CHAPTER

Lung-on-a-chip platforms for modeling disease pathogenesis

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Introduction

The global prevalence and major burden of lung disease on human health and economy highlight the importance of understanding lung pathology to advance treatment. In the ongoing endeavor to improve the tools and methodologies in the field, we can now create artificial cellular microenvironments that closely mimic those found in the human body. This will potentially revolutionize the way in which human biology is investigated, in the form of organ-on-a-chip (OOC) devices. The novel capabilities of microengineering systems can be leveraged to mimic lung functions, which involve a complex and hierarchical milieu of fluid and solid mechanical stresses. These platforms are termed lung-on-a-chip (LOC) and will be the focus of this chapter. We provide an overview of lung physiology and common pathologies, along with the conventional in vitro approaches to study them. We then introduce LOC development and illustrate the uses of these devices and the effects they have had on lung pathology research. An outline of the current state of LOC devices is given alongside foreseen improvements that we deem critical to the future success of LOC devices as effective research tools.

Anatomy and physiology of the respiratory system

The respiratory system supplies oxygen and removes carbon dioxide via gas exchange across the capillary–alveolar interface. The respiratory system also maintains blood pH, filters xenobiotics, and serves as a blood reservoir within the pulmonary vasculature. The system is composed of nose, pharynx, larynx, trachea, bronchi, and lungs. The trachea bifurcates into two primary bronchi and

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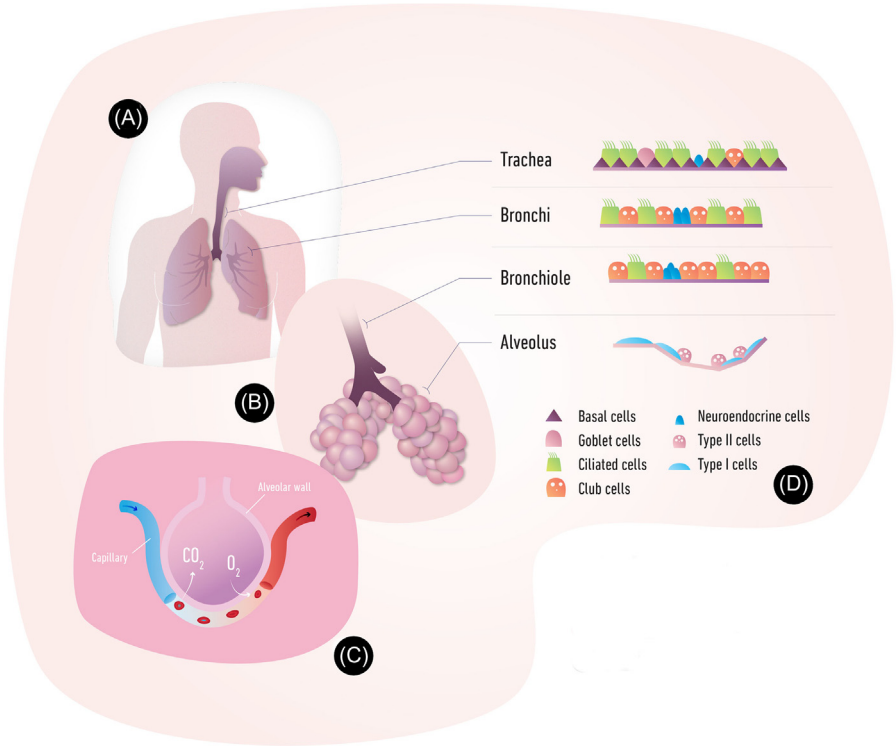


FIGURE 4.1

(A) Simplified schematic of the anatomy of the respiratory system; (B) respiratory airways include the bronchioles and the alveoli; (C) physiology of gas exchange at the alveolar–capillary interface; and (D) a schematic model of distribution of the main cell types of the respiratory organs.

continues to branch into lobar and segmental bronchi, bronchioles, and terminal bronchioles (Fig. 4.1A).

The terminal bronchioles in the lungs are cone-shaped, spongy organs located in the thoracic cavity, further separate into respiratory bronchioles, alveolar ducts, and alveolar sacs (Fig. 4.1B). Alveoli are grape-like structures with a diameter of approximately 250 μm. Gas exchange with the blood occurs through the alveolar membrane, at the alveolar–capillary interface (Fig. 4.1C). The adult human lung has around 300 million alveoli, with a surface area of up to 100 m^2 (Anjali and Mahto, 2016; Fonseca et al., 2017; Siegel et al., 2017; Wang et al., 2016).

The airways are coated by epithelium, which appears as a pseudostratified, ciliated layer across the trachea, and the bronchi, where it is mainly composed of basal cells, ciliated cells, and goblet cells, which are responsible for mucus secretion, with a protective function. In the bronchioles the epithelium is thinner and

cuboidal and is composed of club secretory cells and few ciliated cells; club cells regulate immune and metabolic activities by secreting surfactants and antimicrobial proteins. The alveolus membrane is layered with two types of pneumocytes, type I (ATI) and type II (ATII) alveolar epithelial cells. Type I cells are flat, squamous cells involved in gas exchange and immune response and compose 90% of the alveolar surface, while type II cells are cuboidal and secrete surfactants to reduce the alveolar surface tension (Anjali and Mahto, 2016; Siegel et al., 2017; Wang et al., 2016) (Fig. 4.1D).

The burden of respiratory diseases: classification, impact on global health, and statistics

Respiratory diseases mainly involve airways, lung tissue, and pulmonary circulation and are commonly classified as (1) obstructive, characterized by narrowed airways with formation of mucus/liquid plugs, (2) restrictive, when the lung volume is reduced, or (3) infectious, caused by microorganisms (Anjali and Mahto, 2016). According to the World Health Organization, respiratory diseases are the major cause of death and disability in the world, especially chronic obstructive pulmonary disease (COPD), asthma, acute lower respiratory tract infection and pneumonia, tuberculosis, and lung cancer (Forum of International Respiratory Societies and European Respiratory Society, 2017; Wang et al., 2016).

COPD and asthma are the two main chronic obstructive diseases and affect 200 and 334 million individuals worldwide, respectively. COPD is the third leading cause of death, with 3 million death per year. Asthma affects 14% of children, representing the leading chronic childhood disease. Mortality from lower respiratory tract infection (more than 4 million individuals annually) exceeds mortality from the human immunodeficiency virus, tuberculosis and malaria combined and is a leading cause of death in children aged under 5 years (Forum of International Respiratory Societies and European Respiratory Society, 2017). Tuberculosis is the most widespread infectious disease worldwide, with more than 10 million new cases and almost 2 million deaths each year (Fonseca et al., 2017). Lung cancer is estimated to be the leading cause of cancer deaths (Siegel et al., 2017), with 1.6 million deaths per year. The social and economic burden of respiratory is high, and the costs of COPD and asthma are estimated to exceed €30 and €15 billion per annum, respectively, in the European Union alone (Blume and Davies, 2013).

Another respiratory disease is cystic fibrosis (CF), a multiorgan pathology caused by a mutation in the CF transmembrane conductance regulator (CFTR) gene that affects more than 70,000 individuals worldwide. This mutation reduces ion transport, causing decreased mucociliary clearance, chronic inflammation, and bacterial colonization at the pulmonary level, with the lung symptoms being the most serious (Lavelle et al., 2016; Wang et al., 2014). The establishment and improvement of study models in research are thus necessary for a better understanding of lung pathophysiology and developing new therapies.

In vitro and in vivo models of respiratory disease pathogenesis

Building appropriate research models is fundamental to investigate the mechanisms of human lung development and pathophysiology. An ideal lung model should hierarchically reproduce morphological and functional aspects. At the cellular level, it should include various human cell types from lung tissue (alveolar and bronchial epithelial cells), vasculature (vascular endothelial cells), immune cells, and neural tissue (nerve cells) to recreate cell−cell interactions and the interplay between cells and the surrounding three-dimensional (3D) microenvironment. At the tissue level, the physiological mechanisms should provide cyclic stretching to mimic the mechanical stimulation via respiration and the controlled flow of both air and blood typical of the respiratory airways (Bajaj et al., 2016; Miller and Spence, 2017). At the system level, the components of the respiratory tract (bronchial, alveolar, etc.) should be integrated for a complete mimicking of the respiratory apparatus. In vivo, ex vivo, and in vitro lung models will be described in this chapter, addressing the main advantages and drawbacks of each approach.

The in vivo approach: animal models of lung diseases

Because of ethical considerations, the use of human models is severely restricted to minimal or no-detriment studies, such as analyses of the immune response (e.g., skin allergy test) or physical outputs such as breathing peak flow rate and heart rate (e.g., exercise), as well as to the study of tissue biopsies (Blume and Davies, 2013; Gordon et al., 2015). Experimental animal models have thus provided most of the current knowledge of lung pathophysiology and are the cornerstone of developing new drugs (Blume and Davies, 2013; Miller and Spence, 2017). Several animal species have been used as lung disease models of asthma, acute respiratory distress syndrome (ARDS), COPD, CF, tuberculosis, and lung cancer; these include small animal models (mice, rats, guinea pigs, hamsters), rabbits, dogs, sheep, and nonhuman primate models (NHPs) (Fonseca et al., 2017; Fricker et al., 2014; Han et al., 2018; Rosen et al., 2018) (Table 4.1). NHPs are considered the best model because their lungs mirror the anatomical, physiological, and immune features of the human lung (Fonseca et al., 2017; Miller et al., 2017), but costs and ethical issues strictly limit their use, especially for large-scale experiments. Owing to economics and ease of scientific manipulation, small animals and specifically mice are the most commonly used model (Fonseca et al., 2017; Marqués-García and Marcos-Vadillo, 2016; Meurs et al., 2008).

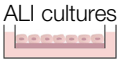
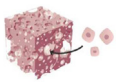
Mice models have primarily been “humanized” through grafting of human cells and tissues (Calderon et al., 2013; Ito et al., 2012) or transgenic, knockout, or knockin approaches (Pérez-Rial et al., 2015) to recreate particular human disease features. Once established, these humanized models are further developed to achieve the appropriate pathophysiological conditions or progression (Table 4.2).

Table 4.1 List of the main aspects achieved/recreated by means of animal (in vivo) models of the common lung pathologies.

Pathology	Aspects achieved/mimicked using animal models
ARDS	• Analysis of the mechanisms of AFC reduction and pulmonary edema (Huppert and Matthay, 2017) • Altered alveolar ion transport and decreased epithelium permeability causes AFC reduction in mice exposed to influenza virus (Chen et al., 2004) • MSC therapy to decrease *Escherichia coli* pneumonia injury (Devaney et al., 2015; Gupta et al., 2012)
Asthma	• Asthma development and exacerbation mechanisms induced by viruses by means of sensitized animal models (Han et al., 2018; Mullane and Williams, 2014) • The target of the T-helper type 2 cells responsible for driving allergic asthma mechanism (Holt et al., 1999; Zosky and Sly, 2007) • Role of airway remodeling in chronic and acute AHR (Meurs et al., 2008)
CF	• CFTR mouse models recreated the altered nasal epithelium typical of human CF (Lavelle et al., 2016; McCarron et al., 2018); • CFTR −/− mutant pigs were used to model electrolyte transport defects and mimic the impaired bacteria clearance mechanisms (Chen et al., 2010; Ostedgaard et al., 2011; Stoltz et al., 2010); • CFTR −/− ferrets were used to recreate mucus plugging and defective ion transport of the tracheal epithelium (cAMP-dependent chloride transport) (Fisher et al., 2013; Sun et al., 2010) • Test of therapeutics [e.g., pH modification for acidic CF tissues (Alaiwa et al., 2016) and gene therapy (Cmielewski et al., 2014; Cooney et al., 2016)]
Lung cancer	• Preclinical models for testing chemotherapeutics, drug combinations (Sandler et al., 2006), and chemopreventive therapies (Herzog et al., 1997; Wang et al., 2009; You and Bergman, 1998) • Xenograft models as tool for personalized medicine and cancer recurrence prediction (Dong et al., 2010; John et al., 2011; Kellar et al., 2015) • GEMMs to assess carcinogenesis, cancer prevention (Kellar), and metastatic mechanisms (Gazdar et al., 2016, 2015)
COPD	• Determine the role of immune cells (macrophages, neutrophils, NKs, T cells) by means of CS-induced models (Beckett et al., 2013; Dhami et al., 2000; Eppert et al., 2013; Fricker et al., 2014; Motz et al., 2010) • Defining the involvement of proteins (chemokines, cytokines), enzymes (protease), and oxidative stress in COPD pathogenesis (Foronjy and D'Armiento, 2006; Fricker et al., 2014) • Create models of bacterial and viral exacerbation (Gaschler et al., 2009; Pérez-Rial et al., 2015; Zhou et al., 2013)
Tuberculosis	• Models of latent TB, TB granuloma formation (guinea pigs, rabbits) (Fonseca et al., 2017; Gupta and Katoch, 2005; Orme and Basaraba, 2014), and necrosis (Kramnik and Beamer, 2016) • Modeling HIV/TB coinfection to test new therapeutics for HIV prophylaxis and treatment (Diedrich and Flynn, 2011; Pawlowski et al., 2012) • Models for testing anti-TB vaccines and drugs (Cardona and Williams, 2017; Zhan et al., 2017)

AFC, *alveolar fluid clearance;* AHR, *airway hyperresponsiveness;* ARDS, *acute respiratory distress syndrome;* CF, *cystic fibrosis;* CFTR, *cystic fibrosis transmembrane conductance regulator;* COPD, *chronic obstructive pulmonary disease;* CS, *cigarette smoke;* GEMM, *genetically engineered mouse model;* HIV, *human immunodeficiency virus;* MSC, *mesenchymal stem (stromal) cell;* NK, *natural killer;* TB, *tuberculosis.*

Table 4.2 List of lung in vitro models and the main outcomes achieved using each of them.

Model	Aspects achieved
2D cultures 	• Study of ciliary structure and ciliary dysfunction (Pifferi et al., 2009; Rutland and Cole, 1980) • Used for clinical diagnostics in TB (acid fast smear assays)
ALI cultures 	• Showing mucociliary differentiation of tracheal epithelial cells (Whitcutt et al., 1988) • Study of pulmonary toxicity: air pollutants (Upadhyay and Palmberg, 2018), cigarette smoke (Li, 2016; Thorne and Adamson, 2013), infectious agents (Miller and Spence, 2017) • Epithelium and ECM remodeling in asthma (Benam et al., 2015) • Cellular effect of CFTR mutation in CF (Miller and Spence, 2017)
Organoids 	• Progenitor and PSC cells can generate functional proximal and distal airway epithelium (Ghaedi et al., 2014; Gotoh et al., 2014; Konishi et al., 2016) • Mimicry of the human fetal lung development and tumorigenesis (Kaisani et al., 2014; Miller et al., 2018) • Screening of proteins responsible for goblet cells metaplasia[a] in diseased lung (Danahay et al., 2015) • Analysis of lung stem cells self-renewal mechanisms and interactions with the niche (Dye et al., 2016; Kretzschmar and Clevers, 2016; Zepp et al., 2017)
Biological (decellularized) scaffolds 	• Ventilated scaffolds enabled pneumocytes differentiation (Doryab et al., 2016; Inanlou and Kablar, 2005) • Effective stem-cell differentiation compared to other matrix-based models (Cortiella et al., 2010; Hoganson et al., 2014; Price et al., 2010) • Large-scale production of alveolar epithelium (Ghaedi et al., 2014) • Chronic lung disease (COPD, IPF) acellular scaffolds as models of disease pathomechanics (Booth et al., 2012; Gilpin and Wagner, 2018; Sava et al., 2017) • Use of airway scaffolds (trachea) for clinical transplants (Delaere et al., 2018; Laurance, 2010; Macchiarini et al., 2008)
Bioengineered scaffolds 	• Formation of alveolar-like structures (Doryab et al., 2016; Douglas et al., 1976) • Alveolar and vascular regeneration (Shigemura et al., 2006) • Tissue-engineered trachea and bronchus (Nichols et al., 2017)

2D, *Two-dimensional;* ALI, *air–liquid interface;* CF, *cystic fibrosis;* CFTR, *cystic fibrosis transmembrane conductance regulator;* COPD, *chronic obstructive pulmonary disease;* ECM, *extracellular matrix;* IPF, *idiopathic pulmonary fibrosis;* PSC, *pluripotent stem;* TB, *tuberculosis.*
[a]*Goblet cells metaplasia is responsible for mucus hypersecretion and airway obstruction, features typical of lung diseases such as COPD, chronic asthma, and CF (Nadkarni).*

In vivo models of the main lung diseases

Exposing mice to cigarette smoke for 3–6 months is a method of reproducing smoke-induced COPD hallmarks of airway remodeling, inflammation, and emphysema (Beckett et al., 2013; Fricker et al., 2014; Pérez-Rial et al., 2015). The cigarette smoke–induced COPD mouse model is also used in combination with bacterial or viral infection to create models of COPD exacerbation and with hypoxia conditions or growth factor inhibitors to induce pulmonary hypertension and emphysema in severe COPD models. Mice sensitized to allergens such as ovalbumin, house dust mite, or *Aspergillus fumigatus* are commonly used to model asthma-related exacerbations and airway hyperresponsiveness, typical of chronic allergic asthma (Benam et al., 2015; Han et al., 2018; Meurs et al., 2008; Mullane and Williams, 2014).

Murine models of influenza pneumonia have contributed to elucidating the mechanism of reduction of the alveolar fluid clearance typical of ARDS, showing the reduced activity of alveolar ion channels and the altered permeability of alveolar epithelium (Huppert and Matthay, 2017). Furthermore, mice infected by *Mycobacterium tuberculosis* have been used to study the mechanisms of tuberculosis dormancy (Alnimr, 2015), granuloma formation (Orme and Basaraba, 2014), and tuberculosis necrosis (Kramnik and Beamer, 2016). CFTR-knockout mice have been used as CF models, as well as under bacteria-challenged conditions, achieved by inoculating mice with *Pseudomonas aeruginosa*. However, this model has demonstrated limited validity because of an inability to spontaneously develop the pathological features of CF. The absence of characteristic lung inflammation and mucus plugs resulted in the development of more accurate CF ferret and pig models (Lavelle et al., 2016; McCarron et al., 2018; Rosen et al., 2018; Wang et al., 2014).

Human tumor xenografts in mice are the most commonly used lung cancer models (Kellar et al., 2015; Ruggeri et al., 2014) in research and in preclinical pharmacology studies. Genetically engineered mouse models also represent a valuable tool in studying lung tumorigenesis and cancer progression. By mimicking the tumor microenvironment, these models can provide more reliable outcomes for drug toxicity tests (Gazdar et al., 2016, 2015).

Limitations of animal models

Despite the contribution of animal models to understanding disease mechanisms, animals and humans are substantially different (Miller and Spence 2017): murine airways differ from human airways in size, airway branching patterns, and anatomy of upper airways (Hofmann et al., 1989; Jong and de Maina, 2010a; Miller and Spence, 2017). Furthermore, human and murine lung development occurs on different time scales (Snoeck, 2015) and via differing molecular pathways and self-renewal of progenitor cells (Nikolić et al., 2018). Mice and humans share several cell types, but these differ in location, distribution, quantity, morphology, and function, effectively creating gaps in recapitulating specific

pathophysiological features (Mullane and Williams, 2014; Nichols et al., 2013). More critically, animals do not necessarily develop human pathologies such as asthma or tuberculosis (Fonseca et al., 2017; Zosky and Sly, 2007); thus in vivo models typically represent limited features of the disease. Moreover, respiratory and heartbeat rates are substantially higher in rodents, resulting in faster physiologic and metabolic processes, a difference that should be taken into account in pharmacokinetic studies (Jong and de Maina, 2010a; Perinel et al., 2017). Thus correlation of test results across species and translation to human clinical trials is still challenging and raises the issue of the validity of in vivo research (Fonseca et al., 2017).

Because of these drawbacks, more than 80% of drugs that pass the preclinical animal testing stage in mice fail in clinical trials, with a substantial loss of time and resources invested, underscoring the need for less expensive, more representative, and higher throughput models (Miller and Spence, 2017; Mullane and Williams, 2014; Perrin, 2014).

Ex vivo approach

The use of ex vivo models allows targeted studies of airway and parenchyma that cannot be ethically conducted in vivo while still providing a 3D tissue architecture (Blume and Davies, 2013; Gordon et al., 2015). Biopsy samples obtained from diseased and healthy (control) patients by bronchoscopy have been used to investigate the pathophysiological mechanisms and cellular immune responses to allergic inflammation in asthmatic patients and to investigate human tissue responses during therapeutic testing and development (Bhowmick and Gappa-Fahlenkamp, 2016; Blume and Davies, 2013). Human precision-cut lung slices have been shown to be useful models for immune responses to allergies, new drug formulation, toxicology assays, and tumor studies (Constant et al., 2015; Gordon et al., 2015). However, these models suffer from short-term viability and lack of reproducibility due to the source variability; in addition, the barrier properties can be compromised by direct exposure to stimulating, challenge agents applied to the tissue pre- or postsample acquisition (Blume and Davies, 2013).

Use of in vitro models

Several cell types can be used to produce in vitro models; these can be classified into three main categories:

1. *Primary human airway epithelial cells*: Primary cells from bronchial brushing and biopsies or from commercial suppliers mimic differentiated aspects of the epithelium, exhibiting the normal pulmonary phenotype under healthy conditions. Normal human bronchial epithelial and small-airway epithelial cells are available for disease and pharmacological studies. Furthermore, immortalization protocols are now available to extend the lifespan of primary

cells without losing the differentiation capability (Gordon et al., 2015). Primary cells also offer the potential for personalized medicine studies, but obtaining patient cells can be difficult.

2. *Cell lines*: Immortalized cell lines are obtained from tumors or by viral transfection and are commonly used in research because of their ease of culturing, the high throughput of proliferation, and the longevity of the culture, which effectively leads to lower costs than those of in vivo studies (Gordon et al., 2015). Human bronchial epithelial cells, such as the adenocarcinoma line Calu-3, have been used to mimic central airways in transport studies, model barrier properties, and recreate disease and infection models of the bronchial epithelium. Peripheral airway models have been recreated mainly using the A549 alveolar adenocarcinoma cell line as a model of alveolar type II cells for drug formulation, transfection, and infection studies (Bhowmick and Gappa-Fahlenkamp, 2016; Gordon et al., 2015). The A549 line has also been used in studying non−small cell lung carcinoma, which represents 80%−85% of lung cancer cases (Gazdar et al., 2010).
3. *Stem cells*: The controlled differentiation of human embryonic stem cells or induced pluripotent stem cells represents a powerful tool with the potential for large-scale production (Gordon et al., 2015). The process commonly used is directed differentiation (Dye et al., 2016a,b), which consists of providing biological cues to cells to drive their differentiation into specific cell types, but there is still a lack of standardized protocols for cell production and characterization. Directed differentiation enables the use of cells from patients genetically predisposed to pathologies and genetic modification of human-induced pluripotent stem cells (Constant et al., 2015; Dye et al., 2016a,b).

In vitro models range in their complexity and accuracy in recreating various aspects of the lungs. The simplest model is the two-dimensional (2D) monolayer media-submerged model, in which cells of the same type are cultured on a dish or a membrane and exposed to media on both the apical and basal sides (Bhowmick and Gappa-Fahlenkamp, 2016; Miller and Spence, 2017). The model is used in clinical diagnostics of ciliary dysfunction and tuberculosis (Miller et al., 2017; Rosenfeld et al., 2014).

The lack of an in vivo−like architecture in 2D in vitro models causes cellular dedifferentiation, and protein expression and cellular response only partially capture the underlying physiological mechanisms (Bhowmick and Gappa-Fahlenkamp, 2016). 3D in vitro models reproduce a microenvironment that can mimic in vivo architecture and functional features. Cells seeded into 3D models have been shown to create functional tight junctions, with the formation of basement membranes, polarization, and a phenotype resembling that of in vivo conditions (Bhowmick and Gappa-Fahlenkamp, 2016; Miller and Spence, 2017). These platforms include air−liquid interface (ALI) 3D organoids, tissue-engineered constructs, and LOC systems. Unlike the submerged model, the ALI model aims to mimic the airway microenvironment by seeding epithelial cells on a permeable

membrane of a Transwell insert, with the apical surface in contact with the atmosphere (air) and the basal side exposed to culture medium (liquid) (Bhowmick and Gappa-Fahlenkamp, 2016; Miller and Spence, 2017; Whitcutt et al., 1988). ALI systems enable the evaluation of barrier properties and have recreated mucus production and epithelial cell responses under both health and pathology conditions (Benam et al., 2015; Gordon et al., 2015). Furthermore, in vivo–like cell–cell interaction can be recreated by using multicellular models, in which different cell types are cocultured to study their interplay under healthy and inflammatory conditions (Gordon et al., 2015; Upadhyay and Palmberg, 2018).

Several commercial ALI models are available: CULTEX (Hannover, Germany) and VITROCELL (Waldkirch, Germany) are in vitro aerosol systems used to investigate cellular responses to particles and gases (Bhowmick and Gappa-Fahlenkamp, 2016; Upadhyay and Palmberg, 2018). EpiAirway (MatTek, Ashland, MA, United States) and MucilAir (Epithelix, Geneva, Switzerland) are ready-to-use ALI systems composed of primary cells from donors with different pathologies (e.g., asthma, COPD, CF) that can reproduce various anatomical sites (e.g. trachea, bronchi). The coculture of human fibroblasts enables the study of complex inflammatory mechanisms, such as in the fibrotic lung (EpiAirwayFT, MucilAir-HF). These commercial in vitro platforms can recreate in vivo barrier features, including cellular morphology, mucus production, and tight junctions, providing a sufficiently complex model to evaluate airway disease mechanisms and perform toxicology, drug delivery, and infection studies. Long-term and chronic studies have also been possible because of the long shelf life of these systems (Bhowmick and Gappa-Fahlenkamp, 2016; Gordon et al., 2015). OncoCilAir, a 3D in vitro lung cancer model, has been used in preclinical studies of anticancer therapies to investigate tumor–stroma interactions (Constant et al., 2015; Mas et al., 2016).

3D organoids are self-assembled aggregates of various cell types grown in extracellular matrix-like gel (e.g., Matrigel) (Bhowmick and Gappa-Fahlenkamp, 2016; Miller and Spence, 2017). Organoids can be formed from primary stem cells or from pluripotent stem cells and can be assembled as tracheospheres, bronchospheres, or alveolospheres to recapitulate the features of lung compartments (Barkauskas et al., 2017; Gkatzis et al., 2018). Their ability to mimic cellular spatial organization and recreate specific organ functions have defined organoids as innovative platforms for modeling lung development, lung disease, and lung cancer (Nadkarni et al., 2016). Organoids have been used to clarify the role of growth factors and signaling pathways in branching morphogenesis (Gkatzis et al., 2018; Nadkarni et al., 2016) and that of genetic alterations and malignant transformation in lung tumorigenesis. Intestinal organoids have been developed successfully to evaluate CFTR mutations and drug formulations in CF treatment (Dekkers et al., 2013). Organoids from stem cells have been used to study pulmonary viral infections (Chen et al., 2017) and to elucidate the complex early-stage mechanisms typical of infectious diseases such as tuberculosis (Bielecka and Elkington, 2018; Fonseca et al., 2017).

Lung tissue engineering enables the development of lung substitutes for transplantation in patients with end-stage lung diseases and is a platform for drug screening and potentially personalized therapeutics (Doryab et al., 2016; Hoganson et al., 2014; Langer and Vacanti, 2016). Engineered scaffolds can integrate both vascular and airway structures into a complex 3D lung construct, reproducing the necessary gas exchange (Hoganson et al., 2014). Two parallel approaches are used: the first consists of engineering synthetic substrates for cell growth and differentiation using either biopolymers (e.g., collagen, Matrigel, gelatin) or biocompatible polymers [e.g., poly(DL-lactic acid), polyglycolic acid, poly-2-hydroxyethyl methacrylate] and blends. The second involves the fabrication of decellularized biological scaffolds from which the cellular components of the organ or tissues are removed using detergents while preserving the anatomical structure and extracellular matrix (Doryab et al., 2016). The device is then recellularized with specific cell types, eventually with the aid of bioreactors (Doryab et al., 2016; Langer and Vacanti, 2016). This method demonstrates the feasibility of recreating functional lung constructs and is potentially applicable for clinical use (Nichols et al., 2017). While a core goal of lung tissue engineering is the ability to replace or restore functional lung tissue, scaffolds are a valuable platform for evaluating lung development, studying disease, and testing therapeutics. Studies using decellularized scaffolds have shown how extracellular matrix from diseased and aged donors influences cell attachment, proliferation, and survival and clarified the role that structural remodeling of the extracellular matrix plays in pathology initiation and progression (Gilpin et al., 2017; Tjin et al., 2017; Wagner et al., 2014).

Despite their advantages over traditional culture models, organoids and lung tissue engineering approaches have some drawbacks, key among which is the difficulty to spatially pattern and organize different cell types, the controlled distribution of biochemical molecules, or the defined mechanical stimulation across the 3D tissue. The LOC technology overcomes these challenges using microfluidic devices that can mimic tissue stretching, physiological flow, and biochemical stimuli (Bajaj et al., 2016; Seo and Huh, 2019).

Current lung-on-a-chip systems

The respiratory system exhibits dynamic behavior of cyclic motion physical stresses, as the lung is fundamentally a mechanical organ. With each breath, the lungs undergo a considerable change in volume and an approximate change in tissue length of 4%−25% (Gump et al., 2001). The resulting macroscale stress constitutes only one part of the complex physical forces (of varying magnitude, direction, and frequency) transmitted down to the microscale in the alveoli, where surface tension and fluid shear stresses from blood and interstitial flow begin to dominate (Fredberg and Kamm, 2006; Guenat and Berthiaume, 2018).

While 3D lung cell culture platforms are significant improvements over their 2D counterparts, they still largely fail to take into account the mechanical cues that are vital to lung development and function, and consequently are inadequate lung analogs for disease modeling (Mammoto et al., 2013). Leveraging the advantages of microfluidics, OOC platforms have enabled researchers to develop more biomimetic systems that recreate both the biochemical and mechanical aspects of cellular microenvironments in human organ systems. A wide variety of organ functions can thus be mimicked.

The versatility of microfluidic systems can be leveraged to model a wide variety of physiological lung conditions, in both healthy and diseased states, at various levels within the hierarchical lung structure. Moreover, by taking a modular approach, individual microfluidic units can be combined to study the complex interactions between the respiratory system's distinct functional zones. Skolimowski et al. (2012a,b) demonstrated this concept with interconnected microfluidic compartments of media at varying oxygen levels to model the anaerobic sinus environment, the microaerobic tracheal and bronchial zone, and the highly aerobic alveolar zone (Fig. 4.2). These compartments were connected with a series of micropumps and valves, which enabled the study of antibiotic efficacy with changes in oxygen tension on bacteria associated with mortality in CF. This system serves as an example of the new capabilities that microfluidics offer to drive discoveries in lung pathology.

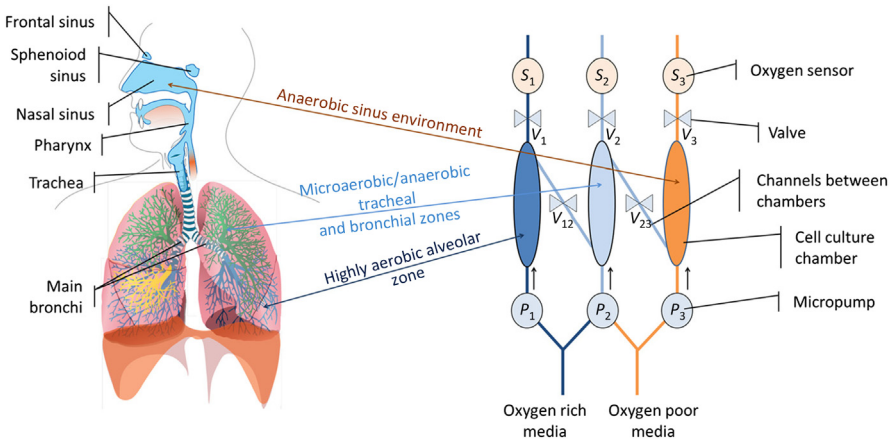


FIGURE 4.2

The modular microfluidic airway model. Each of the interconnected compartments is maintained at varying levels of oxygen concentration, corresponding to different zones throughout the human airway.

Reprinted from Skolimowski, M., Weiss Nielsen, M., Abeille, F., et al., 2012. Modular microfluidic system as a model of cystic fibrosis airways. Biomicrofluidics 6 (3), 1–11, with the permission of AIP Publishing.

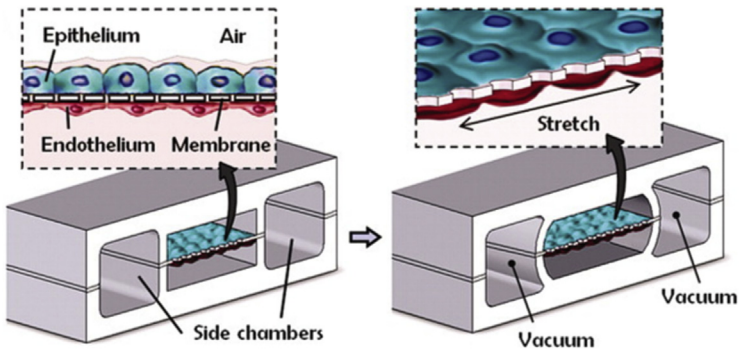


FIGURE 4.3

Mechanically active lung-on-a-chip device. The three-layer PDMS device consists of two central chambers separated by a thin porous membrane seeded with alveolar epithelial cells on one side and vascular endothelial cells on the other. An air–liquid interface is established by flowing air in the upper, alveolar channel. As illustrated, vacuum can be applied to the two-side chambers in order to deform the elastic PDMS walls, and thus the membrane. This serves to unidirectionally stretch the cells and simulates the stretching that is undergone during the expansion and contraction of the alveoli in the human body. *PDM*, Spolydimethylsiloxane.

From Huh, D., Matthews, B.D., Mammoto, A., Montoya-Zavala, M., Hsin, H.Y., Ingber, D.E., 2010. Reconstituting organ-level lung functions on a chip. Science 328, 1662–1668. doi:10.1126/science.1188302. Reprinted with permission from AAAS.

In this section, we discuss from a structural and microfluidic standpoint, innovative and noteworthy LOC systems and how their designs mimic a variety of lung functions and conditions. This chapter considers OOC devices to be systems that can recreate organ-level function and response, most often via tissue–tissue interfaces, unlike simpler, gel-based 3D cell cultures or single-tissue cultures.

Mechanically active alveolar–capillary interface

The pioneering work by Huh et al. (2010) in simulating the mechanical stresses on lung cells was the first to mimic the cyclic stretching of the alveolar membrane at an ALI in a microfluidic device. The multicompartmental polydimethylsiloxane (PDMS) device (Fig. 4.3) consisted of two primary media channels separated by a thin, extracellular matrix-coated porous membrane, one side of which was seeded with alveolar epithelial cells and the other with microvascular endothelial cells. After cells reached confluence, an ALI was established by flowing air through the epithelial, or alveolar, channel and cell culture media through the endothelial, or vascular, channel, recreating the ALI found at the alveolar–capillary interface. To simulate the cyclic strain that this interface experiences in vivo, the group used an innovative actuation method employing lateral

microfluidic channels, through which vacuum could be applied and subsequent unidirectional stretching of the elastomeric membrane could be achieved. With this setup, endothelial cell alignment responses mimicked those of actual endothelium and in vivo blood vessels (Iba and Sumpio, 1991; Thodeti et al., 2009), differing from cell behavior in 3D culture systems that do not incorporate an ALI or mechanical actuation (Pampaloni et al., 2007).

The platform was then evaluated from an immunological standpoint to verify that the addition of cyclic mechanical stresses in an in vitro lung model could produce more complex and biomimetic organ-level responses. Pulmonary inflammation response was first demonstrated using a medium containing the proinflammatory cytokine tumor necrosis factor α (TNFα) into the vascular channel of the microfluidic device. The endothelial expression of intercellular adhesion molecule 1 (ICAM-1) and subsequent adhesion and transmembrane migration of fluorescently labeled neutrophils were monitored as a measure of the system's inflammatory response. The inclusion of mechanical strain on the alveolar membrane produced no changes in immune response, but this was not the case when nanoparticles were used as the stimulant. Silica nanoparticles, often used in airborne particle toxicity studies (Lin et al., 2006; Napierska et al., 2009), were deposited as a thin liquid layer onto the epithelial side of the membrane and their absorption across the membrane increased in the presence of physiologically similar levels of membrane strain. The immune response, determined again by ICAM-1 expression, also increased. This response was similar to that observed in murine lungs exposed to silica nanoparticles and consistent with in vivo evidence, suggesting nanoparticle toxicity attributable to cross-membrane absorption (Nel et al., 2006). This is in contrast to the lower levels of absorption shown in static in vitro Transwell-based systems or the same PDMS device used without mechanical actuation.

This biomimetic platform was the first demonstration of a mechanically active system generating more realistic in vitro lung environments and subsequent immune responses than conventional static systems and has since become a foundation in microfluidic configuration for further development of LOC devices. Some of these are discussed in detail in Organ-on-a-chip systems for modeling pathological conditions section.

Mimicking the pulmonary parenchymal environment

Building on the microengineered device that Huh et al. pioneered, Stucki et al. aimed to reproduce an even more biomimetic alveolar strain regime on a chip (Stucki et al., 2015). Where many systems recreate strain in either a linear (Huh et al., 2012) or 2D manner (Tschumperlin and Margulies, 1998; Vlahakis et al., 1999), an alveolar sac expands and contracts in vivo in three dimensions, not unlike a balloon. While there is still much to be learned about the mechanobiology of the lung (Waters et al., 2012), it follows that the strain applied on in vitro systems should aim to closely replicate the 3D stretching occurring in vivo, and

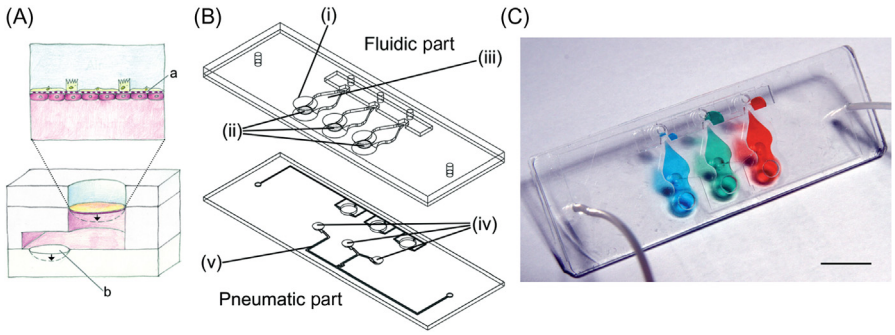


FIGURE 4.4

(A) LOC system incorporating an artificial alveolar membrane (a) onto which epithelial and endothelial cells can be seeded, and that can be three-dimensionally stretched with mechanical actuation of the diaphragm-inspired lower membrane (b) by an electro-pneumatic pump. (B) Schematic of the device separated into a fluid part and a pneumatic part. The fluid part contains three cell culture wells (i) above porous, flexible membranes (ii). The basolateral chambers (iii), to be filled with cell culture media, can be found directly beneath the membranes. The pneumatic component features a microdiaphragm (iv) at the base of each of the basolateral chambers and directly adjacent to pneumatic microchannels (v) in order to apply the vacuum that causes their deformation. (C) Photograph of the device with colored fluid inserted into the basolateral channels for visualization (scale bar: 10 mm). *LOC*, Lung-on-a-chip.

Reprinted (adapted) with permission from Stucki, A.O., Stucki, J.D., Hall, S.R.R., Felder, M., Mermoud, Y., Schmid, R.A., Geiser, T., Guenat, O.T., 2015. A lung-on-a-chip array with an integrated bio-inspired respiration mechanism. Lab Chip 15, 1302–1310. doi:10.1039/C4LC01252F – Published by The Royal Society of Chemistry.

studies have indeed shown that strain profiles affect cell responses (Berry et al., 2003; Deng et al., 2009; Gould et al., 2012; Park et al., 2004). This device (Fig. 4.4) employs two PDMS membranes to achieve 3D stretching. The first is a porous membrane that acts as an alveolar barrier, similar to the mechanically active alveolar–capillary interface device described above, with epithelial and endothelial cells seeded onto the apical and basal sides of the membrane, respectively. The second membrane is situated below the cellularized membrane and a media compartment and serves as a diaphragm-inspired mechanical actuator. Supplying vacuum below this microdiaphragm deforms it, transmitting a negative pressure through the incompressible cell culture medium onto the artificial alveolar membrane. The resulting membrane deformation thus mimics the 3D stretching observed in the alveoli and can be precisely tuned to specific mechanical inputs such as strain and frequency.

Another important objective of the work of Stucki et al. was to address the constraints of robustness and reproducibility of LOC-type devices, considerations often deemed vital to the eventual adoption of OOC platforms on a larger scale

(Esch et al., 2015). From a biological standpoint, one issue of reproducibility arises during the cell-seeding stage of an OOC, where the limited control over cells, once they are within the microfluidic device, can often result in imprecise or nonhomogeneous cell distributions. Unlike most LOC devices, which cannot be disassembled after fabrication nondestructively, the LOC developed by Stucki et al. was composed of two principal parts that could be reversibly bonded to one another with sufficient applied pressure, allowing the device to be readily opened and closed more than once. Thus when the device was disassembled, both sides of the artificial alveolar membrane could be accessed, facilitating straightforward and controlled cell seeding via conventional pipetting methods. This approach improves LOC ease of use and cellular reproducibility and represents an important step toward wider scale use of LOC devices.

Simulating surface tension stresses

One aspect of simulating the lung microenvironment that has been only briefly discussed is recreating the in vivo fluid mechanical stresses (as opposed to solid mechanical stresses that result from cyclic stretching, of which the previous two systems have been exemplars). Fluid stresses, or more specifically surface tension-related fluid stresses, are critical role lung diseases such as ventilator-induced lung injury, ARDS, and neonatal respiratory distress syndrome, where the propagation of pathological air−liquid menisci with each expansion of the alveoli imposes a variety of stresses on the underlying epithelial cells (Bilek et al., 2003). The difficulty in recreating the complex interplay of both the solid and fluid mechanical stresses is reflected in the fact that previous models investigating these types of lung diseases simulated either the mechanical stretching (Tschumperlin et al., 2000) or the surface tension-related effects of air−liquid plugs (Huh et al., 2007) in isolation, but not simultaneously.

Douville et al. were the first to bridge this gap with a LOC that allowed the study of the combined effects of the solid and fluid stresses observed in surface tension-related diseases (Douville et al., 2011). The PDMS device consisted of two chambers (termed alveolar and actuation chambers) separated by a thin membrane (Fig. 4.5). The upper (alveolar) chamber was filled with cell culture medium, so that human alveolar basal epithelial cells could be seeded onto the upper side of the membrane, and the lower (actuation) chamber was put under vacuum to stretch the membrane in a 3D manner, similar to the membrane deformation achieved by Stucki et al. After epithelial cell confluence was reached in the device's horizontal configuration, the device could be positioned vertically to orient the liquid meniscus perpendicular to the cell layer. Upon stretching of the membrane, the meniscus could be propagated along the cell layer, exposing it to the fluid mechanical stresses from surface tension effects. With careful tuning of the vacuum applied to the actuation channel, the propagation speed and frequency can be controlled to model a range of stress scenarios. This combinatorial approach resulted in cell morphology and death that were significantly different

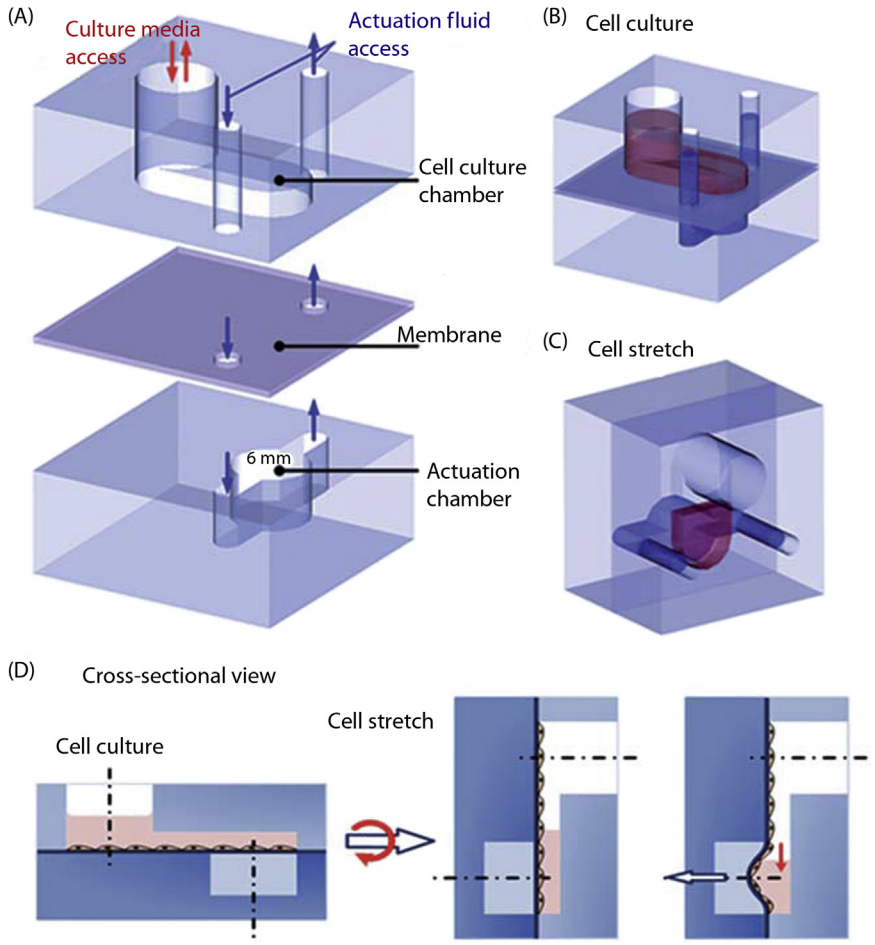


FIGURE 4.5

(A) "Alveoli-on-chip" microfluidic device composed of three principal parts: an upper piece with a semiopen cell culture chamber; a thin, porous membrane onto which epithelial cells are seeded; and a lower piece with the actuation chamber, to which vacuum can be applied to deform the membrane. Cell culturing is done with the device in its horizontal orientation (B), then it is rotated into its vertical orientation for study with a combination of solid and fluid mechanical stresses. (D) Cross-section schematic illustrating the device's two orientations. In its vertical orientation deformation of the membrane leads to a propagation of the air–liquid interface, exposing the adjacent cells to surface tension-related stresses.

Reprinted with permission from Douville, N.J., Zamankhan, P., Tung, Y.C., Li, R., Vaughan, B.L., Tai, C.F., et al., 2011. Combination of fluid and solid mechanical stresses contribute to cell death and detachment in a microfluidic alveolar model. Lab Chip 11, 609–619, reproduced by permission of The Royal Society of Chemistry (http://www.rsc.org/).

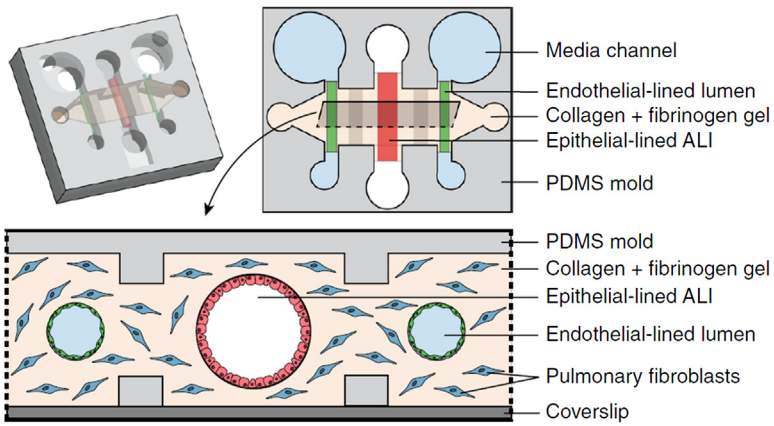


FIGURE 4.6

Organotypic LOC device featuring two media-filled endothelial-lined lumens and one central epithelial-lined lumens, where an ALI is present. The lumens run through a collagen and fibrinogen gel matrix, incorporating pulmonary fibroblasts. *ALI*, Air–liquid interface; *LOC*, lung-on-a-chip.

Reprinted (adapted) with permission from Barkal, L. J., et al., 2017. Microbial volatile communication in human organotypic lung models. Nat. Commun. 8 (1).

than when the system was used to model cyclic membrane stretching. The findings were in agreement with hypotheses drawn from clinical studies (Chu et al., 1967; Hirschl et al., 1998, 1996) that suggested that surface tension forces play a critical role in pathologies such as neonatal respiratory distress syndrome and ARDS and that modeling cyclic stretching alone is insufficient.

Complex organotypic cocultures

More recently, Barkal et al. (2017) introduced an organotypic LOC model of the terminal bronchiole, whose biological complexity and innovative design features represent a step toward mimicking in vivo biology and a more user-friendly and versatile platform. The system (Fig. 4.6) is based on a fibroblast and collagen gel matrix structure that supports epithelial and endothelial cell monolayers. This type of matrix is rarely integrated into in vitro lung platforms, where most often a microporous polymer membrane is used without a fibroblast-integrated support matrix, whether in Transwell-based systems (Yamaya et al., 2002) or more recent LOC devices (Benam et al., 2016a,b). During the fabrication process, three PDMS rods are encased in the hydrogel matrix and, once removed, leave behind channels with circular cross-sections. These three parallel channels, one to model the bronchial airway and two to model lateral vascular capillaries, offer a biologically relevant geometry onto which epithelial and endothelial cells can be

cultured and result in distinctly different cell behavior that contrasts with the traditionally flat cell monolayers used in other in vitro models (Bischel et al., 2014). Furthermore, the ability to control the shape and size of the lumina with precision allowed the team to create lumina that matched the average dimensions of the terminal bronchioles and neighboring vascular capillaries in the human body (Anderson and Foraker, 1962; Hansen and Ampaya, 1975). This microscale design and fabrication approach, along with the use of primary human cells, can create a truly organotypic device to mimic the complex immunoinflammatory environment present in the lung.

Innovation in the physical design of a LOC chip device must also be matched by careful consideration of how it can be used simply and effectively to create a robust experimental platform. In the LOC of Barkal et al., each lumen can be accessed through pipette-compatible ports, allowing easy and specific insertion and removal of material for chemical analysis. This enables evaluation under two distinct pathological conditions: First, pathogens could be introduced into the bronchial lumen to investigate lung response to direct pathogen contact. Second, a complementary module was designed to facilitate host−pathogen communication via volatile compounds, which has been shown to produce unique host infection responses (Briard et al., 2016; Koo et al., 2014). The external microbial culture module could be positioned over the LOC in a sealed dish and facilitate bacteria and fungi-derived volatile compound interactions with the bronchiole model, a largely unexplored phenomenon that has particular relevance in CF patients colonized by multiple microbial species (Amin et al., 2010). This type of modular approach will likely prove to be indispensable in expanding OOC capabilities and helps this device stand out as a comprehensive LOC.

Organ-on-a-chip systems for modeling pathological conditions

LOC technology has enabled in vitro studies of lung pathology under dynamic, more in vivo−like conditions. Microfluidic size scales aid the mimicking of ALI phenomena, rendering them ideal platforms for small-airway pathophysiological models. This technology is expected to contribute significantly to understanding the mechanisms, causes, and symptoms of pulmonary diseases and developing treatments.

In this section, we organize systems by components of the airway hierarchy, primarily focusing on the ALI of small airways and alveoli. The majority of these systems use a cellularized membrane, similar to the work done by the Ingber group (Huh et al., 2010). However, we first highlight the precursor microfluidic systems that provided the framework for studying on-chip lung pathophysiologies.

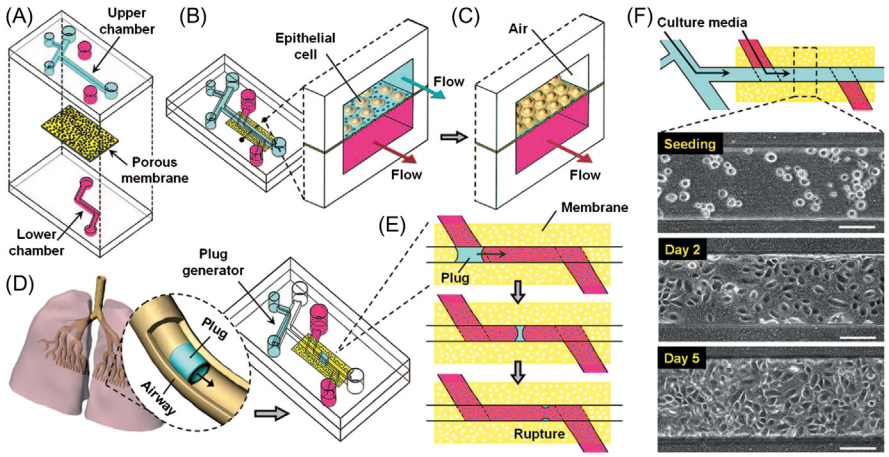


FIGURE 4.7

A microfluidic device recapitulating the formation of liquid plug formation and propagation in the small airways.

Reprinted (adapted) from Huh, D., Fujioka, H., Tung, Y.-C., Futai, N., Paine, R., Grotberg, J.B., et al., 2007. Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. PNAS 104 (48), 18886–18891. © 2007 National Academy of Sciences, U.S.A.

Microfluidic precursors as lung pathology models: liquid plugs in small airways

The Takayama group (Huh et al., 2007) (Fig. 4.7) was among the first to demonstrate an on-chip small-airway pathological condition, by replicating the formation and propagation of mucus plugs in the airway lumen. These plugs are similar to those that form in vivo and are associated with pulmonary diseases and conditions such as COPD, asthma, pulmonary edema, and bronchiolitis (Cassidy et al., 1999). Liquid plugs form abnormally following dysregulation of surfactant levels or a dysfunction of the surfactant itself, resulting in a viscous film coating of the small-airway epithelium that increases the probability of air–liquid instabilities and plug generation. The plugs block the airways, inhibiting gas exchange and air flow. During inhalation, as the lung inflates, the liquid plug is driven further down along the airway lumen, until it eventually ruptures and reopens the airway. This rupturing causes a breathing sound termed crackles (Piirilä and Sovijärvi, 1995). Listening for crackles is a clinical detection method for associated pulmonary diseases. This microfluidic model showed that the mechanical forces resulting from plug rupturing cause damage to airway epithelial cells, and the on-chip plug rupture produced sounds to the clinical phenomenon. Of note, the formation of plugs is also observed during artificial ventilation of patients undergoing medical interventions, and this model system could recreate plug formation. The model was subsequently leveraged to systematically produce and monitor on-chip liquid

plugs for the focused evaluation of plug characteristics and airway pressure changes. This was accomplished by integrating a liquid plug generator to introduce a controlled air−liquid segmented flow in the airway lumen of the chip (Tavana et al., 2010). Furthermore, the addition of pulmonary surfactants to reduce surface tension in the liquid plugs resulted in less damage to the epithelial cells (Tavana et al., 2011). The application of surface phenomena captured epithelial responses and highlighted microfluidics as an important technology platform for LOC modeling.

Modeling lung inflammation, asthma, and chronic obstructive pulmonary disease in small-airway chips

A more recent small-airway-on-a-chip device was developed to model human lung inflammatory diseases and identify new antiinflammatory therapies (Benam et al., 2016a,b) (Fig. 4.8). This system mimicked the effect of epithelial−endothelial crosstalk on lung inflammation in a microfluidic device, as a platform for obtaining organ-level responses to pathological processes.

A functional, healthy small airway was reconstructed on a microfluidic chip containing human airway epithelial cells and endothelial cells, using similar device architecture. Permeability measurements confirmed the barrier integrity of the cellularized membrane, while immunofluorescence confocal microscopy identified the mixed tissue composition (ciliated, goblet, club, and basal cells, representative of a healthy small-airway epithelium). The ciliary beating frequency on the apical surface of the endothelial cells was similar to that observed in healthy human cilia in vivo. An asthma-like state was induced in the chip by perfusing the cell culture medium with interleukin 13, a mediator of allergic asthma, which increased the number of goblet cells and secretion of proinflammatory cytokines in the vascular compartment. A decrease in ciliary beating frequency was also observed, mirroring the ciliary beating behavior in asthma patients (Thomas et al., 2010).

This airway chip was also used to model inflammatory responses to pathogenic infections in the small airways that trigger exacerbation of asthma and COPD using a viral mimic, polycytidylic acid. This immunostimulant was perfused through the chip to induce a severe asthma exacerbation-like state, resulting in the secretion of several proinflammatory cytokines. Interestingly, a monoculture of epithelial cells seeded onto the chip under the same culture conditions and the same stimulus exhibited significantly less secretion of cytokines than the epithelial−endothelial coculture. The additive effect on cytokine secretion in the cocultured LOC suggests a synergistic inflammatory dynamic between the endothelial and epithelial layers. The system was able to detect cytokine expression as a pathophysiological outcome and to recreate neutrophil recruitment when flowing neutrophils through the lower, “vascular” channel. This mirrors the initial adhesion and rolling of neutrophils underflow in microvessels when circulating

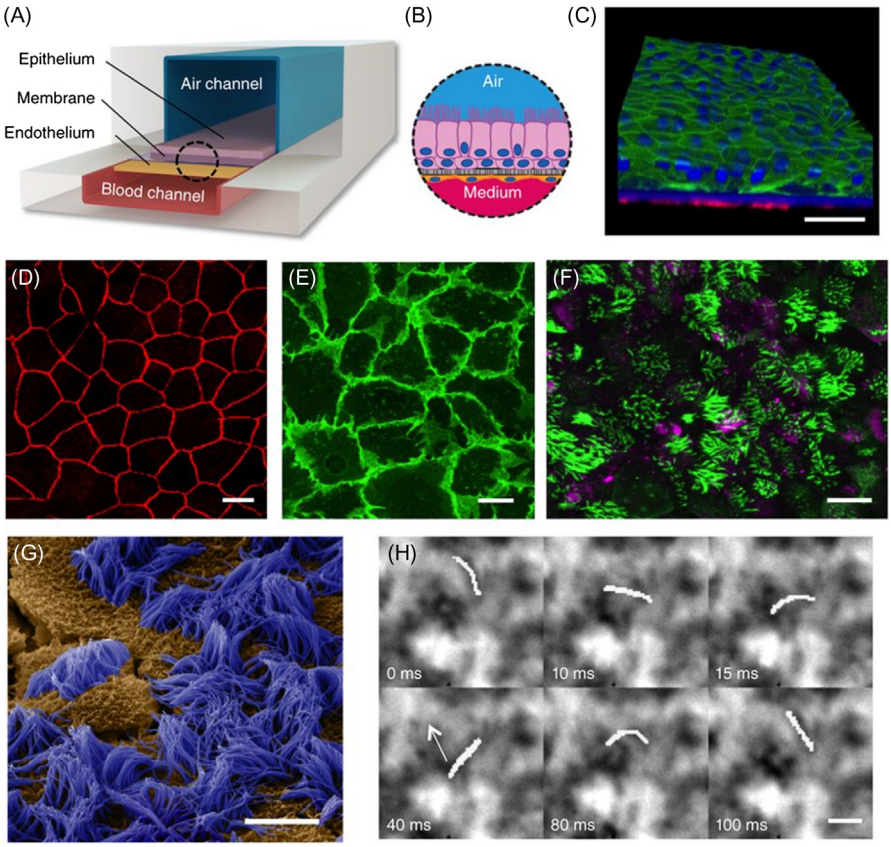


FIGURE 4.8

A functional small airway was reconstructed on a microfluidic chip, containing differentiated human cells to model human lung inflammatory diseases, expressing actively beating cilia function.

Reprinted by permission from Benam, K.H., Villenave, R., Lucchesi, C., Varone, A., Hubeau, C., Lee, H.-H., et al., 2016a. Small airway-on-a-chip enables analysis of human lung inflammation and drug responses in vitro. Nat. Methods 13, 151–157.

through inflamed living tissues (Lawrence and Springer, 1993). Proinflammatory responses were also produced in devices with epithelial cells harvested from COPD patients. A similar immunostimulation regime was performed with either polycytidylic acid or the bacterial-derived stimulant lipopolysaccharide endotoxin (LPS) to model the inflammatory effects of COPD exacerbation. Clinical disease features of COPD exacerbation, specifically upregulation of the proinflammatory cytokine macrophage colony–stimulating factor secretion, were recaptured in chips seeded with cells from COPD patients but not in from healthy controls.

The ability to model such functional responses suggested that the model could be used for drug discovery, and this hypothesis was confirmed by assessing whether drugs could reverse the interleukin 13-induced phenotype. A Janus kinase inhibitor restored the epithelium to normal conditions, suggesting that the drug could be used as an antiinflammatory and that the microfluidic model could be leveraged to evaluate therapeutic responses.

This work established a comprehensive template for modeling on-chip lung inflammation, but also highlighted the utility of LOC devices for running parallel studies while modulating the cultured cells or stimulation regimes. This ability to parallelize on a relatively small footprint extends the functionality of air−liquid in vitro culture platforms while adding control of flow and environmental elements, bridging the gap with the more dynamic environment of the lung.

Cigarette smoke is known to cause damage to multiple organs in the body, especially the lungs (United States Surgeon General, 2014), but electronic cigarettes and other tobacco products have been less characterized. The effective modeling of lung inflammation in the airway-on-a-chip system was leveraged to study smoke-induced pathophysiology in vitro by connecting the device to a smoking machine (Benam et al., 2016a,b). The small-airway microdevice was seeded with human epithelium cells from either COPD patients or healthy individuals and exposed to smoke from various tobacco products. A microrespirator was integrated into the device to apply smoke directly into the airway of the chip, mimicking realistic inhalation of smoke by cyclically "breathing" in and out of the chip with representative frequency and volumes. Unlike previous in vitro models where the cells were submerged in medium containing diluted smoke extract, effectively skewing the impact of smoking conditions, this model exposed the cells to whole smoke. Key smoke-generated phenotypes of the epithelium, such as increased oxidative stress, were reproduced under the whole-smoke regimes. Furthermore, as the cells were exposed to high levels of smoke (nine cigarettes over a single 75-minute period), they exhibited certain features seen in chronic smokers, such as expression of most oxidation−reduction genes. By implementing a pre-conditioned smoking regime, this model can rapidly create phenotypic disease features of chronic smokers, paving the way for future studies of cellular alterations associated with chronic smoking. A major advantage of this model is the ability to run side-by-side comparisons between cells from the same COPD patient, enabling a true-match comparison. This comparison, coupled with single-cell resolution, high-speed imaging, and subsequent statistical analysis of the apical ciliary beating, revealed that smoking had a heterogeneous impact on ciliary beating patterns: beating frequency was only reduced over some areas of the epithelial layer. In contrast, this true-match comparison did not find a significant alteration following exposure to electronic cigarettes. Overall, this demonstrates how the model can be used to account for interindividual differences. The limitation of this study was that the model was not used at its full potential, as only epithelial cells were exposed instead of an epithelial/endothelial coculture.

Pulmonary edema and intravascular thrombosis modeled in alveolus-on-a-chip devices

The mechanical activation of lung cells in LOC systems was most notably shown by Huh et al. to model the alveolar–capillary interface (Huh et al., 2010). The coupling of mechanical forces generated from a repetitive breathing motion and the device architecture mimicked a lung epithelium-like barrier in which the more instructive disease process of inflammation could be evaluated. Building on this "breathing" LOC system modeled interleukin 2–induced pulmonary edema (Huh et al., 2012). Pulmonary edema is a complex condition in which vascular fluid accumulates in the airspace of the alveoli as a result of leakage from the pulmonary vessels (Miles, 1977). A less common form of pulmonary edema can develop as a chemotherapeutic side effect, when interleukin 2 is administered to patients with melanoma or renal cancers. Cells in the LOC device were mechanically stimulated by cyclic stretching to produce the appropriate barrier and physico-similar properties. Once achieved, clinical doses of interleukin 2 were introduced into the vascular channel, provoking responses similar to those observed in patients (Conant et al., 1989), including vascular leakage and fibrin deposition.

Separately, the Ingber group–stimulated inflammatory mechanisms and platelet activation in the pulmonary vascular endothelium to model thrombus formation within pulmonary vessels (Jain et al., 2018) using a two-chamber LOC platform similar to the classic model (Fig. 4.9) (Huh et al., 2010). In this modified version, the upper (air) chamber was seeded with primary human lung alveolar

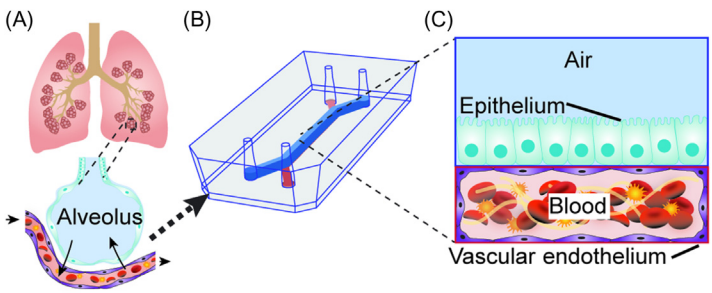


FIGURE 4.9

Intravascular thrombosis modeled in alveolus-on-chip. (A) A structural sketch showing the ALI of the alveolus that was modeled in the device. (B) A principal sketch of the device architecture. (C) Illustration of the cellular compartments of the device showing the epithelial cells in the air compartment and the endothelial cell lining the walls of the liquid compartment, forming the "vascular tube." *ALI*, Air–liquid interface.

Reprinted from Jain, A., Barrile, R., van der Meer, A., Mammoto, A., Mammoto, T., De Ceunynck, K., et al., 2018. Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. Clin. Pharmacol. Ther. 103, 332–340, with the permission of John Wiley and Sons.

epithelial cells. A principal difference from the previous model was that the lower (vascular) compartment was lined on all four extracellular matrix-coated walls with human vascular endothelial cells to form a vascular tube, instead of being limited to the basal surface of the ALI (Jain et al., 2018). This enabled perfusion of whole blood through the vascular tube, mimicking healthy human microvessels, without spontaneous platelet aggregation and thrombus formation (Furie and Furie, 2008). The ability to perfuse whole blood in this model is an important step toward a more individualized assessment of disease features or drug responses using lung cells and blood from the same patient. This model system demonstrated three important aspects of disease modeling in OOC technology: in vivo–like responses of intravascular thrombosis, organ-level contributions to inflammation-induced thrombosis, and evaluation of potential antithrombotic therapeutics. First, the model captured a prothrombotic disease state by stimulating the endothelialized channel with perfusion of the inflammatory cytokine TNFα. The endothelium responded in a concentration-dependent manner, with increasing TNFα levels corresponding to more inflammation in the endothelium. Concomitantly, increased platelet aggregation and subsequent thrombus formation occurred. Implementing a synthetic biology approach, this system decoupled the endothelial/epithelial cellular interaction, enabling the analysis of the independent and collective responses of the lung epithelium and endothelium to determine that the prothrombotic effects were escalated as a function of the epithelial–endothelial coculture. LPS, a bacterial endotoxin known to induce pulmonary thrombosis, was introduced into the endothelial tube to study thrombus formation in a monoculture of endothelial cells and in an endothelial–epithelial coculture. Surprisingly, LPS did not induce any thrombus formation when the endothelium was stimulated in the absence of the epithelium, but the same treatment in the epithelial–endothelial coculture increased barrier permeability and platelet aggregation in the vessel. As these responses were only observed in the device with a more complete cellular milieu, it appeared that LPS-induced thrombosis occurred through indirect stimulation of the epithelium. These findings demonstrate one of the major advantages of OOC devices, as a similar study cannot be performed in vivo without the ability to control and manipulate cellular constituents individually. Another major advantage of this system is the ability to sample by collecting the outflow from the vascular channel and analyzing the cytokines produced within the LPS-stimulated alveolus-on-a-chip device. Effective sampling within LOC devices provides more depth in evaluating and modeling pathophysiology progression by measuring shifts in cytokines with functional changes. It is critical to note that when performing this thrombotic evaluation, the model did not mimic the breathing motion of the lungs, although the system can incorporate dynamic stimulation. The cyclic strain from breathing can affect lung pathology, and its incorporation in future prothrombotic studies will augment the physiological relevance of this model. Furthermore, this system has the potential to become a precursor test platform for evaluating thrombolytic or antithrombolytic therapeutics.

Modeling cystic fibrosis in a microfluidic device

The multiorgan nature of CF (Elborn, 2007) makes it difficult to develop comprehensive in vitro models of the disease, which is why animal models are commonly used to study CF development and progression. Microfluidic pulmonary models can mimic the accumulation of viscous mucus, trapping bacteria to result in recurrent infections and other complications. Skolimowski et al. used a two-chamber microfluidic device and an alginate hydrogel to model the thick mucus layer in the bronchi of CF patients (Skolimowski et al., 2012a,b); the hydrogel was formed on top of a membrane by introducing sodium alginate into the top channel and flowing calcium chloride in the bottom channel. A human subbronchial gland cell line (Calu-3) was seeded on the bottom of the membrane in the lower compartment. To model bacterial infection, *P. aeruginosa* laboratory strain PAO1 bacteria were added into the top channel, to form a biofilm in the hydrogel. This model was used to study the efficacy of antibiotic treatment by introducing antibiotic drugs in relevant concentrations and cycles into the medium flowing in the bottom channel. The same group developed a modular multicompartment microfluidic system (described in Current lung-on-a-chip systems section) to model CF conditions in the various compartments of the airway system (Skolimowski et al., 2012a,b). The segmentation of the system into a pulmonary-like hierarchy, and the effective inoculation of the model with *P. aeruginosa*, served as a test platform of bacterial response to antibiotic treatments. Testing bacterial responses at different oxygen levels can be achieved in a more conventional system, but the footprint and scalability of an interconnected system adds a new aspect and provides a holistic approach that may identify feedback loops that would be nonexistent in singular testing modules using one oxygen concentration at a time. This interconnected modular approach could be used to understand metabolism, cytokine secretion, and other cellular- and tissue-level responses and could be extended into an integrated LOC module for pathophysiological study.

Lung tumor-on-a-chip devices

As previously mentioned, lung cancer is estimated to be the most prevalent lethal cancer, making the search for suitable therapeutics against lung tumors one of the foremost challenges facing modern healthcare. 3D in vitro systems have allowed researchers to study lung tumors in a more complex microenvironment that resembles the physiological environment better than static 2D models (Asghar et al., 2015). Tumor-on-chip systems emerged over the past 10 years, aiming to model the local tumor environment during various stages of the cancer cascade. Traditionally, tumor-on-chip models have recreated the general tissue microenvironment in which tumors form, rather than an organ-specific environment. These models enable long-term studies, providing sufficient time for the slow growth of the on-chip tumor, which can take weeks to establish in vitro. In addition to gaining a general understanding of the disease complexity at the organ level, the

development of tumor-on-a-chip platforms is motivated by their potential in preclinical drug evaluation, with better control of individual parameters than animal models.

Interfacing cancer progression with the microenvironmental cues of an organ system may provide new perspectives on progression mechanisms and as such, insight into therapeutic targets. One of the most sophisticated microfluidic orthotopic lung tumor models (Hassell et al., 2017) built on an alveolus-on-a-chip device (Jain et al., 2018) (Fig. 4.9, Section 4.3), demonstrated the influence of mechanical cues from the cyclical process of breathing on tumor growth and vascular invasion. The epithelial tissue layer in the model was injected with human non−small cell lung cancer cells that express high levels of fluorescent proteins to optically monitor tumor growth. Upon subjecting the chip to cyclic mechanical stretching, cell proliferation was inhibited by 50% and the tumor cells grew centered in smaller areas over the epithelium instead of spreading over larger areas. This work suggested the potential of a positive feedback loop dependent on motion, in which a mechanical strain associated with unencumbered breathing decreases epithelial growth factor receptor (EGFR) phosphorylation, while the loss of motion from increased cell proliferation in the alveoli enhances tumor growth, with a concomitant increase in phosphorylated EGFR levels. This feedback loop additionally highlights the adverse effect that enhanced EGFR phosphorylation has on rociletinib treatment, underscoring the importance, superiority, and utility of models that exhibit physiological relevance (Jain et al., 2018).

In addition to gaining a general understanding of the disease complexity of cancer at the organ level, tumor-on-a-chip devices have potential as preclinical screening platforms for drugs. Advances in the field of nanotechnology have provided a range of novel nanotherapeutics, and their safety and efficacy must be evaluated. A platform to assess drug sensitivity for the treatment of lung cancer was developed to mimic the 3D tumor microenvironment (Xu et al., 2013) (Fig. 4.10). Non-small-cell lung cancer cells and stromal cells were exposed on-chip to chemotherapeutic drugs with different gradient concentrations to assay an appropriate concentration and single- and combined-drug chemotherapy regimes. By using patient-specific cells, this platform may personalize effective clinical cancer treatments.

Improvements needed in lung-on-a-chip platforms for disease modeling and lung regeneration

Limitations of current organ-on-a-chip systems

OOC approaches hold great promise for the future development of in vitro studies of lung pathophysiology. Microfluidic models increase our knowledge of pulmonary diseases by their ability to mimic dynamic, physiologically relevant features of the human lung and approach cell culture studies using synthetic biology

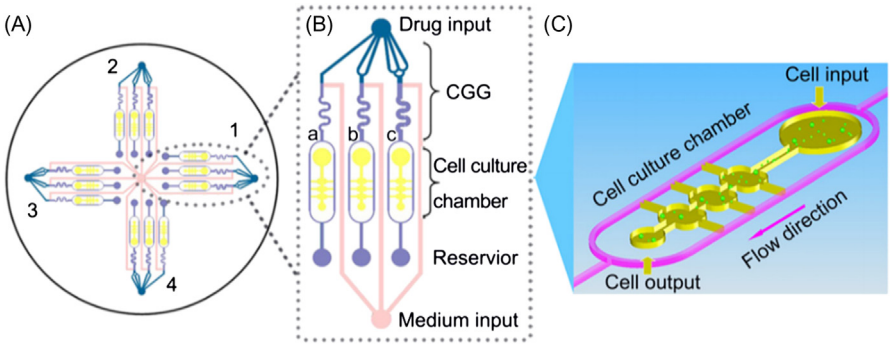


FIGURE 4.10

A microfluidic platform to assess drug sensitivity for the treatment of lung cancer. The device contains parallel cell culture chambers with nonsmall cancer cells and stromal cells.

Reprinted from Xu, Z., Gao, Y., Hao, Y., Li, E., Wang, Y., Zhang, J., et al., 2013. Application of a microfluidic chip-based 3D co-culture to test drug sensitivity for individualized treatment of lung cancer. Biomaterials 34, 4109–4117. © 2013, with permission from Elsevier.

methods. Some of the major accomplishments in disease modeling using LOC platforms have been described in this chapter. Another important accomplishment is the ability to perform a true-match comparison between the responses from normal cells and disease-exposed cells from the same patient, which is a step toward the development of individualized treatments. The development of these systems over the past decade illustrates the ongoing efforts of creating LOC platforms to model pulmonary diseases, predict clinical outcomes, and evaluate drug efficacy. However, technical challenges remain. Although microfluidic technology allows multiplexing and automation of cellular assays with high throughput, a general hurdle is to render the design useful, easy to handle, and compatible with the equipment available in biology laboratories. With increasing device complexity, design aspects and usability become important considerations, so as to avoid producing nontranslatable, nonadoptable technologies. Furthermore, with miniaturization, small variations can still exert a great impact, so high robustness and reproducibility are required of these devices. In this section, we discuss the limitations of the current state-of-the-art of LOC devices and the necessary improvements, followed by an outline of future directions of in vitro lung pathology modeling.

Improvements to organ-on-a-chip technology

Proof-of-concept studies have demonstrated the usefulness of LOC technology, but further research is needed to develop even more sophisticated systems that better mimic human organ functions and expand the usability of the devices.

To date, LOC devices have not reached their full potential in terms of biological complexity. The current OOC platforms still lack high fidelity of cellular components and are oversimplified to only a few main cell types assumed to be sufficiently representative of the whole tissue (Takebe et al., 2017). Thus an important next step toward expanding the use of LOC devices should be the incorporation of other cell types, especially immune cells such as macrophages and lymphocytes, to study the crosstalk between cells. For example, in the breathing LOC model developed by Huh et al. (2012), the findings suggested that interleukin 2–induced toxicity may be mediated by lymphocytes or other specific immune cells. Even though the experiments were not performed, the device can incorporate immune cells to study their particular contribution to responses, a feature that is not possible in animal models. In addition, the use of human-induced pluripotent stem cells can recreate the complex lung tissue functionality and architecture by deriving various types of lung cells from both proximal and distal airways and by mimicking the branching morphogenesis process directly on a microfluidic platform (Dye et al., 2016a,b; Gordon et al., 2015). Moreover, even though a simplified vascular component has already been integrated into LOC platforms (Jain et al., 2018), the incorporation and innervation of mesenchymal and neural tissue are fundamental to fully recreate the in vivo microenvironment.

There are also several engineering challenges that must be addressed in device complexity and material composition, to both improve these systems and increasing their accessibility for end-users. PDMS is currently the most widely used material in fabricating LOC devices, because of its ease of use, rapid fabrication process, optical clarity, and elasticity. However, as it is hydrophobic and gas-permeable, small hydrophobic molecules can be readily absorbed by PDMS, complicating toxicology studies (Gomez-Sjoberg et al., 2010). While the gas permeability of PDMS is beneficial for culturing cells under optimal gas conditions, the small scale and permeability can result in hyperoxia. Achieving a tissue- and disease-specific gas environment may require changing the culture conditions (i.e., incubator gas content) to match in vitro oxygen tension levels to that of in vivo tissue levels. Controlling specific oxygen environments increases the complexity of culturing these systems, as PDMS is too gas-permeable to allow the cultured tissue to consume oxygen through cellular respiration or to modulate oxygen tension levels. Other materials traditionally used for cell culture experiments, such as polystyrene, can overcome this problem, but they are much stiffer, with a Young's modulus three to four orders of magnitude greater than that of PDMS, which far exceeds that of biological tissues. In addition, they cannot be stretched like PDMS to emulate breathing. The elasticity of PDMS membranes enables the application of mechanical strain on the cells using vacuum pressure applied to specific channels in the devices.

Another critical drawback of PDMS is that it presents challenges in larger scale manufacturing, limiting the scale-up of production and distribution to end-user researchers and clinicians. Injection molding could enable high-throughput fabrication of PDMS devices. However, the process would be expensive to

implement and difficult to make robust in order to control batch to batch variability based on the premixing and curing of PDMS. Unless there is a highly profitable application, it is unlikely that investments into mass production of PDMS devices will be made (Klapperich, 2009).

Furthermore, the fabrication of ultrathin membranes to mimic the basement membrane of lung alveoli and bronchiolar epithelium remains an obstacle. This membrane, onto which cells are seeded, must be ultrathin to recreate appropriate cell communication through the basal membrane, concomitantly capturing in vivo–like responses and functions. One solution for constructing such a membrane is to directly deposit it on the device. Yang et al. demonstrated the use of this technique to model an alveolar tumor and evaluate the anticancer drug gefitinib (Yang et al., 2018), using a biocompatible poly(lactic-*co*-glycolic acid) electrospun nanofiber membrane as cellular scaffold prepared directly on the PDMS layer. The scaffold provided a 3D environment for cell growth and the thickness was controllable to a few microns by modifying the spinning time. Although the microchip was cultured as a static system, the study provides a good example of how tissue engineering techniques can be integrated into OOC technology. Improving device materials or using a composite of various materials can capture microenvironmental parameters such as the integration of chemical signals or mechanical properties that are more in line with in vivo stiffness. This will benefit the OOC field by capturing more physiologically relevant cellular responses in these surrogate in vitro model systems.

One of the greatest promises of microfluidic in vitro systems is the potential to integrate on-chip microsensors for real-time detection. This would enable control and manipulation of parameters during the cell assay. Electrochemical or optical sensors can be incorporated using techniques from microtechnology to monitor environmental conditions such as local temperature and oxygen levels (Murphy and Atala, 2016) or cell behavior and mechanical, electrical, and chemical stimuli. Quantitative information about the cell culture can then be obtained as a functional readout on cell behavior or to benchmark to other systems or other experiments. To date, LOC research has largely depended on fluorescence microscopy, which is a qualitative analytical method. The lack of quantitative standardized parameters is a general problem in the OOC field. So far, microsensors have not been widely integrated into LOC systems. In barrier systems, such as models of the lung alveolus, small airway, or blood–brain barrier, it is imperative to monitor barrier formation and function for the formation of a confluent barrier. Quantitative information on the barrier can be obtained by measuring transepithelial electrical resistance (TEER). Proof-of-concept studies have measured TEER in LOC systems (Henry et al., 2017), but the technique has not been widely implemented, perhaps because the technology has not until recently been robust enough for accurate readouts. In addition, TEER measurements in ALI cultures are difficult, because of the need to have both channels filled with liquid on either side of the membrane. While this enables the TEER measurement, if cells are submerged in liquid for too long, the cells can become highly stressed and suffer from damages.

Other, better defined sensors for microscale integration include gas-based sensors for nitric oxide, oxygen, and carbon dioxide, and pH sensors. These environmental sensors can provide insight into the metabolic activity of the LOC and how it impacts the functional outcomes being evaluated. These sensors, along with TEER readings, are noninvasive and enable continuous nonendpoint analysis, enhancing the study of lung pathophysiology.

The small dimensions of microfluidic devices are the main strength of the technology, offering lower reagent consumption and fast analysis. However, the importance of scaling and relative dimensions in biology has not been widely discussed in the LOC field (Bajaj et al., 2016). Several approaches have been suggested for scaling, including allometric scaling by the relative mass of organs and functional scaling by factors such as blood flow or metabolic rate. Developing allometrically scaled models of the lung on the microscale is physically challenging, as it is impossible to recreate realistic ratios of ALI surface area, dimensions, and blood volumes (Bajaj et al., 2016). Proportional scaling and relevant dimensions become increasingly important when developing multi-OOC or body-on-a-chip devices (Moraes et al., 2013) to mimic the interactions between organs or disease or drug response on a multiorgan level. Body-on-a-chip systems have already been used to model processes such as pharmacokinetic clearance in drug-toxicity studies (Prantil-Baun et al., 2018) and are discussed in further detail in the chapter entitled "Human body-on-a-chip systems."

Body-on-a-chip systems would be well-positioned to model diseases that simultaneously affect the lungs and other organs, such as CF or smoke-induced injuries; these multicompartmental systems could have a great impact on our understanding of disease mechanisms and their interplay. In addition, crosstalk between lung components or functions could theoretically be modeled using a multicompartmental system and appropriate scaling to capture the in vivo hierarchy.

The LOC systems that have been developed to date are largely represented by barrier-function models rather than parenchymal tissue-function models. In addition to gas exchange, the lung is responsible for controlling pH balance in the blood, preventing embolisms, protecting against infection, enabling speech, and providing a blood reservoir. Future LOC systems should include parenchymal tissue functions modeled with spheroid or organoid cultures, as discussed in the following sections.

Innovative strategies and fabrication methods for improving standard microfluidic devices

Organoids-on-a-chip and of the need for multiorgan platforms

Researchers agree that the use of 3D models is fundamental to recapitulate human pathophysiology. Organoids have emerged as a promising tool for addressing the limitations of common 2D approaches (In vitro and in vivo models of respiratory

disease pathogenesis section) (Devarasetty et al., 2018; Wilkinson et al., 2017). Using a synergistic approach, it is possible to combine OOC devices and organoids to enable a higher throughput readout for organoids and a more in vivo like cellular composition for OOC devices (Takebe et al., 2017). This integrated technology has been used to create multi-OOC platforms to study interorgan interactions (Skardal et al., 2016; Takebe et al., 2017): Skardal et al. fabricated a modular device composed of a microfluidic circulatory system that connected bioprinted lung, heart, and liver organoid microreactors to evaluate drug responses both in individual organoids and systemically (Skardal et al., 2017, 2015). The lung module was fabricated by culturing vascular endothelial cells, lung bronchial epithelial cells, and fibroblasts over a porous membrane, creating a layered 3D organoid. TEER and electrophysiological sensors were used to monitor the organoids and their CFTR activity. Similarly, the Advanced Tissue-Engineered Human Ectypal Network Analyzer program designed by the Los Alamos National Laboratory is a system composed of lung, heart, liver, and kidney organoids of millimetric size that uses standard clinical diagnostic tools (Skardal et al., 2019). Leveraging 3D organoid cultures in a dynamic controllable microenvironment will recreate structure−function relationships with more complexity, such as organoid alveolar sacs or bronchospheres at the end of bronchiole-like microfluidic tubes. This combinatorial approach may close the hierarchical, geometric, and fundamentally biological gaps between in vitro and in vivo models and position the next generation of OOC systems.

Three-dimensional printing for microfabrication and bioprinting

3D printing methods are a valuable tool in overcoming the main limitations of conventional microfabrication techniques, which are time-consuming and require expensive equipment and special facilities (Amin et al., 2016; Bhattacharjee et al., 2016; Ho et al., 2015). This technology has been used to fabricate master molds, microfluidic devices, and microfluidic components for generating gradients, mixing, and sensing, for a wide range of biological and cellular applications (Amin et al., 2016; Knowlton et al., 2016b; Lee et al., 2016; Zhou, 2017). Unlike standard techniques in microfluidic device fabrication, 3D printing enables the production of complex 3D structures such as microvascular networks (Bertassoni et al., 2014; Hasan et al., 2014; Wu et al., 2010), which are fundamental to ensure vascularized tissues.

While 3D printing can produce complex structures using plastic polymers, bioprinting enables high spatial control in positioning and patterning cells and biomaterials to form heterogeneous biological structures (Ho et al., 2015; Murphy and Atala, 2014; Verhulsel et al., 2014; Yi et al., 2017). Moreover, the ability to print a wide range of biomaterials as alternatives to PDMS circumvents the issue of PDMS absorption of small hydrophobic molecules (Halldorsson et al., 2015; Wang et al., 2012). Optimizing the biomaterials used for printing can help capture tissue- and organ-specific properties such as mechanical characteristics and chemical cues. The technology has been used to fabricate tissue constructs such as

bone (Young Park et al., 2015), liver (Lee et al., 2016), skin (Kim et al., 2017), kidney (Homan et al., 2016; Sochol et al., 2016), nervous system (Johnson et al., 2016), heart (Zhang et al., 2016), and lung. Park et al. harnessed 3D cell printing to fabricate a vascularized airway-on-a-chip using an extracellular matrix bioink encapsulating endothelial cells and fibroblasts and print a 3D vascular network integrated with an airway epithelium model (Park et al., 2018). The platform was used to investigate the role of cytokines in asthma and asthma exacerbation and closely recreated in vivo the pathophysiological mechanisms.

Furthermore, 3D printing can also be used to fabricate both the microfluidic device and the OOC components (Knowlton et al., 2016a); Lee et al. produced an OOC device in a single-step process by printing poly(ε-caprolactone) as the platform material and encapsulated various cell types in an extracellular matrix-based hydrogel, eliminating the cell-seeding step. The 3D bioprinted device was used to create a liver-on-a-chip platform, exhibiting enhanced liver function and confirming the versatility and potential of rapid prototyping techniques (Lee and Cho, 2016). By providing a variety of rapid, scalable, and reproducible fabrication methods, 3D printing can improve LOC design and engineering, while bioprinting can enable the spatial control of a more complex cellular milieu native to the lung tissue, comprehensive of the mesenchymal and neural tissue components.

Conclusions

Harnessing microfluidic systems for precision control and monitoring of cellular microenvironments is a logical and necessary progression in modeling diseases in vitro. While microfluidic devices can potentially revolutionize research in this field, there are numerous challenges that must be overcome before broad adoption and use of these devices, and eventual replacement of conventional disease modeling techniques can be expected. As discussed throughout this chapter, the challenges that LOC devices face generally fall into two broad categories: biological complexity and robust engineering design. It is the opinion of the authors that at the time of writing, the most significant factors limiting LOC devices, and where the most innovation is required, are engineering design challenges. Biomimetic models cannot truly proliferate until a robust engineering base is established. By focusing on creating highly reproducible and easy-to-use microfluidic systems, greater adoption and use of LOC devices can be realized and their capabilities pushed further.

The next steps in LOC technology must involve a push for more interdisciplinary thinking and awareness, echoing the development of microfluidics in general, a field defined by a complex interplay of multidisciplinary foundations and technological requirements driven by members of the biology, materials science, chemistry, electronics, and engineering communities. Parallel advancements in organoid synthesis and 3D printing will have great implications for LOC devices,

so breakthroughs will require creative amalgamations of discrete techniques and expertise. OOC devices and LOC systems, in particular, are largely still in their infancy, and much progress is required before they become a truly transformative technology. Although the techniques and execution are not yet optimized, the notable advantages of LOC platforms should be viewed as an inspiration for continued progress.

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