Molecular and Biophysical Mechanisms of Lung Cell Injury

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Respiratory System

Upper Respiratory Airways (Eustachian Tube)
Otitis Media (Ear disease)

Pulmonary Airways/Alveoli
Acute Lung Injury

Goal: Utilize engineering, mathematical and biological techniques to understand the pathogenesis of these disorders and develop novel treatments.
**Structure of Pulmonary Airways**

- Complex bifurcating geometry of pulmonary airways.

- **Rigid** vs. **Compliant** regions of the lung.

- Alveolar contact with circulating blood.

Kitoka, Takakai, Suki, JAP 1999
**Pulmonary Mechanics – How do we breath?**

Breathing mechanics:
- Negative pressure breathing
- Contraction of Diaphragm
- Deformation of Lung Tissue
- Generates negative pressure which “sucks” air in.
- Elastic recoil during exhalation

Alveolar Expansion/Compression
Infant Respiratory Distress Syndrome

- Lung Immaturity → Surfactant Insufficiency
- Surfactant Insufficiency → Atelectasis
- Atelectasis → Epithelial Injury
- Epithelial Injury → Alveolar Leakage
- Alveolar Leakage → Hyaline membranes
- Hyaline membranes → Fibrosis

• ↑Reopening Pressure → Cell Injury/Fluid Leakage
• Surfactant Function → ↓γ and reopening pressure

Courtesy, D.P. Gaver, Tulane Univ.
**Acute Lung Injury**

- Infections → Necrosis and Detachment of alveolar epithelial cells.
- ↑ permeability of alveolar-capillary barrier → Flooding of small airways and alveoli
- ↓ gas exchange, severe hypoxia
- Standard of care: Mechanical Ventilation
  - Weaning, Sedation, *Ventilator Induced Lung Injury*
- 30% to 40% mortality rates

S. Ghadiali, Lehigh University
Ventilation Induced Lung Injury (VILI)

- Low Volume Injury (Atelectrauma)
  - Injury due to mechanical stresses exerted during the opening of fluid-filled airways
  - Mechanisms of injury are not well known!

- High Volume Injury (Volutrauma)
  - In-vitro cell-culture studies used to identify mechanism of stretch-induced injury

\( V_T: 12 \text{ ml/kg} \rightarrow 6 \text{ ml/kg} \)

Mortality: 39.8% → 31.0%
**How do Airways Close during ALI?**

- Original Theory: Pulmonary edema $\rightarrow$ ↑lung weight $\rightarrow$ airway collapse
- Surfactant Inactivation Experiments (Schiller et. al.):
  - Sub-pleural photomicrographs indicate alveolar instability
  - Limitation: no disruption of epithelium
- Oleic Acid Injury
  - Fluid-filled of non-collapsed regions


Figure 1. *In situ* photomicrographs of alveoli during tidal ventilation in a normal lung (CONTROL) and after acute lung injury by Tween lavage (TWEEN). Photomicrographs at end expiration (EXP-...
Fluid Forces During Airway Reopening

• Summary of Computational Fluid Dynamic Modeling

Computational Fluid Dynamic Model

Evolution Eqn
\[ \frac{\partial h^*}{\partial t^*} = - \frac{\partial q^*}{\partial x^*} \]

Kinematic Condition
\[ q^* = \frac{\gamma_{eq} h^*}{3 \mu} \frac{\partial k^*}{\partial x^*} \]

Stokes Flow
\[ \nabla P^* = \mu \nabla^2 u^* \]

LUBRICATION THEORY

BOUNDARY ELEMENT

Stress Balance
\[ \tau_{eq} = \gamma_{eq} k^* \hat{n} \]

Governing Parameter:
\[ Ca = \frac{\mu U}{\gamma} \]
Hydrodynamic Stresses

Epithelial Cells (not to scale)

Air bubble

Bubble Velocity

Occlusion fluid
Velocity, $u$
Viscosity, $\mu$
Surface tension, $\gamma$

Maximum stress values (dimensional):

$$\left(\frac{dP}{dx}\right)_{\text{max}} = 0.34 \left(\frac{\gamma}{H^2}\right)Ca^{-0.29}$$

$$\left(\tau_s\right)_{\text{max}} = 0.69 \left(\frac{\gamma}{H}\right)Ca^{0.36}$$

$$\left(\frac{d\tau_s}{dx}\right)_{\text{max}} = \left(\frac{\gamma}{H^2}\right)^* \left(0.22 + 1.2Ca^{0.75}\right)$$
Mechanobiology of Reopening

Mechanical Response:
• Cell Deformation
• Plasma Membrane Disruption
• Cell Detachment

Hydrodynamic Stresses

Biological Response:
• Protein/Gene expression

Cell Necrosis
Barrier Disruption

Apoptosis
Inflammatory Mediators

Current Research Focus:

*How do different biophysical properties (fluid stresses, cell morphology, cell microrheology) influence cellular deformation and injury during airway reopening?*
**Experimental Flow System**

- Protocol:
  a) Fill chamber with occlusion fluid (PBS or Cell Culture Media, F12K).
  b) Retract fluid to generate bubble propagation.
  c) Fill chamber with live/dead (Calcein / Ethidium) stain and visualize *in-situ*.

- System can accommodate:
  a) Various microchannel geometries
  b) Flexible membranes

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Effect of Airway Diameter and Bubble Velocity

- 4-5 day culture of Rat L2 Epithelial cells (ATCC) till 100% confluent.
- Bubble propagation with high surface tension fluid (PBS)

- More cellular necrosis at slower bubble velocities and in smaller channels
- Implications - High frequency ventilation protocols may help protect the cells in distal regions of the lung.
- Why less death at slower velocities?

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Correlation of Hydrodynamics with Cell Death

- Correlate with maximum value of each stress component during re-opening.
  - Strong correlation with \((dP/dx)_{\text{max}}\)
  - Weak correlation with \((d\tau_{S}/dx)_{\text{max}}\)
  - No correlation with \((\tau_{S})_{\text{max}}\)

\[
(dP/dx)_{\text{max}} = 0.34(\gamma / H^2)Ca^{-0.29}
\]

\[
(d\tau_{S}/dx)_{\text{max}} = (\gamma / H^2)* (0.22 + 1.2Ca^{0.75})
\]

\[
(\tau_{S})_{\text{max}} = 0.69(\gamma / H)Ca^{0.36}
\]
Emergent Phenomena

- Knowledge of the components of a system (reductionism) is not sufficient to understand system behavior.
  - Example 1: Paint Set -> Monet Painting?
  - Example 2: Gene sequence -> Life patterns?
  - Example 3: Different types of Mechanical Stresses (shear, normal, etc.) -> Cell Injury Patterns?

Understanding the emergence of pressure gradients required engineering approaches (i.e. coupling computational and experimental techniques).
**Effect of Confluence on Cellular Injury**

- 1 day or 4 day culture of Rat L2 cells (ATCC) (25% and 100% confluence).
- Bubble propagation in 0.5mm height channel with cell culture media at 25°C

- Why is there less death for the 100% confluent cells?
- Hypothesis: Cell morphology influences the amount of cell deformation/death.
Quantification of Cellular Morphology

- Laser scanning confocal images of cells with cytoplasmic stain (Calcein AM).
- Cells in 100% monolayer are flatter and thinner.
Fluid-Structure Models of Cell Deformation

- Model cells as viscoelastic medium with linear-elastic plasma membrane.

\[ \sigma_{ij}^s = G' \varepsilon_{ij} + G'' \frac{\partial \varepsilon_{ij}}{\partial t} \]

- Apply transient hydrodynamic stresses due to bubble propagation (Ghadiali et al., JFM, 2003).

\[ n_j \sigma_{ij}^s = n_j \sigma_{ij}^f \]

\[ n_i v_i = n_i \frac{\partial d_i^s}{\partial t} \]

\[ d_i^f = d_i^s \]
Effect of Morphology on Membrane Strains

Subconfluent

Confluent

Given the same loading and mechanical properties, subconfluent cells develop higher effective strains.

Cell morphology is an important factor for cellular injury.

\[ G'_{cell} = 500 \text{ dyn/cm}^2 \]
\[ G'_{mem} = 1E8 \text{ dyn/cm}^2 \]
100% monolayers contain cell-cell contacts and tight actin networks.

25% monolayers exhibit significant changes in cytoskeletal networks (dividing cell).

Indicates that the microstructural (or micromechanical) properties may play a role in a cell’s susceptibility to injury.
Effect of Cytoskeletal Structure on Cell Injury

- Alter cytoskeletal structure and expose cells to equivalent reopening conditions.

- Jasplakinolide → rigid cells → expect less necrosis. √

- Latrunculin → softer cells → expect more necrosis X?

- Hypothesis: ↑ viscous dampening of transient hydrodynamic loads → less deformation.

- Question: Are Latrunculin cells more viscous?
Measurement of Cell Mechanics

- Oscillating Optical Tweezers
  - Forced oscillation of external bead attached to the cytoskeleton at $\omega = 1$ to 1000Hz
  - Obtain elastic and viscous components of $G^*$: $G^*(\omega) = G'(\omega) + iG''(\omega)$
  - Advantages: Total displacement is $\sim 100$nm – no plastic deformations

\[
G'(\omega) = \frac{k_{OT}}{4\pi a} \left( \frac{3}{2 \sin \theta} + \frac{\cos \theta}{\sin^3 \theta} \right) \left( \frac{A \cos \delta(\omega)}{D(\omega)} - 1 \right)
\]

\[
G''(\omega) = \frac{3k_{OT}}{16a \sin \theta} \left( \frac{A \sin \delta(\omega)}{D(\omega)} \right)
\]
• Power-law rheology: $\beta=0.18$ for untreated cells is consistent with other intracellular measurements (Fabry, PRL, 2001)

• $\beta$ is related to elastic vs. fluid like properties, $\beta=0 \rightarrow$ elastic, ↑$\beta \rightarrow$ more viscous
  • Normal cells: $\beta = 0.18 \pm 0.07$
  • Latrunculin-treated cells: $\beta = 0.24 \pm 0.08$ (p<0.03)
Viscous Dampening during Bubble Flows

- Peak hydrodynamic stresses are applied quickly (~100-200 ms).
- ↑ viscous relaxation time ($t_R = \eta/G_{cell}$) mitigates deformation/injury
- Fluidization of cell may explain ↓ necrosis in latrunculin treated cells!
Cell Adhesion Studies

- Bubble propagation with different fluids.
  - Serum-free cell culture media, $\gamma = 57 \text{ dyne/cm}$
  - PBS, $\gamma = 72 \text{ dyne/cm}$

- $\uparrow \gamma$ results in significant detachment for sub-confluent cells.

- Why is there more detachment for 25% monolayer?

- Hypothesis: Cell morphology influences forces at and rupture of focal adhesions bonds.

**Bubble Velocity**

<table>
<thead>
<tr>
<th>Confluence</th>
<th>Control</th>
<th>0.3 mm/s</th>
<th>30 mm/s</th>
</tr>
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<tbody>
<tr>
<td>25%</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>100%</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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<tr>
<td>25%</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
</tbody>
</table>

- **Media**
  - PBS ($\uparrow \gamma$)
  - Media ($\downarrow \gamma$)

Ghadiali et al., submitted JAP, 2007
Multi-scale Modeling of Cell Adhesion

- Model FA complex with linear adhesion springs

- Spring constant, rupture distance based on integrin-Fn system (Chan et. al., Biomaterials, 1999)
  - $\sigma \sim 1.0$ dyne/cm
  - $x_{\text{rupture}} \sim 10$ nm

- Location of Focal Adhesions?

\[ f_{\text{bond}} = \sigma (x_m - \lambda) \]
Focal Adhesion Distribution

- TIRF Microscopy
  - Excitation at $\theta > \theta_c$
  - Evanescent wave propagates 100-200 nm into sample
  - High resolution imaging of focal adhesion sites.

- Peripheral organization of actin (green) and vinculin (red).

- Investigate effect of heterogeneous spring properties (interior vs. exterior).
Effect of Morphology on Cell Detachment

\[ k_i = k_p = 0.6 \]
\[ G'_\text{cell} = 2500 \text{ dyn/cm}^2 \]
\[ E_{\text{mem}} = 1E8 \text{ dyne/cm}^2 \]

Subconfluent cell peeled 50%
Confluent cell peeled 32%

- Sub-confluent cells are more susceptible to detachment due to change in morphology.
- Clinical implication: Injured lungs (subconfluent conditions) are highly susceptible to further lung damage (cell detachment)
Microbubble Induced Cell Detachment

Increasing Pressure Gradient

\[ \Delta P = 380 \]
\[ \Delta P = 780 \]
\[ \Delta P = 1180 \]

Increasing Shear Gradient

\[ \Delta \tau_s = 500 \]
\[ \Delta \tau_s = 1000 \]
\[ \Delta \tau_s = 1500 \]

Microbubble flows can peel cell off surface

Z-Displacement Magnitude [\( \mu m \)]

0 1 2 3 4 5

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Effect of Membrane Mechanics

Increasing Membrane Stiffness → Less Peeling

Subconfluent

<table>
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<th>Membrane Stiffness</th>
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<tbody>
<tr>
<td>$E_{\text{mem}} = 1E7$</td>
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<td>67%</td>
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</table>

Confluent

<table>
<thead>
<tr>
<th>Membrane Stiffness</th>
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<tbody>
<tr>
<td>$E_{\text{mem}} = 1E7$</td>
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<td>45%</td>
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</table>

Displacement Magnitude [µm]

$k_f = k_p = 0.6 \text{ dyn/cm}$

$G'_{\text{cell}} = 2500 \text{ dyn/cm}^2$
Conclusions

- Hydrodynamic stresses, Cell morphology, Cell mechanics, Molecular mechanics may all play a role in cellular injury.

- Coupling of mathematical models and experimental data is critical for understanding the mechanisms of cell injury during airway reopening
  - ↑Pressure gradients is responsible for ↑death at low bubble velocities
  - Changes in cell morphology is partly responsible for ↑death in sub-confluent cells
  - ↑Viscous dampening may be responsible for decreases in cell deformation/death.

- Advanced modeling techniques are required to understand complex emergent phenomena (effect of dP/dx and morphology on cell injury/detachment).
Integrating Life Science and Engineering

Biological/Physiological System are very complex!

Biologist/Physiologist provide powerful tools to probe these systems.

Engineers provide mathematical tools which can be used to understand how the different components interact.