TISSUE BARRIERS

Taylor & Francis
Taylor & Francis Group

Tissue Barriers

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ktib20

Models for barrier understanding in health and disease in lab-on-a-chips

J. Ponmozhi, S. Dhinakaran, Dorottya Kocsis, Kristóf Iván & Franciska Erdő

To cite this article: J. Ponmozhi, S. Dhinakaran, Dorottya Kocsis, Kristóf Iván & Franciska Erdő (2024) Models for barrier understanding in health and disease in lab-on-a-chips, Tissue Barriers, 12:2, 2221632, DOI: 10.1080/21688370.2023.2221632

To link to this article: https://doi.org/10.1080/21688370.2023.2221632

Published online: 09 Jun 2023.

TISSUE BARRIERS
2024, VOL. 12, NO. 2, e2221632 (32 pages)
https://doi.org/10.1080/21688370.2023.2221632

Taylor & Francis
Taylor & Francis Group

REVIEW

Models for barrier understanding in health and disease in lab-on-a-chips

J. Ponmozhi[a], S. Dhinakaran[b], Dorottya Kocsis[c], Kristóf Iván[c], and Franciska Erdő[c]

[a]Microfluidics Laboratory, Department of Mechanical Engineering, IPS Academy-Institute of Engineering Science, Indore, India; [b]The Centre for Fluid Dynamics, Department of Mechanical Engineering, Indian Institute of Technology Indore, Indore, India; [c]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary

ABSTRACT

The maintenance of body homeostasis relies heavily on physiological barriers. Dysfunction of these barriers can lead to various pathological processes, including increased exposure to toxic materials and microorganisms. Various methods exist to investigate barrier function in vivo and in vitro. To investigate barrier function in a highly reproducible manner, ethically, and high throughput, researchers have turned to non-animal techniques and micro-scale technologies. In this comprehensive review, the authors summarize the current applications of organ-on-a-chip microfluidic devices in the study of physiological barriers. The review covers the blood-brain barrier, ocular barriers, dermal barrier, respiratory barriers, intestinal, hepatobiliary, and renal/bladder barriers under both healthy and pathological conditions. The article then briefly presents placental/vaginal, and tumour/multi-organ barriers in organ-on-a-chip devices. Finally, the review discusses Computational Fluid Dynamics in microfluidic systems that integrate biological barriers. This article provides a concise yet informative overview of the current state-of-the-art in barrier studies using microfluidic devices.

ARTICLE HISTORY
Received 3 February 2023
Revised 30 May 2023
Accepted 31 May 2023

KEYWORDS
blood-brain barrier; computational fluid dynamics (CFD); dermal barrier; hepatobiliary barrier; ocular barriers; organ-on-a-chip; physiological barriers; renal and intestinal barriers; respiratory barrier; tumor barriers

Introduction

The analysis of body fluids has a rich history dating back to ancient times. Bedside diagnosis tools, such as matching the color and appearance of urine with physiological states, were commonly used. However, with advancements in science, more precise and standardized equipment was developed, which led to diagnosis moving away from the patient and into laboratories. Fortunately, with the emergence of microfluidic devices, the possibility of point-of-care testing has become a reality. Microfluidic devices were first fabricated in the late 1960s by key players such as the Finnigan Instrument Corporation, Stanford and Purdue University, Siemens, and IBM[1]. These devices have made it possible to revisit the bedside diagnosis, allowing for efficient and accurate testing at the patient's side.

In 1993, Harrison and his colleagues designed glass chips measuring 1 by 2 centimeters for electrophoresis-based chemical analysis, marking a significant milestone in the field[2]. Another major breakthrough occurred in 1998, with the invention of rapid prototyping of microfluidic systems using polydimethylsiloxane, which allowed for the fabrication of microfluidic chips in less than a day[3]. Meanwhile, the technology of 3D printing has continued to evolve, making it easier to create large-scale, integrated, and multi-layered microfluidic devices. This advancement has greatly enhanced the study of cell- and organ-on-a-chip constructs, allowing for more realistic modeling of physiological and pathological conditions[1].

According to a PubMed search, the number of annual publications on Lab-on-a-Chip has been steadily increasing since exceeding 100 in 2003, reaching over 1000 in 2015 (see Figure 1). Notably, the first publication in the database regarding "organ-on-a-chip" was in 2010, describing a chip that reconstitutes the alveolar-capillary interface of the human lung[4]. In the past decade, there has been a significant increase in the number of papers published on organ-on-a-chip technology, and the proportion of "organ-on-a-chip" publications relative to all "lab-on-a-chip" publications has been steadily increasing as well, as shown in Figure 1C-D, respectively.

CONTACT Franciska Erdő erdo.franciska@itk.ppke.hu Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Práter u. 50a. 1083 Budapest, Hungary

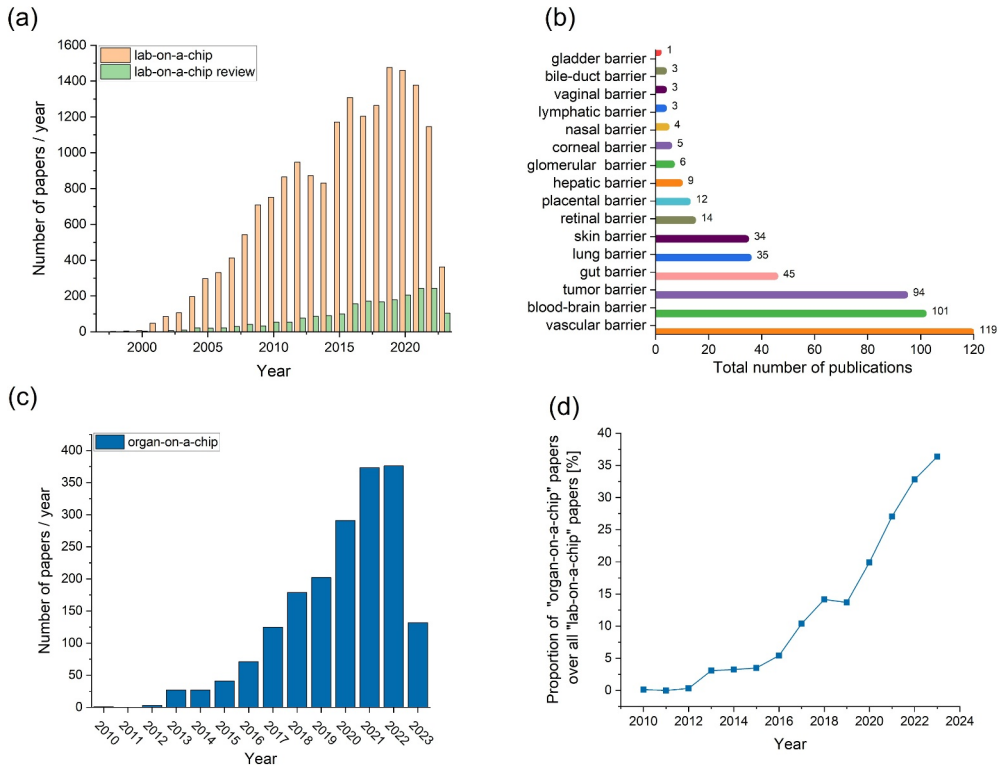

Figure 1. (a) the number of publications in the PubMed database as a function of year with the search term "lab-on-a-chip", showing all hits (orange) and exclusively review articles (green). (b) the total number of publications on lab-on-a-chip studies testing different barriers. (Search term: "(lab-on-a-chip) and (xx barrier)".) (c) the number of publications with the search term "organ-on-a-chip". (d) Changes in the proportion of "organ-on-a-chip" studies over all "lab-on-a-chip" articles over time. (The PubMed search was completed by April 17, 2023.).

The purpose of this review article is threefold: firstly, to provide a comprehensive overview of the applications of microfluidic chips in studying normal and pathological biological barriers; secondly, to showcase the diverse methodological approaches employed in this research area; and thirdly, to highlight the various parameters that can be monitored in these dynamic, physiologically relevant *in vitro* or *ex vivo* systems. By analyzing the studies discussed in this review, scientists working in this field can enhance their own models and achieve more predictive and informative results. Ultimately, this can facilitate the translation of findings from microscale models to human conditions, thereby advancing the development of novel treatments and therapies

Organ-on-a-chips: fabrication technologies, materials, and cell sources

Throughout the evolution of technology, numerous fabrication methods and materials have been employed in the production of microscale microfluidic devices, such as organ-on-a-chips (as detailed Table 1). However, each fabrication method has its advantages and limitations, which are also summarized in Table 1. When compared to traditional 2D and 3D in vitro cell culture assays in culture dishes or in vivo animal models, the use of new organ-on-a-chip devices has several pros and cons (outlined in Table 2 and Table 3). Organ-on-a-chip models must replicate certain tissue and organ structures and functions; hence, appropriate cell types and sources must be prepared and provided. Primary cells isolated from specific organs without any gene modification are considered ideal cell sources for organ-on-a-chips, as they exhibit comparable functions to in vivo environments[11]. However, in vitro culture of primary cells can be challenging for certain types, such as neurons and cardiomyocytes, and they may only be stably available for a limited time[12]. The emergence of human-induced pluripotent stem cells (iPSCs) provides a potential alternative

Table 1. Techniques and materials for fabrication of microfluidic devices (modified from Cao et al.[124]).

Fabrication Techniques	Materials used	Advantages	Limitations
Photolithography	Resin Glass Elastomers (e.g. PDMS, polyurethane) Thermoplastic (e.g. PMMA) Cyclic olefin polymers	High precision over feature geometry Comparatively fast Used on different materials such as glass and silicon	Require a cleanroom for fabrication High initial tooling and machinery costs Work only on perfectly flat substrates
Soft lithography	PDMS Liquid metal Polystyrene	Capable of mass production of sophisticated microstructures Cost effective Relatively easy setup High throughput Cleanroom free operation	Need aid from another lithography method to fabricate the stamp master
Injection moulding	Polystyrene (PS) PMMA Cyclic olefin copolymer (COC)	High repeatability and reliability once the mould tool is made Capable of mass production at a low cost Relatively short cycle time Allows for complex geometries with tight tolerances Little plastic waste	High initial tooling and machinery costs Long initial lead times Difficult and expensive for design changes
Hot embossing process	PMMA Polycarbonate (PC) COC Polystyrene (PS) Polylactic acid (PLA)	Cost-effective High efficiency Capable on mass replication High structural precision	Difficult to control the optimal process parameters Require thermoplastic materials to be used Great care needed when de-embossing the products
Etching technique	Poly (2-hydroxyethyl methacrylate) P-HEMA Silk fibroin PDMS	Capable of mass production of fine-detailed and complex microdevices Rapid prototyping High precision fabrication Production of burr-free parts with no stress to the metal	High initial tooling and machinery costs Require high expertise to operate specialized equipment Require using some corrosive gases
Laser cutting process	paper	Non-contact carving method, Excellent accuracy and precision A relatively short development time	Expensive initial and maintenance costs Produce harmful pyrolysis by-products when burning plastics Require compressed air or water wash after finishing
3D printing	PDMS Glass polyethylene-glycol(PEG) Natural biopolymers (e.g. collagen, silk fibroin, gelatin, alginate and chitosan)	Flexible designing and modeling Excellent precision, smooth surface resolution and watertight tolerances depending on printing types Utilize various materials Rapid prototyping Minimal waste Cost-effective	Restricted building part sizes Require post-processing methods such as water jetting, sanding, chemical soaking and air-drying

Table 2. Organ-on-a-chip technologies pros and cons.

Pros	References	Cons	References
Cheap manufacturing	5	Presence of surface effect	6
In-house fabrication	5	Small volumes, sensitive analysis required	6
Many drugs/dosages can be tested at the same time	6	Laminar flow within the microchannels/not proper mixing	6
Do not meet ethic concerns	6	Special other instruments needed	7–9
Physiologically relevant tissue microenvironment	6		
Low variability in replicates	6		
3D structure	6		
Test reliability	6		
User friendly/portable	6		
Parallelization (saving space, money and time)	6		
Dynamic systems, mimic shear stress	10		

for organ-on-a-chip cell sources[13,14]. iPSCs can be induced to differentiate into various organ-specific cells, and commercially available organ-specific cells derived from iPSCs exist[15]. Additionally, iPSCs from patients carry their genetic information, allowing for the creation of disease models for these patients. Furthermore, gene editing technology can be utilized to recreate and study organ-specific functions affected by mutations, which has significant applications[16]. Table 4 summarizes the primary cell types employed in various barrier-on-a-chip systems

Table 3. Comparison of 2D/3D cell culture, animal models and organ-on-a-chips (modified from Koyilot et al., 2022[125] and Yu et al., 2019[126].).

2D/3D cell culture	Animal models	Organ-on-a-chip
High throughput screening (HTS) system	Mimic cellular/tissue environment complexity	Replicate physiological/pathophysiological environment Ability to integrate with various sensors and actuators HTS is possible with integration of automatization and smart analysis system
Limited cell-cell interaction (2D) Necrotic cells (3D) Limited physiological relevance Static condition Inefficient nutrient and waste transport	Limited throughput Ethical and legal issues Difficulties with the translation and correlation with humans	It is not scalable with the human organs Require external pumps, tubing, connectors, and valve to operate

Table 4. Cell types frequently used in different organ-on-a-chip barrier models.

Barriers on-a-chip	Most frequently used cell types	References
Blood-brain barrier	endothelial cells, pericytes, astrocytes	17–23
Neurovascular unit barrier	endothelial cells, pericytes, astrocytes, neurons, microglia cells	17
Visual system barrier	endothelial, epithelial cells, fibroblasts, neuroblastoma cells, mesenchymal stem cells	24–32
Respiratory system barrier	epithelial cells, endothelial cells, fibroblasts, macrophages,	33–51
Dermal barrier	keratinocytes, fibroblasts, melanocytes, endothelial cells	52–59
Intestinal barrier	enterocytes, intestinal epithelial cells, colon carcinoma cells, microbiome bacteria	60–68
Liver barrier	hepatocytes, endothelial cells, cholangiocytes	69–87
Kidney barrier	glomerular endothelial cells, visceral epithelial cells (podocyte), renal carcinoma cells, fibroblasts, macrophages	85,88–97
Vaginal/placental barrier	vaginal epithelial cells, uterine stromal fibroblasts, lactobacilli, other bacteria, placental villous endothelial cells, trophoblasts, macrophages	98–104
Tumor barrier	fibrosarcoma cells, endothelial cells, macrophages, breast ductal carcinoma cells, other tumor cells	105–113
Multiorgan barrier	endothelial cells, liver cells, small intestine cells and lung tumour cells, bone cells, neurons, astroglia, muscle, breast cancer cells, colon cancer cells, connective tissue cells	114–123

Table 5. Tumor-on-a-chip models and their applications.

tumour-on-a-chip model	cells employed	Visualizing method	Application	References
Tumour microenvironment on a chip	Murine macrophage cell RAW 264.7 and tumour spheroids coculture	confocal laser scanning microscope	investigating migration of drug-carrying macrophages towards tumor, their tumor infiltration, and the corresponding drug responses in tumor spheroids.	145
3 D Liver tumour on a chip model	hepatoma parental and resistant cells		Drug resistant evaluation on different tumour stages and diverse medication periods	146
Breast and prostate tumour on a chip model	Viable breast and prostate Patient-derived xenograft tissue slices	Light microscopy	Cisplatin and apalutamide chemotherapeutic probes sensitivity to breast and tumour xenograft models.	147
Single breast cancer cell and its extracellular vesicles	Michigan Cancer Foundation 7 (MCF-7), Sloan Kettering Breast Cancer 3 (SkBr-3) cells, and Human Embryonic Kidney (HEK293) were obtained from ATCC	total internal reflection fluorescence microscope	Cancer cell extravasation	148

Physiological barriers

Physiological barriers are natural defense mechanisms that protect organisms, organs, and organ systems from harmful environmental stimuli. These barriers can take different forms, such as physical, chemical, or biological barriers, and their nature can vary based on the organism's environment and lifestyle. For instance, the skin is a primary physical barrier that shields against dehydration and infection, while mucous membranes are chemical barriers that produce antibacterial substances. By providing an initial line of defense against potentially harmful agents, physiological barriers play a crucial role in maintaining the health and integrity of an organism's internal environment. Barriers can take different forms, ranging from a single cell layer (such as monolayers) to more complex cell cultures (like blood-brain barrier models composed of endothelial cells, pericytes, and astrocytes) or complete tissues (such as excised skins, epidermis, or full-thickness skins). These barriers serve as critical interfaces that control the passage of substances and protect the

underlying structures from damage. In modern biomedical research, various biological barriers can be integrated into microfluidic lab-on-a-chip or organ-on-a-chip devices, enabling precise modeling and analysis of the physiological barriers in a controlled and realistic setting. This approach can facilitate the development of novel drug delivery systems, disease models, and tissue engineering applications.

Before conducting an organ-on-a-chip experiment using a microfluidic chip that integrates cell culture, it is essential to assess the barrier function by measuring the transepithelial/endothelial electrical resistance (TEER). TEER is a widely accepted quantitative technique used to measure the integrity of tight junction dynamics in cell culture models of endothelial and epithelial monolayers. High TEER values indicate strong barrier function and tight junction integrity, making it a useful tool to evaluate the barrier properties of cells before the transport of drugs or chemicals. TEER measurements can be performed in real-time without causing cell damage and are generally based on measuring ohmic resistance or impedance across a wide spectrum of frequencies. The blood-brain barrier (BBB), gastrointestinal (GI) tract, and pulmonary models are some examples of barrier models that have been widely characterized using TEER measurements. However, it is worth noting that TEER values can vary depending on certain factors, such as temperature, medium formulation, and passage number of cells, leading to high inter- and intra-laboratory differences in TEER values. Therefore, it is important to carefully control these factors to ensure accurate and reproducible TEER measurements[127].

Current commercial devices for TEER measurement are limited to static and macroscopic cellular systems, which are not suitable for use in organ-on-a-chip devices due to their small cell culture areas that cannot accommodate electrode placement. Immobilizing and integrating the electrodes directly within the chip close to the cellular monolayer can reduce electrical resistance and improve the signal-to-noise ratio. Electrode size should also be miniaturized to fit organ-on-a-chip systems. To ensure a uniform current density, the electrode design for chip applications can be combined with electrical simulation and modeling. In contrast to traditional cell culture systems, organ-on-a-chips offer the advantage of allowing the study of cells under physiologically relevant conditions, such as continuous fluid flow and consequent shear stress on the cells, and cell-cell interactions. This approach has the potential to provide more accurate and reproducible results in drug screening and disease modeling[128,129]. The following section provides some examples of TEER measurements in microfluidic environments for in vitro modeling of the blood-brain barrier, gastrointestinal barrier, and airway barrier.

Booth et al. (2011) developed a multi-layered microfluidic device for microscale modeling of the blood-brain barrier (BBB), which comprised of four PDMS substrates, two glass layers, and a porous polycarbonate membrane sandwiched between PDMS layers[130]. This device also integrated thin-film electrodes that were fabricated by depositing layers of Cr, Au, and Ag on glass, followed by chlorination of the silver surface to generate an electrochemically active AgCl surface. The electrodes were connected to a commercial EVOM system, and the TEER of b.End3 (endothelial) cells in co-culture with C8D1A cells (astrocytic) was increased from 20 to 250 Ωcm^2 by exposing the cells to dynamic conditions that provide shear stress. Kim et al. developed a cell-based gastrointestinal system in a microfluidic system, where TEER values between 3000 to 4000 Ωcm^2 were observed, whereas control cultures under static conditions exhibited TEER values between 700 to 800 Ωcm^2[131]. Such systems may allow investigating the influence of shear stress on the barrier function of in vitro GI tract tissues[132,133]. However, it's important to note that these high values of TEER are not physiologic[133].

A functional small airway microfluidic model was developed by culturing human alveolar epithelial (A549) cells on Transwell inserts with an air-liquid interface[134]. TEER values were measured over the course of 6 days using a MilliCell-ERS system from Millipore AG. The TEER values continuously increased and reached a maximum of 128 Ωcm^2 and 152 Ωcm^2 on the liquid and air interfaces, respectively[134]. This highlights the potential of microfluidic environments to improve the barrier function of in vitro systems due to the effect of shear stress on the cells. Over the past few

decades, different devices have been developed to test the barrier function and integrity of normal or disease-specific models. In the following sections, we will explore the most important applications of organ-on-a-chip devices for testing the functional integrity of various physiological barriers.

Blood-brain barrier and neurovascular units

The microvessels within the central nervous system play a critical role in not only transporting energy substrates and waste products but also in tightly regulating the movement of ions, molecules, and cells between the bloodstream and the brain. The blood-brain barrier (BBB) is a compact structure consisting of capillary endothelial cells surrounded by pericytes and astrocyte endfeet. This structure is responsible for protecting the brain from toxins and pathogens, and the properties of the BBB greatly influence the development of neurological disorders. However, this barrier also prevents the entry of drugs targeting the central nervous system. The microenvironment plays a significant role in BBB function, and fluid flow, along with precise composition, is critical in maintaining barrier function[135].

Microphysiological systems aim to replicate the complex parameters of the BBB. BBB-on-a-chip devices typically consist of endothelial cells, astrocytes, and pericytes, while neurovascular-units-on-a-chip (NVU) devices also include neurons, as shown in Figure 2. Numerous BBB-on-a-chip systems have been developed, ranging from planar structures to various 3D models. 2D chips usually consist of two chambers separated by a porous membrane made from polycarbonate, polyester, or polydimethylsiloxane, which enables interaction between different types of cells (i.e., endothelial cells in one compartment and astrocytes with pericytes in the other)[17]. However, the major drawback of this construct is the lack of contact co-culture. The term '2.5D models' is used for structures where a 3D extracellular matrix compartment contains astrocytes and pericytes, and the endothelial cell monolayer is inside a predesigned structure such as a rectangular or square channel (as shown in Figure 2[18]. This model enables a more physiologically accurate co-culture architecture, although it is technically challenging, and comparing different models is not straightforward. In the 3D models, a cylindrical, perfusable endothelial cell layer that is 100–800 µm wide is embedded into a 3D matrix containing astrocytes, pericytes, neurons, and microglia. The construction is even more challenging, and compared to the 2D model, the disadvantage of the 2.5D and 3D devices is the difficulty in measuring permeability through the barrier, which is now mostly limited to fluorescent markers[18].

Based on literature data, the physiological TEER value for brain microvasculature ranges between

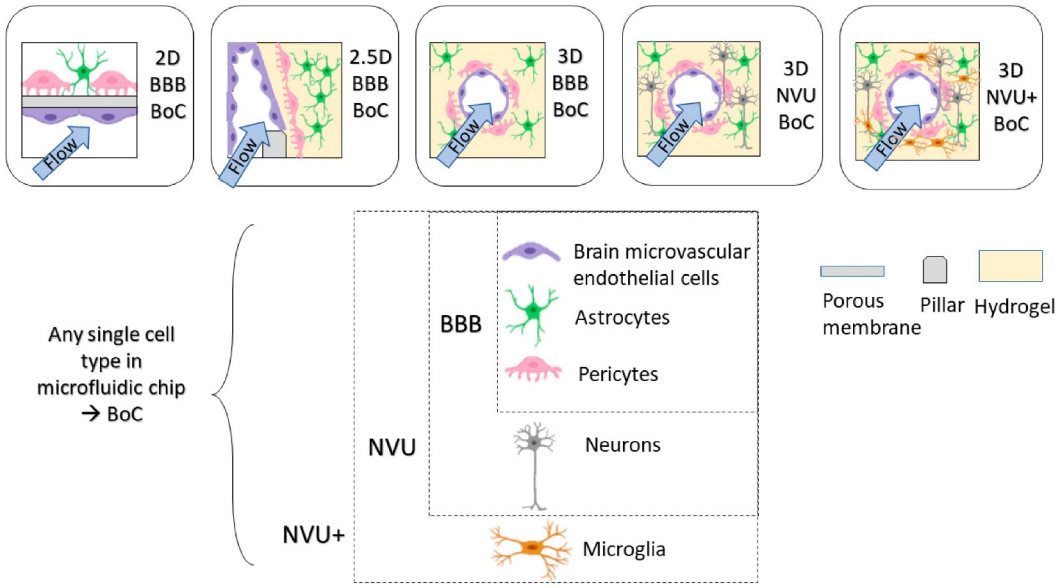

Figure 2. Classification of brain-on-a-chip devices (BoC) based on construct geometry and cell types[17].

1500 and 8000 Ωcm[217]. However, the TEER values of BBB-chips vary greatly, ranging between 200 and 24,000 Ωcm[217]. BBB-on-a-chip devices have been developed to study various neurological disorders such as Alzheimer's disease[19], brain cancers including glioblastoma[20], ischemic stroke[21], as well as infectious diseases such as meningitis caused by Cryptococcus neoformans[22] and SARS-CoV-2 virus[23]."

Barriers in the visual system (retinal and corneal barriers)

The retina, the innermost and light-sensitive region of the eye, comprises multiple layers of specialized neurons, as well as glial cells, endothelial cells, and epithelial cells. Due to its sensitivity, it is essential to protect it against harmful agents that may enter the bloodstream. The retina receives blood supply from two distinct vascular beds: the retinal vasculature, which supports the inner retina, and the choroidal vasculature, which supports the outer retina. As a result, the blood-retinal barrier (BRB) is composed of two separate barriers. The inner BRB is formed by specialized retinal vascular endothelial cells with well-developed tight junctions, while the outer BRB is composed of the retinal pigmented epithelium, which regulates the flux from the choriocapillaris[24].

Several devices have been developed to model the outer BRB, with the simplest planar case involving the culture of retinal pigment epithelium (e.g., ARPE-19) and endothelial cells (e.g., HUVEC) separated by a porous membrane or a fibrin matrix[24]. However, the use of HUVEC cells in the retina model is actually inadequate. Devices that better model the physiological spatial arrangement have also been created, such as circular structures of microvessels occupied by HUVEC cells located below an ARPE-19 cell layer[25]. To further improve the model, Chen and coworkers used human lung fibroblasts (NHLF) as supporting cells in addition to the epithelial (ARPE) and endothelial (HUVEC) cells[26]. Comparison of the ARPE-HUVEC-NHLF culture's TEER values to the ARPE monolayer showed that the outer BRB model had higher values (although still below 100 Ωcm^2), possibly due to the presence of fibrin gel and the influence of microvessels[26].

Various constructs have been developed to mimic the inner BRB, such as those made of tubular human retinal microvascular endothelial cells (hRMVEC) and extracellular matrix components[136]. In addition, a wider range of retinal layers has been combined on a single chip to establish the inner BRB (primary human retinal endothelial cells), middle neuronal layers (SH-SY5Y human neuroblastoma cells), and outer BRB (ARPE-19)[27]. The created oBRB models have led to studies on the pathomechanism of several diseases leading to blindness, such as wet type age-related macular degeneration, as choroidal neovascularization is the first step in many disorders[28]. On the other hand, iBRB models have been used to investigate the response of barrier function to leakage mediators[136].

The cornea is a transparent, avascular part of the eye that consists of epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium[29]. It maintains the metabolic activities of the ocular surface and protects the inner part of the eye. Tight cellular barriers are formed by corneal epithelium and endothelium in the anterior and posterior parts, but according to some research, the main barrier function is played by the epithelial cells[30]. Organ-on-a-chip devices that model the corneal barrier include a porous membrane embedded into the microfluidic platform that separates the apical and basal sides. In some cases, only corneal epithelial cells (such as HCEpi or HCE-T) are cultured on the membrane[31], while in other investigations, endothelial cells (such as HCEnd) are also involved, cultured on the other side of the membrane, as shown in Figure 3[29].

Yu et al. were able to achieve TEER values of 600–800 Ωcm^2 and ~ 900 Ωcm^2 by culturing HCEpi alone and HCEpi together with HCEnd on the membrane, respectively[29]. In vivo conditions involve shear stress, which is important in several biological barriers, including the corneal epithelium. To model the constantly changing properties of the corneal epithelium due to eye blinking, microfluidic devices that can create dynamic conditions are necessary. For example, bidirectional and unidirectional flows can be applied above and below the epithelial cells, respectively[32]. These

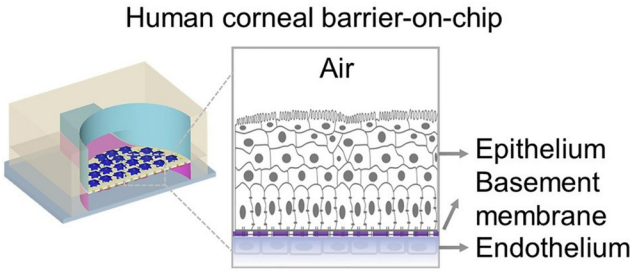

Figure 3. Cross-sectional schematic diagram of the cornea-on-a-chip. Endothelial and epithelial cells are cultured on the opposite sides of the membrane[29].

devices are useful for studies on eye blinking shear stress and in ophthalmic drug development. The chamber below the cells is also suitable for sample analysis, such as detecting biologically relevant extracellular metabolites and other molecules penetrating across the corneal epithelium, as performed by Abdalkader and Kamei[32]. In addition, Yu et al. used the cornea-on-a-chip for epithelial wound healing studies, where extracellular vesicles derived from bone marrow-derived mesenchymal stem cells significantly enhanced corneal epithelial wound healing[29].

Respiratory barrier

The airway epithelium plays a crucial role in maintaining the barrier function of the airway tract, which is composed of three major components: mucociliary clearance, intercellular apical junctional complexes that regulate paracellular permeability, and antimicrobial peptides secreted by airway epithelial cells. These components work together to clear inhaled pathogens, allergens, and particulate matter without inducing inflammation and to maintain tissue homeostasis[137]. Recently, Cohen and colleagues developed a multi-compartment airways-on-chip model of the human respiratory tract to demonstrate the feasibility of delivering microspheres for therapeutic applications[33]. This model anatomically mimics the flows in respiratory zones and includes three compartments with different inlet channels: nasal (4 mm diameter), bronchial branches (between 2.2 and 1.25 mm diameter), and alveoli (100 µm height x 170 µm width) (Figure 4). The authors used this system to simulate the intranasal administration of encapsulated immune cells (epithelial TC-1 cells and MH-S macrophages) in microspheres in fluid and to analyze microsphere delivery along the respiratory tract. This study demonstrates the feasibility of using hydrogel-based topical microspheres in the context of pulmonary damage and tissue repair.

Exposure to air pollution and particulate matter (PM) is associated with various respiratory diseases in humans. However, there are few studies exploring how multicellular networks communicate within a tissue microenvironment after PM exposure[34]. Byun et al. developed a three-dimensional (3D) respiratory mucosa-on-a-chip model in vitro, composed of human nasal epithelial cells, fibroblasts, and endothelial cells, to investigate the effects of urban particulate matter (UPM) on the human respiratory mucosa (Figure 5)[34]. The researchers exposed human nasal epithelial cells to UPM and observed a disruption in the respiratory mucosa's integrity. They found that UPM decreased the expression of zonula occludens-1 in both the epithelium and endothelium and induced vascular endothelial cadherin expression in the endothelium.

Figure 5 shows the respiratory mucosa-on-a-chip designed by Byun and colleagues[34], which consists of three layers: human nasal epithelial cells (hNEC), fibroblasts, and endothelial cells. In this study, nasal cells were exposed to urban particulate matter (UPM), which resulted in disruption of cell

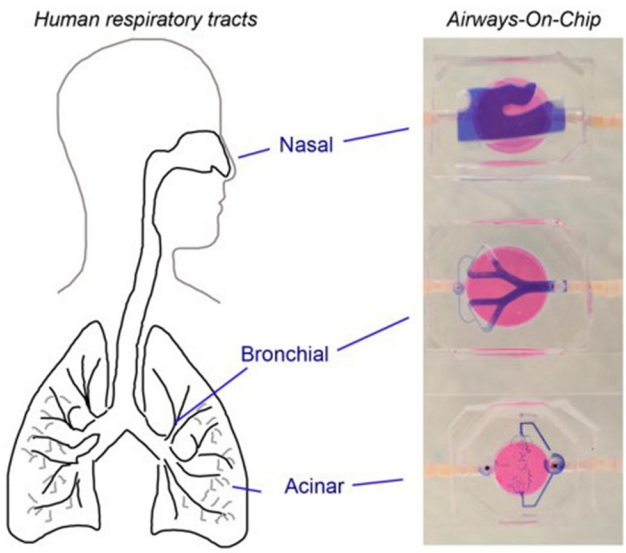

Figure 4. The Airways-on-a-chip design developed by Cohen and co-workers[33].

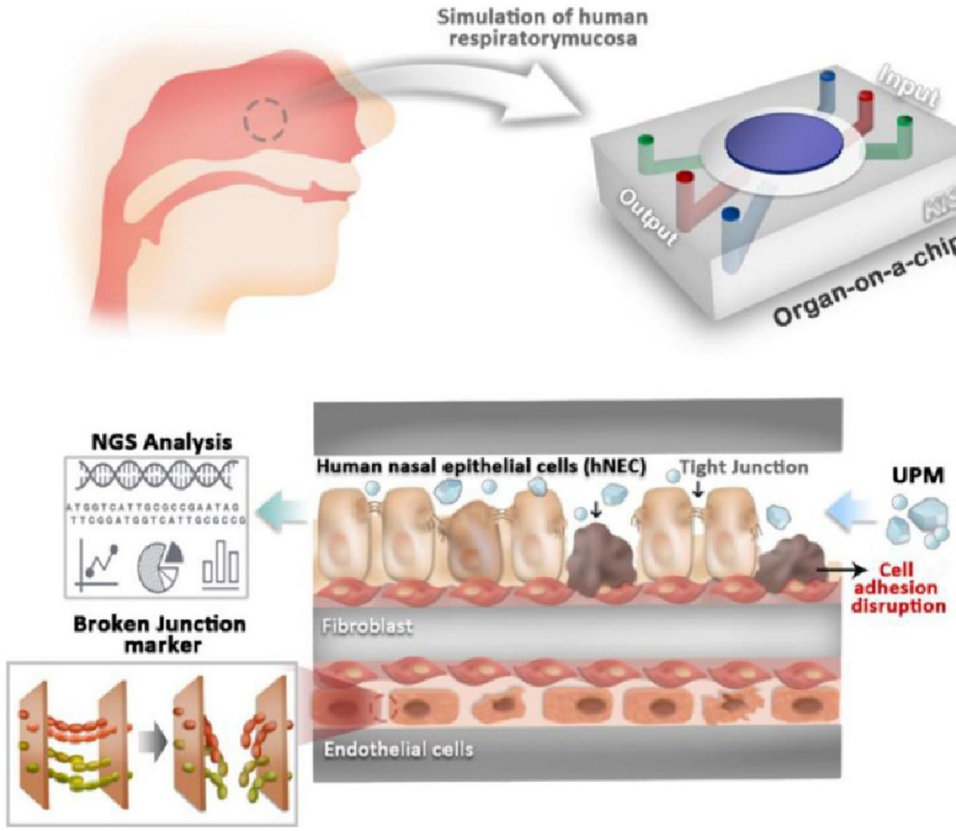

Figure 5. Shows the respiratory mucosa-on-a-chip designed by Byun and colleagues [35], which consists of three layers: human nasal epithelial cells (hNEC), fibroblasts, and endothelial cells. In this study, nasal cells were exposed to urban particulate matter (UPM), which resulted in disruption of cell junctions due to alterations in gene expression. This disruption led to pro-inflammatory responses in the endothelial cells, further disrupting endothelial junction proteins.

junctions due to alterations in gene expression. This disruption led to pro-inflammatory responses in the endothelial cells, further disrupting endothelial junction proteins.

Zhu et al.[35] developed a lung-on-a-chip system that allows for visualization of breathing by inducing cyclic deformation of pulmonary alveoli through regular airflow, mimicking the expansion and contraction of the lung. The resulting deformation of the cells was monitored using an array of colors and compiled into a color atlas that parallels pulmonary disease symptoms. This lung-on-a-chip system has the potential to be used for disease monitoring and drug development against lung disorders.

Over the past decade, several lung-on-a-chip devices have been developed to model the complex structure and function of the human lung. In a review by Francis and colleagues, the utility of lung-on-a-chip technology in testing various lung disorders and therapeutic interventions was summarized[36]. The authors demonstrated that these devices were used to model a wide range of lung diseases, including COPD (chronic obstructive pulmonary disease)[37], COPD induced by cigarette smoking[38], asthma[37,39], lung cancer[40–42], fibrosis[43,44], COVID-19[45–47], tuberculosis[48], influenza-induced pneumonia and fungal infections[49], pulmonary toxicity[50], and pulmonary thrombosis[51].

Dermal (skin) barrier

The epidermis, which is the uppermost layer of the skin, serves protective and defensive functions through a unique differentiation end product of keratinocytes known as the stratum corneum (SC)[138]. One of the most critical functions of the SC is the creation of a permeability barrier, which prevents transcutaneous evaporative water loss and

allows for survival in a potentially dehydrating external environment. The SC is a multi-layered tissue made up of flattened, anucleate corneocytes surrounded by multiple planar lamellae sheets that are rich in ceramides, cholesterol, and free fatty acids (FFA)[139,140]. Below the epidermis is the dermis, which contains various cell types such as fibroblasts, hair follicles, stem cells, vascular, immunological and neuronal elements, and sweat ducts. In skin-on-a-chip models, either the epidermis or full-thickness skin (epidermis plus dermis) can be tested.

In a recent review, Filaire and colleagues analyzed the main in vitro models used for safety testing of cosmetic products, focusing on skin sensitization, skin corrosion, skin irritation, and skin absorption, as well as their advantages, limitations, and regulatory requirements. The review also highlighted recent innovative technologies, such as Organ-on-a-Chip (OoC) models, for toxicology and efficacy testing[52].

Several research groups have analyzed and developed in vitro skin models on a chip with different levels of complexity[53–59,141,142]. Some studies have described skin aging models[142] and dermatological disease models on a chip[59,141]. Others have investigated the effect of therapeutics, such as Coenzyme Q10[56]. Here, we present some recent results in more detail.

The skin undergoes both intrinsic aging, caused by metabolic processes, and extrinsic aging, caused by environmental factors. However, replicating the intrinsic aging process in vitro is challenging due to its long-term progression. In a recent study, Jeong and colleagues accelerated aging on a full-thickness skin equivalent by applying periodic mechanical stimulation and mimicking the circadian rhythm for 28 days. They developed a full-thickness, three-dimensional skin equivalent by culturing human fibroblasts and keratinocytes and using a flexible skin-on-a-chip. Periodic compressive stress led to a reduction in epidermal layer thickness (as shown in Figure 6), contraction rate, and secretion of Myb, while increased galactosidase gene expression, secretion of reactive oxygen species, and transforming growth were observed. This in vitro aging skin model can be used to accelerate drug development for skin diseases and cosmetics that cannot be tested in animals[142].

In a recent study, Jones and coworkers described the development of a novel skin-on-a-chip model that integrates primary and immortalized human cells to create a full-thickness skin equivalent[55]. The model was housed in a microfluidic device in which a microvasculature had been previously established. The study assessed the impact of the chip design on the quality of the microvascular networks formed and developed a methodology to harvest tissues from embedded chips after 14 days of culturing. This allowed analysis of the impact of culture conditions and vascularization on the morphology and stratification of the skin equivalents' epidermis. The results showed that vascularization enhanced the stratification and differentiation, including thickness, architecture, and expression of terminal differentiation markers such as involucrin and transglutaminase-1. As a result, the matured skin substitutes were formed in the microfluidic chips (Figure 7)[55].

Kim and colleagues demonstrated a pumpless skin-on-a-chip system in their paper[56]. They cultured a human skin equivalent (HSE) composed of the epidermis and dermis on the chip and achieved the desired flow rate through gravity alone, without the need for a pump or external tube connection. To test the efficacy of Coenzyme Q10, an anti-aging and antioxidant, it was added to the culture solutions. The authors measured the relationship between the contraction rate of the HSE and the secretion of transforming growth factor TGF-1 by performing enzyme-linked immunosorbent assay (ELISA). By increasing the concentration of coenzyme Q10, the number of cells per unit area and the thickness of the epidermal layer increased proportionally. The level of filaggrin expression and the contraction rate of the full-thickness HSE were also found to be proportional to the secretion of TGF-1.

Atopic dermatitis (AD) is a complex and multifactorial disorder that is still not well understood, and there is currently no appropriate disease model available for its analysis[59]. To address this issue, Kim and colleagues stimulated a human skin equivalent (HSE) in a pumpless skin-on-a-chip system using interleukins IL-4 and IL-13, which are cytokines that play a significant role in AD[59]. The researchers investigated the morphological changes, gene expression,

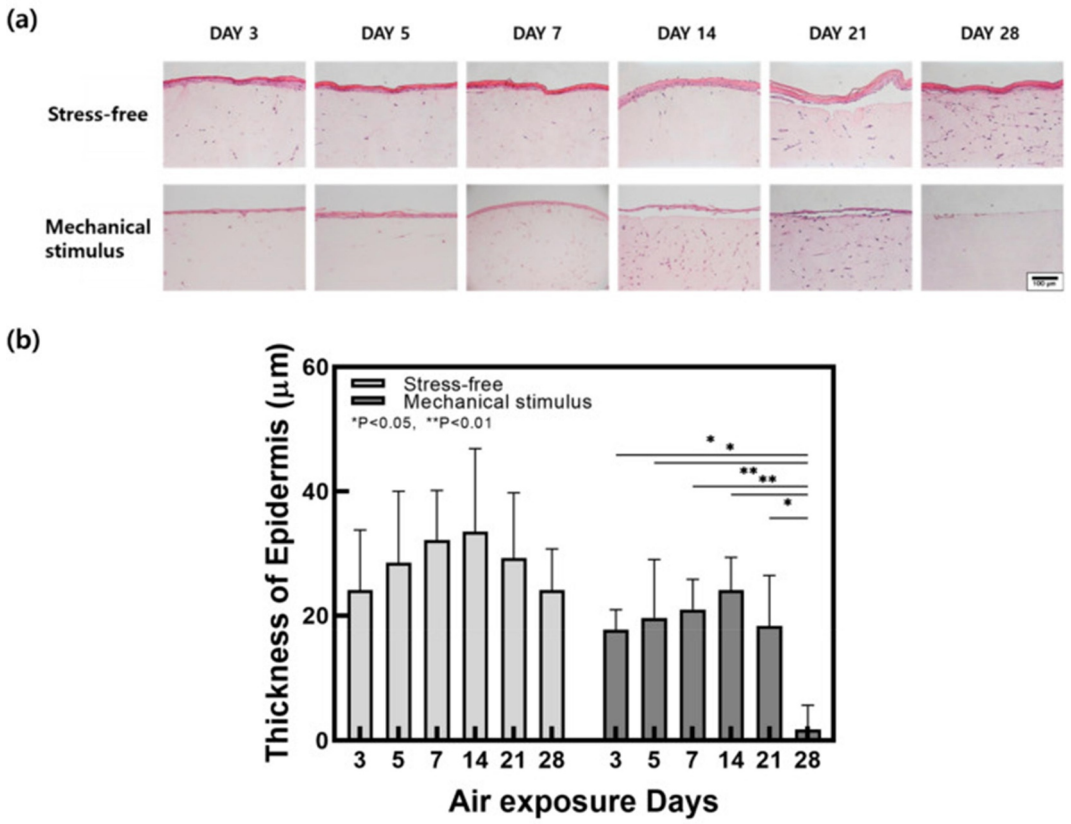

Figure 6. Changes in epidermal thickness with and without compressive stimulation in a flexible skin-on-a-chip[142]. (a) Hematoxylin-eosin stained sections as a function of air exposure days. Scale bar = 100 μm, n= 5. (b) Average thickness of epidermis with error bars (SEM), * p<0.05; ** p<0.01.

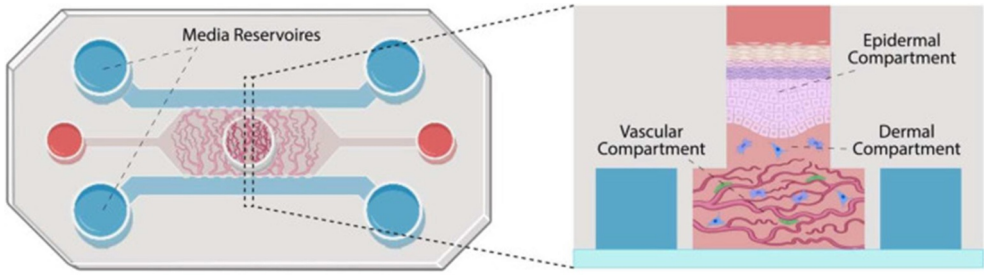

Figure 7. Schematic representation of skin-on-a-chip model proposed by Jones et al. The chip is composed of three parallel channels which are separated by microposts and connected to the media reservoirs. A central well is positioned in the centre of the central channel. The vascular compartment (red: endothelial elements, green: pericytes, blue: fibroblasts) is formed in the central channel. Dermal fibroblasts are introduced within the second hydrogel compartment above the vascular compartment. Keratinocytes (pink) are seeded above the dermal layer, and they are allowed to stratify[55].

cytokine secretion, and protein expression in the stimulated HSE. Spongy formations similar to those observed in AD lesions were seen, and the expression of barrier-related genes and proteins, such as filaggrin, loricrin, and involucrin, induced by IL-4 R signaling, decreased. However, carbonic anhydrase II (CAII), a gene specifically expressed in patients with AD, was upregulated[59]. Based on these results, the skin-on-a-chip model of AD developed by Kim and colleagues seems to be a suitable tool for testing markers of AD pathology and evaluating the efficacy of various therapeutic interventions.

Intestinal (gut) barrier

The intestinal barrier, which includes enterocytes on the gut's apical surface, is a semipermeable structure that allows the absorption of vital nutrients and facilitates immune sensing, while preventing the passage of pathogenic molecules and bacteria. Both structural and molecular components work together to accomplish this complex yet crucial function of the gastrointestinal tract. The intestinal microbiome has an impact on several biological functions in the body. While animal models are a powerful tool to study the relationship between the host and microbe, there is still an unmet need for a physiologically relevant in vitro human intestinal system[60].

De Gregorio and coworkers proposed an immune-competent gut-microbiota axis to replicate the architecture and vertical typography of the microbiota, along with a complex extracellular microenvironment. This system was developed as a microbiota-human intestine-on-chip (MihI-oC) (Figure 8) and proved to be a valuable platform for studying inflammation, including ileitis, inflammatory bowel disease (IBD), Crohn's disease, and zonulin-mediated gut disease. A microbiota chamber (MC) was integrated into the chip for cultivating the various microbial species of the intestinal microbiota (both microaerophilic and anaerobic). The proposed MihI-oC could be used for testing drugs or evaluating active food digestion, and it could be combined with other modules that mimic various organs, such as the blood-brain barrier (microbiota gut-brain axis), adipose tissue for obesity studies, or the liver for assessing drug toxicity[61].

Jing and coworkers[62] also developed a novel human gut-on-a-chip microsystem, but for a different purpose. They aimed to investigate the regulatory effects of chitosan oligosaccharides (COS) on the occurrence and development of human enteritis. The researchers created an intestinal injury model on the chip using dextran sodium sulfate (DSS) and found that COS was able to decrease intestinal epithelial injury by promoting the expression of the mucous layer. Additionally, an inflammatory bowel disease model was established on the chip using E. coli 11,775. It was demonstrated that COS could protect the intestinal epithelial and vascular endothelial barriers by inhibiting the adhesion and invasion of E. coli 11,775[62].

The gastrointestinal tract can potentially be exposed to inorganic nanomaterials present in food. In vitro gut-on-chip systems are better suited for testing compound toxicity than static models due to the added shear stresses caused by the flow of the medium. In a recent study, intestinal epithelial Caco-2 cells were exposed to TiO2 (E171) and ZnO (NM110) nanomaterials, and the cells' responses were monitored at the gene expression level under dynamic and static conditions[63]. The gut-on-chip system resulted in higher sensitivity of the cells and is proposed to be used for toxicological hazard characterization of nanomaterials.

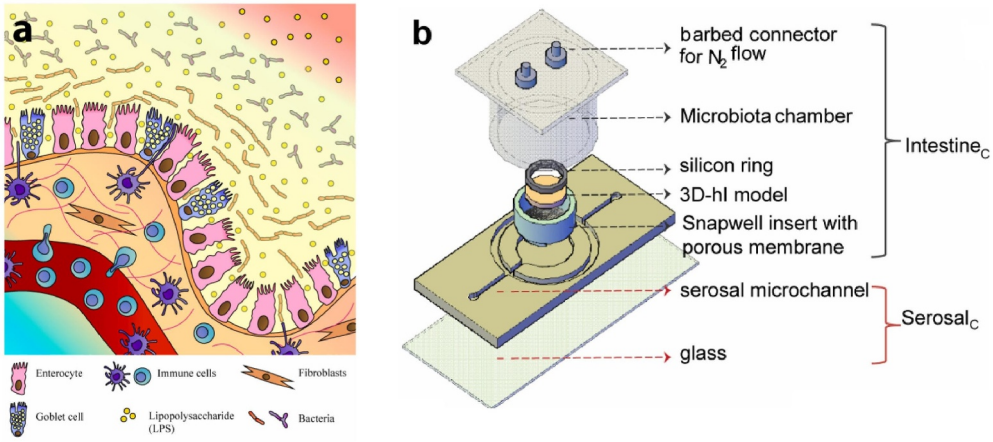

Figure 8. (a) Schematic representation of injured intestinal tissue model; (b) experimental setup of MihI-Oc highlights the microbiota chamber that seals the Intestine compartment (IC) in which was accommodated the 3D-human intestine[61].

Milani and coworkers investigated the interplay of Caco2 cells in co-culture with HT29 cells, cultivating them in a gut-liver organ-on-a-chip system[64]. The intestinal compartment and single-donor primary hepatocytes in the hepatic compartment were used to analyze intestinal permeability, metabolism (intestinal and hepatic), and potential interactions of those processes. The prodrug mycophenolate mofetil was tested, and the conversion of mycophenolate mofetil to the active drug mycophenolic acid, as well as further metabolism to a glucuronide metabolite, were assessed over time. This methodology may be applied to other drugs wherever quantitative knowledge of changing drug concentration with time enables better understanding of the biological effect.

In a review by Moossavi and coworkers[65], research on the intestinal microbiome was categorized into three main areas: (i) diet-microbiome and drug-microbiome interactions; (ii) microbiome-targeted therapeutic pharmacology; and (iii) mechanistic studies of the gut microbiome and microbiome-targeted interventions in extraintestinal pathologies. Gut-on-chips were highlighted as biomimetic systems that can replicate intestinal physiology, allowing for in vitro investigation of the bidirectional effects of the host and microbiome.

Other studies have also shown that microfluidic devices can provide a biomimetic microenvironment where cells are arranged in specific patterns and are exposed to fluidic and mechanical forces[66]. Sasaki et al. conducted a study to evaluate drug transport across Caco-2 cell layers in microfluidic devices and examined the influence of fluid flow on drug transport and metabolism[66]. The microfluidic device consisted of two blocks of polydimethylsiloxane (PDMS) and a polyethylene terephthalate membrane sandwiched in between with 3.0 μm diameter pores. Under dynamic fluidic conditions, Caco-2 cells developed microvilli and formed multilayered structures, in contrast to static conditions. The basal-to-apical transport of rhodamine 123 (a P-glycoprotein substrate) was greater than apical-to-basal transport in both Transwell and microfluidic cultures, and the presence of tariquidar (a specific P-glycoprotein inhibitor) completely eliminated the asymmetrical transport. Additionally, the researchers found that the dynamic conditions had little effect on the gene expression of transporters and metabolic enzymes. Based on these results, Sasaki and colleagues suggested the microfluidic system as a useful tool for drug transport and metabolism studies.

Shin and coworkers utilized multiple imaging techniques to characterize a novel microfluidic platform and conventional setups for culturing Caco-2 or intestinal organoid epithelial cells[67]. Their study investigated the regeneration of functional intestinal microarchitecture and in vitro morphogenesis during physiologically relevant shear stress and mechanical motions. This proposed protocol has the potential to greatly impact biomedical research, as it provides a method to regenerate in vitro 3D intestinal epithelial layers for biomedical, clinical, and pharmaceutical purposes.

Sontheimer-Phelps and her research team studied colonic mucus physiology by integrating primary human colonic epithelial cells in a microfluidic organ-on-a-chip device[68]. The Colon Chip generated a mucus layer with thickness and bilayered microstructure similar to that of the human colon (as shown in Figure 9).

Hepatic and bile-duct barriers

The blood-bile barrier (BBIB) or hepatic barrier is situated in the liver and is mainly composed of tight junctions present at the apical membrane domain of hepatocytes. This restricts the mixing of sinusoidal blood and bile. The development of physiologically relevant and broadly applicable liver cell culture platforms is crucial for drug development and disease modeling. Organ-on-a-chip systems provide a promising alternative to conventional, static 2D cultures by offering much-needed cues such as perfusion, shear stress, and 3D cell-cell communication. However, these devices vary greatly in their complexity, both in manufacturing and implementation (for a review, see Dalsbecker et al.[69]). This review article describes a comparison of different types of liver-on-a-chip devices based on their culture format: static platforms[70], gravity-driven platforms[71], pump-driven 2D platforms[72], membrane-based 2D platforms[73], 3D mass culture

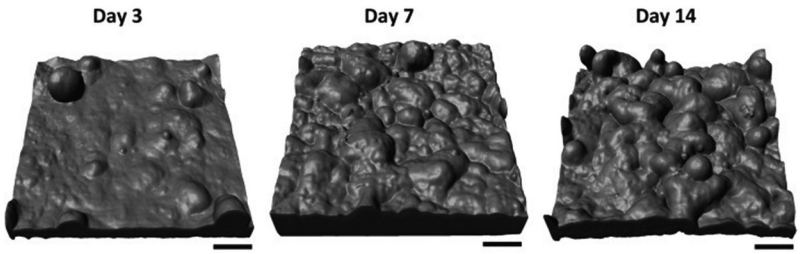

Figure 9. Epithelium development in the Colon Chip after 3, 7, and 14 days of culture after monolayer formation. Three-dimensional confocal microscopic reconstruction of z-stack images of the epithelial cells based on F-actin staining. Images are representative of two independent experiments. Scale bars: 100 µm[68].

in sinusoid mimetic[74–78], 3D mass culture in lobule mimetic[79], spheroid chip[80–82], and liver slice chip[83].

Kwon and coworkers have developed a dynamic liver acinus (LADY) chip that replicates a key functional structure of the liver acinus and hepatic zonation[84]. The LADY chip generates a gradual flow of oxygen and glucose-carrying culture medium into the HepG2 cell chamber, corresponding to the blood flow from portal triads to the central vein in vivo in the liver. They have analyzed zonal protein expression patterns in periportal zone-1 and perivenous zone-3, demonstrating liver zonation. The LADY chip could be valuable in drug development studies to examine drug-induced zonal hepatotoxicity.

Liu and colleagues successfully created bilayered microspheres from vascularized liver tissue. Their study focused on reconstructing tissue-tissue interfaces and designing a tri-vascular liver-on-a-chip (TVLOC) with a hepatic artery, portal vein, and central vein (as shown in Figure 10)[85]. The team analyzed the distribution of velocity and

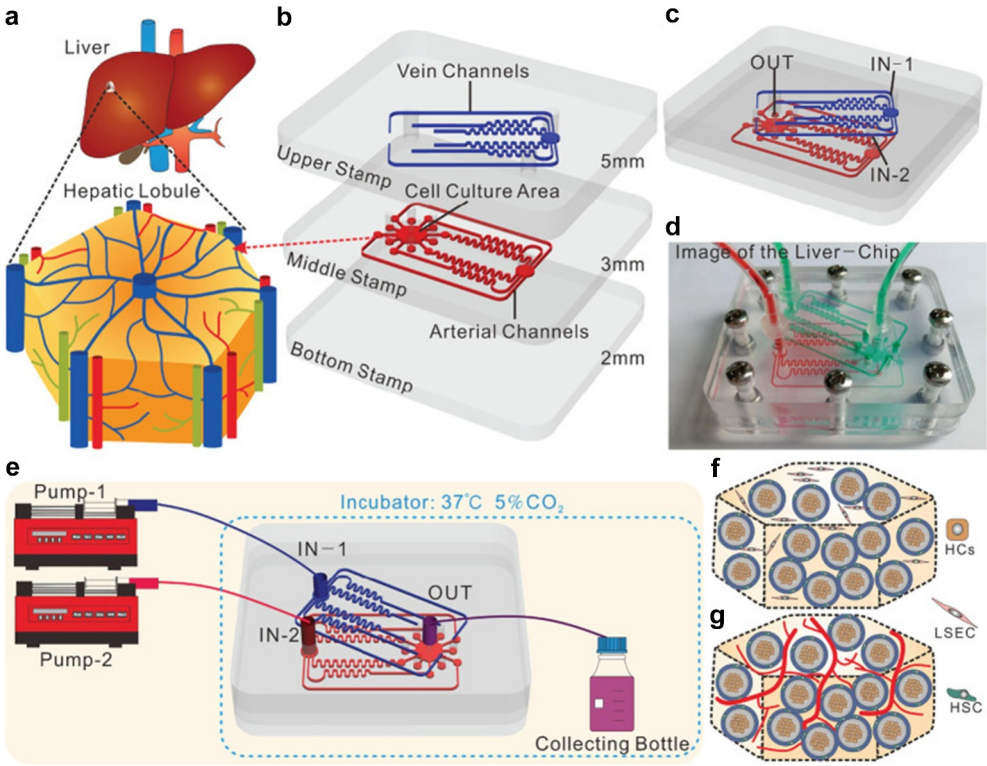

Figure 10. Design and operation of tri-vascular liver on-a-chip proposed by Liu and co-workers[85]. (a) In vivo hepatic lobulus. (b) Components of the proposed liver-on-a-chip. (c) Assembled liver-on-a-chip. (d) Photo on the assembled liver-on-a-chip. (e) Schematic of the liver-on-a-chip operating set up. (f) Early stage of hepatocytes (HCs) and liver sinusoidal endothelial cells (LSEC). (g) Formation of the vascular liver tissue.

concentration fields in the culture area, providing valuable insights into the TVLOC's functionality.

Chronic cholestatic liver diseases, such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), often lead to damage of the biliary epithelium's barrier function. To address this, Du and coworkers developed a bile duct-on-a-chip system that mimics both the 3D tubular structure of the bile duct and its barrier functions[86]. This device can be created using primary murine extrahepatic cholangiocytes, which are functionally similar to cholangiocytes in vivo, and stably express biliary markers, junctional molecules, and bile salt transport proteins, as well as developing cilia on their apical surfaces. The bile duct-on-a-chip system allows experiments to be performed using genetically modified cholangiocytes, and human cholangiocytes (including those derived from induced pluripotent stem cells) can also be studied using this microfluidic device.

The same research group developed a more advanced system called the bile duct-on-a-chip (BDOC), which mimics the open-ended 3D tubular structure of bile ducts when cultivated with either a cholangiocyte cell line or primary cells. This device exhibits a barrier function similar to bile ducts in vivo, making it an ideal in vitro platform to investigate the pathophysiology of bile ducts using cholangiocytes[87].

Renal and urinary barriers

The glomerular filtration barrier is a specialized interface for blood filtration that allows for the passage of small and mid-sized solutes in plasma while remaining relatively impermeable to macromolecules. Its integrity is maintained through a physicochemical and signaling interplay among its three primary components: the glomerular endothelial cells, the basement membrane, and the visceral epithelial cells, or podocytes[97]. Adverse drug effects can arise due to direct toxicity, which may be specific to certain organs such as the kidneys, or indirectly through effects such as vascular damage or the deposition of crystals in the kidneys. The early prediction and identification of these potential effects are essential for ensuring the safety of new drugs entering the market[88].

Human-derived kidney proximal tubule cells (HRPTECs) maintained their epithelial polarization characteristics when cultured on a kidney-on-a-chip device[89]. This dual-channel device enables drug administration in a physiologically relevant compartment. In a study by Nieskens and coworkers, they successfully replicated cell polarization-dependent cisplatin toxicity in a kidney proximal tubule-on-a-chip[89]. The use of this model in drug discovery has great potential for identifying safe and effective novel drugs, ultimately contributing to the reduction of attrition rates caused by drug-induced kidney injury.

Human-derived kidney proximal tubule cells (HRPTECs) maintained their characteristics of epithelial polarization in vitro when cultured on a kidney-on-a-chip device. This dual-channel device enabled drug administration in a physiologically relevant compartment[89]. In a study reported by Nieskens and coworkers, cell polarization-dependent cisplatin toxicity was successfully replicated in a kidney proximal tubule-on-a-chip[89]. The use of this model in drug discovery has the potential to aid in the identification of safe novel drugs, and to contribute to the reduction of attrition rates due to drug-induced kidney injury.

Human-derived kidney proximal tubule cells (HRPTECs) were injected into dual-channel Nortis chips and perfused for seven days. The expression of tight junction protein 1 (zona occludens-1), lotus lectin, and primary cilia at the apical membrane was observed, indicating an intact proximal tubule brush border. The gene expression of MATE1 (SLC47A1) and MATE2-k (SLC47A2), as well as the megalin endocytosis receptor, increased significantly in chip cultures compared to classical 2-dimensional cultures. This system can be utilized to improve preclinical prediction of drug-induced kidney toxicity and reduce kidney-related adverse effects.

Nieskens and coworkers reported on the evaluation of a recently developed human-derived kidney proximal tubule-on-a-chip for replicating SPC5001 antisense oligonucleotide-induced toxicity and a clinically relevant kidney injury biomarker response[90]. They observed a concentration-dependent release of kidney injury biomarkers, including KIM-1, NGAL, clusterin, osteopontin, and vascular endothelial growth factor (VEGF),

into the tubule perfusate, demonstrating the translational value of this kidney-on-a-chip model.

In a recent study, the renoprotective effect of adenosine was investigated in a human in vitro renal ischemia/reperfusion injury model using a coculture of a proximal tubule and blood vessel on-a-chip. This innovative platform allows for the robust and efficient evaluation of pathophysiological research and the development of novel therapeutic compounds due to its high throughput capabilities[91]. The results from this study show promise for the potential use of adenosine as a renoprotective agent and highlight the benefits of using microphysiological systems to model and study kidney injury.

Weber and coworkers also utilized a micro physiological system, the human kidney 3D Single-channel Nortis system, to investigate the toxicity and safety properties of a class of nephrotoxic antibiotics known as polymyxins[92]. The use of this system allowed for the sensitive detection of urinary biomarkers, including KIM-1 and miRNAs, which are indicative of acute kidney injury. The implementation of new technologies, such as the human kidney 3D MPS, provides a more accurate and efficient means of detecting and evaluating drug-induced kidney injury.

Kim and coworkers conducted an analysis of a three-dimensional kidney-on-a-chip model to assess the physicochemical factors of contrast media in contrast media-induced nephropathy (CIN)[93]. The novel CIN model accurately reflected in vivo situations under shear-stress conditions. The authors demonstrated the role of viscosity-induced nephrotoxicity under high-shear-stress conditions, which differed from conventional in vitro studies. They found that low osmolarity contrast media (iopromide) exhibited higher cytotoxicity than iso-osmolar contrast media (iodixanol) under physiological shear-stress conditions. However, under high-shear-stress conditions, iodixanol caused renal tubular cell damage comparable to iopromide, decreasing the urine flow rate and exposing cells to high shear-stress for a longer duration.

The mammalian bladder maintains high electrochemical gradients between urine and blood, which allows the kidney to alter body chemistries through urinary excretion. The urothelium plays a critical role in maintaining a strict permeability barrier to carry out this function. However, when this barrier is breached, urine components flow into the deeper bladder layers, leading to symptoms of cystitis.

The urothelium serves as a barrier to ions, solutes, water flow, and pathogens[94]. A microfluidic device made of PDMS and matrigel combined with extracellular matrix (ECM) was used to culture a muscle-invasive bladder cancer cell line (RT4) and a superficial human bladder carcinoma cell line, in order to better understand metastatic bladder cancer. After two weeks, observation using a confocal and fluorescent microscope revealed increased expression of the cell adhesion molecule CD44 in the RT4 cell line[95].

A PDMS microchannel with versatile capabilities was developed to facilitate precision medicine. The microchannel can accommodate four different cell types (T24 cells, macrophages (Raw 264.7), fibroblasts (BJ-5Ta), and HUVECs) in separate chambers to simulate the bladder tumor micro environment and test their response to clinical chemotherapeutic drugs (gemcitabine (G), cis-diammineplatinum dichloride (C), methotrexate (M)). Fluorescence microscopy was utilized to measure cancer cell mortality using acridine orange ethidium bromide after administering individual medications as controls. Several combinations (CMV, MVAC, and GC) were also used for comparison. The researchers reported that MVAC had a stronger impact, and that the tumor-mimicking platform exhibited high expression of the macrophage effector molecule Arg-1, which is responsible for the phenotypic change process of stromal cells[96].

Vaginal and placental barrier

Lactobacilli and other microorganisms protect the vagina from reproductive infections, premature delivery, and other negative health effects caused by a polymicrobial imbalance. However, there have been few studies conducted using human epithelial cells in a microfluidic chip containing both beneficial lactobacilli and harmful pathogens.

Human vaginal epithelial cells and human uterine stromal fibroblasts were cultured on both sides of a 50 μm thick porous membrane and housed on

a microfluidic substrate to replicate a vagina-on-a-chip with an in vivo-like microenvironment. After 72 hours of co-culturing with L. crispatus and its consortia, the proinflammatory cytokines IL-6, IL-8, IL-1α, IL-1β, and interferon-inducible protein-10 (IP-10) were downregulated. This indicates that even in the absence of immune cells, L. crispatus directly influenced the epithelium to lower the production of inflammatory cytokines. On the other hand, when co-cultured with G. vaginalis in the same environment, the vaginal chip revealed epithelial damage, a rise in pH, and elevated expressions of inflammatory cytokines[104,104].

Placental barrier dysfunction caused by infection can result in preterm birth, neonatal morbidity and mortality, fetal inflammatory response syndrome, sepsis, and other short- and long-term consequences throughout the phases of fetal development.

In order to mimic placental inflammation caused by bacteria, BeWo cells for trophoblasts and HUVECs for endothelial cells were cultured inside a microfluidic device with a membrane separating them, as illustrated in Figure 11A. Despite the pore size of the membrane being 0.4 μm, which prevents E. coli (with dimensions of 2 μm in length and 0.5 μm in width) from crossing the membrane, the bacteria sent to the mother's side cells caused cell death in both types of cells (with more HUVECs found to be dead). Quantitative real-time PCR revealed high relative mRNA expressions for the inflammatory molecules IL-1α, IL-1β, IL-6, IL-8, and TNF-α (with TNF-α being the highest). To observe the elevated levels of inflammatory molecules, E. coli was injected into BeWo cells for six hours and human macrophages (THP-1) were added and evaluated 30 minutes later using fluorescence microscopy. The study found that more THP-1 attached to the BeWo cells and that co-culture increased the expression of inflammatory markers IL-1α, IL-1β (with the highest for co-culture), IL-6, and IL-8[98]. Similar to this study, Mandt et al. developed a microstructure using two-photon polymerization, a high-resolution 3D technique[99]. They created a semipermeable barrier that allows small molecules like glucose to pass through while retaining compounds with a high molecular weight.

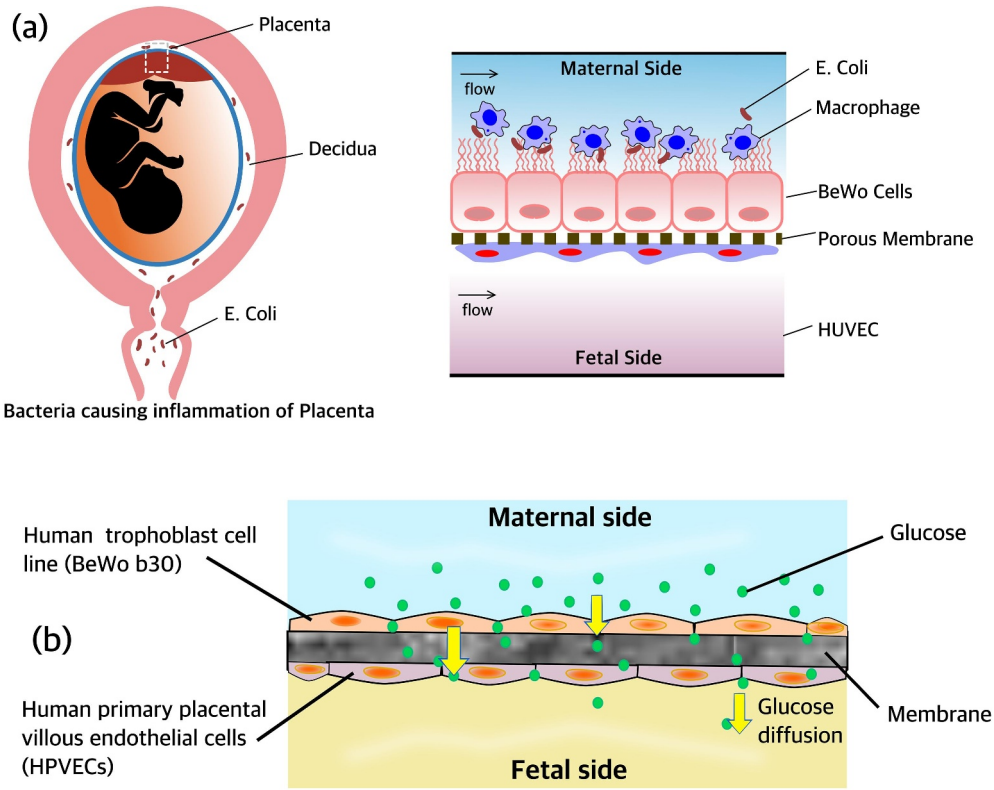

Figure 11. (a) Schematic of the placental barrier. E. coli causing inflammation of the placenta is shown.[98] (b) Sketch of the placental barrier showing the maternal and foetal side. Glucose diffusion taking place across the barrier is also shown.[100.]

Blundell and his team investigated glucose transport across the membrane in a microphysiological model of the human placental barrier using monoculture cells, co-culture cells, and a membrane without cells[100]. They evaluated glucose transport by creating a gradient of glucose concentration, where 10 mM glucose was perfused with the culture medium on the maternal side and 5.5 mM glucose was perfused on the fetal side for 2 hours, as shown in Figure 11B. Glucose transfer rates and glucose permeability coefficient studies were also conducted using vitrified membrane alone, JEG-3 monoculture (maternal side cells), HUVECs monoculture, and co-culture[101].

The complex placental responses to hazardous nanoparticles (NPs) (TiO2–50 μm) exposure were investigated in a physiological 3D microenvironment with flow between fetal and maternal cells, and extracellular matrix serving as the membrane. Oxidative stress, cell apoptosis, barrier permeability, and maternal immune cell behavior with macrophages (THP-1) were examined for both cell types. The amount of reactive oxygen species was found to be elevated at a concentration of 200 μg/ml of NPs[102].

In their review, Shojaei et al. present a comprehensive overview of placenta-on-a-chip models designed for evaluating nanoparticles to treat pregnancy-related disorders[103]. The authors highlight the benefits of these models, including their dynamic microfluidic chip design with placenta cells, which are ethical, cost-effective, repeatable, and biomimetic compared to animal models. The review provides insights into the diverse and dynamic designs of placenta-on-a-chip models for evaluating nanoparticles, emphasizing their potential in advancing research on pregnancy-related disorders[103].

Tumour barrier

One of the major challenges in cancer treatment is the blood-tumor barrier (BTB), which limits the penetration of many therapeutic agents into tumors, making it difficult to effectively treat brain metastases and other types of cancer. The BTB increases the difficulty of treating brain metastasis by preventing accumulation of chemotherapy within metastases at therapeutically effective concentrations. Traditional therapies such as surgical resection, radiotherapy, and chemotherapy have shown poor efficacy, with a low median survival rate of 5–8% after post-diagnosis. Therefore, it is of primary importance to investigate and enhance the permeability of the tumor barrier for different drugs and new drug formulations in order to improve the effectiveness of anticancer therapy[113]. There are many developments to test tumors on-a-chip with different applications. For some examples see Table 5.

A tumor-on-a-chip is a necessary tool for investigating key aspects of cancer such as cell proliferation, migration, intravasation, and angiogenesis. These activities are influenced by tumor cell interactions with interstitial flow and diffusion of morphogens to the nearby microvascular network. A wide review is made on the recent developments with microfluidics to understand the cancer microenvironemnt undergoing metastatic cascade with microscopy by Hakim et al., and Del Piccolo et al.[143,144].

To better understand cancer cell intravasation and the presence of tumor cells in the blood, a 3D model of the tumor-vascular interface was created. In this model, endothelial cells and fibrosarcoma cells (HT1080) were cultured in two parallel channels, with the ECM matrix in between. The mobility of cancer cells was compared with and without macrophages on the endothelium, and it was observed that cancer cells intravasated the HUVEC monolayer at a higher rate in the presence of macrophages. The study also looked at the effect of TNF-α on cancer cells' motility and the time it took them to traverse 60 μm in the ECM matrix[105].

In another study, researchers developed a breast cancer-on-a-chip model that replicated the microarchitecture of breast ductal carcinoma in situ (DCIS). The DCIS was embedded inside the mammary duct, with a stromal layer impregnated with breast fibroblast on the underside of the ECM membrane to mimic the vascular compartment of capillaries in mammary stroma in vivo (Figure 12). Mammary epithelial cells were cultivated on the opposite side of the membrane, and five DCIS spheroids per μl of the corresponding media were introduced into the upper channel. To test the effectiveness of paclitaxel, a 20 nM drug concentration was administered for 24 hours into the lower

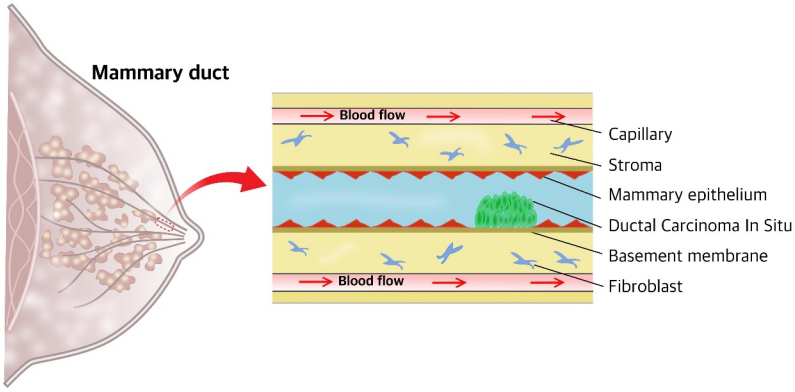

Figure 12. Schematic of a human breast cancer-on-a-chip. Also shown is the Ductal Carcinoma in situ embedded in a mammary duct consisting of the mammary epithelium and a basement membrane surrounded by stromal tissue that contains fibroblasts[106].

microchannel. The study found that the spheroids multiplied more in the absence of an anticancer medication. This platform allowed for precise parametric control of the cell type and drug concentration and helped visualize biological responses[106].

To develop precision medicine for different tumors, Carvalho and coworkers developed a colorectal tumor-on-a-chip to determine the efficacy of onco-nanomedicine. They developed microfluidic platforms to investigate progression and drug sensitivity for colorectal, breast, and lung cancer cells[107,108].

Tian's group conducted a review on various tumor-on-a-chip (TOC) devices that have been developed to assess the effectiveness of nanodrugs targeting different types of tumors[109]. The review highlighted the development of improved TOCs that more accurately mimic the in vivo environment of the tumor site, making them promising for preclinical trials. The applications of TOCs in evaluating nanoparticle (NP) delivery systems were also explored, including the effects of flow rate, Polyethylene Glycol (PEG) modification, anti-angiogenic effects, enhanced permeability and retention (EPR) effects on the endothelial and ECM barriers, active targeted drug delivery, and drug resistance. These TOC platforms have emerged as a means to overcome the limitations of past preclinical trials with other platforms that failed in clinical trials[109]. Notable highlights of the review included a heart-cancer-on-a-chip platform for measuring biomarkers after treatment with doxorubicin (DOX)[110], and a breast cancer-on-a-chip for determining the efficiency of photodynamic therapy[111]. Another review on tumor-on-a-chip reported on the multiplexed drug screening, transport and delivery of nanoparticles, and analysis of transcription[112].

The tumor microenvironment in the chip helps for the study of various applications such as testing drug efficacy to develop patient specific precision medicine, to visualize the cancer cell proliferation and intravasation rate, drug diffusion in the cancer site, cytotoxicity of drugs on the cancer cells as evident from the following table. The following table describes the different tumor-on-a-chip works carried out as alternative to animal experiments. More works related to other types of cancer is carried out and in the near future.

Multi-organ barrier

Initially, organ-on-a-chip analysis was carried out using mono-culture of cells. Subsequently, co-culture techniques were developed to create tumor-on-a-chip models, followed by multi-organ-on-a-chip systems to study tumor progression and test the efficacy of new drugs. This clinically relevant platform is highly versatile and has enabled researchers to simulate the complex metabolism of whole-body organs, allowing for a more comprehensive understanding of organ function and drug response. As multi-organ toxicity and decreased efficacy due to metabolic activity are significant challenges in drug development, the development of comprehensive multi-organ barrier models is essential for testing drug efficacy and toxicity across multiple organs. As the field continues to advance,

it is anticipated that these models will become increasingly sophisticated, enabling researchers to better simulate and study the complex physiology of human organs and the body as a whole.

Perestrelo and coworkers have compiled available literature on body-on-chips and their applications, such as ADME profiling, drug quantification in different body parts, testing drug/nanoparticle concentration and toxicity, interaction with all organ barriers, and their side effects[114]. A guide to organ-on-a-chip provides up-to-date information on mono-cell culture, co-culture, multi-organ culture, disease modeling, toxicity testing, drug bioactivation, and device fabrication techniques for all types of organ-on-a-chip[115]. Picollet-D'hahan has provided an overview of existing multi-organ-on-a-chip devices, along with integrated body-on-a-chip devices[116]. Systemic and cross-organ communication is crucial for deciphering and emulating the temporal processes involved in physiological functions[116]. Kimura and colleagues have successfully replicated microphysiological circulation and organ ratio, and evaluated the activity of anticancer drugs with liver cells (HePG2), small intestine cells (Caco-2), and lung tumor cells (A549)[117]. To evaluate cancer growth and metastasis processes in distant organs, Xu and colleagues tracked lung cancer cell metastasis to brain, bone, and liver barriers on a multi-organ-on-a-chip device in 2016[118].

Kong and his team developed a multi-organ-on-a-chip using PDMS to track breast tumor cell preference for bone, lung, muscle, and liver with chemokine stimulation[119]. They reported that breast tumor cells showed metastatic potential and preferred to migrate to lung, liver, bone, and then muscle[119]. Satoh and his team emulated the processes of absorption in the intestinal barrier, metabolism on the liver barrier, and cell killing for tumor cells and connective tissues to evaluate the efficacy of an anticancer drug[120]. They developed a PDMS microfluidic platform with a PC membrane to house Caco-2 cells, HepaRG cells, HCT-116 cells, and TIG-121 connective tissue cells[120]. Edington established a multi-organ-on-a-chip platform with 4, 7, and 10 organ models, similar to a physiome-on-a-chip, for quantitative pharmacological studies[121]. Other multi-organ-on-a-chip platforms have been developed to study reproductive medicine and metabolic multi-organ diseases with a microfluidic motherboard for the evaluation of ovarian hormone control of downstream human female reproductive tract and liver-peripheral tissues[122], and a model for type II diabetes with high glucose stimulation, which releases insulin and enhances glucose uptake in the presence of insulin[123].

In the event of human organ diseases, drug treatment becomes challenging due to strong barriers that prevent drugs from passing through and reaching the site of the disease. The effectiveness of a drug on a diseased organ is determined by its concentration at the site. To predict the impact of drugs, it is essential to have quantitative information on drug concentration. The blood-brain barrier, for instance, poses a challenge to the treatment of brain cancer as it is a strong barrier to drug entry. In the case of blood-brain barrier studies in LOC devices, injecting drugs of different concentrations into the microchannels results in physical phenomena such as fluid flow and mass transfer. However, it is difficult to experimentally measure fluid velocities or drug concentrations across any barrier in in vitro studies due to small channel sizes and complex geometries in many cases. Therefore, a noninvasive technique that can provide all necessary information at the desired location and time with greater ease is needed.

Computational fluid dynamics in the study of biological barriers

The use of mathematical models and appropriate numerical techniques to solve microfluidic flow models is a widely used method for obtaining physical variables. Mathematical modeling and simulations can provide quantitative data on flow velocities, mass concentration, and other variables at any point within the microchannel of a LOC device. Compared to experiments, mathematical modeling and simulations have several advantages, such as faster results, lower costs, greater understanding of problems, and the ability to simulate actual conditions. However, mathematical modeling and simulations have their own disadvantages. Numerical errors are inherent in numerical solutions, and the accuracy of numerical solutions depends entirely on the mathematical model of real-world processes. One must have a proper mathematical model to

make accurate predictions. For example, suppose one wants to model and simulate the flow through porous tissues to obtain flow velocities at any location. In that case, it is essential to know the values of porosity and permeability of the tissues to predict flow velocities accurately. These values are usually determined through experiments, and if they are unknown or not measured accurately, the resulting numerical solution will not match experimental results. Despite these drawbacks, mathematical modeling and simulations have gained popularity due to the aforementioned advantages.

The following paragraph discusses mathematical modeling of flow and mass transfer in biological barriers and computational fluid dynamics (CFD) techniques for solving governing equations of fluid flow or mass transfer. We also review related studies on biological barriers conducted using CFD techniques.

Mathematical modeling involves the use of differential equations to describe physical phenomena such as fluid flow, heat transfer, mass transfer, or chemical reactions taking place in any domain of interest. For example, the continuity and Navier-Stokes equations govern fluid flow in the region of the microchannel where clear fluid flows in a LOC device comprising a porous layer sandwiched between two layers of channels (refer to Figure 13)[149].

The governing equations are:

$$\Delta.v = 0 \tag{1}$$

$$\rho\left[\frac{\partial v}{\partial t} + v.\nabla v\right] = -\nabla P + \mu\nabla^2 v + \rho g \tag{2}$$

Here, ρ is the density of the fluids, v is the velocity vector, t is the time, P is the pressure, μ is the fluid viscosity, and g is the acceleration due to gravity.

For the porous region (such as the porous substrate where cells are placed), the Darcy's law, which is widely used for flow in porous media is applicable and is given by[150]:

$$v = -\frac{K}{\mu}\nabla P \tag{3}$$

Here, K is the permeability of the porous media and is related to its porosity as

$$K = \frac{d_p^2 \in^3}{180(1- \in)^2}, \tag{4}$$

where, d_p is the particle diameter, ϵ is the porosity of the medium. An extension to the Darcy's equation is the Brinkman equation that accounts for transitional flow between boundaries, given by[150]:

$$\nabla P = -\frac{\mu}{K}v + \underline{\mu}\nabla^2 v. \tag{5}$$

Here, the coefficient μ˜ is the effective viscosity.

The mass transfer equation for the transport of drugs, glucose, oxygen, or any other component is given by[149]:

$$\frac{\nabla C}{\nabla t} + u.\nabla C = D\nabla^2 C, \tag{6}$$

where, C is the concentration and D is the diffusion coefficient. In Eqn (1) - (6), ρ, μ, ϵ, K and D are experimentally obtained quantities.

For simple geometries such as a straight microchannel, analytical solutions can be obtained by simplifying the governing equations. For example, the Navier-Stokes equations can be simplified for one-dimensional flow in a channel, allowing for analytical solutions for flow velocities and concentration to be derived. However, analytical solutions are not possible for complex, two- or three-dimensional geometries, such as LOC devices with channels of varying cross-section. In such cases, numerical simulations using computer methods are required. Moreover, the highly non-linear nature of the above-mentioned equations necessitates an iterative solution, making manual computations impractical. In the following section, we will provide a brief overview of computational fluid dynamics.

Computational fluid dynamics (CFD) is a subfield of fluid mechanics that employs computers to solve problems related to fluid flow, heat and mass transfer, chemical processes, and more. The CFD method involves the creation of a CAD model, the selection of appropriate governing equations, their solution, and post-processing. To determine variables such as flow velocity or concentration distribution in a microfluidic chip, the following steps are taken:

- A computer model of the microchannel is created.
- The geometry is then divided into several cells/volumes, a process known as meshing.
- A suitable mathematical equation, called the mathematical model, is selected for each cell/volume to represent the physical phenomena of fluid flow.
- The discretized equations are then solved using a computer.
- The resultant pressure, velocity, temperature, or concentration measurements are displayed and evaluated in a technique known as post-processing.

Figure 13 illustrates the CFD simulation process, which enables the theoretical simulation of physical phenomena. CFD simulations are advantageous compared to experiments because they require a shorter execution time and enable flow visualization in locations where experimental visualization is impossible. Additionally, CFD simulations are relatively cost-effective, as they only require computers and software, unlike expensive experimental

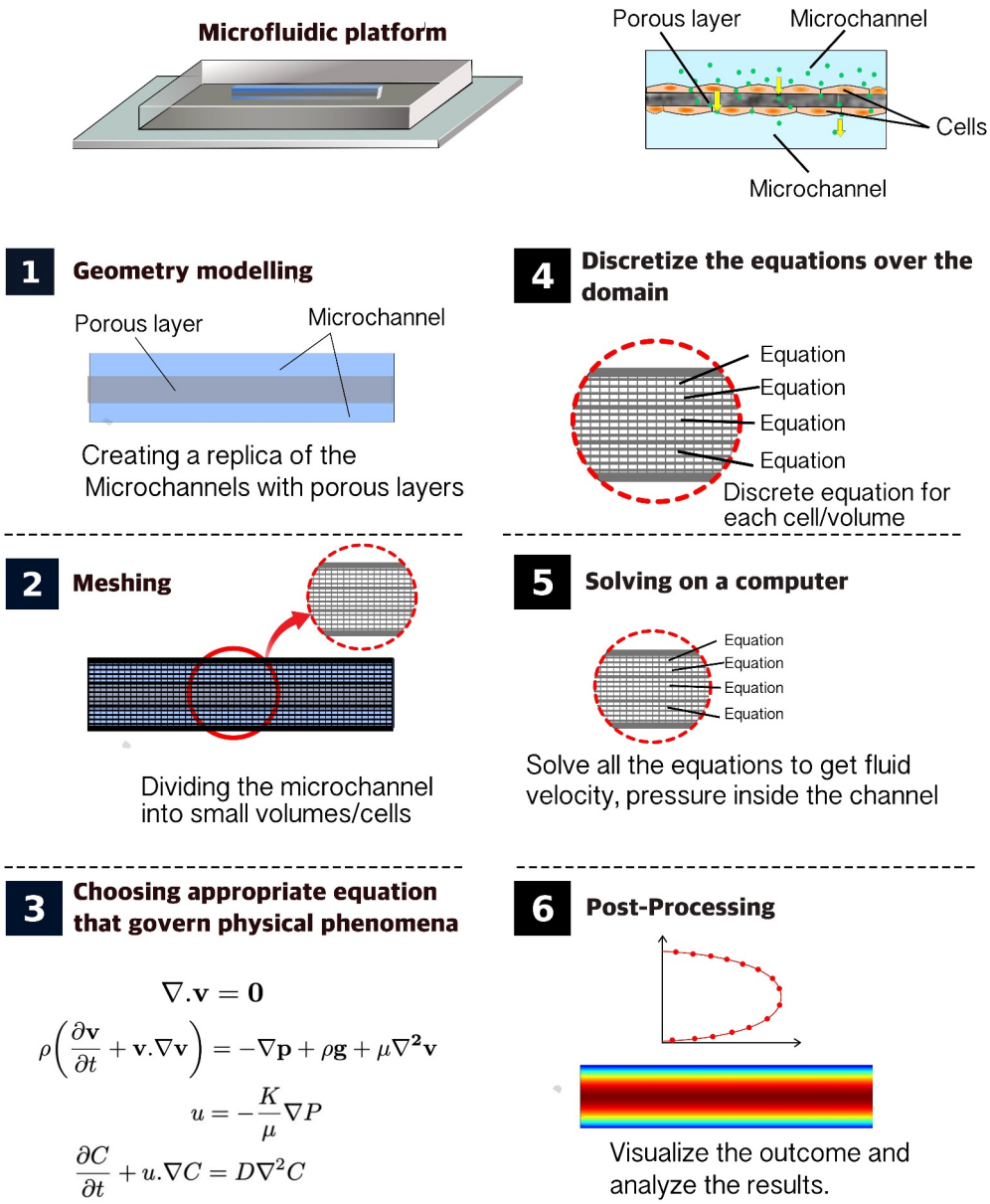

Figure 13. Steps involved in a Computational Fluid Dynamics simulation in a microfluidic chip with channels above and below a porous layer. The top-right figure illustrates the diffusion of glucose or any other substance in a porous layer containing a layer of cells.

setups. In the field of biomedical engineering, CFD is a powerful technology that is becoming increasingly prevalent, with applications including the study of drug delivery, nasal airflow, arterial blood flows, and blood flow in stented blood arteries, among others. In microfluidic Lab-on-a-Chip devices, CFD is primarily utilized for microfluidic device optimization, mixing, and drug diffusion. Despite numerous experimental investigations on biological barriers, there is a lack of computational fluid dynamics studies. In the next paragraph, we will review studies involving CFD in various biological barriers.

Fluid mechanics plays a crucial role in the endothelial microenvironment, where endothelial cells are subjected to various flow fields, such as unsteady, pulsatile, or waveform flow types in the carotid artery. In vitro models, like microfluidic chips, can apply these flow types to study the cells' response to 3D flow fields. Bouhrira et al. performed an experimental and computational study of fluid flow in a 3D microfluidic model of a blood-brain barrier to analyze the velocity field and shear stress caused by flow separation, using Star-CCM+ software for the simulations[151]. They identified the location of the stagnation point for separated flow from the calculated shear stress profiles and measured the velocity field using μPIV experiments, which closely agreed with the CFD predictions.

Lee et al. developed a bladder cancer model using a multilayered tissue-on-a-chip device (MToC) for their study of Bacillus Calmette – Guérin immunotherapy[152]. The authors conducted both experimental and computational investigations, solving the continuity and Navier-Stokes equations for the 3D steady-state laminar flow in the MToC device. Nutrient-containing fluids were allowed to flow through the chip while waste products were drained. However, excessive flow velocity could cause physical stress on the cells, so the authors used CFD simulations to determine the optimal flow rates that correspond to shear stress in the device but do not adversely affect the cells in the MToC device. The calculated results revealed the optimal flow rates for cell growth.

In the realm of drug transport or the transport of other substances through a placental barrier, Mosavati et al. devised a 3D placenta-on-a-chip model[153]. The team created a placental interface between fetal and maternal blood within a microfluidic chip by culturing human umbilical vein endothelial cells and trophoblast cells on the top and bottom of a porous polycarbonate membrane with a porosity of $\epsilon = 0.01$ as specified by the manufacturer. Employing CFD simulations, the authors computed the flow field and glucose concentration distribution. They utilized the Navier-Stokes equation (Eqn. 1 and 2) for the unobstructed flow regions and the Darcy model (Eqn. 5) for the porous membranes.

In the context of treating eye-related diseases, such as diabetic retinopathy or other illnesses or infections, Davies et al. conducted a study involving both experiments and computations to investigate drug delivery across the retinal barrier[154]. The authors examined the transport of dextran and ibuprofen from silicone oil over the retinal barrier and validated their mathematical flow and mass transfer model using the Brinkmann model (Eqn. 5) with measured permeability and diffusion coefficients from static experiments. The authors confirmed their numerical model with experimental investigations, where the drug concentration profiles were observed to be within 5% and 18% for acellular and cellular membranes, respectively. Numerous other studies involving CFD simulations of flow and mass transfer across biological barriers exist in the literature, but for brevity, they are not presented here. Table 6 provides an overview of the CFD simulations conducted for different barriers.

Summary and outlook

The human body is equipped with a range of physiological barriers that act as a defense mechanism against diverse stimuli. A comprehensive review has been carried out on the numerous Lab-on-a-Chip (LOC) devices that are currently available for investigating biological barriers in both healthy and pathological states. These devices enable researchers to acquire valuable micro-scale data and obtain real-time visualization of various parameters related to the barrier function.

The lab-on-a-chip (LOC) technology is versatile and can address unmet needs without the use of human or animal models. This review discusses 15 different physiological barriers that can be studied

Table 6. Overview of CFD analysis carried out for different biological barrier.

Objective	Barrier	CFD Analysis	Reference
To investigate the effect of disturbed flow on the integrity of the BBB using a 3D, perfusable bifurcation model consisting of a co-culture of endothelial cells with mural and glial cells.	Blood-brain	Flow field in 3D vessels patterned inside microchannels. Helped in choosing the flow rate for different geometries of microchannel to create a reproducible flow rate	[151]
To develop a multilayered Tissue-on-a-Chip (MToC) to Simulate Bacillus Calmette–Guérin (BCG) Immunotherapy for Bladder Cancer Treatment	Bladder	CFD was used to build a microfluidic device and flow analysis was performed to determine the optimal flow rate that creates shear stress levels that do not harm the cells.	[152]
To develop organ-on-a-chip device to study placental pathologies	Placental	CFD simulations were carried out to calculate flow field and glucose concentration distribution. The authors used the Navier-Stokes equation for the clear flow region and the Darcy model for porous membrane	[153]
To study the drug transport of silicon oil across outer blood-retinal barrier	Blood-Retinal	Continuity, Navier-Stokes, and the species transport equations were solved in a cell culture chamber. The Brinkman equations were solved for the porous matrix. Flow field in the chamber as well as Dextran transport across the barrier was investigated.	[154]
To investigate the drug transport across *in vitro* cornea	Corneal	Transcorneal drug permeation in a rabbit cornea model was studied. The continuity, momentum and energy equations were solved.	[128]
To design biomimetic airway models of the epithelial barrier	Lung	CFD simulations were performed to design microfluidic channels.	[129]
To develop an *in vitro* 3D liver chip model to study the hepatic-cellular interactions in micro-environment	Hepatic	CFD simulations of shear flow past hepatic cells placed over porous substrate sandwiched between microchannels were studied. Darcy law is used to model the flow in porous substrates.	[155]

using LOC microfluidic systems. The review covers topics such as physiological functions, transport mechanisms, drug delivery, cancer cell progression, and toxicity on healthy and diseased cells. The review also discusses co-culturing multiple cell types in the same device and the use of various membranes to simulate multi-organ-on-a-chip systems. As precision medicine gains traction, there is an increasing interest in developing human-on-a-chip systems to study the effects of novel drugs on multiple organs. This review can guide a multidisciplinary team in developing such a device by simulating transport processes between different organ barriers and monitoring drug levels absorbed in different organs. Mathematical modeling and simulation of fluidic processes can also contribute to the development of more sophisticated and relevant microfluidic systems, which can help in optimizing and expediting the testing of new drug candidates without *in vivo* experiments.

Recent advancements in technology have made modern microfluidic systems more accessible to researchers across various fields of biotechnology. These systems are characterized by high-throughput analysis, portability, and parallelization, providing several advantages over traditional methods. Microfluidic devices are capable of reducing chemical and sample consumption, improving energy and mass transfer, and considering the dynamic flow and shear stress within tissues. Furthermore, these devices have a relatively small size, making them versatile and easily integrated into existing lab setups. Consequently, microfluidic systems have the potential to revolutionize every sub-field of biotechnology, including healthcare, pharmaceuticals, and industry.

Microfluidic devices are widely utilized in various sub-fields of biotechnology, which are categorized based on their applications. Red biotechnology primarily focuses on pharmaceutical and medicinal use, while green biotechnology is used in agriculture. Yellow biotechnology is applied in food science, white biotechnology finds industrial use, and blue biotechnology is utilized in marine and freshwater applications[156–158]. The physiological barriers within microfluidic chips belong to the red biotechnology sub-field, although other sub-fields can also benefit from the use of microfluidic systems as organ-on-a-chips. Unfortunately, most published microfluidic barrier-on-a-chip systems remain in the proof-of-concept phase and are not yet established commercial tools. The lack of standardization and difficulties with sensor miniaturization pose challenges, along with the complexity of

manufacturing and parallel cell culturing. Additionally, a lack of appropriate bio-analytical methods to accompany sample collection and evaluate transport and drug delivery studies in real-time presents further challenges. Overcoming these obstacles will be critical to realizing the full potential of microfluidic devices in biotechnology.

Measuring or visualizing transport phenomena across layers of cells or tissues grown inside LOC lab-on-a-chip devices can sometimes be impractical. Computational simulations of transport phenomena can aid in understanding fluid flow and mass transfer processes along the tortuous paths between cells or through tissues. This review also briefly reviews Computational Fluid Dynamics (CFD) and outlines the steps involved in simulating flow and mass transfer in a LOC device using this methodology. In a barrier-on-a-chip device, a cell layer grows on the surface of a substrate, and flow across this substrate resembles flow in a medium. The Darcy and Brinkman models are fundamental porous media models that can be used to calculate flow velocities and mass transfer, such as drug concentration, across layers. CFD is a versatile methodology that is gaining popularity across various disciplines. It is anticipated that experimentalists, such as clinicians and biologists, developing LOC devices will adopt this method or collaborate with engineers to gain a deeper understanding of transport processes in barrier-on-a-chip systems and optimize their experiments.

While organ-on-a-chip systems have already provided several important and valuable results, this review has also revealed several technical issues that must still be addressed. To ensure the continued progress of barrier studies on lab-on-a-chip devices, close collaboration between experts from different disciplines and research groups is fundamentally important. Such collaboration will facilitate the adaptation and refinement of already published systems, as well as the development of more physiologically relevant, easy-to-fabricate, and user-friendly models of tissues, organs, or perhaps even entire bodies in the future. The success of these efforts will depend on effective communication and cooperation between researchers, and on the application of cutting-edge technologies and techniques in the field. By working together, experts can overcome the technical challenges facing organ-on-a-chip systems and unlock the full potential of these innovative tools for advancing biotechnology and medical research.

Acknowledgments

This work was supported by the Ministry of Innovation and Technology, Hungary with support from the National Research Development and Innovation Fund under the TKP2021 programme through grant no. TKP2021-EGA-42; Science and Engineering Research Board (SERB), Government of India, under grant no. CRG/2021/008464. The authors are grateful to Barnabás Bánfi for editing the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Ministry of Innovation and Technology, Hungary with support from the National Research Development and Innovation Fund under the TKP2021 programme through grant no. TKP2021-EGA-42 and Science and Engineering Research Board (SERB), Government of India, under grant no. CRG/2021/008464.

ORCID

Franciska Erdő http://orcid.org/0000-0001-6265-3777

Author contributions

Conceptualization, writing original draft: F.E., J.P., S.D., D.K., K.I.; funding acquisition K.I. (Hungary), J.P. & S.D (India). All authors have read and agreed to the published version of the manuscript.

References

1. Castillo-León J. Microfluidics and Lab-on-a-Chip Devices: history and Challenges. In *Lab-on-a-Chip Devices and Micro-Total Analysis Systems: a Practical Guide*. Castillo-León, J., Svendsen, W.E. Eds. Springer International Publishing : Cham. 2015pp. 1–15. ISBN 9783. ISBN 9783319086873. 319086873. 10.1007/978-3-319-08687-3_1
2. Harrison DJ, Fluri K, Seiler K, Fan Z, Effenhauser CS, Manz A. Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a

chip. Science. 1993;261(5123):895–897. doi:10.1126/science.261.5123.895.
3. Duffy DC, McDonald JC, Schueller OJA, Whitesides GM. Rapid prototyping of microfluidic systems in Poly(Dimethylsiloxane). Anal Chem. 1998;70(23):4974–4984. doi:10.1021/ac980656z.
4. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. Science. 2010;328(5986):1662–1668. doi:10.1126/science.1188302.
5. Abu-Dawas S, Alawami H, Zourob M, Ramadan Q. Design and fabrication of low-cost microfluidic chips and microfluidic routing system for reconfigurable multi-(organ-on-a-chip) assembly. Micromachines. 2021;12(12):1542. doi:10.3390/mi12121542.
6. Danku AE, Dulf E-H, Braicu C, Jurj A, Berindan-Neagoe I. Organ-on-A-Chip: a survey of technical results and problems. *Front Bioeng Biotechnol*. 2022;10:10. doi:10.3389/fbioe.2022.840674.
7. Mauriac H, Pannetier C, Casquillas GV. Organs on chip review. Elveflow. 2020. [accessed 2023 June 08].https://www.elveflow.com/microfluidic-reviews/organs-on-chip-3d-cell-culture/organs-chip-review/
8. Bein A, Shin W, Jalili-Firoozinezhad S, Park MH, Sontheimer-Phelps A, Tovaglieri A, Chalkiadaki A, Kim HJ, Ingber DE. Microfluidic organ-on-a-chip models of human intestine. Cell Mol Gastroenterol Hepatol. 2018;5(4):659–668. doi:10.1016/j.jcmgh.2017.12.010.
9. Sontheimer-Phelps A, Hassell BA, Ingber DE. Modelling cancer in microfluidic human organs-on-chips. Nat Rev Cancer. 2019;19(2):65–81. doi:10.1038/s41568-018-0104-6.
10. Bajza Á, Kocsis D, Berezvai O, Laki AJ, Lukács B, Imre T, Iván K, Szabó P, Erdő F Verification of P-Glycoprotein function at the dermal barrier in diffusion cells and dynamic "skin-on-a-chip" microfluidic device. *Pharmaceutics* 2020, *12*, E804, doi:10.3390/pharmaceutics12090804.
11. Jiang L, Li Q, Liang W, Du X, Yang Y, Zhang Z, Xu L, Zhang J, Li J, Chen Z, et al. Organ-on-A-Chip database revealed—achieving the human avatar in silicon. Bioengineering. 2022;9(11):685. doi:10.3390/bioengineering9110685.
12. Wang YI, Carmona C, Hickman JJ, Shuler ML. Multi-organ microphysiological systems for drug development: strategies, advances and challenges. Adv Healthcare Mater. 2018;7(2):1701000. doi:10.1002/adhm.201701000.
13. Lind JU, Busbee TA, Valentine AD, Pasqualini FS, Yuan H, Yadid M, Park S-J, Kotikian A, Nesmith AP, Campbell PH, et al. Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing. Nat Mater. 2017;16(3):303–308. doi:10.1038/nmat4782.
14. Ellis BW, Acun A, Can UI, Zorlutuna P. Human IPSC-Derived myocardium-on-chip with capillary-like flow for personalized medicine. Biomicrofluidics. 2017;11(2):024105. doi:10.1063/1.4978468.
15. Kanamori T, Sugiura S, Sakai Y. Technical aspects of microphysiological systems (MPS) as a promising wet human-in-vivo simulator. Drug Metab Pharmacokinet. 2018;33(1):40–42. doi:10.1016/j.dmpk.2017.11.006.
16. Lee SH, Jun B-H. Advances in dynamic microphysiological organ-on-a-chip: design principle and its biomedical application. J Ind Eng Chem. 2019;71:65–77. doi:10.1016/j.jiec.2018.11.041.
17. Cameron T, Bennet T, Rowe EM, Anwer M, Wellington CL, Cheung KC. Review of design considerations for brain-on-a-chip models. Micro (Basel). 2021;12(4):441. doi:10.3390/mi12040441.
18. Katt ME, Shusta EV. In Vitro models of the blood-brain barrier: building in physiological complexity. Curr Opin Chem Eng. 2020;30:42–52. doi:10.1016/j.coche.2020.07.002.
19. Yoon J-K, Kim J, Shah Z, Awasthi A, Mahajan A, Kim Y. Advanced human BBB-on-a-Chip: a new platform for alzheimer's disease studies. Adv Healthcare Mater. 2021;10:e2002285. doi:10.1002/adhm.202002285.
20. Yi H-G, Jeong YH, Kim Y, Choi Y-J, Moon HE, Park SH, Kang KS, Bae M, Jang J, Youn H, et al. A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. Nat Biomed Eng. 2019;3(7):509–519. doi:10.1038/s41551-019-0363-x.
21. Wevers NR, Nair AL, Fowke TM, Pontier M, Kasi DG, Spijkers XM, Hallard C, Rabussier, van Vught, Vulto, et al. Modeling ischemic stroke in a triculture neurovascular unit on-a-chip. Fluids Barriers CNS. 2021;18(1):NA–NA. doi:10.1186/s12987-021-00294-9.
22. Kim J, Lee K-T, Lee JS, Shin J, Cui B, Yang K, Choi YS, Choi N, Lee SH, Lee J-H, et al. Fungal brain infection modelled in a human-neurovascular-unit-on-a-chip with a functional blood–brain barrier. Nat Biomed Eng. 2021;5(8):830–846. doi:10.1038/s41551-021-00743-8.
23. Buzhdygan TP, DeOre BJ, Baldwin-Leclair A, Bullock TA, McGary HM, Khan JA, Razmpour R, Hale JF, Galie PA, Potula R, et al. The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in-vitro models of the human blood–brain barrier. Neurobiol Dis. 2020;146:105131. doi:10.1016/j.nbd.2020.105131.
24. Ragelle H, Goncalves A, Kustermann S, Antonetti DA, Jayagopal A. Organ-on-A-Chip technologies for advanced blood–retinal barrier models. J Ocul Pharmacol Ther. 2020;36(1):30–41. doi:10.1089/jop.2019.0017.
25. B Arık Y, Buijsman W, Loessberg-Zahl J, Cuartas-Vélez C, Veenstra C, Logtenberg S, M Grobbink A, Bergveld P, Gagliardi G, den Hollander AI, et al. Microfluidic Organ-on-a-Chip model of the outer blood–retinal barrier with clinically relevant read-outs

for tissue permeability and vascular structure. *Lab Chip*. 2021;21(2):272–283. doi:10.1039/D0LC00639D.
26. Chen L-J, Raut B, Nagai N, Abe T, Kaji H. Prototyping a versatile two-layer multi-channel microfluidic device for direct-contact cell-vessel co-culture. Micro (Basel). 2020;11(1):79. doi:10.3390/mi11010079.
27. Yeste J, García-Ramírez M, Illa X, Guimerà A, Hernández C, Simó R, Villa R. A compartmentalized microfluidic chip with crisscross microgrooves and electrophysiological electrodes for modeling the blood–retinal barrier. Lab Chip. 2017;18(1):95–105. doi:10.1039/C7LC00795G.
28. Chung M, Lee S, Lee BJ, Son K, Jeon NL, Kim JH. Wet-AMD on a chip: modeling outer blood-retinal barrier in vitro. Adv Healthcare Mater. 2018;7(2):7. doi:10.1002/adhm.201700028.
29. Yu Z, Hao R, Du J, Wu X, Chen X, Zhang Y, Li W, Gu Z, Yang H. A human cornea-on-a-chip for the study of epithelial wound healing by extracellular vesicles. iScience. 2022;25(5):104200. doi:10.1016/j.isci.2022.104200.
30. Mun EA, Morrison PWJ, Williams AC, Khutoryanskiy VV. On the barrier properties of the cornea: a microscopy study of the penetration of fluorescently labeled nanoparticles, polymers, and sodium fluorescein. Mol Pharmaceutics. 2014;11(10):3556–3564. doi:10.1021/mp500332m.
31. Abdalkader R, Chaleckis R, Wheelock CE, Kamei K. Spatiotemporal determination of metabolite activities in the corneal epithelium on a chip. Exp Eye Res. 2021;209:108646. doi:10.1016/j.exer.2021.108646.
32. Abdalkader R, Kamei K. Multi-corneal barrier-on-a-chip to recapitulate eye blinking shear stress forces. Lab Chip. 2020;20(8):1410–1417. doi:10.1039/C9LC01256G.
33. Cohen N, Vagima Y, Mouhadeb O, Toister E, Gutman H, Lazar S, Jayson A, Artzy-Schnirman A, Sznitman J, Ordentlich A, et al. PEG-Fibrinogen hydrogel microspheres as a scaffold for therapeutic delivery of immune cells. Front Bioeng Biotechnol. 2022;10:905557. doi:10.3389/fbioe.2022.905557.
34. Byun J, Song B, Lee K, Kim B, Hwang HW, Ok M-R, Jeon H, Lee K, Baek S-K, Kim S-H, et al. Identification of urban particulate matter-induced disruption of human respiratory mucosa integrity using whole transcriptome analysis and organ-on-a chip. J Biol Eng. 2019;13(1):88. doi:10.1186/s13036-019-0219-7.
35. Zhu Y, Sun L, Wang Y, Cai L, Zhang Z, Shang Y, Zhao Y. A biomimetic human lung-on-a-chip with colorful display of microphysiological breath. Adv Mater. 2022;34(13):2108972. doi:10.1002/adma.202108972.
36. Francis I, Shrestha J, Paudel KR, Hansbro PM, Warkiani ME, Saha SC. Recent advances in lung-on-a-chip models. Drug Discov Today. 2022;27(9):2593–2602. doi:10.1016/j.drudis.2022.06.004.
37. Felder M, Trueeb B, Stucki AO, Borcard S, Stucki JD, Schnyder B, Geiser T, Guenat OT. Impaired wound healing of alveolar lung epithelial cells in a breathing Lung-On-A-Chip. *Front Bioeng Biotechnol*. 2019;7:7. doi:10.3389/fbioe.2019.00003.
38. Benam KH, Villenave R, Lucchesi C, Varone A, Hubeau C, Lee H-H, Alves SE, Salmon M, Ferrante TC, Weaver JC, et al. Small Airway-on-a-Chip enables analysis of human lung inflammation and drug responses in vitro. Nat Methods. 2016;13(2):151–157. doi:10.1038/nmeth.3697.
39. Nesmith AP, Agarwal A, McCain ML, Parker KK. Human airway musculature on a Chip: an in vitro model of allergic asthmatic bronchoconstriction and bronchodilation. Lab Chip. 2014;14(20):3925–3936. doi:10.1039/c4lc00688g.
40. Hassell BA, Goyal G, Lee E, Sontheimer-Phelps A, Levy O, Chen CS, Ingber DE. Human organ chip models recapitulate orthotopic lung cancer growth, therapeutic responses, and tumor dormancy in vitro. Cell Rep. 2017;21(2):508–516. doi:10.1016/j.celrep.2017.09.043.
41. Khalid MAU, Kim YS, Ali M, Lee BG, Cho YJ, Choi KH. A lung Cancer-on-Chip platform with integrated biosensors for physiological monitoring and toxicity assessment. Biochem Eng J. 2020;155:107469. doi:10.1016/j.bej.2019.107469.
42. Xu Z, Gao Y, Hao Y, Li E, Wang Y, Zhang J, Wang W, Gao Z, Wang Q. Application of a microfluidic chip-based 3D Co-Culture to test drug sensitivity for individualized treatment of lung cancer. Biomaterials. 2013;34(16):4109–4117. doi:10.1016/j.biomaterials.2013.02.045.
43. Barkal LJ, Procknow CL, Álvarez-García YR, Niu M, Jiménez-Torres JA, Brockman-Schneider RA, Gern JE, Denlinger LC, Theberge AB, Keller NP, et al. Microbial volatile communication in human organotypic lung models. Nat Commun. 2017;8(1):1770. doi:10.1038/s41467-017-01985-4.
44. Asmani M, Velumani S, Li Y, Wawrzyniak N, Hsia I, Chen Z, Hinz B, Zhao R. Fibrotic microtissue array to predict anti-fibrosis drug efficacy. Nat Commun. 2018;9(1):2066. doi:10.1038/s41467-018-04336-z.
45. Si L, Bai H, Rodas M, Cao W, Oh CY, Jiang A, Moller R, Hoagland D, Oishi K, Horiuchi S, et al. A human-airway-on-a-chip for the rapid identification of candidate antiviral therapeutics and prophylactics. Nat Biomed Eng. 2021;5(8):815–829. doi:10.1038/s41551-021-00718-9.
46. Zhang M, Wang P, Luo R, Wang Y, Li Z, Guo Y, Yao Y, Li M, Tao T, Chen W, et al. Biomimetic human disease Model of SARS-CoV-2-induced lung injury and immune responses on organ chip system. Adv Sci. 2021;8(3):2002928. doi:10.1002/advs.202002928.
47. Tang H, Abouleila Y, Si L, Ortega-Prieto AM, Mummery CL, Ingber DE, Mashaghi A. Human Organs-on-Chips for virology. Trends Microbiol. 2020;28(11):934–946. doi:10.1016/j.tim.2020.06.005.

48. Thacker VV, Dhar N, Sharma K, Barrile R, Karalis K, McKinney JD A Lung-on-Chip infection model reveals protective and permissive roles of alveolar epithelial cells in tuberculosis 2020, 2020.02.03.931170.
49. Deinhardt-Emmer S, Rennert K, Schicke E, Cseresnyés Z, Windolph M, Nietzsche S, Heller R, Siwczak F, Haupt KF, Carlstedt S, et al. Co-infection with Staphylococcus Aureus After Primary Influenza Virus Infection Leads To Damage Of The Endothelium In A Human Alveolus-on-a-Chip Model. Biofabrication. 2020;12(2):025012. doi:10.1088/1758-5090/ab7073.
50. Huh D, Leslie DC, Matthews BD, Fraser JP, Jurek S, Hamilton GA, Thorneloe KS, McAlexander MA, Ingber DE. A human disease model of drug toxicity–induced pulmonary edema in a lung-on-a-chip microdevice. Sci Transl Med. 2012;4(159):159ra147. doi:10.1126/scitranslmed.3004249.
51. Jain A, Barrile R, van der Meer AD, Mammoto A, Mammoto T, De Ceunynck K, Aisiku O, Otieno MA, Louden CS, Hamilton GA, et al. Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. Clin Pharmacol Ther. 2018;103:332–340. doi:10.1002/cpt.742.
52. Filaire E, Nachat-Kappes R, Laporte C, Harmand M-F, Simon M, Poinsot C. Alternative in vitro models used in the main safety tests of cosmetic products and new challenges. Int J Cosmet Sci. 2022;44(6):604–613. doi:10.1111/ics.12803.
53. Alberti M, Dancik Y, Sriram G, Wu B, Teo YL, Feng Z, Bigliardi-Qi M, Wu RG, Wang ZP, Bigliardi PL. Multichamber microfluidic platform for high-precision skin permeation testing. Lab Chip. 2017;17(9):1625–1634. doi:10.1039/C6LC01574C.
54. Lukács B, Bajza Á, Kocsis D, Csorba A, Antal I, Iván K, Laki AJ, Erdő F. Skin-on-a-Chip device for ex vivo monitoring of transdermal delivery of drugs—design, fabrication, and testing. Pharmaceutics. 2019;11(9):E445. doi:10.3390/pharmaceutics11090445.
55. Jones CFE, Di Cio S, Connelly JT, Gautrot JE. Design of an Integrated Microvascularized Human Skin-on-a-Chip Tissue Equivalent Model. *Front Bioeng Biotechnol*. 2022;10:915702. doi:10.3389/fbioe.2022.915702.
56. Kim J, Kim K, Sung GY. Coenzyme Q10 efficacy test for human skin equivalents using a pumpless skin-on-a-chip system. Int J Mol Sci. 2020;21(22):8475. doi:10.3390/ijms21228475.
57. Tárnoki-Zách J, Mehes E, Varga-Medveczky Z, Isai DG, Barany N, Bugyik E, Revesz Z, Paku S, Erdo F, Czirok A. Development and evaluation of a human skin equivalent in a semiautomatic microfluidic diffusion chamber. Pharmaceutics. 2021;13(6):910. doi:10.3390/pharmaceutics13060910.
58. Varga-Medveczky Z, Kocsis D, Naszlady MB, Fónagy K, Erdő F. Skin-on-a-chip technology for testing transdermal drug delivery—starting points and recent developments. Pharmaceutics. 2021;13(11):1852. doi:10.3390/pharmaceutics13111852.
59. Kim K, Kim H, Sung GY. An interleukin-4 and interleukin-13 induced atopic dermatitis human skin equivalent model by a skin-On-A-Chip. Int J Mol Sci. 2022;23(4):2116. doi:10.3390/ijms23042116.
60. Jeon MS, Choi YY, Mo SJ, Ha JH, Lee YS, Lee HU, Park SD, Shim J-J, Lee J-L, Chung BG. Contributions of the microbiome to intestinal inflammation in a gut-on-a-chip. Nano Converg. 2022;9(1):8. doi:10.1186/s40580-022-00299-6.
61. De Gregorio V, Sgambato C, Urciuolo F, Vecchione R, Netti PA, Imparato G. Immunoresponsive microbiota-gut-on-chip reproduces barrier dysfunction, stromal reshaping and probiotics translocation under inflammation. Biomaterials. 2022;286:121573. doi:10.1016/j.biomaterials.2022.121573.
62. Jing B, Xia K, Zhang C, Jiao S, Zhu L, Wei J, Wang ZA, Chen N, Tu P, Li J, et al. Chitosan oligosaccharides regulate the occurrence and development of enteritis in a human gut-on-a-chip. *Front Cell Dev Biol*. 2022;10:877892. doi:10.3389/fcell.2022.877892.
63. Kulthong K, Hooiveld GJEJ, Duivenvoorde LPM, Estruch IM, Bouwmeester H, Zande M. van der Comparative study of the transcriptomes of caco-2 cells cultured under dynamic vs. static conditions following exposure to titanium dioxide and zinc oxide nanomaterials. Nanotoxicology. 2021;15(9):1233–1252. doi:10.1080/17435390.2021.2012609.
64. Milani N, Parrott N, Ortiz Franyuti D, Godoy P, Galetin A, Gertz M, Fowler S. Application of a gut–liver-on-a-chip device and mechanistic modelling to the quantitative in vitro pharmacokinetic study of mycophenolate mofetil. Lab Chip. 2022;22(15):2853–2868. doi:10.1039/d2lc00276k.
65. Moossavi S, Arrieta M-C, Sanati-Nezhad A, Bishehsari F. Gut-on-chip for ecological and causal human gut microbiome research. Trends Microbiol. 2022;30:710–721. doi:10.1016/j.tim.2022.01.014.
66. Sasaki Y, Tatsuoka H, Tsuda M, Sumi T, Eguchi Y, So K, Higuchi Y, Takayama K, Torisawa Y, Yamashita F. Intestinal permeability of drugs in caco-2 cells cultured in microfluidic Devices. Biol Pharm Bull. 2022;45(9):1246–1253. doi:10.1248/bpb.b22-00092.
67. Shin W, Kim HJ. 3D in vitro morphogenesis of human intestinal epithelium in a gut-on-a-chip or a hybrid chip with a cell culture insert. Nat Protoc. 2022;17(3):910–939. doi:10.1038/s41596-021-00674-3.
68. Sontheimer-Phelps A, Chou DB, Tovaglieri A, Ferrante TC, Duckworth T, Fadel C, Frismantas V, Sutherland AD, Jalili-Firoozinezhad S, Kasendra M, et al. Human colon-on-a-chip enables continuous in vitro analysis of colon mucus layer accumulation and physiology. Cell Mol Gastroenterol Hepatol. 2020;9(3):507–526. doi:10.1016/j.jcmgh.2019.11.008.
69. Dalsbecker P, Beck Adiels C, Goksör M. Liver-on-a-chip Devices: The Pros And Cons Of Complexity.

Am J Physiol Gastrointest Liver Physiol. 2022;323(3): G188–G204. doi:10.1152/ajpgi.00346.2021.
70. Khetani SR, Bhatia SN. Microscale culture of human liver cells for drug development. Nat Biotechnol. 2008;26(1):120–126. doi:10.1038/nbt1361.
71. Chen Z, He S, Zilberberg J, Lee W. Pumpless platform for high-throughput dynamic multicellular culture and chemosensitivity evaluation. Lab Chip. 2019;19 (2):254–261. doi:10.1039/C8LC00872H.
72. Kang YBA, Eo J, Mert S, Yarmush ML, Usta OB. Metabolic patterning on a chip: towards in vitro liver zonation of primary rat and human hepatocytes. Sci Rep. 2018;8(1):8951. doi:10.1038/s41598-018-27179-6.
73. Prodanov L, Jindal R, Bale SS, Hegde M, McCarty WJ, Golberg I, Bhushan A, Yarmush ML, Usta OB. Long-term maintenance of a microfluidic 3D human liver sinusoid. Biotechnol Bioeng. 2016;113(1):241–246. doi:10.1002/bit.25700.
74. Toh Y-C, Lim TC, Tai D, Xiao G, van Noort D, Yu H. A microfluidic 3D hepatocyte chip for drug toxicity testing. Lab Chip. 2009;9(14):2026–2035. doi:10.1039/B900912D.
75. Lee PJ, Hung PJ, Lee LP. An artificial liver sinusoid with a microfluidic endothelial-like barrier for primary hepatocyte culture. Biotechnol Bioeng. 2007;97(5):1340–1346. doi:10.1002/bit.21360.
76. Nakao Y, Kimura H, Sakai Y, Fujii T. Bile canaliculi formation by aligning rat primary hepatocytes in a microfluidic device. Biomicrofluidics. 2011;5 (2):22212. doi:10.1063/1.3580753.
77. Boul M, Benzoubir N, Messina A, Ghasemi R, Mosbah IB, Duclos-Vallée J-C, Dubart-Kupperschmitt A, Le Pioufle B. A versatile microfluidic tool for the 3D culture of heparg cells seeded at various stages of differentiation. Sci Rep. 2021;11(1):14075. doi:10.1038/s41598-021-92011-7.
78. Ong LJY, Chong LH, Jin L, Singh PK, Lee PS, Yu H, Ananthanarayanan A, Leo HL, Toh Y-C. A pump-free microfluidic 3D perfusion platform for the efficient differentiation of human hepatocyte-like cells. Biotechnol Bioeng. 2017;114(10):2360–2370. doi:10.1002/bit.26341.
79. Banaeiyan AA, Theobald J, Paukštyte J, Wölfl S, Adiels CB, Goksör M. Design and FABRICATION OF A SCALABLE LIVER-LOBULE-ON-A-CHIP MICRO PHYSIOLOGICAL PLATForm. Biofabrication. 2017;9 (1):015014. doi:10.1088/1758-5090/9/1/015014.
80. Ma L-D, Wang Y-T, Wang J-R, Wu J-L, Meng X-S, Hu P, Mu X, Liang Q-L, Luo G-A. Design and fabrication of a liver-on-a-chip platform for convenient, highly efficient, and safe in situ perfusion culture of 3D hepatic spheroids. Lab Chip. 2018;18(17):2547–2562. doi:10.1039/C8LC00333E.
81. Wagner I, Materne E-M, Brincker S, Süssbier U, Frädrich C, Busek M, Sonntag F, Sakharov DA, Trushkin EV, Tonevitsky AG, et al. A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. Lab Chip. 2013;13(18):3538–3547. doi:10.1039/c3lc50234a.
82. Kim J-Y, Fluri DA, Kelm JM, Hierlemann A, Frey O. 96-well format-based microfluidic platform for parallel interconnection of multiple multicellular spheroids. J Lab Autom. 2015;20(3):274–282. doi:10.1177/2211068214564056.
83. van Midwoud PM, Groothuis GMM, Merema MT, Verpoorte E. Microfluidic biochip for the perifusion of precision-cut rat liver slices for metabolism and toxicology studies. Biotechnol Bioeng. 2010;105(1):184–194. doi:10.1002/bit.22516.
84. Kwon D, Choi G, Park S-A, Cho S, Cho S, Ko S. Liver acinus dynamic chip for assessment of drug-induced zonal hepatotoxicity. Biosensors. 2022;12(7):445. doi:10.3390/bios12070445.
85. Liu J, Feng C, Zhang M, Song F, Liu H. Design and fabrication of a liver-on-a-chip reconstructing tissue-tissue interfaces. *Front Oncol*. 2022;12. doi:10.3389/fonc.2022.959299.
86. Du Y, Khandekar G, Llewellyn J, Polacheck W, Chen CS, Wells RG. A bile duct-on-a-chip with organ-level functions. Hepatology. 2020;71 (4):1350–1363. doi:10.1002/hep.30918.
87. Du Y, Polacheck WJ, Wells RGBDO-AC. Methods mol biol. 2022;2373:57–68. doi:10.1007/978-1-0716-1693-2_4.
88. Faria J, Ahmed S, Gerritsen KGF, Mihaila SM, Masereeuw R. Kidney-based in vitro models for drug-induced toxicity testing. Arch Toxicol. 2019;93 (12):3397–3418. doi:10.1007/s00204-019-02598-0.
89. Nieskens TTG, Persson M, Kelly EJ, Sjögren A-K. A multicompartment human kidney proximal tubule-on-a-chip replicates cell polarization–dependent cisplatin toxicity. Drug Metab Dispos. 2020;48 (12):1303–1311. doi:10.1124/dmd.120.000098.
90. Nieskens TTG, Magnusson O, Andersson P, Söderberg M, Persson M, Sjögren A-K. Nephrotoxic antisense oligonucleotide SPC5001 induces kidney injury biomarkers in a proximal tubule-on-a-chip. Arch Toxicol. 2021;95(6):2123–2136. doi:10.1007/s00204-021-03062-8.
91. Vormann MK, Tool LM, Ohbuchi M, Gijzen L, van Vught R, Hankemeier T, Kiyonaga F, Kawabe T, Goto T, Fujimori A, et al. Modelling and prevention of acute kidney injury through ischemia and reperfusion in a combined human renal proximal tubule/blood vessel-on-a-Chip. Kidney360. 2022;3(2):217–231. doi:10.34067/KID.0003622021.
92. Weber EJ, Lidberg KA, Wang L, Bammler TK, MacDonald JW, Li MJ, Redhair M, Atkins WM, Tran C, Hines KM, et al. Human kidney on a chip assessment of polymyxin antibiotic nephrotoxicity. JCI Insight. 2018;3(e123673):123673. doi:10.1172/jci.insight.123673.
93. Kim K, Jeong B, Lee Y-M, Son H-E, Ryu J-Y, Park S, Jeong JC, Chin HJ, Kim S. Three-dimensional

kidney-on-a-chip assessment of contrast-induced kidney injury: osmolality and viscosity. Micro (Basel). 2022;13(5):688. doi:10.3390/mi13050688.
94. Lavelle J, Meyers S, Ramage R, Bastacky S, Doty D, Apodaca G, Zeidel ML. Bladder permeability barrier: recovery from selective injury of surface epithelial cells. Am J Physiol Renal Physiol. 2002;283(2):F242–253. doi:10.1152/ajprenal.00307.2001.
95. Eojin L, Chunga K, Hyungseop H, Jimin P, Yu-Chan K, Myoung-Ryul O, Hyun-Kwang S, Hojeong J. Bladder cancer-on-a-chip for analysis of tumor transition mechanism. Front Bioeng Biotechnol. 2016;4. doi:10.3389/conf.FBIOE.2016.01.00831.
96. Liu P, Cao Y, Zhang S, Zhao Y, Liu X, Shi H, Hu K, Zhu G, Ma B, Niu H. A bladder cancer microenvironment simulation system based on a microfluidic co-culture model. Oncotarget. 2015;6(35):37695–37705. doi:10.18632/oncotarget.6070.
97. Menon MC, Chuang PY, He CJ. The Glomerular Filtration Barrier: Components And Crosstalk. Int J Nephrol. 2012;2012:1–9. doi:10.1155/2012/749010.
98. Zhu Y, Yin F, Wang H, Wang L, Yuan J, Qin J. Placental barrier-on-a-chip: modeling placental inflammatory responses to bacterial infection. *ACS Biomater Sci Eng*. 2018;4(9):3356–3363. doi:10.1021/acsbiomaterials.8b00653.
99. Mandt D, Gruber P, Markovic M, Tromayer M, Rothbauer M, Kratz SRA, Ali SF, Hoorick JV, Holnthoner W, Mühleder S, et al. Fabrication of biomimetic placental barrier structures within a microfluidic device utilizing two-photon polymerization. Int J Bioprint. 2018;4(144). doi:10.18063/IJB.v4i2.144.
100. Blundell C, Tess ER, Schanzer ASR, Coutifaris C, Su EJ, Parry S, Huh D. A microphysiological model of the human placental barrier. Lab Chip. 2016;16(16):3065–3073. doi:10.1039/c6lc00259e.
101. Lee JS, Romero R, Han YM, Kim HC, Kim CJ, Hong J-S, Huh D. Placenta-on-a-chip: a novel platform to study the biology of the human placenta. J Matern Fetal Neonatal Med. 2016;29(7):1046–1054. doi:10.3109/14767058.2015.1038518.
102. Yin F, Zhu Y, Zhang M, Yu H, Chen W, Qin J. A 3D human placenta-on-a-chip model to probe nanoparticle exposure at the placental barrier. Toxicol In Vitro. 2019;54:105–113. doi:10.1016/j.tiv.2018.08.014.
103. Shojaei S, Ali MS, Suresh M, Upreti T, Mogourian V, Helewa M, Labouta HI. Dynamic placenta-on-a-chip model for fetal risk assessment of nanoparticles intended to treat pregnancy-associated diseases. Biochim Biophys Acta, Mol Basis Dis. 2021;1867(7):166131. doi:10.1016/j.bbadis.2021.166131.
104. Mahajan G, Doherty E, To T, Sutherland A, Grant J, Junaid A, Gulati A, LoGrande N, Izadifar Z, Timilsina SS, et al. Vaginal microbiome-host interactions modeled in a human vagina-on-a-chip. Microbiome. 2022;10(1):201. doi:10.1186/s40168-022-01400-1.
105. Zervantonakis IK, Hughes-Alford SK, Charest JL, Condeelis JS, Gertler FB, Kamm RD Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. *Proceedings of the National Academy of Sciences* 2012, *109*, 13515–-13520, doi:10.1073/pnas.1210182109.
106. Choi Y, Hyun E, Seo J, Blundell C, Kim HC, Lee E, Lee SH, Moon A, Moon WK, Huh D. A microengineered pathophysiological model of early-stage breast cancer. Lab Chip. 2015;15(16):3350–3357. doi:10.1039/C5LC00514K.
107. Carvalho MR, Barata D, Teixeira LM, Giselbrecht S, Reis RL, Oliveira JM, Truckenmüller R, Habibovic P. Colorectal tumor-on-a-chip system: a 3D tool for precision onco-nanomedicine. *Sci Adv*. 2019;5(5):eaaw1317. doi:10.1126/sciadv.aaw1317.
108. Shirure VS, Bi Y, Curtis MB, Lezia A, Goedegebuure MM, Goedegebuure SP, Aft R, Fields RC, George SC. Tumor-on-a-chip platform to investigate progression and drug sensitivity in cell lines and patient-derived organoids. Lab Chip. 2018;18(23):3687–3702. doi:10.1039/c8lc00596f.
109. Tian C, Zheng S, Liu X, Kamei K-I. Tumor-on-a-chip model for advancement of anti-cancer nano drug delivery system. J Nanobiotechnology. 2022;20(1):338. doi:10.1186/s12951-022-01552-0.
110. Lee J, Mehrotra S, Zare-Eelanjegh E, Rodrigues RO, Akbarinejad A, Ge D, Amato L, Kiaee K, Fang Y, Rosenkranz A, et al. A heart-breast cancer-on-a-chip platform for disease modeling and monitoring of cardiotoxicity induced by cancer chemotherapy. Small. 2021;17(15):e2004258. doi:10.1002/smll.202004258.
111. Yang Y, Yang X, Zou J, Jia C, Hu Y, Du H, Wang H. Evaluation of photodynamic therapy efficiency using an in vitro three-dimensional microfluidic breast cancer tissue model. Lab Chip. 2015;15(3):735–744. doi:10.1039/C4LC01065E.
112. Tsai H-F, Trubelja A, Shen AQ, Bao G. Tumour-on-a-chip: microfluidic models of tumour morphology, growth and microenvironment. *J R Soc Interface*. 2017;14(131):20170137. doi:10.1098/rsif.2017.0137.
113. Arsiwala TA, Blethen KE, Wolford CP, Panchal DM, Sprowls SA, Fladeland RA, Kielkowski BN, Pritt TA, Wang P, Wilson O, et al. Blood-tumor barrier opening by MRI-guided transcranial focused ultrasound in a preclinical breast cancer brain metastasis model improves efficacy of combinatorial chemotherapy. Front Oncol. 2023;13:1104594. doi:10.3389/fonc.2023.1104594.
114. Perestrelo AR, Águas ACP, Rainer A, Forte G. Microfluidic organ/body-on-a-chip devices at the convergence of biology and microengineering. Sensors (Basel). 2015;15(12):31142–31170. doi:10.3390/s151229848.
115. Leung CM, de Haan P, Ronaldson-Bouchard K, Kim G-A, Ko J, Rho HS, Chen Z, Habibovic P, Jeon NL, Takayama S, et al. A guide to the organ-on-a-chip. Nat Rev Methods Primers. 2022;2(1):1–29.

doi:10.1038/s43586-022-00118-6.
116. Picollet-D'hahan N, Zuchowska A, Lemeunier I, Le Gac S. Multiorgan-on-a-chip: a systemic approach to model and decipher inter-organ communication. *Trends Biotechnol*. 2021;39(8):788–810. doi:10.1016/j.tibtech.2020.11.014.
117. Kimura H, Ikeda T, Nakayama H, Sakai Y, Fujii T. An on-chip small intestine–liver model for pharmacokinetic studies. J Lab Autom. 2015;20(3):265–273. doi:10.1177/2211068214557812.
118. Xu Z, Li E, Guo Z, Yu R, Hao H, Xu Y, Sun Z, Li X, Lyu J, Wang Q. Design and construction of a multi-organ microfluidic chip mimicking the in vivo microenvironment of lung cancer metastasis. ACS Appl Mater Interfaces. 2016;8(39):25840–25847. doi:10.1021/acsami.6b08746.
119. Kong J, Luo Y, Jin D, An F, Zhang W, Liu L, Li J, Fang S, Li X, Yang X, et al. A novel microfluidic model can mimic organ-specific metastasis of circulating tumor cells. Oncotarget. 2016;7(48):78421–78432. doi:10.18632/oncotarget.9382.
120. Satoh T, Sugiura S, Shin K, Onuki-Nagasaki R, Ishida S, Kikuchi K, Kakiki M, Kanamori T. A multi-throughput multi-organ-on-a-chip system on a plate formatted pneumatic pressure-driven medium circulation platform. Lab Chip. 2017;18(1):115–125. doi:10.1039/C7LC00952F.
121. Edington CD, Chen WLK, Geishecker E, Kassis T, Soenksen LR, Bhushan BM, Freake D, Kirschner J, Maass C, Tsamandouras N, et al. Interconnected microphysiological systems for quantitative biology and pharmacology studies. Sci Rep. 2018;8(1):4530. doi:10.1038/s41598-018-22749-0.
122. Xiao S, Coppeta JR, Rogers HB, Isenberg BC, Zhu J, Olalekan SA, McKinnon KE, Dokic D, Rashedi AS, Haisenleder DJ, et al. A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. Nat Commun. 2017;8(1):14584. doi:10.1038/ncomms14584.
123. Bauer S, Wennberg Huldt C, Kanebratt KP, Durieux I, Gunne D, Andersson S, Ewart L, Haynes WG, Maschmeyer I, Winter A, et al. Functional coupling of human pancreatic islets and liver spheroids on-a-chip: towards a novel human ex vivo type 2 diabetes model. Sci Rep. 2017;7(1):14620. doi:10.1038/s41598-017-14815-w.
124. Cao UMN, Zhang Y, Chen J, Sayson D, Pillai S, Tran SD. Microfluidic organ-on-a-chip: a guide to biomaterial choice and fabrication. Int J Mol Sci. 2023;24(4):3232. doi:10.3390/ijms24043232.
125. Koyilot MC, Natarajan P, Hunt CR, Sivarajkumar S, Roy R, Joglekar S, Pandita S, Tong CW, Marakkar S, Subramanian L, et al. Breakthroughs and applications of organ-on-a-chip technology. Cells. 2022;11(11):1828. doi:10.3390/cells11111828.
126. Yu F, Hunziker W, Choudhury D. Engineering microfluidic organoid-on-a-chip platforms. Micromachines. 2019;10(3):165. doi:10.3390/mi10030165.
127. Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ. TEER measurement techniques for in vitro barrier model systems. J Lab Autom. 2015;20(2):107–126. doi:10.1177/2211068214561025.
128. Pak J, Chen ZJ, Sun K, Przekwas A, Walenga R, Fan J. Computational modeling of drug transport across the in vitro cornea. Comput Biol Med. 2018;92:139–146. doi:10.1016/j.compbiomed.2017.11.009.
129. Tenenbaum-Katan J, Artzy-Schnirman A, Fishler R, Korin N, Sznitman J. Biomimetics of the pulmonary environment in vitro: a microfluidics perspective. Biomicrofluidics. 2018;12(4):042209. doi:10.1063/1.5023034.
130. Booth R, Kim H. A MULTI-LAYERED MICROFLUIDIC DEVICE for in vitro BLOOD-BRAIN BARRIER PERMEABILITY STUDIES. Washington, USA: Seattle; 2011.
131. Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip. 2012;12(12):2165–2174. doi:10.1039/c2lc40074j.
132. Yee S. In Vitro permeability across caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man–fact or myth. Pharm Res. 1997;14:763–766. doi:10.1023/a:1012102522787.
133. Fleischer D. Biological transport phenomena in the gastrointestinal tract: cellular mechanisms. CRC Press. ISBN 978-0-429-22188-0; 1999. pp. 163–200. doi:10.1201/9780203909478.
134. Tavana H, Zamankhan P, Christensen PJ, Grotberg JB, Takayama S. Epithelium damage and protection during reopening of occluded airways in a physiologic microfluidic pulmonary airway model. Biomed Microdevices. 2011;13(4):731–742. doi:10.1007/s10544-011-9543-5.
135. Cui B, Cho S-W. Blood-brain barrier-on-a-chip for brain disease modeling and drug testing. BMB Rep. 2022;55(5):213–219. doi:10.5483/BMBRep.2022.55.5.043.
136. Ragelle H, Dernick K, Khemais S, Keppler C, Cousin L, Farouz Y, Louche C, Fauser S, Kustermann S, Tibbitt MW, et al. Human retinal microvasculature-on-a-chip for drug discovery. *Adv Healthcare Mater*. 2020;9(21):e2001531. doi:10.1002/adhm.202001531.
137. Ganesan S, Comstock AT, Sajjan US. Barrier function of airway tract epithelium. Tissue Barriers. 2013;1(4):e24997. doi:10.4161/tisb.24997.
138. Elias PM. Stratum corneum defensive functions: an integrated view. J Invest Dermatol. 2005;125:183–200. doi:10.1111/j.0022-202X.2005.23668.x.
139. Elias PM. Skin Barrier Function. Curr Allergy Asthma Rep. 2008;8(4):299–305. doi:10.1007/s11882-008-0048-0.
140. Elias PM, Menon GK. Structural and lipid biochemical correlates of the epidermal permeability barrier. Adv Lipid Res. 1991;24:1–26. doi:10.1016/b978-0-12-024924-4.50005-5.
141. Kocsis D, Horváth S, Kemény Á, Varga-Medveczky Z, Pongor C, Molnár R, Mihály A, Farkas D, Naszlady BM,

Fülöp A, et al. Drug delivery through the psoriatic epidermal barrier—A "skin-on-a-chip" permeability study and ex vivo optical imaging. Int J Mol Sci. 2022;23:4237. doi:10.3390/ijms23084237.
142. Jeong S, Kim J, Jeon HM, Kim K, Sung GY. Development of an aged full-thickness skin model using flexible skin-on-a-chip subjected to mechanical stimulus reflecting the circadian rhythm. Int J Mol Sci. 2021;22:12788. doi:10.3390/ijms222312788.
143. Hakim M, Kermanshah L, Abouali H, Hashemi HM, Yari A, Khorasheh F, Alemzadeh I, Vossoughi M. Unraveling cancer metastatic cascade using microfluidics-based technologies. Biophys Rev. 2022;14:517–543. doi:10.1007/s12551-022-00944-8.
144. Del Piccolo N, Shirure VS, Bi Y, Goedegebuure SP, Gholami S, Hughes CCW, Fields RC, George SC. Tumor-on-chip modeling of organ-specific cancer and metastasis. Adv Drug Deliv Rev. 2021;175:113798. doi:10.1016/j.addr.2021.05.008.
145. Wang H-F, Liu Y, Wang T, Yang G, Zeng B, Zhao C-X. Tumor-microenvironment-on-a-chip for evaluating nanoparticle-loaded macrophages for drug delivery. *ACS Biomater Sci Eng*. 2020;6(9):5040–5050. doi:10.1021/acsbiomaterials.0c00650.
146. Shao C, Yu Y, Lei X, Cao J, Zhao Y, Ye F. Organ-on-a-chip for dynamic tumor drug resistance investigation. Chemical Engineering Journal. 2023;460:141739. doi:10.1016/j.cej.2023.141739.
147. Chakrabarty S, Quiros-Solano WF, Kuijten MMP, Haspels B, Mallya S, Lo CSY, Othman A, Silvestri C, van de Stolpe A, Gaio N, et al. A microfluidic cancer-on-chip platform predicts drug response using organotypic tumor slice culture. Cancer Res. 2022;82(3):510–520. doi:10.1158/0008-5472.CAN-21-0799.
148. Nikoloff JM, Saucedo-Espinosa MA, Dittrich PS. Microfluidic platform for profiling of extracellular vesicles from single breast cancer cells. Anal Chem. 2023;95(3):1933–1939. doi:10.1021/acs.analchem.2c04106.
149. Versteeg HK, Malalasekara W. Introduction to computational fluid dynamics, an: the finite volume method. Harlow, England: Pearson Education Limited; 1995.
150. Nield DA, Bejan A. Convection in porous media 3rd. New York: Springer; 2006. ISBN 978-0-387-29096-6.
151. Bouhrira N, DeOre BJ, Sazer DW, Chiaradia Z, Miller JS, Galie PA. Disturbed flow disrupts the blood-brain barrier in a 3D bifurcation model. Biofabrication. 2020;12(2):025020. doi:10.1088/1758-5090/ab5898.
152. Lee S, Kim JH, Kang SJ, Chang IH, Park JY. Customized multilayered tissue-on-a-chip (MToC) to simulate bacillus Calmette–Guérin (BCG) immunotherapy for bladder cancer treatment. Biochip Journal. 2022;16(1):67–81. doi:10.1007/s13206-022-00047-2.
153. Mosavati B, Oleinikov AV, Du E. Development of an organ-on-a-chip-device for study of placental pathologies. Int J Mol Sci. 2020;21(22):8755. doi:10.3390/ijms21228755.
154. Davies AE, Williams RL, Lugano G, Pop SR, Kearns VR. In vitro and computational modelling of drug delivery across the outer blood–retinal barrier. Interface Focus. 2020;10(2):20190132. doi:10.1098/rsfs.2019.0132.
155. Du Y, Li N, Yang H, Luo C, Gong Y, Tong C, Gao Y, Lü S, Long M. Mimicking liver sinusoidal structures and functions using a 3D-Configured microfluidic chip. Lab Chip. 2017;17(5):782–794. doi:10.1039/C6LC01374K.
156. Enders A, Grünberger A, Bahnemann J. Towards small scale: overview and applications of microfluidics in biotechnology. Mol Biotechnol. 2022. doi:10.1007/s12033-022-00626-6.
157. Kafarski P. Rainbow code of biotechnology. Chemik. 2012;66:814–816.
158. Barcelos MCS, Lupki FB, Campolina GA, Nelson DL, Molina G. The colors of biotechnology: general overview and developments of white, green and blue areas. FEMS Microbiol Lett. 2018;365. doi:10.1093/femsle/fny239.