CHAPTER 24

Engineering Pulmonary Epithelia and Their Mechanical Microenvironments

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24.1 Introduction

The lung has an anatomically unique structure consisting of a branching network of conducting tubes that enable convective gas transport to and from alveolar compartments, where gas exchange occurs. Throughout development and adult life, the respiratory system experiences a variety of physical forces imposed by structural changes in surrounding tissue, continuous passage of fluids, and cyclic mechanical deformation of the basement membrane and extracellular matrix (ECM). As the most essential cellular constituent of the respiratory system, the epithelial cells comprising the luminal surface of airways and alveoli are known to sense and respond to this dynamic mechanical environment. During fetal lung development, mechanical forces generated by airway distension and intermittent fetal breathing movements have been shown to profoundly influence the proliferation, apoptosis, and differentiation of pulmonary epithelial cells [1, 2]. Mechanical perturbations in the mature lung also play a critical role in regulating the structure, function, and metabolism of the epithelial cells [3]. Unusual changes in the mechanical environment of the respiratory system often contribute to the progression and exacerbation of various pulmonary disorders by eliciting abnormal biological responses of epithelial tissue in airways and alveoli. For example, large deformations of the alveolar basement membrane due to overdistension of the lung during mechanical ventilation can induce injury of alveolar epithelial cells and upregulate inflammatory responses, which can lead to ventilator-induced lung injury [4]. In asthmatic airways, compressive mechanical forces resulting from smooth muscle constriction have been shown to cause airway epithelial cells to communicate with neighboring mesenchymal cells to initiate and amplify airway remodeling [5].

Over the past two decades, discovery and elucidation of these mechansensitive events in various physiologic and pathologic situations have been of paramount importance and interest in respiratory biology and physiology. Experimentally investigating the effects of mechanical forces on pulmonary epithelial cells, however, is often challenging for several reasons: (1) Due to the complex architecture and mechanical properties of the lung and marked spatial heterogeneity of pulmonary epithelial cells, ex vivo and in vivo models based on whole-organ systems are difficult to use for studying the mechanical responses of airway and alveolar tissue at the cellular level. Instead, in vitro cell-culture systems can be used as an alterna-
tive approach. Unlike other traditional culture methods, however, in vitro culture of pulmonary epithelial cells often requires permeable supports for cell attachment and independent access to both basal and apical sides of a cellular monolayer to allow the cells to polarize. Moreover, after reaching confluence, the cells need to be grown at an air-liquid interface to differentiate and gain morphological and biochemical phenotypes that match those found in native tissue. (2) It is difficult to precisely reproduce in vivo–like dynamic mechanical microenvironments of pulmonary epithelial cells in vitro. This usually entails careful design, fabrication, and engineering of mechanical tools capable of generating different types of physical forces that can modulate the interactions of the epithelial cells with culture substrata or fluid flows to simulate various physiologic and pathologic conditions. Also, the need for the ability to systematically control the level of applied forces and to quantify actual mechanical stresses acting on the cells remains an important issue in such systems. (3) Mechanical forces in the respiratory system can induce not only immediately detectable changes in the morphology and viability of pulmonary epithelial cells but also very transient signaling events mediated by soluble molecules such as cytokines and growth factors. This can trigger complex intra- and intercellular signal transduction, leading to longer-lasting effects on the cells. Therefore, a detailed understanding of the cellular responses to mechanical stimulation requires accurate and quantitative measurements and analysis of the biochemical environments of pulmonary epithelial cells. Such tasks usually call for the development of biological assay systems with high sensitivity and specificity.

These challenges and requirements have limited our understanding of the behavior of lung cells in their mechanically active microenvironment and, in turn, driven the development of new methodologies to address the technical limitations arising from the lack of appropriate tools for cellular studies. In this chapter, we describe recent research efforts directed toward developing new experimental models that enable in vitro engineering of pulmonary epithelial cells, re-creation of their dynamic mechanical environment, and measurements of their response to various physiologic and pathologic mechanical forces. Specifically, we review the literature pertaining to cellular-level experimental studies based on in vitro systems that can (1) induce and optimize the growth and differentiation of primary pulmonary epithelial cells in biomimetic culture environments, (2) recreate mechanical stresses originating from matrix deformation and tissue remodeling to investigate cell-surface or cell-cell interactions, (3) generate various pulmonary flows to simulate cell-fluid interactions, and (4) measure and characterize cytokine production induced by mechanically stimulating pulmonary epithelial cells.

Figure 24.1 shows the flow of research for microtechnology-based tissue engineering of the pulmonary epithelia and their microenvironments to analyze cellular-level responses to physiologic and pathologic mechanical stimuli. Hypotheses (dark grey block in the first row) concerning the effect of various mechanical microenvironments are formulated and classified into specific physical parameters (light grey blocks in the second row). These parameters are imposed upon the cells of interest (green oval in the fourth row; for this chapter, the cells of interest are pulmonary epithelial cells) using microtools (white blocks in the third row). The responses of cells are also measured and analyzed by various microtools (white blocks in the fifth row) to characterize changes in biochemical and cellular parameters (light grey
24.2 The Lung and Pulmonary Epithelial Cells

The complex hierarchy of branching airways is a major determinant of the internal structure of the lung that provides sufficient surface area for gas exchange to meet the need for oxygen uptake and carbon dioxide elimination. During inspiration, air passes through either the nose or mouth into the larynx, which opens into a long tube called the trachea. The trachea branches into two primary bronchi, one of which enters each lung. Within the lung, there are more than twenty generations of branching, resulting in the formation of intrapulmonary bronchi, bronchioles, alveolar ducts, and alveolar sacs. The lumens of these airways and alveoli consist of various types of epithelial cells that greatly differ in morphology and function, depending on their location within the airway tree. Also, there is considerable
interspecies variation in the cellular composition of the pulmonary epithelium. In this section, we provide an overview of the different types of epithelial cells identified beyond the larynx in the human respiratory system.

### 24.2.1 Tracheobronchial Epithelial Cells

The trachea and bronchi have ciliated pseudostratified columnar epithelium with a thick basement membrane. This epithelium and its underlying layer of loose connective tissue called the lamina propria make up the tracheobronchial mucosa. Two principal cellular components of the tracheobronchial epithelium are ciliated cells and goblet cells [Figure 24.2(a)]. Ciliated cells represent approximately 80 percent of the epithelial cells residing on the luminal borders of large airways. They extend through the full thickness of the epithelium and are best characterized by beating cilia. Goblet cells are interdispersed among the ciliated cells and secrete mucus, a viscous fluid composed primarily of highly glycosylated proteins called mucins suspended in electrolyte solutions. Mucus acts as a fluid barrier to protect airway epithelium by trapping foreign particulates entering the lung, which are then removed from the airway toward the throat by a coordinated sweeping motion provided by the beating cilia of ciliated cells. This process is known as mucociliary clearance and serves as an important first line of defense in the respiratory system.

![Figure 24.2 Pulmonary epithelial cells](image)

**Figure 24.2** Pulmonary epithelial cells: (a) Scanning electron micrograph (top) of bronchial epithelium and its ultrastructure (bottom) viewed by transmission electron microscopy. G, C, and B represent goblet cells, ciliated cells, and basal cells, respectively. (b) Nonciliated and dome-shaped Clara cells (CC) are predominant in bronchiolar epithelium. Ciliated cells (C) are sporadically situated between Clara cells. (c) Alveolar epithelium consists of large cuboidal type II epithelial cells and thin squamous type I cells. (Source: [6–11], reproduced with permission.)
Other cell types present in tracheobronchial epithelium include basal cells and brush cells. Basal cells are distributed mainly along the basement lamina and are smaller in size compared to other cells. The main function of these cells is to attach the columnar epithelium to the basement membrane. They can also undergo mitosis and play an important role in the growth, maintenance, and replacement of the epithelial layer. Brush cells have a columnar structure and are characterized by the presence of microvilli on the apical surface. Their basal surface is in synaptic contact with afferent nerve endings, which suggests the possible role of these cells as chemoreceptors involved in general sensation.

### 24.2.2 Bronchiolar Epithelial Cells

Bronchioles are smaller branches of bronchi and distinguished by a prominent smooth muscle layer and the absence of cartilage around the outer walls. The branching of bronchioles eventually leads to the formation of terminal bronchioles, which then divide into respiratory bronchioles that have alveoli sporadically distributed on their walls. Bronchiolar epithelium consists mainly of ciliated cells and nonciliated secretory cells known as Clara cells [Figure 24.2(b)]. Ciliated cells have a simple columnar shape but become more cuboidal in distal bronchioles. With increasing airway generation, the number of basal cells, ciliated cells, and goblet cells decreases dramatically, and Clara cells become a predominant population in the bronchioles. Clara cells are dome-shaped, produce watery proteinaceous secretions called Clara cell secretory proteins, and usually protrude into the bronchiolar lumen. These secretory cells are unique to the bronchiolar region of the lung and differ from goblet cells in that their secretory granules are smaller in size and number and they contain abundant granular endoplasmic reticulum. Also, Clara cells are known as one of the most multifunctional and heterogeneous cell types in the mammalian lung, and their function has not yet been fully established [12]. Several lines of evidence suggest that they are involved in the production of surfactant proteins and may play an important role in the regulation of pulmonary inflammation. Also, it has been demonstrated that Clara cells are responsible for detoxifying harmful airborne substances inhaled into the lung using the isoenzymes called cytochrome p450 present in their smooth endoplasmic reticulum. Finally, they are mitotically active and have been shown to serve as the progenitor of ciliated cells as well as themselves, contributing to the regeneration of bronchiolar epithelium.

Although the presence of basal cells in the distal parts of the airway system is uncommon, they persist in small airways, but are absent in terminal bronchioles. Bronchiolar basal cells are flattened, triangular, or trapezoidal in shape and have different organelle features from those of bronchial basal cells. They act as progenitor cells for bronchiolar epithelium under certain conditions and can differentiate into Clara cells or ciliated cells.

### 24.2.3 Alveolar Epithelial Cells

The alveoli are the final branchings of the airway tree and serve as the gas exchange units of the lung. They have hollow sacs whose open ends are continuous with the lumens of the airways. The alveolar epithelium comprises two epithelial cell types
with highly specialized functions: type I and type II alveolar epithelial cells [Figure 24.2(c)]. Type I cells are large (~40 µm in diameter) squamous cells with long and thin cytoplasmic extensions that spread out along the walls and comprise the thin alveolar epithelium; in regions of gas exchange in alveoli, these cells may measure as small as 0.1 µm in thickness. They constitute ~35 percent of all alveolar epithelial cells and cover ~93 percent of the alveolar epithelial surface area. Type I cells are responsible for gas exchange, and their small thickness greatly facilitates gas diffusion across the blood-air barrier. They are also known to regulate transport of physiologically important solutes and water between circulating blood and the alveolar space, contributing to maintaining alveolar fluid balance.

Type II cells are small cuboidal cells (~10 µm in diameter) that comprise ~7 percent of the alveolar epithelial surface area and ~65 percent of all alveolar epithelial cells. These cells are located in the alveolar corners and are identified by intracellular lamellar bodies that serve as storage granules for surfactant and by the presence of organelles and microvilli on their apical membrane. Their major function is to synthesize, secrete, and recycle surfactant, a surface-active lipoprotein that serves to reduce surface tension at the air-liquid interface in the alveolar epithelium. Other major functions include the synthesis of immune effector molecules and the regulation of fluid balance through transport of ions and water across transmembrane channels. Type II cells also maintain alveolar homeostasis by mediating repair to the injured alveolar epithelium; when type I cells are damaged and removed, type II cells can proliferate and differentiate into type I cells to replace the injured cells. Therefore, type II cells play a critical role in normal pulmonary function, as well as in the response of the lung to damage.

24.3 In Vitro Production and Engineering of Pulmonary Epithelium

In vitro culture of pulmonary epithelial cells has been an indispensable tool for advancing our understanding of the complex cellular and molecular processes underlying normal function and disease development in the respiratory system. The most important requirement for successful in vitro culture is the establishment of optimal conditions that enhance the proliferative capacity of cells and facilitate the expression of differentiated characteristics found in the native pulmonary epithelium. This is achieved mainly by defining the growth media, optimizing ECMs, and using an air-liquid interface for culture. Here, we discuss the use of these approaches to engineer in vitro culture of primary pulmonary epithelial cells and to reproduce the essential morphological and biochemical properties of in vivo airway or alveolar epithelium.

24.3.1 Engineering of Primary Airway Epithelial Cell Culture

A primary factor that affects the viability of freshly harvested airway epithelial cells is the culture media. The most widely used media formulations for primary airway epithelial cells are based on the Lechner’s Laboratory of Human Carcinogenesis basal medium and the Dulbecco’s modified Eagle’s medium/Ham’s F-12 [13]. For long-term culture, the exposure of the cells to serum is generally limited to short
periods because blood-derived serum contains transforming growth factor (TGF)–β, which can inhibit the proliferation of primary airway epithelial cells [14, 15]. Small amounts of serum, however, have been shown to stimulate morphological differentiation of distal airway epithelial cells [16]. Other important supplements for modulating the proliferative capacity of airway epithelial cells include retinoic acid, epidermal growth factor (EGF), bombesin, and fibroblast growth factor [15, 17–21]. EGF also induces the morphological transformation of human bronchial epithelial cells in culture by causing them to generate filapodia extensions [22].

ECMs are also important in primary airway epithelial cell culture because they have a profound influence on cytoplasmic receptors and cellular architecture, which can change gene expression and eventually the presentation of specific phenotypes. Yamaya et al. studied the differentiated structure and function of primary human tracheal epithelial cells on different types of collagen culture supports, including human placental collagen, vitrogen gels, and ECM produced by bovine corneal endothelial cells [21]. Based on transepithelial electrical-resistance measurements, electron microscopic imaging, and immunohistochemistry, they showed that the cells cultured on vitrogen gel produced airway epithelium with cytological and electrical properties that closely mimicked native epithelium. Also, their method was effective for culturing primary cystic fibrosis epithelial cells and retaining their essential disease characteristics in vitro. Wu et al. investigated the differentiation of human tracheobronchial epithelial (HTBE) cells on collagen gels [23]. They demonstrated that the epithelial cells synthesized mucin and formed mucous granules in culture when they were maintained on collagen gel substrata but not on plastic tissue-culture plates or thin collagen-coated surfaces. In a follow-up study, Robinson and Wu also examined the effect of collagen gel (CG) thickness on the growth and differentiation of HTBE cells [24]. They found that the attachment and proliferation of the cells were independent of CG thickness, but the synthesis and secretion of mucin was elevated with an increasing CG thickness. A mixture of collagen, fibronectin, and bovine serum albumin has been used by Lechner et al. to induce clonal growth of human bronchial epithelial cells without using feeder cells [25]. Collagen gels can also be used to establish coculture of airway epithelial cells with lung fibroblasts. Infeld, Brennan, and Davis cocultured human lung fibroblasts between the layers of type I collagen gel with HTBE cells on the upper collagen lattice [26]. In this system, the fibroblasts were observed to migrate preferentially toward HTBE cells, suggesting that HTBE cells directed fibroblast migration. In another study using similar coculture systems, they also showed that HTBE cells penetrated collagen matrices when cultured with human fetal lung fibroblasts and that the invading clusters of the epithelial cells formed tubular structures undergoing dichotomous branching [27]. These studies provided important insights into airway morphogenesis during development, repair after lung injury, and the pathogenesis of bronchial neoplasms. Mio et al. also developed coculture of human bronchial epithelial cells (HBEC) and human lung fibroblasts in collagen gels to study connective-tissue contraction during wound healing and fibrosis [28]. Their results showed that HBEC might modulate fibroblast activities by causing them to generate higher levels of traction force and to induce the contraction of collagen gel.
The most critical step necessary to induce the differentiation of airway epithelial cells is to expose proliferated cells to an air-liquid interface (ALI). During this procedure, cells are grown on a semipermeable membrane that separates two independent chambers placed in a culture well, and medium is added to the basolateral side only. This configuration results in the formation of a layer of liquid with a thickness of ~15 µm and an increased level of aerobic respiration in the cells [29]. Dejong et al. demonstrated that ALI culture led to the formation and maturation of cilia in HBEC culture, regardless of the type of substratum used in the study (de-epidermized dermis and collagen membrane) [30]. Also, it was found that the cells maintained in submerged cultures showed no sign of ciliogenesis, evidencing the important role of ALI culture in morphological differentiation of airway epithelial cells. Using a canine bronchial epithelial cell-culture system, Johnson et al. showed that ALI culture conditions caused a significant increase in active transport of sodium ions compared to standard submerged culture methods [29]. It was also noted that cellular metabolism shifted from anaerobic to aerobic. These results indicate that increased oxygen availability to the epithelial cells in ALI culture produced a higher rate of transepithelial active sodium transport. Based on the use of ALI, Davidson et al. established a novel primary culture system of differentiated murine tracheal epithelial cells that can potentially facilitate the studies of tracheal epithelium from transgenic mouse models of human pulmonary disease such as cystic fibrosis [31]. Their technique allowed for the generation of confluent and polarized epithelial cultures with high transepithelial resistance (an index of tight junction formation), minimal contamination with nonepithelial cell types, differentiation into ciliated cells and goblet cells, and genetic expression/electrophysiological profile characteristic of murine tracheal epithelium. Similar examples can be found in the work by You et al., where they created primary culture of mouse tracheal epithelial cells using ALI that can undergo rapid proliferation and differentiation into ciliated cells, mucus-secreting cells, Clara cells, and progenitor-like cells [32]. Whitcutt, Adler, and Wu designed a simple and disposable culture chamber called a Whitcutt chamber where culture conditions could be switched between submerged culture and ALI culture by the movement of a transparent and permeable gelatin membrane [33]. Using this chamber, they demonstrated in vitro production of fully differentiated guinea pig tracheal epithelial cells exhibiting various mucociliary functions. Differentiated primary airway epithelial cells are known to rapidly dedifferentiate and lose their proliferative capacity and secretory/morphological properties during subculture [34–36]. To address this issue, Widdicombe et al. developed a simple technique for expanding primary culture of human tracheal epithelial cells and demonstrated that the epithelial cells could be passaged several times without losing the ALI-culture-induced differentiation characteristics [36].

24.3.2 Engineering of Primary Alveolar Epithelial Cell Culture

In vitro culture of primary type I alveolar epithelial cells is difficult to establish and maintain [37, 38]. For this reason, researchers have been dependent mainly upon primary culture of type II alveolar epithelial cells for cellular studies. It has been shown that primary type II cells acquire morphological and biochemical properties of type I cells over several days in culture [39, 40]; during differentiation, type II cells
lose their cuboidal shape, form a monolayer with high transepithelial resistance, lose microvilli and lamellar bodies, and display thin cytoplasmic extensions away from a protruding nucleus. This process is also accompanied by the decreased synthesis of surfactant phospholipids/surfactant proteins and an increased reactivity to type I–specific membrane components. These type I–like cells have gained acceptability as an in vitro model of type I alveolar epithelium for investigating transport of solute, water, and drugs in alveoli [40]. Based on the extensive phenotypic changes of primary type II cells in culture, Elbert et al. established in vitro alveolar epithelium comprising type I–like cells [41]. The use of low-serum growth medium and polyester filter supports coated with fibronectin and collagen promoted the differentiation of type II cells into type I–like cells and enabled the production of monolayers with tight junctions and desmosomes. Fuchs et al. further characterized the differentiation process in the same culture system and showed the transition of a cell population consisting mainly of type II cells to an epithelial monolayer composed of both type I and type II cells [42]. They also found that the type I–like cells exhibited invaginations of plasma membrane resembling caveolae and the increased synthesis of the structural protein caveolin-1, both of which are believed to play an important role in macromolecule transport across the blood-air barrier form by type I cells in vivo [43]. The effect of ECMs on the differentiation of type II cells into type I–like cells was evaluated by Olsen et al. [44]. Using collagen, fibronectin, and laminin-5 (Ln5), they revealed that early in culture, type II cells lost their characteristic properties and developed intermediate type I–type II phenotypes, regardless of individual matrix components. Beyond this stage, the cells showed ECM-dependent behavior; type II cells on matrices consisting of collagen/fibronectin, collagen alone, or Ln5 alone continued a loss of their characteristics, whereas the cells on collagen/fibronectin/Ln5 or collagen/Ln5 regained their original phenotypes.

Under appropriate conditions, type II cells have been shown to maintain their phenotypic characteristics without differentiating into type I–like cells [39] and have been used as an in vitro model for examining the metabolism and function of type II alveolar epithelial cells. These techniques include the addition of growth factors, culture on various ECM substrata, and the use of an air-liquid interface. Borok et al. demonstrated that keratinocyte growth factor (KGF) could modulate and partially maintain the characteristic phenotype of type II cells by preventing and reversing the expression of aquaporin-5 (a water channel present in vivo on the apical surface of type I cells only) in primary culture of rat type II cells [45]. In another study, they also showed that the use of culture medium supplemented with KGF or rat serum inhibited or reversed the expression of T1α, a gene expressed exclusively by type I cells in the adult rat lung [46]. This verifies that the phenotype of type II cells can be retained in primary culture and that the differentiation between type I and type II cells is partially reversible. KGF was also shown to mediate epithelial-mesenchymal interactions in coculture of rat type II cells and lung fibroblasts, which then allowed the type II cells to maintain their ability to produce surfactant proteins [47]. Nonplastic culture substrata have proven effective for preserving the characteristics of type II cells. Geppert, Williams, and Mason used floating collagen membranes to prevent rat type II cells from spreading and undergoing subsequent morphological changes [48]. Laminin-rich matrix gel derived from Engelbroth-Holm-Swarm sarcoma was shown to prolong the retention of lamellar bodies [49]
and to promote surfactant-protein synthesis [50] in type II cells. Organotypic culture systems based on gelatin sponge matrices enabled the formation of alveolar-like structures composed of surfactant-secreting rat type II cells with lamellar bodies, as well as long-term maintenance of their phenotype [51]. Although not as commonly practiced as in the case of primary airway epithelial cell culture, ALI culture contributes significantly to the maintenance of the differentiated characteristics of type II alveolar epithelial cells. Dobbs et al. corroborated this by showing that human type II cells cultured in ALI were cuboidal in shape, contained lamellar bodies, and had high content of mRNA for surfactant proteins, whereas the cells maintained in submerged culture differentiated into type I–like squamous cells [52]. Using ALI culture of human type II cells, Alcorn et al. demonstrated that increased oxygen availability to the cells due to the presence of ALI increased cell polarization and contributed to promotion and maintenance of type II cells without a loss of their essential properties, as well as the expression of surfactant proteins [53]. ALI culture combined with the use of defined serum-free medium and porous polycarbonate membranes coated with Matrigel was employed in coculture of rat type II cells with bovine microvascular endothelial cells to recreate the blood-air barrier in vitro [54].

24.4 Engineering of Cell-Matrix and Cell-Cell Interactions

Expansion and relaxation of the lung during respiration or mechanical ventilation generate mechanical deformation of the basement membrane of pulmonary epithelial cells, which in turn imposes mechanical stresses on the cells. Respiratory diseases with tissue remodeling often accompany chronic mechanical perturbations to the interactions between pulmonary epithelial cells themselves or between the cells and the underlying subepithelial layer. In this section, we categorize these mechanical forces and stimulations into different types depending on their essential features and discuss the resulting responses of pulmonary epithelial cells.

24.4.1 Cyclic Stretch in Two-dimensional Culture

The Flexercell strain unit is a commercially available mechanical device that can impose cyclic stress (tension or compression) on cells by using vacuum to induce the deformation of an elastic membrane to which cells are attached [Figure 24.3(a)] [55]. Using fetal rat II alveolar epithelial cells cultured in this system, Sanchez-Esteban et al. demonstrated that cyclic stretch simulating breathing movements during lung development promoted the differentiation of fetal type II cells, as assessed by the increased production of surfactant phospholipids and the elevated expression of surfactant proteins B and C [56]. In primary culture of fetal rabbit type II alveolar cells, cyclic deformation also increased proliferation, surfactant-related phospholipid synthesis, and cAMP levels, suggesting that airway distension in the developing lung is involved in the maturation of type II cells [57]. In the edematous lung, the alveolar epithelium plays a crucial role in edema clearance by transporting Na⁺ and liquid out of the air spaces. Waters et al. used the Flexercell system to examine the role of cyclic stretch in the regulation of Na⁺-K⁺-ATPase (mediator of active Na⁺ transport) in murine alveolar epithelial cells and showed that short-term cyclic
mechanical stress stimulated its activity by increasing intracellular Na\(^+\) and recruiting Na\(^+\)-K\(^+\)-ATPase subunits to the basement membrane [58]. Short-term cyclic stretch simulated by the Flexercell also induced apoptosis and secretion of the major lipid component of pulmonary surfactant known as phosphatidylcholine in primary culture of rat alveolar type II cells [59]. Vlahakis et al. used A549 type II alveolar epithelial cells cultured on the Flexercell system and demonstrated that cyclic stretch enhanced the gene expression and release of interleukin (IL)–8, illustrating that epithelial deformation can trigger inflammatory signaling [38]. The Flexercell unit was also used to reveal that cyclic mechanical strain inhibited repair of epithelial wounds in human airway epithelial cell culture [60].

One drawback of the Flexercell system is that the cyclic inflation or deflation of its circular cell-culture substratum generates nonuniform loading conditions and causes the deformation of cells to vary significantly depending on its radial position [64, 65]. This limits quantitative analysis of cellular responses and prevents one from making detailed correlations between the amount of deformation and functional change. To overcome these limitations, Tschumperlin and Margulies devel-

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**Figure 24.3** In vitro systems for generating mechanical forces that regulate cell-matrix and cell-cell interactions: (a) In the Flexercell strain unit, cells are cultured on an elastic membrane and stretched by negative pressure imposed by vacuum. (b) Cell-stretching system for exposing cells to uniform and equibiaxial mechanical strain. The device uses a silicone membrane as a cell-culture substratum deformed by the vertical motion of a circular indentor. (c) Alveolar epithelial cells exhibit injurious response to applied cyclic stretch in a dose-dependent manner (% represents the percent change in the membrane surface area). (d) Transwell-based cell-culture system used to apply an apical-to-basal transmembrane pressure gradient across bronchial epithelial cells grown at an air-liquid interface on a permeable membrane. (e) Scanning electron micrograph of cultured rabbit tracheal epithelial cells (left) and light micrograph showing mechanical stimulation of the apical surface of a single cell using a microprobe (ci: cilia, cs: cellsurface, m: microprobe, sc: stimulated cell). *(Source: [5, 61–63], reproduced with permission.)*
opoped a stretching device capable of subjecting cells to uniform equibiaxial strains [Figure 24.3(b)] [61]. Using this system, they demonstrated the injurious response of primary rat type II alveolar epithelial cells to applied cyclic stretch in a dose-dependent manner [Figure 24.3(c)]. Also, they studied the vulnerability of the cells at different stages of proliferation and revealed that the level of cellular injury decreased with increasing time elapsed after seeding. Their findings suggest that morphological and phenotypic alterations with time in culture fundamentally change the susceptibility of alveolar epithelial cells to mechanical deformation. In a separate study using the same type of cells and device, they showed that the extent of injury responses of the cells varied depending on the nature of applied membrane deformation, such as frequency, time, and magnitude [66]. It was noted that cyclic deformation was significantly more damaging than static stretch and that cellular injury was independent of the rate of deformation during single stretch and was elevated with increasing frequency and deformation amplitude. Waters et al. also constructed a pneumatically driven mechanical system to impose homogenous biaxial cyclic deformation to cells on elastomeric membranes [67]. In their study, they demonstrated that the inhibition of spreading and migration of airway epithelial cells during wound closure was dependent upon the magnitude of applied strain.

24.4.2 Static Stretch or Pressure in Two-dimensional Culture

Wirtz and Dobbs devised a stretching system where hydrostatic pressure induced mechanical stretching of flexible silastic membranes [68]. Applying static stretch to primary rat type II alveolar epithelial cells grown in this system, they revealed the mechanism by which deep inflation of the lung stimulates surfactant secretion; mechanical stretch of type II cells caused a transient (<60 s) increase in cytosolic Ca\(^{2+}\), and this was followed by a sustained (approximately fifteen- to thirty-minute) stimulation of surfactant secretion in a dose-dependent fashion.

The epithelium of asthmatic airways has a mechanically unique cellular microenvironment, where smooth muscle constriction leads to the buckling of the airway wall and the folding of airway epithelium into deep crevasses [5]. As a result, the epithelial cells along the folds are pushed up against each other and, thus, experience prolonged compressive mechanical stresses. Ressler et al. recreated this situation in vitro by applying static pressure selectively to the apical surface of primary rat tracheal epithelial cells cultured on a porous membrane in the Transwell system [Figure 24.3(d)] [69]. They found that the apical-to-basal transmembrane pressure gradient induced the expression of early growth factor–1 (transcription activator of airway remodeling genes), endothelin-1 (potent bronchoconstrictor), and TGF-β1 in a magnitude- and time-dependent manner. The same system was employed by Tschumperlin et al. to reveal that compressive mechanical stresses similar to those occurring in vivo during airway constriction gave rise to increased release of endothelin protein and TGF-β2 from HBECs [70]. These peptides were found to promote fibrotic protein synthesis by fibroblasts, which was consistent with subepithelial fibrosis characteristic of chronic asthma. In another study based on the same method, they also demonstrated that the pressure gradient applied across the airway epithelium at in vivo levels enhanced phosphorylation of extracellular signal-regulated kinase [71]. Swartz et al. created cocultures of HBECs and human
lung fibroblasts in the Transwell system (epithelial cells in the upper chamber and fibroblasts in the lower chamber) and examined the communication of compressive forces between the two cell types [72]. Their study showed that the mechanical stresses applied to the airway epithelial cells were communicated to fibroblasts to induce integrated biological responses that mimicked the key features of airway remodeling found in asthma.

24.4.3 Mechanical Stretch in Three-dimensional Culture

Skinner developed a solenoid-operated programmable stretch device where the movement of a metal bar induced by applying a magnetic field to the solenoid could generate the elongation of three-dimensional sponge matrices in a controllable manner [73]. Using this system, Liu et al. demonstrated that intermittent mechanical stretch simulating physical forces induced by fetal breathing movements enhanced DNA synthesis and division of fetal rat alveolar epithelial cells cultured in a gelatin sponge [74]. They also showed that the proliferative response of the fetal cells to mechanical stress did not take place in two-dimensional monolayer culture systems and was unique to three-dimensional culture [75]. In separate studies, they provided evidence that the strain-induced cellular proliferation was mediated by growth factors [76] and that mechanical stimulation upregulated the gene expression and protein synthesis of platelet-driven growth factor (PDGF)–B and PDGF β-receptor [77]. Intermittent mechanical strain inducing the proliferation of fetal lung cells was also shown to differentially regulate the expression of various ECM molecules and to selectively increase collagen and biglycan content [78]. Xu et al. used the same stretch device to examine the responses of fetal rat lung cells and fibroblasts cultured in a three-dimensional matrix, as well as the effect of the interactions between the two cell types on stretch-induced cell proliferation [79]. Their results showed that lung epithelial cells and fibroblasts responded to mechanical strain by increasing DNA synthesis when each cell type was subjected to strain separately, but DNA synthesis was suppressed when the epithelial cells were cocultured with fibroblasts having different gestational ages. The mechanical device used for stretching three-dimensional cell culture in these studies was developed into a commercially available computerized apparatus known as the Bio-Stretch System [80].

24.4.4 Direct Mechanical Stimulation of Single Cells

Precisely controlled motion of microstructures has been adopted as a unique strategy for mechanically stimulating pulmonary epithelial cells at the single-cell level to study their mechanical responses or cell-cell communication. Isakson et al. developed a method where a glass micropipette with a tip diameter of 1 μm was piezoelectrically deflected downward for a short period (~150 ms) to locally deform the apical membrane of a single cell [81]. Using this approach combined with coculture of rat type I and type II alveolar epithelial cells established on collagen/fibronectin/laminin-5 coated coverslips, they investigated intercellular signaling between the two cell types. From this study, it has been shown that mechanically induced Ca^{2+} signaling between type I and type II cells is coordinated by unique profiles of the gap-junction protein connexin. A similar approach to mechanical stimu-
lation of single cells was reported by Sanderson and Dirksen [62]. Their system was also based on the piezoelectric vertical movement of a microprobe that caused the dimpling of a single cell [Figure 24.3(e)]. This mechanical actuation scheme was implemented to study the mechanosensitive response of ciliated airway epithelial cells. They demonstrated that mechanical stimulation resulted in a significant increase in ciliary beating frequency in a dose- and Ca^{2+}-dependent manner and that the locally applied mechanical stress could be transmitted to adjacent cells through cell-cell communication.

### 24.5 Engineering of Cell-Fluid Interactions

Pulmonary epithelial cells are constantly exposed to moving fluids in development, health, and disease. Recent evidence shows that during gestation, spontaneous peristaltic airway constrictions in the fetal lung propel liquid through the airway tree [2]. After birth, the microenvironment of pulmonary epithelial cells changes drastically due to the formation of an air-liquid interface over the pulmonary epithelium and convective transport of air generated by respiration. As a result of the formation of the air-liquid interface, surface tension becomes a dominant force in regulating the dynamics of pulmonary flows and the interaction of epithelial cells with fluids. In this section, we present in vitro systems that reproduce surface-tension-driven interfacial phenomena observed in the respiratory system to investigate the effect of the resulting fluid mechanical stresses on pulmonary epithelial cells. We also describe cell-culture devices that can recreate air-flow-induced shear stresses or apply fluid flows containing various gaseous compounds or soluble chemicals to pulmonary epithelial cells.

#### 24.5.1 In Vitro Airway Reopening Models

Pulmonary airways are coated with a thin, viscous liquid film produced by secretory cells in the airway epithelium. In a wide range of respiratory diseases that can accompany surfactant deficiency or inactivation, the liquid layer lining the epithelial surface of small airways becomes more susceptible to surface-tension-driven instability that leads to the formation of an occluding liquid plug across the airway lumen and the obstruction of air flow in the peripheral respiratory units [82]. During respiration, the liquid plug propagates along the airway due to a pressure gradient until the plug volume decreases to the point at which it ruptures to reopen the blocked airway [83]. This reopening process, however, has been shown through computational simulations to generate abnormally high levels of mechanical stresses [84], which may exert deleterious mechanical forces on pulmonary epithelial cells.

To investigate the vulnerability of pulmonary epithelial cells to fluid mechanical-stress-induced injury experimentally, Bilek, Dee, and Gaver created an in vitro model of airway reopening where rat alveolar epithelial cells in a liquid-filled parallel-plate chamber were subjected to the steady progression of a semi-infinite air bubble [Figure 24.4(a)] [85]. Using this system, they showed the injurious response of the epithelial cells to mechanical stresses imposed by the movement of an air-liquid interface that simulated plug propagation during airway reopening. Also, they dem-
onstrated the role of pulmonary surfactant in mitigating the detrimental effect of fluid mechanical stresses on the cells. Through computational simulations, it was concluded that the steep pressure gradient near the front meniscus of the air bubble was the most likely cause of cellular injury. In their follow-up study based on the same system, it was found that human alveolar epithelial cells exhibited the same pattern of cellular injury in response to the propagation of semi-infinite air bubbles and that flow conditions mimicking repetitive airway reopening caused the denudation of the cellular monolayer [86]. More importantly, by varying the viscosity of
liquid in the culture chamber, they showed that the magnitude of pressure gradient, not the duration of mechanical stimulation, determined the extent of cellular injury.

Although these studies provided important insights into the mechanism of airway injury during airway reopening, the experimental models looked only at the effect of the propagation of semi-infinite air fingers (this is relevant to the first breath of a new born) and failed to recreate the essential fluid dynamic characteristics of in vivo airway reopening, such as the propagation of liquid plugs with finite lengths and plug rupture. Also, they relied on submerged culture of transformed alveolar epithelial cells and thus had limited capabilities to reproduce in vivo–like airway epithelium. To address these issues, Huh et al. developed a compartmentalized three-dimensional microfluidic small airway system integrated with computerized air-liquid two-phase microfluidics [87]. This device produced fully differentiated airway epithelium through air-liquid-interface culture of primary human small airway epithelial cells in a biomimetic culture environment [Figure 24.4(b)] and recreated propagation and rupture of finite liquid plugs by dynamically switching air-liquid two-phase flows in polymeric microchannels [Figure 24.4(c)]. Using this system, they demonstrated that fluid mechanical stresses generated by plug propagation and rupture led to significant cellular damage in a dose-dependent manner, even with differentiated airway epithelia, which are structurally more robust than nondifferentiated epithelial monolayers [Figure 24.4(d)] and that the injury of the cells during plug propagation resulted mainly from the mechanical stresses produced by the front meniscus of a moving liquid plug. Furthermore, they revealed that plug rupture caused more severe damage than plug propagation alone and that transient pressure waves generated by plug rupture enabled acoustic detection of injury events as crackling sounds similar to respiratory crackles.

### 24.5.2 Application of Air-Flow-Induced Shear Stresses to Pulmonary Epithelial Cells

During tidal breathing, the flow of air through airway tubes exerts shear stresses on airway epithelial cells in vivo. Tarran et al. developed an in vitro airway epithelial cell-culture system where the physiological shear stresses were reproduced by the rotational motion of culture plates mounted on a motorized stage [88]. Using this device, they examined the effect of shear stress on the regulation of the volume of airway surface liquid (a critical component of the host defense mechanism mediated by mucociliary clearance) in normal and cystic fibrosis human nasal epithelial cell cultures. They found that the height of the surface liquid layer in normal epithelia increased significantly when the cells were subjected to shear stresses. This study also revealed that applied shear stresses induced the cellular release of ATP, which allowed cystic fibrosis airway epithelia to maintain airway surface liquid at physiological volumes and to sustain mucus transport over prolonged periods, whereas static culture without shear stresses resulted in the rapid depletion of the surface liquid layer.

Even-Tzur et al. created a modular device for subjecting airway epithelial cells cultured at an air-liquid interface to shear stresses produced by air flow [89]. In this system, airway epithelial cells are first cultured in custom-designed wells with permeable membranes that support proliferation and air-liquid-interface-induced dif-
differentiation. For mechanical stimulation, the differentiated cells are transferred to a flow chamber, where they are exposed to steady air flows that generate shear stresses uniformly distributed over the apical surface of the cellular monolayer. This system was used to demonstrate that wall shear stresses induced by physiological air flows affected mucus secretion and intracellular structures of differentiated human nasal epithelial cells.

24.5.3 Exposure of Pulmonary Epithelial Cells to Gaseous Compounds

Tarkington et al. created a flow system for generating and guiding ozone-containing gas streams to the apical surface of pulmonary epithelial cells cultured on a permeable membrane [90]. The operation of this device is based on serially connected flow vessels that can cooperatively control, monitor, and manipulate the pressure, ozone concentration, temperature, and humidity of gaseous samples delivered to the cells maintained in a culture incubator. Using this system, they demonstrated the deleterious effect of ozone exposure on human tracheobronchial epithelial cells. They also revealed that the reduction in the viability of cells after ozone exposure was inversely proportional to the volume of liquid covering the apical side of a monolayer, illustrating the protective role of epithelial liquid film. Sun et al. used this flow system to demonstrate significantly decreased cellular viability, inhibition of cellular metabolism and replication, and compromised membrane integrity resulting from the exposure of HBECs to side-stream cigarette smoke [91].

Aufderheide and Mohr designed a new type of cell-culture system known as CULTEX that allowed for direct exposure of pulmonary epithelial cells maintained at an air-liquid interface to air flows containing various gaseous substances and complex mixtures [92]. This system consists of (1) a modular culture unit based on the Transwell system where pulmonary epithelial cells are cultured on a porous membrane and intermittently exposed to an air-liquid interface at certain time intervals without a loss of viability, and (2) a specially designed tube system for transporting and uniformly distributing gaseous test samples to a monolayer of cells maintained at an air-liquid interface. This device is also equipped with computer-controlled sensing systems capable of regulating culture conditions such as temperature, CO₂ content, and humidity, eliminating the need for cell-culture incubators. This system was used to analyze cellular viability and metabolic activity after the exposure of HBECs to synthetic air, nitrogen dioxide, and ozone [93]. Knebel, Ritter, and Aufderheide investigated the effect of diesel exhaust fumes on HBECs and revealed dose-dependent cytotoxic responses of the cells using the CULTEX system directly connected to a diesel engine [94]. Wolz et al. extended the application of the CULTEX system to the examination of biological responses of HBECs to cigarette smoke [95]. They demonstrated that the exposure of the cells to side-stream cigarette smoke induced DNA damage in a dose-dependent manner. Based on the principle of sedimentation, Fukano, Yoshimura, and Yoshida modified this system to expose the cells to both vapor and particulate phases of mainstream smoke [96]. Using this system, they showed that cigarette-smoke exposure led to the increased expression of mRNA for the sensitive oxidative stress enzyme known as heme oxygenase–1, which has been implicated in the pathogenesis of various respiratory diseases.
Cell culture analog (CCA) bioreactors are in vitro culture systems that consist of interconnected compartments, each of which has different types of cells to represent different organs and to mimic their characteristic metabolism [97]. These systems are designed to allow multiple cell types to interact with each other through recirculating culture media. This makes CCA bioreactors more attractive for predicting integrated biological responses to chemicals or pharmaceuticals than conventional in vitro culture methods that are often limited in reproducing the communication between various cell types constituting different organs.

Researchers have used pulmonary epithelial cells as a cell type representative of the lung in several CCA devices and studied their response to metabolites delivered from other tissue compartments through fluid flows. Sweeney et al. designed a CCA bioreactor consisting of liver and lung chambers lined with rat hepatoma and alveolar epithelial cells, respectively [98]. Using this system, they showed that the circulation of reactive naphthalene metabolites from the liver to the lung compartments induced the cytotoxicity of the alveolar epithelial cells and the depletion of glutathione (GSH). Ghanem and Shuler improved this system to obtain physiologically more relevant operating conditions (e.g., liquid residence time, the ratio of lung to liver cells) by culturing cells on microbeads packed into tissue chambers [99].

Efforts to reduce the volume requirements of conventional CCA bioreactors have led to the development of microfabricated CCA devices. Compared to their macroscopic counterparts, microscopic CCA systems are more amenable to recapitulating physiological flow conditions and accurately modeling the physical parameters of physiological systems, such as the ratio of liquid to cells and liquid residence times in each organ [97]. Owing to miniaturization, they require much smaller amounts of reagents and cells, making them more advantageous for testing drugs or culturing cells that are expensive and limited in supply. Furthermore, the small size of microscale CCA makes it possible to realize high-throughput testing and analysis using multiple CCA devices operating in parallel. The development of a microfabricated CCA bioreactor with integrated oxygen sensors was reported by Sin et al. [100]. To enable real-time monitoring of cell metabolism, they constructed a microfluidic cell-culture system combined with fluorescence lifetime-based sensing of dissolved oxygen. The device comprises interconnected microscale chambers that are fabricated in silicon and sustain microfluidic culture of different types of cells such as lung epithelial cells and liver cells. Using this device, they demonstrated that the microscale CCA system provided a more physiologically realistic tissue-culture environment and might potentially enable more accurate and physiologically relevant toxicity testing. The microfabricated CCA was also shown to be capable of monitoring gas exchange of individual cell cultures in real time using integrated oxygen-sensing components, offering significant advantages over traditional cell-culture techniques that generally take endpoint measurements. Viravaidya et al. used this system to probe naphthalene toxicity and demonstrated that the exposure of alveolar epithelial cells to fluid flows containing naphthalene metabolites produced in the liver compartment led to the depletion of GSH in the epithelial cells [101]. In a separate study, the design of the microfabricated CCA system was modified to have four chambers to include culture of differentiated adipocytes that could...
mimic the key functions of fat tissue [102]. The new device was used to study the bioaccumulation, distribution, metabolism, and toxicity of compounds such as fluoranthene, naphthalene, and naphthoquinone. This investigation revealed that fluoranthene preferentially accumulated in the fat chamber and that the presence of adipocytes in the system significantly reduced GSH depletion of alveolar epithelial cells induced by naphthalene and naphthoquinone. Based on these findings, the microscale CCA system was suggested to hold great potential for assessing the absorption, distribution, metabolism, elimination, and toxicity characteristics of drug candidates prior to animal or human trials.

24.6 Measurements of Mechanically Induced Inflammatory Responses

Acute respiratory distress syndrome (ARDS) and acute lung injury are often fatal inflammatory lung conditions that occur when a traumatic event such as pneumonia, shock, sepsis, or massive aspiration leads to inflammation, increased pulmonary vascular permeability, and extravasation of fluid and inflammatory cells into the pulmonary interstitium and alveolar spaces [103]. Both conditions are characterized by a massive systemic inflammatory response involving a cascade of both pro- and anti-inflammatory molecules. In this section, we describe (1) inflammatory mediators, and (2) the measurements of mechanically induced inflammatory responses.

24.6.1 Inflammatory Mediators

24.6.1.1 Cytokines

Cytokines are small, soluble, regulatory proteins (8 to 80 kDa) produced by cells during the inflammatory process and function as messengers in cell-to-cell communication. They are active at picomolar and femtomolar concentrations and principally act in an autocrine or paracrine fashion to initiate, amplify, and perpetuate local and systemic inflammatory responses. Cytokines are produced in cascades where initial signals are amplified by the target cells. Many cytokines are pleiotropic in that they have overlapping functions, with one cytokine having effects that are synergistic or antagonistic with other cytokines. Due to this interdependency, the composition of the “cytokine milieu” is more important than a single cytokine [104]. This sophisticated network involving complex feedback mechanisms is believed to play a central role in homeostasis of the immune system and the coordination of its responses [105].

24.6.1.2 Cytokines and Lung Injury

An imbalance in the production of cytokines can have profound effects on the body and is implicated in various diseases. Scientists have recognized that cytokine concentrations in biological fluids are often a useful indicator of the presence or severity of a disease. For example, in patients with ARDS, both pro- and anti-inflammatory cytokines are detected in the bronchoalveolar lavage fluid. Following lung injury,
the early response cytokines [i.e., tumor necrosis factor (TNF)–α and IL-1β] are released by the alveolar macrophages [106]. These cytokines then act in a paracrine fashion to stimulate other cellular components of the alveolar-capillary wall to produce more effector molecules [107] that involve both lung injury (IL-8) and repair [TGF-β1, hepatocyte growth factor (HGF), IL-6, IL-10]. After the onset of ARDS, there is a major anti-inflammatory response that peaks and exceeds the proinflammatory response involving both cytokines (IL-10) and cytokine antagonists (IL-1 receptor antagonist, soluble IL-1 receptor II, soluble TNF receptors I and II) to downregulate and attenuate the proinflammatory response [108]. Often it is the balance of pro- and anti-inflammatory mediators that determines the outcome of ARDS.

The ability to measure these soluble mediators in immune regulation can help us to understand the role of cytokines in the immune system and disease pathogenesis, to identify markers to predict the outcome of diseases, and to develop preventive and curative therapies to treat lung injury [105]. The successful development of these therapeutic strategies to suppress harmful inflammatory responses depends on understanding the steps leading to the activation of the inflammatory response and an understanding of the regulatory mechanisms that are involved in the response.

24.6.2 Cytokine Release Induced by Mechanical Stimulation

During lung injury, the cells of the alveolar epithelium are stimulated to secrete IL-8, a potent chemoattractant for neutrophils. Mechanical signals, such as deformation, that distress the alveolar epithelium can also lead to an increase in IL-8 (Figure 24.5). Neutrophils in the vascular component undergo extravasation (along the IL-8 gradient) into the tissue interstitium. Once there, the activated neutrophils release reactive oxygen metabolites, proteolytic enzymes, and additional cytokines in an acute inflammatory response [109]. This propagates into a cycle of inflammation leading to local tissue injury and biotrauma, where compartmentalization of the alveolar cytokines is lost, leading to their release into the pulmonary circulation and a subsequent systemic inflammatory response that can lead to multiple system organ failure and death [109].

It has been proposed that the structural disruption present is due to two factors: (1) lung overdistension in small airways and alveoli due to increased tidal volumes (volutrauma), and (2) shear forces generated from the repetitive opening and closing of atelectatic regions filled with fluid (edema), which causes severe wall stress and surfactant depletion (atelectrauma) [111]. Overdistension is mainly due to the end-inspiratory volume and is believed to degrade surfactant, disrupt the blood-gas barrier, and increase the levels of cytokines and inflammatory cells in the lung. The cyclic opening and reopening of injured alveoli can cause shear stress on the epithelial cell layers, and recent studies have suggested that the rapid rate of lung distension may be as important as the magnitude of distension in lung injury. Other factors to consider include the frequency of the stretch and the duration of stretch (inspiratory time) [112, 113].
24.6.2.1 Cytokine Release by Alveolar Epithelial Cells

Many researchers have used A549 type II alveolar epithelial cells to represent the alveolar epithelium and its responses to mechanical stimulation. Vlahakis et al. used A549 cells cultured on the Flexercell system and concluded that cyclic stretch enhanced the gene expression and release of IL-8 in a dose-dependent manner when varying the amplitude of stretch [38]. Stimulating the cells with TNF-\(\alpha\), a proinflammatory signaling molecule known to cause gene expression and protein release by A549 cells [107, 114], further increased the release of IL-8 [38]. Yamamoto et al. also used the Flexercell system with A549 cells and demonstrated that cyclic stretch increased the expression of HGF, IL-8, and TGF-\(\beta\) at both the mRNA and protein levels [115, 116]. Quinn et al. used a cell-stretch device to apply a uniform biaxial strain to A549 cells grown on flexible fibronectin-coated silicone membranes [117]. Cycles of 5 percent and 15 percent strain were shown to induce IL-8 release, as well as transcription of IL-8 mRNA. Both were accompanied by an increase in the activity of the mitogen-activated protein kinases: stress-activated protein kinase and p38 [117].

Previous studies have also used primary type II alveolar cells subjected to mechanical stretch. Hammerschmidt et al. exposed type II cells to cyclic stretch using the Flexercell system and determined that cyclic mechanical stretch alters cell
mediator release toward a proinflammatory pattern [118]. There was an increased production of eicosanoids, a decreased generation of the anti-inflammatory cytokine IL-10, and unchanged production of proinflammatory cytokines. Levine et al. isolated rat alveolar epithelial cells from healthy rats and from septic rats. The cells were exposed to high levels of cyclic equibiaxial stretch. The cells isolated from the septic rats were more vulnerable to the mechanical stretch and showed higher rates of cell death [119].

24.6.2.2 Cytokine Release by Alveolar Macrophages

Alveolar macrophages attach to the alveolar epithelium and can also play an important role in lung injury through the release of inflammatory mediators in response to mechanical stimulation (stretch) and chemical stimulation (inflammatory stimuli such as bacterial endotoxin). Pugin et al. developed an in vitro lung model where alveolar macrophages cultured on a silastic membrane are exposed to prolonged cyclic pressure-stretching strain. The macrophages responded by secreting IL-8 and matrix metalloproteinase–9 [120]. Lang, Barnetta, and Doylea cultured alveolar macrophages onto IgG-coated silastic membranes and applied a repetitive sinusoidal 30 percent mechanical strain [121]. Physical strain, in combination with lipopolysaccharide (LPS) enhanced macrophage secretion of TNF-α.

Because of the close proximity of alveolar macrophages to the pulmonary epithelium, it is believed that Type II alveolar epithelial cells respond to the alveolar macrophage-derived cytokines by releasing other pro- and anti-inflammatory cytokines such as IL-8 and IL-6. In fact, Standiford et al. have shown that LPS-stimulated alveolar macrophage media induce the increase of IL-8 in A549 epithelial cells [107]. Crestani et al. have found that A549 cells incubated with alveolar-macrophage-conditioned medium increased secretion of IL-6 [122].

24.7 Conclusion

Experimental studies outlined in this chapter highlight the remarkable breadth of mechanosensitive events mediated by pulmonary epithelial cells in the respiratory system. Undoubtedly, mechanical forces are essential for regulating the structure, function, and metabolism of pulmonary epithelial cells in a wide array of developmental, physiological, and pathological processes. As is evident from the studies described here, understanding various cellular responses to the dynamic mechanical environment of the respiratory system has been greatly facilitated by in vitro systems that can reproduce, manipulate, and measure the interaction of pulmonary epithelial cells with physiological and pathological mechanical forces.

Advancing our knowledge of the mechanically regulated behavior of lung cells will inevitably require careful design and engineering of new experimental tools that allow us to better mimic the geometrical and structural properties of the pulmonary epithelium and to precisely recreate the essential nature of tissue deformation and fluid flows occurring in vivo. Assay systems capable of detecting mechanotransduction signaling molecules with high sensitivity and specificity will also make a significant contribution to such efforts by providing a means to study
the molecular mechanisms of observed mechanosensitive events. The development of these new tools will likely involve contributions from various scientific and engineering fields. Based on these predictions, we suggest the following as examples of important future research directions: (1) Existing in vitro models have limited capabilities to examine cellular responses to mechanical stresses originating from surface-tension-driven interfacial phenomena that are prevalent in small airways and alveoli. This is mainly due to the technical difficulties associated with recreating the dynamic and complex microscale flow configurations found in the distal parts of the lung. These limitations may be resolved by microfabricated systems that can exploit the advantages of small length scales and low Reynolds number to create in vivo–like microscale gas-liquid two-phase flows in a controllable and reproducible fashion. Also, such microsystems can be readily integrated with microfluidic cell culture to enable on-chip engineering and mechanical stimulation of pulmonary epithelial cells in a single device. (2) Most in vitro systems aimed to investigate the mechanosensitive behavior of lung cells often neglect to consider the effect of other important stimulating factors (e.g., inflammatory mediators, hormones, chemokines, and growth factors) that can act in concert with mechanical forces to strongly affect cellular responses. For example, it has been shown that the remodeling of asthmatic airways is increased by a synergistic combination of mechanical stresses with inflammatory mediators [123]. Therefore, future experimental models need to be designed to enable in vitro re-creation and study of the interplay between mechanical stimulation with other confounding factors in various combinations. Also, study of paracrine and autocrine effects may benefit from microscale culture systems that better mimic physiological cell-fluid ratios. (3) Recent progress in microtechnology offers new opportunities to develop microfluidics-based miniaturized assay systems with more advanced analytical and sample-handling capabilities. Assays performed in microfluidic systems are advantageous over standard assay methods for many reasons, including compatibility with flow-based systems, small reagent/sample consumption, simplified automation and procedures, parallel analysis of multiple samples, and low-cost mass production. These systems will greatly assist future studies by enabling rapid, efficient, and quantitative measurements of molecular profiles regulating the mechanosensitive response of pulmonary epithelial cells.

The ability to engineer and characterize pulmonary epithelia and their mechanically regulated microenvironments will enhance our fundamental understanding of the role of physical forces in normal lung function and the development of respiratory diseases. Because of various difficulties associated with studying this topic in vivo and using conventional in vitro systems, what is required are multidisciplinary approaches based on the engineering of integrated microtools capable of regulating multiple microenvironmental factors (integration of effector microtools in Figure 24.1) and performing real-time quantitative measurements of multiple cellular responses (integration of readout microtools in Figure 24.1). Although most of the components exist, integration is still a technological challenge as well as an area of opportunity. The effective design, construction, and application of such integrated systems will also require in-depth biological and clinical insights (top row in Figure 24.1), together with the bioengineering expertise to result in the highest impact and most useful knowledge (bottom row in Figure 24.1).
References


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