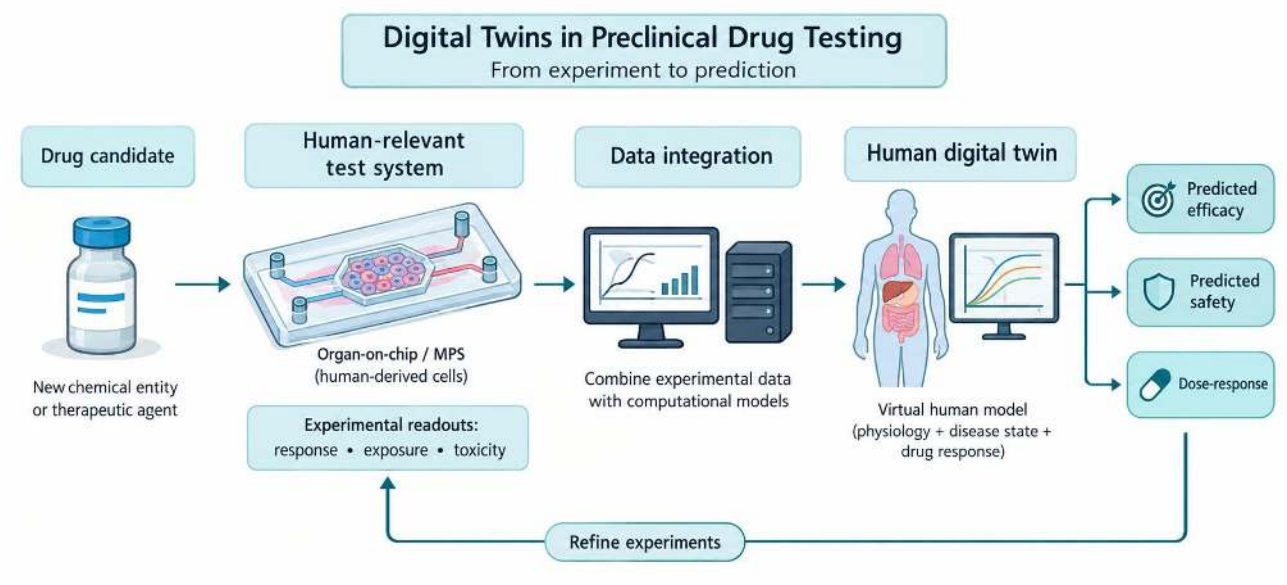


Figure 12: Representation of Digital Twins in Preclinical Drug Testing [118]

In the context of preclinical drug testing, this multi-scale approach provides a framework for connecting experimental models such as cell cultures and organ-on-chip systems with computational representations of biological behavior. The following sections therefore discuss the potential applications of digital twins across different levels of preclinical modelling, from **cell → cell culture → tissue → organ → animal model**, (as shown in figure 12) with emphasis on their role in drug response prediction, safety assessment, disease modelling, and reduction of dependence on conventional experimental approaches. [118].

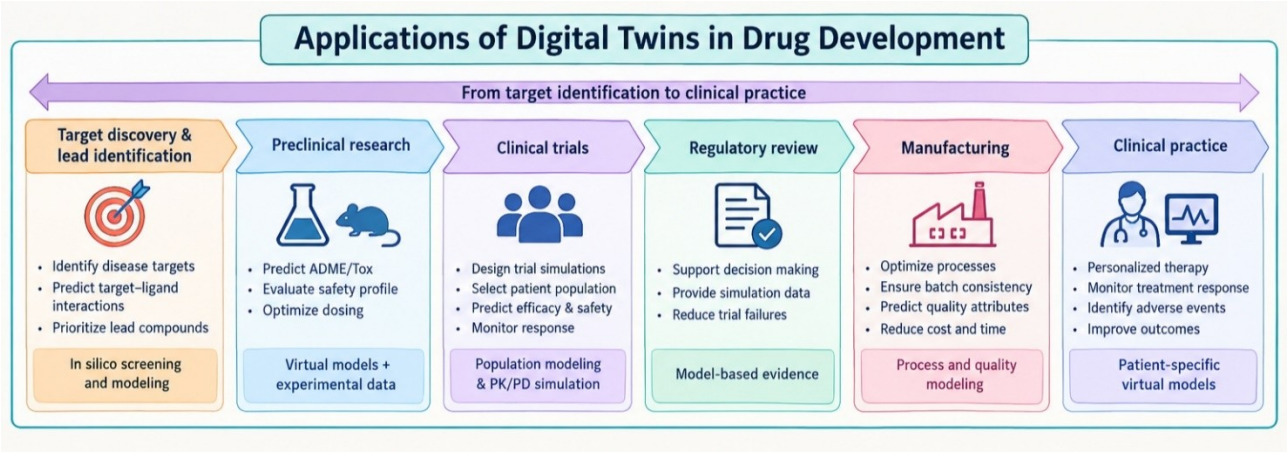


Figure 13: Representing Application of Digital Twins in Drug Development [118]

7.4 Application:-

8.Challenge of organ on chip technology and digital twins:-

Biological completeness: No chip reproduces the entire human body. Important endocrine, neural, immune and systemic interactions may remain missing. Multi-organ designs increase realism but also complexity.

Cell quality and maturity: Primary cells vary between donors, while iPSC-derived cells may differ in maturity and phenotype. Reproducibility therefore requires defined cell-characterization criteria.

Device materials and drug adsorption: Microfluidic materials can absorb or bind compounds, potentially changing the effective exposure. This requires material selection and compound-specific controls.

Scale and throughput: Highly sophisticated chips may be difficult to operate at screening scale. Automation and standardized readouts are needed for industrial adoption.

Model uncertainty: Digital twins can produce precise-looking predictions that are nevertheless wrong if the model is misspecified. Verification, validation and uncertainty quantification are essential.

Data standards and interoperability: Different chip manufacturers and laboratories may generate incompatible data structures. Common metadata, reference compounds and reporting standards are necessary.

Regulatory acceptance: Regulators increasingly consider NAMs, but acceptance depends on context of use and validation. A new method cannot be assumed to be acceptable solely because it is technically advanced.

Cost and expertise: Successful integration needs microengineering, cell biology, analytical chemistry, pharmacology, pharmacometrics and computational modelling expertise. This can be challenging for academic laboratories, particularly in resource-limited settings.

Ethical and governance issues for digital twins: Patient-level models require careful handling of privacy, model bias, transparency and responsibility for decisions based on predictions. [118].

9.Future perspectives

1. Personalized and precision medicine:

The combination of patient-specific induced pluripotent stem cells (iPSCs) with customized scaffold design may facilitate the development of disease-specific organ-on-chip (OoC) models. Such models could be used for individualized drug screening and optimization of therapeutic strategies. A data-driven approach may further support the design of patient-specific scaffolds by incorporating information obtained from medical imaging and functional assessments, thereby contributing to more personalized and precise therapeutic applications.

2. Smart and responsive scaffolds:

Future scaffold systems are expected to incorporate materials capable of responding to biological signals, such as pH and enzymes, or to external stimuli, including light, magnetic fields, and temperature. These responsive materials could provide controlled regulation of cellular behavior, drug release, and scaffold remodeling according to the requirements of the tissue microenvironment. Examples include thermoreversible hydrogels such as PIC, photocleavable hydrogels, and enzyme-sensitive crosslinking systems. Incorporating such dynamic scaffolds into OoC platforms could improve their ability to reproduce changing physiological conditions and increase their experimental flexibility.

3. Multimaterial and gradient scaffold design:

Advanced manufacturing approaches, particularly multimaterial 3D printing and gradient fabrication, can be used to produce scaffolds with spatial variations in composition, architecture, and mechanical properties. Such heterogeneous structures may more closely reproduce tissue interfaces and the complex microarchitecture observed in different organs [119].

4. Integrated sensing and real-time monitoring:

The incorporation of microsensors within scaffold-based OoC systems offers an opportunity for continuous monitoring of important microenvironmental parameters. Sensors capable of detecting temperature, pH, oxygen levels, glucose, and other relevant variables can provide real-time information about cellular activity and tissue responses. This approach could generate more detailed dynamic datasets and improve the monitoring of biological functions within the chip [120].

5. Green manufacturing and sustainable development:

Sustainability is likely to become an important consideration in the future development of OoC technologies. Environmentally responsible fabrication methods and recyclable or biodegradable materials could help reduce dependence on organic solvents and minimize energy consumption during manufacturing. Such approaches may contribute to reducing the environmental impact associated with the production and operation of OoC platforms

6. Integration of organoids with organ-on-chip platforms:

Combining organoid technology with OoC systems may provide a more physiologically representative approach to tissue modelling. Organoids possess intrinsic self-organizing capabilities, while OoC platforms offer controlled regulation of factors such as fluid flow, mechanical forces, and the cellular microenvironment. Their combined use could therefore support the development of more complex tissue structures and improve the functional relevance of organ-specific models [121].

CONCLUSION:-

Organ-on-chip technology and digital twins represent complementary components of a broader shift toward human-relevant, data-driven pharmaceutical development. Organ chips can reproduce selected organ-level functions and provide experimentally measured responses from human cells under controlled dynamic conditions. Digital twins and mechanistic computational models can integrate those observations with prior physiological knowledge, simulate untested scenarios and support precision dosing or virtual study design. Evidence from Liver-Chip toxicology, multi-organ pharmacokinetic modelling and recent digital-twin/MPS studies indicates that the integration is technically plausible and scientifically promising. The most defensible position is not that these technologies have already eliminated the need for all animal experiments, but that they can progressively replace, reduce or refine animal studies when validated for a specific context of use. Regulatory initiatives from the FDA and ongoing international work on NAM qualification make the integrated physical–computational paradigm an especially timely topic for pharmaceutical science.

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