Image-based finite element modeling of alveolar epithelial cell injury during airway reopening

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Dailey HL, Ricles LM, Yalcin HC, Ghadiali SN. Image-based finite element modeling of alveolar epithelial cell injury during airway reopening. J Appl Physiol 106: 221–232, 2009. First published November 13, 2008; doi:10.1152/japplphysiol.90688.2008.—The acute respiratory distress syndrome (ARDS) is characterized by fluid accumulation in small pulmonary airways. The reopening of these fluid-filled airways involves the propagation of an air-liquid interface that exerts injurious hydrodynamic stresses on the epithelial cells (EpC) lining the airway walls. Previous experimental studies have demonstrated that these hydrodynamic stresses may cause rupture of the plasma membrane (i.e., cell necrosis) and have postulated that cell morphology plays a role in cell death. However, direct experimental measurement of stress and strain within the cell is intractable, and limited data are available on the mechanical response (i.e., deformation) of the epithelium during airway reopening. The goal of this study is to use image-based finite element models of cell deformation during airway reopening to investigate how cell morphology and mechanics influence the risk of cell injury/necrosis. Confocal microscopy images of EpC in subconfluent and confluent monolayers were used to generate morphologically accurate three-dimensional finite element models. Hydrodynamic stresses on the cells were calculated from boundary element solutions of bubble propagation in a fluid-filled parallel-plate flow channel. Results indicate that for equivalent cell mechanical properties and hydrodynamic load conditions, subconfluent cells develop higher membrane strains than confluent cells. Strain magnitudes were also found to decrease with increasing stiffness of the cell and membrane/cortex region but were most sensitive to changes in the cell’s interior stiffness. These models may be useful in identifying pharmacological treatments that mitigate cell injury during airway reopening by altering specific biomechanical properties of the EpC.

Flow-induced cell injury; epithelial cell mechanics; orthotropic membrane; ADINA

CERTAIN PATHOLOGICAL CONDITIONS such as pneumonia and sepsis can lead to the development of the acute respiratory distress syndrome (ARDS), which is the most severe manifestation of acute lung injury (ALI). This condition is characterized by increased permeability of the alveolar-capillary barrier leading to fluid accumulation in the distal airways (43). In many cases, mechanical ventilation is necessary to stabilize patients with ARDS. However, ventilators can generate injurious mechanical forces that exacerbate the existing lung trauma. These mechanical forces, which are typically applied to lung epithelial cells (EpC), can cause cell necrosis (6, 46), further barrier disruption (9, 10, 46), and upregulation of inflammatory pathways (36). These mechanical and biological responses lead to additional lung injury known as ventilator-associated lung injury (VALI) (12). Although recent advances in ventilation protocols, including low tidal volume (37) and high positive end-expiratory pressure (PEEP) (17), have decreased the iatrogenic effects of mechanical ventilation, mortality rates for ARDS/VALI patients remain high, with estimates ranging from 25 to 50% (12, 42, 43).

Traditional clinical perspectives on the management of ARDS and VALI involve controlling organ-level parameters such as the ventilation tidal volume and pressure (36) or molecule-level treatments such as surfactant therapy (43). However, the symptoms of ARDS and VALI originate in cell-level responses to mechanical loads including stress failure of the epithelial cell membranes (42) and cell detachment from the basement membrane (46), as well as a variety of mechanotransduction processes (12). Inflammation and disruption of the epithelium leads to alveolar flooding, which mediates additional lung injury through two principal mechanisms: overdystension of the remaining aerated alveoli (i.e., stretch-induced injury or volutrauma) and the reopening of fluid-filled airways via bubble propagation (i.e., flow-induced injury or atelectrauma) (42).

Flow-induced injury, or cell damage induced by moving air-liquid interfaces, has been implicated in the pathogenesis of ARDS/VALI. Several investigators have presented experimental and theoretical models of airway reopening via bubble propagation through fluid-occluded channels. Theoretical investigations have explored mechanical and hydrodynamic coupling during bubble propagation through flexible airways (18, 33, 34), with some results suggesting that surfactant administration may reduce the cellular strains during reopening of a collapsed airway. Unfortunately, surfactant administration may have limited clinical applicability because of surfactant deactivation in the presence of plasma proteins (8). Experimental studies in rigid-walled channels have largely focused on the link between epithelial cell injury and the various components of the hydrodynamic stress field induced by the moving air-liquid interface. For example, Bilek et al. (6) and Kay et al. (26) used computational and experimental techniques to demonstrate a correlation between cell damage and the large pressure gradient near the bubble tip during airway reopening. Jacob and Gaver (22) investigated the effect of cell morphology using a simplified computational model of the epithelium (i.e., a 2-dimensional corrugated channel wall) and showed that cells with different heights but similar height-to-width aspect ratios will experience nearly identical peak stresses during...
bubble passage. If cellular injury/necrosis were determined only by the magnitude of the applied hydrodynamic loads, one would expect cells with similar aspect ratios to exhibit similar death and injury rates under experimental conditions.

To test this hypothesis, Yalcin et al. (46) conducted airway reopening experiments on CCL-149 rat EpC cultured to obtain low- (25%) and high-confluence (100%) monolayers. They used confocal microscopy to characterize the morphology of these EpC by measuring cell height, length, and width and found that subconfluent cells were taller but that both cell types had similar aspect ratios. After bubble passage, the subconfluent cells exhibited significantly higher death and injury rates compared with confluent cells for a range of reopening bubble speeds. This result suggests that hydrodynamic stresses alone cannot explain the difference in injury rates between the subconfluent and confluent cell types. Therefore, in this study we used computational techniques to further investigate the role of cell morphology and mechanics in determining cell injury during airway reopening.

The collective contributions of the studies discussed above have been to elucidate some of the fluid-mechanical and biological processes involved in lung injury. However, none of these studies directly measured intracellular deformation or strain during flow-induced injury. The goal of this study was to use computational models to help fill this gap in our understanding of the mechanisms responsible for cell injury by quantifying the strains experienced by EpC during airway reopening. We used confocal microscopy images to develop three-dimensional (3-D) finite element models of in vitro cell geometries and used these models to explore the influence of cell morphology and cell mechanics on the risk of cell injury during airway reopening.

MATERIALS AND METHODS

Cell Culture and Confocal Imaging

The computational models presented in this study are based on direct measurements of in vitro cell morphologies. Human A549 alveolar EpC (CCL-185; American Type Culture Collection, Manassas, VA) were cultured at passages 20–30 and were maintained in Ham’s F12K medium with 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% fungizone (Invitrogen, Carlsbad, CA). The cell culture medium was changed every 2–3 days. Cells were harvested with 0.125% trypsin (Invitrogen), counted with trypan blue exclusion (Invitrogen), and seeded onto 30-mm-diameter coverslips placed in six-well plates by adding 2 ml of a 8 × 10^6 cells/ml solution to each well. Cells were grown under standard culture conditions (37°C, 5% CO_2 and 95% air) for 1 day to obtain a low-confluence (25%) monolayer or for 4 days to obtain a high-confluence (100%) monolayer. Cells in the low-confluence monolayer were tall and rounded, whereas cells in the high-confluence monolayer were flat and closely packed. These two characteristic morphology groups were chosen to serve as models for the thin/spread morphology of type I EpC (high-confluence group) and the larger/taller morphology of type II EpC (low-confluence group). Cells in both the high-confluence and low-confluence groups were chosen at random from their respective confocal images without foreknowledge of cell morphometry or proximity to neighboring cells.

When the cell monolayers achieved the desired confluence, they were visualized using laser scanning confocal microscopy (LSCM). Confocal images of the whole cell morphology were produced by staining the cells’ cytoplasm with a fluorescent dye. Cells were exposed to a solution of serum-free cell culture medium and 2 μM calcein AM, a stain that has excitation and emission wavelengths of 494 and 517 nm, respectively, incubated in the dark for 10 min, and then viewed with a Zeiss LSM 510 Meta confocal microscope at ×40 magnification. Each confocal image was created by stacking 20–40 sequential 0.2-μm-thick scans, depending on the height of the cell layer. Total scanning time for each image was ~2 min. See Fig. 1A for a comparison of confluent and subconfluent monolayer scans. Accuracy of this visualization technique was confirmed by imaging several 15-μm-diameter fluorescent microspheres (FocalCheck; Invitrogen). The mean x-, y-, and z-direction diameters were all within 2.6% of 15 μm, and the mean values for the ratio of x to z and y to z diameters were all within 0.4% of unity. Therefore, this technique does not produce any out-of-plane distortion.

Finite Element Models

After LSCM visualization, we extracted a series of parallel cross-sectional images from the EpC monolayer such that each image represented a transverse slice of the cells, from the substrate to the apex. The images were then imported into the Rhinoceros (Seattle, WA) computer-aided design package. For each image, we generated boundary curves that defined the cells’ apical surfaces using cubic nonuniform rational B-splines. After processing all cross sections, we created a network of curves for each cell in the monolayer and generated surfaces based on those curve networks. Using this method, we produced 3-D surface maps of the EpC in both confluent and subconfluent monolayers (see Fig. 1 for step-by-step images of model creation). Quantitative morphology data for the subconfluent and confluent cell groups is given in Table 1.

To generate the finite element models, we exported the cell geometry bodies to the ADINA 8.4 (Watertown, MA) finite element package. We modeled eight cells from the confluent monolayer and eight cells from the subconfluent monolayer. Each cell was meshed with four-node tetrahedral isotropic solid elements for the cell body and four-node triangular shell elements for the cell membrane. The maximum element edge length was chosen to be d_{max} = 0.1 μm, which captured the essential features of the cell’s geometry (see detailed mesh pictures in Figs. 1, 4, and 8). The confluent cell models had on average 9,590 ± 3,417 solid elements and 1,963 ± 410 shell elements. The subconfluent cell models had on average 28,889 ± 8,650 solid elements and 3,572 ± 1,438 shell elements. We report averages as means ± SD.

Hydrodynamic loads and boundary conditions. In this study, we were interested in the maximum deformation EpC experience during the propagation of an air bubble in a fluid-occluded parallel-plate flow chamber (Fig. 2). Several previous experimental and computational studies have implicated the large pressure gradients near the bubble tip as the likely cause of cell damage during airway reopening (22, 26, 46). In particular, Bilek et al. (6) showed that cell damage increases.
with decreasing bubble velocity, which also correlates with increasing the maximum pressure gradient near the bubble tip. These authors developed relationships for the maximum instantaneous hydrodynamic stresses and stress gradients near the bubble tip as a function of the capillary number, \( Ca \), or dimensionless bubble speed.

\[
\frac{(\tau_{\text{max}})}{\gamma / H} = 0.69Ca^{0.36} \tag{1}
\]

\[
\frac{(d\tau / dx_{\text{max}})}{\gamma / H^2} = 0.22 + 0.2Ca^{0.75} \tag{2}
\]

\[
\frac{(dP / dx_{\text{max}})}{\gamma / H^2} = 0.34Ca^{-0.29} \tag{3}
\]

where \( Ca = \mu U / \gamma \), \( \mu \) is the fluid viscosity, \( U \) is the bubble speed, and \( \gamma \) is the surface tension of the air-liquid interface. \( \tau \) and \( d\tau / dx \) are the shear stress and shear stress gradient, \( dP / dx \) is the pressure gradient, \( H \) is the channel half-height, and \( x \) is the axial coordinate in the flow direction. To quantify the maximum deformation of EpC during airway reopening, these maximum instantaneous hydrodynamic loads were applied at the cells’ apical surfaces as a superposition of normal and tangential stress tractions. Tangential shear stresses were applied in the direction of flow (x-direction). Spatial gradients in pressure and shear stress were defined with respect to the flow direction. Pressure magnitudes were controlled by an upstream reference value, \( P_{\text{ref}} = 0.3\gamma / H \). System scaling variables were chosen to match typical experimental conditions: \( H = 0.05 \text{ cm} \), \( \mu = 0.007 \text{ g/cm} \cdot \text{s} \), and \( \gamma = 70 \text{ dyn/cm} \). Cell-cell attachments were neglected, and the cells were rigidly fixed at the basal surface. The membrane/cortex and cell interior were coupled with a no-slip boundary condition.

**Cell mechanical properties.** Cell deformation in the ADINA finite element models is governed by a standard stress balance relationship and tangential stress tractions. Tangential shear stresses were applied in the direction of flow (x-direction). Spatial gradients in pressure and shear stress were defined with respect to the flow direction. Pressure magnitudes were controlled by an upstream reference value, \( P_{\text{ref}} = 0.3\gamma / H \). System scaling variables were chosen to match typical experimental conditions: \( H = 0.05 \text{ cm} \), \( \mu = 0.007 \text{ g/cm} \cdot \text{s} \), and \( \gamma = 70 \text{ dyn/cm} \). Cell-cell attachments were neglected, and the cells were rigidly fixed at the basal surface. The membrane/cortex and cell interior were coupled with a no-slip boundary condition.

**Table 1. Quantitative morphology data for the confluent and subconfluent cells used in this study**

<table>
<thead>
<tr>
<th>Morphology Parameter</th>
<th>Confluent Cells</th>
<th>Subconfluent Cells</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H_{\text{max}}, \mu \text{m} )</td>
<td>3.1 ± 0.51</td>
<td>9.8 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( A, \mu \text{m}^2 )</td>
<td>913.8 ± 292.6</td>
<td>1562.3 ± 936.0</td>
<td>NS</td>
</tr>
<tr>
<td>( V, \mu \text{m}^3 )</td>
<td>1424.6 ± 366.9</td>
<td>7261.6 ± 2907.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( L, \mu \text{m} )</td>
<td>43.0 ± 7.1</td>
<td>51.7 ± 16.2</td>
<td>NS</td>
</tr>
<tr>
<td>Aspect ratio ( H / L )</td>
<td>0.08 ± 0.02</td>
<td>0.21 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n = 8 \) in each group. Cell length (L) was calculated measured in the direction of cell elongation, according to the procedure of Yalcin et al. (46). \( H_{\text{max}} \), maximum height; \( A \), projected area; \( V \), volume. Statistical significance of confluent-subconfluent population differences were checked using a homoscedastic \( t \)-test. NS, nonsignificant results.
We used a linear elastic material model with the following constitutive relationship (3):

$$\sigma_{ij} = \frac{E}{(1 + \nu)(1 - 2\nu)} \varepsilon_{ij} \delta_{ij}$$  (5)

$$\varepsilon_{ij} = \frac{1}{2} \left( \frac{\partial d_i}{\partial x_j} + \frac{\partial d_j}{\partial x_i} \right)$$  (6)

where $\varepsilon_{ij}$ is the Lagrangian strain tensor, $\varepsilon_{kk} = \varepsilon_{11} + \varepsilon_{22} + \varepsilon_{33}$ is the strain invariant, and $E$ is the Young's modulus. All equations are written in standard indicial notation. Estimates of cell stiffness, $E_{\text{cell}}$, by various experimental microrheology techniques have shown significant variability, with published stiffness values ranging from tens of pascals to a few kilopascals (21) (see also the results summarized in Table 2). To account for this wide range of possible stiffness values, we modeled the cell interior as an elastic isotropic medium with a broad range of cell stiffness, $200 < E_{\text{cell}} < 10,000 \text{ dyn/cm}^2$.

Membrane mechanical properties. Previous investigators have demonstrated that structural/mechanical differences between the deep cytoskeleton and the submembrane cortical region in alveolar EpC may result in different mechanical properties (i.e., stiffness) for these two regions (27, 29). In this study, we modeled the membrane region of the cell as an elastic shell and considered two models for its mechanical properties. The first model included only the mechanical contributions of the lipid bilayer, which has a thickness $h \approx 5 \text{ nm}$ (7). In the second model, we added the contribution of the actin cortex, which has a thickness $h \approx 100 \text{ nm}$ (47). In both cases, the membrane/cortex region can be thought of as a material that resists extension, bending, and shear. The moduli that describe this resistance to deformation ($k_c$, $k_b$, and $k_s$) can be estimated from the literature as shown in Table 3. We incorporated these experimental values in our computational models by noting the following relationships between $k_c$, $k_b$, and $k_s$ and the Young’s modulus, $E$, and shear modulus, $G$, of the membrane. These relationships are derived from classical plate theory (38, 41).

$$k_c = \frac{E_h}{2(1 - \nu)}$$  (7)

$$k_b = \frac{E_h^3}{12(1 - \nu^2)}$$  (8)

where $h$ is the thickness of the membrane and $\nu$ is the Poisson ratio. The extension modulus (Eq. 7) is similar to a surface tension (dyn/cm) and reflects the force per unit length required to induce a unit area expansion of the surface. The bending modulus (Eq. 8), which is also commonly referred to as the flexural rigidity, has units of torque (dyn·cm) and reflects the bending moment required to induce a unit change in curvature. Finally, the shear modulus (Eq. 9) also has units of tension (dyn/cm) and reflects the force per unit length required to induce a unit angular deformation. We assumed the cells are incompressible materials, so $\nu = 0.49$. For materials with isotropic mechanical properties, the shear modulus and Young’s modulus are related:

$$G = \frac{E}{2(1 + \nu)}$$  (10)

Applying this isotropic formulation to the membrane/cortex would overestimate the shear resistance by six orders of magnitude. To match the $k_c$ more closely, we modeled the membrane/cortex as an elastic orthotropic shell with the following stress-strain relationship (1):

$$\begin{bmatrix}
\varepsilon_{xx} \\
\varepsilon_{yy} \\
\varepsilon_{zz} \\
\gamma_{xy} \\
\gamma_{yz} \\
\gamma_{xz}
\end{bmatrix} =
\begin{bmatrix}
\frac{1}{E_x} & -\frac{1}{E_y} & 0 & 0 & 0 & 0 \\
-\frac{1}{E_y} & \frac{1}{E_x} & 0 & 0 & 0 & 0 \\
0 & 0 & \frac{1}{E_z} & 0 & 0 & 0 \\
0 & 0 & 0 & \frac{1}{2G_{xy}} & 0 & 0 \\
0 & 0 & 0 & 0 & \frac{1}{2G_{yz}} & 0 \\
0 & 0 & 0 & 0 & 0 & \frac{1}{2G_{xz}}
\end{bmatrix}
\begin{bmatrix}
\sigma_{xx} \\
\sigma_{yy} \\
\sigma_{zz} \\
\tau_{xy} \\
\tau_{yz} \\
\tau_{xz}
\end{bmatrix}$$  (11)

where $\varepsilon_{ij}$ are the strain components and $\sigma_{ij}$ are the stress components. Unlike typical orthotropic engineering materials such as composites, we assume the membrane/cortex does not have direction-dependent mechanical properties, so $E_x = E_y = E_z = E_{\text{mem}}$, $G_{xy} = G_{yz} = G_{xz} = G_{\text{mem}}$, and $\nu_{xy} = \nu_{yz} = \nu_{xz} = \nu_{\text{mem}} = \text{constant}$.

To match $E_{\text{mem}}$ and $G_{\text{mem}}$ to the experimental values reported in Table 3, we started by selecting values for the extension modulus, $k_c$. Knowing $k_c$ and $h$ determined $E_{\text{mem}}$ (Eq. 7). The bending modulus is also a function of $E_{\text{mem}}$ and $h$ (Eq. 8), so choosing $k_c$ determined $k_b$.

### Table 2. Reported values for stiffness of alveolar epithelial cells by various experimental microrheology techniques

<table>
<thead>
<tr>
<th>Source</th>
<th>Experiment</th>
<th>Cell Type</th>
<th>Reported Values*</th>
<th>Scaled Values†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alvarez et al. (2)</td>
<td>AFM</td>
<td>A549</td>
<td>$G' \approx 600 \text{ Pa}$</td>
<td>$E \approx 18,000 \text{ dyn/cm}^2$</td>
</tr>
<tr>
<td>Ballard et al. (4)</td>
<td>OT</td>
<td>A549</td>
<td>$G' = 420 \text{ Pa}$</td>
<td>$E = 12,600 \text{ dyn/cm}^2$</td>
</tr>
<tr>
<td>Berrios et al. (5)</td>
<td>MTC</td>
<td>Primary rat ATII</td>
<td>$G' = 50–300 \text{ dyn/cm}^2$</td>
<td>$E = 150–900 \text{ dyn/cm}^2$</td>
</tr>
<tr>
<td>Laurent et al. (20)</td>
<td>MTC,OT</td>
<td>A549</td>
<td>$E_{\text{OT}} = 34–58 \text{ Pa}$</td>
<td>$E = 300–2,600 \text{ dyn/cm}^2$</td>
</tr>
<tr>
<td>Trepet et al. (39)</td>
<td>MTC</td>
<td>A549</td>
<td>$G' = 722 \pm 83 \text{ Pa/\mu m}$</td>
<td>$E = 2,600 \text{ dyn/cm}^2$</td>
</tr>
<tr>
<td>Trepet et al. (40)</td>
<td>MTC</td>
<td>A549</td>
<td>$G'_{\text{RGD}} = 418 \pm 38 \text{ Pa/\mu m}$</td>
<td>$E = 100–1,500 \text{ dyn/cm}^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$G'_{\text{act-LDL}} = 27.9 \pm 1.6 \text{ Pa/\mu m}$</td>
<td></td>
</tr>
</tbody>
</table>

AFM, atomic force microscopy; MTC, magnetic twisting cytometry; OT, optical tweezers. *Values typically reported in terms of the complex shear modulus, $G' = G'' + iG''$, where $G'$ and $G''$ are the storage and loss moduli of the cell. When reported values have been estimated from a figure, reference frequency $\omega_0 = 1 \text{ Hz}$. †Values have been scaled to consistent CGS units using $E = 2G'(1 + \nu)$, where $E$ is elasticity and $\nu$ is the Poisson ratio. ‡Scaling for values from Trepet et al. (39, 40) was accomplished by multiplying the reported modulus by the reported bead displacement, $d \approx 120 \text{ nm at } \omega = 1 \text{ Hz}$.

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Table 3. *Reported values for the mechanical properties of the cell membrane and cortex by various experimental techniques.*

<table>
<thead>
<tr>
<th>Source</th>
<th>Experiment</th>
<th>Cell Type</th>
<th>Region</th>
<th>Scaled Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily and Elson (11)</td>
<td>Cell poking</td>
<td>RBC</td>
<td>Membrane</td>
<td>(k_e = 179-348 \text{ dyn/cm})</td>
</tr>
<tr>
<td>Earnshaw and Crawford (13)</td>
<td>Capillary waves</td>
<td>GMO bilayer</td>
<td>Lipid Bilayer</td>
<td>(k_e = 95 \text{ dyn/cm})</td>
</tr>
<tr>
<td>Evans (15)</td>
<td>Micropipette</td>
<td>RBC</td>
<td>Membrane</td>
<td>(k_e = 1.8 \times 10^{-12} \text{ dyn/cm})</td>
</tr>
<tr>
<td>Waugh et al. (45)</td>
<td>Tethering</td>
<td>Lipid vesicles</td>
<td>Lipid Bilayer</td>
<td>(k_e = 1.2 \times 10^{-12} \text{ dyn/cm})</td>
</tr>
<tr>
<td>Zhelev et al. (47)</td>
<td>Micropipette</td>
<td>Neurophil</td>
<td>Cortex</td>
<td>(k_e = 2 \times 10^{-11} \text{ dyn/cm})</td>
</tr>
<tr>
<td>Evans and Waugh (14)</td>
<td>Micropipette</td>
<td>RBC</td>
<td>Cortex</td>
<td>(k_e = 450 \text{ dyn/cm})</td>
</tr>
<tr>
<td>Hénon et al. (19)</td>
<td>Optical tweezers</td>
<td>RBC</td>
<td>Cortex</td>
<td>(k_e = 2.5 \times 10^{-3} \text{ dyn/cm})</td>
</tr>
<tr>
<td>Liu et al. (31)</td>
<td>Optical stretch</td>
<td>RBC</td>
<td>Cortex</td>
<td>(k_e = 4.611 \times 10^{-3} \text{ dyn/cm})</td>
</tr>
<tr>
<td>Waugh and Evans (44)</td>
<td>Micropipette</td>
<td>RBC</td>
<td>Cortex</td>
<td>(k_e = 6.6 \times 10^{-3} \text{ dyn/cm})</td>
</tr>
<tr>
<td>Lenormand et al. (30)*</td>
<td>Optical tweezers</td>
<td>RBC skeletons</td>
<td>Cortex (no bilayer)</td>
<td>(k_e = 2.4 \times 10^{-3} \text{ dyn/cm})</td>
</tr>
</tbody>
</table>

*Reported shear modulus, \(k_s\), of cortex skeleton with no lipid bilayer is comparable to results with bilayer present, indicating that shear resistance does not arise from the lipid bilayer. \(k_e\), extension modulus; \(k_b\), bending modulus.

in our orthotropic shell model. For the lipid bilayer, the resulting \(k_b\) value was close to the experimental observations, whereas the cortex \(k_b\) value was slightly overestimated. Finally, we used the experimentally determined shear modulus to set \(G_{\text{mem}}\) in our model. Note that lipid bilayers exhibit very little resistance to shear, so \(k_b\) of the bilayer reported in Table 4 is an arbitrary value chosen to be an order of magnitude less than \(k_b\) of a cortex skeleton, as reported by Lenormand et al. (30). For reference, the target moduli \(k_s\), \(k_b\), and \(k_e\) as well as the resulting modeling parameters \(E_{\text{mem}}\), \(G_{\text{mem}}\), and \(h\) are given in Table 4 for the lipid bilayer only and for the membrane plus the actin cortex region.

Data Inquiry and Statistics

For all of the results presented in this study, we quantified cell deformation in terms of effective strain (\(\varepsilon_{\text{eff}}\)) in the cell membrane.

\[
\varepsilon_{\text{eff}} = \frac{2}{3} \varepsilon_{ij} \cdot \varepsilon_{ij} = \left( \frac{2}{3} (\varepsilon_{xx}^2 + \varepsilon_{yy}^2 + \varepsilon_{zz}^2 + 2(\varepsilon_{xx}^2 + \varepsilon_{yy}^2 + \varepsilon_{zz}^2)) \right)^{1/2}
\]

(12)

where \(\varepsilon_{ij}\) is the strain components calculated in the finite element analysis. For the purposes of discussion, we queried the maximum effective strain, \(\varepsilon_{\text{eff,max}}\), to quantify localized peak strains within the cells. We also calculated the area-averaged strain, \(\varepsilon_{\text{eff,avg}}\), to quantify whole cell effects. Statistical analysis was performed using SPSS 15.0 (Chicago, IL) assuming a log-normal probability distribution. The log-normal distribution of \(\varepsilon_{\text{eff,max}}\) and \(\varepsilon_{\text{eff,avg}}\) in the subconfluent and confluent cell populations was confirmed by a Kolmogorov-Smirnov test (all \(P \geq 0.483\)).

RESULTS

We began by comparing the subconfluent and confluent cell populations with two different membrane models (lipid bilayer only or bilayer plus cortex). Figure 3 shows maximum and area-averaged effective strains in the membrane for subconfluent and confluent cells with the lipid bilayer membrane model or the cortex membrane model. In all cases, cells were exposed to bubble-induced stresses at \(Ca = 5E-5\), which is typical for experimental airway reopening (6, 26, 46). As expected, increasing cell stiffness decreased the amount of deformation experienced by the cells. Comparing the two cell populations (Fig. 3) shows the subconfluent cell models predicted higher strains than the confluent cell models. ANOVA indicated that cell type (confluent vs. subconfluent) and cell stiffness were both statistically significant factors (all \(P < 0.001\) for both the lipid bilayer and cortex models, using maximum and area-averaged strain data). Among confluent or subconfluent cells only, differences between the lipid bilayer and cortex models were statistically significant (all \(P < 0.001\) by ANOVA). For all data shown in Fig. 3, all \(E_{\text{cell}}\) treatment levels were significantly different [all \(P < 0.001\) by a least significant difference (LSD) post hoc test]. Contour plots showing strain localization patterns for representative confluent and subconfluent cells with both membrane models are shown in Fig. 4.

As previously discussed, several experimental and computational studies have implicated the large pressure gradient near the moving bubble tip as the primary mechanism of cell injury during airway reopening (6, 22, 26, 46). To investigate this hypothesis, we performed a series of simulations in which pressure and shear stresses were applied independently (as opposed to simultaneously) for a range of capillary numbers. The results presented in Fig. 5 show bubble-induced strain with pressure loads only (A) or shear loads only (B). Note that these results are for the confluent cell population only. For both pressure-only and shear-only loading, \(Ca\) and the membrane model were statistically significant factors (all \(P < 0.001\) by ANOVA). Maximum pressure-induced strains (Fig. 5A) were ~1.7 times lower in cells with cortex model than in cells with the lipid bilayer model (significant at all \(Ca\) values with all \(P \leq 0.028\) by homoscedastic t-test). Maximum shear-induced strains (Fig. 5B) were ~3.2 times lower with the cortex model than with the lipid bilayer (significant at all \(Ca\) values with all \(P \leq 0.001\) by t-test). Results for area-averaged strains were similar (data not shown). Furthermore, for the slow bubble speeds typically used in airway reopening experiments (\(Ca < 5E-4\)), pressure-induced strains were one to two orders of magnitude larger than shear-induced strains (compare Fig. 5, A and B).

Table 4. *Mechanical properties of the baseline membrane and cortex models in terms of measured moduli and modeling parameters.*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lipid Bilayer Only</th>
<th>Membrane + Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_e)</td>
<td>150 dyn/cm</td>
<td>450 dyn/cm</td>
</tr>
<tr>
<td>(k_b)</td>
<td>(4 \times 10^{-12}) dyn/cm</td>
<td>(5 \times 10^{-9}) dyn/cm</td>
</tr>
<tr>
<td>(k_s)</td>
<td>(1 \times 10^{-4}) dyn/cm</td>
<td>(2.5 \times 10^{-3}) dyn/cm</td>
</tr>
<tr>
<td>(H)</td>
<td>5 nm = 0.005 (\mu)m</td>
<td>100 nm = 0.1 (\mu)m</td>
</tr>
<tr>
<td>(E_{\text{mem}})</td>
<td>3E8 dyn/cm²</td>
<td>4.5E7 dyn/cm²</td>
</tr>
<tr>
<td>(G_{\text{mem}})</td>
<td>200 dyn/cm²</td>
<td>250 dyn/cm²</td>
</tr>
</tbody>
</table>

Values are mechanical properties of the models in terms of measured extension, bending, and shear moduli, as well as the modeling parameters \(E_{\text{mem}}\) (Young's modulus) and \(G_{\text{mem}}\) (shear modulus), calculated using Eqs. 7–9.
As bubble speed increased (higher $Ca$), shear forces also increased, but shear-induced strains remained $<1\%$ in the experimentally relevant regime ($Ca < 5E^{-4}$). This finding is in agreement with previous studies that have implicated the large pressure gradients near the bubble tip in cell injury during airway reopening (6, 22, 26).

Up to this point, we have been comparing results from our two baseline membrane models: the lipid bilayer only and the bilayer with an actin cortex. In these two models, the membrane thickness, Young’s modulus, and shear modulus were simultaneously changed to produce the desired bending, extensional, and shear moduli (see Table 3). To quantify the role of each mechanical property specified in our models, we systematically varied our three independent parameters: $G_{mem}$, $E_{mem}$, and $h$ (see Figs. 6, 7, and 9). In each case, variations were performed from the baseline cortex model by using the confluent and subconfluent cell populations with $E_{cell} = 1,500$ dyn/cm$^2$.

![Fig. 3](image1.png)

Fig. 3. Maximum ($\varepsilon_{eff,max}$) and area-averaged strain ($\varepsilon_{eff,avg}$) for subconfluent and confluent cells with the baseline lipid bilayer model (A) and the baseline cortex model (B). Strain decreases with increasing cell modulus ($E_{cell}$). Data are geometric means ± 70% confidence intervals.

![Fig. 4](image2.png)

Fig. 4. Strain localization contour plots for representative confluent cells (A and B) and subconfluent cells (C and D). For each cell type, cells are shown with the baseline lipid bilayer model (A and C) and the baseline cortex model (B and D). Results shown are for $Ca = 5E^{-5}$, $E_{cell} = 2,000$ dyn/cm$^2$. Cells with the cortex model (B and D) had fewer “hot spots” of strain localization.
In Fig. 6, we varied the shear modulus, $G_{\text{mem}}$, keeping $h$ and $E_{\text{mem}}$ at the baseline cortex values. The lower limit was defined by the $G_{\text{mem}}$ value required to match the published experimental value for $k_s = 0.0025 \text{ dyn/cm}$, and the upper limit was prescribed by an equivalent isotropic membrane model ($k_s = 150 \text{ dyn/cm}$). Increasing the shear modulus of the membrane region generally decreased membrane strain. ANOVA indicated that $G_{\text{mem}}$ was a statistically significant factor for confluent cells ($P = 0.035$ and $P = 0.010$ based on maximum and area-averaged strains) and subconfluent cells ($P < 0.001$ based on maximum and area-average strains), but an LSD post hoc test showed that this effect was only significant when very low shear moduli were compared with very high shear moduli. In fact, no statistically significant changes in both maximum and average strain were found for single order-of-magnitude changes in $G_{\text{mem}}$ among confluent cells. However, $\varepsilon_{\text{eff,max}}$ did show significant changes with single order-of-magnitude changes in $G_{\text{mem}}$ among subconfluent cells only, which suggests that subconfluent cells may be more sensitive to the membrane shear modulus than confluent cells.

In Fig. 7, we varied the Young’s modulus of the membrane, $E_{\text{mem}}$, keeping $h$ and $G_{\text{mem}}$ at the baseline cortex values. We varied the cortex stiffness to include several orders of magnitude above and below the baseline value of $E_{\text{mem}} = 4.5E7 \text{ dyn/cm}^2$. Results indicate that increasing $E_{\text{mem}}$ generally decreased the membrane strain magnitudes, and this effect was most significant for the subconfluent cells. ANOVA indicated that $E_{\text{mem}}$ was a statistically significant factor for both maximum and area-averaged strain ($P < 0.001$ in all cases). We also examined the change in strain localization patterns within the cells as a function of $E_{\text{mem}}$ (Fig. 8). When the cortex had low stiffness ($E_{\text{mem}} = 4.5E4 \text{ dyn/cm}^2$; Fig. 8, A and C), the strain patterns indicated that the cell experienced relatively uniform deformation in the direction of flow. The cell flattened out and bulged over the downstream edge, creating large regions of high strain. This type of bulging deformation was previously hypothesized to lead to membrane rupture and cell death (6), and our models have confirmed that this behavior could arise under the influence of the large pressure gradients found near the bubble tip. However, as $E_{\text{mem}}$ increased, the strain localization patterns were significantly altered. When the cortex stiffness matched the experimental baseline ($E_{\text{mem}} = 4.5E7 \text{ dyn/cm}^2$; Fig. 8, B and D), the cells did not exhibit the downstream bulge and strain localization patterns did not have a distinct upstream-downstream trend. As expected from Fig. 7, these strain localization trends were most pronounced in the subconfluent cells.

Finally, as shown in Fig. 9, we varied cortex thickness, $h$, keeping $E_{\text{mem}}$ and $G_{\text{mem}}$ at the baseline cortex values. The $h$ range was chosen to include a lower limit of double the thickness of a lipid bilayer ($h = 0.01 \mu m$) and an upper limit of five times the experimental baseline ($h = 0.5 \mu m$). Increasing cortex thickness generally decreased the maximum strain magnitudes, and $h$ was a statistically significant factor for both maximum and averaged strains ($P \leq 0.001$ in both cases by ANOVA). Again, we repeated our examination of the strain localization behavior and observed a decrease in the magnitude of the peak strains with increasing cortex thickness but no noteworthy changes in the strain localizations patterns (images not shown). For these simulations, the chosen baseline $E_{\text{mem}}$ value prevented whole cell deformation patterns like those shown in Fig. 8, A and C.
DISCUSSION

Cell Morphology Affects Injury Risk

The first aim of this study was to examine the influence of cell morphology on the risk of cell injury during airway reopening. Our cell models indicated that given the same hydrodynamic loading and mechanical properties, subconfluent cells develop higher membrane strains than confluent cells (Fig. 3). If we assume that membrane strain correlates positively with the risk of cell injury, our results are in agreement with the experimental findings of Yalcin et al. (46), who saw higher death rates for subconfluent cells than confluent cells. Based on measurements of the cell aspect ratio, Yalcin et al. (46) concluded that hydrodynamic stress amplification was not the source of the population differences in cell death and postulated that cell morphology and cytoskeletal changes might be the reason for the observed differences between the confluent and subconfluent death rates. The results presented in this study support the idea that cell morphology alone can have a significant effect on strain (i.e., risk for cell injury). To illustrate the morphological differences between the two groups of cells, Fig. 10 presents top and side views of all cells used in this study. Note that the confluent cells are flattened and cobblestone-like, whereas the subconfluent cells are taller and more rounded. Our results indicate that these shape characteristics contributed to the increased susceptibility of subconfluent monolayers to bubble-induced injury. In the strain localization images (Figs. 4 and 8), we noted similar patterns.
between the subconfluent and confluent cells, but the trends were significantly amplified in the subconfluent cell group.

This finding has interesting implications for the health of the epithelium and lung mechanics during ARDS. In this study, we used type II A549 alveolar EpC. However, depending on the degree of confluency of the monolayer, A549 cells exhibit significant differences in characteristic morphology. In a low-confluence monolayer, A549 cells are tall and rounded, which corresponds to the typical morphology of type II cells. However, as the monolayer approaches 100% confluence, the morphology of the cells changes to a more thin and spread profile, which is consistent with the morphology of type I cells (refer to Fig. 10). The computational models presented in this study indicate that subconfluent cells with type II morphology develop higher strains than confluent cells with type I morphology. If we correlate higher strain with an increased risk of injury, this data suggests that due to their morphology, type II cells may be at higher risk for injury than type I cells during airway reopening. Morphology-dependent preferential injury of type II cells could lead to a loss of surfactant secretion and additional challenge to the ARDS-stressed lung.

**Cell Mechanics Affect Induced Strain**

The second aim of this study was to examine the influence of cell mechanics on the risk of cell injury during airway reopening. Our models showed a clear decrease in effective strain with increasing cell stiffness (Fig. 3) and a statistically significant dependence of strain on the membrane model (lipid bilayer vs. bilayer plus actin cortex, see Fig. 3). These results suggest that altering the microstructure and mechanical properties of the cytoskeleton and the membrane/cortex region may be an effective way to reduce bubble-induced cell injury during the ventilation of patients with ARDS. However, it might be difficult to identify effective therapies using only experimental approaches due to the complexity and heterogeneity of the cell’s structural and mechanical environment. To help identify which structural/mechanical components of the cell have the greatest influence on bubble-induced injury, we performed a sensitivity analysis on the independent parameters in our cell mechanics model. Order-of-magnitude sensitivity ratios were defined as follows, taking care to include only statistically significant changes in $\varepsilon_{\text{eff,max}}$ with the given parameter:

\[
\Delta E_{\text{cell}} = \frac{\varepsilon_{\text{eff,max}} \text{ at } E_{\text{cell}} = 1E3 \text{ dyn/cm}^2}{\varepsilon_{\text{eff,max}} \text{ at } E_{\text{cell}} = 1E4 \text{ dyn/cm}^2}
\]

\[
\Delta E_{\text{mem}} = \frac{\varepsilon_{\text{eff,max}} \text{ at } E_{\text{mem}} = 4.5E4 \text{ dyn/cm}^2}{\varepsilon_{\text{eff,max}} \text{ at } E_{\text{mem}} = 4.5E5 \text{ dyn/cm}^2}
\]

\[
\Delta h = \frac{\varepsilon_{\text{eff,max}} \text{ at } h = 0.01\mu m}{\varepsilon_{\text{eff,max}} \text{ at } h = 0.1\mu m}
\]

where $\Delta E_{\text{cell}}$, $\Delta E_{\text{mem}}$, and $\Delta h$ were calculated from the data in Figs. 3, 7, and 9, including only confluent cells with variations on the baseline cortex model. No sensitivity analysis was performed for $G_{\text{mem}}$ because there were no statistically significant changes in $\varepsilon_{\text{eff,max}}$ with single order-of-magnitude changes in $G_{\text{mem}}$. Results of this analysis suggest that confluent and subconfluent cells have similar sensitivities to $E_{\text{mem}}$ and $h$.
(confluent $\Delta E_{\text{mem}} = 1.28 \pm 0.19x$ and $\Delta h = 1.83 \pm 0.61x$; subconfluent $\Delta E_{\text{mem}} = 1.31 \pm 0.14x$ and $\Delta h = 1.76 \pm 0.57x$). In contrast, the sensitivity to $E_{\text{cell}}$ was much higher (confluent $\Delta E_{\text{cell}} = 10.99 \pm 1.55x$; subconfluent $\Delta E_{\text{cell}} = 6.98 \pm 2.85x$). These results indicate that the magnitude of the maximum strains induced by airway reopening stresses was more sensitive to the stiffness of the cell interior (cytoskeleton) than to the cortex stiffness or cortex thickness. On the basis of this analysis, altering the mechanics of the cytoskeleton (i.e., $E_{\text{cell}}$) may have a larger protective effect than altering the membrane/cortex region.

Link Between Morphology and Mechanics

Throughout this study, we have observed a relationship between morphological and mechanical factors in the risk for cell injury during airway reopening. As previously discussed, our results demonstrated that subconfluent cells develop higher strains than confluent cells for equivalent hydrodynamic loads and mechanical properties. In the preceding sensitivity analysis, we saw that $\Delta E_{\text{cell}}$ for the confluent cells was nearly double $\Delta E_{\text{cell}}$ for the subconfluent cells. Although we have been discussing the sensitivity to $E_{\text{cell}}$, these results also can be interpreted as sensitivity to the mechanical contribution of the membrane/cortex. As the cytoskeleton becomes softer (low $E_{\text{cell}}$), the role of the membrane/cortex becomes more dominant. Thus the sensitivity analysis on $E_{\text{cell}}$ also can be interpreted as evidence that due to their morphology, confluent cells are more sensitive than subconfluent cells to the mechanical contribution of the membrane/cortex region.

Throughout this study, in our comparisons between subconfluent and confluent cells, we have assumed that the two populations have identical mechanical properties. However, previous investigators observed microstructural differences between subconfluent and confluent cells, particularly in the redistribution of actin (46). These structural differences may be associated with differences in the mechanical properties of the cytoskeleton or cortex region. For example, Yalcin et al. (46) observed a diffuse, relatively homogeneous actin network in their confluent EpC and a redistribution of actin to the cell periphery in the subconfluent EpC. The analogous data sets from this study would be confluent cells with the lipid bilayer and subconfluent cells with the thicker cortical region (see Fig. 11 and compare Fig. 4, A and D). These results indicate that even when the membrane/cortex mechanical contribution is chosen to maximize strain in the confluent cells and minimize strain in the subconfluent cells, the subconfluent group still exhibits higher maximum and area-averaged strains. Statistically significant differences between the two cell populations were observed with $P < 0.001$ for both $\varepsilon_{\text{eff, max}}$ and $\varepsilon_{\text{eff, avg}}$ by ANOVA. Clearly, a better assessment of the mechanics of subconfluent and confluent cells via experimental microrheology techniques such as atomic force microscopy, magnetic twisting cytometry, or optical tweezing would add to our understanding of experiments such as those performed by Yalcin et al. (46) and our ability to accurately study these cells with computational models. However, even without that information, the results of this study have demonstrated that cell morphology plays a significant role in the risk for cell injury.

Model Limitations

The in vitro model of airway reopening (Fig. 2) involves the motion of an air-liquid interface that generates transient hydrodynamic stresses on the EpC lining the channel wall. In this study, we utilized a series of simplifying assumptions to make this complex fluid-structure interaction problem more tractable. First, in modeling the EpC using the finite element method, we have used a continuum mechanics assumption for the cell interior, which is in reality a highly inhomogeneous structure. However, a large body of previous studies have established a precedent for modeling cells as homogeneous continua (16, 23–25, 28, 32, 35). Given the correspondence between published experiments (46) and our modeling results, we believe our models can effectively elucidate the role of cell morphology and membrane mechanics in cell injury, despite the limitations inherent in using continuum mechanics.

Another complication in modeling the A549 cells is that, to our knowledge, no direct experimental measurements of membrane/cortex mechanics are currently available for alveolar EpC, so the parameters reported in Table 3 have been gleaned from other cell types. To address the uncertainty arising from a lack of direct measurements for our cell type, we conducted a parameter variation with respect to the membrane mechanical properties, $k_m$, $k_b$, and $k_c$, that spanned several orders of magnitude and almost certainly encompassed the true values for alveolar EpC. In addition, we observed that the effect of cell morphology on the risk of injury was consistent throughout the large variations in membrane mechanical properties.

We also have made limiting assumptions with regard to the interaction between the cells and the air-liquid interface. In this study, we developed a decoupled fluid-structure interaction model of cell deformation during airway reopening. As previously discussed, the hydrodynamic stresses were derived from

![Fig. 11. Even when membrane models were chosen to maximize strains in the confluent cells (lipid bilayer model) and minimize strains in subconfluent cells (cortex model), the subconfluent group still had higher strains than the confluent group due to their morphology. Differences between subconfluent and confluent groups were statistically significant (all $P < 0.001$ by ANOVA).](http://jap.physiology.org/)
flat wall bubble propagation computations as described by Bilek et al. (6), which neglects the stress amplification caused by nonplanar cell topology. For our simulations, we chose $Ca = 5 \times 10^{-5}$ to correspond to experiments. At this $Ca$ value, the cells on the wall may deform the bubble and alter the hydrodynamic stress field. Jacob and Gaver (22) showed that this effect amplifies the maximum stresses given in Eqs. 1–3 and that the degree of amplification is determined by the cell aspect ratio. As shown in Table 1, the average aspect ratio for the cell type used in this study (i.e., A549) was larger in the subconfluent population than in the confluent population. Based on the relationships developed by Jacob and Gaver (22), these aspect ratios would result in normal stress amplification factors of approximately two for confluent cells and approximately six for subconfluent cells. However, the stress field associated with the bubble is strongly dependent on air-liquid interface curvature, and we should not assume that stress amplification depends only on cell aspect ratio and not on cell shape (e.g., flat, cobblestone-like confluent cells vs. rounded subconfluent cells). In an effort to minimize the number of variable parameters in this study and focus only on the contribution of cell morphology and mechanics to stress and strain distribution within the cell, we have chosen to neglect the stress amplification associated with nonplanar wall topologies at very low $Ca$. It should be noted that because we expect subconfluent cells to induce more stress amplification than confluent cells, we also expect that including this effect would produce even higher strains in the subconfluent cell group than in the confluent cell group and that the conclusions would be consistent with the results we have presented.

We also simplified the complex transient airway reopening stresses by considering only the instantaneous maximum stresses and stress gradients. We have confidence in the assumption that transient effects can be neglected for this study because Kay et al. (26) observed that cell damage during airway reopening is correlated with the magnitude of the pressure gradient, not with the duration of exposure to damaging stresses. Thus we have neglected cell viscoelasticity and have performed static simulations with elastic cell and membrane/cortex constitutive models. It is important to note that this assumption requires that the dynamic rheological properties of the cells are unchanged, as was the case for the Kay et al. study. However, during other experimental conditions, such as the treatment of cells with cytoskeletal agents, both the cell stiffness magnitude and the dynamic rheological behavior can change, and this assumption will no longer be valid. Therefore, correlations of computational models with experiments in which dynamic rheological properties are not constant will require the development of fluid-structure interaction, transient dynamic models of this complex system.

Conclusions

In summary, we have developed image-based finite element models of alveolar EpC to examine the influence of cell morphology and mechanical properties on cell deformation during airway reopening. We considered two cell populations, confluent and subconfluent cells, and conducted a parameter variation study on cell and membrane/cortex mechanics. We have presented the following conclusions:

- **Cell morphology.** Given the same hydrodynamic load conditions and cell mechanical properties, subconfluent cells develop higher membrane strains than confluent cells and have a higher risk of injury (Fig. 3). This finding agrees with previous experimental observations of cell injury during airway reopening (46).

- **Cell mechanics.** Membrane strain decreases with increasing cell stiffness, $E_{\text{cell}}$; membrane stiffness, $E_{\text{mem}}$ and $G_{\text{mem}}$; and membrane thickness, $h$ (see Figs. 3, 6, 7, and 9). A sensitivity analysis also suggested that due to their morphology, confluent cells may be more sensitive than subconfluent cells to the mechanical contribution of the membrane/cortex region.

These results not only improve our understanding of experimental studies for which membrane stress-strain visualization is intractable but also suggest areas for future investigation. For example, our results indicate that cortical mechanics may play an important role in determining the differences between subconfluent and confluent cell injury risk during airway reopening. This observation suggests that experiments are needed to characterize the changes in deep cytoskeletal and submembrane cortical mechanics associated with subconfluent and confluent cells and with cytoskeleton-altering agents. Incorporating these types of measurements into the image-based cell models developed in this study and comparing computational results with experimental data on cell injury during airway reopening would be an effective way to evaluate how different drug agents might be used to prevent cell injury during airway reopening.

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