Microfluidics, Lung Surfactant, and Respiratory Disorders

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DOI: 10.1309/LMJWE0WO65VFWPAS
The lungs are the major organs of the respiratory system and contain a branching network of airway tubes that become shorter, narrower, and more numerous as they penetrate deeper into the lung. The tracheobronchial tree comprises 3 major types of airways: cartilaginous bronchi, membranous bronchioles, and gas exchange ducts consisting of respiratory bronchioles and alveolar ducts. Upper airways and terminal bronchioles act merely as conduits for the passage of gas, whereas respiratory bronchioles and alveolar ducts carry out both conducting and gas exchange functions. Airways less than 1 to 2 mm in diameter that include membranous, terminal, and respiratory bronchioles as well as alveolar ducts are known as distal airways. These airways have a very large total cross-sectional area and relatively low resistance to the flow of gas (~10% to 20% of the total lung resistance). Due to their small size and high compliance, distal airways are prone to instabilities and closure at low lung volumes (eg, at the end of expiration). To prevent these fine structures from collapse (atelectasis), secretory cells of alveolar and neighboring airway epithelium produce pulmonary surfactant that distributes at the surface of the liquid lining layer of distal lung epithelium. Functional surfactant reduces surface tension at the luminal air-liquid interface and, thus, maintains the patency of airways and alveoli at low lung volumes. The presence of hysteresis in the pressure-volume curve of the lung indeed reflects this point (Figure 1): the pressure required to support a certain volume (V) of the lung units during deflation (P2) is smaller than that when lungs are inflated (P1). During deflation, the surfactant film is compressed and presumably a more compact layer of surfactant lipid molecules is present at the air-liquid interface, which results in a lower surface tension. Active surfactant at the luminal air-liquid interface imparts patency to the distal lung units and allows cyclic ventilation of the lung without collapse of airways and alveoli.

**Distal Lung and Surfactant Dysfunction**

Many respiratory disorders are accompanied by surfactant dysfunction. Abnormalities in biochemical and biophysical properties of surfactant arise in lung diseases such as acute respiratory distress syndrome (ARDS), asthma, and cystic fibrosis through different pathways. Following are two examples: (1) In ARDS, the integrity of the alveolo-capillary barrier is compromised due to such events as severe chest trauma, serious infection in the blood or other tissues, and bacterial or viral insult to the lung. Consequently, a proteinaceous fluid leaks from capillaries into the interstitium and floods alveoli. Furthermore, inflammatory cells, neutrophils, and macrophages infiltrate alveoli and airways. The presence of fluid and inflammatory mediators alters concentration and composition of the lung surfactant and accounts for surfactant malfunction in ARDS. (2) Asthma is a chronic disease of the respiratory system that can be triggered in various ways (eg, by airborne allergens). In a complex cascade of events, allergen-induced proliferation of lymphocytes causes release of various cytokines (interleukin [IL]-4 and IL-5) and toxic mediators such as eosinophil cationic protein (ECP) and leukotrienes. IL-4 and IL-5 interfere with the normal function of surfactant proteins SP-B and SP-C and the surface tension-lowering property of surfactant. In addition, ECP directly damages the epithelium, induces structural changes to surfactant vesicles, inhibits surfactant function in a concentration-dependent manner, and causes airway inflammation. Leakage of plasma proteins such as albumin and fibrinogen into airways aggravates inhibition of surface activity of the lung surfactant.

Although initiating molecular mechanisms of surfactant inactivation may differ in various forms of respiratory disorders, a common downstream event is fluid accumulation within the noncartilaginous distal lung units and instability of the luminal air-liquid interface. This scenario often results in the closure of airways and obstruction of airflow to the lung periphery and thus impedes the gas transfer process.

**Airway Closure Models**

Depending on the deformability of airway walls, two different modes of airway closure may take place. The first mode, known as the “meniscus occlusion,” involves formation of a liquid plug across the airway lumen without deformation of airway walls (Figure 2A). During inflation of the lung, which may be facilitated by normal breathing or mechanical ventilation, the pressure associated with the inspired air pushes the plug downstream into the airway. The plug leaves behind a trailing film as it propagates, becomes thinner, and eventually ruptures. The second type of airway closure is referred to as the “compliant collapse,” which is initiated by drainage of liquid into regions of larger film thickness within the airway lumen (Figure 2B). Due to the pressure difference across the airway wall, air-liquid interfaces of the opposite sides of the airway lumen come in contact and form a liquid bridge. This mode of airway closure involves large deformations of airway walls. Given that the trailing film is thicker than the precursor film, the plug propagates under the inspired air pressure and eventually ruptures. Thus, normal airflow is reestablished. Both modes are associated with the generation of fluid mechanical forces that will be discussed below.

**Distal Airways Obstruction**

Because of the challenging nature of studying distal airways, their role in various forms of respiratory failure has not been evaluated in great detail and less is understood about their importance in lung pathogenesis as compared with large airways. For instance, asthma was originally described...
as an inflammatory disease involving mainly central airways. However, pathological and physiological evidence from surgically resected lung tissues, transbronchial biopsies, and autopsy lung specimens indicated that the inflammatory process extends beyond the central airways to the peripheral airways and the lung parenchyma and more severe inflammatory and structural changes occur in the distal airways of asthmatic patients. Small airways have been recognized as a predominant site of airflow obstruction in asthmatic persons with more severe inflammation than in large airways. Furthermore, histological data from animal studies shows that tidal airway closure is detrimental to peripheral airways and causes the alveolo-airway attachments to rupture and denudes the epithelium. On the basis of available evidence, distal airways are evidently implicated in lung pathogeneses.

**Numerical Approaches**

To evaluate fluid mechanical forces exerted on airway epithelium during the airway closure and reopening process, computational methods have been employed to model propagation and rupture of liquid plugs in airways. These models enable systematic evaluation of the effect of various parameters such as fluid inertia and liquid plug length in channels pre-wetted with different liquid lining thicknesses both in the presence and absence of surfactant. These studies suggest that during propagation of the plug, abnormally large shear stress, pressure, and their gradients are exerted on airway walls both in the front and rear menisci of the liquid plug. In particular, when the plug length becomes sufficiently smaller than the airway diameter, much larger gradients of shear stress and pressure are developed at the front meniscus of the progressing plug. These forces that are amplified with fluid inertia were suggested as the primary cause of damage to airway epithelial cells. Including physiological levels of surfactant in the liquid abates the mechanical forces and may serve to protect the airway epithelium.

**Animal Models and Human Studies**

Much of the available information regarding the contribution of distal airways to various pulmonary disorders and the downstream effect of nonphysiologic flow conditions on distal lung units is largely due to animal studies. For example, it has been shown that repeated closure and reopening of distal airways of lungs for up to 4 hrs in normal rabbits results in an increase in flow resistance and desquamation of epithelial cells of terminal and respiratory bronchioles. Such investigations enhance understanding of distal lung pathogenesis and reveal a wealth of information from organ level (eg, lung function) to tissue and cell level (eg, cell morphology); nevertheless, care must be taken in correlating such data with human lung diseases. This is because no animal model replicates the pathogenesis of a disease observed in humans and, in fact, marked discrepancies between numerous successful studies in asthma animal models and very few clinical trials in patients reflects this notion. Consequently, drugs that show efficacy in animal models may be of little clinical benefit to humans.

Studies of lung pathology in human subjects rely on different techniques, including noninvasive high-resolution computed tomography (HRCT) and evaluation of dynamic compliance and hysteresis, to direct measurements through broncoscopic methods. These studies provide evidence about damage and remodeling of peripheral airways due to cyclic closure and reopening of the airways. Availability of human subjects, complications due to large variations of measured responses to similar stimuli from one subject to another, and ethical issues are a number of disadvantages of studies involving human subjects.

**Microengineered In Vitro Airway Models**

Given the complexity of in vivo studies of pulmonary diseases, development of reliable in vitro human airway models capable of mimicking characteristics of airways tissue in vivo is essential. Bilek and colleagues considered this approach and cultured rat pulmonary epithelial cells in a parallel-plate flow chamber that represented an idealized airway model (Figure 3). To mimic airway occlusion, the chamber was filled with a surfactant-deficient fluid. Reopening was generated by the progression of a semi-infinite air bubble through the chamber. It was shown that a significant number of cells die due to the propagation of only a single bubble. Replacing the fluid with
1 mg/mL Infasurf surfactant eliminated cell injury. Computational analysis of the reopening process showed that bubble propagation generates a complex cycle of mechanical forces including a steep pressure gradient near the bubble front (rear of liquid blob) in the vicinity of the channel walls. These abnormal mechanical stresses were suggested to compromise cellular viability.

Over the past decade, microfluidic systems have been used widely as platforms for the culture of mammalian cells to study various physiologic and pathologic conditions in vitro. The possibility of re-creating physiologic-like microenvironments where cells exhibit phenotypes found in their native tissue inside the body has made microfluidic cell-based settings increasingly popular. To enable in vitro modeling of the meniscus occlusion mode of airway closure and subsequent airway reopening, our group designed a compartmentalized microfluidic device that consists of 2 polymeric chambers made of polydimethylsiloxane (PDMS) separated by a porous membrane (Figures 4A and 4B). The top and bottom compartments correspond to the luminal and basal sides of airway epithelium, respectively, and were fabricated using soft lithography technique. The dimensions of microchannels, 300 μm × 100 μm, approximate the size of small airways. A thin polyester membrane containing pores of 400 nm is sandwiched between the upper and lower chambers and plays the role of a basement membrane to support the attachment and growth of cells. Primary human small airway epithelial cells (SAECs) were seeded into the upper chamber and allowed to attach to the membrane under static culture for 4 to 6 hrs. To promote cellular growth, both chambers were perfused with culture media using a syringe pump. After several days, a confluent monolayer of cells was formed with a cellular viability of ~90% (Figure 4C). In the next step, culture media was removed from the top chamber and the apical surface of the cells was exposed to air. To supply cells with nutrients, the lower chamber was perfused with culture media. Maintaining this air-liquid interface culture condition for about 3 weeks caused SAECs to differentiate. This was confirmed by detection of the Clara cell secretory 10-kDa protein (CC10), which showed increasing levels during the air-liquid interface culture, and expression of the tight junctional transmembrane protein occludin. Thus, an in vitro microengineered airway model was created that exhibits morphological and secretory phenotypes similar to those found in vivo.

A great advantage of microfluidic platforms is the possibility of integrating various components. To generate liquid plugs on-chip, the microfabricated airway system was integrated with...
a liquid plug generator that operates based on a two-phase flow configuration (Figure 5A and 5B). First, liquid is pumped at 10 mL/hr into the K-shaped top channel and is focused by a compressed air stream to form a stable stratified air-liquid flow (Figure 5B-1). Airflow rate is adjusted by a flow meter to 20 to 70 cm$^3$/min. Using a pinch valve to close tubing that connects the air source to the plug generator, the airflow is blocked for a very short period of time (≥30 min). This allows the liquid to spread and progress into the culture chamber (Figure 5B-2). The blockage time of the airflow is accurately controlled by a computer code. Recovery of airflow reestablishes the stratified flow and pinches off a liquid plug that propagates downstream the culture chamber under air pressure (Figure 5B-3). Size and propagation speed of liquid plugs is determined by liquid and air flow rates.

Figure 6 represents a typical response of the microfluidic airway to repeated propagation of liquid plugs. Green and red fluorescence represent live and dead cells stained with Calcein AM and ethidium homodimer (EthD-1) fluorescent dyes, respectively. Exposing SAECs to 10, 50, and 100 events of plug propagation caused significant damage to cells and reduced cellular viability to 61%, 44%, and 22%, respectively. Numerical simulation (see above) revealed the existence of large gradients of pressure and shear stress in the front meniscus of the plug where it converges to a precursor film on the airway wall. These stresses and their corresponding gradients at the front meniscus were much larger than those at the rear meniscus of the liquid plug as well as the stresses generated at the front tip of the air bubble (that resembles the rear meniscus of a liquid plug) experiment of ref. 28. Most
likely, abnormal mechanical forces disrupt the plasma membrane of cells beyond their ability to reseal and result in cell death. This finding implies that, in vivo, significant damage to airway epithelium due to repeated reopening of occluded airways beyond the potential of cell repair mechanisms may cause airway fibrosis and overall poor lung function.

**Conclusion**

In vitro models of normal and diseased human lung provide a promising direction that can enhance the current understanding of pulmonary disorders and complement studies involving animals and human subjects. Our integrated microfluidic platform enabled re-creation of human distal airways on a chip and generation of pathologic flow conditions that result in the obstruction of surfactant-deficient airways. This in vitro model provided physiologic culture conditions for primary human cells of small airways such that cells exhibited secretory and morphological phenotypes of the airway epithelium in vivo and formed an airway “tissue” whose integrity was preserved under abnormal mechanical forces. It was shown that significant cellular-level lung injury occurs due to the reopening of occluded airways. This versatile microtechnological platform can potentially facilitate systematic study of respiratory disorders in vitro, enable testing safety and efficacy of new pharmaceuticals and evaluating cellular-level responses, and can contribute to the design of strategies for treating and preventing lung injuries.

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