

# Human-on-a-Chip Technology: A View on Novel Approaches for Precision Medication Therapy

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## ABSTRACT

Traditional drug discovery relies heavily on animal models and conventional in vitro systems; however, their ability to accurately predict human responses remains limited. Human-on-a-chip (HOC) technology has emerged as an advanced microphysiological platform that integrates multiple organ-on-a-chip (OOC) systems within a microfluidic network to replicate human physiological functions and inter-organ interactions. Unlike single OOC models that focus on individual organ functions, HOC systems enable the study of complex systemic processes, including absorption, distribution, metabolism, and excretion (ADME) of therapeutic agents.

HOC platforms combine microfluidics, microsystem engineering, and cellular biology to recreate physiologically relevant human microenvironments in vitro. Progression from two-dimensional (2D) to three-dimensional (3D) culture systems and ultimately to HOC technology has substantially improved the ability to model human responses to drugs and diseases. These platforms offer significant advantages over conventional preclinical models by enhancing predictive accuracy, reducing reliance on animal testing, and supporting personalized medicine approaches.

This review discusses the fundamental concepts of HOC technology, including design strategies, microfabrication and bioprinting methods, biological applications, and current challenges. Furthermore, it highlights the potential of HOC systems in drug discovery, disease modeling, toxicity assessment, and precision medicine. Although technical and standardization challenges remain, HOC technology has demonstrated considerable promise in improving preclinical screening efficiency and reducing research and development costs. Continued advances in fabrication methods, validation protocols, and regulatory acceptance are expected to accelerate the clinical and industrial adoption of HOC platforms.

## Keywords

Human-on-a-chip technology; Body-on-a-chip; in vitro models; microfluidics; Organ-on-a-Chip; personalized medicine

## INTRODUCTION

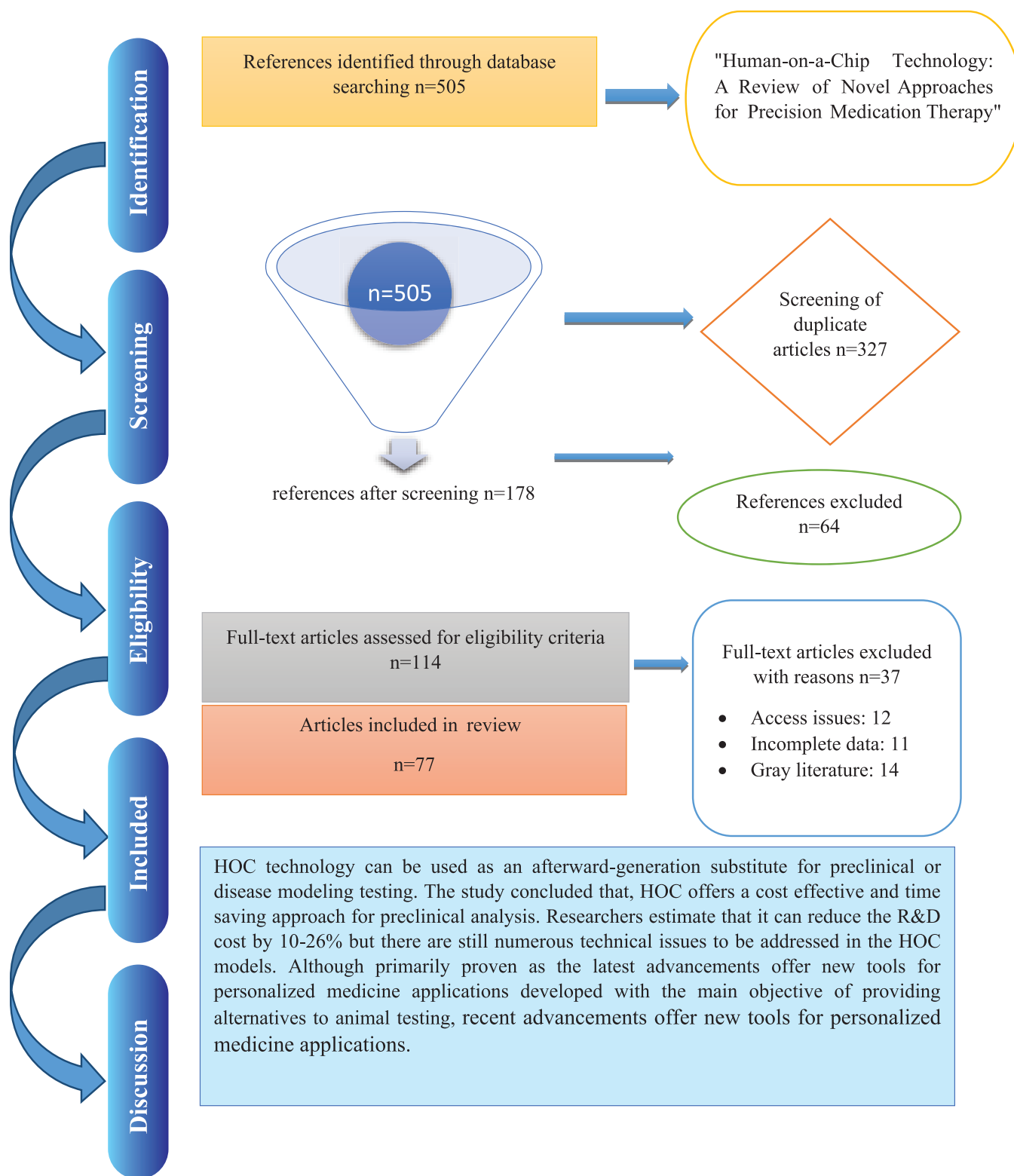
The discovery and development of new therapeutic agents remain complex, costly, and time-consuming processes. Despite substantial advances in biomedical research, the pharmaceutical industry continues to face high attrition rates during clinical development. It is estimated that nearly 90% of drug candidates that demonstrate promising results during preclinical testing ultimately fail during clinical trials, with failure rates exceeding 95% in oncology-related drug development. These failures are largely attributed to the limited ability of conventional preclinical models to accurately predict human physiological responses<sup>(1-3)</sup>.

Traditionally, drug discovery has relied on two principal experimental approaches: in vitro cell culture systems and animal models. Although these methods have significantly contributed to biomedical research, both possess inherent limitations. Conventional in vitro models often fail to reproduce the complex cellular interactions and dynamic microenvironments present in living tissues, whereas animal models frequently exhibit species-specific physiological differences that limit their predictive value for human responses. Consequently, there is an increasing need for more physiologically relevant preclinical platforms capable of bridging the translational gap between laboratory findings and clinical outcomes.<sup>(1-4)</sup>

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**Graphical Abstract/ Methodology**

Recent advances in tissue engineering, microfluidics, biomaterials, and cellular biology have led to the development of micro physiological systems known as Organ-on-a-Chip (OOC) technologies.<sup>4,8</sup> An OOC is a micro engineered platform designed to mimic the structure and function of a specific human organ by recreating its cellular architecture, biochemical signaling, and mechanical microenvironment. These systems have successfully been developed for several organs, including the lung, liver, kidney, heart, intestine, skin, and brain.<sup>8-13</sup>

While single-organ models provide valuable insights into organ-specific physiology and disease mechanisms, they cannot fully reproduce the complex interactions that occur among multiple organ systems within the human body. To address this limitation, researchers have developed Human-on-a-Chip (HOC) technology, also referred to as multi-organ-on-a-chip systems<sup>3,25</sup>. HOC platforms integrate multiple interconnected organ models within a single microfluidic network, enabling the simulation of systemic physiological processes and inter-organ communication. Such systems allow researchers to investigate absorption, distribution, metabolism, and excretion (ADME) pathways, drug efficacy, toxicity, and disease progression in a more comprehensive and physiologically relevant manner.<sup>3,25,26</sup>

The evolution of HOC technology has been driven by progressive improvements in in vitro culture systems.<sup>5,7</sup> Early two-dimensional (2D) cell cultures provided a simple and cost-effective method for studying cellular behavior but lacked the structural complexity of native tissues. Subsequent development of three-dimensional (3D) culture systems, spheroids, and organoids significantly improved the physiological relevance of in vitro models by promoting enhanced cell-to-cell and cell-to-matrix interactions.<sup>6,7</sup> Building upon these advancements, microfluidic technologies introduced dynamic fluid flow and mechanical stimulation, ultimately enabling the development of sophisticated organ-on-chip and human-on-chip platforms.<sup>21-24</sup>

In addition to improving the predictive accuracy of preclinical studies, HOC systems have emerged as promising tools for precision medicine. By incorporating patient-derived cells, these platforms can facilitate personalized drug screening, disease modeling, and therapeutic optimization while reducing reliance on animal experimentation. Furthermore, HOC

technologies offer opportunities to decrease research and development costs and accelerate the identification of safe and effective therapeutic candidates.

This review provides a comprehensive overview of Human-on-a-Chip technology, including its scientific foundations, microfluidic principles, design strategies, fabrication approaches, and biomedical applications. Particular emphasis is placed on the role of HOC systems in drug discovery, disease modeling, toxicological assessment, and personalized medicine. Current challenges, limitations, and future perspectives are also discussed to highlight the potential of this rapidly evolving technology in transforming preclinical research and healthcare innovation.

## **From 2D to 3D Cell Cultures: *Modelling Human Tissue Physiology***

### **Two-Dimensional (2D) Cell Culture**

Two-dimensional (2D) cell culture systems represent the earliest and most widely used in vitro model for studying cellular behavior. In this approach, cells are grown as a monolayer on flat, rigid substrates such as plastic or glass in the presence of nutrient-rich culture media. This configuration allows uniform exposure of cells to nutrients and experimental compounds, making it a simple and cost-effective tool for basic biological studies and high-throughput screening.<sup>1,4</sup>

However, 2D cultures do not accurately reproduce the structural, mechanical, and biochemical complexity of native tissues.<sup>5,7</sup> The lack of three-dimensional cell-cell and cell-extracellular matrix (ECM) interactions significantly alters cellular morphology, polarity, and gene expression, thereby limiting their physiological relevance and predictive accuracy in drug testing.<sup>6</sup>

### **Three-Dimensional (3D) Cell Culture**

To overcome the limitations of 2D systems, three-dimensional (3D) cell culture models were developed. In these systems, cells are grown within scaffolds, hydrogels, or low-adhesion environments that promote aggregation into spheroids or tissue-like structures. These configurations enhance cell-cell and cell-matrix interactions, resulting in improved replication of in vivo-like tissue architecture and function.<sup>5-7</sup>

Despite their improved physiological relevance, 3D spheroids often suffer from diffusion limitations. In larger spheroids, restricted oxygen and nutrient transport can lead to hypoxic cores and necrotic

regions, which may affect experimental consistency and reproducibility. Additionally, static 3D cultures fail to fully replicate the dynamic mechanical forces and fluid flow present in living tissues.<sup>12-15</sup>

### Organoids

Organoids are advanced 3D self-organizing structures derived from stem cells or tissue-resident progenitor cells. These systems recapitulate key structural and functional features of native organs under appropriate biochemical and biomechanical conditions. Organoids derived from induced pluripotent stem cells (iPSCs) or tissue-derived cells (TDCs) have been successfully developed for multiple organs, including the brain, liver, kidney, intestine, and lung.<sup>14-16</sup>

Although organoids provide a higher level of biological complexity than conventional 3D cultures, they remain limited by the absence of vascularization and systemic physiological interactions. As a result, they cannot fully replicate dynamic processes such as blood flow, immune interactions, and inter-organ communication.<sup>7,9</sup>

### Transition toward Organ-on-a-Chip Systems

The limitations of static 2D and 3D culture systems, including organoids, have driven the development of microfluidic-based platforms known as Organ-on-a-Chip (OOC) technologies. These systems integrate living cells within micro engineered channels that provide controlled fluid flow, mechanical stimulation, and tissue-specific microenvironments.<sup>10-12</sup>

By incorporating dynamic perfusion and mechanical cues, OOC platforms more closely replicate in vivo physiological conditions compared to static culture systems. (14) This advancement represents a critical transition from simplified cell culture models toward more predictive and functionally relevant micro physiological systems, forming the foundation for Human-on-a-Chip (HOC) technology.<sup>15</sup>

### Organ-on-a-Chip (OOC) Technology

Organ-on-a-Chip (OOC) technology refers to micro engineered in vitro platforms designed to replicate the structural, functional, and mechanical properties of individual human organs. These systems combine living cells with microfluidic channels to recreate organ-specific microenvironments, including biochemical gradients, mechanical forces, and dynamic fluid flow.<sup>8</sup>

A landmark development in this field was the lung-on-a-chip model, which demonstrated the ability to mimic

human respiratory function using a microfluidic device composed of parallel channels separated by a porous membrane.<sup>10</sup> The upper channel is lined with alveolar epithelial cells, while the lower channel contains vascular endothelial cells.<sup>11</sup> Cyclic mechanical stretching simulates breathing motions, enabling the recreation of key physiological functions of the human lung, including gas exchange and inflammatory responses.<sup>12</sup> This breakthrough established the foundation for subsequent development of OOC models for other organs such as the heart, liver, kidney, intestine, skin, brain, and placenta.<sup>13</sup>

### Microfluidic Systems in OOC Platforms

Microfluidics forms the technological backbone of OOC and Human-on-a-Chip systems. It involves the precise manipulation of small volumes of fluids (typically micro- to nanoliter scale) through channels with dimensions ranging from 10 to 100 micrometers. These systems enable controlled delivery of nutrients, removal of waste products, and application of mechanical and chemical stimuli to cultured cells.<sup>21-24</sup>

Microfluidic devices are typically composed of materials such as polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA), silicon, glass, and paper-based substrates. Among these, PDMS is most widely used due to its optical transparency, biocompatibility, gas permeability, and ease of fabrication.<sup>23</sup>

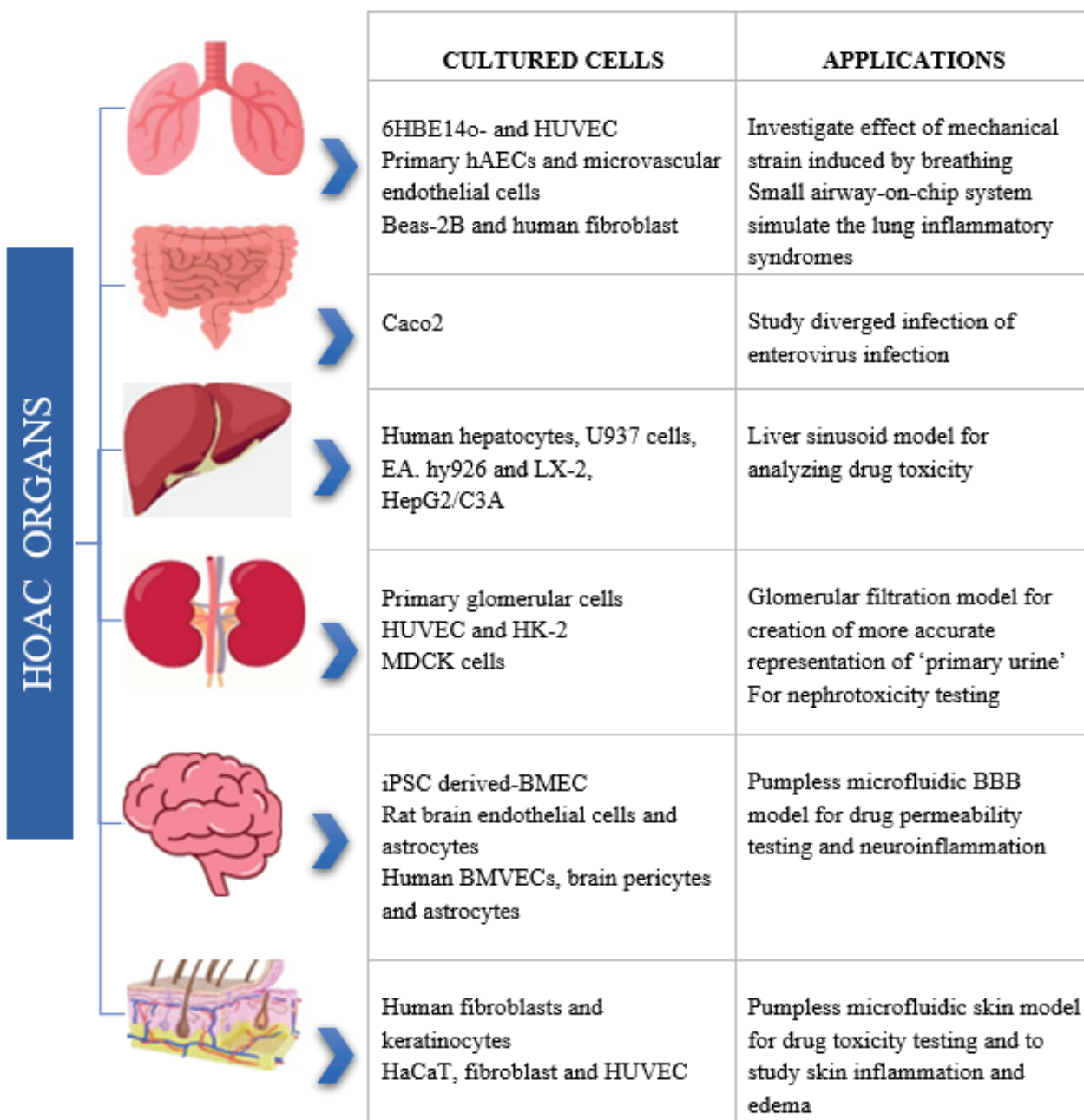
Key advantages of microfluidic systems include reduced material consumption, high-throughput experimental capability, integrated multiple processes on a single chip, enhanced control over micro environmental conditions (including shear stress and concentration gradients) and potential for cost-effective and scalable biomedical applications.

These characteristics have enabled microfluidic platforms to transition from experimental laboratory tools to advanced systems for biomedical research, diagnostics, and drug screening applications. Further, the integration of multiple organ-specific microfluidic modules has led to the emergence of Human-on-a-Chip (HOC) systems. These platforms allow interconnection of different tissue models through a shared circulating medium, enabling the study of inter-organ communication and systemic drug responses. As a result, HOC systems provide a more comprehensive representation of human physiology compared to single-organ OOC models.

## Microfluidic Chip Technology

Microfluidic technology has emerged rapidly and is characterized by a variety of micro-domain effects and its position in application-based streams extending from microarrays to cellular-level biophysics<sup>24</sup>. This microfluidic chip technology is further characterized as Organ-on-a-chip or Human-on-a-chip technology.

These microfluidic chips are customized as miniaturized devices for configuration to precisely control physicochemical reactions of the contained fluid. The greater surface area of chip is due to its dense size which ultimately results in high transfer of mass and high throughput. The channels are veined on silicon or polymer layers characteristically to facilitate proficient mixing<sup>24</sup>.



**Fig.1:** Human Organs-On-A-Chip with Their Cultured Cell Sources and Applications

## Multi-organ Chip Technology: Human-on-a-chip

Human-on-a-chip (HOC) denotes an in-vitro model to simulate the functioning of the entire human physiological system within a microfluidic system<sup>22</sup>. Pharmaceuticals and biomedical sectors significantly get assistance for drug testing and development from these multi-organ-on-a-chip eventually termed as Human-on-a-chip. HOC is a favorable substitute for animal-based testing models with the greater significance of high throughput besides high content screening at a small scale. HOC technology offers outstanding control over spatial uniformity, chemical gradients, time-dependent biochemical stimuli, and substrate automatic properties, etc.<sup>25</sup>

This HOC technology is crucial for development of well-designed micro tissue and organoids of evocative in vivo physiology and symbolizes an auspicious technology for screening and development of drug and novel medicaments. It addresses the scalability issues, batch-to-batch variability, and poor predictability that limits the pace of clinical translation of Nano therapeutics<sup>26</sup>.

The *fig.01* (27) represents the multifaceted organ systems assimilated into microdevice that imitate physiology of different organs such as brain, heart and even teeth. OOCs utilizes a strategic mix of multicellular arrangement (primary cells, cancer cell lines, and stem cells) which is essentially required to emulate the complex structural and functional reality of human tissues and dynamic microenvironments for accurate cancer modeling and drug screening.<sup>27</sup>. The employment of these inter-connected organs in chip modules to form HOC, helps in depth insights into complicated human physiology on an entirely diverse scale.

HOC systems emulate essential functional components of biological barriers through the incorporation of 3D microchannels that function as culture substrates for diverse cell types. To accurately replicate intricate functioning of biological barrier, these in vitro systems must possess:

- Precise spatial arrangement of cells to form micro tissue architectures.
- A biochemical microenvironment specific to the respective organ
- Effective interfaces between functional

tissue-tissue and tissue-micro environment interactions.

By embodying these features, these platforms can assist to invaluable in vitro models, facilitating the investigation of intricate biological and pharmacological processes at different biological barriers<sup>28</sup>.

## Microfluidic-Based Platforms to Simulate Physiological Biological Barrier:

To establish a proper in vitro model to simulate a physiological biological barrier<sup>28</sup> through engineering approaches, it is crucial to understand the molecular and physiological basis of biological barriers. It includes;

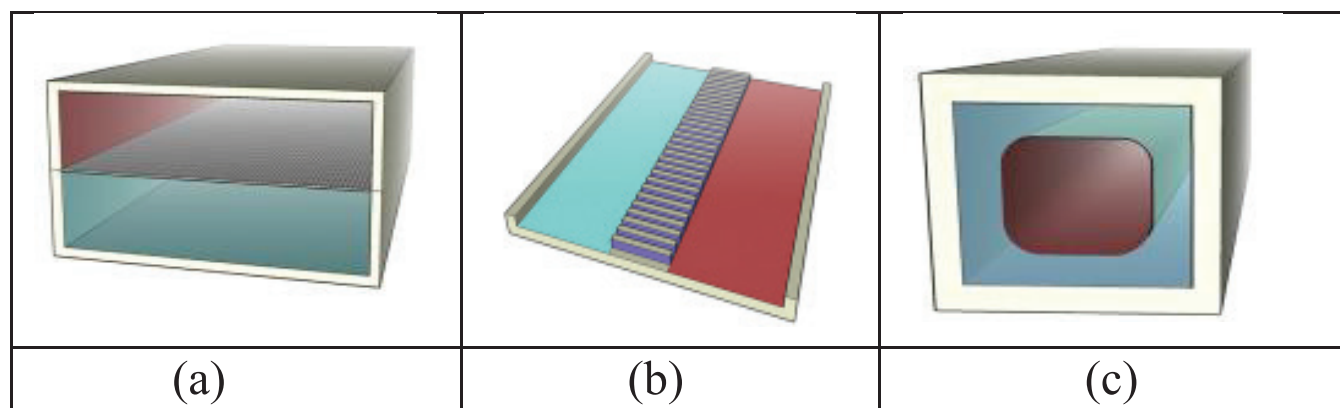
- Structural and biochemical properties of active transport and metabolic barrier.
- Barrier dysfunctioning in diseased state.
- Possible strategies to mend drug delivery at the barrier.

With 3D microchannels, microfluidic HOC simulates the features of functional units of biological barriers, which acts as culture substrate for different cell type. These in vitro models have;

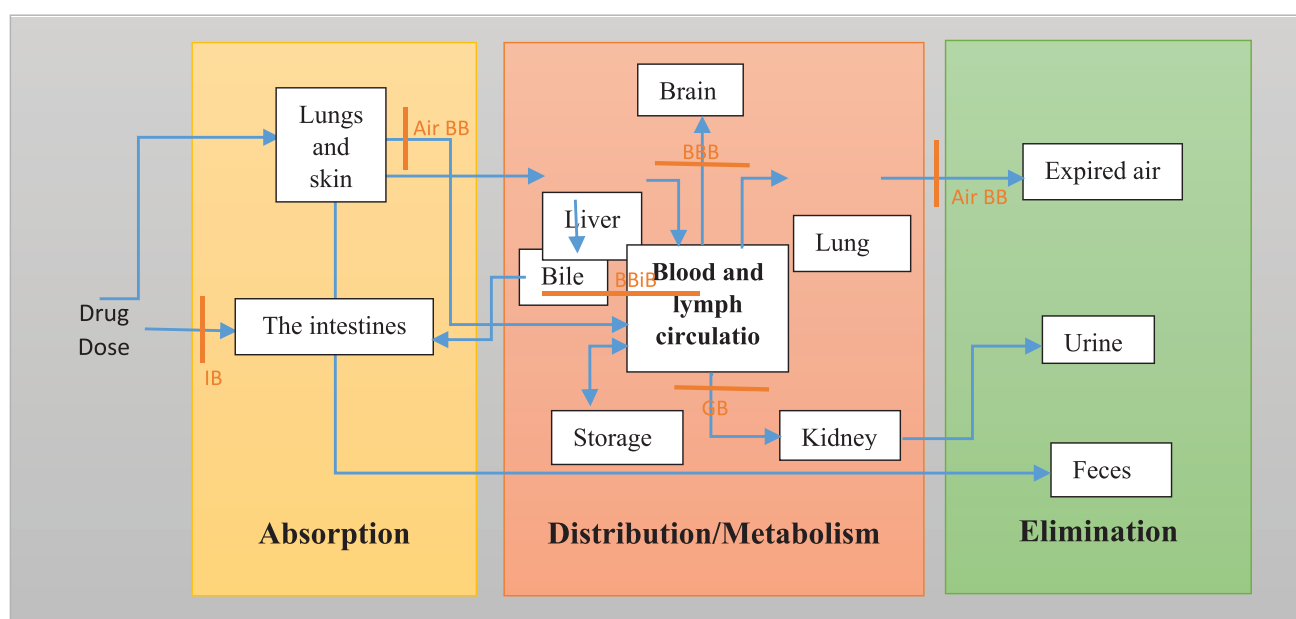
- Proper spatial dissemination of cells forming micro-tissue architecture.
- A biochemical microenvironment for specified organ.
- Functional tissue-tissue and tissue-microenvironment interfaces.

These techniques have been commonly used for creating microfluidic barrier including Porous membrane, Micro fabricated channels and Hydrogel sacrificial mold as seen in *fig.02*.

Human-on-a-chip (HOC) technology significantly advances screening and development of drugs by providing a sophisticated and physiologically relevant platform for mimicking pharmacokinetic and dynamic parameters. The micro channels in the chip mimic blood vessels, allowing for the study of drug transport, metabolism, and distribution between different organs. The 3D micro channels in HOC devices create a dynamic microenvironment that replicates physiological conditions more accurately. This dynamic setup allows the study of time-dependent drug responses and the assessment of long-term effects along with toxicity testing to determine the efficacy of drug and its action



**Fig.2:** Biological Barriers in Microfluidic Chip (a) Porous Membrane (b) Microfabricated Channels (c) Hydrogel Sacrificial Mold



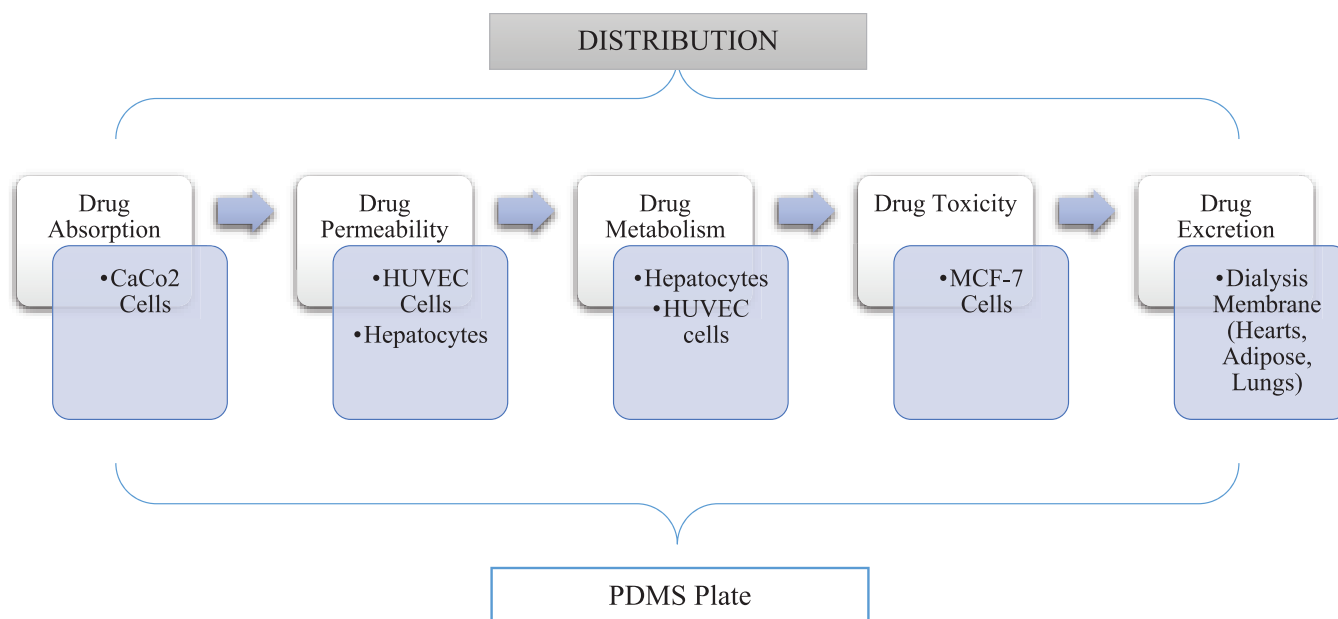
*BB= Air Blood Barrier, IB= Intestinal Barrier, BBiB= Blood Biliary Barrier, GB= Glomerular Barrier, BBB= Blood Brain Barriers.*

**Fig.3a.** Pictorial Demonstration of Pharmacokinetic Parameters Distribution in Human-On-A-Chip

### Human-on-a-chip Working Principle

Human-on-a-chip (HOC) is the favorable 3D microfluidic cellular culture platform to modernize the practice and procedures of existing in-vitro cell culture models<sup>30</sup>. HOC system simulates or mimics the human physiological system or human body in a miniaturized system. This technology has equipped a vast area on land of research since past 20 years. For this technology the cells are developed in microchambers or on surface

of porous membrane which are then perfused constantly with medium passing through numerous microchannels with dimension of about 10-100  $\mu\text{m}$ <sup>30</sup>. The media flow maintains precisely and is controlled to sustain the cell in its healthy state and provide the significant shear stress to prevent the build-up of metabolic waste product. The fluid shear stress be influenced by the velocity of fluid and dimensions of channels. The gravitational flow or capillary driven system flows media with the help of



**Fig.3b.** Represents Different Aspects of Drug Development or Testing

active pump from one compartment to other <sup>25</sup>. Ease of manipulation of micro-liter volume of liquids has made the HOC models a platform to achieve scaling and dynamic cross talk between cells <sup>26</sup>. The common sources of cell used in HOC are: Immortal cell lines or tissue biopsies, Stem cells, and Induced Pluripotent stem cells are shown in *fig.04*.

- *Fresh tissue biopsies* significantly simulate the in-vitro and in-vivo physiology, but dearth the wide convenience for assemble from patients.
- *Primary cells* derived by biopsies can be preserved in culture for longer duration and allows more comprehensive studies; however, the 3D structure of in-vivo organs rebuilt for development of demonstrative functional assays.
- *Pluripotent Stem cells* include different type of cells derived from embryos and embryo related tissue, or can be obtained by reprogramming of adult somatic cells (human induced pluripotent stem cells, hiPS; represents unlimited potent human cell sources to use, derived from patient by minimal invasive procedures) <sup>25</sup>.

## DESIGN STRATEGIES

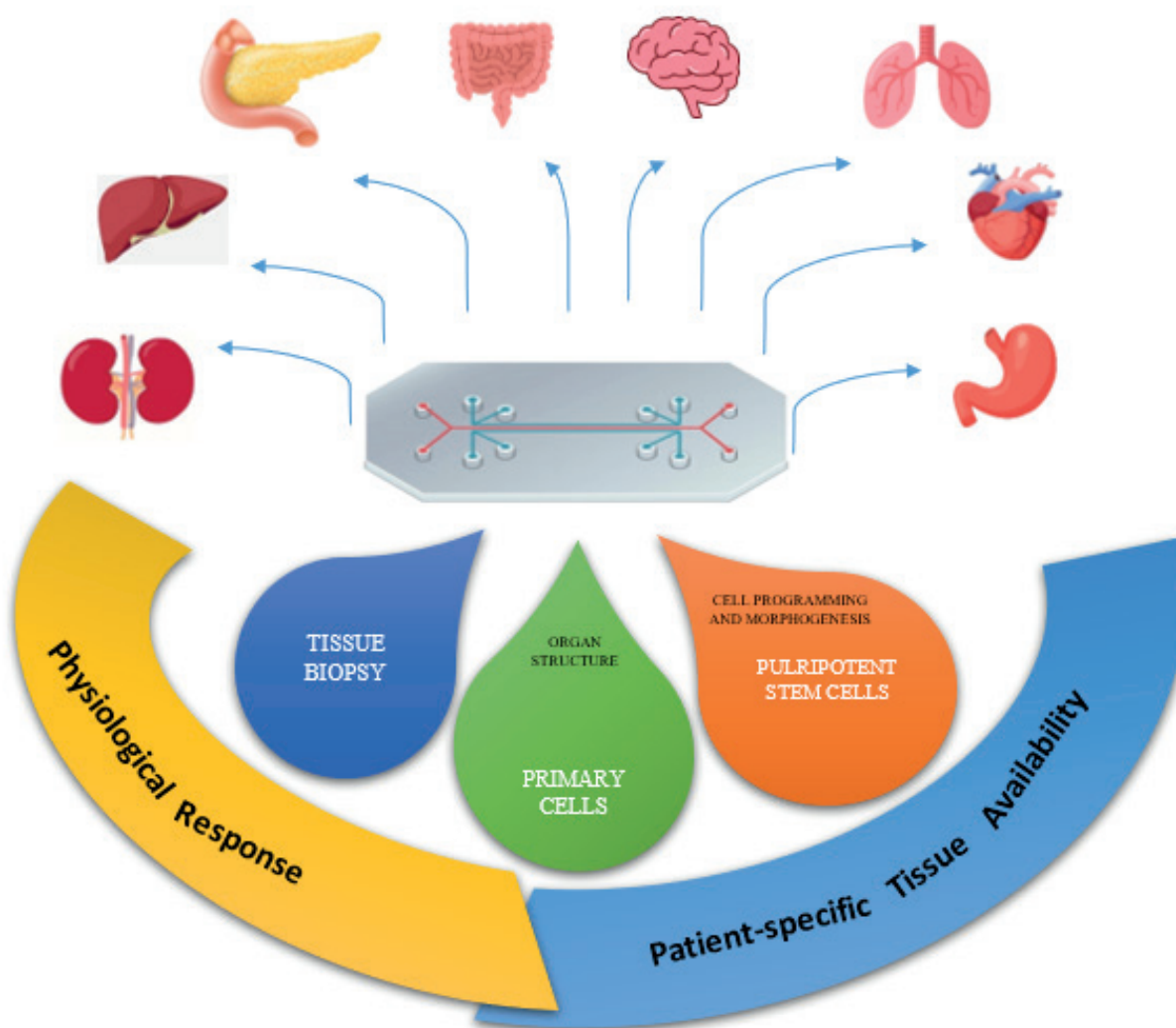
The design of Human-on-a-Chip (HOC) systems requires the integration of biological, mechanical, and engineering principles to recreate physiologically relevant microenvironments. A well-designed platform must ensure controlled fluid dynamics, appropriate cellular organization, and efficient inter-organ communication. <sup>28,31</sup>

Key design requirements include precise spatial arrangement of cells to form tissue-like architectures, maintenance of organ-specific biochemical environments, and establishment of functional tissue–tissue interfaces. <sup>34</sup> In addition, dynamic perfusion is essential to replicate vascular flow, regulate nutrient supply, and remove metabolic waste products.

Modern HOC platforms often incorporate microfluidic networks with embedded sensors for monitoring parameters such as oxygen concentration, pH, and cellular metabolic activity. These integrated systems enhance experimental control and allow real-time assessment of biological responses. <sup>39</sup>

## Methods of Microfabrication:

Microfabrication refers to a set of engineering processes used to construct structures at the micrometer scale.



**Fig. 4:** Sources of Cells for HOC Development

These techniques are fundamental to the development of microfluidic devices and HOC platforms, enabling precise control over channel geometry and cellular microenvironments.<sup>35,40</sup>

Common fabrication approaches include soft lithography, photolithography, and laser-based structuring methods. Each technique offers distinct advantages and limitations depending on the required resolution, material compatibility, and scalability.

#### **Soft Lithography:**

Soft lithography is a widely used technique for fabricating microfluidic devices due to its simplicity, low cost, and compatibility with biological applications.

It typically employs polydimethylsiloxane (PDMS) to replicate micro-scale patterns from a master mold. PDMS is favored for its optical transparency, flexibility, and gas permeability, making it suitable for cell culture systems.<sup>42</sup>

However, limitations include challenges in large-scale production, difficulty in achieving complex three-dimensional structures, and restricted compatibility with certain materials.<sup>47</sup>

#### **Photolithography:**

Photolithography enables high-resolution patterning of microstructures using light-sensitive materials. It is widely used in cleanroom-based fabrication processes

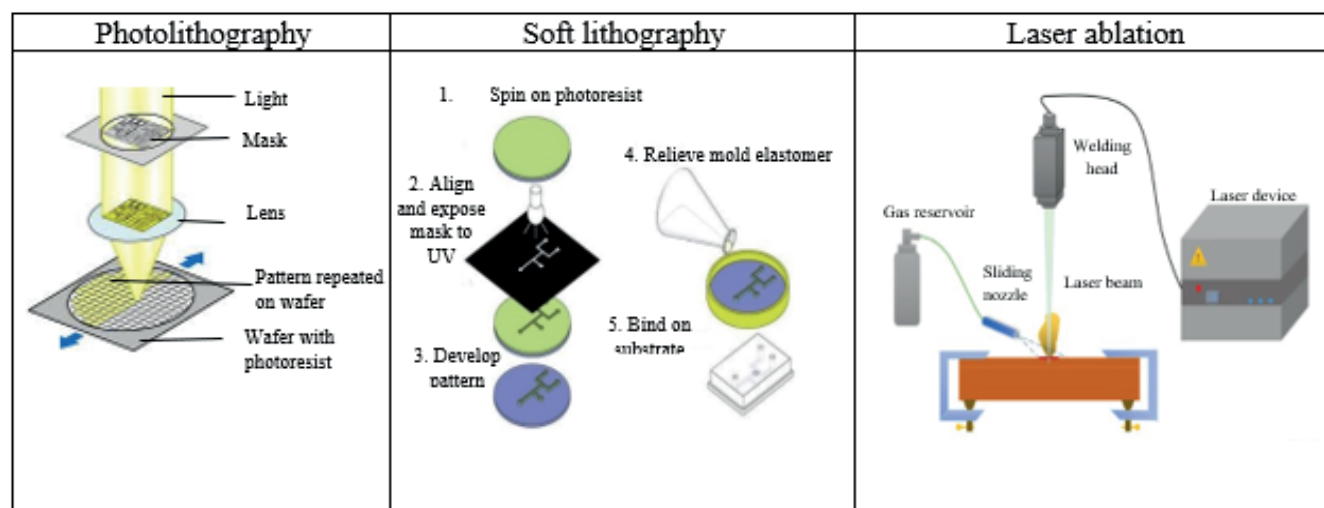
and provides excellent reproducibility and precision. Despite its advantages, it requires expensive equipment, specialized facilities, and is generally limited to planar structures and photo-cross linkable materials.<sup>44,45</sup>

### Laser Ablation:

Laser ablation techniques use focused laser energy to remove material and create microchannels with high precision. This method allows flexible patterning without the need for physical masks. However, it may

introduce thermal damage, surface irregularities, and limitations in fabricating complex 3D geometries. The importance of laser ablation includes; wide acceptance of its work piece materials which may be thin, elastic, and require zero mechanical strength. This method is restricted due to the non-uniform depth of the cavity and slanted side walls, and requirement of multiple laser shots to reach the desired depth, and the inconvenience of achieving the size of focus<sup>32</sup>.

**Table 1:** Schematic Processing of Different Microfabrication Techniques.



**Table 2:** Fabrication Methods with Their Materials, Advantages, Disadvantages and Limitations

| Fabrication Method | Materials                                                                       | Advantages                                                                                                     | Disadvantages                                                                                                                   | Limitations                                                                                                                                                                                                                                     | Ref.                 |
|--------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Soft Lithography   | Polymers (PDMS, SU-8), Collagen, Alginate, Fibrinogen, PEG, PCL, PVA, PGA, PLA. | Time-saving, Cost-effective, Reusable molds, Easy to handle, High throughput, Wide range of resolution (nm-mm) | Continuous structure is required, and a Beam is needed to fabricate the stamp.                                                  | Limited material biocompatibility and high resolution are challenging to achieve, not reproducible on a large scale, hard to construct 3D structures, skilled personnel id required, and integration of diverse functional groups is difficult. | (35)<br>(36)<br>(32) |
| Photolithography   | PDMS, PMMA, PEG, PEO, PVA, PU, Gelatin, Collagen, and Fibrinogen.               | Strong reproducibility, Wide resolution ranges (micron -ml).                                                   | Costly equipment and skilled personnel are required, Time consuming, and restricted to the use of photocrosslinkable materials. | Expensive, limited surface properties control, not used directly on biological components, inaccessible.                                                                                                                                        | (37)<br>(38)<br>(33) |
| Laser Cutting      | Polymers, hydrogels, cells.                                                     | Robust time saving, alternative to replace PDMS,                                                               | Expensive, Ventilated rooms or instruments are required to remove harmful gases while laser cutting of plastics.                | Limited adaptability in material selection, potential to thermal damage to delicate structures, difficulty in creating 3D structures, time consuming.                                                                                           | (39)<br>(40)<br>(41) |

PDMS: Polydimethylsiloxane, PEG: Polyethylene Glycol, PCL: Polycaprolactone, PVA: Polyvinyl Alcohol, PGA: Polyglycolic Acid, PLA: Polylactic Acid, PMMA: Polymethyl Methacrylate, PEO: Polyethylene Oxide, PU: Polyurethane.

### 3D BIOPRINTING TECHNIQUES:

Three-dimensional (3D) bioprinting has emerged as an advanced fabrication approach for constructing complex and biomimetic tissue architectures for Human-on-a-Chip (HOC) systems. Unlike conventional microfabrication techniques, bioprinting enables the precise deposition of living cells, biomaterials, and bioactive molecules in a layer-by-layer manner, facilitating the creation of heterogeneous and functional tissue constructs.

The integration of bioprinting with microfluidic platforms has significantly enhanced the ability to reproduce physiologically relevant tissue structures with controlled spatial organization, mechanical properties, and cellular microenvironments.

The 3D bioprinting can be carried out in either one step or more than one steps of fabrication. In one-step fabrication, the complex structure can be built and heterogeneity can be achieved by marginal administration of polymer and bio-ink along with cells is deposited to craft HOC. While, in two-step fabrication, the cells carrying bio-inks are deposited on the pre-fabricated platform, after that, they then gather with a glass cover holding channels<sup>30</sup>.

The molecular mass of drug molecules must be smaller than 500Da (Dalton) to permeate the component of the medium and drugs and reach the interior of cells. Rhodamine dye is used as a representative protein molecule with a molecular mass of 479 Da<sup>43</sup>. The absorption ratio is calculated by sampling the inlet and outlet to compare dye concentrations. Lower absorption in the channel indicates reduced wall-leaching. With PDMS exhibiting a three-fold higher dye absorption value than PCL, PCL retains molecules in the fluidic stream, making it superior for HOC platforms.<sup>43</sup>. Hence, 3D bioprinting not only suitable for the development of multifaceted heterogeneous HOC devices, but also diminishes the labor-intensive intervention, labor expenditures and assist in designing with more accuracy and reproducibility<sup>30</sup>.

#### Bioinks and Biomaterials

Bioinks are hydrogel-based formulations that encapsulate living cells and provide structural support during and after the printing process. Common natural biomaterials include alginate, collagen, gelatin, fibrin, and agarose, while synthetic polymers such as polycaprolactone (PCL) and polyethylene glycol (PEG) are also widely used.

An ideal bioink should exhibit suitable viscosity for printing, high biocompatibility, mechanical stability, and the ability to support cell viability and function. The selection of bioink is critical for ensuring structural fidelity and biological performance in HOC platforms.

#### Inkjet Bioprinting:

The inkjet bioprinting technique is also stated as drop-on-demand or drop-by-drop printing. The first generation of inkjet bioprinting was carried out by modified conventional printer used for printing on papers. Now biomaterials, cells, and substrates are being used in place of inks and papers<sup>44</sup>. The inkjet-based Bioprinting is based on either a powder or a photopolymer. In this method the pattern or design layer is created by the printer at once by distributing a layer of powder and inkjet printing binder in a cross-section. Post-printing finishing work is necessary, in which the surrounding powder is blow off, thermoset polymer impregnation and wax is used for strengthening the bonded powder, and a photopolymer system is used to deposit the small volume to construct and sustain material and in consecutive layers to form an entity in XYZ direction with resolution (~30 micrometers) comparable to a laser printer.<sup>45</sup>. The thickness of a layer is around 100 micrometers (0.1 mm), while certain printing machines can print layers as thin as 16 micrometers<sup>32</sup>. With high precision, the droplets of biomaterials (of about a few nanoliters) with or without cells are deposited on a substrate.<sup>45</sup>

The droplet is formed through vaporization and dispensed through extruded actuators (thermal or piezo-electric modality) on a platform in a layer-by-layer manner for the fabrication of 3D structures. In certain printers, the 3D structure in XYZ direction is constructed by rotating extruders on a fixed printing platform.<sup>45</sup>

The technique depends upon factors include various physical properties of inks (such as; viscosity, surface tension), or process dependent parameters (such as; nozzle diameter, feed rate and layer thickness) collectively impacts the ultimate diameter of drop and resolution of structure printed<sup>44</sup>. The positioning of jet on substrate is require which can be done by optimizing the conciliation between the applied force and distance between nozzle and substrate.

In this technique, the concurrent loading and depositing multiple cells and biochemical materials spatially during the process and it is significant for construction

of composite and multifunctional and gradient structure like tissue interfaces.

The major drawback of this technique is the “only low viscosity” bioink utilization by this method, the highly viscous bioinks could delay the cross-linking of hydrogels at each layer, significantly for large structures. Also, the obstruction of nozzle limits the density of cells of bioinks and offers high cell viability.<sup>44, 45</sup>

The bioinks with low viscosity (~0.1Pa) is suitable for bioprinting. The shape conformity of vertical construct is poorer with inkjet than other printing techniques. Due to all these factors, the use of inkjet bioprinting technique limited for the fabrication of chip platform containing multiple tissues and biomaterials with higher viscosity.<sup>45</sup>

### **Laser Assisted Bioprinting (LAB):**

LAB is non-interactive with complex hardware, a commonly used, but costly, the laser-based process of patterning, also known as laser-direct-write, and now more increasingly used in tissue engineering<sup>46</sup>. Wide variety of materials with high resolution-deposition and bio-inks of various compositions, such as; bio-polymers peptides, DNA, and Nano-tubes are used<sup>44</sup>.

LAB entails a pulsed laser beam, a converging system, a donor slide containing two layers; one is an energy absorbing layer, and other is biomaterial layer, and a collector substrate slide. The bubbles are generated by absorption of laser on substrate (either gold or titanium), which afterwards impel the cell containing materials from the donor slide onto the collector slide by generating waves of shock.<sup>42, 46</sup>

The bioprinting design is constructed by deposition of droplet with pre-designed patterns layer-by-layer to construct 3D structure. The droplet is pre-coated with hydrogel layer to reduce its impact<sup>47</sup>. The various factors that influence the resolution of LAB includes laser energy, pulse frequency, viscosity and consistency of biomaterial layer, air gap between donor and collector side along with wettability of substrate slide<sup>42, 46</sup>.

The significance of LAB technique is that, it is a nozzle free processing thereby avoiding the complications associated with its blockage and greater shear stress on its tip. Consequently, it enables the patterning with high cellular densities while maintaining the high cell viability (>95%)<sup>42</sup>.

The drawbacks of this technique is its high cost, along

with lengthy preparation time and trouble in precise targeting and cells deposition<sup>46, 47</sup>. In terms of volume (bioprinting per minute) and sample preparation, this technique is one of the least productive. The cellular suspension is loaded on a bottom of donor slide to maintain humidity (nearly 100%) throughout all the process. The most challenging is the homogenous distribution of cell suspension on the slide over a short time (10min)<sup>42</sup>. The trouble in loading of suspension homogeneously are due to the effect of gravity on cell layers, which results in formation of aggregates. The 10% glycerol is also added to counter the evaporation of suspension, so the duration for deposition of droplet is enhanced<sup>42</sup>.

Certain advances in technical development are under consideration to overcome the hurdles of bioprinting technologies such as; resolution, speed, cell viability, cell densities, and proper cross-linking method. The two-photon polymerization (2PP) is a laser assisted bioprinting technique which allows the cross-linking of high accuracy by selective irradiation. Because of its high resolution, the construction ranges between few hundred microns to few milliliters. 2PP is mainly employed in construction of 3D platform that are post sowed with cells, the cells directly embedded in resin now a days<sup>46, 47</sup>.

### **Extrusion Bioprinting:**

Extrusion bioprinting is a widely used bioprinting method. It denotes that materials flow constantly from the reservoir over a nozzle fixed on an automated-controlled collector in a layer-by-layer fashion<sup>44</sup>. The extrusion-based process possesses a higher printing speed than other fabrication processes and is more convenient to use due to its simplicity. The parameters that need to be focused in this method is the optimization of conformity of shape and cell viability. Smaller the diameter of the needle increases the conformity of the shape of the structure printed, but it can be harmful to the cells due to high shear stress. The sensitivity of the cells varies with shear stress<sup>45</sup>. The feeding material followed the mechanism of either a pneumatic or mechanical (piston or screw-based) system. The dissemination of extruded monofilaments following contrary effects on shape conformity is challenging for Extrusion bioprinting, and the requirement of elevation in viscosity for rapid gelation of extruding inks to fabricate configuration with satisfactory geometric precision. To overcome the margins arising due to high viscosity requirements,

various approaches have been established, which include the use of sacrificial secondary mediums, co-axial outlet extrusion structures, and carrying out of pre or post-crosslinking approaches for soft hydrogels <sup>44</sup>.

The extrusion-based bioprinting method is capable of printing the arrangement of biomaterials, such as; composite bio-inks analogous to natural tissues, that's why it is suitable for the fabrication of multiple organs on a chip and extracellular matrix components. In this method, observing the printing temperature which is controlled by heating or cooling the thermal jacket is crucial <sup>45</sup>. Other parameters which are needed to be observed are; consistency, rheological properties, cross-linking mechanism, and shear uniformity behavior of hydrogel inks that direct the sustainability of cell encapsulation and printing by this method.

The drawbacks of the extrusion method of small resolution and slow printing speed besides low cellular bulks are due to the exposure of cells to high shear stress while deposition through the nozzle <sup>44</sup>. The resolution property of printing is based upon the rheology or viscosity (resistance to flow) of bio-inks and storage modulus (a measure of energy stored in the elasticity of bio-inks) <sup>30</sup>. High dispensing pressure and shear stresses imposed on the cells in a viscous fluid and lessening the cell viability results in loss of resolution and speed. It is important to note the printing temperature which is controlled by heating or cooling the thermal jacket, <sup>45</sup>

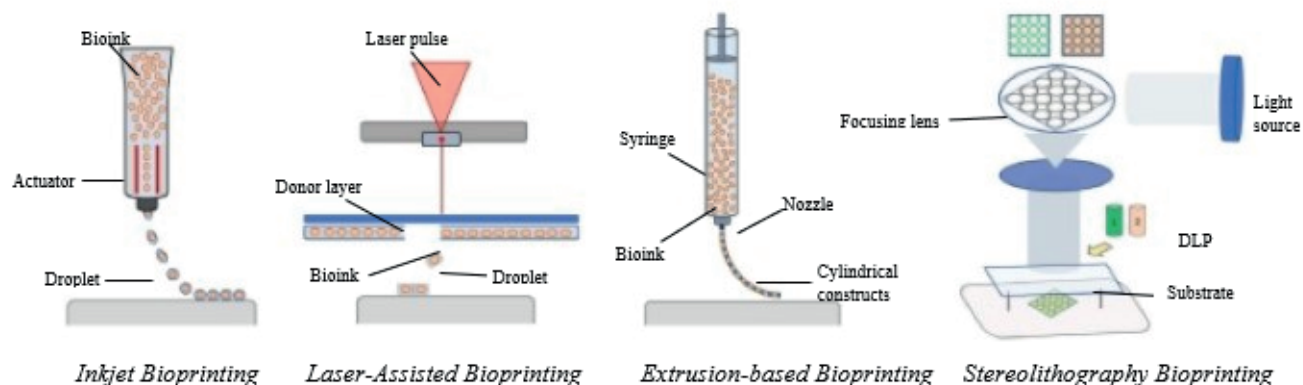
Due to their support for cell viability and function of invitro, the hydrogels formed from proteins such as; collagen or matrigel are considered as gold-standard biomaterials for 3D cell culture. However, these molecules are challenging to fabricate at room temperature or 37°C. In this method, the bio-inks must remain in the liquid phase to prevent clogging of nozzle, but highly viscose bio-inks can damage the cell membranes and negatively impact on cell viability. Nevertheless, the bio-inks must be sufficiently viscous to adhere to the substrate and maintain the desire shape. <sup>4,30</sup>.

### **Stereolithography (SLA):**

Stereolithography (SLA) is the most rapid Microfabrication technique developed in the 1980's. This technique is based on light-based structuring method that often uses UV light to treat light-sensitive bio-inks and hydrogels (biocompatible with photo-initiators to facilitate photo-polymerization of bio-inks) and influences the photo-reactive chemistry of particular

polymers to obtain 3D configurations with high speed and structural resolution. Like extrusion bioprinting, SLA method fabricates 3D tissue constructs in a layer-by-layer fashion <sup>4</sup>. UV laser polymerizes photosensitive resin selectively to form a solid layer to build a platform in a 3D direction, the process is repeated until the construction is completed. <sup>45</sup> Each layer is produced by digitally light-controlled projection on the surface of a collector. Digital light projection (DLP) is the most frequently used technique, in which the whole layer can be cured at once or in a short interval (44). In the SLA-based bioprinting technique, photo-polymerization is induced by regulating light intensity, irradiation time, and concentration of photo-initiators <sup>45</sup>. Either beaming scanning or mask-image projection could be the source of light. In beam scanning, the selective scanning of the focused laser beam is done to achieve continuous drawing and solidification of a flat layer. After the hardening of the flat layer, another layer is added to the previous one and the beaming-scanning process begins again <sup>4</sup>. In the mask-image-projection process, the DLP method is employed to design and define the mask image, useful in the solidification of all layers of bio-ink in a single projection, which results in rapid curing of the bio-ink layer. This structuring technique allows the construction with greater resolution than the extrusion technique <sup>4</sup>. The polymerization of resin can be precisely controlled by positions of laser focus to achieve selective polymerization. Additionally, the vertical printing constructed by SLA has good quality. (45) In comparison to the inkjet bioprinting technique, SLA-based techniques don't endure shear stress of the nozzle, and high cell viability. The crosslinking density of each layer influence by the amount of radiation energy and the time of exposure <sup>4</sup>.

Besides its advantages of SLA-based bioprinting for providing greater printing speed and elevated resolution of microfluidic channel of capillary diameter ( $\geq 50$   $\mu$ m), there are some drawbacks of this technique which includes the release of radicals during photo-crosslinking SLA which might result in elevated cytotoxicity. Further, the SLA used polymerized photo-resin that have poor mechanical properties, and it is restricted to use one biomaterial at a time i.e. it doesn't reflect heterogeneous nature and manages the synchronic activity of various types of cell and organ-functions <sup>4</sup>. The high radiation and toxicity associated with photo initiators used within the hydrogels damages the cells.

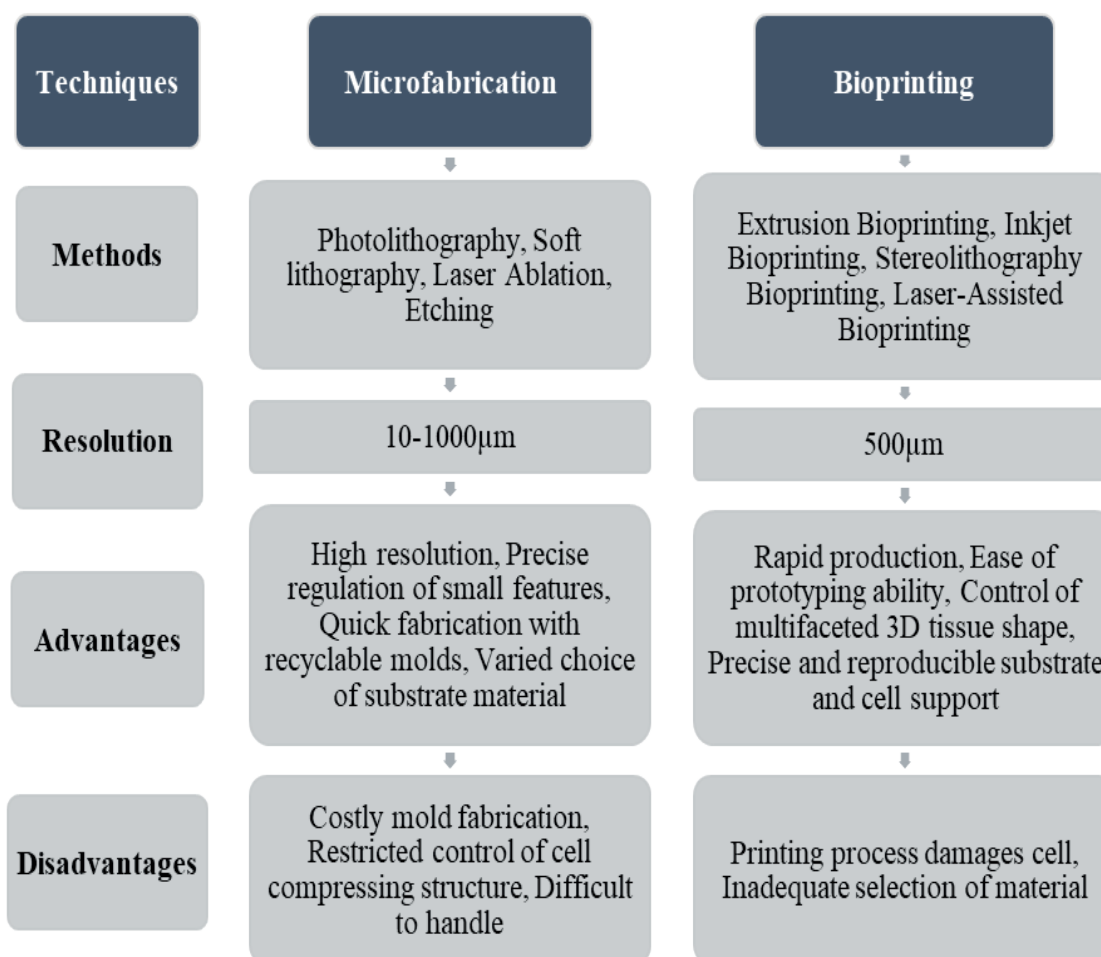


**Fig. 5:** Represents Schematic Illustration of 3D Bioprinting Techniques for Development of HOC

**Table 3:** Represents Bioprinting Methods with Their Materials, Advantages, Disadvantages and Limitations

| Bioprinting Techniques            | Materials and Hydrogels                                                                                                                                                                                                  | Advantages                                                                                                            | Disadvantages                                                                                                       | Limitations                                                                                                                                                                                                                                              | Ref.                 |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| <b>Inkjet Bioprinting</b>         | 3D printed PCL scaffold coated with SAPH, Alginate, Gelatin, Fibrin, Collagen, PLGA, PCL, PEG, and GelMA.                                                                                                                | High output, speed accuracy, reproducible, widely available.                                                          | Provision of thermal and mechanical stress by thermal droplet formation, chances of damage of membrane.             | Only the bio-inks with greater consistency are used, blockage of the nozzle limits the cellular density of bio-inks.                                                                                                                                     | (44, 45, 48)         |
| <b>Extrusion Bioprinting</b>      | PLCL, ceramic doped with various ins (Si, Li, SrPO, SiO, Mo, Cu, Ca, Mn, Mg, Sr), bredigite ceramic.                                                                                                                     | Broad range of materials, economical, wide availability, versatility, can be customized.                              | Resolution limits to 100 $\mu$ m, cell viability only 40% to 80%, inappropriate for complicated network structures. | Excessive shear force impacts cell viability, optimized bio-inks essential for cell adhesion and rheological properties, limited resolution of $\approx$ 100 $\mu$ m, not reproducible.                                                                  | (44, 45, 49, 50, 51) |
| <b>Stereolithography</b>          | Resin, MMP, and photo-polymerizable bio-inks, dECM, GelMA, exosomes, PEG, and Gelatin.                                                                                                                                   | Elevated resolution, viability of cell up to 90%, suitable for multiple scale network channels.                       | Exclusive, Inadequate Bio-compatibility, Greater Cytotoxicity.                                                      | Poor mechanical properties of polymerized photo-resin, limited usage of one biomaterial, not reproducible, increased, due to release of radical species during photo cross-linking causes cytotoxicity.                                                  | (4, 44, 45, 49, 52)  |
| <b>Digital Light Processing</b>   | Alginate, Gelatin, Collagen, Human cells specific to tissue and organs, customized bio-inks, Growth factors, Cytokines, ECM, photo-polymerizable cross-linking material, Cell culture media, and nutrients.              | High printing speed, exceptional automatic properties and organizational integrity, and printing precision.           | Exclusive resin materials, small machine-driven property, danger of cracks or failing over period.                  | Limited resolution and precision, potential cytotoxicity due to photo-initiators, difficulty in achieving heterogeneity between multiple materials, longer printing time, difficulty in designing precise small features, and limited bio-compatibility. | (53, 54)             |
| <b>Laser Assisted Bioprinting</b> | ECM, Proteins, Collagen, Fibrinogen, Gelatin, Alginate, Hyaluronic acid, Cell-laden bio-inks (such as hepatocytes and cardiomyocytes), Photo-initiators, cell culture media (contains nutrients and growth factors etc). | High resolution ranges from pico-meter to micro-meter scale, Printing speediness, and High cellular action precision. | Expensive due to laser usage.                                                                                       | Limited reproducibility, increased cytotoxicity, poor mechanical properties of polymerized photo-resin, inability to design accurate micro environment.                                                                                                  | (49, 55)             |

ECM: Extracellular matrix, dECM: De-cellularized extracellular matrix, MMP: Matrix metalloproteinase, PCL: Poly ( $\epsilon$ -caprolactone), SAPH: Self-assembled peptide hydrogel, PLGA: Poly (lactic-co-glycolic acid), PEG: Polyethylene glycol, GelMA: Gelatin methacrylate, PLCL: Poly(L-lactide-co- $\epsilon$ -caprolactone)



**Fig. 6:** Represents Schematic Comparison Between Microfabrication and Bioprinting Methods for Development of HOC Model

## APPLICATIONS OF HUMAN ON CHIP

Human-on-a-Chip (HOC) technology has emerged as a versatile platform with wide-ranging applications in biomedical research, pharmaceutical development, and personalized healthcare. By integrating multiple organ-level functions within a controlled microfluidic environment, these systems provide physiologically relevant models for studying complex human biological processes.

### Drug Discovery and Development

HOC platforms offer a promising alternative to conventional preclinical models for drug discovery and development. Traditional approaches rely heavily on animal testing and static cell cultures, which often fail to accurately predict human responses. In contrast, HOC systems replicate key physiological conditions,

enabling more reliable assessment of drug efficacy, toxicity, and metabolism.<sup>3,26</sup>

These platforms are particularly useful for evaluating pharmacokinetic and pharmacodynamics profiles, including absorption, distribution, metabolism, and excretion (ADME). By simulating inter-organ interactions, HOC systems allow researchers to study drug transport pathways and metabolic transformations in a more integrated manner.

### DISEASE MODELING

HOC technology enables the development of physiologically relevant disease models that replicate human pathological conditions. These models provide valuable insights into disease progression and underlying mechanisms that are difficult to study using conventional systems.

Multi-organ chip platforms have been used to investigate complex diseases such as cancer metastasis, metabolic disorders, and inflammatory responses. Additionally, interconnected organ systems allow the study of endocrine and immune interactions, offering a more comprehensive understanding of disease pathology.<sup>60,63</sup>

For example, organ-chip networks have been utilized to model insulin regulation in diabetic conditions and to investigate organ-specific toxicity mechanisms of pharmacological agents. Such applications highlight the potential of HOC systems in advancing translational research.<sup>63</sup>

### Precision and Personalized Medicine

One of the most significant applications of HOC technology lies in precision medicine. By incorporating patient-derived cells, these systems can replicate individual-specific physiological responses, enabling tailored therapeutic strategies.<sup>71</sup>

HOC platforms can be used to evaluate patient-specific drug responses, identify optimal treatment regimens, and predict adverse drug reactions. This approach reduces reliance on trial-and-error prescribing and enhances treatment effectiveness.<sup>73</sup>

The integration of induced pluripotent stem cells (iPSCs) further enhances the potential of HOC systems in personalized medicine by enabling the generation of patient-specific organ models for disease simulation and drug testing.

### Toxicology and Safety Assessment

HOC systems provide a reliable platform for toxicity testing by simulating human-specific metabolic pathways. Unlike animal models, these systems can better predict hepatotoxicity, nephrotoxicity, and cardiotoxicity by replicating inter-organ metabolic interactions.<sup>75,77</sup>

This capability makes HOC technology particularly valuable for early-stage safety assessment in drug development, reducing late-stage clinical failures and associated costs.

### Future Research and Translational Potential

The continued development of HOC technology is expected to significantly impact biomedical research and healthcare innovation. Integration with biosensors, artificial intelligence, and advanced imaging systems may further enhance their predictive accuracy and

functionality.<sup>76</sup>

Despite ongoing progress, challenges related to standardization, scalability, and regulatory acceptance must be addressed before widespread clinical and industrial adoption can be achieved.

In the long term, HOC technology has the potential to shift biomedical research toward truly patient-specific modeling. The use of patient-derived stem cells and personalized organ chips could enable individualized drug screening and disease modeling, supporting a transition from population-based treatment strategies to precision medicine approaches.

Overall, Human-on-a-chip systems are poised to become an essential component of next-generation biomedical research, bridging the gap between experimental models and human clinical outcomes.

### Conclusion and future Perspectives:

Human-on-a-chip (HOC) technology represents a major advancement in micro physiological systems, offering a more human-relevant alternative to traditional in vitro and animal-based models. By replicating key aspects of organ-level structure and function within microfluidic platforms, HOC systems have significantly improved the study of human physiology, disease mechanisms, and drug responses. Across drug discovery, disease modeling, toxicology, and precision medicine, HOC platforms provide more predictive and integrated experimental environments compared to conventional methods. Their ability to simulate inter-organ communication and dynamic physiological conditions makes them particularly valuable for improving preclinical screening and reducing late-stage drug failure.

Future developments are expected to focus on the integration of HOC systems with advanced technologies such as real-time biosensors, artificial intelligence, and automated microfluidic control. These integrations may enable continuous monitoring, predictive modeling, and higher-throughput drug testing with improved accuracy.

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## Author Contributions

AA, BM and SG conceived and designed the study, conducted research, provided research materials, and collected and organized data. SG analyzed, interpreted data and supervised. All authors wrote initial and final draft of article, have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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