Biosensors and Bioelectronics 231 (2023) 115271

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Organs-on-chips technologies – A guide from disease models to opportunities for drug development

Anna Grazia Monteduro [a,1], Silvia Rizzato [a,1], Giusi Caragnano [a], Adriana Trapani [b], Gianluigi Giannelli [c], Giuseppe Maruccio [a,*]

[a] Omnics Research Group, Department of Mathematics and Physics "Ennio De Giorgi", University of Salento and Institute of Nanotechnology, CNR-Nanotec and INFN Sezione di Lecce, Via per Monteroni, 73100, Lecce, Italy
[b] Department of Pharmacy-Drug Sciences, University of Bari "Aldo Moro", Bari, Italy
[c] National Institute of Gastroenterology IRCCS "Saverio de Bellis", Research Hospital, Castellana Grotte, Bari, Italy

ARTICLE INFO

Keywords:
Organ on chip
Microphysiological systems
Multiorgan models
Lab on chip
Drug screening
Drug research
Disease models
Cancer
Microfluidics
Biosensors

ABSTRACT

Current in-vitro 2D cultures and animal models present severe limitations in recapitulating human physiopathology with striking discrepancies in estimating drug efficacy and side effects when compared to human trials. For these reasons, microphysiological systems, organ-on-chip and multiorgans microdevices attracted considerable attention as novel tools for high-throughput and high-content research to achieve an improved understanding of diseases and to accelerate the drug development process towards more precise and eventually personalized standards. This review takes the form of a guide on this fast-growing field, providing useful introduction to major themes and indications for further readings.

We start analyzing Organs-on-chips (OOC) technologies for testing the major drug administration routes: (1) oral/rectal route by intestine-on-a-chip, (2) inhalation by lung-on-a-chip, (3) transdermal by skin-on-a-chip and (4) intravenous through vascularization models, considering how drugs penetrate in the bloodstream and are conveyed to their targets. Then, we focus on OOC models for (other) specific organs and diseases: (1) neurodegenerative diseases with brain models and blood brain barriers, (2) tumor models including their vascularization, organoids/spheroids, engineering and screening of antitumor drugs, (3) liver/kidney on chips and multiorgan models for gastrointestinal diseases and metabolic assessment of drugs and (4) biomechanical systems recapitulating heart, muscles and bones structures and related diseases. Successively, we discuss technologies and materials for organ on chips, analyzing (1) microfluidic tools for organs-on-chips, (2) sensor integration for real-time monitoring, (3) materials and (4) cell lines for organs on chips. (Nano)delivery approaches for therapeutics and their on chip assessment are also described. Finally, we conclude with a critical discussion on current significance/relevance, trends, limitations, challenges and future prospects in terms of revolutionary impact on biomedical research, preclinical models and drug development.

1. Introduction

Fighting human diseases and improving life expectations are key challenges for the modern society. In these efforts, two major routes can be identified: on one side, a better understanding of disease mechanisms and the involved pathological processes; on the other, the development of effective therapeutic strategies for disease treatment with limited side effects and impact on life conditions. Organs-on-chips (OOC) technologies have the ambition to provide a resource-efficient response to this demand in the form of miniaturized microphysiological systems for biomedical research.

In this review, we provide an overview on the current status and latest trends in on-chip disease models with special attention to opportunities for drug development. As sketched in the graphical summary in Fig. 1, we will start with **(1)** an analysis of OOC technologies with applications for testing the major drug administration routes: **(1.1)** intestine-on-a-chip (oral/rectal route), **(1.2)** lung-on-a-chip (inhalation), **(1.3)** skin-on-a-chip (transdermal) and **(1.4)** vascularization

* Corresponding author.
E-mail address: giuseppe.maruccio@unisalento.it (G. Maruccio).
[1] These authors equally contributed to the work.

https://doi.org/10.1016/j.bios.2023.115271
Received 13 December 2021; Received in revised form 24 November 2022; Accepted 26 March 2023
Available online 31 March 2023
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models (intravenous). Here, we will consider how drugs can penetrate in the bloodstream, directly or across relevant blood organ barriers and be conveyed to their targets, including analysis of inflammatory processes.

Then, we will focus on **(2)** microphysiological systems for (other) specific organs, processes and diseases studies: **(2.1)** neurodegenerative diseases (with brain models and blood brain barriers), **(2.2)** tumor models (including their vascularization, spheroids/organoids, engineering and screening of antitumor drugs), **(2.3)** biomechanical systems recapitulating heart, muscles and bones structures and related diseases, **(2.4)** liver/kidney on chips and **(2.5)** multiorgan models for gastrointestinal diseases and metabolic assessment of drugs.

In section 3, we will discuss **(3)** technologies and materials for organ on chips, analyzing **(3.1)** microfluidic tools for organs-on-chips, **(3.2)** sensor integration for real-time monitoring, **(3.3)** materials and **(3.4)** cell lines for organs on chips. Finally, we conclude with a critical discussion on current significance/relevance, trends, limitations, challenges and future prospects in terms of revolutionary impact on biomedical research, preclinical models and drug development.

This review is not intended to provide exhaustive information on the OOC research (an impossible task for this fast-growing field) but takes

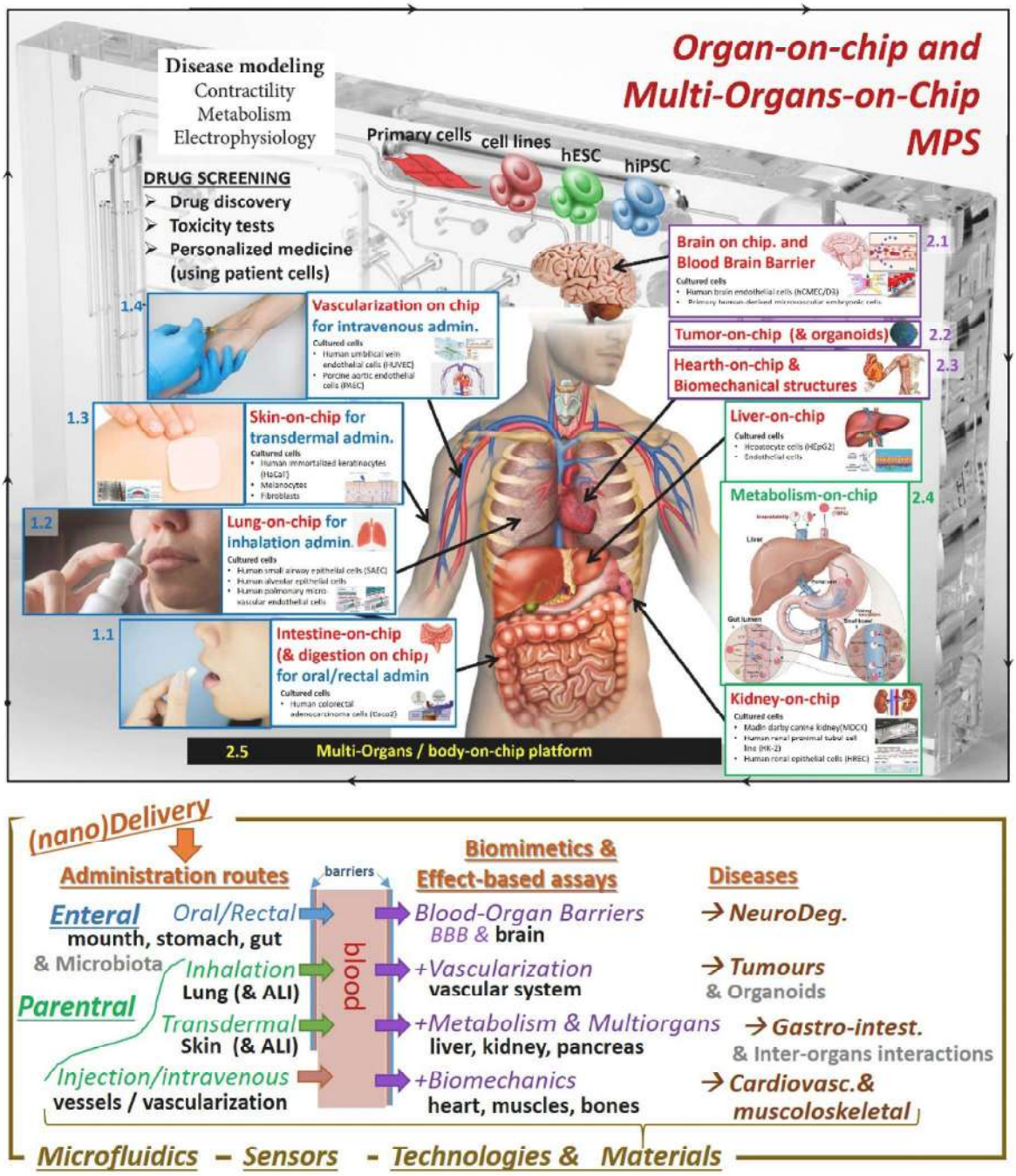


Fig. 1. Graphical summary and main topics covered by the present review, from enteral (oral/rectal) and parenteral (inhalation, transdermal, injection/intravenous) administration routes with relevant organ models (see Section 2) to biomimetics and effect-based assays with relevant organs and related diseases (see Section 3). These sections include discussion also on aspects related to relevant blood organ barriers to cross, vascularization, organoids, metabolisms, multiorgans models. In the external frame are then reported the microfluidics and sensor technologies and materials available for the development of organs-on-chips and automated platforms for high-throughput screening (see Section 4).

the form of an atlas for both researchers approaching the topic and advanced users, providing useful introduction to major themes and indications for further readings.

2. Microphysiological systems for testing drug administration routes

The development of new drugs from discovery within proper candidate libraries to preclinical and clinical phases is a quite inefficient, long and costly process (Fig. 2 A). Indeed, establishing one new drug typically requires several years of research and hundreds of millions of dollars/euros, with the involvement of specialized personnel and a validation procedure characterized by strictly regulated clinical phases and high risk in front of such huge investments. The success rate of new compounds is as low as 5% resulting in significant resources' loss every year. In addition, in the search for novel drugs, another crucial issue to improve therapeutic efficacy concerns the availability of effective routes for pharmaceuticals delivery in patients, e.g. by oral/rectal, inhalation, transdermal or intravenous administration (Fig. 2 B). Organ-on-chip devices can provide useful support in both these directions, since they allow to recapitulate intrinsic and extrinsic features of an organ/tissue/construct, its microenvironment and biological barriers and enable testing drug efficacy, solubility, permeability, targeted delivery and toxicity in a more appropriate and reliable way (Low et al., 2021; Bhatia and Ingber, 2014; Huh et al., 2010a, 2011; Skardal et al., 2016; Caplin et al., 2015a; Benam et al., 2015).

Today, drug research relies on the use of conventional in-vitro 2D cell cultures and animal experiments which are not able to properly predict clinical efficacy, toxicity and side effects of therapeutics in humans since they are inadequate to reproduce human physiology. For these reasons, organs-on-chips technologies recently attracted considerable attention as alternative platforms for drug development with research grown tremendously worldwide. The interest is motivated by their potential use for high-throughput, high-content and resource-efficient screening (Fig. 3). This represents a paradigm change and is important to review recent innovations and advances in the field in order to catch the opportunities provided by most appropriate disease models which are becoming more and more accepted by pharmaceutical companies as novel tools able to accelerate the drug development process towards more precise and eventually personalized standards (Dhiman et al., 2019).

Before starting to review cases relevant for various organ and diseases, it is worth noticing that a general classification of OOC architecture can be done in terms of ECM dimensionality distinguishing among 2D, 2.5D and 3D models (Bennet et al., 2021). In 2D models, a synthetic membrane separates two vertically-stacked compartments and ECM provides coating, favors cell adhesion/growth and participates in defining the barrier and transport properties with respect to nutrients and drugs. 2.5D models are realized either using multiple ECM coated membranes or replacing the membrane with a thin hydrogel film. Instead, in 3D models, thick 3D hydrogel layers are used so that stromal cells can be incorporated to better recapitulate the interstitial matrix (Bennet et al., 2021).

2.1. Intestine-on-a-chip for testing oral/rectal administration

A major route for drug administration relies on oral/rectal delivery. Thus, in this section, we focus on intestine-on-a-chip models. Key challenges in this case are represented by gastro-intestinal drug solubility and permeability since drugs must enter the blood circulation upon intestinal absorption in order to become effective. Moreover, potential side effects must be evaluated, in particular on the involved organs (see Section 2.4 and 2.5 for metabolism-on-chip, digestion-on-chip and toxicity studies involving liver, kidney and multiorgans microphysiological systems).

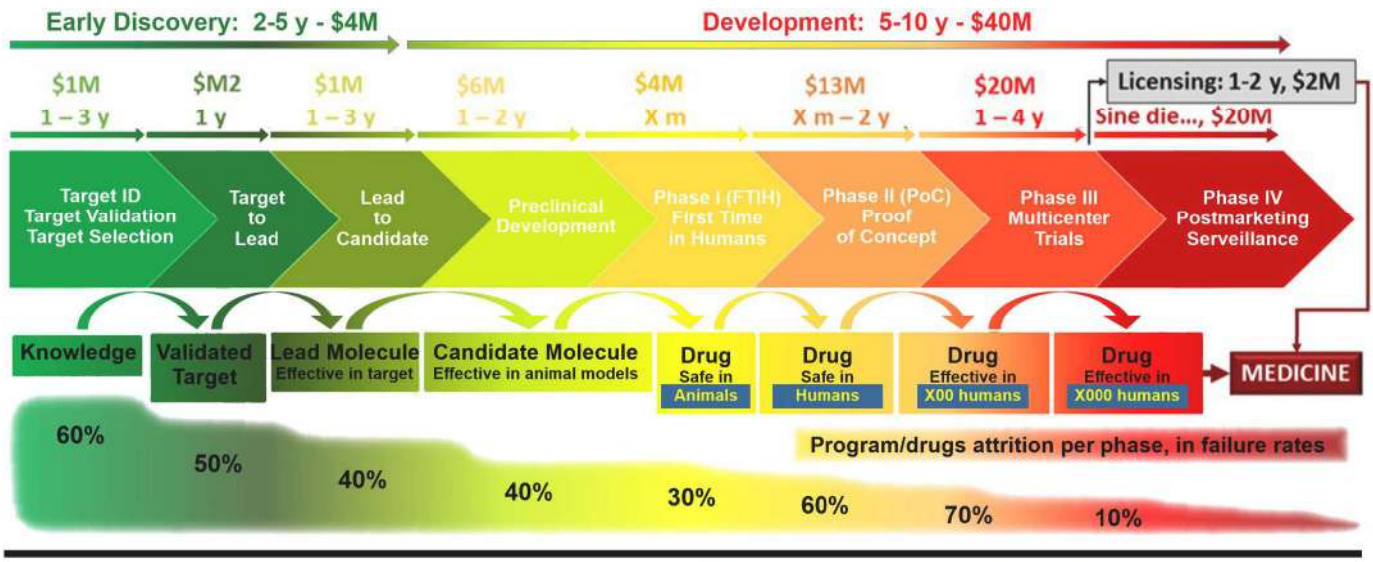
The intestine plays a fundamental role mediating nutrient, water and drug uptake, performing a critical immunological function and hosting a complex pattern of commensal and mutualistic microorganisms (gut microbiota). Thus it is a relevant model to reproduce in terms of barrier function (from 2D cell cultures to 3D villi microstructures) and physiological conditions including oxygen gradient, shear stress and mechanical deformations (Ashammakhi et al., 2020a; Fois et al., 2019; Hewes et al., 2020; Marrero et al., 2021a).

Various intestine-on-a-chip/gut-on-a-chip designs were reported in literature integrating 3D compartmentalized systems, perfusable chambers, 3D hydrogel scaffolds and stretchable materials for mimicking peristaltic movements (Fois et al., 2019; Hewes et al., 2020; Bein et al., 2018; Maurer et al., 2019; Marrero et al., 2021b). 3D compartmentalized systems with upper and bottom chambers separated by a porous membrane provide a simple, popular strategy for organ-on-a-chip layout and the implementation of biological barrier models. Ingber group employed this approach modifying their previously reported breathing lung-on-a-chip (Huh et al., 2010a) (see Section 1.2) in the form of gut-on-a-chip (Kim et al., 2012). In particular, Kim et al. (2012) integrated an ECM-coated polydimethylsiloxane (PDMS) membrane among two PDMS microfluidic channels to obtain two vertically stacked chambers (Fig. 4 A1-A3). Caco-2 cells were used as human intestinal epithelial cells. The addition of two vacuum chambers on the channel sides permitted to mimic the peristaltic motions, while a strain of Lactobacillys rhamnosus was added to simulate intestinal native microbes. Polarized Caco-2 cells were instead seeded by Kimura et al. to better mimic human intestine (Kimura et al., 2008) since they express morphological and functional characteristics of mature small intestinal enterocytes with better barrier functions.

Focusing on drug delivery, the oral uptake of the chemotherapeutic agent SN-38 (7-ethyl-10-hydroxycamptothecin) was investigated by Pocock and coworkers using an intestine-on-chip model (Pocock et al., 2017) which improves conventional Caco-2 Transwell approach by better mimicking the biological barrier function (Fig. 4 B1–B3). In more detail, they differentiated an epithelial cell monolayer using external mechanical stimuli and obtained a 3D rippling morphology mimicking microvilli expression. Then, they investigated the structure permeability for SN38 modified with fatty acid esters of different lengths and at different positions, demonstrating that lipophilic prodrugs can contribute to tackle low oral bioavailability issues. These models are also applicable to nanoformulations and biological entities.

The transport of both high- and low-permeability drug compounds across the intestinal barrier was studied by Kultong et al. (Kulthong et al., 2020) with a dynamic gut-on-chip model (Fig. 4 C1–C3). Specifically, the investigated compounds were antipyrine, ketoprofen, digoxin, and amoxicillin with concentrations up to 500 μM, 300 μM, 250 μM, and 500 μM, respectively, for 24 h on a differentiated monolayer of human colorectal adenocarcinoma cells (Caco-2). The authors compared the apparent permeability (P_{app}) values of the four compounds and observed that for antipyrine and ketoprofen, P_{app} values were lower in Caco-2 cells under dynamic flow conditions than under static conditions in transwell systems. These differences may be due to the effect of the chip design, the material composing the diffusion membrane, and the presence of laminar flow. For amoxicillin which is a low permeability compound, the P_{app} values are instead similar under both dynamic and static flow conditions. Based on a comparison of OOC approach with the static transwell model, Kultong et al. concluded that their gut-on-chip model was adequate to study drug transport (Kulthong et al., 2020).

Notably, gut-on-a-chip models were likewise employed to study (with improved efficacy) the response to nutrients and to emulate gut inflammation, host-microbiota interplay as well as interactions with environmental factors where the imbalance between pro-inflammatory and anti-inflammatory cytokines and alterations of the composition and function of the gut microbiota (dysbiosis) play a pivotal role (Maurer et al., 2019; Beaurivage et al., 2019; Kim et al., 2016; Neurath, 2014). As a result, OOC can provide valuable insights on intestinal



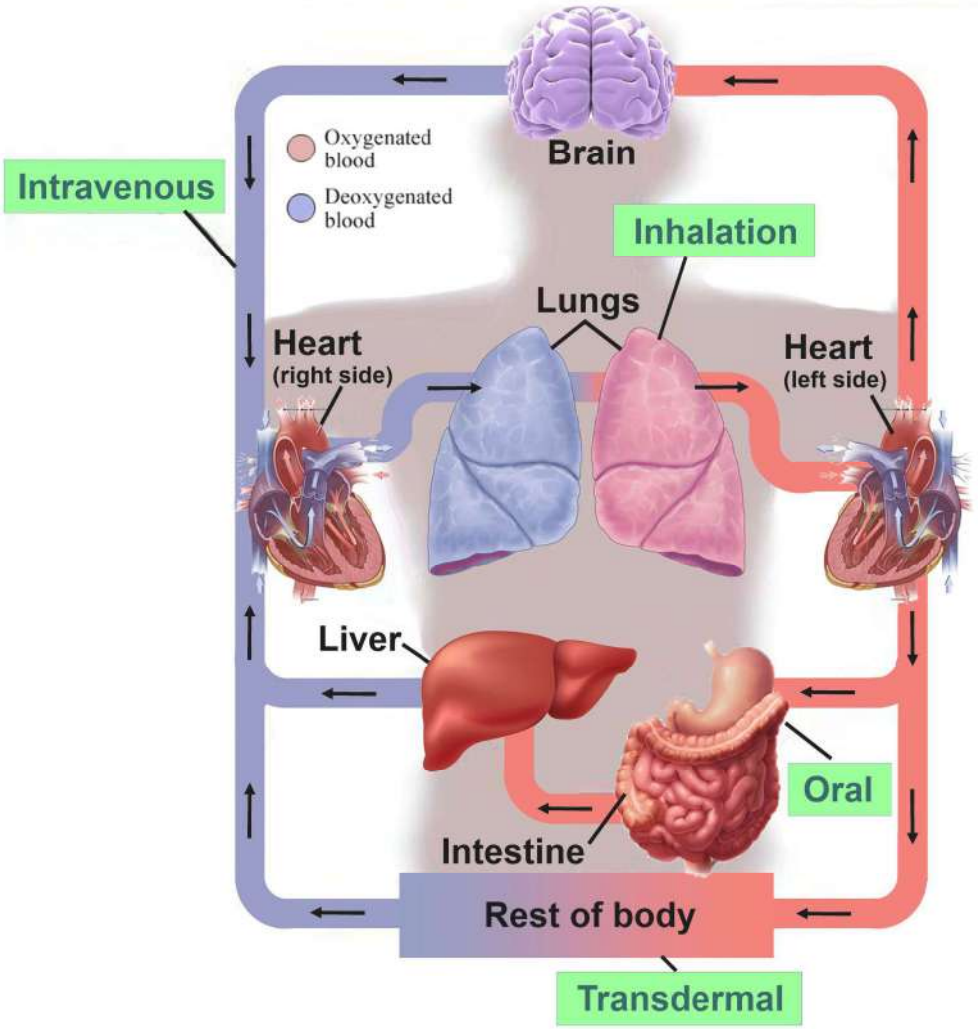


Fig. 2. A. The drug discovery process [https://doctortarget.com/machine-learning-applied-drug-discovery/] and major drug administration routes (beyond the involved organs/barriers, also the time until effect changes with the administration routes from short to long (30–60 s for intravenous administration; 2–3 min for inhalation; 5–30 min for rectal; minutes to hours for transdermal). **B** Routes of drug administration.

Model properties/ applications	Animals	2D cell cultures	3D cell cultures	Spheroids/ Organoids	Single OOC	Multiple OOC
Biomimetics / Recapitulation	−	✗	−	✓	✓	+
Complexity	✓	✗	−	✓	✓	+
Disease models	−	−	−	✓	✓	✓
Drug research	−	−	−	✓	✓	+
Cell-cell interactions	✓	✗	✓	✓	✓	✓
Organ-organ interactions	✓	✗	✗	✗	✗	✓
Vascularization	✓	✗	✗	−	−	✓
Integration of biosensors for real time	−	✓	✓	✓	✓	✓
Throughput	✗	✓	✓	✓	✓	✓
Costs	✗	✓	✓	−	−	−
Ethics	✗	✓	✓	✓	✓	✓

Fig. 3. Advantages of single and multiple OOC platforms in comparison to other methodologies.

dysfunction and physiopathology, the involved pathways and their relation to severe and chronic gut diseases with complex etiology from early stage to full manifestation and eventual worsening (Hewes et al., 2020; Marrero et al., 2021a; Bein et al., 2018; Maurer et al., 2019). In this respect, OOC are expected to overcome animal models which often fail when extrapolated to humans due to the differences in microbiota composition and immune system. Furthermore, OOC models are expected to overcome limitations of 2D and 3D cultures, such as the lack of cell-matrix interaction and mechanical stimuli respectively (Wang et al., 2021; Ashammakhi et al., 2020b).

2.2. *Lung-on-a-chip for testing inhalation administration*

Administration by inhalation provides another important route which is particularly relevant for respiratory and pulmonary diseases including viral and bacterial lung infections, chronic obstructive pulmonary disease (COPD), pulmonary edemas, tuberculosis and lung cancer which are among the top 10 causes of death according to World Health Organisation (World Health Organisation [WHO]). Against these diseases, inhaled drugs can be advantageous for rapid delivery/action and in terms of targeted instead than systemic exposure with drugs directly deposited within the airways. In this case, the absence of interaction with liver or kidney can result in reduced toxicity and side effects and thereby improve therapeutic efficacy, patient outcomes and patient quality of life (Cidem et al., 2020; Borghardt et al., 2018). By reproducing human in vivo pulmonary microenvironment, the air-liquid interface and the lung-blood barrier for inhaled agents, lung-on-chip platforms facilitate research (Huh et al., 2010a; Bennet et al., 2021; Ainslie et al., 2019; Khedoe et al., 2020; Barros et al., 2021; Campillo et al., 2021).

Major difficulties in recapitulating lungs airways lies in their morphological and histological complexities with the presence of different types of cells and epithelium in addition to mucus and the relevance of branching and breathing movements. Remarkably, in their seminal work, Huh et al. (2010a) demonstrated a breathing lung-on-a-chip (Fig. 5 A1-A2) having the form of a compartmentalized system with vertically-stacked chambers separated by a porous membrane. Specifically, the upper and bottom chambers were seeded with human alveolar epithelial cells and lung capillary endothelial cells respectively. An air-liquid interface (ALI) mimicking the alveolar-capillary barrier was then reproduced by depleting the cellular media in the upper compartment. Two side vacuum chambers were also realized to reproduce the cyclic strain and mechanical forces on the culture membrane associated to breathing (Fig. 5 A1). This architecture then became very popular and extensively used in OOC models, beyond the lung-on-a-chip case (Frost et al., 2019; Ishahak et al., 2020).

Known biomarkers (e.g. fluorescent albumin, transferrin and dextrans) are habitually employed to evaluate the barrier permeability and the influence of shear stress on paracellular and transcellular transport, i.e. between epithelial/endothelial cell junctions or through cell lacking

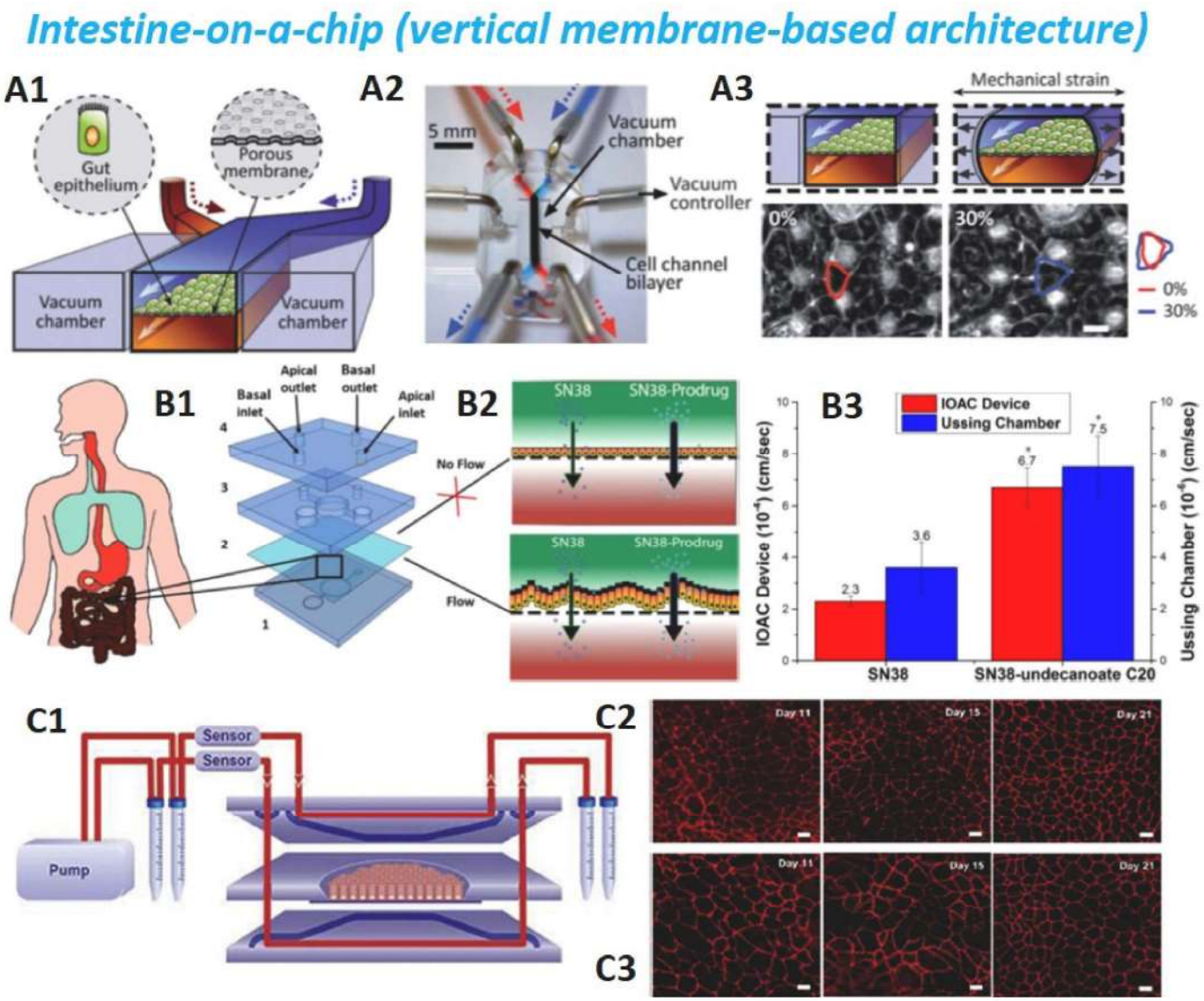


Fig. 4. ***(A1)*** *Schematic representation of the gut-on-a-chip device in which the ECM membrane is covered by gut epithelial cells and cross the central microchannel between two vacuum chambers.* ***(A2)*** *A photo of the gut-on-a-chip device. Blue and red dyes flow through tubing to the upper and lower microchannels, respectively, to visualize these channels.* ***(A3)*** *Schematic (top) and phase contrast images (bottom) of intestinal monolayers cultured within the gut-on-a-chip in the absence (left) or presence (right) of mechanical strain. Reproduced from Ref.* (Kim et al., 2012). ***(B1)*** *Structure of the intestine-on-a-chip reported by Pocock* et al. *with apical and basal chambers separated by a PC membrane.* ***(B2)*** *Scheme of the performed permeability assay for chemotherapeutic agent SN38 through Caco-2 cell monolayers.* ***(B3)*** *Comparison among results from the OOC platform and rat intestinal mucosal membrane mounted in an Ussing Chamber. Reproduced from Ref.* (Pocock et al., 2017). ***(C1)*** *Microfluidic gut-on-chip realized by Kulthong* et al. *and consisting of three re-sealable glass slides resulting in two microfluidic chambers separated by a polyester (PET) membrane where Caco-2 cells were cultured. The flow was injected by use of a multichannel air pressure pump. Confocal microscope images of top view of the Caco-2 cells layer, evidencing tight junction patterns (ZO-1/TJP1) in red, cultured for 21 days in a static Transwell system* ***(C2)*** *or a gut-on-chip system* ***(C3)*** *under a continuous flow of* 100 μL/h. *Reproduced from Ref.* (Kulthong et al., 2020).

the required active transporters (Huh et al., 2010a; Frost et al., 2019). In this respect, confocal microscopy is a valuable technique since it permits localization of the biomarkers in the different compartments to investigate transport across the barrier. In general, the alveolar barrier permeability was found to be significantly smaller than in the case of liquid cultures (Huh et al., 2010a). Trans-epithelial electrical resistance (TEER) measurements provide an useful alternative to confocal microscopy for evaluating the barrier characteristics since the resistance correlates to the tightness of the cell junctions (Huh et al., 2010a; Henry et al., 2017; Stucki et al., 2018) (Fig. 5 A3).

A model of drug toxicity–induced pulmonary edema was also implemented by Ingber group using a similar alveolar-capillary interface with human pulmonary epithelial and endothelial cells that experience both air and fluid flow. In this study, the applied cyclic mechanical forces (Huh et al., 2012) mimicking the breath were found to play a relevant role in the edema formation and the authors had the possibility to test some therapeutic approaches.

This membrane-based architecture, however, does not exhaust all the proposed approaches. Various models and interfaces have been reported using different architectures and types of cell culture. They span from simple monocultures for the epithelium (Felder et al., 2019; Huang et al., 2021) to co-cultures for epithelial-endothelial (in alveolar-capillary (Huh et al., 2010a; Douville et al., 2010; Jain et al., 2018a; Stucki et al., 2015; Zamprogno et al., 2021) and small-airway models (Benam et al., 2016)) and epithelial-mesenchymal (Humayun et al., 2018) interfaces. Tri-cultures with epithelial, fibroblast and

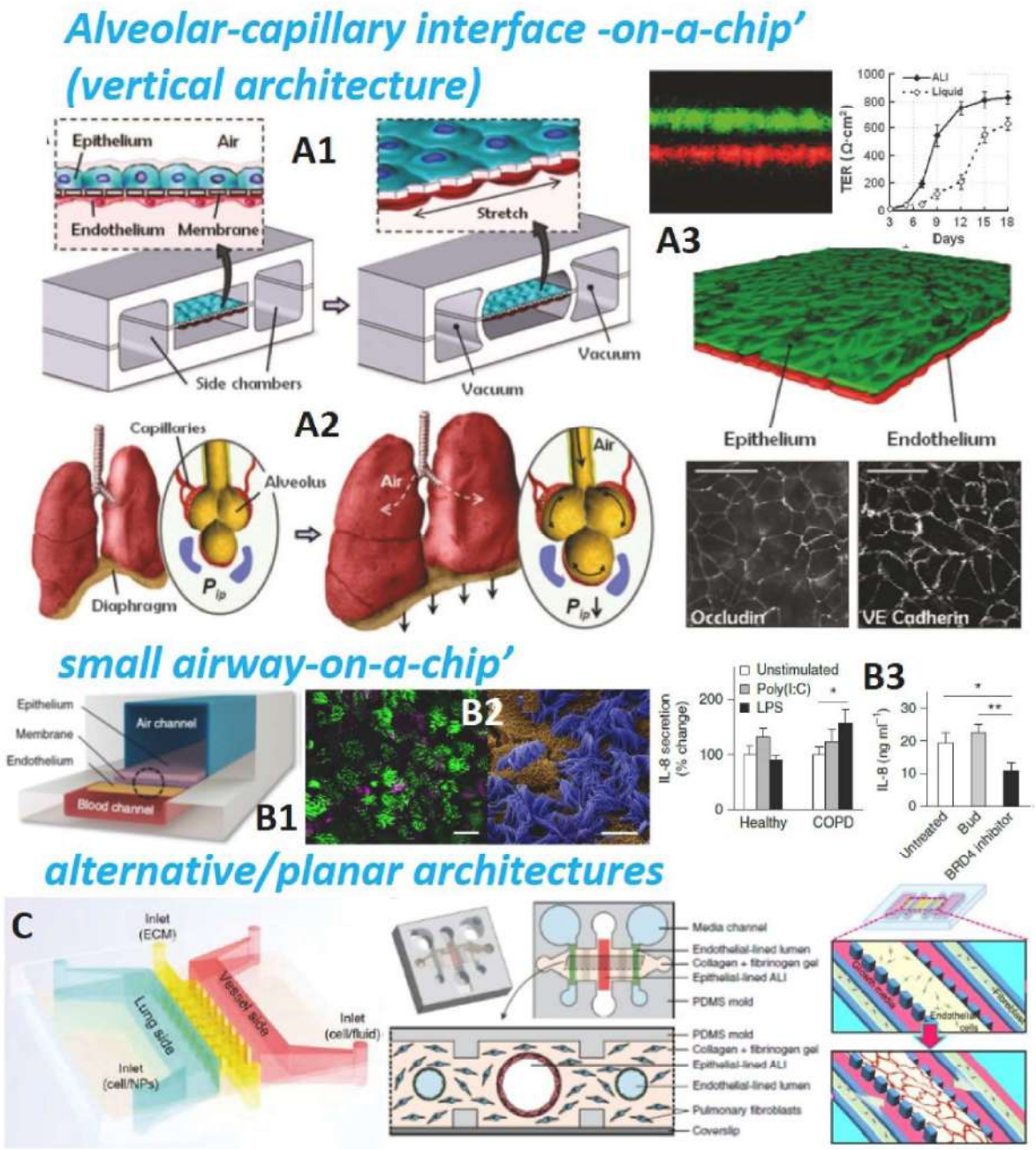


Fig. 5. **(A1-A2)** Alveolar air-liquid interface model which uses a cyclic pressure to mimic breathing. **(A3)** Assessment of the interface by TEER measurements and fluorescent staining. Reproduced from Ref. Huh et al. (2010a). **(B1)** Air-liquid interface model workflow with seeding of endothelial cells (LMECs) and then alveolar epithelial cells (AECs) on a transwell system. **(B2)** Human airway epithelium grown on chip (scale bar, 20 μm) and cilia (blue) on the apical surface of the airway epithelium (scale bar, 10 μm). **(B3)** Effects on production of the cytokines IL-8 on small airway chips lined by either normal or chronic obstructive pulmonary disease epithelial cells (left) and upon therapeutic modulation of cytokine inflammation (right). Reproduced from Ref. (Benam et al., 2016). **(C)** Schematic of structures of lung-on-a-chip with alternative/planar architecture. Reproduced from Ref. (Barkal et al., 2017; Park et al., 2018; Zhang et al., 2018a).

endothelial cells were instead employed for recapitulating epithelial-stromal/vascular tissue interfaces (Sellgren et al., 2014; Barkal et al., 2017; Park et al., 2018). In this way, through an appropriate cell selection, lung barrier function, inflammation, immune response and injury were modeled in lung-on-a-chip format providing unprecedented tools for physiopathological investigations, drug development, inhalation assays, exposure studies and airborne toxicological assessment (Huh et al., 2010a; Bennet et al., 2021; Khedoe et al., 2020; Lin et al., 2022; Zhang et al., 2018a).

In particular, microphysiological models of human asthmatic and COPD airways are relevant for better understanding and contrasting diseases which have a high personal, societal and economic impact and are associated to inflammatory processes affecting the whole respiratory tract, from central to peripheral (small) airways (van den Berge et al., 2011) which have sizes <2 mm in internal diameter and include bronchioles and alveoli. Remarkably, an organotypic small-airway-on-chip was reported by Ingber group using membrane-separated chambers and microvascular endothelium and mucociliary bronchiolar epithelium from patients (Fig. 5 B1–B3) (Benam et al., 2016). Interestingly, the authors analyzed the exposure to interleukin-13 (IL-13, which has a key role in asthma), viral mimic poly(I:C) (an analogue of dsRNA produced by cells infected by respiratory viruses) and lipopolysaccharide

endotoxin (LPS, a bacterial wall derived component) in terms of effect on inflammatory cytokine/chemokine secretion, decreased ciliary function, globet cell hyperplasia and neutrophil recruitment. Then, they used the same platform for drug discovery applications and in particular testing therapeutic agents capable to contrast the inflammation. Viral-induced exacerbation of asthma was investigated by Nawroth et al. too (Nawroth et al., 2020). Stucki et al. instead employed micro-diaphragms and an electro-pneumatic setup to stretch the alveolar barrier and showed the effect of these mechanical cues on metabolic activities and cytokine secretion (Stucki et al., 2015). A layout with three vertically stacked chambers with arrayable suspended gels was realized by Humayun et al. to examine the interactions among airway epithelial cells and smooth muscle cells which are relevant in chronic lung diseases (Humayun et al., 2018).

Injuries, alveolar epithelial wound healing under mechanical strain and intravascular thrombosis were another subject of study for the development of new anti-inflammatory therapeutic agents (Felder et al., 2019; Jain et al., 2018b). The inclusion of fibroblasts in these models is important to contemplate their interactions with epithelial and immune cells and their role in airway repair through ECM deposition and degradation, once triggered by epithelial cells after an injury (Bennet et al., 2021). In this respect, Sellgren et al. employed an architecture with three vertically stacked chambers to produce an airways model with epithelial cells, lung fibroblasts and polarized microvascular endothelial cells (Sellgren et al., 2014); while Bovard et al. combined lung/liver-on-a-chip for toxicity studies (Bovard et al., 2018) (for multi-organ on chips models see Section 2.5). Other recent reports focused on modeling fibrotic diseases (Mejias et al., 2020), investigated endotheliitis and vascular damage during SARS-CoV-2 infection (Thacker et al., 2021), and intriguingly the spontaneous evolution of influenza viruses (Si et al., 2021).

Beyond the membrane-based approach, different architectures with adjacent chambers were recently reported with the target to provide improved 3D models able to better mimic in vivo structures (Barros et al., 2021; Barkal et al., 2017; Park et al., 2018; Zhang et al., 2018a). In particular, Zhang et al. realized three parallel channels (Fig. 5 C) with a lung and a vessel side respectively lined with human pulmonary alveolar epithelial cells and vascular endothelial cells. The middle ECM channel was then filled with Matrigel to recapitulate the alveolar capillary barrier. This platform was employed for nanotoxicity tests (Zhang et al., 2018a). The bronchiole model by Barkal and colleagues has some similarities and differences. This approach offers more physiologically relevant microarchitectures in terms of sizes, geometries and 3D interactions as compared to flat conformations with porous membranes. Furthermore, the various compartments can be addressed separately and the cell types are exposed to different conditions thanks to gel polymerization in a stable 3D structure and the presence of appropriate ports for injection/removal/sampling. The counterpart is the inherent difficulty in collecting gel-embedded cells since device disassembly for gel extraction and subsequent digestion is required. This process became even harder in multicellular hydrogel-based models (Park et al., 2018), when cell collection is necessary before digestion or requires cell sorting method.

3D cell printing was employed by Park et al. to fabricate an airways model comprising a blood vessel network made of human dermal microvascular endothelial cells and human lung fibroblasts in decellularized ECM. Primary human tracheal epithelial cells were instead seeded on a Transwell insert containing an ECM membrane (Park et al., 2018). The presence of a vascular network improves the physiological relevance of this model, although the co-culture of endothelial cells and fibroblasts complicate the analysis (Bennet et al., 2021). Recently, in the search for more physiologically relevant models and materials, alternative architectures were proposed using nanofiber membranes (Yang et al., 2018), reverse engineered hydrogels with inverse opal structure (Huang et al., 2021) and stretchable collagen-elastin biomembranes.

2.3. Skin-on-a-chip for testing transdermal administration

Skin implements several functions for the body: (i) thermoregulation through sweat glands, (ii) heat, pressure and strain sensing through several sensory receptors, (iii) vitamin D synthesis. As a physical barrier, it preserves from dehydration, maintains gas concentration gradients (for O_2, CO_2, N_2) and protects the body against exposure to external agents and threats (including microorganisms, ultraviolet radiation, toxic and mechanical agents). Within the therapeutic field, transdermal drug administration provides a mean for administration of systemically acting drugs through vessels or adipose tissue after overcoming the skin barrier.

Human skin-on-a-chip (SOC) models (van den Broek et al., 2017; Cui et al., 2022; Nitsche et al., 2022; Zoio and Oliva, 2022; Risueno et al., 2021; Mathes et al., 2014) attracted significant attention for dermatological studies, wound healing, risk assessment for external agents/chemicals, evaluation of cosmetic products and their ingredients and transdermal drug administration research. In this case, differences with animal skin are considerable in terms of structural and biochemical properties, lipid profile, hair density and stratum corneum thickness (Cui et al., 2022). As the more external layer consisting of protein-rich dead cells in a lipid matrix, the stratum corneum influences drug permeation with its thickness and lipid composition contributing in establishing the barrier penetration characteristics. 2D cell cultures suffer in appropriately recapitulating attachments and growth kinetics and the lipid profile and, for example, can exhibit limitations in evaluating permeability of hydrophilic compounds depending on the use of non-lipid or lipid-based membrane models. 3D skin-on-a-chip models can better mimic in vivo physiological conditions.

Human skin consists of three structural compartments. The external, avascular epidermis layer is mainly composed of keratinocytes producing keratin as a protective protein. The intermediate dermis compartment is a connective tissue with fibroblasts as the primary cell component in a collagen microenvironment, elastin fibers providing elastic properties and hyaluronic acid involved in skin hydration. It is pervaded by blood and lymph vessels and contains nerves, sensory receptors, sweat and sebaceous glands, hair follicles and shafts. The inner subcutaneous tissue is predominantly made of adipocytes (fat cells) in addition to fibroblast and macrophages and is characterized by larger nerves and blood vessels. For widening the range of functions and possible investigations, it can be relevant to include additional cell types (e.g. melanocytes and Langerhans cells in the epidermis) (Zoio and Oliva, 2022). Both skin biopsies or models generated off-chip were integrated in chip models.

Progress in the field came through the development of more and more appropriate and complete models. We can distinguish different categories of in vitro skin models (Fig. 6 A) with increasing physiological relevance and complexity and skin-on-a-chip followed a similar development (van den Broek et al., 2017; Cui et al., 2022; Nitsche et al., 2022; Risueno et al., 2021; Mathes et al., 2014):

- reconstructed human epidermis (RHE) models typically uses only one cell type (keratinocytes): they provide a well-established and reproducible platform for risk assessment and were employed in skin irritation, corrosion and sensitization studies in toxicological and cosmetic research as well as transdermal drug delivery, phototoxicity, metabolization assays (Gibbs et al., 2013). With the incorporation of melanocytes, RHE models allow skin lightning and pigmentation assays (Jain et al., 2012). However, more advanced models are needed for drug efficacy tests, recapitulating cross talk with other cell types;
- dermo-epidermal human skin equivalents (HSE) (Mathes et al., 2014; Asbill et al., 2000) are cultivated in serum and include the dermis compartment with collagen I and fibroblast cells in proximity to the keratinocytes which are seeded in a second phase and cultivated at the air-liquid interface to form the adjacent epidermal

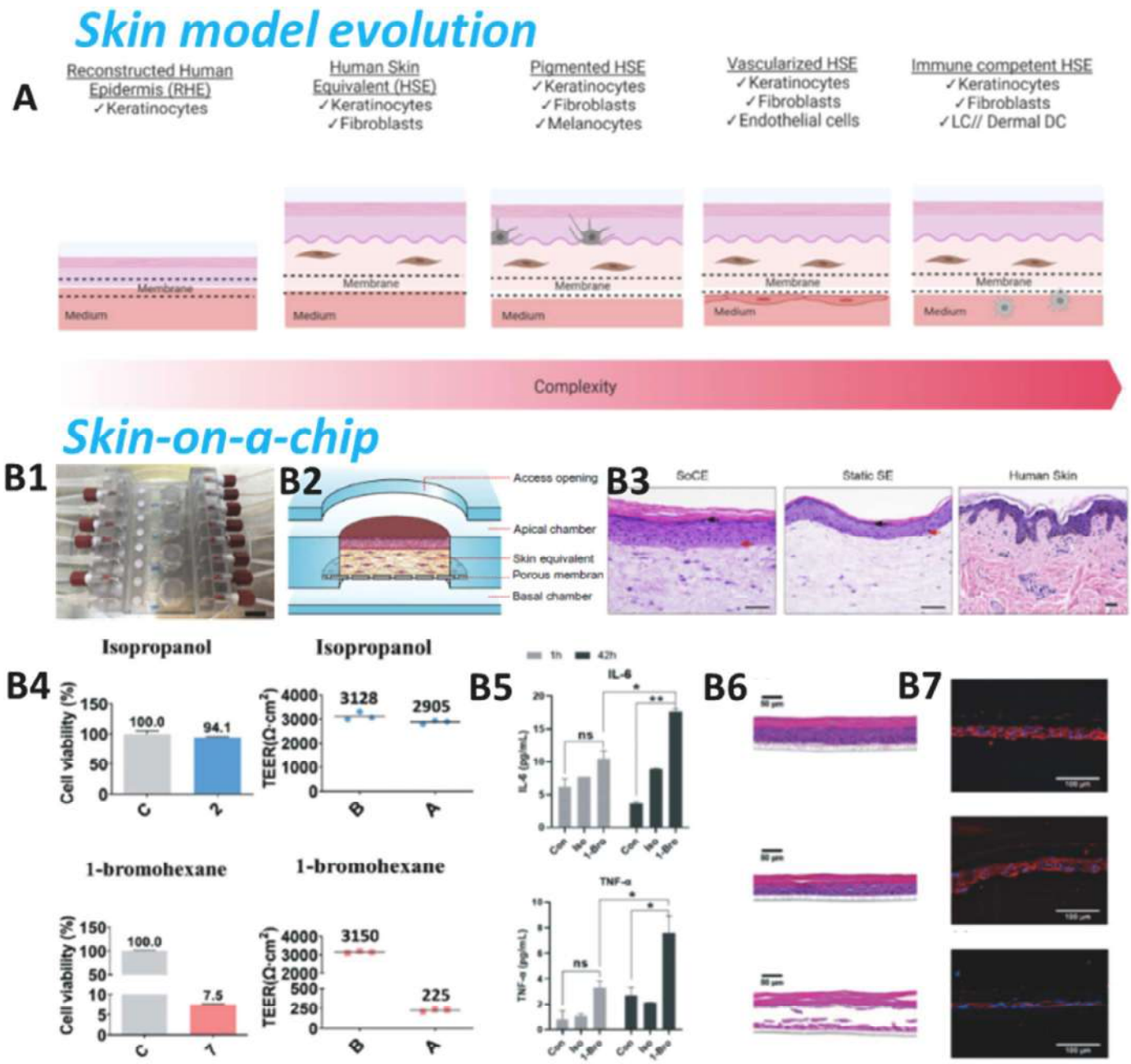


Fig. 6. (A) *Summary of selected 3D* in vitro *skin tissue models, depicted with increasing biological complexity from left to right and their research applicability and predictability for NGRA using an open access OOC device for air–liquid culturing. Reproduced from Ref.* (Nitsche et al., 2022). ***(B1–B2)*** *Photographs and scheme of skin-on-chip equivalent (SoCE);* ***(B3)*** *Histological images representing epidermal morphogenesis in skin-on-a-chip equivalent compared to static human skin equivalents. Reproduced from Ref.* (Sriram et al., 2018) ***(B4)*** *MTT Cell viability (C = negative control) and TEER values of the skin-on-chips before and after 42h exposure to isopropanol and 1-bromohexane irritation (label B means before, A means after).* ***(B5)*** *Inflammatory cytokine release following isopropanol and 1-bromohexane irritation.* ***(B6)*** *Hematoxylin–eosin staining of integrated epidermis-on-chip (iEOC) system:(from top to bottom) control, isopropanol irritation and 1-bromohexane irritation.* ***(B7)*** *Tight junction maker ZO-1 staining of integrated epidermis-on-a-chip: control, isopropanol irritation and 1-bromohexane irritation. Scale bars:* 100 μm. *Reproduced from Ref.* (Zhang et al., 2021)

compartment. These models allow to investigate cell-cell crosstalk and provide more biomimetic barrier properties. In vitro, they exhibit high reproducibility and standardization and enable evaluation of wound healing and bacterial adhesion. Major drawbacks concern the limited lifespan and the limited cell types employed.

- human skin equivalents integrating other cell types to perform investigation of basic melanogenesis and vitiligo pathogenesis (incorporating melanocytes), innate immune response, irritant exposure and allergen assessment (incorporating immune or Langerhans cells); epidermal development, wound healing, pigmentation disorders and autologous transportation (incorporating stem cells) (Mathes et al., 2014; Duval et al., 2012; Ouwehand et al., 2011; Nissan et al., 2011). Dorsal root ganglion neurons were also incorporated to reconstruct a peripheral skin nerve system (Risueno et al., 2021). Models with trans-appendageal moieties were furthermore developed, e.g. hair follicles for substance penetration studies (Michel et al., 1999). Limitations include lower standardization and limited tissue survival.
- vascularized human skin equivalents are the next technological step which allows nutrients and oxygen supply and waste removal

resulting in extended tissue survival. This approach enables the assessment of transdermal penetration of drugs in the bloodstream, angiostatic therapies and adipose metabolism, impact of drugs on adipose tissue and angiogenesis. While long term cultivation becomes feasible, this technology is less standardized than the previous ones.

- skin-on-a-chip models promise to provide the ultimate step of technological innovation in the form of miniaturized microfluidic platforms which allow high throughput physiologically relevant studies at reduced costs by applying different physical and biochemical stimuli (including shear stress, mechanical forces and chemical gradients). Skin fragments have been either transferred to the chip from a biopsy or a HSE (Abaci et al., 2015; Kim et al., 2019a) or directly generated in situ (in an open structure or in channels as tissue-holding compartments) (Lee et al., 2017; Song et al., 2017, 2018a; Jeon et al., 2020; Kim et al., 2020; Sriram et al., 2018; Ramadan and Ting, 2016; Wufuer et al., 2016; Au - Risueño et al., 2021).

SOC advances underwent similar development phases from RHE (Abaci et al., 2015; Mohammadi et al., 2016) to HSE and integration of additional cell types, components and vascularization (Cui et al., 2022). RHE-based SOC employed young and mature keratinocytes with different level of stratification and junctional tightness, exhibited stability above 24 h and were employed for in vivo irritation assays (Zhang et al., 2021). HSE-based SOC exhibited better barrier functions due to an improved cell viability and were used for transdermal/topical drug permeation studies (Fig. 6 B1–B7) (Sriram et al., 2018; Alberti et al., 2017) and wound healing (Li et al., 2015; Biglari et al., 2019) and skin microbiome investigations (Applied In Vitro Toxicology, 2015). A 3D vinyl-based bilayer tissue model made of an epithelial and a stromal component was reported by Valencia et al. (2021) using immortalized human skin keratinocytes (hKCs, HaCaT cell line) and primary human dermal fibroblasts (hFBs). A microporous (polycarbonate) membrane was again employed to separate the skin model from a blood vessel channel used to recapitulate dynamic perfusion and drug delivery. Varone et al. introduced two vacuum channels around the epithelium chamber to apply mechanical forces (Varone et al., 2021).

Hair follicles were incorporated in SOC models by Atac et al. (2013). Integration of explants in SOC can provide access to all cell types and diseased skin but suffer for limited availability and donor variability. Multi-organ on chips integrating skin with other models (e.g. liver, intestine and kidney) to investigate their crosstalk are the next frontier (Wagner et al., 2013a; Maschmeyer et al., 2015a, 2015b) (see Section 2.5). Microfluidic arrays of dermal spheroids were implemented by Chen et al. as a screening platform for skincare products ingredients (Chen et al., 2021).

2.4. Vascularization models for testing intravenous administration

The vascular system has a critical role for maintaining homeostasis and organ-specific functions (Pradhan et al., 2020). Tissue survival in vivo is entirely dependent on delivery of nutrients through blood vessels, while vascular dysfunctions are associated to various chronic or acute disorders. Furthermore, several therapeutic agents are directly administered intravenously instead than reaching the bloodstream across other biological barriers/organs. Compared to oral administration, this route allows shorter times until effect and, importantly, permits to overcome impediments related to low gastro-intestinal solubility and permeability (Pocock et al., 2017).

Thus, it is not surprising that the recapitulation of micro-tissues and organoid vascularization became focus of intense research as a key tool for providing suitable disease models with superior biomimetic performance in investigating pathophysiology and testing drug efficiency. In this respect, it is worth noting that vascularization additionally consents to increase lifespan of OOC models and, for this reason, it is often integrated in various microphysiological systems (as already mentioned in some examples before). On the other hand, on chip models are relevant for the investigation of specific diseases of the vascular system such as atherosclerosis and deep vein thrombosis (Benam et al., 2015).

Various techniques were exploited and, in some cases, combined to produce network of perfused microvessels, vascularized micro organs (VMO) and micro tumors (VMT) (Osaki et al., 2018a; Dellaquila et al., 2021; Lin et al., 2019). Vasculogenesis and angiogenesis are the biological processes responsible for in vivo formation of new blood vessels leading respectively to de novo formation of vascular system and growth of capillaries from pre-existing vasculature (Vailhé et al., 2001; Risau, 1997). Driving these processes in vitro provides an effective strategy for a (random) formation of vessels by seeding precursors, stem or endothelial cells inside extracellular matrix and exposing them to vascular growth factor (e.g. VEGF) (Sekine et al., 2013). This approach permits to generate random networks of sub-100 μm vessels but presents limitations when perfusable vascular lumens are the target.

In this case, microfluidics, soft lithography, 3D printing and bioprinting provide useful alternatives and support. For example, soft lithography allows to realize perfusable microchannel networks by replica molding following an appropriately engineered CAD design. In this case, the (already discussed) membrane-based layout is a widespread strategy upon the pioneering work of Ingber group (Huh et al., 2010a) and was employed in various organ-specific models including lung, gut, liver, kidney, hearth, brain and blood-brain barrier. In Fig. 7 A, the case of application to liver is reported (Du et al., 2017). In particular, in the liver-on-a-chip model by Du et al., the four major hepatic cell lines were grown in two membrane-separated chambers to recapitulate liver functions (namely from base to top: hepatocytes, hepatic stellate cells, the liver sinusoidal endothelial cells and Kupffer cells).

Other architectures are based on lateral/planar microchannels (e.g. interconnected by sub-networks of smaller sizes) (Dellaquila et al., 2021). External pumps with valves or hydrostatic pressure consent to drive the flow in the perfusable vascularized systems. In all these cases, the use of scaffolds with predetermined geometries enables high control on lumen sizes, network interconnections, flow rates and imposed shear stress. Direct micromachining of microfluidic networks was also performed by laser patterning techniques with advantages in realizing layer-by-layer architectures but at higher cost (Fig. 7 B).

Templating and sacrificial molding methods are another option (Fig. 7 C). In this respect, 3D printing offers a cost-efficient approach through the use of cytocompatible sacrificial templates in engineered tissues but presents limitations in terms of minimal sizes (no less than ≈100 μm) (Miller et al., 2012). As examples, in skin-on-a-chip field, Abaci et al. and Mori et al. (Abaci et al., 2016; Mori et al., 2017) exploited respectively micropatterned alginate sacrificial layers or nylon threads successively removed in order to obtain hollow channels to be covered by endothelial cells (Abaci et al., 2016; Mori et al., 2017). A combination of 3D printing for the device layout and 3D bioprinting of the cellular layers was exploited by some authors as a mean to achieve high structural control (Kim et al., 2019b).

In various reports, biological processes and engineered-based fabrication techniques have been successfully combined to exploit the advantages of both worlds (Dellaquila et al., 2021; Kim et al., 2013; Wang et al., 2016) leading to microfabricated vessel scaffolds by lining microfluidic channels with endothelial cells (Fig. 7). For example, Hughes and Lee group realized perfusable microvascular networks connecting microfluidic channels without noticeable leakage. The adopted strategy was to pass through different stages of vascular development from vasculogenesis, to endothelial cell (EC) lining, sprouting angiogenesis and anastomosis (Fig. 7 D) (Wang et al., 2016). Then, they employed this approach to support the in vitro growth of 3D (vascularized) microtumors and to investigate the effect of drugs targeting growth factors in terms of regressing the vasculature (Sobrino et al., 2016a). Tumor cell extravasation dynamics was also investigated