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Role of Airway Recruitment and Derecruitment in Lung Injury

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Abstract

The mechanical forces generated during the ventilation of patients with acute lung injury causes significant lung damage and inflammation. Low-volume ventilation protocols are commonly used to prevent stretch-related injury that occurs at high lung volumes. However, the cyclic closure and reopening of pulmonary airways at low lung volumes, i.e., derecruitment and recruitment, also causes significant lung damage and inflammation. In this review, we provide an overview of how biomedical engineering techniques are being used to elucidate the complex physiological and biomechanical mechanisms responsible for cellular injury during recruitment/derecruitment. We focus on the development of multiscale, multiphysics computational models of cell deformation and injury during airway reopening. These models, and the corresponding *in vitro* experiments, have been used to both elucidate the basic mechanisms responsible for recruitment/derecruitment injury and to develop alternative therapies that make the epithelium more resistant to injury. For example, models and experiments indicate that fluidization of the cytoskeleton is cytoprotective and that changes in cytoskeletal structure and cell mechanics can be used to mitigate the mechanotransduction of oscillatory pressure into inflammatory signaling. The continued application of biomedical engineering techniques to the problem of recruitment/derecruitment injury may therefore lead to novel and more effective therapies.

Keywords

microbubble flows; cell mechanics; surface tension forces; ventilation-induced lung injury; power law rheology; pulmonary mechanobiology

I. INTRODUCTION

A. Ventilation-Induced Lung Injury

The lung is a highly dynamic and complex structure that is responsible for the oxygenation of red blood cells in the circulatory system and the concurrent removal of carbon dioxide.¹ The complex bifurcating structure of the pulmonary airway tree is designed to maximize the surface area of the alveolar-capillary barrier and thereby facilitate diffusive gas exchange between the pulmonary alveoli and the pulmonary microvasculature. The alveolar-capillary barrier consists of a layer of microvascular endothelial cells contacting the blood, a basement membrane, and a layer of pulmonary epithelial cells contacting the airspace.

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Under normal conditions, the flat type I alveolar epithelial cells, which cover ~90% of the surface area, exist in a tight monolayer and therefore prevent fluid from entering pulmonary airspaces.² In addition, the cuboidal type II epithelial cells, which typically exist in alveolar corners,³ help maintain lung stability by secreting surfactant⁴ and by actively clearing edema fluid from pulmonary airspaces.⁴ However, during severe pulmonary infections and/or septic conditions, bacterial/viral toxins or other noxious stimulants cause significant injury and detachment of the type I epithelial cells, which in turn leads to flooding of the alveolar and airway spaces.² As a result, the acute phase of these conditions, i.e., acute lung injury (ALI), is characterized by the influx of a protein-rich edema fluid into the alveolar airspace due to an increased permeability of the alveolar-capillary membrane.⁵ In addition to airway/alveolar flooding, protein components in the plasma can deactivate surfactant,⁶ and this deactivation leads to the collapse of unstable airways and alveoli.⁷ The derecruitment of lung regions (i.e., airway and alveolar closure) leads to severe hypoxia, and as a result patients must be treated with mechanical ventilators in order to survive.

Acute respiratory distress syndrome (ARDS) was first documented in 1967,⁸ and mechanical ventilation has been the most common and effective method available to reduce mortality in patients with ALI/ARDS. The main goal of mechanical ventilation is to recruit (i.e., open) collapsed regions of the lung and thereby allow for adequate blood oxygenation. Although the primary objective of mechanical ventilation is blood oxygenation and CO₂ elimination, it is now well recognized that mechanical ventilators may also magnify the severity of lung injury by causing macroscopic air leaks and triggering inflammatory signaling cascades.^{9,10} The additional injury caused by the ventilator is known as ventilation-induced lung injury (VILI). Many therapies and strategies such as vascular-based therapies, prone position therapy, exogenous surfactant therapy, anti-inflammatory therapies, and antioxidant therapies have been utilized to try and prevent/minimize VILI, improve oxygenation, or accelerate the resolution of lung injury.^{11,12} However, none of these approaches have had a significant impact on mortality rates. In addition, although extracorporeal and intravascular membrane systems can be used to artificially oxygenate the blood, these devices can only be used as an adjunct to mechanical ventilation in patients with ALI.^{13,14} Since mortality rates for ALI/ARDS are very high (~30%) and since mechanical ventilation is required for survival in these patients, the development of improved ventilation protocols or other treatments for ALI is a major public health issue. As a result, there is an increasing need to better understand the pathophysiological, biomechanical, and immunological mechanisms responsible for VILI.

Historically, ALI patients were ventilated with relatively high tidal volumes ($V_t \sim 12$ mL/kg) since this ensured recruitment of a majority of lung regions and therefore provided adequate blood oxygenation. However, this high-volume ventilation protocol also exposes the epithelium to large stretching deformations that can cause significant additional damage to the epithelium.^{15–17} Large stretching deformations within the epithelium can cause plasma membrane disruption (i.e., cell necrosis),^{18,19} alterations in the cell's mechanical properties (i.e., cell rheology),^{20,21} disruption of the alveolar-capillary barrier,^{22,23} changes in gene expression,²⁴ the release of inflammatory proteins (i.e., cytokines),²⁵ and the secretion of surfactant molecules.²⁶ Based on these laboratory studies, several clinical trials^{26,27} utilized low-lung volume techniques to minimize the amount of stretch-induced ventilator-associated lung injury. In particular, the seminal ARDSNet study²⁷ clearly demonstrated that the use of low-tidal volume results in a lower mortality compared to higher volumes. However, several studies indicate that even low-volume techniques can result in significant lung damage.^{28,29}

B. Low-Volume Injury and Atelectrauma

During low-volume ventilation, dependent regions of the lung may “close” or become “derecruited” by two distinct but related mechanisms, namely, compliant collapse of small pulmonary airways and alveoli, or fluid occlusion of noncollapsed airways/alveoli.³⁰ There is currently direct and indirect evidence for both the compliant collapse mechanism and fluid-occlusion mechanism of airway/alveolar derecruitment. For example, CT data in patients with ARDS indicate that pulmonary edema in dependent regions leads to an increased lung weight locally, which in turn causes collapse of pulmonary airways/alveoli.³¹ Other investigators have used optical microscopy of the pleural surface of the lung to show that during surfactant deactivation, alveoli undergo different degrees of compliant collapse, and that inflection points on the pressure-volume curve are due to several factors in addition to alveolar recruitment and reexpansion.³² In contrast to the collapse mechanism, other investigators have used direct experimental data to support the fluid-occlusion mechanism. For example, Martynowicz et al.^{33,34} used a parenchymal marker technique, which directly monitors regional changes in lung volume and lobar expansion, to show that during oleic acid-induced lung injury, small pulmonary airway/alveoli do not collapse and do not experience cyclic collapse and reopening. In addition, subpleural imaging of alveoli with laser scanning confocal microscopy³⁵ indicate that alveoli in injured lungs become occluded with edema fluid rather than collapsing. Thus, the mechanisms of derecruitment may be a function of the type of injury (i.e., surfactant dysfunction versus acid-induced changes in alveolar-capillary barrier permeability). However, both mechanisms likely occur in clinical situations due to the multifactorial etiology of ALI.

The recruitment of collapsed or fluid-filled airways and alveoli will involve the movement of an air-liquid interface or microbubbles of air that displaces the surrounding fluid (see Fig. 1).³⁰ Specifically, recruitment may involve either the progression of an air bubble in a noncollapsed airway (Fig. 1A) or the oscillatory penetration of an air bubble into a fluid-filled alveolus (Fig. 1B). The movement of these air-liquid interfaces results in fluid flow and a complex, nonuniform stress field on the epithelial cells lining the airway/alveolar walls. These hydrodynamic stresses may include normal pressure stresses, tangential shear stresses, and spatial and temporal gradients in these stresses.^{36,37} As with the stretching deformations that occur during high-volume ventilation, the hydrodynamic forces generated during cyclic airway/alveolar recruitment/derecruitment at low lung volumes can also cause significant cellular injury. As described in Section II.B. below, these hydrodynamic forces can result in plasma membrane disruption and cell necrosis,^{36,38–40} disruption of membrane-cytoskeleton interactions,⁴¹ and alveolar-capillary barriers,^{39,40} as well as activation of inflammatory pathways.⁴² Although both large stretching deformation and air-liquid flows can cause physical injury to the alveolar barrier, Douville et al.⁴³ have recently used a novel microfluidic system to demonstrate that the stresses associated with air-liquid flows are more damaging to the epithelium than stretching deformation. Nonetheless, minimizing VILI likely requires the prevention of both atelectrauma during cyclic recruitment/derecruitment and volutrauma during lung overdistension.

Several investigators have attempted to use an “open-lung” strategy⁴⁴ to prevent cyclic recruitment/derecruitment, and therefore the associated atelec-trauma, in which one either specifies a positive endexpiratory pressure (PEEP) to prevent derecruitment or uses a recruitment maneuver to open up the lung. Theoretically, higher PEEP values would prevent airway/alveolar closure and would therefore prevent the cellular injury caused by cyclic recruitment and derecruitment. Although some studies indicate that higher values of PEEP may prevent lung injury,²⁶ more recent studies do not indicate that the use of high PEEP results in lower mortality.⁴⁵ In fact, studies in normal lungs indicate that high PEEP is actually associated with increased lung inflammation.⁴⁶ One factor that might contribute to lung injury during high-PEEP ventilation is the development of high transmural pressures.

These higher pressures might be sensed by the epithelium and transduced into inflammatory signaling and cytokine secretion.⁴² In addition to further lung damage, this mechanoinflammatory response may lead to systemic inflammation and multiple system organ failure, a common cause of death in ALI patients. Another reason why PEEP has not been shown to significantly reduce mortality is that the optimal PEEP for a given patient is often difficult to define.⁴⁷ Although investigators have proposed using the lower inflection point (LIP) on the pressure-volume curve to define PEEP, as stated above, the LIP is not a highly specific indicator of airway/alveolar recruitment^{32,48} and may have several other physical determinants such as elastic forces in the parenchyma and chest wall. In addition, Ward et al.⁴⁹ indicate that identifying clear inflection points in a clinical setting is difficult, and therefore the appropriate setting of PEEP is challenging. The challenges associated with setting appropriate PEEPs were demonstrated in the ARDSNet ALVEOLI trial⁴⁵ where the use of high PEEP did not significantly reduce mortality. Recently, Gattinoni et al.⁵⁰ demonstrated that the amount of recruitable lung is highly variable and depends on PEEP, and Talmor et al.⁵¹ have shown that customizing the level of PEEP based on esophageal pressure measurements (an indicator of transpulmonary pressure) leads to improved oxygenation and lung compliance. Therefore, large-scale clinical trials that explore the benefit of patient-specific specification of PEEP are warranted. However, to date only clinical trials focusing on lower tidal volumes have been successful in reducing mortality during VILI and ALI.²⁷

Although the settings necessary to reduce volutrauma are well established, it is not clear what the optimal settings are to prevent cellular injury due to cyclic recruitment/derecruitment (i.e., atelectrauma). Furthermore, it may not be possible in a clinical setting to establish ventilator settings that eliminate atelectasis and the injurious hydrodynamic forces generated during airway reopening (i.e., pressure and shear stress). One potential way to reduce the magnitude of the hydrodynamic forces generated during airway reopening is to reduce the air-liquid surface tension via the use of exogenous surfactant therapy. Unfortunately, surfactant replacement therapy has had limited clinical success,^{52,53} since the leak of plasma proteins into the airway/alveolar space often deactivates surfactant.⁵⁴ Instead of trying to reduce these forces via altered ventilator settings or surfactant therapy, our laboratory is actively pursuing alternative strategies to reduce the cellular injury associated with atelectrauma. In our approach, changes in the epithelial cell's cytoskeletal and biomechanical properties are used to make these cells less susceptible to the hydrodynamic forces associated with airway reopening and atelectrauma. As described in this paper, the pursuit of this strategy has been aided by the concurrent development of multiphysics computational models of cell mechanics/deformation during microbubble flows and *in vitro* experiments to both validate these models and test model predictions.

C. Cell Mechanics

Although the development of VILI may be due to a variety of mechanisms, most investigators have focused on volutrauma during high-tidal volume ventilation where distension of the basement membrane is translated into deformation of the attached epithelial cells. These stretching deformations can cause significant cellular injury, and the effects of stretching on cellular responses have been elegantly reviewed by previous authors.^{55,56} In particular, large stretching deformations can result in plasma membrane disruption, cell necrosis, and disruption the alveolar-capillary barrier. As noted above, the hydrodynamic forces associated with cyclic airway recruitment and derecruitment can also result in significant cellular necrosis and barrier disruption. However, interpreting the current literature on cell injury in this field requires one to address a fundamental difference in the mechanics of stretch-induced versus hydrodynamic force-induced injury. Specifically, *in vitro* stretching protocols are designed to impose a specific amount of strain (i.e.,

deformation) to the cells regardless of the cell's mechanical properties. As a result, changes in cellular mechanics and cytoskeletal structure cannot, by definition, affect the amount of cellular deformation under these conditions. It is important to note, however, that cytoskeletal alterations may influence other biological processes that contribute to cell necrosis. For example, Vlahakis et al.¹⁹ demonstrated that disruption of the cytoskeleton and cooling cells from 37°C to 4°C results in reduced lipid trafficking, which normally helps repair plasma membrane breaks. Interestingly, these authors report that disrupting the cytoskeleton leads to a “softer” cell, while cooling leads to an increase in cell stiffness. Although these results suggest that during stretching cell stiffness is not an important determinant of injury, it is important to remember that under these conditions the cell undergoes a predetermined amount of strain.

Unlike the direct specification of cell strain that occurs during cell-stretching experiments, the mechanics of cellular deformation and injury during recruitment and derecruitment is more complex since the amount of cell strain is not known a priori. Specifically during microbubble flows, the epithelial cells are exposed to different types of hydrodynamic forces. Under these conditions, cellular deformation depends on numerous factors, including the magnitude of the applied hydrodynamic forces (which can be changed by altering the bubble velocity, the air-liquid surface tension, or the airway diameter), cellular mechanical and/or rheology properties, and the cell's morphology. Clearly, increased hydrodynamic loads would be expected to cause more cell deformation and injury. However, as described by several previous studies,^{36,38,39} only increases in the applied pressure gradient have been shown to significantly influence injury and necrosis. Similarly, increases in cell stiffness, as reflected by the cell's elastic or Young Modulus, would be expected to result in less deformation and injury. However, recent studies from our lab indicate that while cell stiffness is not an important determinant of injury, changes in viscoelastic properties (i.e., fluid- versus solidlike characteristics) are critical in governing deformation and injury.⁴⁰ Finally, changes in cell morphology may alter the way hydrodynamic forces are distributed within the cell, and may therefore alter the amount of deformation that occurs. Given that cellular deformation is proportional to injury, we hypothesized that changes in cell mechanics might be an effective way to prevent damage during microbubble flows. As discussed in this paper, we have tested this hypothesis by developing computational models of cellular deformation during microbubble flows,^{2,57,58} and conducting *in vitro* experiments in which the cell's rheological properties are modified by altering cytoskeletal structure.^{40,42} Results indicate that changes in cell mechanics and cytoskeletal structure may indeed be an effective way to prevent injury during recruitment/derecruitment. We are currently exploring the translation of this information into clinically relevant pharmacological agents.

D. Mechanotransduction

Recently, it has become increasingly clear that biological cells are exquisitely sensitive to both internal and external mechanical forces, and that these mechanical forces can significantly alter biochemical signaling and cell behavior via a process known as mechanotransduction.^{56,59,60} For example, researchers have demonstrated that biomechanical forces play an important role in embryonic development,^{61,62} in branching morphogenesis,⁶³ and in stem cell differentiation.⁵⁹ In particular, Engler et al.⁵⁹ demonstrated that culturing human mesenchymal stem cells on a highly compliant matrix results in preferential differentiation into neurons, while culturing on rigid substrates results in differentiation into osteocytes. In the past decade, researchers have also begun to elucidate the mechanisms by which cells sense mechanical forces and transduce them into biochemical signals.⁶⁰ For example, it is well established that transmembrane integrin receptors are a key signaling site and act as mechanoreceptors. Integrins typically cluster at focal adhesions sites and in addition to anchoring the cell to the extracellular matrix (ECM),

these structures also play a key role in sensing internal and external forces and transducing these mechanical stimuli into biochemical signals. For example, force-induced activation of integrin receptors can influence a variety of biochemical pathways including the Rho-ROCK contractility pathway⁶⁴ and the NF- κ B inflammation pathway.⁶⁵ Since integrins at focal adhesions are linked with the cytoskeleton, stimulation of the contractility pathway can generate additional traction forces at these focal adhesions, which in turn can lead to additional biochemical signaling. This positive mechanical feedback plays an important role in generating the dynamic changes in cell mechanics required for cancer cell migration and invasion.⁶⁶

Mechanotransduction processes have also been shown to play an important role in lung physiology and pathophysiology. For example, previous investigators have demonstrated that cyclic stretching of lung epithelial cells results in alterations in protein and gene expression,⁶⁷ upregulation of inflammatory pathways, and the release of cytokines.²⁵ The reader is referred to an excellent recent review by Tschumperlin et al.⁵⁵ for more information about the diverse effects of lung distension and cyclic stretch on cell behavior. In addition to cyclic stretching, lung epithelial cells are subjected to other complex mechanical forces during ventilation, including high intrathoracic pressures with PEEP, and shear stresses and pressure gradients during microbubble flows.³⁰ In addition to the physical injury caused by microbubble flows, such as necrosis and barrier disruption, the mechanotransduction of hydrodynamic forces has also been demonstrated. For example, exposing respiratory epithelial cells to shear stresses can alter mucus secretion⁶⁸ and paracellular permeability,⁶⁹ while static pressures can alter early growth response-1 (EGR1) and transforming growth factor- β 1 (TGF β 1) gene expression.⁷⁰ The mechanotransduction of these forces into inflammatory activation and cytokine secretion may be particularly important in the context of VILI. As discussed in Section III, our lab has recently demonstrated that exposing alveolar epithelial cells to dynamic oscillatory pressures can activate the NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) transcription factor, which controls a variety of inflammatory pathways including those responsible for interleukin-6 and -8 (IL6 and IL8) cytokine/chemokine production.⁷¹ Given the need to control exacerbations of inflammation during mechanical ventilation, previous investigators have attempted to use different types of anti-inflammatory therapies to minimize VILI and acute lung injury.¹² For example, peroxisome proliferators activated receptor (PPAR) agonists have been shown to attenuate lipopolysaccharide (LPS)-induced lung inflammation in rats, and these agonists normally work by inhibiting transcription factors that regulate inflammation (i.e., NF- κ B).⁷² However agents with broad anti-inflammatory properties, such as corticosteroids, have been found to be either ineffective or even harmful during early acute lung injury.¹²

Since the actin cytoskeleton serves an important role in mechanotransduction and the transmission of externally applied forces to various signaling sites within the cell, we recently hypothesized that changes in cytoskeletal structure may be an effective way to mitigate mechanically induced inflammation in lung epithelial cells. For example, disruption of the cytoskeleton would in theory reduce the amount of force transmitted to signaling sites and would therefore reduce the amount of mechanically induced inflammation. In addition to force transmission, changes in cytoskeletal structure will also alter cellular mechanical properties such as stiffness and viscoelasticity.⁴⁰ As discussed above, changes in cell mechanics may significantly influence the amount of global cell deformation and local strain within the cell. These deformation patterns may play an important role in mechanically induced inflammation. As described in Section IV, our laboratory has recently demonstrated that disruption of the actin cytoskeleton can indeed mitigate mechanically induced inflammation.⁴² However, this effect is highly dependent on both the frequency of the applied oscillatory load and the viscous properties of the cells themselves. Based on this

data, we are currently exploring how clinically relevant pharmacological agents influence mechanically induced inflammation.

In this paper, we first provide an overview of the *in vivo* and *ex vivo* studies that have clearly demonstrated the injurious nature of cyclic airway recruitment/derecruitment. We then summarize several *in vitro* studies from our laboratory that have elucidated some of the biophysical and biostructural mechanisms responsible for both cell injury and activation of inflammatory pathways. We then provide a quick overview of how mathematical and computational modeling techniques have been applied to the recruitment/derecruitment problem. In addition to simulating the complex physics of cell deformation and injury during airway reopening, several of these computational models also make important predictions about how changes in cell mechanics can be used to prevent injury. We describe several experimental studies that confirm these predictions, and conclude with a description of how future studies will focus on translating these results into clinically relevant therapeutics.

II. INFLUENCE OF RECRUITMENT/DERECRUITMENT ON CELL INJURY AND INFLAMMATION

A. *In Vivo* Animal Studies

Several *in vivo* and *ex vivo* studies have demonstrated that cyclic recruitment and derecruitment of pulmonary alveoli can exacerbate lung injury and inflammation. Muscedere et al.⁷³ used an *ex vivo* model where surfactant inactivation initiates derecruitment, and the lung is then ventilated at low volumes and zero PEEP. Under these atelectatic conditions, morphological measurements indicated significant lung injury, which was due to recruitment/derecruitment, as opposed to overdistension of pulmonary alveoli. Similarly, other investigators have used *ex vivo* studies in rats to show that cyclic recruitment/derecruitment can cause increased histologic injury and release of pro-inflammatory cytokines.^{74,75} D'Angelo and colleagues^{28,76,77} have used a low-tidal volume, zero PEEP ventilation in normal rabbits to investigate the effect of cyclic recruitment/derecruitment on lung injury. In these studies, cyclic recruitment/derecruitment causes significant peripheral airway injury, epithelial cell necrosis, sloughing of epithelial cells, and the rupture of alveolar-bronchiolar attachments. Zero PEEP/low-tidal volume ventilation also caused inflammatory damage as evidenced by an increase in polymorphonuclear (PMN) leukocytes in alveolar septa. Additional studies by this group indicate that the amount of lung injury depends on inflation rates, where high inflation rates result in more PMN leukocytes than low inflation rates.⁷⁶ As expected, increasing the surface tension in these models resulted in both physical cell injury and an increased number of PMN leukocytes.⁷⁷ Interestingly, a recent *in vivo* study in open-chested rats⁷⁸ indicates that moderate airway closure due to zero PEEP does not cause significant cytokine release, while the use of negative PEEP or surfactant dysfunction along with low tidal volumes does result in significant cytokine release. Other studies⁷⁹ have demonstrated that mechanical ventilation with large VT or zero PEEP induced marked cytokine productions such as tumor necrosis factor alpha (TNF- α) and IL-6, which may contribute to the development of a systemic inflammatory response. Similarly, Cheng et al.⁸⁰ showed that negative end-expiratory pressure, even at low VT, can accentuate the cytokine response. This data suggests that pulmonary and systemic inflammation during ventilation is due to stress-related damage to pulmonary epithelia and endothelia.

B. *In Vitro* Models

Originally, the high shear stress generated between open and closed alveoli were thought to be the main factor responsible for cell injury during cyclic recruitment and derecruitment of

the lung.⁸¹ Several investigators have used microfluidic *in vitro* cell culture models to more precisely investigate how the complex mechanical forces exerted on epithelial cells during cyclic airway reopening influence injury and adhesion.^{36,38–41} As noted in Section I, tangential shear stresses and compressive pressure, as well as their corresponding spatial and temporal gradients, may influence cell injury during airway reopening. Bilek et al.³⁶ were the first to use *in vitro* systems to investigate the relative importance of these forces on epithelial cell necrosis. These authors used indirect correlations between computational fluid dynamic models of microbubble flows and experimental data to demonstrate that cell necrosis is primarily due to spatial gradients in pressure. In a follow-up study,³⁸ the Gaver group also demonstrated that the amount of cell necrosis does not depend on the duration of exposure to hydrodynamic stress. Although these authors focused on how changes in reopening velocities influence cell injury, changes in airway diameter and cell morphology may also influence cell injury by altering the magnitude of hydrodynamic forces generated during reopening. To investigate the relative importance of airway diameter and cell morphology, our laboratory designed an adjustable-height microfluidic system that exposes epithelial cells to well-defined microbubble flow conditions.³⁹ For these studies, a rat pulmonary epithelial cell line (CCL-149, ATCC) was cultured to either 100% confluency or 25% confluency, and then exposed to reopening conditions using a surfactant-deficient, high-surface tension fluid. Reopening conditions investigated include bubble velocities of 30, 3.0, and 0.3 mm/s and channel heights (which correspond to airway diameter) of 0.5, 0.8, and 1.7 mm. This system was therefore more representative of terminal airway structure and dynamics. Using this system, Yalcin et al.³⁹ demonstrated that both bubble velocity and channel height have significant effects on cell necrosis after only one reopening event. In line with the results from Bilek et al.,³⁶ decreasing the bubble velocity results in increased necrosis. In addition, smaller channel heights also lead to a significant increase in necrosis. This data indicates that dependent regions of the lung with smaller airway diameters and small reopening velocities are highly susceptible to injury during recruitment. Yalcin et al.³⁹ analyzed their data by plotting the percent cell necrosis as a function of three different hydrodynamic stresses, namely, pressure gradient, maximum shear stress, and shear stress gradient. For this analysis, stress magnitudes were calculated according to the computational models developed by Bilek et al.³⁶ and pertain to airway reopening in a flat 2D channel. Results indicate a very strong correlation between cell necrosis and the applied pressure gradient, a weak correlation between necrosis and shear stress gradients, and no correlation between necrosis and the maximum shear stress. In addition to confirming the pressure gradient mechanism of cell injury, these data indicate that spatial gradients in stress are the primary determinants of cell injury. Finally, Yalcin et al.³⁹ also used the microfluidic system to compare the effect of a single airway reopening event versus the effect of multiple reopening events. Interestingly, the initial recruitment event did not cause significant detachment of confluent, type I epithelial cells, while five sequential recruitment/derecruitment maneuvers resulted in significant detachment. Multiple reopening events also resulted in progressive increases in necrosis that plateaus after five reopening events.

During ALI, sloughing of type I epithelial cells results in a subconfluent monolayer of primarily type II epithelial cells.² In addition to the loss of cell-cell contacts, the differences in type I versus type II morphology may make subconfluent monolayers more susceptible to microbubble-induced injury. Type II cells exhibit a rounded or cuboidal morphology, while type I cells exhibit a flatter, cobblestone morphology. Previous computational models⁸² indicate that cells with large height-to-width ratios may result in amplification of the hydrodynamic stresses generated by the microbubble. As a result, it is logical to hypothesize that subconfluent monolayers of type II cells, which may have larger aspect ratios, are more susceptible to injury than confluent monolayers of type I cells. Our laboratory tested this hypothesis by culturing rat epithelial cells to 100% and 25% confluency, and exposing these cells to different reopening conditions.³⁹ This study clearly indicates that for all reopening

velocities, subconfluent cells experience more cell necrosis than confluent cells. In addition, one reopening event causes significant detachment of subconfluent cells compared to minimal detachment in the confluent monolayer.³⁹ Interestingly, although confocal microscopy clearly documented changes in morphology between subconfluent and confluent cells consistent with type II versus type I morphology, analysis of these data indicate that the subconfluent and confluent cells exhibited similar height-to-width ratios that were not statistically different. Therefore, amplification of hydrodynamic forces did not occur in this system and does not explain the increased injury in subconfluent cells. As discussed in Section III, computational models of cell deformation during microbubble flows indicate that the rounded morphology of type II cells alters the way apically applied forces are distributed within the cells, leading to high localized membrane strains. These higher strains would lead to more membrane rupture and thus cell necrosis. As a result, changes in stress distributions rather than changes in hydrodynamic force magnitude are responsible for increased necrosis in type II cells. Since type II cells represent an important repair source and govern fluid transport,⁴ damage to such cells during airway reopening may be an important cause of lung injury during low-volume mechanical ventilation.

In addition to physical injury, the hydrodynamic forces generated during cyclic recruitment/derecruitment may be sensed and transduced by the epithelial cells into inflammatory signaling. Although the inflammatory response of epithelial cells to infectious agents⁸³ and stretching deformations⁵⁶ are well documented, the inflammatory response of the epithelium to the forces generated during airway reopening are not well established. A pilot study in our laboratory demonstrated that exposure of alveolar epithelial cells to 5 dyn/cm² shear stress for 1 h resulted in the nuclear translocation of a primary transcription factor, NF- κ B, that controls various pro-inflammatory genes.³⁰ Although this study used immunofluorescent techniques to track NF- κ B location, we have also conducted a more thorough investigation of how static and oscillatory pressures activate NF- κ B.⁴² For these studies, human alveolar epithelial cells (A549) were exposed to either static pressure for 1 h or oscillatory pressure for .5 h. ELISA techniques were used to quantify NF- κ B activation and binding to DNA. For the oscillatory studies, a triangular pressure waveform was used with peak amplitudes of 10, 12, and 14 cm H₂O and frequencies of 0.125 and 0.18 Hz. As shown in Fig. 2A, oscillatory pressures activated NF- κ B in a magnitude- and frequency-dependent fashion. It is interesting to note that maximum NF- κ B activation occurs at 12 cm H₂O, possibly reflecting the viscoelastic nature of the epithelial cells, since it is possible to have less deformation at higher loading rates even though the pressure magnitude is higher.^{42,84} Less deformation when the rate of pressure loading is larger might result in lower levels of NF- κ B activation. In this study, we also used Western blotting techniques to demonstrate that static pressures activate NF- κ B via the canonical pathway that involves degradation of the protein I κ B α , which normally inhibits NF- κ B activation and nuclear translocation. As shown in Fig. 2B, we have also investigated the role of calcium signaling during mechanically induced inflammation and pressure activation of NF- κ B. Although previous studies indicate that stretch-induced activation of inflammation is dependent on the influx of extracellular calcium,⁶⁷ we found that treatment with the extracellular calcium chelator, EGTA, had no effect on NF- κ B activation (see Fig. 2B). Conversely, treatment with the intracellular calcium chelator, BAPTA-AM, resulted in a complete block of pressure-induced inflammation and NF- κ B activation. The implication is that pressure loading does not open or activate ion channels in the plasma membrane, but rather stimulates release of calcium from intracellular stores such as the smooth endoplasmic reticulum. As such, blocking ion channels in the plasma membrane may not be effective in mitigating pressure-induced inflammation. As described in Section IV, we have also conducted studies to investigate what types of changes in the cytoskeleton may be useful in mitigating pressure-induced inflammation and biotrauma during ventilation.

III. MATHEMATICAL MODELS OF RECRUITMENT/DERECRUITMENT

A. Phenomenological

As described above, the process of airway/alveolar recruitment and derecruitment is very complex. In addition to the complex physical factors that govern airway reopening, such as surface tension, parenchymal tethering, airway geometry, and surfactant transport dynamics, the heterogeneous nature of the lung makes it difficult to model the biophysical aspects of recruitment/derecruitment on the whole-lung scale. However, Bates and colleagues^{85–88} have developed several phenomenological models of recruitment/derecruitment to analyze and interpret experimental data during ALI. These models typically use parameters that describe the opened or closed status of airways and alveoli stochastically. These parameters depend on several factors including the local airway pressure and time. More recently, this group has enhanced the model to account for the effects of airway tree branching on recruitment, derecruitment, and inflation history.⁸⁸ However, it is difficult to link parameter values in these phenomenological models to specific biophysical factors that represent treatment targets.

B. Biophysical Models

Gaver and colleagues^{82,89–91} have pioneered the use of computational biomechanical models to understand the complex biophysics of airway opening and closing. This group used boundary element techniques to simulate the propagation of an air bubble in a fluid-filled channel. In addition to precisely modeling the physical mechanisms of airway reopening, these models provide detailed information about several parameters that are directly related to cellular injury, such as hydrodynamic stress values at the airway wall. For example, these models have been used to describe how hydrodynamic stresses vary as a function of reopening speed and wall topography.^{82,90} Krueger et al.⁹² developed models that account for surfactant transport during alveolar recruitment, and Ghadiali et al.^{93,94} developed models that account for the complex physicochemical interactions that influence the mechanics of airway reopening. Heil et al.^{95,96} have developed finite element models of airway reopening in collapsible vessels, while Naire et al.⁹⁷ have accounted for the unsteady nature of airway reopening. Finally, Grotberg and colleagues have developed several models to account for liquid plug formation, motion, and rupture.^{98–101} Although these biophysical models have significantly advanced our understanding of the fluid mechanics of airway recruitment/derecruitment, they do not directly simulate cellular deformation during reopening, and can therefore only infer potential injury mechanisms. Since the injury response of epithelial cells is clearly related to the amount of deformation, our laboratory has developed several unique and sophisticated models of cellular deformation during airway reopening that account for complex morphological, biomechanical, and fluid-structure interactions.

C. Cellular Models

As described above, experimental data from our lab³⁹ indicates that subconfluent monolayers of epithelial cells experience more plasma membrane rupture and cell necrosis than confluent monolayers. This study also demonstrated that subconfluent and confluent cells exhibit similar aspect ratios and therefore experience similar hydrodynamic stress magnitudes during airway reopening. We therefore hypothesized that the rounded morphology of subconfluent type II cells alters the way apically applied forces are distributed within the cell. This altered distribution could in turn lead to more strain (and rupture) in the plasma membrane. Since it is difficult to measure small strains (i.e., <10%) at the cellular level during rapid airway reopening, we have developed image-based finite element models of cell deformation that account for cell-specific morphology.⁵⁸ As shown in Fig. 3A, subconfluent and confluent monolayers of epithelial cells were treated with a

cytoplasmic fluorescent stain and imaged using a laser scanning confocal microscope (LSCM) (Zeiss). A computer aided design software package (Rhino-3D) was then used to define cross-sectional boundary curves (Fig. 3B), which define the cell's apical surface. These curves were then used to generate 3D solid models of the epithelial monolayer (Fig. 3C). The solid models for individual cells were then meshed and analyzed with a finite element software package (ADINA) to calculate maximum cell deformation during reopening. For these static simulations, the maximum hydrodynamic stress values for pressure gradient and shear stress formulated by Bilek et al.³⁶ were applied simultaneously to the apical surface of the cell. As shown in Fig. 3D, for equivalent hydrodynamic loads, the subconfluent cells, which are taller and rounder than the confluent cells, always exhibit more strain in the plasma membrane (red area in lower panel). As described in Dailey et al.,⁵⁸ a total of eight cells in each group (confluent and subconfluent) were analyzed assuming a variety of material properties for the cell cytoplasm and the cortex/membrane region. Even when material parameters were selected to maximize strain/deformation in the confluent cells and minimize deformation in the subconfluent cells, the subconfluent cells still experienced more membrane strain and deformation. These cell-specific computational models therefore confirmed our hypothesis that stress distribution within the cells is highly dependent on cell morphology, and that rounder/taller cells are more susceptible to microbubble-induced injury. Nonetheless, these models did not account for the transient nature of airway reopening. Thus, we have also developed more sophisticated fluid-structure interaction models to account for time-dependent cell mechanics.⁵⁷

Although the transient hydrodynamic stress field during airway reopening can be readily obtained from boundary element simulations,^{89,94} modeling the transient deformation response of cells to such stresses requires specification of an appropriate constitutive viscoelastic model. It is now clear that cells exhibit complex nonlinear rheology in which the storage/elastic modulus follows power law behavior as a function of frequency.^{84,102–104} Such rheology implies that deformation is not governed by a single intrinsic time constant, but rather can occur on many time scales. Models that account for large ranges of time constants might therefore be more appropriate. In a recent study,⁵⁷ our laboratory modeled the deformation response of epithelial cells to airway reopening stress using both a standard one-time constant Maxwell material model and a power law material model. For this study, imaged-based cell-specific models for confluent cells were utilized and a power law material model was formulated using a Prony-Dirichlet series. The resulting complex modulus G^* has the following form, where α is the slope of G^* versus frequency on a log-log scale, and $\alpha = 0$ represents a purely elastic material, while increases in α represent a more fluidlike material (i.e., fluidization):

$$|G^*(\omega)| = G_0 \left(\frac{\omega}{\omega_0} \right)^\alpha \quad (1)$$

Here, G_0 is an arbitrarily chosen reference value at the frequency ω_0 .

In addition to capturing transient deformations during microbubble flows as shown in Fig. 4B, correlation of these models with experimental observations has highlighted the importance of including power law rheology in these simulations. Specifically, our lab and others^{36,39} have demonstrated that the amount of cell necrosis during airway reopening is dependent on the applied pressure gradient. We investigated if the Maxwell and/or power law rheology model simulations are consistent with this observation by plotting the maximum membrane strain (a marker of cell necrosis) obtained during reopening as a function of the pressure gradient applied during different simulations. Note that for these simulations, the pressure gradient was modified by changing both the bubble velocity and

the channel height, and this correlation is therefore analogous to the correlation performed by Yalcin et al.³⁹ As shown in Fig. 4C, when a Maxwell model with time constant of 1 s was used, only a weak correlation was observed ($r^2 = 0.22$). In contrast, when a power law model with $\alpha = 0.2$ is implemented (Fig. 4D), we obtain a very strong correlation between maximum strain and pressure gradient ($r^2 = 0.93$). Previous experimental studies³⁸ indicate that cell injury is not a function of exposure time. The study by Dailey et al.⁵⁷ also demonstrated that only power law models capture this insensitivity of cell injury to exposure time. These results indicate that the intrinsic power law rheology of epithelial cells is the reason they are susceptible to pressure gradient-induced injury. In addition, the computational models predicted that changes in power law rheology, such as increasing the slope parameter α (fluidization), should have a cytoprotective effect. As described below, we have recently conducted experimental studies⁴⁰ validating this prediction. Thus, the development of complex, integrative mathematical models accounting for biophysical, cell-specific morphology and complex cell rheology/mechanics has led to important predictions about how to prevent cell injury during cyclic recruitment/derecruitment.

IV. IMPORTANCE OF TIME SCALES IN RECRUITMENT/DERECRUITMENT INJURY

A. Alveolar Recruitment

The importance of time scales on recruitment dynamics has been recently highlighted by an interesting study by Albert et al.⁸⁵ These authors used *in situ* microscopy to measure the dynamics of both recruitment of individual alveoli and the recruitment of larger lung regions. This study clearly demonstrated that although recruitment does not happen instantaneously, most of the gross recruitment occurs within the first 2 s of recruitment. The authors then correlated their experimental observations with a mathematical model, and results indicate that opening pressures in the lung may not follow a Gaussian distribution. These wide distributions in opening pressures may have important clinical implications, since they may complicate the setting of PEEP or the timing of a recruitment maneuver. Given the complex viscoelastic properties of lung tissue strips and pulmonary epithelial cells, it is interesting to note that time scales appear to play an important role at multiple scales, including cellular, tissue, and whole-organ levels. The development of advanced multiscale models that link temporal dynamics across these multiple scales may significantly aid in our understanding of recruitment/derecruitment dynamics. Furthermore, it is important to note that the time scale of recruitment (~ 2 s), which has also been measured noninvasively using computer tomography,¹⁰⁵ may be a function of the degree and type of injury. Specifically, Albert et al.⁸⁵ demonstrated that lung injury leads to changes in both recruitment and derecruitment dynamics. Therefore, assessment of recruitment dynamics on a patient-specific basis may be important for determining optimal PEEP and recruitment maneuvers.⁴⁷ In addition to the temporal dynamics of recruitment, the temporal dynamics of derecruitment have been modeled by Ma and Bates.⁸⁸ Their model suggests that although recruitment normally increases the fraction of open lung, it may also lead to larger derecruitment after the recruitment maneuver. This increased derecruitment may have biophysical origins related to surfactant dysfunction, Rayleigh-Taylor instabilities, and liquid plug formation. Therefore, it may be useful to develop more advanced biophysical models of the derecruitment process that account for these mechanisms.

B. Cell Fluidization and Mechanotransduction

Although a better understanding of recruitment and derecruitment dynamics might aid in establishing optimal ventilator settings, our group has been investigating an alternative mechanism of lung injury prevention that involves the alteration of time scales at the cellular level. The rationale for this approach is that even if optimal PEEP and recruitment

maneuvers are identified, it may be difficult to completely eliminate the mechanical forces that cause cellular injury and the activation of inflammatory pathways. For example, the heterogeneous distribution of opening pressures within the lung⁸⁵ will result in overexpansion of some lung regions (i.e., baby lung) and cyclic collapse and reopening of other regions. Thus, the heterogeneous nature of lung injury and inflation/deflation dynamics may make the generation of injurious mechanical forces inevitable. Rather than trying to eliminate all injurious mechanical forces, our laboratory is interested in altering the cellular response to these mechanical forces (i.e., the mechanotransduction response), and is particularly interested in using changes in cytoskeletal structure and cell mechanics to achieve this objective.

As described in Section III, our computational models of cell deformation during airway reopening⁵⁷ indicate that the complex time scale-invariant properties of lung epithelial cells might be responsible for their susceptibility to pressure gradient forces. These models also clearly indicate that “fluidization” of the cell, i.e., an increase in the power law exponent α , might be cytoprotective. As shown in Fig. 5, we have recently used *in vitro* experimental techniques to confirm this prediction.⁴⁰ For this study, confluent monolayers of epithelial cells were treated with the cytoskeleton disrupting agent Latrunculin and exposed to cyclic airway reopening conditions. An optical tweezer microrheometer⁸⁴ was used to measure the power law rheology under different treatment conditions. Latrunculin caused widespread disruption of the actin cytoskeleton and, as shown in Fig. 5B, these cells were softer and more fluidlike (increase in power law slope) compared to untreated cells. These “fluidized” cells experienced less cell necrosis after both one and five reopening events (Fig. 5A), thus confirming our computational predictions. Experiments were also conducted at different temperatures, and cells held at 37°C were softer and more fluidlike than cells held at 23°C. Cells at 37°C also experienced significantly less necrosis during cyclic airway reopening, further confirming the protective role of fluidization.

In addition to reducing the amount of physical injury, it may be even more important to reduce mechanically induced inflammation during ventilation. Although inhibiting specific inflammatory signaling pathways is possible, the precise signaling mechanisms responsible for the mechanotransduction of pressure and shear stress into inflammatory signaling are not known. Given the important role of the actin cytoskeleton in mechanosensing, force transmission, and mechanotransduction, we hypothesized that changes in cytoskeletal structure may be useful in mitigating the transduction of pressure into inflammatory signaling.⁴² As shown in Fig. 2A, the application of ± 12 cm H₂O pressure at a frequency of 0.18 Hz, i.e., breathing frequency, causes activation of NF- κ B. In addition, the application of ± 14 cm H₂O of static pressure also caused significant activation of NF- κ B. To investigate the role of the actin cytoskeleton, these experimental conditions were repeated in cells that were either treated with Latrunculin A, which disrupted the cytoskeleton, or Jasplakinolide, which caused actin redistribution toward the perinuclear region. Importantly, treatment with these cytoskeletal agents alone (i.e., no mechanical stimulation) either caused a minor decrease (Latrunculin) or a significant increase (Jasplakinolide) in NF- κ B activation. These data indicate that NF- κ B activation may depend on the prestress within the cell. However, confirmation of this hypothesis would require direct measurement of prestress via techniques such as traction force microscopy and finite element analysis.¹⁰⁶ The amount of NF- κ B activation in the Latrunculin- and Japlakinolide-treated cells were also measured after exposure to both static (Fig. 6A) and oscillatory (Fig. 6B) loading conditions. In order to identify how changes in cytoskeletal structure influence the mechanotransduction of pressure into NF- κ B activation, we normalized our data with the amount of activation in an unloaded but drug-treated control sample. Although we initially expected that disruption of the actin cytoskeleton in Latrunculin-treated cells would mitigate mechanotransduction processes, we found that under static loading conditions, Latrunculin-treated cells actually

exhibited increased NF- κ B activation in response to pressure. Conversely, Jasplakinolide-treated cells exhibited slightly less NF- κ B activation during static loading. Although Latrunculin cells are more fluidlike than untreated cells (i.e., higher slope in Fig. 5B), these cells are also “softer.” Therefore, under static loading, Latrunculin-treated cells would actually deform more than untreated cells. Similarly, the “stiffer” Jasplakinolide-treated cells would deform less. This data supports the hypothesis that the amount of mechanotransduction is directly related to the amount of cell deformation.

To further investigate this hypothesis, Latrunculin- and Jasplakinolide-treated cells were also exposed to oscillatory pressure conditions. As shown in Fig. 6B, both the Latrunculin- and Jasplakinolide-treated cells did not exhibit any statistically significant change in normalized NF- κ B activation compared to unloaded controls. As noted in Fig. 5B, the Latrunculin-treated cells are more viscous or fluidlike, and would therefore deform slower in response to an applied oscillatory load. At sufficiently high loading frequencies, these viscous cells do not have enough time to deform. A reduced deformation of viscous cells under oscillatory loading would be consistent with the observed reduction in mechanotransduction for Latrunculin-treated cells. We note that data from Huang et al.⁴² indicate that at 0.125 Hz, Latrunculin-treated cells exhibit increased NF- κ B activation compared to unloaded controls. Therefore, at this lower frequency, the Latrunculin cells apparently have enough time to deform, and their reduced stiffness allows them to undergo more deformation and more mechanotransduction. The limitation of these studies is that cell deformation was never directly or indirectly assessed. Future studies that quantify cell deformation under these conditions using experimental or computational approaches are warranted. Nonetheless, the study by Huang et al.⁴² clearly indicates that changes in the actin cytoskeleton can be used to alter the mechanotransduction of pressure into inflammatory signaling. However, the effectiveness of this approach is highly dependent on the type of cytoskeletal change, the time scales associated with the cell's mechanical properties, and the time scales of the mechanical loading conditions.

V. IMPLICATIONS AND CONCLUSIONS

We have provided a review of the complex issues surrounding the mechanisms of recruitment and derecruitment in injured lungs during mechanical ventilation. It is clear that cyclic recruitment/derecruitment can cause significant physical injury and inflammatory exacerbation, and is a significant cause of ventilation-induced lung injury (VILI). In addition to animal studies, biomedical engineers have applied their skills in microfluidic devices, mathematical and computational modeling, and cell mechanics to elucidate some of the important mechanisms of recruitment/derecruitment injury. In particular, phenomenological models of recruitment/derecruitment have provided important information about what ventilation settings might minimize atelectasis. In addition, computational models from our lab have also provided important information about how changes in cell mechanics and cytoskeletal structure may be used to make the epithelium more resistant to the mechanical forces generated during cyclic airway reopening. Importantly, many of the computational predictions about the types of cytoskeletal changes that would be cytoprotective have been experimentally validated *in vitro* using gross cytoskeletal agents such as Latrunculin. The clinical translation of this basic science knowledge will require the use of more discriminating pharmacological agents. For example, the common pharmaceutical Simvastatin has been shown to have effects on the cytoskeleton such as the attenuation of actin stress fibers¹⁰⁷ that, according to data from our lab, would be cytoprotective. As a result, investigating the effectiveness of Simvastatin on recruitment/derecruitment injury is warranted. In addition, although changes in cytoskeletal structure to prevent cellular injury and the mechanical activation of inflammatory pathways seems promising, ALI and ventilation-induced lung injury are complex diseases that will likely require more than just

injury prevention. For example, the cell migration processes that govern epithelial repair are critical to restoring normal lung function. In addition to insufficient wound healing, inappropriate repair or transitions from epithelial to mesenchymal phenotypes may exacerbate the fibrotic conditions that can occur in the later stages of VILI.¹⁰⁸ The amount of cell migration and wound repair has also been shown to depend on the cells' biomechanical properties.^{109,110} Future studies that use computational modeling and experimental techniques to understand how changes in cellular biomechanics promote wound repair are therefore warranted. Such information may lead to novel treatments strategies that use different pharmacological agents during the various stages of VILI to either prevent additional injury or promote appropriate repair of the lung.

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FIGURE 1.

Schematic diagram of recruitment dynamics and air-liquid motion in pulmonary airways (A) and alveoli (B); in both cases, the movement of air-liquid interfaces results in the application of complex hydrodynamic forces on the epithelial cells lining airway/alveolar walls.

FIGURE 2.

(A) Effect of oscillatory pressure on NF- κ B activation at different frequencies and pressure magnitudes, where * indicates statistically significant differences with unloaded controls ($p < 0.01$) and ^ indicates statistically significant differences ($p < 0.05$) between the ± 12 cmH₂O and ± 10 or ± 14 cm H₂O pressure levels; (B) influence of intracellular (BAPTA-AM) and extracellular (EGTA) calcium chealators on pressure induced NF- κ B activation, where * indicates statistically significant differences with unloaded controls ($p < 0.01$) and ^ represents statistically significant differences with activation in the no treatment group ($p < 0.01$); all data are mean $\pm 95\%$ confidence interval (reproduced from Huang et al.,⁴² *Cell Mol Bioeng*, 2010, Springer, used with permission).

FIGURE 3.

(A) Confocal microscopy of confluent (top) and subconfluent cells (bottom) alveolar epithelial cells; (B) 3D reconstruction of a representative cell [red circle in (A)] using cross-sectional boundary curves that define the cell's apical surface; (C) surface map of cell height in the monolayer; (D) finite element models showing solutions for effective strain in the membrane given equivalent loading conditions; results indicate that subconfluent cells developed higher strains than confluent cells (reproduced from Dailey et al.,⁵⁸*J Appl Physiol*, 2009, Am Physiol Soc, used with permission).

FIGURE 4.

(A) Transient dynamic pressures applied to the epithelium during microbubble flows as a function of dimensionless velocity (Ca); (B) effective membrane strains in a representative cells at locations 1, 2, and 3 during transient microbubble flows; correlation of normalized membrane strain with the applied pressure gradient in cells models with a Maxwell (C) and power law (D) material model; only the power law models capture the strong correlation observed experimentally (reproduced from Dailey et al.,⁵⁷*Biomech Mechanobiol Bioeng*, 2010, Springer, used with permission).

FIGURE 5.

(A) Effect of Jasplakinolide and Latrunculin treatment on amount of cell necrosis in adhered cells following one or five reopening events; data are means $\pm$ SE, and * represents statistically significant differences compared with no treatment ($p < 0.05$); (B) optical tweezer measurements of the shear modulus as a function of oscillation frequency under no treatment, Jasplakinolide, and Latrunculin treatment; data indicates that Latrunculin cells are softer and more fluidlike (reproduced from Yalcin et al.,⁴⁰*Am J Physiol Lung Cell Mol Physiol*, 2009. Am Physiol Soc, used with permission).

FIGURE 6.

(A) Effect of static pressure and cytoskeletal agents on NF- κ B activation; (B) effect of cytoskeletal agents on NF- κ B activation at an oscillation frequency of 0.18 Hz; all data are mean $\pm$ 95% confidence interval and represent fold change in activation with respect to unloaded controls in each treatment group; * indicates statistically significant differences with unloaded controls ($p < 0.05$) ^ represents statistically significant differences with activation in the no treatment group ($p < 0.05$) (reproduced from Huang et al.,⁴²*Cell Mol Bioeng*, 2010, Springer, used with permission).