Review

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Review

Biological Barrier Models-on-Chip: An Innovative Tool for Studying Diseases and Discovering New Drug Therapies

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Abstract: The exploration of alternatives to the use of animal models and cell cultures has culminated in the creation of organ-on-a-chip systems in which organs in physio-pathological conditions and their reactions to the presence of external stimuli are simulated. In addition, they support the recreation of tissue interfaces such as tissue-air, tissue-liquid and tissue-tissue, which are very similar to those present in vivo, even through the presence of biomechanical stimuli. In this way they are best suited to mimic biological barriers, such as the skin, placenta, blood-brain barrier and others, which are characterized by tissue interface and their functioning is important to ensure the homeostasis of the organism. This review shows the different biological membranes that we can simulate within an organ-on-chip, also using induced pluripotent stem cells to act in the direction of personalized medicine. Different methods that can be used to detect barrier formation, including the integration of electrodes for real-time monitoring, are also explained, highlighting advantages and challenges.

Keywords: organ-on-chip; biological barrier; skin-on-chip; gut-on-chip; BBB-on-chip; placenta-on-chip; airway-on-chip

1. Introduction

Several biological barriers can be found in the human body, and some of these may separate the external environment from the internal environment, such as the skin, cornea, pulmonary and digestive systems, while others separate different tissues or compartments within the body itself, such as the placenta, the blood-brain barrier, the blood-retinal barrier and the testes. The presence of biological barriers is necessary for the homeostasis of the human body, as they regulate the passage of biomolecules, drugs, ions and organisms, and their malfunction can lead to disease [1–4]. Traditional methods, such as animal models and 2D cultures, can be used to study pathologies that result from the malfunction of biological barriers or to investigate the passage of drugs through them. Chen et al. demonstrated through the use of animal models that palmitoylation enzyme activity is essential for skin barrier integrity [5] and Li et al. used Zebrafish as an animal model to study the transport of nanoparticles used in drug transport across biological barriers such as the blood-brain barrier the blood-retinal barrier and the gastrointestinal barrier [6]. In the last decade, a new approach to studying the complexity of human pathophysiology has emerged, and this is represented by Organ-on-chips (OoC) technology [7–9]. These are microfluidic devices consisting of channels and chambers that range from the sub-micron to millimetre scale and within which different cell types

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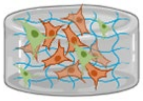
are introduced to go on to mimic the functional unit of an organ [10,11]. These platforms can also be integrated with sensors to monitor cellular activity in the extracellular environment in real-time [12–16]. Moreover, Organ-on-chips go beyond several limitations of animal models, such as ethical issues, limited translatability of results between animals and humans, and variability in drug response, as shown in Table 1 [7–9]. This new technology is also able to go further some problems of 2D cultures, such as the absence of cellular heterogeneity and the absence of compartmentalization, and also overcome some critical issues of 3D cultures such as the lack of physical and mechanical stimuli that can be mechanisms of compression, tension, and shear stress that affect organ development in vivo [12,17]. To address the problem of variability in drug response among different individuals of the same species, it would be useful to tailor drug therapy to identify the most suitable drug treatments for the individual patient and to limit side effects as well [18–20]. An example of personalized therapy may be the technology of iPSCs, induced pluripotent stem cells, which are obtained from skin biopsy or blood collection and stem cells are generated that retain the genetic characteristics of the donor [21–23]. Combining the technology of iPSCs and Organ-on-chips could be a promising application in the field of personalized medicine to enable more effective therapy for each patient [18,19,24]. In the next sections, we will discuss the different Organ-on-chips used to mimic the various biological barriers of the human body, such as the blood-brain barrier, skin, placenta, gastrointestinal barrier and others, while also integrating the technology of iPSCs. [18,19,74]

Table 1. Comparison of different study models for diseases.

	Animal model	Cell culture 2D	Cell culture 3D	Organ-on-chip
				
Translatability of results	**LOW**	**MEDIUM**	**HIGH**	**HIGH**
Ethical issues	**HIGH**	**LOW**	**LOW**	**LOW**
Recapitulate disease model	**MEDIUM**	**LOW**	**MEDIUM**	**HIGH**
Drug discovery	**MEDIUM**	**MEDIUM**	**HIGH**	**HIGH**
Biosensor equipment incorporation	**LOW**	**HIGH**	**HIGH**	**HIGH**
Cell-cell interaction	**HIGH**	**LOW**	**HIGH**	**HIGH**

2. Blood-Brain Barrier (BBB) on-a-Chip

The blood-brain barrier is a component of the central nervous system and is composed of cerebral capillaries and thus endothelial cells and the perivascular pedicels of astrocyte. Endothelial cells are connected by occluding junctions, such as zonula occludens-1 (ZO-1) and claudin, which regulate the passage of substances, molecules, and ions from blood to brain fluids and vice versa, thus making the membrane very selective so as to protect the brain from pathogens and dangerous substances and ensure proper brain homeostasis [25]. Another barrier present in the brain is the blood-liquid barrier, which is located in the choroid plexuses and consists of the choroid capillaries and the cells of the choroid epithelium. This barrier also has tight junctions that determine the physiological impermeability between blood and cephalorachidian fluid (CSF) [26–29]. However, the blood-brain barrier also makes it difficult for the passage of drugs for the treatment of neurodegenerative diseases such as Alzheimer's or Parkinson's disease, for example, which are

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increasing more and more as life span grows. One way to study how to allow drugs to pass through the blood-brain barrier may be by making an blood-brain- barrier-on-chip integrated with biosensors to monitor various biomolecules and compounds involved in neurodegenerative diseases so that they can also be useful in the development of new drug therapies [28–32] . Badiola-Mateos et al. [33] have created a system that can simulate and monitor the integrity of the blood-brain barrier in real-time. It is a Lab on chip(LOC) device consisting of two pairs of cyclin-olefin polymer (COP) layers each of which has polycarbonate membranes inside. In addition, the device is integrated with small electrodes that enable electrical impedance spectroscopy (EIS) measurements at multiple positions. The cells that are used are brain microvascular endothelial cells line (hCMEC/D3) that are seeded on the bottom layer and bovine pericytes that are seeded on the top layer. After 48 h, the cells are treated with D-mannitol to induce the destruction of the barrier formed in the chip. Then the evolution of barrier reformation at multiple locations is monitored using multiple-frequency trans-endothelial electrical resistance coupled with machine learning techniques. These data were also confirmed by immunochemical staining techniques, as shown in Figure 1B and 1C. This approach is thus able to identify BBB regeneration in the device following drug treatment, and this could pave the way in the future for optimizing drug treatments for neurodegenerative diseases. Xu et al. [34] developed a BBB-on chip to study the functional responses of the blood-brain barrier under normal and pathological conditions and particularly in brain metastasis and glioblastoma. They recreated the architecture of the BBB using a co-culture of endothelial cells and astrocytes within a 3D extracellular matrix and in the presence of flow to simulate the mechanical stimuli present in the blood vessel system. It is noteworthy that tumour cells to give metastasis in the brain must cross the blood-brain barrier. Using this microfluidic platform it has been seen that there is an interaction between cancer cells and the BBB and in particular there is a passage through the barrier of lung cancer cells, breast cancer cells and melanoma cells but not liver cancer cells. In addition, it has been shown with the use of this platform that despite the aggressiveness of glioblastoma it cannot cross the blood-brain barrier and give metastasis in the vascular system surrounding the barrier. Partyka et al. [35] showed through their studies that the presence of mechanical stimuli, due to the presence of a flow within a BBB-on chip, favours the formation of tight junctions at the endothelial level and this was verified by measuring transendothelial electrical resistance and through dextran perfusion. The chip is characterised by two compartments connected by a hydrogel reservoir containing type I collagen, hyaluronan, hCMEC/D3 cells and astrocytes subjected to fluid flow and cyclic deformation caused by hydrostatic pressure,as shown in Figure 1G. The presence of shear and tension stress facilitates the formation of the endothelial barrier both in the presence of astrocytes in the extracellular matrix and its absence [35].

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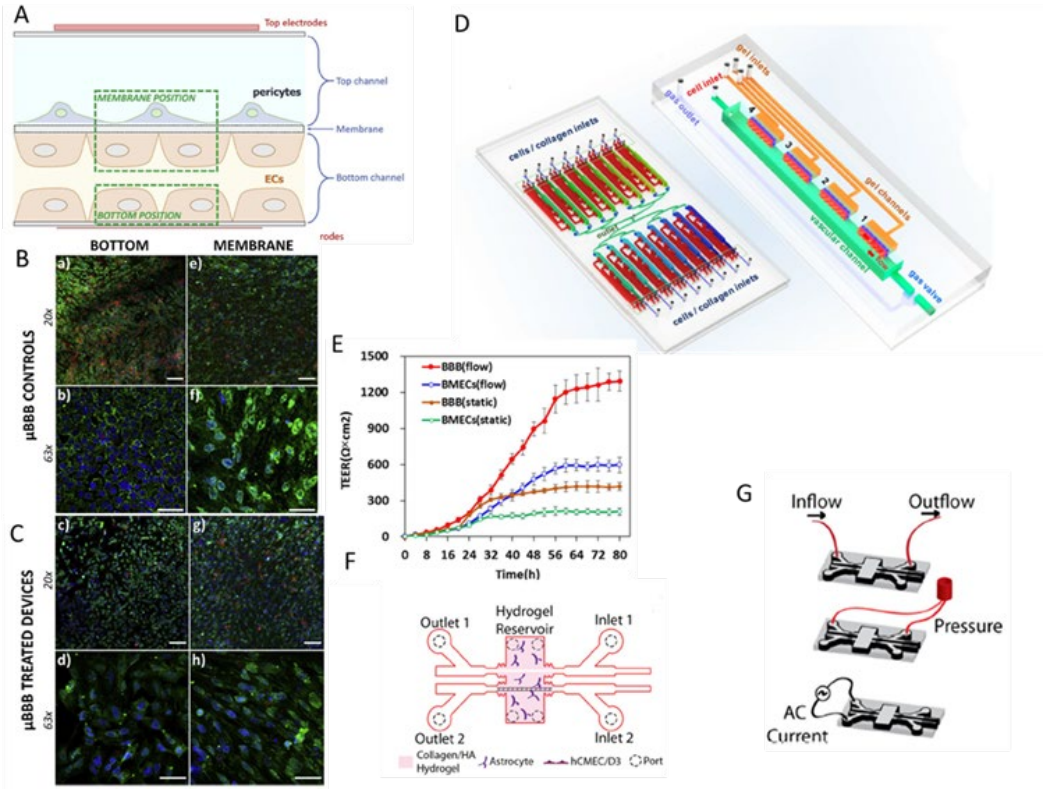

Figure 1. A) Schematic representation of the horizontal section of BBB-on chip: pericytes are seeded on the polycarbonate membrane in the upper channel while the hCMEC/D3are seeded on the bottom channel. Interdigitated electrodes are placed on both the lower and upper channels so as to perform MTEER measurements [33]. B) and C) Confocal microscopy images of BBB-untreated (a,b,e,f) and mannitol-treated BBB after 24 h(c,d,g,h). Immunochemical staining was performed on the side of the membrane comprising pericytes and endothelial cells (e-h) and on the lower position comprising endothelial cells only (a-d): nuclei are stained blue, ZO-1 tigh junctions are stained green and VE-cadherin adherens junctions are stained red. Scale bars are 100 μm for 20X images and 50 μm for 63X images [33]. D)The left image shows the chip design, which consists of 16 independent units connected by a neuronal microchannel, and the right image shows a single enlarged unit with a vascular channel, a gas channel, a gas valve and four gel channels [34]. E) TEER measurement of the barrier function in the BBB group and microvascular endothelial cells (BMECs) alone under static and flow conditions [34]. F) 3D blood brain barrier model by Partyka et al. [35] G) Representation of fluid flow, cyclic deformation and TEER measurements in the BBB-on chip [35].

3. Blood-Retinal Barrier (BRB)-on-Chip

The blood-retinal barrier consists of the retinal pigment epithelium (RPE) and is responsible for protecting the retinal environment by separating it from the systemic circulation. The blood-retina barrier is divided into the outer blood-retina barrier and the inner blood-retina barrier. The outer barrier separates (oBRB) the fenestrated choriocapillaris from the retina, thus controlling the passage of substances from the retina to the choroid and is composed of pigmented epithelium cells (RPE) whereas the inner barrier (iBRB) has retinal vascular endothelial cells, which limit the transport of solutes and molecules from the vascular circulation. (Figure 2)

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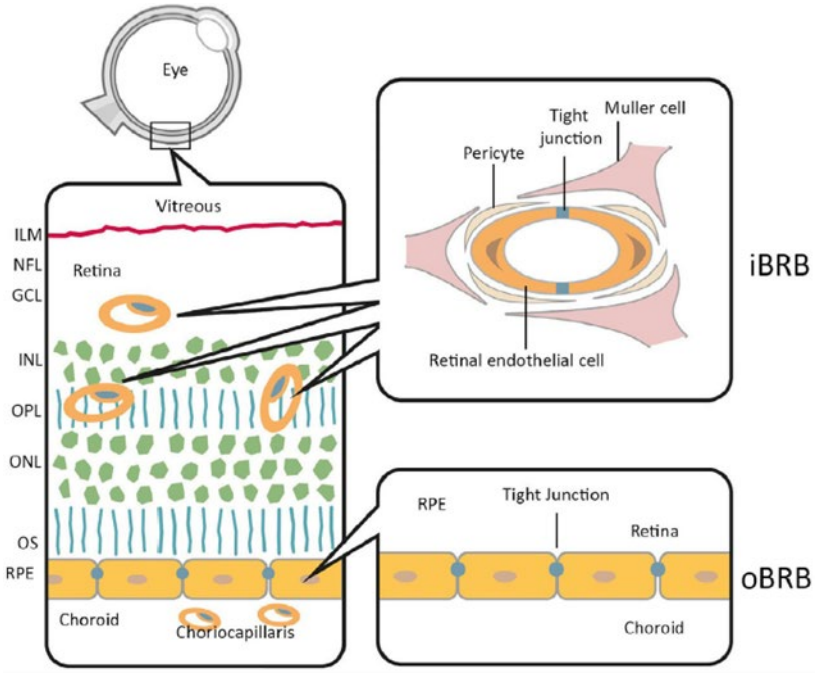

Figure 2. Schematic representation of the inner and outer blood-retinal barrier. The iBRB is made up of endothelial cells coating the vessels of the internal retina. The oBRB is constituted by retinal pigment epithelial (RPE) cells and regulates the exchange of material from the retina to the choroid. ILM: inner limiting barrier, GCL: ganglion cell layer, INL: outer nuclear layer, OS: outer segments [36].

Disruption of the blood-retinal barrier can lead to eye diseases such as age-related retinopathy and diabetic retinopathy resulting in loss of neuronal signal and thus loss of vision. The systems that are used to study these diseases are classical 2D cell culture systems, which, however, are unable to reproduce the architecture of the BRB, and animal models, which are also suboptimal due to the excessive time and cost involved in carrying out studies on them. One solution may be to realise organ-on-chip and thus recreate the BRB inside a chip to introduce mechanical stimuli present physiologically, recreate the three-dimensionality of the barrier's architecture and study physiological and even pathological situations under optimal conditions [36–38]. Yeste et al. [39] constructed a compartmentalised microfluidic chip to study the blood-retinal barrier by co-culturing primary human retinal endothelial cells (HRECs), to simulate iBRB a human neuroblastoma cell line (SH-SY5Y) and a human retinal pigment epithelium cell line (ARPE-19) to simulate (oBRB), in several parallel channels (Figure 3E). In addition, the chip also has electrodes on the substrate, in contrast to other chips that have electrodes on each side of the cell barrier, which enable the monitoring of occluding junctions through the TEER. The novelty of this chip is that the electrodes are only on the substrate unlike most where they are on each side of the barrier. Furthermore, the formation of the endothelial and epithelial barrier is also demonstrated by permeability and immunofluorescence studies. This device could be useful in the study of multi-barrier models and in particular in the study of retinal diseases. In most developed countries, age-related macular degeneration (ADM) is the main cause of blindness in people over 65 years old. ADM involves choroidal neovascularisation, which is a consequence of irregular secretion of growth factors including vascular endothelial growth factor (VEGF) which is one of the most important factors involved in angiogenesis. Chen et al. [40] developed a microfluidic device to study ADM. It consists of two layers of PDMS: in the upper layer human retinal pigment epithelial cells (ARPE-19) are seeded and in the lower layer human umbilical vein endothelial cells (HUVEC), and they are separated by a porous PDMS membrane to mimic the Bruch's membrane, present in vivo, that separates retinal cells from the choroid. The chip allows the cells to be kept in culture for a long period and the presence of angiogenic conditions in vitro has made it possible to study changes in the retinal pigment epithelium microenvironment, the effects of

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glucose concentration and chemical hypoxia on cell-cell interactions, as shown in Figure 3B. This device could also be used to study the process of angiogenesis in both physiological and pathological conditions such as tumours.

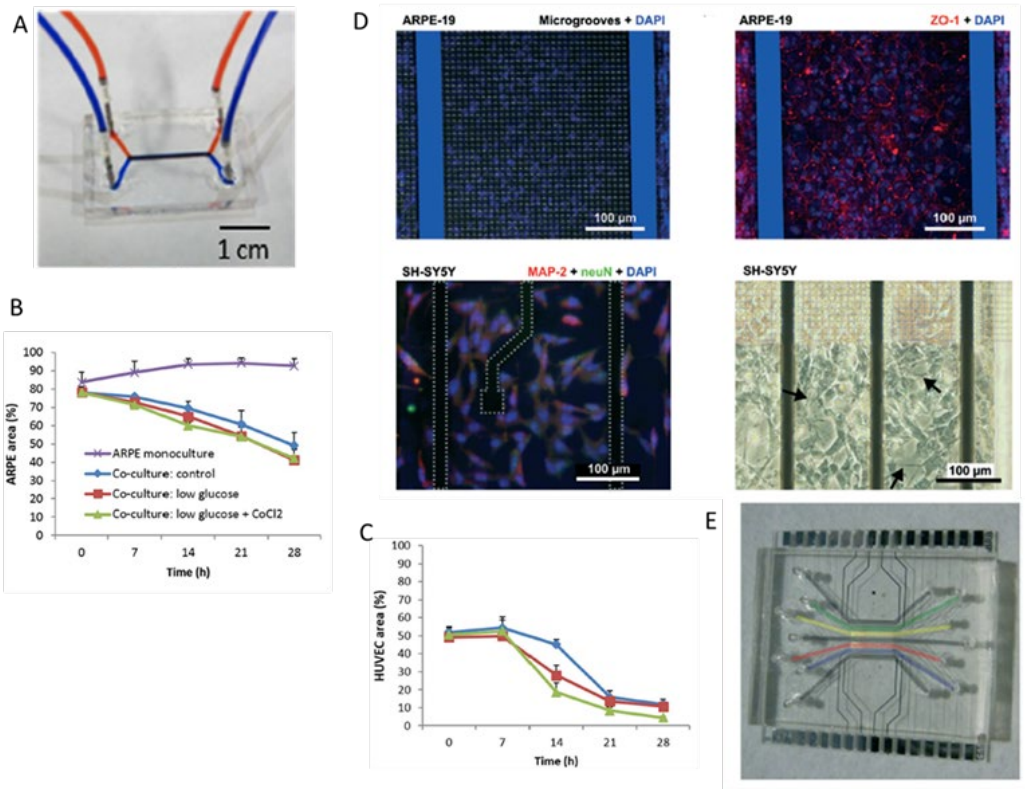

Figure 3. A) Photograph of the microfluidic device with blue and red dye channels by Chen et al. [40] B) and C) show graphs in which the growth area is quantified at different time points in the case of a control situation, low glucose concentrations in the culture medium and in the case of the addition of $CoCl_2$ to harden the cell hypoxia for the two cell types cultured in the chip. In particular, Figure B shows the time-dependent growth area of ARPE-19 even in a monoculture situation, whereas Figure C analyses the time-dependent growth area of HUVECs. We can see that there was a reduction in the growth area of ARPE-19 after 14h, but not as noticeable as was the case for HUVEC at the same time point. Based also on the information obtained from permeability tests in which they saw that the cell monolayer of ARPE-19 is present even under low glucose concentration and/or hypoxic conditions, the researchers speculated that the cause of the reduction in ARPE-19 growth was not due to the conditions of the culture medium but to the possible invasion by HUVECs that favoured the detachment of ARPE-19 [40]. D) Four images from the experiments carried out by Yeste et al. [39] are shown. The top left image shows the cell monolayer (ARPE-19) of the retinal epithelial barrier within the microchannels of the device. The cell nuclei are stained blue with DAPI. In the second upper right image, the ZO-1 protein is shown in red, which is distributed along the epithelial monolayer and demonstrates together with the permeability tests and TEER the barrier formation in the microfluidic device grid. Both upper images are of confocal microscopy at 10X magnification. The lower image on the left is a fluorescence image in which the SH-SY5Y, inside the microfluidic platform, is stained blue with DAPI and neuronal markers are stained green (neuN) and red (Map-2). The lower right image shows the SH-SY5Y under the light microscope within the microchannels of the device and the black arrows indicate the neuritic extensions. E) Photograph of the microfluidic device described by Yeste et al. [39].

4. Skin-on-Chip

The skin is the largest organ of the human body and represents an important defence barrier against pathogens and chemicals. It consists of three layers: starting with the outermost layer, we

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have the epidermis which includes keratinocytes, melanocytes and cells of the immune system (Langerhans cells); the dermis, in which there are blood vessels, sensory nerves, lymph vessels, sebaceous glands, sweat glands, hair follicles and fibroblasts; and finally, in the innermost layer, there is the subcutaneous tissue or hypodermis, which includes blood vessels, lymphatics, sensory nerves, collagen and adipose tissue. The realisation of a microfluidic platform, which mimics the cell-cell and cell-matrix interaction that exists in the three skin layers of the skin, simulating the vascular, immune and nervous systems present in it, integrated with biosensors to monitor the functions of this organ. Skin-on-chip systems could be an alternative to in vitro or engineered skin models that still lack some important components to best mimic the physio-pathology of the skin [41–44]. The skin also has its microbiome that contributes to protecting the skin from pathogens and to the development of the immune system. It has been seen that in different areas of the skin, different types of bacteria would lead to different types of diseases [45–47]. For example, Quan et al. have constructed a interfaced-controlled-skin-on-chip (IC-SoC), as shown in Figure 4E, to study the effect of a bacterium that is Propionibacterium acnes (P.acnes), which colonises the surface of the skin resulting in acne, and to investigate drugs to reduce the inflammation caused by this bacterium and drugs to help repair the skin barrier have also been tested. The microfluidic device consists of 3 layers made of polydimethylsiloxane (PDMS): a bottom layer with a channel for the cell culture medium, a porous PET membrane, an upper layer with a channel for air and a culture chamber, and a sort of lid where there are inlets and outlets for air and culture medium. The cells used in this platform are immortalised human keratinocytes (HaCaT) that are added in a solution of type I collagen hydrogel and dermal fibroblasts. Due to the presence of a flow of air in the device, the differentiation and stratification of keratinocytes are promoted, just like in vivo. Immunofluorescence tests, transepidermal electrical resistance measurements and permeability tests were then performed to verify skin barrier functions and compare the results with experiments performed in Transwell. They then used *Propionibacterium acnes* to produce inflammation on the surface of the skin barrier formed in the platform and simulate a pathological condition occurring in vivo. They subsequently also tested the efficacy of two drugs used in the treatment of acne, namely polyphyllin H and dexamethasone. They found that the effect of these two drugs reduces the effect of pro-inflammatory cytokines, confirming previous studies carried out in vitro. Therefore the use of this IC-SoC proved effective in simulating an inflammatory condition useful for drug testing [48]. The skin-on-chip device can also be used to investigate the infection caused by the Herpes simplex virus, which infects the skin and mucous membranes [49,50]. Sun et al. used a skin-on-chip platform in which, by combining the microfluidic approach and vascularisation, the architecture of the human skin is simulated so that the immune response of the host in case of herpes simplex virus infection and the effect of antiviral drugs can be examined [50]. Other researchers have created skin-on-chips integrated with nerve fibres, which are present in vivo and are important for the perception of pain, temperature and other mechanical stimuli. They studied the growth of neurites from the dermis layer to the epidermis and this was demonstrated by immunofluorescence experiments. Later, to demonstrate the sensory function of the tissue, Martorina et al. used topical applications of capsaicin, which is a transient receptor protein-villanoid -1 (TRPV1) channel agonist, and the use of this compound results in the formation of calcium currents that show sensory function in the epidermis [47,51].

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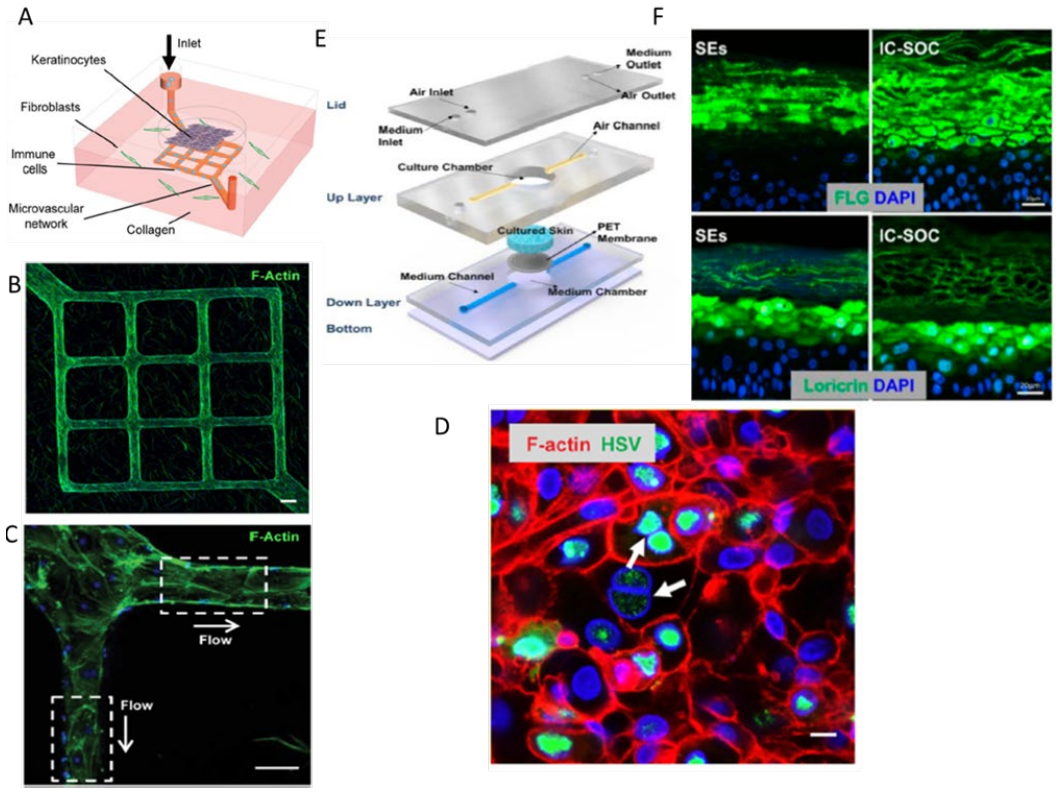

Figure 4. A) Skin-on-chip image by Sun et al. [50] represents the design of the device and the cellular components inside it. B) e C) Confocal microscopy images where in B) is shown in the grid the presence, after two weeks of culture, of the endothealised microvascular network in the dermis and outside the grid the presence of collagen. Figure C is an enlargement of part of Figure B and shows the direction of flow indicated by the white arrows and in detail the cell monolayer present in the grid. In both images, the actin filaments are coloured green and the cell nucleus is blue (DAPI) (Scale bar 100μm) [50]. D) Confocal microscopy image showing infection in the epidermis by herpes simplex virus (HSV) in the skin-on-chip. HSV is shown in green and the actin filaments are in red. White arrows indicate multi nuclei formation, nucleus enlargement and chromatin margination. (Scale bar 20 μm) [50]. E) Exploted view of the skin-on-chip system realized by Quan et al. [48] F) Fluorescence images show the comparison between the expression of filaggrin (FLG) and loricrin (stratum corneum proteins) in the static skin equivalent (SE) model and the controlled skin interface (IC-SoC). It can be seen that in the IC-SoC the stratum corneum is thicker and has the greater structural integrity of the dermal-epidermal junction (DEJ) than in the SE model due to the dynamic conditions of the IC-SoC. (Scale bar 20 μm.) [48].

5. Cornea-on-Chip

The cornea is the main lens of the eye and allows light rays to pass into the eye by converging the rays towards the retina; it also protects the eye from pathogens and injuries. It represents a barrier between the outside world and the eyeball and is composed of five layers, from the outermost inwards we find the corneal epithelium, Bowman's lamina, the stroma, Descemet's membrane and finally the endothelium.The transcorneal route represents the main route for the administration of ophthalmic drugs, which are predominantly for topical use. Therefore having a model that closely simulates the physiology of the cornea in vivo can help in the screening of ophthalmic drugs for local use. Animal models, such as rodents have significant differences in their visual apparatus compared to humans, for example, they do not possess the fovea, a retinal structure present in humans, and 85% of their optic nerves decay on the other side of the brain compared to humans. An animal model that best simulates the human eye is the eye structure of monkeys, which are, however, complicated to breed [52,53]. Today, 2D cell cultures and 3D models are more commonly used, but these have limitations. In recent years, however, new microfluidic platforms are being used that are also

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integrated with organoids that are better capable of mimicking the pathophysiology of the eye [54–57]. A group of researchers made a cornea on a chip to study the repair of epithelial wounds through extracellular vesicles. Zitong Yu et al. fabricated a chip consisting of two PDMS layers each containing a microfluidic channel and in the centre a circular structure called the culture zone, to simulate the structure of the human cornea, which has a porous polycarbonate membrane covered with extracellular matrix elements(Figure 5A). In addition, there is also a chamber for measuring transepithelial electrical resistance (TEER). In the chip, human corneal epithelial cells (HCEpi) were cultured in the upper channel and human corneal endothelial cells (HCEnd) in the lower channel. After verifying with immunofluorescence techniques and with TEER the formation of the corneal barrier, a corneal wound was simulated. Its repair was studied using vesicles derived from mesenchymal stem cells that produce a series of anti-inflammatory cytokines and also reduce the concentration of pro-inflammatory cytokines such as matrix metalloprotease 2 (MMP-2), which was analysed in this study [58]. Through the cornea-on-chip, it is also possible to mimic the blinking and study its mechanism, and this was seen by Seo et al. who created an in vitro dry eye disease model that features a 3D cell culture scaffold consisting of primary human keratinocytes representing the subepithelial stroma within a hydrogel. The scaffold is attached to a perfusion chamber and a biomimetic eyelid, as shown in Figure 5C, that can be mechanically operated, and the conjunctival and corneal epithelium was reproduced on the scaffold using a 3D cell modelling technique that allows for the assembly of different multilayer cell structures. This device may pave the way for the study of human ocular mechanisms and the discovery of new ophthalmic drugs [59].

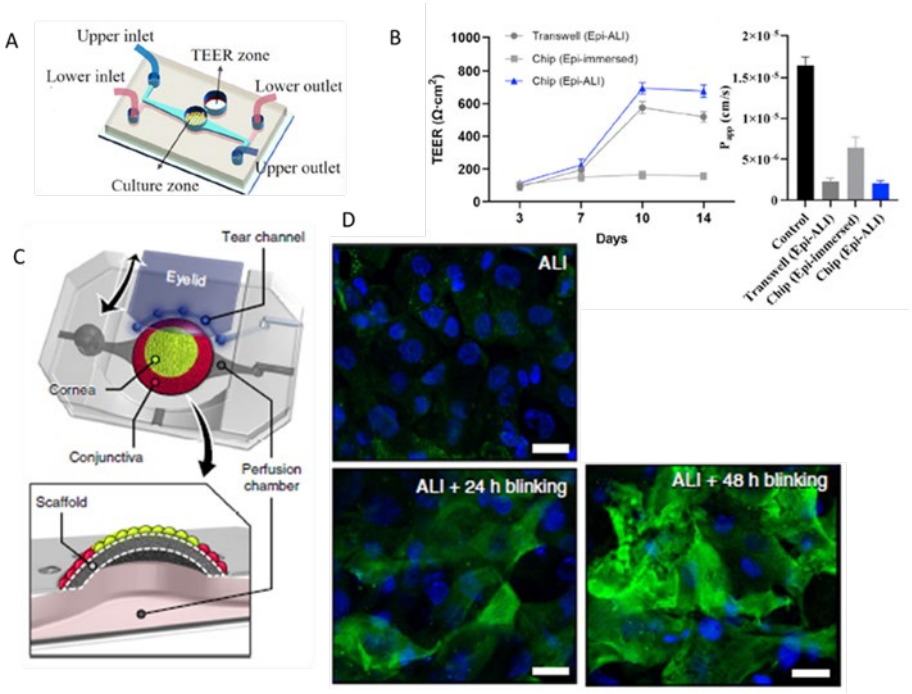

Figure 5. A) Graphic representation of the cornea-on-chip by Yu et al. [58] B) The left picture shows a graph with TEER values as a function of days: the permeability of HCEpi cells is measured under three different conditions, transwell EPI(epithelial) -ALI(air-liquid interface), EPI-Immersed chip, EPI-ALI chip at day 3, day 7, day 10 and day 14. The image on the right shows a graph obtained by measuring the permeability coefficient (Papp) of the corneal epithelium using 5kDa FITC-dextran in three different situations (transwell EPI-ALI, EPI-Immersed chip EPI-ALI and a control situation without the cells). Both graphs show that cell permeability and thus barrier integrity is higher in the EPI-ALI chip [58]. C) Image of an eye-on-a-chip by Seo et al. [59] D) Fluorescence microscopy images showing corneal epithelial cells cultured at the air-liquid interface to simulate the organ environment in vivo. The upper left image shows a situation where cells are subjected to submerged culture for two days followed by three days of ALI culture without blinking. Only the nucleus can be seen in blue (DAPI). In the lower image on the left the cells are in submerged culture for two days followed by one day of ALI culture

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without blinking and one day of ALI+ blinking, cytokeratin 3/12 (CK- 3/12) which is a protein specific to terminally differentiated corneal epithelial cells is highlighted in green. In the lower right image, on the other hand, the cells are in submerged culture for two days followed by one day of ALI culture without blinking and two days of ALI culture with blinking, and it can be seen that the presence of CK-3/12 is greater, indicating greater differentiation of the corneal epithelial cells (Scale bar 20μm.) [59].

6. Airway-on-Chip

Another biological barrier of the human body is represented by the respiratory system, which includes the nose, pharynx, larynx, trachea, lungs, pleura, bronchi and bronchioles, which not only allow gaseous exchanges between the external environment (oxygen) and the human being (carbon dioxide), but also protect him or her from dust particles, soot, and other pathogenic microorganisms that can settle on the airways. Among the protective mechanisms are the cilia present on the cells of the airways that together with a layer of mucus trap pathogens and particles to prevent access to the lungs, and the macrophages present on the surface of the alveoli that phagocytose and kill potentially dangerous microorganisms [60–62].

Animal models, 2D and 3D in vitro cell culture models with spheroids and organoids are used to investigate lung disease, but each has limitations such as the difficulty in mimicking the mechanical stimulus of respiration, the absence of perfusion, an air-liquid interface (ALI) and the inability of the animal model exposed to smoke to reproduce disabling lung disease. To overcome these problems, microfluidic platforms capable of mimicking the functional unity of the lung and integrating mechanical stimuli and the presence of an air-liquid interface (ALI), or the alveolar-capillary interface (ACI), come to the rescue. Moreover, they can also be integrated with biosensors that can detect analytes (biomolecules and microorganisms) and thus monitor the progress of pathology [63–66].

Henry et al. developed a polycarbonate organ-on-chip device, integrated with electrodes, which has two channels separated by a porous PET membrane. The electrodes, two for the upper layer and two for the lower layer allow real-time measurement of the transepithelial electrical resistance of the monolayer of primary human airway epithelial cells (hAECs) for up to 62 days of culture [67].

Khalid et al. instead developed a platform for lung cancer on a chip made of two layers of glass covered with indium tin oxide (ITO) electrodes for TEER impedance sensing to assess the cytotoxicity of certain drugs used to treat lung cancer. In addition, the chip is also connected to a sensor for pH monitoring. In the upper layer of the chip is a microfluidic channel where human lung adenocarcinoma cells (NCI-H1437) were seeded and treated with doxorubicin and docetaxel, which are two anti-cancer drugs. It has been seen that doxorubicin is more toxic to cells than docetaxel and this has been demonstrated with the TEER impedance response by assessing the cellular index (CI), cell pH monitoring and cell viability assays. This sensor-integrated microfluidic device could be used to assess the cytotoxicity of any new drug or compound [68].

In the last two years, the presence of Sars-CoV-2 has led to an increased demand for new approaches and methodologies to discover effective prophylaxis and therapies against it [69–71]. Longlong Si et al. [70] realised a microfluidic bronchial-airway-on-chip device to study the pulmonary response following Sars-CoV-2 and influenza A infection and to test drugs to be used to treat viral infections or to prevent them. Longlong Si et al. realised a microfluidic bronchial-airway-on-chip device to study the pulmonary response following Sars-CoV-2 and influenza A infection and to test drugs to be used to treat viral infections or to prevent them.

The chip has two layers of polydimethylsiloxane (PDMS), each containing a microfluidic channel separated from the other by a porous PET membrane. The upper layer is called the air channel,in which the primary basal stem cells of the human lung bronchial airway epithelium have been seeded and in the lower channel, called the blood channel the endothelial cells (Figure 6A). After creating the air-liquid interface (ALI) in the chip to restore physiological lung conditions in vivo, infection by the influenza A PR8 (H1N1) virus expressing the GFP protein is simulated by introducing it into the air channel. The researchers noted using of fluorescence microscopy techniques, that following infection by the PR8 virus, epithelial damage, destruction of occluding junctions and loss of cilia

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occur, and the extent of infection is more significant in the on-chip ALI than in the undifferentiated basal epithelium before formation of the air-liquid interface. They then compared the immune response occurring in the chip, caused by different influenza strains such as H5N1, H3N2 and H1N1 and saw that the viruses causing a more severe pathogenesis with a greater release of cytokines and chemokines were H3N2 and H5N1 . They then compared the immune response occurring in the chip caused by different influenza strains such as H5N1, H3N2 and H1N1 and saw that the viruses causing more severe pathogenesis with a greater release of cytokines and chemokines were H3N2 and H5N1. Exploiting the characteristics of the airway chip Si et al. tried to identify drugs to block the entry of Coronavirus: they used the hepatocyte-derived carcinoma cell line (Huh-7) and viral particles that have a pseudo-S-protein, which serves the virus to enter host cells. After seeing that these particles can mimic Coronavirus infection, they tested several antiviral drugs including verapamil, arbidol, amiodarone, hydroxychloroquine, chloroquine and amodiaquine and the results showed that the most potent inhibitor of Sars-CoV-2 infection is amodiaquine together with its metabolite.

The airway-on-chip microfluidic platform could accelerate the discovery of useful prophylactic procedures to stop the severe acute respiratory syndrome coronavirus (Sars-CoV-2) in the world [70,71].

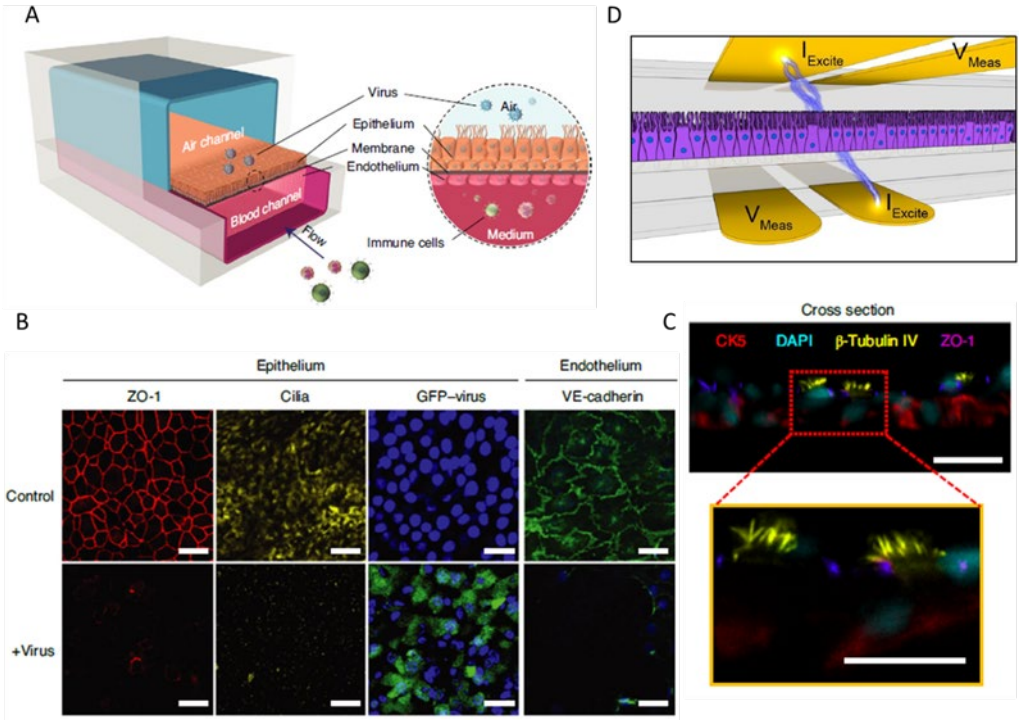

Figure 6. A) Drawing of a cross-section of the airway chip [70]. B) Immunofluorescence images showing cell-cell adherens junctions such as ZO-1 (red) in the epithelium and VE-cadherin in the endothelium (green) of the airway chip both in the absence (top) and presence (bottom) of influenza virus, which expresses green fluorescent protein (GFP). Also shown in the image are the cilia (yellow) of the epithelial cells and we can see that in the presence of the virus, there is an injury to the epithelium and endothelium with loss of cilia and damage to the adherens junctions. [70] C) Immunofluorescence images showing a cross-section of the pseudostratified epithelial layer of the human airway with cells expressing cytokeratin 5 (CK-5) in red and beta-tubulin IV in yellow as well as DAPI in blue and ZO-1 in purple. [70] D) Schematic representation of the TEER-chip.A current is applied between two electrodes (Iexcite) positioned above and below the cell culture and the potential drop between two other electrodes (Vmas) is measured [67].

7. Gastrointestinal Barrier-on-Chip

The gastrointestinal barrier protects what we introduce from outside with our food, thus acting as an active and extremely precise filter. The defence mechanism of the digestive system already takes

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place in saliva, thanks to an antibacterial enzyme called lysozyme, which has a protective action against possible microorganisms introduced with food. Going down into the stomach, there is gastric acid, which with its low pH allows the breakdown of the bacterial cell barrier. Finally, in the intestine, there is a barrier, consisting of intestinal epithelial cells connected by tight junctions and covered with mucus, that blocks the passage of potentially dangerous substances such as microorganisms and allergens [72]. In addition, the protective action of the gut is also exerted by the collection of 'good' bacteria (intestinal microbiota) and the gut-associated lymphoid tissue (GALT), which represents the immune system in the digestive tract that has the complex task of tolerating the presence of commensal bacteria and responding to pathogenic microorganisms. [73–76]

2D animal models and cell cultures have also been used in the gastrointestinal barrier to understand the molecular mechanisms underlying diseases and to discover new drug treatments. However, researchers are aware that these tools have some disadvantages: 2D cultures cannot mimic intestinal peristalsis and do not replicate the 3D architecture of the organ, and for animal models, we may have inconsistencies between animal and human responses to the same drug. Organ-on-chip can come to the rescue to overcome these limitations. [77–80]

Jeong et al. developed a polydimethylsiloxane device to mimic the human stomach through the use of organoids and microfluidic techniques. The platform has two layers each containing a channel separated by a porous polyethylene terephthalate (PET) membrane, in the upper channel, epithelial cells derived from human antral organoids (hAOs) are seeded, and in the lower channel, primary gastric mesenchymal stromal cells (gMSCs). The organoid-derived cells provide gastric stem cells that can to differentiate and are an important resource for the formation of the gastric mucosal barrier, in addition, the presence of fluid flow and communication between the two cell lines mimics gastric homeostasis and mucosal function. After successfully reproducing the gastric microenvironment, they simulated Helicobacter pylori infection by introducing the bacterium into the upper compartment and in the lower channel instead, they seeded human peripheral blood mononuclear cells (PBMCs). Due to the crosstalk between the gastric cells and immune system cells, the results showed increased expression of cytokines and nuclear factor KB(NF-KB) compared to in vitro assays, demonstrating that this platform can be used to study gastric defence mechanisms and for the development of pharmacological therapies [81].

To study the interactions between the various types of bacteria that can be found in the gut, a team of researchers led by Maurer used a microfluidic device supplied by ChipShop that has two chambers separated by a PET membrane to recreate the gut architecture and to simulate a fungal infection by C. albicans.

They recreated a multi-layered epithelium-endothelium tissue using human colorectal carcinoma epithelial cells (Caco-2) in the lower channel of the chip and human umbilical cord vein endothelial cells (HUVEC) in the lower channel and characterised the barrier formation with immunochemical and electron microscopy techniques. They then introduced Lactobacillus rhamnosus (L. rhamnosus), which is a probiotic bacterium that populates the intestine, and Candida albicans (C.albicans), which is a harmless yeast that can populate the intestinal tract but could also become pathogenic in some situations, into the chip. They saw how the presence of L. rhamnosus can limit the growth of C.albicans and can also reduce the yeast's passage to the vascular compartment. Maurer et al., therefore, demonstrated the usefulness of the gut on a chip to study the intestinal homeostasis exerted by the bacteria present in it [82].

In the intestine, there is an increasing concentration of oxygen from the inside (from the lumen) to the outside (intestinal mucosa) and the maintenance of this oxygen gradient is important to allow the intestinal barrier to perform its protective functions. In the event of a loss of this balance, which can occur through inflammation of the intestine or bacterial infection, free radicals are formed which are harmful to cells [83]. Shah et al. recreated a microfluidic platform (HuMiX), as shown in Figure 7E, to ensure the cross-talk between intestinal epithelium (Caco-2) and the different types of bacteria living in the gut, which can live under aerobic conditions, such as Lactobacillus rhamnosus, or anaerobic conditions such as Bacteroides Caccae. Unlike the other gut-on-chips described above,

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oxygen sensors are also integrated here to monitor the oxygen gradient in real time, generated with oxic and anoxic culture media, to allow the growth of aerobic and anaerobic bacteria. In this way, the HuMiX platform proved to be a useful tool for investigating the relationship between gut and microbiota, which is also important for the study of intestinal diseases [84].

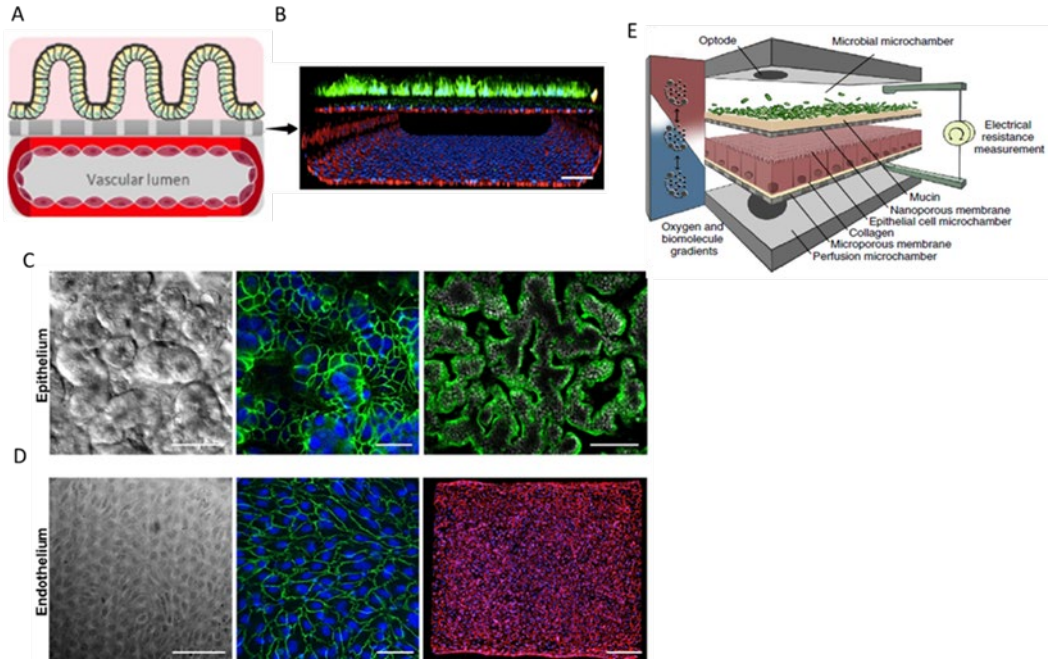

Figure 7. A) Graphic representation of the intestinal villi grown on the membrane of the upper channel and the endothelial lumen formed in the lower channel [83]. B) Confocal microscopy image of a cross-section of the gut-on-chip showing the epithelial villi in green, the endothelium stained red for the presence of VE-cadherin to show adherent junctions, and the nuclei in blue (bar 100μm) [83]. C) DIC image on the left showing the morphology of the villi of the intestinal epithelium consisting of Caco-2 cells kept in culture for 5 days in the gut-on-chip. (bar 50 μm). Centre and right fluorescent microscopy images showing ZO-1 occluding junctions (green, centre, bar 50 μm) and villin (green, right, bar 100 μm). The nucleus is shown in blue (DAPI) [83]. D) Phase-contrast microscopy image of the endothelium kept in culture for 5 days in the gut-on-chip (bar 50 micrometres). Middle and left images of fluorescence microscopy show cell junction-associated proteins including PECAM-1 (green, middle, bar 50 micrometres) and VE-cadherin (red, right, 200 micrometres) while the nuclei are shown in blue [83]. E) Exploted device design by Humix et al. containing a co-culture of human epithelial cells and gastrointestinal tract bacteria [84].

8. Testis-on-Chip

The blood-testicular barrier also known as the Sertoli cell barrier is located in the seminiferous tubules and consists of Sertoli cells and occluding junctions. Its function is to separate the blood vessels from the seminiferous tubules of the testicles to prevent the reflux of immature cells between the basal zone, where the primary spermatocytes and spermatogonia are found, and the adluminal zone where the secondary spermatocytes are located. In addition, the blood-testicular barrier protects the germ cells from possible toxic substances in the blood, preventing them from passing through and preventing substances from the germ cell maturation process that would be attacked by the immune system from passing through the blood [85–87]. The need to develop a testis-on-chip arises from the fact that it simulates the microenvironment of testicular tissue optimally, by introducing physical and chemical stimuli, continuously supplying nutrients, and hormonal stimuli and removing metabolic products through perfusion of the chip by simulating the vascular system [88]. This could be useful to better study the process of spermatogenesis and provide a solution for pre-pubertal cancer survivors for whom there is no hope of becoming parents. Sharma et al. [85] explored

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a microfluidic platform's capacity to study an ex vivo culture of seminiferous tubules of prepubertal marmosets. The device consists of a special chamber in PDMS for ex vivo tissue culture of seminiferous tubules that separates, using of pillar arrays, a perfusion channel simulating the vascular system. The researchers stimulated, using continuous perfusion, the seminiferous tubule tissue of prepubertal marmosets with high and low doses of gonadotropins, hormones produced by the adenohypophysis that regulate the development of the male and female genital organs. They then analysed the tissue response through histological analysis and by determining serum testosterone and oestradiol levels with ELISA tests. What they observed is that when stimulated with high doses of gonadotropins, the epithelium of the seminiferous tubules appears more organised and more complete with Leydig cells, Sertoli cells and germ cells, demonstrating the importance of hormonal stimulation on the endocrine capacity of the seminiferous tubules of prepubertal marmosets and the ability of the microfluidic platform to also integrate the vascular system to better mimic the physiology of the testicular apparatus [85]. One way to study the effect of drugs and their metabolites at the testicular level may be to create a multi-organ platform in which the testicular and hepatic compartments are mimicked, where metabolic activation by enzymes such as cytochrome P450 (CYP450) occurs for many drugs. Baert et al. [89] performed a co-culture, in a microfluidic device, between human liver spheroids consisting of HepaRG cells and primary human liver stellate cells and human testicular organoids obtained from patients with complete spermatogenesis undergoing bilateral orchiectomy. The chip features a circuit consisting of a larger central compartment for the testis connected via microfluidic channels to two smaller lateral compartments one for the liver and the other for the culture medium (Figure 8C) and also features a peristaltic micropump on the chip capable of generating a continuous pulsatile flow. The researchers studied the effect of cyclophosphamide, a chemotherapy drug, on spermatogonia, which are the precursors of spermatozoa. Cyclophosphamide is metabolised by the liver to 4-hydroxycyclophosphamide, enters cells and binds to DNA causing replication inhibition and thus cell apoptosis. However, this molecule causes cell death not only of cancer cells but also of healthy cells, including spermatozoa, and causes gonadotoxicity. In this experiment, a reduction in germ cells was seen following treatment with cyclophosphamide in the situation where there is a co-culture between germ cells and liver cells, which activate cyclophosphamide. In contrast, in the single germ cell culture, it has been shown that more cells survive following cyclophosphamide treatment. This study shows the importance of the multi-organ platform, especially in the case of testing prodrugs, which are metabolised in the liver before acting on the target organs. Without the presence of the multi-organ (liver and target organ), one could make incorrect assessments that do not take into account physiological reality and do not take into account possible side effects as well as benefits [89].

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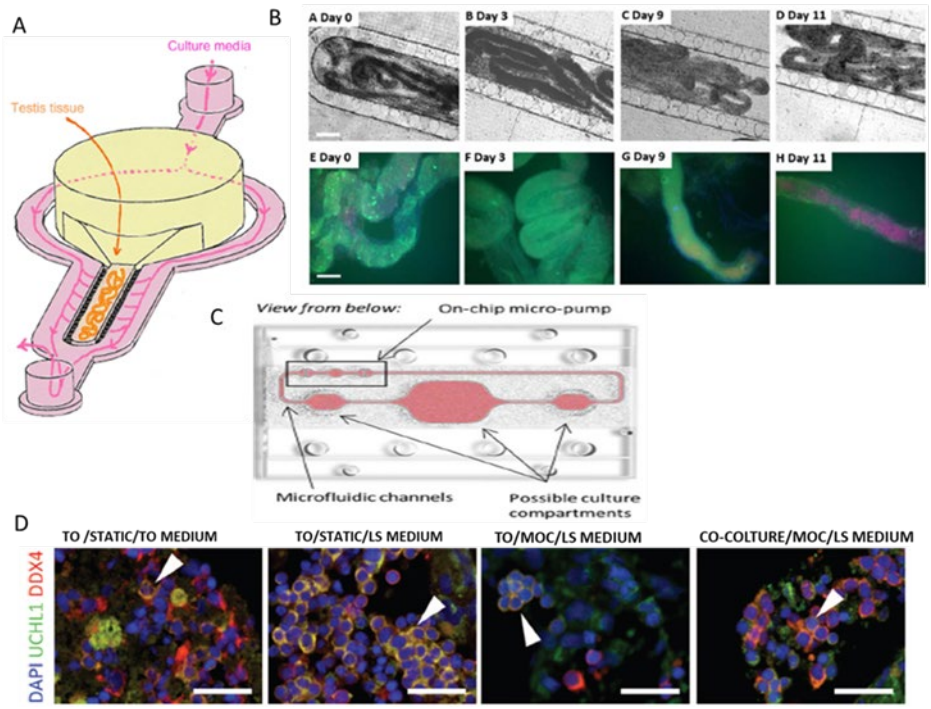

Figure 8. A) Sketch representation of the testis-on-chip device developed by Sharma et al. [85] to study primate testicular tissue. It has a central chamber containing the tissue (yellow) surrounded by a perfusion channel representing the vascular system (pink). The chip is continuously perfused and the two structures are separated by a series of pillars. B) Images A to D are from light microscopy and show the maintenance of tissue integrity in the chip up to day 11. Images E to H show a live/dead assay with calcein (green) highlighting live cells and propidium iodide (red) indicating dead cells. This assay was used to highlight the cell viability of the primate seminiferous tubules on the chip [85]. C) Bottom view of the multi-organ platform including liver and testes by Baert et al. [89] D) Fluorescence microscopy images of testicular organoids (TO) under static conditions and in multi-organ-on-chip (MOC) containing liver spheroid medium (LS). The DAPI in blue indicates the nucleus while the staining of UCHL1 (green) was combined with that of DDX4 (red), a germ cell marker, for the detection of spermatogonia (shown by the white arrows) [89].

9. Placenta-on-Chip

The placenta is an organ that forms in the uterus during pregnancy and it has several functions: it allows gaseous exchanges between the blood of the mother and that of the foetus, it provides nutrients to the foetus, it allows the passage of antibodies and blocks the passage of certain pathogens and other harmful substances, although some molecules and viruses can pass through it and cause damage to the foetus. We can define the placenta as a kind of barrier made up of several layers, which are composed of different types of cells. These include cells that originate from the blastocyst and are subtypes of trophoblasts such as syncytiotrophoblasts, extravillous trophoblasts, trophoblast giant cells and villous cytotrophoblasts. In addition, there are also decidua cells originating from the uterine endometrium and placental macrophages [25,90–92].

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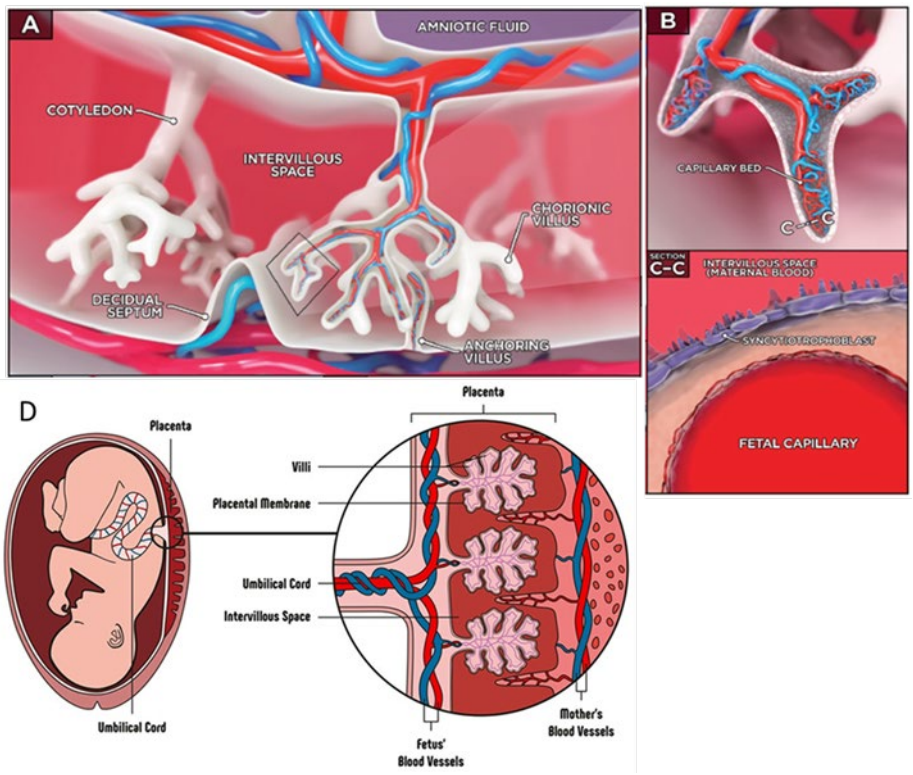

Figure 9. A) Three-dimensional image of some elements that make up the human placenta. Cross section of the cotyledon, chorionic villus and anchor villus [93]. B) Zoom of a cross- section showing the fetal capillaries contained in the chorionic villus [93]. Section C-C) Placental barrier separates the fetal capillaries from the maternal intervillous space and consists of the endothelial cells of the fetal capillaries, a layer of interstitial tissue, and the syncytiotrophoblast [93]. D) On the right image of a fetus inside the placenta connected to it via the umbilical cord. The left schematic representation of a part of the placenta showing maternal blood vessels, chorionic villi, intervillous space, fetal blood vessels and umbilical cord [94].

Exposure of the foetus to drugs or other molecules is a major problem during pregnancy. To study the effects of a drug taken during pregnancy, tests can be carried out on animals, but these can give inconclusive results due to the different structures of the human placenta compared to the animal placenta [93]. Placental transport studies have also been tested on humans, but they are time-consuming and always represent a risk to the foetus. Cell cultures are also not effective enough for placental studies as they cannot faithfully reproduce the placental environment and physiological tissue architecture [91]. One approach to solving these limitations is the realization of the placenta-on-chip, a microfluidic device that mimics the physiological system of the placenta by using human cells to simulate drug transport across the placental barrier [93,95–97]. Blundell et al. [93] created a placenta-on-chip model that simulates the transport through the breast cancer resistance protein (BCRP), which is located on the apical side of the trophoblast cells of the placenta, of a drug used in pregnancy to treat diabetes, glyburide, a substrate of BCRP. The device is composed of two PDMS layers, an upper and a lower one in between which is a semi-permeable polycarbonate membrane coated with fibronectin. Within these compartments, a co-culture is performed: human trophoblasts seeded in the upper layer (maternal) and human placental endothelial villous cells in the lower layer (fetal) undergo continuous perfusion to ensure the formation of a trophoblast-endothelium interface similar to that present in the human placenta in vivo. After verifying the presence of a functional and structural barrier by immunofluorescence staining, permeability tests and TEER, Blundell et al. investigated glyburide transport through the BCRP by performing it both in the presence of trophoblasts on the membrane and in the absence. They saw that the amount of drug perfused remained unchanged in the maternal compartment (upper layer) without cells. In contrast, in the presence of trophoblast cells, the glyburide concentration was lower, suggesting uptake of the drug

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by cells in the upper layer. They, therefore, demonstrated the use of placenta-on-chip can be used for the analysis of drug transport across the placental barrier. However, this model represents the structure of the human placenta at the final stage of pregnancy and is not sufficient to investigate drug transport during the first months of gestation because the placental tissue has a different architecture during this stage than during the last months. The placenta-on-chip may also help study inflammation of the placenta, which can be caused by bacterial infections, and which often leads to preterm deliveries of the newborn, that is, before 37 weeks, which is the predominant cause of neonatal morbidity and mortality. Placental inflammation is characterized by loss of placental function and the presence of inflammatory substances that can damage fetal organs. Zhu et al. [98] created a dynamic placenta-on-chip platform to examine the inflammatory reaction to placental bacterial infection triggered by Escherichia Coli (E.Coli). The device is made of PDMS and has an upper channel where there are BeWo (human trophoblast cell line) cells that mimic the maternal environment and a porous polycarbonate membrane that separates it from the lower compartment where HUVECs (human endothelial cells) have been seeded, which simulate the fetal microenvironment. They inserted E.Coli into the maternal compartment to simulate infection and human macrophages (THP-1) on the trophoblast layer of the placental-on-a-chip. They saw that trophoblasts produced some inflammatory cytokines including IL-1α, IL-1β, and IL-8 and there was also activation of maternal macrophages, thus confirming the cross-talk, which occurs in vivo during gestation, between trophoblasts and maternal macrophages. In addition, when there is placental inflammation, there is also elevated production of inflammatory cytokines in fetal blood vessels. Researchers have seen that even using a microorganism (E.coli) that cannot cross the membrane, endothelial cells release inflammatory cytokines and this is an increase in the presence of trophoblasts, going to confirm the communication that there is between these two cell types. This device can be used to simulate bacterial infections that can occur in the human placenta and be used as a tool for the discovery of new drugs against the treatment of placental infections. However, an upgrade of the device could be the use of pluripotent stem cells to obtain trophoblasts as was tested by Lermant et al. [24], and this could improve the characteristics of the platform. Other immune cells such as granulocytes and T cells could be included in the device to make the model more predictive [98].

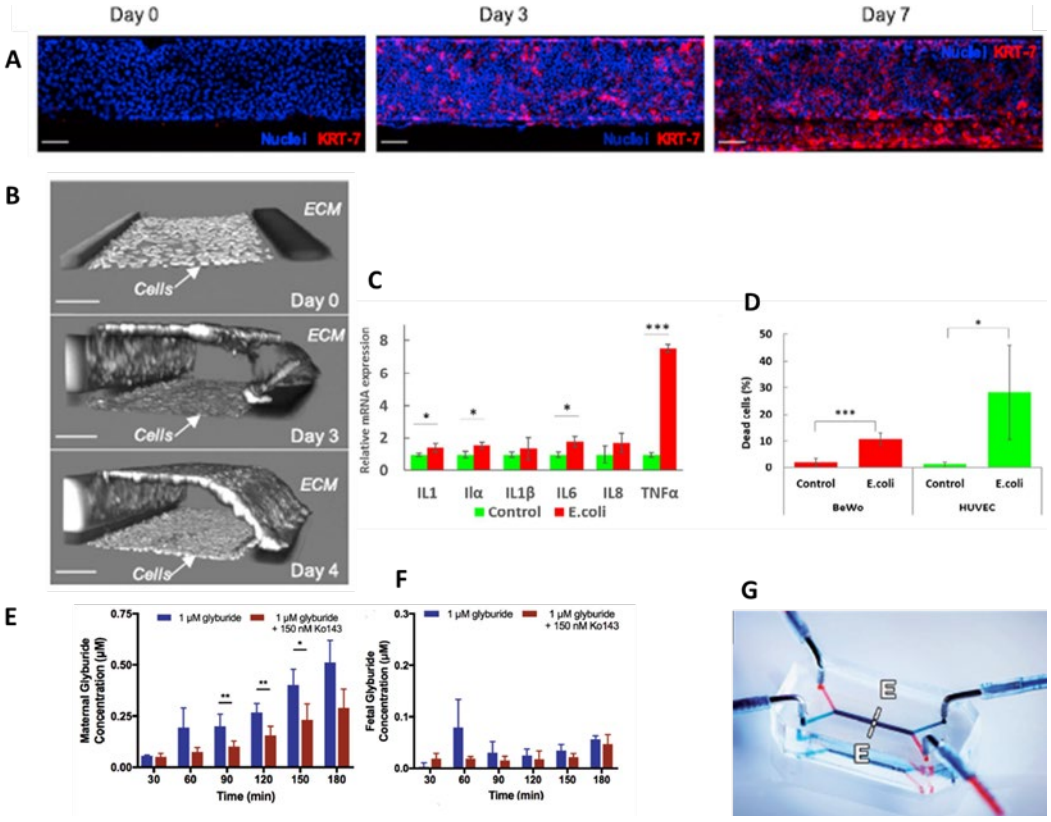

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Figure 10. A) Fluorescence microscope image of cells fixed at day 0, day 3 and day 7 following BAP treatment. Nuclei were labelled with Hoechst, in blue, and KRT-7, a marker of trophoblasts, in red [24]. B) 3D confocal microscope image at day 0, day 3 and day 4 following BAP treatment, which is bone morphogenetic protein 4 (BMP4) treatment of human induced pluripotent stem cells (hiPSCs) coupled with inhibition of basic fibroblast growth factor (FGF-2) and activin/nodal signalling. The nuclei were labelled with Hoechst (scale bar 100um) [24]. C) Graph illustrating a quantitative real-time PCR showing relative expression with E. coli and without. * $P < 0.05$, *** $P < 0.001$. [98]. D) Graphical representation showing the percentage of dead cells (trophoblasts and HUVECs) in the presence of E. coli and its absence within the chip [98]. E) To test the active transport function of BCRP, Blundell et al. administered a BCRP inhibitor, Ko143, and saw that there was a decrease in maternal glyburide levels compared with the control situation (without Ko143) [93]. F) In contrast, fetal glyburide levels in the presence of an inhibitor are not significantly different from those in the control group without an inhibitor [93]. G) Photo of the micro-engineered model representing the placenta-on-chip [93].

Table 2. Summary of the main characteristics, cell lines, preclinical applications and various problems of Organ-on-chips discussed above.

BARRIERS-ON-CHIP	KEY FEATURES	CRITICAL ISSUES	CELL LINES	PRECLINICAL APPLICATIONS
Blood–brain barrier-on-chip	• Blood-brain barrier generation with endothelial cells(hMEC/D3), astrocytes and bovine pericytes in a 3D extracellular matrix. [17–19] • Presence of mechanical stimuli to simulate flow in blood vessels. [18,19] • Presence of biosensors to monitor the integrity of the blood-brain barrier. [15–17]	• Lack of standardization for quantitative assessment of blood-brain barrier function [15].	Endotelial cell: hCMEC/D3 Astrocytes Bovine pericytes. [17–19]	Optimization of drug passage through the BBB for studies of neurodegenerative diseases and tumor. [15–17]
Blood-retinal barrier-on-chip	Generate an endothelial and an epithelial compartment to simulate iBRB and oBRB, respectively. [20,21] • Presence of electrodes to monitor the formation of occluding junctions through TEER. [39,40]	• Difficulty maintaining a cell culture for a long time to day so as to study chronic diseases, such as maculopathies [20,21]. • Lack of standardization makes it difficult to compare different models. [20,21]	• Primary human retinal endothealial cells (HRECs) [39]. • Human neuroblastoma cell line (SHSY5Y) [39]. • Human retinal pigment epithelium cell line (ARPE-19). [39,40] • Human Umbilical Vein Endothelial Cells (HUVEC) [40].	• Study of diabetic retinopathy, age-related retinopathy [39]. • Study of age-related macular degeneration. [39] • Study of angiogenesis under physiological and non-physiological conditions. [40]
Skin-on-chip	• Making of the different layers that make up the skin,	• Lack of important components, such	• Immortalised human keratinocytes (HaCaT). [48]	Pharmacological study for the treatment of acne [48].

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	using heterogeneous cell types. [48,50] • Presence of cell-matrix interaction. [25,41,48] • Presence of airflow for keratinocyte differentiation. [48]	as vascular or immune [48]. • Optimization of culture time and medium composition. [47,51]	• Dermal fibroblasts. [48] • Immune cells. [50]	• Study of viral infections [50]. Pharmacological study of the skin sensory system. [47,51]
Cornea-on-chip	Formation of an epithelial and an endothelial corneal compartment, comprising elements of the extracellular matrix. [58]. • Presence of a biomimetic eyelid to mimic the blinking of eyelashes. [59].	• Lack of stromal behavior may not help in treating more severe lesions [58].	Human corneal epithelial cells (HCEpi) [58] • Human corneal endothelial cells (HCEnd) [58] • Primary human keratinocytes [59].	• Study of ocular mechanisms and discovery of new ophthalmological drugs. [59]. • Corneal epithelial wound repair investigation. [58]
Airway-on-chip	• Presence of mechanical stimulus to mimic breathing and presence of air-liquid interface(ALI). [63,64,67,68,70] Presence of biosensors [63,64,67,68]. • Presence of two compartments, to mimic the epithelium(subjected to the presence of air)-endothelium interface. [67,68,70]	• Biomechanical ventilation generation [99]. • Differentiation process of hAECs in 62 days of culture [36].	• Primary human airway epitelial cells (hAECs). [36] • Human lung adenocarcinoma cells (NCI-H1437). [68].	• Monitoring the cytotoxicity of drugs used to treat lung cancer [68].. • Drug testing for the treatment of viral infections or to prevent them (ie, Sars-CoV-2). [70]
Gastrointestinal barrier-on chip	• Presence of two membrane-separated chambers to simulate cross-talk between primary gastric cells and antral epithelial cells [81]. • Presence of multilayer to recreate the intestinal epithelial-endothelium [82,84]. • Integration of sensors to monitor oxygen concentration for growth of anaerobic bacteria in the gut. [84]	• Challenges in gastric organoid formation. [81] • Ensure anaerobic conditions for the growth of anaerobic bacteria. [84]	• Primary gastric mesenchymal stromal cells (gMSCs) [81]. • Epitelial cells derived from human antral organoids (hAOs) [81]. • Human peripheral blood mononuclear cells (PBMCs) [81] . • Human umbilical cord vein endothelial cells (HUVEC) [82].	• Investigate gastric defence mechanisms and develop drug therapies [81]. • Simulating fungal infections in the gut to identify new drug therapies. [41] • Study of interactions between microbiota and gut to better understand gut diseases. [84]

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Testis-on-chip	• Existence of multiple compartments to simulate the blood-testicular interface. [85]	• Difficulty in creating an on-chip multi-organ that involves the testicular apparatus and another tissue (e.g.,liver). [85]	• Human colorectal carcinoma epithelial cells(Caco-2) [82]. • Ex vivo tissue culture of seminiferous tubules of prepubertal marmosets [85]. • human liver spheroids(HepaRG cells). [89] • Primary human liver stellate cells [89]. • Human testicular organoids [89].	• Analysis of the effect of drugs(i.e.,chemotherapeutics) and their metabolites at the testicular level [89]. • Ex vivo tissue studies to understand the effect of hormonal stimulation. [85]
Placenta-on-chip	Presence of the trophoblast-endothelium interface across two compartments separated by a membrane. [93,98]	• Lack of cell lines characterizing the early stage of gestation [93]. • Difficulty in carrying out studies of drug transport across the placenta during the early months of gestation [93].	• Human trophoblasts(BeWo) [93,98]. • Human placental endothelial villous [93]. • Human umbilical cord vein endothelial cells (HUVEC) [98]. • Human macrophages (THP-1) [98] • Pluripotent stem cells [24].	• Study of the effects of a drug on the fetus [93]. • Investigate bacterial infections at the placental level that can lead to preterm fetal death [98].

10. Conclusion

Biological barriers are crucial in the organism as they help maintain the homeostasis of the body, and the malfunction or destruction of them can lead to a diverse range of diseases. Traditional methods, such as animal models or 2D cell cultures, can be used to study different biological barriers and to investigate drug therapies to be used for diseases due to issues involving barriers [1–4]. However, these tools have some limitations, which can be overcome by the use of organ-on-chips, which allow for better mimics of tissues by also introducing mechanical stimuli and the possibility of taking advantage of co-cultures of different cell lines and even with bacteria and immune system cells [10–12,17]. In addition, biosensors can be integrated into these microfluidic systems to monitor cellular activity in real-time. However, nowadays, the evolution of on-chip organs requires biosensors with higher performance, specificity and sensitivity [99]. Moreover, due to the different designs that each microfluidic platform possesses to best represent the biological barrier of interest, it is difficult to compare the results obtained between two different organs-on-chip [2]. Another limitation of organs-on-chips is the lack of industrial-scale production [91]. Regarding other advantages of these devices, there is the possibility of integrating induced pluripotent stem cells

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(iPSCs), which are derived from the patient, to obtain useful models for the study of personalized medicine and individualized therapies [2,18,19,100]

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