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Recent advances in lung-on-a-chip models

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With the global burden of respiratory diseases, rapid identification of the best therapeutic measures to combat these diseases is essential. Animal models and 2D cell culture models do not replicate the findings observed *in vivo*. To gain deeper insight into lung pathology and physiology, 3D and advanced lung-on-a-chip models have been developed recently. Lung-on-a-chip models more accurately simulate the lung's microenvironment and functions *in vivo*, resulting in more-accurate assessments of drug safety and effectiveness. This review discusses the transition from 2D to 3D models and the recent advances in lung-on-a-chip platforms, their implementation and the numerous challenges faced. Finally, a general overview of this platform and its potential applications in respiratory disease research and drug discovery is highlighted.

Keywords: Organ-on-a-chip; Lung-on-a-chip; Lungs; Respiratory system; Respiratory diseases

Introduction

Human lungs are vital body organs responsible for oxygen and carbon dioxide exchange across the alveoli–capillary network.[1] This exchange of air with the blood occurs at the alveoli, which are the smallest functional units of the respiratory system.[2] Through inhalation, the lungs are exposed to various toxic chemicals, particles, bushfire smoke, cigarette smoke, bacteria and viruses that can cause chronic respiratory conditions such as acute respiratory diseases, asthma, chronic obstructive pulmonary disease (COPD), lung cancer and infections such as COVID-19, influenza and tuberculosis.[3-7] Lung failure ranks third globally among the leading causes of mortality because of these manifestations.

Researchers rely on preclinical models to study the aetiopathogenesis of respiratory diseases and identify effective therapeutics.[8,9] These include animal models, 2D models and 3D cell culture models.[5,10] However, animal models usually do not reproduce exact human biological responses to diseases and drugs. The primary reason for this is that the species exhibit vastly different disease courses, pathogenesis, symptoms, coexisting medical conditions and genetic influences.[11] Furthermore, they are expensive, tedious and often fraught with ethical issues. Meanwhile, 2D models fail to express tissue-specific physiological functions, interactions and lack physiochemical cues and often require *in vivo* animal model validation.[12] The preferable 3D organoids are also unable to replicate complex geometric and mechanical characteristics of the human lungs.[13] As such, preclinical models that better mimic the *in vivo* human lung architecture, microenvironment and functions are required to fill the gaps in existing models.

With the advent of microfabrication techniques, researchers have created advanced cell culture models called 'organ-on-

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chips' (OOCs) that mimic the *in vivo* conditions more closely. OOCs have emerged as a groundbreaking tool that can provide additional insights into human lung pathophysiology and functionality by reproducing organ-level functions.[14] These models require delicate fabrication utilising knowledge of bioengineering, microengineering, microfluidics and material sciences.[15] More specifically, these models successfully replicate the physiology and pathology of the human lungs to culture immortalized cell lines or primary human cells from patients (Fig. 1).[16] This review provides an overview of different models and investigations carried out using the lung-on-a-chip (LOC) platform and highlights recent advances.

Existing models and their limitations

Animal models

Animal models such as rats and mice are widely used for studying respiratory disease pathophysiology, identifying new biomarkers, drug targets and toxicity studies.[17] New chemical entities can be preclinically assessed using animal models, which is vital for new drug discovery. Animal models are crucial for studying lung diseases[18] such as acute respiratory distress syndrome, asthma, COPD, lung cancer, pulmonary fibrosis, cystic fibrosis and respiratory infections.[5,10,19] However, animal models do not really represent the human physiological, pathological and genetic characteristics and thus fail to accurately predict the response of drugs in humans.[20] This inconsistency ultimately hampers the effective development and success of drug compounds tested in subsequent human clinical trials.[21] Animal models are also associated with ethical concerns, high cost and low throughput. Therefore, more-accurate preclinical models for disease modeling and drug testing are required to increase the success of clinical trials and bring effective drugs to the market.

2D models

2D cell culture models are widely used for many biological studies owing to their advantages such as their broad acceptance, manufacturing inexpensiveness, ease of handling and manipulation.[20,22] Moreover, they offer a simplified and controlled platform for cell observation, quantification and response to drugs and toxins.[23] However, most conventional 2D models comprise only one cell type with some complimentary cells on culture plates. This limits their ability to accurately mimic the complex human tissue–tissue structure, interactions and organ-level functions. Another limitation of 2D models is their static condition, which results in the production and accumulation of toxic waste in 2D cell culture as cells differentiate and grow. The waste is accompanied by nutrition depletion, which reduces the supportiveness of the environment surrounding the cells resulting in their destruction and death. Also, cells in 2D models are not exposed to the normal physiological mechanical cues such as mechanical strain, tension, compression and fluid shear stress[24] that are essential for *in vivo* cell growth, proliferation and motility.[25] Therefore, more-complex culture models that recapitulate the complex human microstructures and physiology are required.

3D models

3D cell culture models have received much attention in overcoming the limitations of 2D culture models by providing *in-vivo*-like microenvironments. Techniques involving 3D culture include cellular matrix scaffold, air–liquid interface (ALI) cultures, perfusion culture chambers or hang-drop cultures. ALI cultures using transwell inserts have been commonly used where cells growing on the apical side are exposed to air whereas the basolateral side is submerged in the culture medium. These transwell inserts are user-friendly, suitable for electrophysical, toxico-

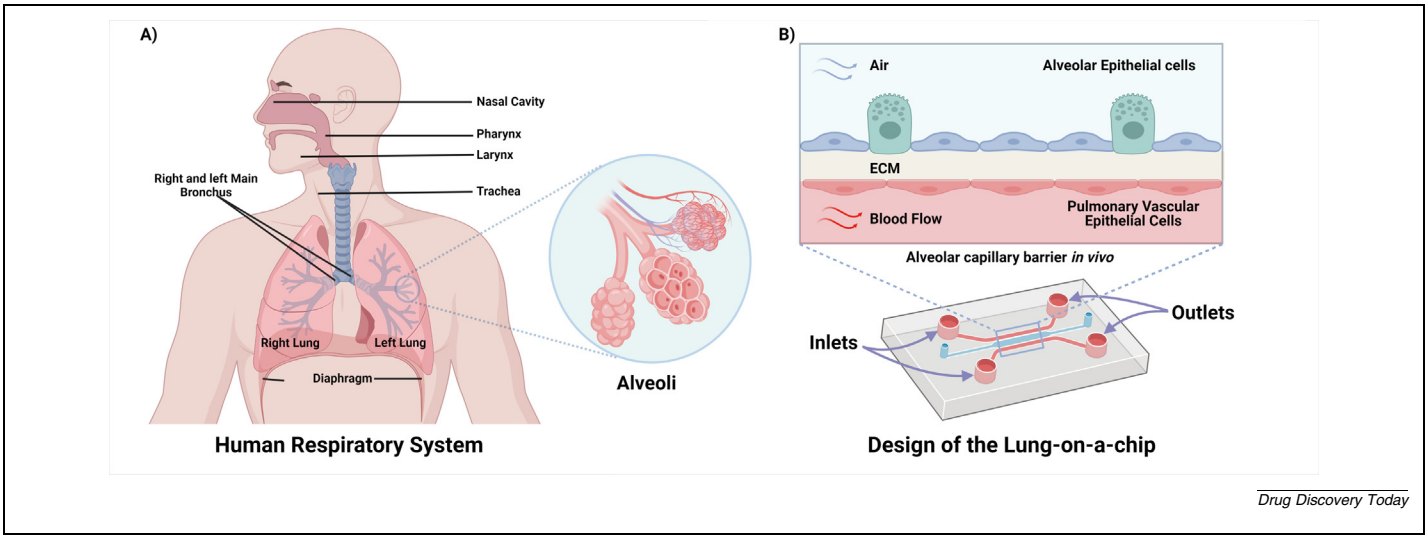

FIGURE 1

Alveolar–capillary barrier *in vivo* mimicked in a lung-on-a-chip model. **(a)** The exchange of oxygen with carbon dioxide takes place in human lungs, specifically in millions of small air sacs called alveoli, which are rich in blood supply. **(b)** Cross-sectional illustration of the microfluidic lung-on-a-chip model with two different channels separated by a thin, porous membrane. Human alveolar epithelial cells and human pulmonary microvascular endothelial cells are cultured at the top and bottom of the extracellular matrix (ECM)-coated membrane, respectively. Once confluent, the media is aspirated from the upper channel to culture the alveolar cells at an air–liquid interface, whereas a syringe pump is connected to the lower channel to continuously infuse media. Figure created on BioRender.com.

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logical and immunological studies, and useful for imaging and drug testing.[26].

Recently, the use of synthetic or natural cell scaffolds (decellularized) where the cells reside and grow in a 3D environment has increased. Synthetic cell scaffolds usually incorporate biocompatible polymer materials, including various hydrogel and fiber scaffolds such as poly-lactic acid and poly-lactic-co-glycolic acid (PLGA).[27] Natural biological extract cell scaffolds are made of extracellular matrix (ECM) gels that contain proteins such as collagen and fibronectin, alginate, gelatin, laminin and elastin, whereas the most commonly used synthetic hydrogels are polyethylene glycol and poly(lactic-co-glycolic acid).[28] Cells can be provided with a physiologically relevant environment by using scaffolds to improve cellular function. Incorporating these hydrogels into LOC enables a better understanding of the *in vivo* environment through replicating 3D cell–cell and cell–ECM interactions, 3D structures and cellular functions.[29] Cells can be randomly scattered in the ECM or agglomerate on top of other cells into 3D cellular clusters known as spheroids or organoids. Organoids can be generated from embryonic and adult stem cells to mimic *in vivo* organ tissue structure.[30] Additionally, patient-derived healthy or tumor tissues can be used to model patient-specific models for testing drugs and for personalized treatment regimens. Although this approach is widely used, the biochemical and biophysical environment for organoid development is hard to control and reproduce.[31] They lack the dynamic vascular supply and depend on passive diffusion for growth, which is insufficient for growing large organoids. Moreover, organoids vary in size, structural organization and gene expression, limiting their use in drug screening and disease modeling.

Organs-on-a-chip

OOCs are advanced microfluidic cell culture devices that mimic human body organs using advanced tissue engineering and microfabrication techniques.[32] An OOC consists of continuously perfused and controlled microchannels lined by living human cells, thus mimicking *in vivo* vascular perfusion, concentration gradients and fluid-flow-induced mechanical forces.[33] This technology provides high spatiotemporal precision that mimics whole organ multicellular architecture, as well as physiological, mechanical and biochemical microenvironmental features, with specific tissue–tissue interactions, cell–ECM interactions, mechanical and fluid forces, and chemical gradients.[29] It reproduces complex organ-level responses to inflammatory cytokines, environmental perturbations, pathogens and drugs by creating *in vivo* replication of disease state pathophysiological responses.[34] Owing to drug biotransformations in several organs, *in vitro* models cannot accurately reproduce these interactions, making it challenging to understand the exact mechanism of action of a drug.[35] OOCs are better at mimicking the *in vivo* physiological conditions and tissue interactions and can predict the response of the drug more accurately. OOC technology addresses many limitations of the conventional cell culture models, as stated in the previous section, and can provide a better platform for drug and toxicity screening. Thus, with further advances, these models can be used as a complementary platform with *in vivo* animal models to cross-validate the findings and improve the predictive power of preclinical research to increase the success rates of human clinical trials.[36].

OOC technology relies on hydrogel, polymer materials and traditional microengineering materials like glass or silicone for tissue attachment and growth.[37] A popularly used polymer is a silicone elastomer, polydimethylsiloxane (PDMS). PDMS is less toxic, inexpensive, easy to process and allows clear visualization and manipulation of cells.[38] However, PDMS is hydrophobic and requires surface modifications before cell seeding.[38] Also, it has high gas permeability and can adsorb small hydrophobic molecules, significantly altering the concentration of bioactive molecules. These issues are often addressed by incorporating biomaterials like collagen, Matrigel®, fibrin, gelatin, chitosan, hyaluronic acid or other polyesters and synthetic hydrogels in the OOC models.[20] Hydrogel chemical composition, porosity and mechanical characteristics make it a suitable material for OOC models.[15] Recently, the use of 3D printing and bioprinting to fabricate OOC models has significantly increased, resolving many of the existing issues and facilitating the development of complex models.[38] Since the first OOC model was developed, different body organs, including lungs, heart, gut, kidney, brain, blood vessels, liver, skin, nerves and bone, have been modeled and studied.[16,33,39-53]. These studies have shown the potential of OOCs to replicate the lung microenvironment, further facilitated by the establishment of numerous start-up firms focusing on these models.[54].

Lung-on-a-chip

LOC models are microengineered multilayered microfluidic devices that reproduce crucial dynamic responses and physiological functions by replicating the *in vivo* 3D lung architecture and cellular environment.[55] Based on the aim of the study and the physiological process to be replicated, different designs of LOC models can be fabricated. The models are fabricated to mimic the functional units of lungs through specific cell types, other structural organization and distinct biophysical and biochemical microenvironments.[31] For example, using a thin membrane, alveolar epithelial cells and pulmonary endothelial cells can be cultured on either side, subjected to air and blood flow, respectively, and mechanically stretched to mimic physiological breathing.[29] LOC designs have successfully been modeled to explore the physiology of human lungs and identify effective therapeutics and diagnostic biomarkers by modeling respiratory diseases and performing toxicological analyses and drug screening studies.[11] A comparison of the LOC model with other existing cell-culture models is illustrated in Fig. 2.

A LOC model was microfabricated and tested for the first time by Huh *et al.* in 2010.[33] This chip contained two parallel microchannels (one on top of the other) separated by a thin, permeable, flexible PDMS membrane coated with ECM proteins to mimic an alveolar–capillary barrier (Fig. 3a). The top layer of the membrane was covered by human alveolar epithelial cells, whereas the bottom layer was covered by human pulmonary endothelial cells. Once the cells reached confluence, the upper channel was aspirated to maintain an ALI and a continuous flow of media was infused into the lower channel. The two lateral hollow chambers enabled the application of a cyclic vacuum that stretched the flexible sidewalls along with the membrane with

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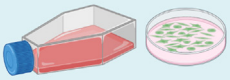
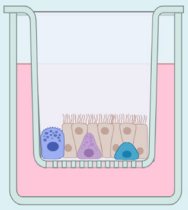
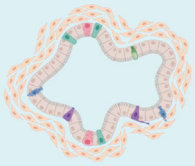
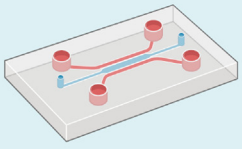
Culture Models		Advantages	Limitations
Static 2D cell culture		-Simple and reproducible -Low cost -High throughput -Real-time monitoring -Long-term cell viability -Patient-specific cells -No ethical issues	-Single cell types -No physiological biomechanics and biochemical cues -No hemodynamic system -Does not mimic 3D tissue architecture
Air Exposed Systems		-Simple and reproducible -Low cost -Co-culture -Cell differentiation -High throughput -Real-time monitoring -Long-term cell viability -Patient-specific cells -No ethical issues	-No Physiological biomechanics -No hemodynamic system -Does not mimic 3D tissue architecture -Inadequate nutrient and waste transport
Organoids		- Phenotypical/physiological relevance -Mimics 3D tissue architecture -Full cell differentiation -Cell-cell and cell-ECM interaction present -Real-time monitoring -No ethical issues	-Lacks immune system -Multiple tissue/organ interface absent - Lacks hemodynamic system -Inadequate nutrient and waste transport -No standard protocols
Animal Models		-3D-tissue architecture -Immune system -Hemodynamic system -Physiological biomechanics and biochemical cues -Multi tissue/organ interaction	-Expensive -Time Consuming -Interspecies variation -Low-throughput -Ethical issues -Findings can be inconsistent in translation to human health
Lung-on-a-chip		-3D-tissue architecture -Controlled microenvironment -Immune system -Hemodynamic system -Physiological biomechanics and biochemical cues -Multi tissue/organ interaction -Patient specific cells -No ethical issues	-No standard protocols -Difficult to scale up -Complex requiring adroit users

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FIGURE 2

Comparison of different cell culture models. Comparison of different *in vitro* and *in vivo* models for disease modeling and drug testing. Figure created on BioRender.com.

adhered cells. The alveolar–capillary interface was physically stretched to replicate physiological breathing.

The models that followed used similar chip designs and cell seeding with modifications and further enhancements for different applications (Table 1). Douville *et al*. designed an alveoli-on-a-chip model to observe the synergistic effects of solid mechanical and fluid stresses present in the alveoli.[56] They studied the impact of 3D cyclic stretching of the alveoli and propagation

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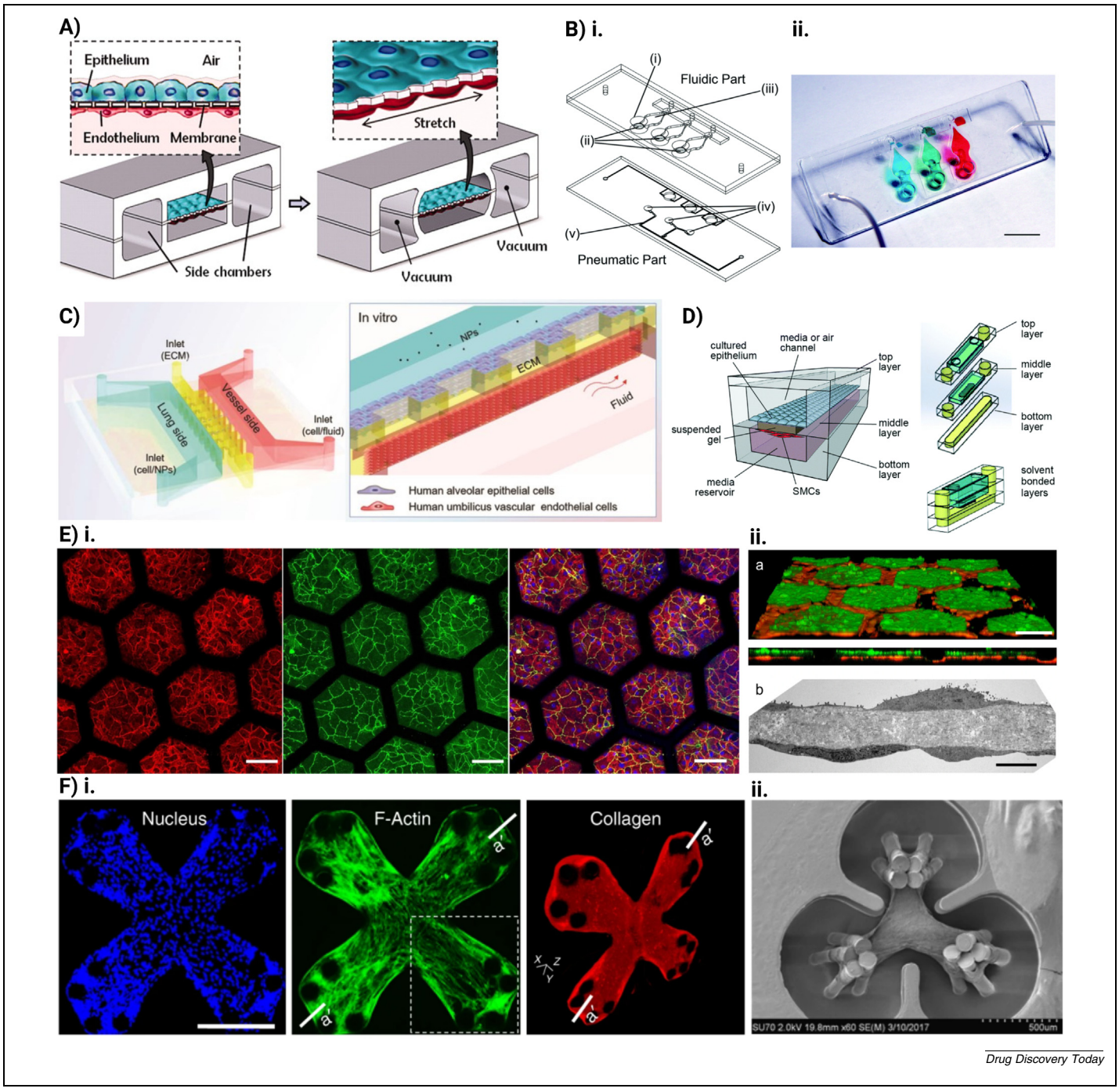

FIGURE 3

Different studies conducted using different lung-on-a-chip designs. **(a)** Model of a breathing lung-on-a-chip fabricated by Huh *et al.* using two channels, separated by polydimethylsiloxane (PDMS) membranes that are thin, flexible and porous. A vacuum applied to the side channels simulates the physiological breathing patterns and mechanically stretches cell membranes. Reproduced, with permission, from [33]. **(b)** The fluidic and pneumatic part of the design **(i)** and a photograph **(ii)** fabricated lung-on-a-chip model filled with food dyes, scale bar: 10 mm. Reproduced, with permission, from [16]. **(c)** Nanotoxicity testing model design using toxic nanoparticles (NPs) to mimic alveolar–capillary interface. The central Matrigel® channel separates the side epithelial and endothelial channels. Media circulates in the endothelial channel mimicking dynamic blood flow. Reproduced, with permission, from [58]. **(d)** Schematic design and exploded view of the chip with three layers of vertically stacked PMMA with a hydrogel chamber and smooth muscle cells (SMCs) in the middle, an airflow chamber at the top and a media reservoir at the bottom. Reproduced, with permission, from [71]. **(e)(i)** Primary human lung alveolar cells (hAEC) used on a lung-on-a-chip model, immunostained for zonula occludens-1 (green), E-Cadherin (red) and merged (Hoechst, blue). Scale bar: 100 μm. **(ii) (A)** Confocal images of co-culture of human primary endothelial cells (Rfp-label in red) and hAEC (E-Cadherin in green) on the CE membrane. Scale bar: 100 μm. **(B)** TEM imaging of hAEpC cells co-cultured with endothelial cells. Scale bar: 5 μm. Reproduced, with permission, from [72]. **(f) (i)** The four-leaflet microtissue mimicking the lung alveolar sac stained for nucleus, F-actin and collagen type-I. Scale bar: 500 μm. **(ii)** SEM image of the human lung-fibroblast-populated microtissue. Reproduced, with permission, from [73].

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TABLE 1

Different diseases studies using lung-on-a-chip models and the aim of the study.

Disease studied	Aim of study	Refs
Chronic diseases		
• COPD	• Small airway-on-a-chip model that mimicked clinical features of COPD and its exacerbation. Tested response of anti-inflammatory compounds	59
	• Cigarette-smoke-induced COPD pathophysiology	60
• Asthma	• Exacerbation of asthma in response to viral infection replicated. Testing of new anti-inflammatory, tofacitinib	59
	• Human rhinovirus and IL-13 induced asthma exacerbation. Testing of CXCR2 antagonist	61
	• Airway musculature-on-a-chip to mimic asthmatic musculature responses on exposure to IL-13	62
• Lung cancer	• Effects of physiological breathing motions on cancer cell growth, invasion and drug resistance	63
	• Testing lung cancer chemotherapy regimens	64
	• Inbuilt sensors to monitor cytotoxicity of anticancer drugs	65
• Fibrosis	• Model fibrotic, αSMA-positive disease phenotype of IPF and further developed a cystic fibrosis model	66
	• Development of pulmonary fibrosis caused by alveolar injuries on exposure to gastric contents	67
Infections		
• COVID-19	• SARS-CoV-2 model to study pulmonary injury and immune response. Tested antiviral, remdesivir	68
	• Reproduced clinically relevant organ-level response. Tested multiple drugs for efficacy	69
• Tuberculosis	• Studied host–pathogen interaction along with the role of surfactant	70
• Pneumonia	• Pathophysiology of pneumonia caused by Staphylococcus aureus and influenza virus	
• Fungal infection	• Inflammatory response to Aspergillus fumigatus	71
Toxicity	• Effects of cigarette smoke and treatment with budesonide, an anti-inflammatory drug	72
	• Effects of inhaled TiO2 and ZnO nanoparticles on epithelial and endothelial cells	57
	• Drug toxicity induced pulmonary edema	56
	• Aflatoxin B1 (AFB1) induced toxicity in a lung/liver-on-a-chip	73
Pulmonary thrombosis	• Studied the pathophysiology of pulmonary thrombosis and tested new antithrombotic	74

of air over the alveolar cells by observing cell death and cell detachment. They highlighted the role of fluid mechanical stresses in developing cell injury and studying clinical therapies to treat surface-tension-related diseases.

A drug-toxicity-induced pulmonary edema model was created by Huh *et al*. using their previous design to replicate the drug toxicity observed in cancer patients receiving interleukin (IL)-2.[57] The findings suggest that mechanical forces associated with breathing patterns triggered vascular leakage that caused pulmonary edema. Stucki *et al*. simulated the *in vivo* pulmonary environment and the 3D contraction and relaxation of the diaphragm with their LOC model.[16] Their model contained a semi-open design for culturing cells and a bottom compartment with a PDMS membrane representing a micro-diaphragm that moved with a negative pressure applied to a small underlying cavity (Fig. 3b). The membrane stretched cyclically, resulting in mechanical strain on the cells as experienced by actual cells in the human lung during physiological breathing. Unlike other models, their model cultured primary human alveolar epithelial cells derived from pneumonectomy patients for lung cancer.

Using a 3D LOC model, Zhang *et al*. studied the pulmonary toxicity of nanoparticles.[58] Their model contained three parallel channels with a central layer of Matrigel® membrane sandwiched between human alveolar epithelium and human vascular endothelium layers to mimic the alveolar–capillary barrier functions and its structural features (Fig. 3c). With the addition of cell–ECM interaction, they used this model to monitor the changes observed in barrier integrity, permeability and expression of junctional proteins after exposure to different concentrations of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles. Yang *et al*. used PLGA electrospinning nanofiber membrane to mimic the 3D cellular environment on a chip.[27] The use of an electrospun membrane allowed them to control its thickness up to a few microns precisely. Their device was used to co-culture epidermal growth factor receptor (EGFR)-targeted epithelial cell line A549 and human fetal lung fibroblasts to test the efficacy of gefitinib – an EGFR-targeted antitumor drug. The effects of physiological breathing patterns on wound healing were studied by Felder *et al*. (2019).[59] They concluded that physiological breathing significantly impaired the alveolar wound repair compared with static conditions.

Recent advances

Recently, researchers have focused on designing disease-specific models of the LOC to study disease pathophysiology. Benam *et al*. developed a human 'small-airway-on-a-chip' to model COPD and asthma and tested therapeutics.[60] They perfused IL-13 into the lower endothelial channel, which led to hypersecretion of inflammatory cytokines, decreased frequency of cilia beating and hyperplasia of goblet cells, which are pathological characteristics of asthma. When the anti-inflammatory drug tofacitinib was added the changes were significantly suppressed. Similarly, they used primary airway cells derived from COPD patients to model COPD and its exacerbation by exposing viral or bacterial pathogens. Nesmith *et al*. used smooth bronchial muscle cells to fabricate a human airway musculature-on-a-chip to evaluate the effects of IL-13 on the asthmatic musculatures.[61].

Hassell *et al*. studied human non-small-cell lung cancer in different microenvironments using their lung cancer model.[62] The physiological breathing cyclic strain on the chip significantly inhibited tumor growth. Khalid *et al*. introduced a novel lung-cancer-on-chip model that used inbuilt sensors to monitor lung tumors in real time.[63] The 3D co-culture model developed by

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Xu *et al.* was used to test the drug sensitivity and identify the most effective chemotherapeutic.[64] Barkal *et al.* introduced a fungus *Aspergillus fumigatus* to the airway channel and studied the inflammatory response.[65] The host interactions and the role of pulmonary surfactant in pulmonary tuberculosis were reviewed by Thacker *et al.* using their LOC infection model[66]; the host was *Mycobacterium tuberculosis*.

With the ongoing crisis of severe acute respiratory syndrome coronavirus (SARS-CoV-2 /COVID19), there is an overwhelming demand for effective therapeutics and prophylactics. A fast and effective way to fight the pandemic is to repurpose drugs already approved for other diseases.[80] The most human-relevant way of doing this is to utilize human OOC technology.[67,68] Si *et al.* used a bronchial-airway-on-a-chip to model type A influenza infection, strain-dependent virulence and inflammatory and immune response.[69] When nafamostat was co-administered with oseltamivir in the chip infected with influenza A virus, the treatment time window for oseltamivir was doubled. The clinically relevant doses of amodiaquine, an antimalarial, successfully inhibited the infection of pseudo-typed SARS-CoV-2 when tested in the chip. However, the inhibition of the infection by hydroxychloroquine and other antiviral drugs observed in static culture was not observed when treated in the chip in a dynamic culture.

Zhang *et al.* modeled a human alveolar chip to investigate the pulmonary injury caused by SARS-CoV-2 and immune response at the organ level.[70] They observed that epithelial cells showed a higher multiplicity of infection (MOI) than endothelial cells. It was shown that the increased levels of inflammatory cytokines, recruitment of immune cells and endothelium detachment were all associated with the exacerbation of inflammation caused by immune cells. They also tested an antiviral, remdesivir, which reduced the disruption of the alveolar–capillary barrier, indicating its potential to treat COVID-19.

Some recent LOC models show high throughput and ease of handling. Shrestha *et al.* developed a microfluidic model to study the effects of continuous positive airway pressure on the nasal airway of obstructive sleep apnea patients by incorporating well-established conventional cell culture models in a 3D-printed system.[13] Humayun *et al.* fabricated an acrylic vertically stacked model using micromilling and solvent-bonding techniques to study interactions between airway epithelium, smooth muscle cells, airway epithelium and ECM[71] (Fig. 3d). Zamprogno *et al.* recently developed a LOC model using collagen and elastin to mimic the *in vivo* alveoli[72] (Fig. 3e). The authors claimed that their membrane was superior to commonly used PDMS membranes in terms of the fabrication method, thickness variation flexibility, stiffness, biodegradability and similarity with a native ECM of the lung parenchyma. They used their model to culture primary human alveolar epithelial cells and replicated the air–blood barrier functions. Similarly, Asmani *et al.* developed a membranous lung microtissue composed of lung fibroblast injected collagen matrix to mimic healthy and fibrotic alveolar tissues[73] (Fig. 3f).

Challenges and future perspectives

Although LOC devices mimic numerous essential functions of the human lungs, they still face various challenges before preclinical applications. With LOC, it is technically impossible to reproduce the entire human lung with its complex structure, architecture and functions. Therefore, it is useful to accurately model specific areas or crucial aspects of a particular tissue. The most important characteristics, such as mimicking the morphological and functional phenotype of the alveolar–capillary interface, can be designed accordingly. Another major issue with LOC is determining the source of the cells to be used in the device, which depends on their availability and the aim of the study. Most research groups use immortalized cell lines or primary cells to represent *in vivo* lung cells and tissues. Cell lines offer lower production costs, higher throughput studies and a longer lifespan.[74] However, immortalized cell lines do not fully represent *in vivo* primary cell types.[75] Thus, recent studies progressively replace cell lines with primary cells isolated directly from human or animal tissues.

Primary cells have a superior ability to reconstitute exact *in vivo* tissue characteristics and faithfully replicate phenotypes (genetically and functionally) of adult state and disease pathology.[76] However, the genetic and epigenetic variability among donors or batches is a significant concern with primary cells. Other problems include the requirement of specialized techniques and media and direct cell donor availability. Recently, the use of stem-cell-derived sources in LOC has increased because they can be obtained from any donor and offer infinite renewable sources.[77] Induced pluripotent stem cells (iPSCs) can model personalized chips using the individual cells to mimic disease phenotypes or can be genetically engineered into a disease-specific mutation. Recently, a new concept of a superior hybrid tool: organoids-on-a-chip, has been put forward where iPSC-based organoids are incorporated into OOC models, with more physiological relevance in terms of functionality and tissue maturity.[31].

The other major issue with LOC is the selection of appropriate cellular scaffolds or extracellular matrix, which play a crucial part in cell–cell interactions.[78] To create a suitable microenvironment for cellular attachment and growth, most models involve decellularized scaffolds or natural or synthetic hydrogels. Hydrogel composition, arrangement and batch variability can significantly affect cellular growth, polarity, vascularization, immune response and visibility.[76] There are no specific protocols or ideal hydrogels to fabricate tissue-specific or organ-specific hydrogels. In addition to hydrogels, the materials used to fabricate the chip itself require attention. As stated before, despite its numerous advantages, PDMS adsorbs and absorbs small hydrophobic molecules and thus requires pretreatment or coating.[79] Materials such as glass, thermoplastics, silicone or 3D-printing resins can be alternatives to fabricate LOC models, based on the type of study, affordability and feasibility of the materials.[76] Model design is another essential factor to be considered during LOC fabrication. Cells are sensitive to the slightest change in their surrounding. Microfluidic channels (length, diameter, inlet and/or outlet angles) should be designed such that the shear forces created by the media flow are similar to the forces experienced *in vivo*.[80].

Multiple OOCs that represent specific organs can be interconnected to replicate the actual interactions between human organs, resulting in a body-on-a-chip (BOC), also referred to as human-on-a-chip. BOC systems can be utilized in the preclinical

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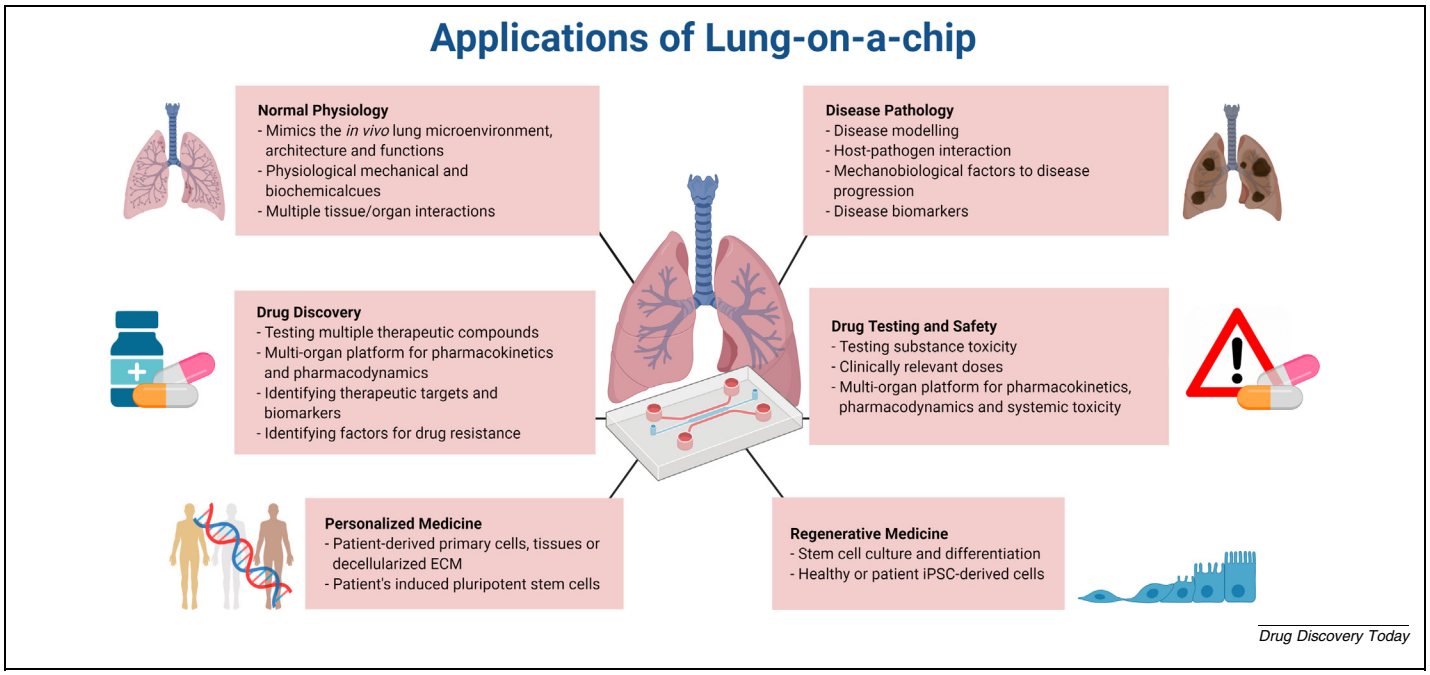

FIGURE 4

Graphical representation of different applications of lung-on-a-chip technology. Lung-on-a-chip platform has already demonstrated its potential with numerous applications across multiple disciplines. With further advances, this platform will assist the healthcare industry and facilitate drug development and personalized medicine. Figure created on BioRender.com.

drug discovery and development processes to predict their efficacy and toxicity.[81] Pharmaceutical companies are currently adapting the OOC technology, and the market need for BOC systems is expected to grow substantially.[82] Despite remarkable progress in the past decade, some aspects still need to be addressed. Injection of cells and samples is a manual process and is often complicated by a short time window, strict sterility maintenance and avoidance of stress. Currently, the manufacturing and implementation of OOCs are relatively expensive. Some OOCs incorporate different materials such as PDMS, glass, PET and PC, resulting in difficulties in obtaining multiplexed units and transitioning to alternative materials.[83] The system further requires methods to ensure compatibility with liquid handling and automated hardware systems. The throughput of OOC models is comparatively low because the injection and/or removal of limited samples, compounds and media into and from the chips require precise control and automation for parallelized experimentation. The sensors used in OOCs are limited in measurable parameters, reproducibility, throughput or sensitivity.[83] Moreover, the wide variety of readouts and vast sets of data gathered from the complex models require sophisticated analytic tools to be developed.

Connecting multiple organ systems to mimic a physiological microenvironment is another major challenge of the OOC system. Sterility, preventing bubbles and controlling different flow rates should be maintained at all times.[84] One major issue is using blood mimetic or universal cell culture medium that fulfills each interconnected tissue's required nutrients and growth factor supply.[76] The physiological relevance of the BOC system can be altered if the cell growth is negatively affected by the use of suboptimal culture media. Single-pass or microformulator systems or recirculation of culture media through design modifications can be a suitable approach to address this issue. Because BOC are highly miniaturized systems of the human body, the correct biological (allometric) scaling of different organs and the transport rates between these organs are required to replicate the maximum physiological responses and pharmacokinetic responses to specific drugs. Also, there are missing organs that directly or indirectly influence the cellular response and drug metabolism. Regardless of the challenges, LOC systems are ever-growing and can serve as an invaluable research tool in studying complex respiratory diseases and identifying the best treatment modalities (Fig. 4).

Concluding remarks

Because air quality is deteriorating globally, the health burden of respiratory diseases is rising.[85] Thus, advanced preclinical models that mimic the *in vivo* human response are essential for developing effective therapeutics to combat respiratory diseases. In some cases, the LOC platform aids the study of complex dynamic properties of *in vivo* cell–cell and tissue–tissue interactions that are necessary to understand human lung pathophysiology. These models have proven to be superior to other existing conventional cell-culturing models because they make the process of drug development more cost-effective, rapid and efficient. With further development, these models could lead to a parallel decrease in the dependency on animal models in drug testing and toxicological studies. Advanced LOC models with the integration of biosensing, imaging and screening systems, combined with meta-analysis of data, can assist pharmaceutical companies, clinicians and researchers in studying disease-specific models.[86] Personalized

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medicine is feasible by integrating patient-specific cells into OOCs for personalized screening and therapies. Moreover, the advanced version of BOC can better replicate the human physiology and pharmacokinetic responses of the whole human body in a single platform.[87] Despite numerous challenges, LOC platforms are anticipated to help the transition from preclinical to clinical studies.[82] Overall, this platform is rapidly growing worldwide and has gathered significant attention from pharmaceutical companies, research organizations and healthcare agencies. This will further widen the adoption of OOC platforms in the healthcare industry and revolutionize drug development and personalized medicine.

Conflicts of Interest

There are no conflicts of interest to declare.

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