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Fabrication of organ-on-a-chip using microfluidics

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ABSTRACT

The use of microfluidic devices represents a significant advancement beyond conventional techniques in the development of innovative *in vitro* assays. Microfluidic chips are specialized devices that precisely control fluids at the microscale level through intricate microchannels, enabling the replication of physical and chemical conditions. When combined with tissue engineering, these chips have evolved into highly specialized tools known as Organ-on-a-Chip (OoC) devices, which can simulate the physiology and functionality of various human tissues and organs. OoC devices are cutting-edge technologies that integrate a biological component representing the target organ with a microfluidic component that mimics blood flow. This combination allows for the replication of biological structures with a more accurate representation of the *in vivo* physiological cellular microenvironment, which can be finely tuned by adjusting the flow rate and composition. As a result, novel microfluidic models for *in vitro* research can overcome the limitations of traditional 2D and 3D static cell cultures, enabling faster clinical translation and more precise predictions of the efficacy, safety, pharmacodynamics, and pharmacokinetics of new drugs. This review will discuss various techniques for fabricating OoCs and their applications in mimicking different physiological microenvironments.

1. Introduction

Recent technological advances in microfluidics have enabled OoCs for precise control of fluids passing through the device [1]. Organs-on-Chips (OoCs) combine microfluidic chips and tissue engineering to mimic the *in vivo* physiological environment and functions of human tissues and organs [2]. Nowadays, drug development is based on traditional *in vitro* cell culture assays, but these lack the complexity needed to describe and reproduce the cell interaction existing in living organs and tissues. Furthermore, *in vivo* assays using animal models are also problematic due to ethical concerns but also because are not representative of human physiology and fail in selecting potential candidate compounds for clinical trials. OoCs are an emerging technology that has gained considerable interest in the pharmaceutical and biotechnology industries in recent years due to their ability to accurately mimic the tissue architecture of an organ in a micrometric device. The main application of OoCs is to optimize novel assays aiming to investigate drug pharmacodynamic and pharmacokinetic profiles.

OoCs contain microfluidic channels and/or chambers coated with human vascular cells and organ-specific cells. Fluids and reagents in milliliter, nanoliter, or picoliter quantities are passed through these channels in a controlled and precise manner, and various sensors and techniques allow monitoring of the cell microenvironment such as glucose or oxygen consumption [3,4]. To date, OoCs have successfully reproduced various organs such as lungs, liver, kidneys, heart, and skin. Moreover, when several OoCs are combined within the same system, a Body-on-a-chip can be created [5]. Therefore, OoC technology is expected to grow and foster the advancement of personalized medicine, as well as to consolidate an alternative to conventional *in vitro* testing, reducing animal use.

This high interest in the development of advanced OoCs is guided by the funding of multiple projects by National Research Agencies such as FDA (Food and Drug Administration), NIH (National Institutes of Health) DARPA (Defense Advanced Research Projects Agency) from the United States [6], ORCHID (Organ-on-Chip development) from the European Union [7] and AMED (Japan Agency for Medical Research and Development) from Japan [8]. In 2016, OoCs made the World Economic Forum's list of top 10 emerging technologies as an innovative device

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that mimics organ physiology by combining cell culture, engineering, and biomedical technology, enabling improved medical research and drug discovery in a more precise and controlled manner [9]. However, the need for new screening platforms that could accelerate the development of new drugs and vaccines did not drive the OoC market until 2019, with the advent of COVID-19. Using a variety of techniques and knowledge, a Lung-on-a-chip infection model for several respiratory viruses was developed with great potential for the development of novel *in vitro* tools to evaluate the therapeutic response of existing antiviral drugs [10].

Following the impact of the SARS-CoV-2 pandemic, the growth of the global OoC market has been driven by the increasing demand for precision and rapid drug detection platforms in the healthcare sector. As a result, research and improvement of OoCs, especially the Lung-on-a-chip for the treatment of lung diseases, which are among the most prevalent diseases in the world, has been promoted. Therefore, the Compound Annual Growth Rate (CAGR) is currently expected to increase by approximately 30 % over the next 5–10 years, and some of the companies making inroads into the OoC market include Emulate Inc, Elveflow, AxoSim, and BiomimX, among others [11,12]. Advances in 3D bioprinting and 3D microfabrication can further drive the refinement and growth of Organ-on-Chip technologies.

In general, the advantages of OoCs over conventional *in vitro* methods are increasing their popularity and they are one of the most promising reference technologies in vaccine and drug development, as they more closely mimic the physiological environment of human cells and provide more accurate and highly relevant clinical data. In addition, the ability to adapt the chip to contain multiple layers of cells and to connect them to other organs can allow simultaneous analysis of the complex interactions between cells and organs and the different responses or reactions that can occur in our body [13].

In this review, we aim to discuss the significance of microfluidic chips in the evolution of OoCs. Several applications of OoCs, which are currently in full growth and improvement for their use in drug development and personalized medicine, as well as the biomaterials utilized, will be covered. Finally, the main types of OoCs, their design and structure, and their main application will be also discussed.

2. Microfluidic chips

Microfluidic chips are technological devices that precisely employ and control very small volumes of fluids flowing through microchannels [9]. Working at the micrometric scale favors laminar flow conditions, fast diffusion, rapid heat transfer, and high relative surface area. These properties extend microfluidic chip applications into areas such as energy harvesting, analytical biochemistry, cell separation, and molecular biology [14]. The small size of the channel diameter and the laminar flow (Fig. 1), which allows parallel fluid streams and diffusion mixing, generate better control of the molecules in terms of the space and time of activity. The large surface-to-volume ratio in these microfluidic systems means that gravitational and inertial forces are insignificant and surface tension predominates. These features have the advantage of requiring less sample volume, being easily portable, and allowing several simultaneous and parallel assays and rapid analysis. In addition, these microfluidic chips can be used to carry out various chemical syntheses such as sample preparation, reaction, separation, and detection.

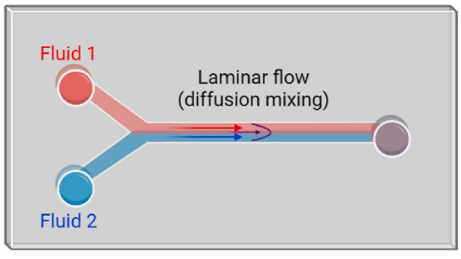

Fig. 1. Laminar flow on a microfluidic chip. The mixing of the two fluids occurs gradually by diffusion.

The development of new drugs involves the synthesis of drug compounds and their subsequent evaluation for efficacy in complex *in vitro* and *in vivo* assays, where conventional techniques generally do not accurately reflect the response that would occur when transferred to a living human organism. Traditional 2D cell cultures are unable to mimic the 3D structure, mechanical properties, and biochemical microenvironment experienced by cells in a living organ, while static 3D cell cultures can overcome some of these limitations (Table 1). Although these methods have made a great contribution to research, the development of microfluidic cell cultured-based platforms can further improve the reproduction of the dynamic, physicochemical, biochemical, and microstructural properties of the living organism's microenvironment to better mimic living organ functions. The use of these microfluidic devices in healthcare can improve the entire drug development process, from drug synthesis and distribution to drug evaluation, reducing the costs and time of preclinical research compared to traditional methods [15–18].

3. Innovative methods of new drug development and evaluation: organ-on-a-chip (OoC)

The discovery of new drugs contributes to the improvement of

Table 1
Advantages and disadvantages of different cell culture methods.

Cell Culture Methods	Advantages	Disadvantages
2D cell culture (Petri dishes)	• Well established protocols • Easy to manage and quantify • Cost-effective • Faster production speed • High feasibility • Easy analysis	• Homogeneous cell layer • No cell-cell and cell-ECM interactions • No 3D tissue structure • Uniformity of nutrients and compounds • Low biological relevance
3D cell culture (Organoids)	• High resemblance to *in vivo* conditions • Self-organization in different cell types • Solubility gradient • Increased physiological relevance • Higher physiological relevance (size and shape similar to *in vivo* cases)	• Lack of fluid flow perfusion and diffusion leading to internal cell death • Absence of immune cells • Static conditions • No control over cell structure • Impossible to separate cells • Limited observation and control • High variability • Low *in vivo* relevance • High sample volume • High cost
Microfluidic chips (Organ-on-chip)	• Different cell types • Control of cell structure and cell maturation • Increased precision and control over the microenvironment • Vascularization • Gradient diffusion of nutrients and drugs • Control of temporal and spatial activity • Low sample volume • Easy integration with sensors and mechanical stimulation • Real-time analysis • Low variability and high *in vivo* relevance • Customised device	• Non-standard protocols • Increased cost and difficulty in manufacturing • Complex design • Bubble formation or clogging of channels by cells

disease treatment, the replacement of ineffective medicines, the advancement of basic and applied science, and the growth of health-related industries [16]. However, this process involves extensive pre-clinical and clinical trials to validate the toxicity and efficacy of the drug, and when issues are encountered at any stage of development, a major financial loss to the pharmaceutical industry can take place if the drug candidate fails which is a critical factor responsible for the decline in the number of new approved drugs each year [13]. The efficiency of developing new drugs approved per billion US dollars spent on R&D has been declining steadily since 1950 [19]. Therefore, OoCs are expected to have a major impact on improving drug development processes by mimicking closer the human microenvironment saving time and money.

OoCs are microfluidic chips containing cell cultures in micro-channels that are continuously perfused to mimic the physiological functions of human tissues and organs. These chips combine the fields of biology and engineering to represent key functions of human physiology and disease processes [20]. The simplest system consists of a single cell type. Advances in tissue and microfabrication and the increasing interest in these devices have allowed the design of more complex structures that connect two or more microchannels through membranes with micro-pores lined on each side of their surface with different cell types, simulating the interactions between different tissues and organs (Fig. 2).

Therefore, OoCs comprise an improvement of *in vitro* assays by representing biological tissues and cells outside the human body with higher accuracy and reproducibility [21,22]. This allows the highly accurate analysis of disease states and therapeutic responses to drug exposure, and the rapid identification of active therapies *in vitro* for their clinical translation to *in vivo* studies (Fig. 3) [10].

The significant discrepancy between animal and human studies translates into a high failure in clinical translation because of low efficacy or high toxicity in animals, which may not be relevant to humans. Even beyond preclinical testing, approximately 40 % of drug candidates fail during clinical trials due to unexpected adverse reactions in humans. This has led to the need for alternative assays that are more predictive of human response, resulting in OoCs. Subsequently, the great expansion of different types of OoCs has been possible by the combination of multiple known technologies, demonstrating that dynamic culture conditions significantly influence the physiological maturation and function of *in vitro* systems, achieving a better representation of the tissue microenvironment and higher throughput of *in vivo* studies, bridging the gap between animal models and human clinical trials.

OoCs are devices ranging from the size of a pen drive to larger systems that connect multiple organs. All of these systems share the following key characteristics:

1) The arrangement of cells within the device and the 3D extracellular matrix.
2) The integration of different cell types to achieve a more physiological cellular balance and to represent cell functionality.
3) The presence of biological and mechanical forces to structure the tissue.

Through the microfluidic channels, the transport and removal of culture medium, metabolites, and formed detritus take place [23,24].

Two approaches have been implemented to build the vascularization of the microfluidic chip:

a) **The bottom-up method** in which cells are seeded into a microenvironment, usually a hydrogel, which develops the vascular networks through angiogenesis and/or vasculogenesis. This method does not require the creation of a microstructure.
b) **The top-down method** in which the microstructure of vessels is created and then the cells are seeded. This method allows greater control over the size and distribution of the OoC features [25].

Another *in vitro* method commonly used for organ mimetic studies is the use of organoids, but they have several limitations such as (i) not considering the effect of red blood cells or tangential tension from blood flow, stroma, and immune cells, which does not allow a global understanding of cellular behavior, (ii) the nutrient supply, gas exchange and waste disposal are more restricted to the interior of the organoids and (iii) the lack of standardized organoids limits uniformity and reproducibility [26].

The fundamental difference between these two methods is that OoC technology uses knowledge of human organs to design a system in which cells and their microenvironment can be precisely and dynamically controlled. In contrast, organoids reproduce the 3D physiological structure of tissues in static conditions (wells or Petri dishes) from primary cells, pluripotent stem cells, or embryonic or adult stem cells (Fig. 4, Table 1) [27]. Some of these limitations can be overcome by reducing the size of the organoid and using bioreactors that improve the distribution of nutrients, oxygen, and waste products. This facilitates the control of the cellular microenvironment and the expansion of 3D tissues *in vitro,* as well as the scale-up and long-term maintenance of cultures [28].

In general, the design of an OoC requires an understanding of the anatomy of the target organ and its simplification down to the elementary functional unit. This is followed by consideration of cell selection and culture, dynamic flow control by perfusion, structural organization, and the specific biochemical and physical microenvironment of the organs [2].

4. Biomaterials used in OoC manufacture

In the fabrication of an OoC, it is necessary to consider the different materials required to produce each of its components. These materials must be selected primarily based on their biocompatibility since they will come into contact with cells and biological components. In this way, we can avoid any interference or adverse effects on tissues and organs. They must also have physicochemical properties appropriate to their applications to fulfill their function. At present, the most widely used materials are polymers that can be modified at the molecular level to stimulate cellular responses.

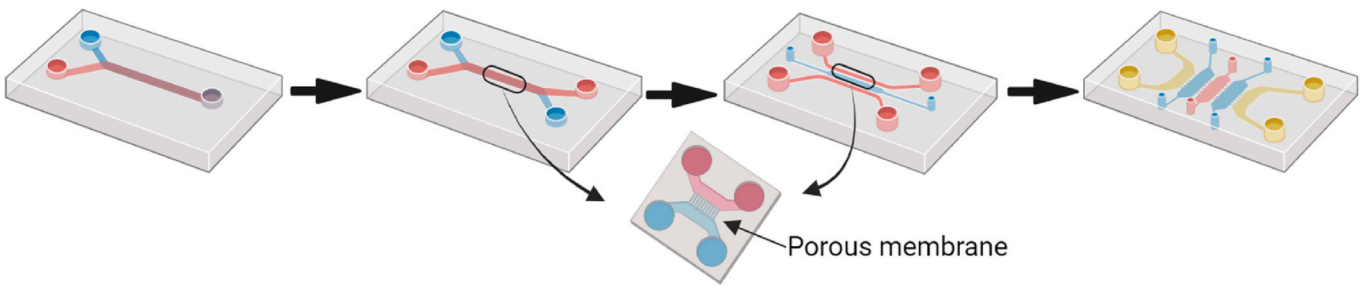

Fig. 2. Representations of single, dual, triple, and multi-microchannel microfluidic chips.

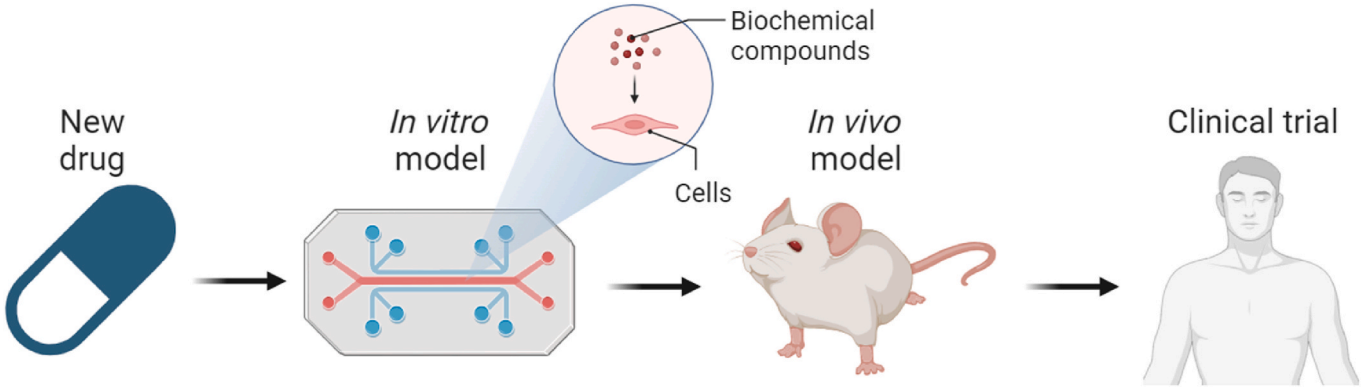

Fig. 3. Roadmap for the preclinical and clinical translation of novel drugs.

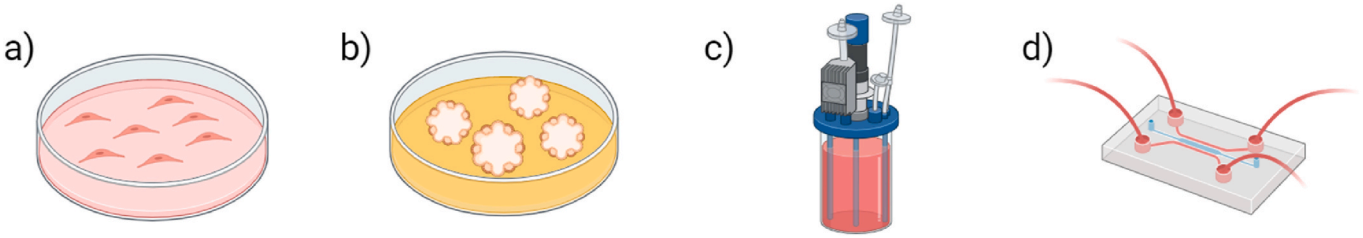

Fig. 4. Representation of the different cell culture methods. a) 2D cell culture with a monolayer of cells. b) Static 3D cell culture with organoids. c) Bioreactor DASbox with cell culture/organoids. d) Organ-on-chip connected to a dynamic flow simulating a more physiological cell microenvironment.

4.1. Microfluidic chip structure

In the microfluidic chip manufacturing process, there is a preference to look for materials that can provide a robust and reliable structure for the device. Initially, silicon and glass were used to create the base structure because of their stability and resistance, but their complex production and high cost may limit their use. Later, other materials such as liquid glass, polymers, papers, and hydrogels were developed. However, polymers are currently the most widely used material due to their high stiffness and cost-effectiveness. Depending on the characteristics of the chip to be manufactured, different types of polymers could be used [29,30].

- **Elastomeric polymers,** such as photocured aliphatic acrylate, which form double-sided acrylic tapes, are one of the most frequently used materials in the development of OoCs, by enabling the formation of complex structures that combine microfluidic and electronic channels robustly and functionally. These also allow the synthesis of more flexible microfluidic devices. Polydimethylsiloxane (PDMS), a silicone-based elastomer produced by soft-lithography or elastomeric micro-molding, is the most used biomaterial due to its biocompatibility, easy application, high transparency (240–1100 nm) allowing easy observation under a microscope, low cost, low autofluorescence and very good deformability allowing the assembly of microchannels on different substrates of different origin. However, PDMS is hydrophobic, and therefore a coating of extracellular matrix (ECM) components is required to allow cellular adhesion to the surface of the microchannels. Moreover, this coated surface may adsorb and/or absorb drugs and their metabolites, so this interaction should be taken into account during their use and the analysis of results [31].
- **Thermoplastic polymers**, such as polystyrene (PS), polycarbonate (PC), poly(methyl methacrylate) (PMMA), poly(ethylene glycol) diacrylate (PEGDA), Teflon, and polyurethane (PU), can be manufactured by injection molding, hot embossing or laser cutting. Some of the advantages of these polymers include their ability to be repeatedly remolded once the glass transition temperature is reached, resistance to small molecule permeation, and greater rigidity compared to elastomers. Additionally, they are lightweight, biocompatible, and easy to manufacture. However, their limitations include: i) not all polymers are transparent (e.g., polypropylene (PP) and polyether ether ketone (PEEK)), which restricts observation; ii) some exhibit high autofluorescence, hindering detection; and iii) their low gas permeability can negatively impact long-term cell cultures.

It should also be noted that sensor systems containing metallic materials are also included in the design of OoCs to allow real-time analysis and test monitoring. These sensors are often made by depositing or integrating metals such as gold and titanium into the device [32].

4.2. Tissue engineering

In the process of tissue formation in Organ-on-a-Chip (OoC) systems, it is essential to culture cells and facilitate the development of the extracellular matrix (ECM), which provides the tissue's *in vivo* structure. Various scaffolds have been designed for this purpose, tailored to the specific tissue being replicated. Ideally, these scaffolds should be biocompatible with cells, possess the mechanical properties necessary to replicate the physiological environment, and be biodegradable to allow replacement by the natural ECM. Thus, selecting the appropriate biomaterial is crucial to achieving optimal scaffold properties. Both natural and synthetic polymers are commonly used in the fabrication of scaffolds for tissue engineering [33].

- **Natural biomaterials** are polymers derived from natural sources. Some of these materials are components of the extracellular matrix (ECM), such as collagen, elastin, proteoglycans, and hyaluronate (HA), or originate from plants and insects, like chitosan, gelatin, dextran, glucose, fibrin, fibroin, and alginate. These polymers are typically elastic, highly biocompatible, and possess high oxygen permeability, making them promising candidates in tissue

engineering, biosensor development, and drug delivery systems. For instance, collagen, the most abundant protein in the body and a key structural component of connective tissues is widely used in scaffolds due to its enzymatic degradability, low immunogenicity, and ability to provide cell adhesion sites.

- **Synthetic biomaterials** are polymers designed to overcome some of the limitations associated with natural biomaterials. Their physical, chemical, and mechanical properties, as well as their degradation rates, can be fine-tuned to better suit specific applications. Another advantage is the minimal batch-to-batch variability compared to natural biomaterials. However, a key drawback is the absence of cell adhesion ligands on their surfaces. Common synthetic polymers used in Organ-on-a-Chip (OoC) systems include polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(L-lactic acid) (PLLA), poly (D-lactic acid) (PDLA), polyglycolic acid (PGA), and poly-caprolactone (PCL).
- **Hybrid biomaterials** combine the advantages of both natural and synthetic biomaterials. These hybrid biomaterials can be synthesized in a more controlled and reproducible manner while also offering cell affinity. Examples of such biomaterials include PLA-chitosan-gelatin, chitosan-siloxane, and PEG-fibrinogen. However, these combinations are still under research and optimization [34].

Recently, *hydrogels* have been introduced as new bio-scaffolds for cell culture. Hydrogels are a 3D network of compact hydrophilic polymeric chains that are embedded in water to form a gel-like structure. Hydrogels can be either natural (e.g., gelatine, collagen) or synthetic (e.g., polyvinyl alcohol (PVA), polyethylene glycol (PEG)) hydrogels. Some of the advantages are: i) diffusion of small molecules; ii) biocompatibility; iii) sensitivity to high temperature; iv) ease of manufacture and v) reduced cost. Most hydrogels have specific cell-binding sites that enhance cell adhesion and proliferation. Even though the advantages above discussed, still are not widely used in OoC due to their low stiffness. Nevertheless, hydrogels have shown good performance in certain applications, such as the development of valves that open or close in response to pH changes, similar to human venous heart valves [32,35].

5. Fabrication methods of OoC

Different microfabrication techniques have been employed for OoC depending on the application and the biomaterials selected. The selected method must take into account the required geometry and dimensions of the chip as well as allow optimal flow control, minimize microfluidic channel clogging, and monitor certain molecules through integrated sensors within the chip [32]. There are different techniques for manufacturing the chip microstructure, being the most commonly used soft lithography, hot embossing, injection molding, and 3D printing.

5.1. Soft lithography

This technique addresses the limitations of conventional photolithography when applied to biological systems and is versatile across a wide range of materials, particularly elastomers like PDMS. Key advantages include rapid prototyping and low cost. The process involves photolithography to create a negative mold (or "master"), into which the elastomer is poured and then cured at high temperatures. The device is subsequently micro-perforated to allow fluid flow. Various patterning techniques can be employed within this method, including replica molding (REM), phase-shifting edge lithography, micro-transfer molding, and capillary molding [36].

5.2. Hot embossing

Hot embossing is a highly suitable and flexible technique for micromanufacturing chips using thermoplastic polymers such as PMMA. The process primarily involves applying force and heat to a master mold. This method offers high efficiency, excellent structural precision, and low cost. However, precise control of temperature and other parameters is essential to ensure high-quality results [32,37].

5.3. Injection molding

This technique is widely used in the mass production of many microdevices and consists of four stages: clamping, injection, cooling, and ejection [38]. To ensure high quality during production, the relationship between temperature, pressure, and injection must be precisely controlled. Due to the difficulty of finding suitable materials, a novel 3D injection-molded plastic matrix (IMPACT) has been developed, showing excellent performance in mimicking angiogenesis from human umbilical endothelial cells (HUVEC) and lung fibroblasts embedded in 3D fibrin gels [39].

5.4. 3D printing

3D printing is an innovative technique for creating microscale components and systems. It also facilitates the production of biomedical parts and tissues by printing with cells, matrices, and biomaterials [32]. Several 3D printing methods are described in the literature, including i) Stereolithography (SLA), which offers the highest precision, enabling intricate features and smooth surface resolution; ii) Selective Laser Sintering (SLS), ideal for complex geometries and microstructures; iii) Fused Deposition Modelling (FDM), suitable for simpler 3D models; and iv) bioprinting, which allows the use of diverse materials such as polymer solutions, hydrogels, and bioactive polymers containing proteins [40].

SLA is one of the most widely used 3D printing techniques, known for its high precision and ability to produce intricate details with smooth surface finishes. This method uses a laser to selectively cure liquid resin layer by layer, allowing for the creation of highly detailed microstructures that are often required for microfluidic devices in OoC applications [18]. In the context of OoC, SLA is particularly advantageous for developing chips with complex geometries, such as microchannels, reservoirs, and valves, which need to precisely mimic the fluidic and mechanical properties of human organs. SLA's high resolution is beneficial for creating the fine-scale structures necessary for proper cellular interaction, nutrient flow, and waste removal, all of which are essential to the physiological relevance of organ models. Furthermore, the ability to create smooth surfaces helps minimize the risk of clogging and reduces shear stress in microfluidic channels, facilitating more accurate fluid dynamics. SLA is particularly suited for the fabrication of chips that require tight control over geometry and surface finish [40].

SLS is another advanced 3D printing technique that uses a laser to selectively sinter powdered material, typically polymer, metal, or ceramic, layer by layer to build up a solid structure [41]. In an OoC context, SLS can be used to create the base structure of the chip, including interconnecting microfluidic channels that facilitate nutrient flow or simulate blood circulation, which are essential for creating realistic tissue models. SLS can be also particularly well-suited for the development of complex geometries and microstructures, which makes it ideal for OoC systems that require intricate networks. The material choices in SLS are broader compared to other 3D printing techniques, as it allows the use of thermoplastic polymers and bio-based materials that can mimic the properties of tissues. This capability is important for creating scaffolds that provide structural integrity while also supporting cell growth and differentiation such as bone tissue engineering [42].

FDM is one of the most commonly used 3D printing techniques due to its simplicity, cost-effectiveness, and availability. In FDM, a thermoplastic filament is heated and extruded through a nozzle to build an object layer by layer [36]. While FDM is often used for less intricate 3D models, it can still play a crucial role in the development of organ-on-a-chip systems, particularly when simple or medium-complexity designs are required. For instance, FDM is

particularly useful for creating structural parts of the chip, such as frames or supports for more complex microfluidic networks created with other techniques. Although FDM does not offer the same level of resolution as SLA or SLS, it can still be used effectively for prototypes or less detail-oriented components. FDM's affordability and ease of use also make it an attractive choice for researchers who are developing early-stage OoC models or experimenting with new designs. Additionally, by utilizing a variety of biocompatible thermoplastic materials, FDM can contribute to the fabrication of OoC models that are suitable for a range of biomedical applications [18,43].

Bioprinting is a revolutionary 3D printing technique that focuses on the deposition of living cells, biomaterials, and bioactive molecules in precise patterns to create functional tissue-like structures. Unlike traditional 3D printing methods, bioprinting utilizes bioinks made from hydrogels, polymer solutions, and bioactive polymers containing proteins to mimic the cellular microenvironment [40]. In the development of OoC models, bioprinting is particularly important as it allows for the creation of complex tissue scaffolds that can incorporate multiple cell types, such as endothelial cells for vascular tissue or neurons for neural tissue. This capability is essential for the fabrication of more advanced OoC models that closely replicate human organs, as it allows for the creation of multi-layered tissue structures with varying cell types. Moreover, bioprinting offers the possibility to directly print microfluidic networks that can be integrated with the printed tissues, providing a seamless and highly functional system for simulating organ-level interactions. The ability to incorporate living cells directly into the printing process opens up new avenues for creating more physiologically relevant models, particularly in disease modeling, drug screening, and regenerative medicine applications [44,45].

While 3D printing allows for the controlled and precise arrangement of cells, certain aspects, such as micrometric fabrication limits and material compatibility, still require further refinement [33,37]. Even though, currently, soft lithography remains the most standardized method, 3D printing is rapidly gaining popularity as an advanced alternative [32]. Incorporating 3D printing techniques into the development of OoC models offers significant advantages in terms of precision, customization, and scalability. Each 3D printing method, from SLA to bioprinting, presents unique capabilities that cater to different aspects of OoC design and functionality. Whether it is the fine resolution of SLA, the complex geometries achievable with SLS, the cost-effective nature of FDM, or the cell-based precision of bioprinting, these techniques together enable the creation of more sophisticated and biomimetic *in vitro* models. As the field continues to evolve, the integration of these 3D printing technologies holds great promise for the future of drug development, personalized medicine, and disease modeling, ultimately advancing our ability to replicate human physiology on a chip.

6. Applications of OoC in different tissue models

OoC systems can be categorized based on the organ they model and further subdivided according to the specific disease being studied. The primary goal of OoC technology is to replicate the target organ in a microenvironment that closely mimics physiological conditions *in vivo*, allowing for easier and more efficient handling. This enables the study of various behaviors that organs and tissues may exhibit when exposed to different internal and external agents. Consequently, OoC systems are continuously being designed and refined based on the organ they represent and their specific applications. In Table 2, information regarding OoC mimicking different tissues is summarized.

Additionally, these systems facilitate the development of personalized medicine tailored to specific patient groups using human-induced pluripotent stem cells [37]. A notable example is the creation and assessment of personalized cellular immunotherapies, where 3D-printed OoCs containing patient-derived tumor cells are used to monitor lymphocyte responses to immunotherapeutic agents in real-time [84].

6.1. *Lung-on-a-chip*

The most well-known OoC is the lung-on-a-chip, a device designed to replicate the functional alveolar-capillary environment of the human lung. This multilayered OoC features a microfluidic system with two parallel microchannels separated by a porous, flexible, and thin (≈10 μm) PDMS membrane, which is coated with extracellular matrix (ECM) proteins like collagen or fibronectin [16]. In this model, alveolar epithelial cells are cultured on the upper surface of the membrane, in contact with the gas channel, while vascular endothelial cells are cultured on the lower surface, adjacent to the vascular channel through which a blood-simulating culture medium flows. Additionally, the device mimics physiological respiratory movements by applying cyclic pressure changes to both sides of the channels, causing the membrane to expand and contract (Fig. 5) [5].

In addition to replicating the physiological and pathological

Table 2
Summary of OoC models.

Organ	Tissue Mimicking Type	Most commonly used cells	Most commonly used Materials	Applications	References
Bone	Osteogenic tissue	Osteoblasts, Osteoclasts, Endothelial cells	PDMS, Hydrogels (Collagen, Alginate)	Bone formation, Drug testing, Osteoporosis studies, bone metastasis	[46–48]
Musculoskeletal	Muscle-tendon interface	Myocytes, Fibroblasts, Tendon cells	PDMS, Gelatin, Collagen, Gelatin methacrylate (GelMA)	Muscle repair, muscular dystrophy, tendon regeneration	[49–51]
Blood Brain Barrier	Endothelial barrier	Human Brain Endothelial cells, Astrocytes, pericytes	PDMS, ECM proteins	Drug penetration, BBB permeability, Disease modeling, cell migration and metastasis, infection brain models	[52–54]
Kidney	Glomerular, Tubular tissue	Podocytes, Tubular epithelial cells, glomerular endothelial cells,	PDMS, Collagen, Alginate	Kidney filtration, Toxicology studies, Drug screening, cancer models	[55–57]
Gut	Intestinal epithelium	Intestinal epithelial cells, Goblet cells	PDMS, Matrigel, Collagen, intestinal mucus model (I-Bac3Gels)	Gut absorption, Microbiome studies, Disease modeling, cnacer, allergies	[58–63]
Blood Vessels	Vascular endothelium	Endothelial cells (eg. Human umbilical vein endothelial cells (HUVECs)), Smooth muscle cells	PDMS, Collagen, Alginate	Vascular disease modeling, angiogenesis, atherosclerosis, Drug testing, ischemia, reperfusion	[64–68]
Lung	Alveolar epithelium	Alveolar epithelial cells, Endothelial cells	PDMS, Gelatin, Collagen, lung proteins, extracellular matrix F127-DA hydrogel membrane	Lung function, Airway diseases, Inhalation toxicology	[69–72]
Liver	Hepatic tissue	Hepatocytes, Kupffer cells, Endothelial cells	PDMS, Matrigel, Collagen	Drug metabolism, Liver toxicity, Disease modeling	[73–75]
Skin	Epidermal layer	Keratinocytes, Fibroblasts, Melanocytes	PDMS, Collagen, Hydrogel	Skin permeability, Drug testing, Disease modeling, wound healing, aging	[76–79]
Body-on-a-Chip	Multi-organ integration	Various primary cells, Stem cells	PDMS, Gelatin, Collagen, Hydrogels	Multi-organ toxicity screening, Drug development	[80–83]

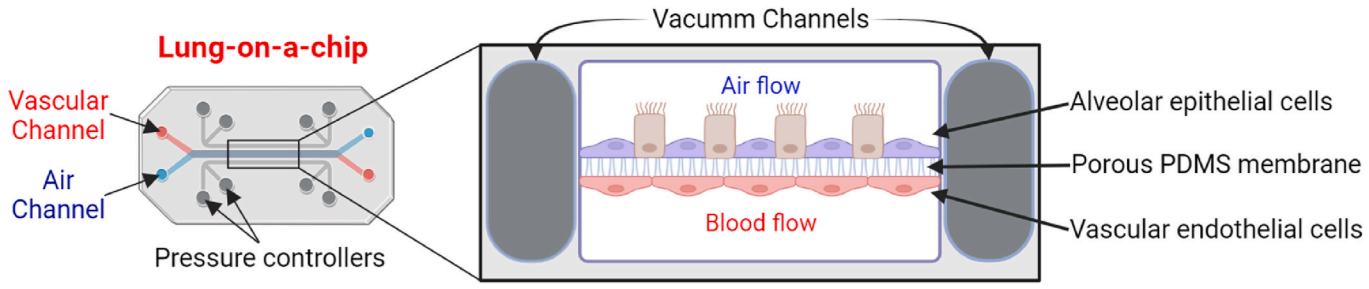

Fig. 5. Simplified basic lung structure for the construction of the Lung-on-a-chip.

microenvironment of the lung, this model can be used to identify effective therapies and diagnostic biomarkers through *in vitro* studies of various disease models, including lung inflammation (asthma, COPD, pulmonary edema), pulmonary fibrosis, lung cancer, pulmonary thrombosis, lung infections, and lung injury [85,86]. Some studies have investigated the inflammatory response to *Aspergillus fumigatus* exposure through the airway channel [87], modeled asthma using IL-13 perfusion [88], and represented COPD by directly using patient-derived cells [89].

However, this lung model has some limitations, including the challenges of manufacturing a flexible, ultra-thin, porous PDMS membrane and the material's tendency to adsorb small molecules, which can impact drug efficacy results in clinical trials [2]. An alternative to PDMS is the use of soft collagen-elastin (CE) membranes, which offer reduced adsorption and superior optical, biodegradation, and stretch properties, making them more comparable to biological membranes [90]. Other drawbacks include the inability to replicate the entire organ and the difficulty in selecting the appropriate cell types, which depends on availability and the specific goals of the study [85].

Despite these limitations, this OoC model serves as a valuable foundation for developing other types of OoCs, such as the Gut-on-a-Chip, which employs a similar approach to simulate intestinal peristalsis. Additionally, these models contribute to advancing and applying alternative biomaterials for lung chip construction, such as PLGA nanofiber membranes and microporous silicon membranes [86].

6.2. *Liver-on-a-chip*

The liver is the major drug-metabolizing organ, and it is therefore important to analyze its response to predict metabolic and detoxification capacity in drug development processes. To this end, a liver-specific microfluidic chip (Liver-on-a-chip) was developed by Deng et al. [91]. Although the liver-on-a-chip is mainly used to assess drug toxicity, it also has great potential to study various liver diseases such as non-alcoholic fatty liver disease or hepatic steatosis [92].

Hepatic sinusoids are crucial for the exchange of substances between hepatic cells and the bloodstream. To model this process, the Liver-on-a-Chip device was engineered with multiple layers to accommodate hepatic sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and hepatocytes. A matrix of micro-columns was used to separate the cell culture from the flow of the culture medium and to confine the liver cells within a central chamber (see Fig. 6). These cells are isolated from the upper and lower PDMS channels by a porous polyethylene (PE) membrane [2]. Additionally, the bile canaliculi, which transport products of cellular biosynthesis, are incorporated into the design. Hepatocytes are aligned in pairs similar to hepatic cords, gradually organizing themselves into bile canaliculi *in vitro*. For assessing hepatic function and drug toxicity, the culture medium is perfused with carboxy dichlorofluorescein diacetate (CDFDA), and the subsequent excretion of carboxy dichlorofluorescein (CDF), metabolized by esterases, into the bile canaliculi is monitored [5].

6.3. *Kidney-on-a-chip*

The kidney-on-a-chip is an advanced *in vitro* model that enables precise identification of nephrotoxic drug compounds during preclinical testing, significantly reducing the reliance on animal models as it remains the only method for assessing renal toxicity. Furthermore, it provides valuable insights into renal disease mechanisms through the representation of various disease models [5].

The kidney, which regulates the body's fluid and electrolyte balance

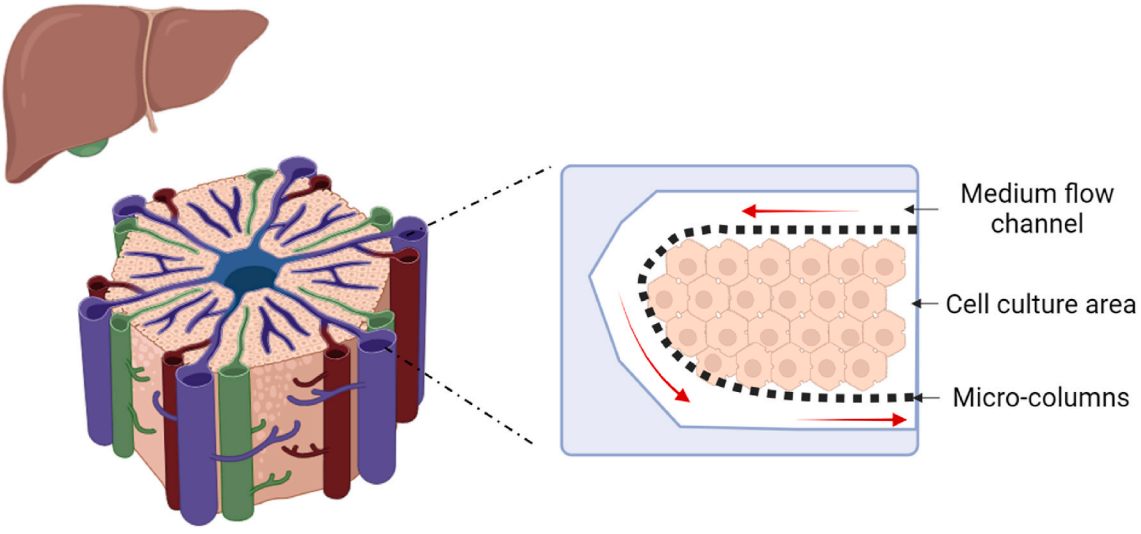

Fig. 6. Representation of a section of the Liver-on-a-chip hepatic sinusoid. The 2 μm space between the micro-columns separates the hepatocytes from the flow of the medium.

while removing waste and toxic substances, operates through the processes of filtration, reabsorption, and secretion within the nephron—the kidney's primary functional unit. To accurately mimic renal structure, the interactions among different cell types, cellular metabolic and endocrine functions, and immune cell recruitment must be considered. This complex system also involves an intricate vascular network and is supported by an extracellular matrix [93]. Although several designs exist for different components of the kidney, a fully integrated kidney-on-a-chip model has yet to be realized (Fig. 7) [94,95].

6.3.1. *Glomerulus-on-a-chip*

The glomerulus is the functional anatomical component of the kidney where the main process of blood filtration takes place. The glomerulus chip consists of a system of two microfluidic channels representing blood and urine flow, respectively, separated by a laminin and collagen-coated membrane. On the membrane, podocytes derived from induced pluripotent stem cells (iPSCs) are cultured on the side facing the urine channel, and endothelial cells on the side facing the blood channel. This device allows for testing of the toxicity of compounds at the glomerular level (e.g., doxorubicin which causes podocyte cell death) and the development of different models to study glomerulopathies such as hypertensive nephropathy [96] or diabetic nephropathy [97].

6.3.2. *Proximal tubule (PT)-on-a-chip*

The PT is the major site of drug elimination from the body being one of the major areas exposed to drug-induced toxicity. The PT-on-a-chip design uses hollow fiber membranes to support and mimic the structure of the proximal renal tubule cells to provide immunoprotection against extra capillary blood flow. The inner surface of the fiber consists of a fibrin-coated hydrogel onto which proximal tubule epithelial cells are seeded, forming a monolayer with an active glucose transport function. It is worth noting that the shear stress of the fluid causes cells to gain polarity, forming the tubular structure and generating functional transporters [98]. Jansen et al. observed that this device resulted in secreted albumin-bound uremic toxins while free albumin through the tubule was reabsorbed [99].

In addition, advances in 3D printing allow the design of more complex functional structures that are more durable over time.

6.3.3. *Distal tubule-/collecting duct-on-a-chip*

The distal tubule and collecting duct are the final parts of the nephron and have the function of maintaining homeostasis and collecting the final urine. In contrast to the previous models, only a handful of studies mimicking the distal tubule and the collecting duct are described in the literature. A multilayer microfluidic chip consisting of fibronectin-coated PDMS microchambers and microchannels with specific renal cells seeded have been engineered. Bearing in mind that the size of human renal tubules is around 50 μm, the fiber diameter was crucial exhibiting better cell functionality those with a smaller fiber diameter (the size of human renal tubules is ~ 50 μm) [94].

6.3.4. *Nephron-on-a-chip*

The nephron is the fundamental structural and functional unit of the kidney whose functions are the ultrafiltration of ions, water, and small molecules from the blood. It has been suggested that a functional kidney could be reconstituted by assembling the nephron's various segments—glomerulus, proximal tubule, loop of Henle, and collecting tubule—thereby enabling countercurrent ion exchange and the diffusion of nutrients, hormones, wastes, and drugs. Culturing MDCK (Madin-Darby canine kidney) renal epithelial cells alongside primary human umbilical vein endothelial cells within alginate-coated hydrogel microchannels and type I collagen, which simulate tubules and vessels, respectively, could successfully replicate the interactions between blood vessel cells and tubule cells (Fig. 7) [100].

Although it is not yet possible to reproduce a complete kidney in an organ-on-chip, the device is expected to help in the study of nephrotoxicity caused by drugs or their metabolites, and in the analysis of various diseases such as cisplatin-induced nephrotoxicity, fibrosis, or lithiasis. The system could also be used to assess rejection and tolerance mechanisms in kidney transplantation [94,101].

6.4. *Gut-on-a-chip*

The gut is the primary organ responsible for the digestion and absorption of nutrients, electrolytes, and drugs. It is distinguished by its extensive surface area and the presence of microbes that facilitate digestion, regulate the immune system and protect against foreign pathogens. Disruptions in the intestinal microbiota are a key factor in many intestinal diseases and can lead to variations in drug efficacy [102].

The gut-on-a-chip model was initially inspired by the lung-on-a-chip design, featuring two microchannels separated by a porous PDMS membrane. Vacuum chambers flanking the channels simulate peristaltic motion through cyclic mechanical stress. To more accurately mimic the *in vivo* microenvironment, it is essential to include a diverse array of cells and establish an appropriate oxygen concentration gradient Alternative gut-on-a-chip designs involve two microchannels divided by an intermediate channel filled with extracellular matrix (ECM) or collagen gel. In this case, the application of physiological fluid flow and shear stress enhances the differentiation of intestinal epithelial cells, promotes villus formation, and improves intestinal barrier function (Fig. 8) [103].

Studies often utilize established cell lines such as immortalized human colorectal carcinoma-derived cells (Caco-2), human umbilical vascular endothelial cells (HUVECs), and human intestinal

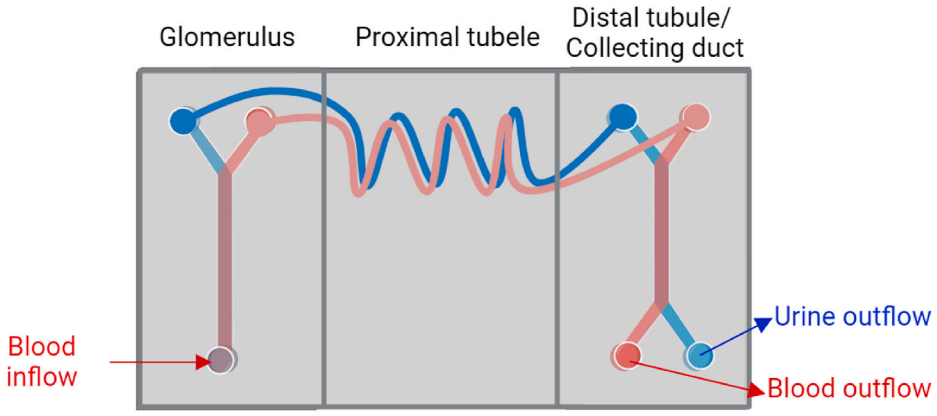

Fig. 7. Simplified representation of the Kidney-on-a-chip. The unification of all nephron components (glomerulus and proximal, distal, and collecting tubules) mimics the physiology of the kidney.

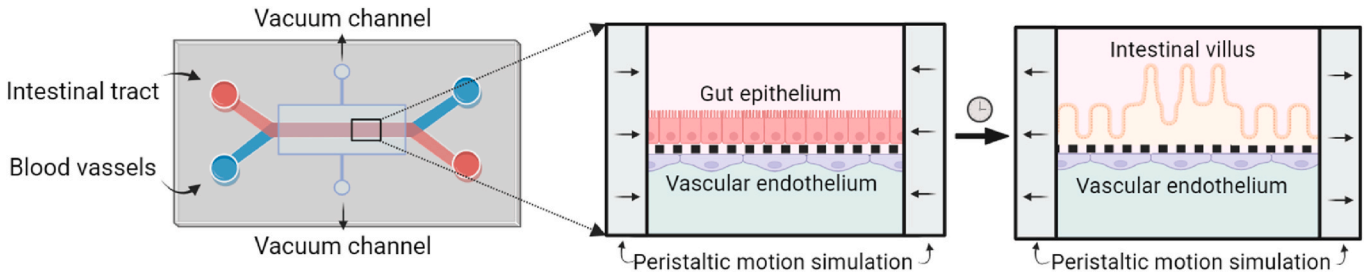

Fig. 8. Gut-on-a-chip design. Simulation of the physiological microenvironment and intestinal motility supports cell differentiation and better intestal representation.

microvascular endothelial cells (HIMECs) to represent the vascular component.

Microorganisms play a crucial role in metabolism and the development of certain diseases. To simulate the human microbiome, bacterial strains like *Escherichia coli*, *Lactobacillus rhamnosus* GG, and *Bacteroides caccae* are also incorporated [104]. Jing et al. investigated the inflammatory response and intestinal injury induced by *E. coli*, as well as the potential impact of subsequent inoculation with human macrophages U937. The introduction of U937 cells did not significantly ameliorate the damage. However, in another experiment, co-inoculation with *Lactobacillus casei* L5 BGB and *E. coli* resulted in reduced secretion of inflammatory factors and improved intestinal barrier function, demonstrating that *L. casei* combined with antibiotics can effectively mitigate intestinal damage and inflammation [105].

6.5. Skin-on-a-chip

The skin, the largest organ in the body, serves as the body's primary protective barrier. Its functions include preventing dehydration, regulating temperature, and shielding against harmful external agents. Developing a physiological skin model is crucial for the pharmaceutical, cosmetic, and chemical industries, particularly for the toxicological testing of new compounds that may cause skin damage. One of the most reliable methods for replicating this organ is the skin-on-a-chip, which enables tissue culture under controlled biochemical and physical conditions such as medium flow, mechanical forces, and chemical gradients [15,106].

The first design that closely mimicked skin architecture utilized a microfluidic chip with three PDMS layers separated by two porous polyethylene terephthalate (PET) membranes. Wufuer et al. co-cultured human epidermal, dermal, and endothelial cells on these membranes while perfusing different types of medium at varying flow rates (Fig. 9). To validate the system's reproducibility, TNF-α was introduced through the channels, which induced inflammation and skin edema. The effectiveness of dexamethasone in reducing inflammation and mitigating endothelial barrier dysfunction was evaluated in this skin-on-a-chip model demonstrating the prevention of tight junction damage after the application of dexamethasone to TNF-α-damaged skin as well as a reduction in the expression of IL-1β, IL-6, and IL-8 [107].

The skin-on-a-chip has demonstrated high potential for a range of studies including (i) diffusion studies of different drugs across the skin, (ii) identification of toxic compounds that may cause skin sensitivity, corrosion, irritation, and phototoxic effects, (iii) drug effectiveness on various skin diseases models such as inflammation, allergy, irritation, and cancer, (iv) wound healing properties to evaluate cell migration and proliferation in tissue damage process, (v) skin aging and evaluation of antioxidant effect, and (vi) mechanisms of angiogenesis [108].

However, skin-on-a-chip has several challenges to overcome, such as mimicking the complex vascular system, appendages, and the different types of secondary skin cells such as immune cells, melanocytes, and adipocytes which are essential for maximum reliability of toxicological tests and skin disease studies [108].

6.6. Bone-on-a-chip

Bones are rigid organs that form the framework of the human body, providing structural support for soft tissues, enabling locomotion, regulating hormones, and housing bone marrow. Bone tissue is distinguished by its dynamic nature, continuously undergoing formation and resorption through the actions of osteoblasts and osteoclasts during growth or in response to injury, ensuring the maintenance of healthy bone [109]. Unlike other tissues and organs, bone has a unique composition, being a rigid, porous tissue composed of various cells (such as osteoblasts, osteoclasts, and osteocytes), inorganic minerals like hydroxyapatite (HA), and other biomaterials. This complexity in composition and structure poses significant challenges in the development of bone-on-a-chip platforms, particularly in terms of design and material selection [46,110].

To design a bone-on-a-chip, a 3D bone scaffold must be first created to mimic the bone structure. This step can be achieved using different techniques such as FDM, which forms large-diameter fibers with low porosity or melt electro-writing (MEW), which forms smaller-diameter fibers with higher porosity, among others [111]. The 3D bone scaffold is subsequently incorporated into a microfluidic cell culture system,

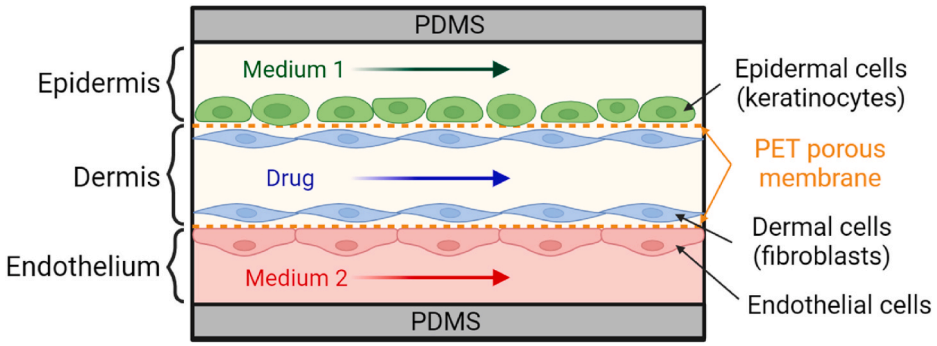

Fig. 9. Skin-on-a-chip design. The longitudinal section of the OoC shows the three main channels simulating the skin structure.

obtaining a bone-on-a-chip by the combination of a scaffold and a microfluidic chip. Galván-Chacón et al. developed a bone-on-a-chip consisting of a central PDMS chamber connected to an inlet and an outlet channel to allow perfusion of the cell culture medium. The central chamber integrated the 3D bone scaffold, which was fabricated by two-photon polymerization (2 PP) laser lithography and then coated with a biomimetic hyaluronic acid layer to mimic the chemistry of the bone mineral phase connected to two additional side channels for the incorporation of calcium phosphate solution (CaP), cell seeding and soluble factors as well as drugs. The side channels were separated from the central chamber by microgrooves and were blocked after the deposition of the CaP layer and cell seeding, creating a static volume reservoir (Fig. 10) [112].

Ceramic scaffolds are commonly used for bone modelling because of their high stiffness, low elasticity, and hard surface. Ceramic scaffolds are biocompatibility and play a major role in bone regeneration promoting osteoblast differentiation and proliferation. However, their application in tissue engineering is limited by the difficulty in controlling its degradation rate. Another alternative approach is the use of collagen scaffolds, a natural biomaterial, but with poor mechanical properties [113].

The bone-on-a-chip model requires an appropriate selection of cells and biomaterials. The geometric structure of the bone matrix is critical for ECM stiffness, compressive and tensile stress as well as fluid shear stress to target a reliable bone model. The bone matrix is a highly organized tissue mainly composed of hydroxyapatite, type I collagen, and other proteins. Combining different materials allows a better simulation of the biological properties of human bone. Different cell types have been used for bone vascularization, bone remodeling or bone metastasis. Mesenchymal stem cells are commonly used cells to represent the bone microenvironment while HUVECs for vascularization [46, 114].

Currently, there are very few studies of successful bone-on-a-chip platforms, which are necessary for a better understanding of the treatment of bone diseases, such as bone tumors, osteoporosis, the regeneration of bone defects, and drug testing [109].

6.7. Blood-brain barrier-on-a-chip

The blood-brain barrier (BBB) is a highly selective permeable membrane that restricts the flow of molecules from the cerebral vasculature into the brain tissue, regulating the central nervous system (CNS) homeostasis and protecting it from neurotoxic substances and pathogens. The BBB also acts as a secretory and metabolic barrier as it contains cells that release certain enzymes such as astrocytes. The functional properties of BBB are manifested by the synergistic contribution of endothelial cells, pericytes, astrocytes, immune cells, microglia, and neural cells [115,116]. Because of this selective and complex structure, the BBB presents a major challenge to the delivery of effective drugs to the CNS and very few effective treatments are available for diseases such as glioblastoma, Alzheimers, and Parkinson's disease.

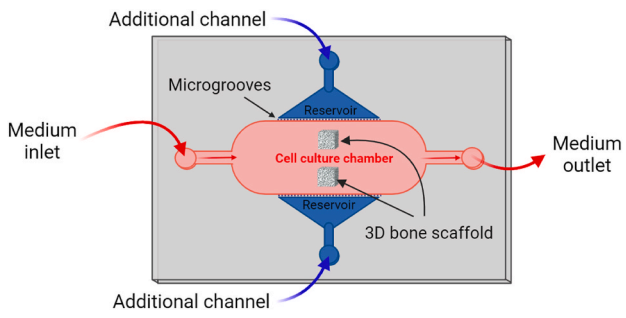

Fig. 10. Representation of the bone-on-a-chip with two 3D bone models incorporated in the main chamber.

One of the first BBB-on-a-chip was based on two parallel microfluidic chambers made of PDMS and separated by a porous membrane. On each side of the membrane were separately seeded brain microvascular endothelial cells, forming the vascular compartment, and astrocytes, pericytes and cortical neurons, forming the brain compartment. Endothelial cells formed a compact monolayer in which tight junction proteins and tissue-specific molecular transporters were expressed, and astrocytes connected both channels through their endfeet, mimicking closely BBB structure and function (Fig. 11) [117]. Another example is the model proposed by Ahn et al. that showed how the use of a 3D Matrigel can promote the self-organization of astrocytes into a 3D network with increased polarised expression of aquaporin-4 and α-syntrophin, and decreased expression of reactive gliosis markers, allowing more sensitive models of neuroinflammation [118].

Recently, a more realistic isogenic model of the human BBB using human induced pluripotent stem cells has been constructed. This design enables the representation of patient-specific models of human BBB disorder, thus advancing the development of precision medicine and the testing of new targeted therapies [119,120]. The use of BBB-on-a-chip has also been proposed to study the cellular alterations that cause brain tumors or neurodegenerative diseases such as Alzheimer's or Parkinson's, and also for the study of the injuries caused by brain trauma [121].

6.8. Vascular tissue-on-a-chip

The fabrication of vascular tissues involves several microfluidic techniques designed to create perfusable, endothelialized blood vessels that mimic the *in vivo* microcirculation. The foundation of vascular tissue engineering in OoC models lies in the design of microfluidic channels that replicate the geometry and flow dynamics of human blood vessels. Microfluidic devices typically feature networks of interconnected channels that are capable of housing endothelial cells and providing the necessary flow to create functional blood vessels. Endothelial cells, which line the inner walls of blood vessels, are typically seeded onto the surface of microfluidic channels to form the lumen of the vessel. To promote endothelial cell adhesion and alignment, the microchannel surfaces are often functionalized with extracellular matrix proteins, such as collagen or fibronectin, which support cell attachment and proliferation. These endothelial cells can then form monolayers that replicate the structural and functional properties of blood vessel linings [122]. In some OoC models, additional cell types such as pericytes, smooth muscle cells, or fibroblasts are co-cultured with endothelial cells to enhance the formation of stable vascular networks.

A key challenge in vascular tissue engineering is the creation of functional, perfusable networks. This is often achieved by introducing a combination of mechanical and biochemical cues that encourage the endothelial cells to self-organize into vessel-like structures. These cues may include controlled shear stress, chemical gradients (e.g., VEGF for angiogenesis), and hypoxic conditions to promote vascularization. In some advanced models, endothelial cells form lumen-like structures, while the inclusion of growth factors or the use of photopatterning can promote branching and anastomosis to form more complex vascular networks [122]. For instance, Nguyen and colleagues employed the needle subtraction method to create two separate channels within a collagen hydrogel to investigate sprouting angiogenesis. Endothelial cells were seeded into one channel and perfused angiogenic factors (HGF, bFGF, MCP-1, VEGF, S1P, and PMA) through the other channel to establish a chemokine gradient, which stimulated angiogenic sprouting. The development and maturation of these sprouts appeared to mirror the mechanics observed *in vivo*, providing a valuable platform for further exploration of the molecular mechanisms underlying angiogenesis [123].

Once endothelial monolayers are formed, it is crucial to establish a continuous blood flow through the microfluidic channels to maintain cell viability and mimic physiological blood vessel function. Blood flow

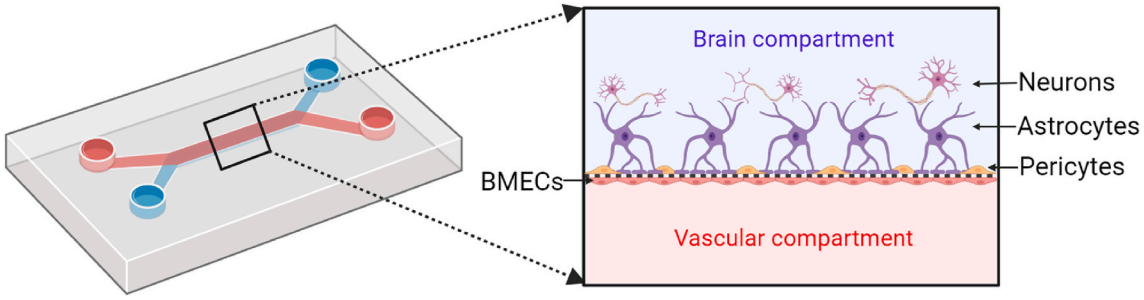

Fig. 11. Design of a BBB-on-a-chip. A compact monolayer of endothelial cells was formed around the membrane and in contact with the astrocyte "endfeet", connecting the two compartments.

generates mechanical stimuli that are continuously sensed by the vasculature, driving its growth and remodeling. As blood moves over the endothelial surface, its viscosity creates shear forces in the direction of flow. At the same time, the pressure inside the blood vessel produces a force perpendicular to the endothelial cells, causing the vessel to stretch. Vessels of different sizes, types (e.g., artery, vein, or capillary), or those in various organs, typically experience distinct pressure and shear stress levels. Variations in shear stress have long been linked to vascular diseases, with areas of altered blood flow near vessel bends and bifurcations often serving as sites for the development of atherosclerotic plaques or aneurysms [124].

To further replicate the functionality of blood vessels, several additional features can be integrated into the microfluidic vascular models. One important aspect is the permeability of the endothelial monolayer, which can be assessed by measuring the passage of small molecules or fluorescent tracers across the vascular barrier. This is crucial for evaluating the effects of drugs, toxins, or inflammatory agents on vascular permeability. Additionally, the ability to induce and measure endothelial responses to shear stress, as well as the migration and proliferation of endothelial cells in response to specific growth factors, can provide valuable insights into vascular biology and disease processes such as angiogenesis or atherosclerosis.

Furthermore, the co-culture of endothelial cells with other cell types such as smooth muscle cells or fibroblasts helps create a more representative tissue model. These cells support vascular maturation, providing structural integrity and regulating the contractility of the vessel wall. The addition of pericytes can also help stabilize the blood vessels and promote the formation of a more robust, physiologically relevant vascular tissue. A robust, multi-cell type 3D vessel-on-chip model entirely based on human induced pluripotent stem cells has been successfully designed. Within a fibrin hydrogel microenvironment, the hiPSC-derived vascular cells self-organized to form stable, reproducible microvascular networks, where the vessels were lumenized and functional, responding to vasoactive stimulation [125].

6.9. *Skeletal muscle tissue in organ-on-a-chip*

Skeletal muscle is a highly dynamic tissue, capable of contraction and responding to various mechanical and biochemical cues. The development of this OoC model is critical for studying muscle physiology, disease, and drug testing to provide a more accurate representation of *in vivo* conditions by incorporating key components such as muscle fibers, supporting cells, and mechanical forces, all within a controlled microfluidic environment. They provide a platform to study a range of muscle-related disorders, including muscular dystrophies, myopathies, and sarcopenia.

The creation of skeletal muscle tissue requires microfluidic platforms that can replicate the mechanical and biochemical conditions for muscle development and function. Typically, these systems incorporate microchannels for nutrient and waste perfusion, as well as for mechanical stimulation. The channels are often made from biocompatible materials such as PDMS or hydrogels, which provide structural support while also allowing for cell culture.

In skeletal muscle OoC models, myoblasts or induced pluripotent stem cells (iPSCs) are used as the starting cell population. These cells are typically seeded onto the surface of a scaffold or embedded in a gel matrix, such as Matrigel or collagen, that supports their growth and differentiation. To promote myogenesis, cells are exposed to specific growth factors (e.g., IGF-1, bFGF) and differentiated conditions. The cells then fuse to form multinucleated myotubes. The formation of muscle fibers is influenced by several factors, including the geometry of the microfluidic channels, the mechanical forces applied, and the biochemical cues provided by the surrounding environment [51].

One of the defining features of skeletal muscle function is its ability to contract in response to electrical or mechanical stimuli [126]. To mimic this in OoC models, mechanical forces are applied to the muscle tissue either through stretching or cyclic contractions. This can be achieved by integrating pneumatic actuators, external force generators, or applying electrical pulses to induce contraction. Stretching or cyclical contraction not only enhances muscle maturation but also improves the alignment of the myotubes, similar to how muscle tissue behaves *in vivo* [127].

While the development of skeletal muscle tissue in OoC models has made significant progress, challenges remain. Achieving mature, fully functional muscle tissue that accurately mimics the complexity of human skeletal muscle is still a work in progress. Key limitations include the difficulty in replicating the structural organization of muscle tissue, the full range of mechanical forces, and the lack of vascularization for long-term culture. Furthermore, the integration of skeletal muscle OoC models with other tissues, such as neural or vascular systems, will provide a more holistic approach to studying muscle function and disease.

6.10. *Body-on-a-chip (BoC)*

Systemic diseases such as cystic fibrosis or diabetes, pathophysiological processes, and pharmacological effects involve multiple organs and therefore cannot be studied in isolation using different types of OoCs [128]. The BoC has been proposed to represent the interactions between the different organs using *in vitro* models, allowing the analysis of a variety of systemic disease models and pharmacokinetic processes such as ADME (Absorption, Distribution, Metabolism and Excretion) processes related to different routes of drug administration and prediction of drug efficacy and toxicity [5]. Using BoC, the pharmacokinetic profile of a drug could be investigated in full taking into consideration the drug absorption in the GUT, the metabolism in the liver, and the excretion in the kidney [83]. BoC can have applications in drug screening, disease modeling, and personalized medicine if models are reproduced using specific cells from the patient.

The BoC is a multi-organs-on-chip platform consisting of two or more OoCs connected by channels or through the co-culture of different organs on a single chip (Fig. 12). This model usually includes the target organ of interest (e.g., tumor, inflammation), organs involved in drug

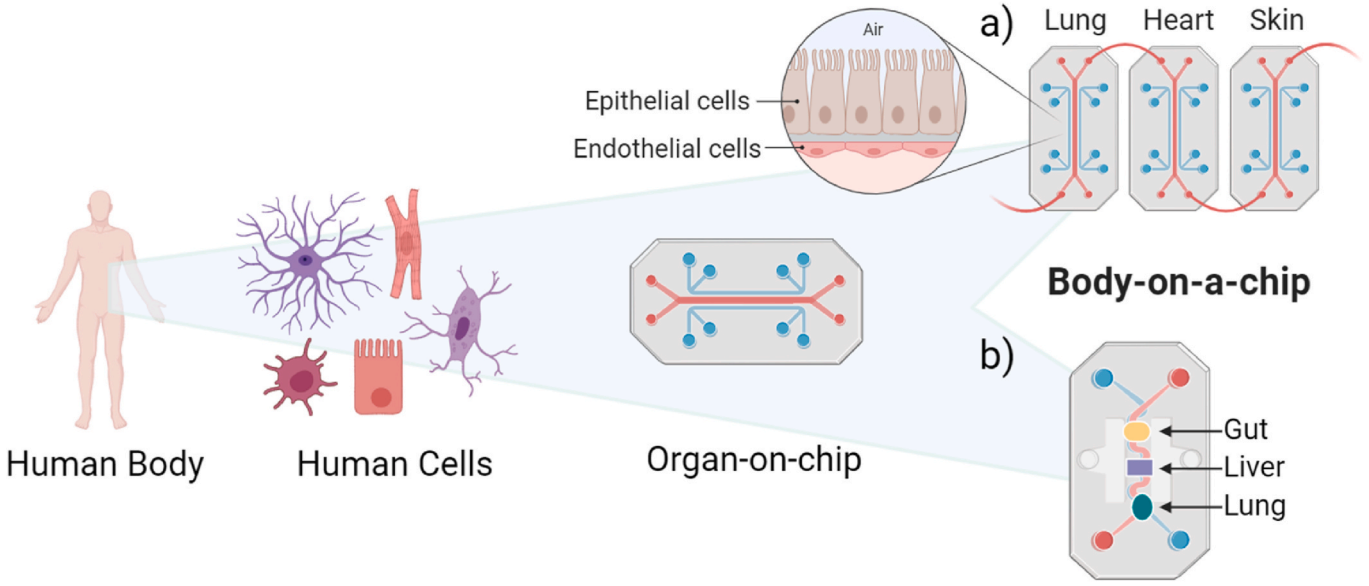

Fig. 12. Development process of a Body-on-a-chip. Key: a) The connection of multiple OoCs; b) The combination of different organs in a single chip.

metabolism and excretion (e.g., liver, kidney) and organs in which toxicity may occur [129]. For the setup of a BoC, a common medium would be selected to circulate between different components of the BoC while different cells should maintain their functionality. However, the use of a common medium is challenging as each tissue has different nutrient and growth factor requirements, which can lead to phenotypic alterations, bubbles and leaks at connections, and an increased risk of contamination [80]. On the other hand, the biomaterial used to fabricate the device must be carefully selected to tailor the microenvironment to the different cell types and to avoid the adsorption and/or absorption of chemicals on the internal surface of the device, which may distort the experimental results. It is also important to take into account fluidic shear stress, organ size scaling, fluid velocity, and the balance between cell volume and fluid in the medium to achieve maximum physiological biomimicry [83].

These models hold great promise for drug development, disease modeling, and personalized medicine, as they provide a more holistic view of how therapies or environmental factors might affect the body as a whole. However, the incorporation of neural innervation and vascular tissue into BoC systems introduces significant challenges that must be addressed for these models to achieve their full (Fig. 13).

One of the most complex issues when integrating neural and vascular tissues into a BoC system is the connection and functional communication between different tissue types. *In vivo*, tissues are not isolated but are interconnected through intricate networks of blood vessels, nerves, and signaling pathways. In a BoC system, the challenge is to recreate these connections in a way that allows for the proper physiological interaction between tissues [130]. For example, the neural innervation of organs such as the heart, gut, or skin is critical for regulating their functions, but replicating these connections *in vitro* is extremely difficult. The neural networks must be carefully incorporated into the microfluidic channels to ensure accurate signal transmission between neurons and target tissues. Neurons are part of a complex, interconnected system that regulates various bodily functions through both electrical impulses and chemical signals. The challenge lies not only in creating a functioning neuronal network but also in ensuring that the electrical and chemical signaling between the nervous system and the tissues it innervates is accurately replicated. For example, in a heart-on-a-chip model, proper neural innervation is essential for coordinating the rhythmic contractions of cardiac tissue. Similarly, in a gut-on-a-chip model, the autonomic nervous system plays a crucial role in regulating motility and secretion. The complexity of establishing these functional connections is compounded by the need to control the microenvironment within the chip, including factors such as pH, oxygen levels, and nutrient gradients, which can all influence the activity of both neural and vascular tissues.

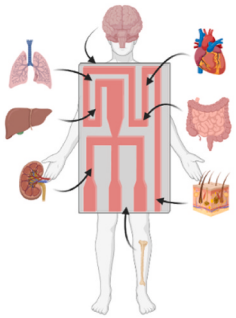

Fig. 13. Schematic representation of a Body-on-a-chip.

Similarly, the vascularization of organs is essential for nutrient and oxygen transport and the removal of metabolic waste. Without functional blood vessels, tissues in a BoC system will quickly become deprived of vital resources, leading to cell death and model failure [131]. Achieving functional vascular networks within OoC platforms has proven to be one of the most daunting challenges. While some progress has been made in creating simple capillary-like structures, fully functional and perfusable vascular networks that replicate the dynamic flow of blood *in vivo* remain elusive. The flow of fluids through the vascular system must be carefully controlled to maintain appropriate pressure, and this is particularly difficult in small-scale microfluidic systems. The internal pressure generated by fluid flow, if not carefully regulated, can cause tissue distortion or even collapse the delicate microvascular networks. Furthermore, the interaction between blood vessels and surrounding tissues is a highly complex process, requiring a balance of shear stress, pressure gradients, and chemical signaling that is challenging to replicate in a microfluidic environment.

Additionally, maintaining the long-term viability of these tissues within the microfluidic chip presents another set of challenges. Neural and vascular tissues have different growth and maintenance requirements compared to other tissue types, and ensuring that they remain viable and functional for extended periods in a static or dynamic microfluidic environment is difficult. Neural tissues require specific

growth factors, and maintaining the integrity of neural synapses and axon growth in a microfluidic channel is a significant hurdle. Vascular tissues, on the other hand, require continuous perfusion with blood-like fluids to sustain function, and any disruption in the fluid dynamics can quickly compromise the system's stability [132].

Finally, scaling up these complex models for high-throughput screening in industrial applications remains a considerable challenge. While small, isolated tissue models on a chip are feasible for research purposes, the integration of multiple tissue types, particularly neural and vascular components, requires careful design and precise engineering. The complexity of maintaining consistent fluid flow, nutrient supply, and waste removal across multiple tissue layers makes scaling these systems for commercial use highly challenging. Additionally, issues related to standardization of protocols, reproducibility, and system validation remain barriers to the widespread adoption of BoC systems in drug development and clinical applications.

In conclusion, while the incorporation of neural innervation and vascular tissue into body-on-a-chip models holds immense promise for creating more physiologically relevant and comprehensive *in vitro* systems, the challenges are considerable. Overcoming these challenges will require advances in microfluidic engineering, tissue engineering, and biomaterials science to create functional and reproducible models. The successful development of fully integrated BoC systems could revolutionize drug development, disease modeling, and personalized medicine by providing a more accurate and scalable alternative to current *in vivo* models. However, addressing issues such as tissue connection, pressure regulation, and long-term viability will be essential to realizing the full potential of these systems.

7. Regulatory challenges

In the U.S., the Food and Drug Administration (FDA) and in EU, the European Medicines Agency (EMA) are responsible for ensuring the safety and efficacy of drugs and medical devices, including new methodologies like OoCs. Both regulatory bodies have recognized the potential of OoC technologies to accelerate drug approval while reducing reliance on animal models. However, their integration into regulatory frameworks presents several challenges. However, before OoC systems can be widely accepted, they need to be standardized and validated to ensure that they provide reliable, reproducible results that accurately predict human drug responses. This includes demonstrating that OoC models perform at least as well as traditional testing models, particularly in terms of human metabolism, toxicity, and efficacy [133,134].

In the U.S., the FDA's 21st Century Cures Act has promoted the use of innovative technologies like OoCs for accelerating the approval of new drugs, emphasizing the importance of non-animal testing and *in vitro* models and providing specific guidance documents on the use of non-clinical data, including the potential incorporation of OoC data into drug development submissions [135]. The agency has established mechanisms for reviewing new drug development tools through programs like the FDA Critical Path Initiative. In September 2024, FDA's Center for Drug Evaluation and Research has accepted the first letter of intent into the Innovative Science and Technology Approaches for New Drugs (ISTAND) Pilot Program for an OoC technology (a type of microphysiological system consisting of four human liver cell types) to study drug-induced liver injury for certain drug candidates [136].

Similarly, the EMA has supported initiatives like the Innovative Medicines Initiative (IMI), which funds research into new technologies including OoCs, to improve drug development and regulatory science. The agency has also encouraged flexible, adaptive licensing pathways for drugs, potentially enabling OoC data to play a role in early-stage approvals [137].

Both agencies, however, face the challenge of ensuring that OoC models are robust enough to be used in regulatory submissions and clinical trial designs. This means extensive validation across different disease models and organs, as well as the establishment of frameworks for interpreting complex data generated from these systems. For the FDA and EMA to accept OoC systems, ongoing collaboration with academic institutions, pharmaceutical companies, and technology developers will be crucial to demonstrate their reliability. Both bodies are beginning to recognize the importance of reducing animal testing and are exploring ways to incorporate OoC models into drug development pathways, though the full integration of these technologies into the regulatory process will require continued research and validation [137]. The growing emphasis on personalized medicine, adaptive trial designs, and regenerative therapies, such as through the FDA's Regenerative Medicine Advanced Therapy (RMAT) designation, and the EU's Horizon 2020 programs, offers a promising future for the incorporation of OoC in regulatory science. As the scientific understanding of OoC technology advances, the FDA and EMA are likely to develop formal pathways for its use, enhancing drug safety and efficacy testing with more human-relevant data.

8. Future perspective and conclusion

In the current era of technological advancement, microfluidic chips have emerged as specialized tools in healthcare, driving the development of *in vitro* organ models such as those replicating the lungs, liver, skin, and gut. These OoC models are designed to mimic the physiological conditions of human organs *in vivo*, offering a more accurate and reliable alternative to traditional drug testing methods. While several OoC models are already being utilized in research, significant efforts are still required to refine their design and optimize manufacturing techniques. Currently, soft lithography is the most widely used fabrication method, though it is gradually being supplemented by 3D bioprinting, which offers enhanced customization and complexity. Additionally, although polydimethylsiloxane (PDMS) is the standard material for chip fabrication due to its flexibility and transparency, its limitations—such as protein and drug adsorption—are driving the development of new, more biocompatible materials suitable for mass production. These innovations could lead to more complex and realistic *in vitro* systems that better replicate human physiology, which is essential for their wider adoption in the pharmaceutical industry, where high-throughput screening and cost reduction are critical.

The recognition of microfluidic technology in the global healthcare market is growing rapidly, especially as OoC platforms promise more accurate and reliable results, along with real-time monitoring. As a result, OoC systems are increasingly being viewed as a viable alternative to reduce animal testing, aligning with the ethical and regulatory pressures on the industry to minimize the use of animals. Furthermore, OoC models are proving invaluable in studying various disease states, offering new insights into disease mechanisms and aiding the development of personalized medicine and targeted therapies. The pharmaceutical industry, driven by the need for more efficient and reliable drug development processes, is beginning to see OoC systems as tools that could shorten development timelines, reduce the costs of clinical trials, and enhance the predictability of clinical outcomes.

One particularly exciting application of OoC technology in the industry is the testing of novel nano- and microparticulate drug formulations, such as nanoparticles, liposomes, and microparticles. These formulations are gaining importance in drug delivery for their ability to enhance bioavailability and target specific tissues [138–140]. Traditional testing methods often struggle to accurately model how these complex formulations interact with human tissues, leading to potential inefficiencies and risks during clinical trials. OoC platforms offer a more accurate, relevant *in vitro* environment to study the pharmacokinetics, cellular uptake, and toxicity of these formulations before moving to *in vivo* testing. For the pharmaceutical industry, this represents a crucial step in optimizing drug development, as it allows for better prediction of a formulation's behavior in human bodies, thus reducing the risk of failure and the need for costly animal experiments. This precision and cost-efficiency are increasingly attractive to pharmaceutical companies

seeking to innovate while managing risk and regulatory hurdles effectively.

Despite these advantages, the widespread adoption of OoC technology in the pharmaceutical industry faces several challenges. From an industrial perspective, one of the major hurdles is the initial investment in OoC technology, as companies must adopt new infrastructure, integrate new materials, and ensure that the models are scalable for high-throughput applications. In addition, the lack of standardized OoC models presents a significant barrier. For industry to embrace these systems fully, the scientific community must establish standardized protocols and validation criteria to ensure that OoC models deliver reproducible and reliable results. Moreover, the pharmaceutical industry often requires a demonstrated track record of success and regulatory acceptance before fully adopting any new technology. This is particularly true for large-scale clinical trials, where the stakes are high, and validation through rigorous *in vivo* studies remains the gold standard. Regulatory bodies like the FDA and EMA will play a crucial role in setting the framework for how OoC data can be integrated into the drug approval process. The regulatory landscape must evolve to provide clear guidelines on how OoC-derived data will be evaluated, ensuring that these models are accepted as reliable predictors of human responses.

Looking forward, the integration of artificial intelligence (AI) into OoC systems could significantly enhance their value in the pharmaceutical industry [141]. AI-driven algorithms could optimize OoC platforms by analyzing complex biological data in real time, improving the accuracy of predictions regarding drug efficacy, toxicity, and pharmacokinetics. Machine learning models could be used to identify correlations and patterns in data that might otherwise be missed, enabling more effective screening of drug candidates. Additionally, AI could streamline the automation of monitoring and testing processes, making OoC systems more scalable and efficient for high-throughput applications. For the industry, this means faster decision-making, reduced costs, and more reliable data to inform clinical trials.

As microfluidics, AI, and advanced biomaterials continue to evolve, next-generation OoC systems will become more sophisticated, reliable, and capable of replicating the complexity of human physiology. For the pharmaceutical industry, the future of OoC technology lies in its ability to offer more accurate preclinical testing, particularly for novel drug formulations such as nanoparticles and microparticles, as well as its potential to reduce the need for *in vivo* testing. However, the success of OoC technology's widespread adoption will depend on the ability to standardize these systems, validate their predictive power, and align them with regulatory guidelines. The collaboration between researchers, industry stakeholders, and regulatory bodies will be crucial in overcoming the technical, economic, and regulatory barriers to ensure that OoC platforms are effectively integrated into drug development pipelines.

In conclusion, organ-on-a-chip technology holds immense potential for the pharmaceutical industry, offering a more accurate and efficient way to test drug formulations, particularly novel nano- and microparticulate systems, before proceeding to *in vivo* studies. With the ongoing advancements in materials, manufacturing techniques, AI integration, and regulatory support, OoC platforms are well-positioned to revolutionize the drug development process. As the industry embraces these technologies, we can expect to see a shift toward more reliable, cost-effective drug testing, ultimately accelerating the development of new therapies and bringing them to market more efficiently.

CRediT authorship contribution statement

S. Ying-Jin: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **I. Yuste:** Writing – review & editing, Methodology, Investigation, Data curation. **E. González-Burgos:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration

During the preparation of this work the authors used ChatGPT in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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