Mechanotransduction & Acute Lung Injury: An Engineering Approach

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**Acute Lung Injury**

**IPF (Fibrosis)**

**Lung Cancer**

**URIs & Otitis Media**

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Acute Lung Injury & Ventilator Induced Lung Injury

• Infections (H1N1, SARS) → Necrosis and Detachment of alveolar epithelial cells.
• Alveolar-capillary barrier disruption, airway flooding, severe hypoxia
• 75k deaths/yr in US (Rubenfeld GD NEJM, 2005)
  Breast + Prostate cancer: 74k deaths/yr (ACS 2011 Cancer Facts)
• Standard of care: Mechanical Ventilation
  • Ventilator Induced Lung Injury
  • 30% to 40% mortality rates

Figure 3. The Normal Alveolus (Left-Hand Side) and the Injured Alveolus in the Acute Phase of Acute Lung Injury and the Acute Respiratory Distress Syndrome (Right-Hand Side).

Mechanical properties of cells govern several biological functions

- Motility, growth, division

Division (Mitosis)


Crawling

klemkelab.ucsd.edu/images/research/proteomics/fig3.gif
Mechanotransduction & Cell Mechanics

- Mechanical properties of cells govern several biological functions
  - Motility, growth, division

- Mechanotransduction: Mechanical Stimuli → Gene Signaling
  - Regulates Lung Morphogenesis (Varner & Nelson, 2014, Development)

- Question: What is the role of Mechanics and Mechanotransduction in Ventilator Induced Lung Injury?

Lung Mechanics & Ventilation Induced Lung Injury (VILI)

- Low Volume Injury (Atelectrauma)
- High Volume Injury (Volutrauma)

- Ventilated ALI
- Normal
- Oxygenation
- Lower inflection point
- Low Volume Injury (Atelectrauma)
- Ventilated

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Deformation-Induced Injury of Alveolar Epithelial Cells
Effect of Frequency, Duration, and Amplitude
DANIEL J. TSCHUMPERLIN, JANE OSWARI, AND SUSAN S. MARGULIES
Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania

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High Stretch causes:
- Cell death (membrane rupture)
- Epithelia and Endothelial Barrier dysfunction
- Activation of pro-inflammatory signaling

VT: 12 ml/kg → 6 ml/kg
Mortality: 39.8% → 31.0%

Atelectasis at Low Lung Volume - Derecruitment

- Surfactant inactivation causes alveolar instability, collapse and reopening (Nieman et al.)

- Barrier Disruption via Oleic Acid Injury causes flooding of non-collapsed alveoli (Hubmayr et al.)

- Airway Recruitment:
  - Complex fluid mechanical stress on epithelium (Ghadiali et al., JFM, 2003)
  - Do these forces exacerbate lung/cell injury?


Low Volume Lung Injury: Atelectrauma

- Simulate Atelectasis in rats and pigs:
  - ZEEP, NEEP or surfactant inactivation (DOSS)

- Reopening causes barrier disruption and pro-inflammatory signaling

- Prediction: Protocols that prevent atelectasis (high PEEP) should prevent lung injury.
Preventing Atelectrauma is Difficult

- Higher PEEP does not improve clinical outcomes
  - ↑ pressures cause barrier disruption and inflammation (Hong CM et al Anesthesia & Analgesia, 2010)
Biomechanics of Cell Injury during Atelectasis

Complex Set of Mechanical Forces on Epithelial Cells

- Pressure
- Pressure Gradient
- Shear Stress


Can changes in the cell’s mechanical properties prevent this type of injury?
Simple Ideas Don’t Always work

- Rigid cells are not protected from injury! (Yalcin, AJPLCMP, 2009)
- Engineering approach for complex systems
  - model the complex system (computational)
  - use model to suggest appropriate intervention.
  - Validate model predictions – clinical translation
Computational models of cell deformation during airway reopening (Ghadiali et al., JFM, 2003, Dailey & Ghadiali, JAP, 2009, Dailey, et al., Biomech Model Mechano Bio, 2009)

Conduct “in-silico” experiments → how to prevent deformation/injury?
• Computational models of cell deformation during airway reopening (Ghadiali et al., JFM, 2003, Dailey & Ghadiali, JAP, 2009, Dailey, et al., Biomech Model Mechano Bio, 2009)

• Conduct “in-silico” experiments → how to prevent deformation/injury?

![Graph showing relationship between power-law exponent and fluidization.](image)

- $G_0 = 195$ dyn/cm$^2$
- $2G_0$
- $3G_0$

$\varepsilon_{eff,max}$ [%]

$\alpha = 0.15$

$\alpha = 0.35$

Dailey, et al., Biomech Model Mechano Bio, 2009
• Confirm computational prediction: ↑ power-law exponent → more fluid-like cell → ↓ deformation/injury.  (Yalcin, AJPLCMP, 2009)

• Clinical Implication: Pharmaceuticals that fluidized the actin cytoskeleton may be useful in preventing airway reopening injury.
Clinically Relevant Fluidization

• Simvastatin – used to control serum cholesterol
  • moderate concentrations fluidize the actin cytoskeleton and reduce cell injury.
  • However, clinical trials and human studies are inconclusive (Kor, Crit Care Med. May 2012)

• Controlling Mechanically Induced Inflammation may be more important!!!

Mechanotransduction & Lung/Systemic Inflammation

Organ Failure occurs when vital organs stop working. It is the leading cause of death in the intensive care unit.

Mechanical Forces in the Lung
↓
Cytokine Release

http://www.sec.gov/Archives/edgar/data/1175151/000114420412033733/v315508_ex99-1.htm
• Air-liquid interface culture of primary human small airway epithelial cells (HAEpC, Promo-cell), polarized epithelium.

• Expose cells to 20 cmH₂O oscillatory pressure 0.2 Hz.

• Models high PEEP inflammation seen in humans.

• Assess IκB, NF-κB and cytokine production / secretion.

Pressure-Induced Inflammation in Primary Human Cells
Pressure-Induced Inflammation in Primary Human Cells

- Mechanically-induced inflammation is magnitude and time dependent.
- Rapid activation: 8-12 hours.
- Does this pressure-induced inflammation occur in-vivo?
Pressure Induced Inflammation In-Vivo

- C57/BALC mice ventilated for 4 hrs at high and low PEEP (3 and 6 cmH2O).
  - ↑ PEEP →
    - Normal Lung Mechanics
    - ↑ inflammatory response
  - Can changes in cell/cytoskeletal mechanics be used to regulate this inflammation?
Cell Mechanics does not Regulate Pressure-Induced Inflammation

- Fluid-like cells exhibit more inflammation!

![Graph showing fold change in NF-kB activation](image)

- *p < 0.05 wrt unloaded control
- ^p < 0.05 wrt no treatment

**No Agent (PBS control)**

**Latrunculin, 0.5uM**

(actin depolymerizer, soft)

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Cell Mechanics does not Regulate Pressure-Induced Inflammation

- Fluid-like cells exhibit more inflammation!

- Simvastatin can reduce inflammation but only at very large dose. Due to pathway inhibition not cell mechanics.

- Is there a biological way to regulate mechanotransduction/inflammatory gene expression?

Mean +/- SEM. * Significant difference (p-value<0.05) vs. no-pressure, and ^ significant difference (p-value<0.05) with respect to other doses evaluated.
MicroRNAs

- Regulate gene expression by suppression of translation or mRNA degradation
- MiRISC recognize target mRNA through **partial sequence complementarity**. Therefore, a single miRNA can mildly down-regulate hundreds of targets.
- miRNAs play an important role in angiogenesis, cancer and immune function (Urbich, Cardiovasc Dis, 2008; Croce, Nat Rev Genet, 2009; Sonkoly, Cancer Biol, 2008).
- Can miRNA’s regulate **mechanically induced inflammation**?
Mir-146a is a Mechanosensitive microRNA

A) B)

- Which miRNAs are mechanosensitive?
- Mechanical Pressure caused changes in several miRNAs
- miR-146a was significantly ↑ and is known to play a role in regulating pathogen induced inflammation.
- PCR screen confirmed that miR-146a was the most de-regulated miRNA.
- Is miR-146a trying to regulate mechanically-induced inflammation?
miR-146a Regulates Mechanically-Induced Inflammation!

Over-expression miR-146a (>100x) results in dramatic decrease in cytokine production.

Question: What targets of miR-146a are regulating mechanotransduction in lung epithelia?
miR-146a targets innate immunity (IRAK1 & TRAF6)
Silencing these targets also regulates mechanically-induced inflammation
Do other miRs that regulate innate immunity play a role in mechanotransduction?
Several other miRNAs (miR-146a, miR-155, miR-33a & miR-181a) can regulate pathogen induced inflammation.

Can these “Inflamma-miRs” regulate mechanically induced inflammation?

Yes! – but miR-146a appears to be the most effective.
Effectiveness of MiR-146a

• miR-146a is effective over a wide range of ventilation pressures (10-30 cmH2O) & frequencies (7-18 breaths/min)

• In-vitro experiments in lung epithelia (type II pneumatocytes and small airway)

• In-vivo translation: need to target right type of inflammation
Acute Lung Infection: Activated Alveolar Macrophage → neutrophil recruitment and bacterial/viral clearance (good inflammation)
Targeted Drug Delivery

• Acute Lung Infection: Activated Alveolar Macrophage → neutrophil recruitment and bacterial/viral clearance (good inflammation)

• Mechanical Ventilation: Mechanical Forces on Lung Epithelium, ↑ inflammation due to mechanotransduction (bad inflammation)

• Goal: Deliver microRNAs to lung epithelial cells only to reduce mechanically-induced lung inflammation.
Nanotechnology Based Drug Delivery

- Collaboration with: Drs. Jim Lee & Yun Wu (OSU NSEC and U Buffalo)

- **Technology: Lipoplex nanoparticles** (Wu et al, Mol Therapy Nuc Acid, 2013; Wu et al, BMES Annual Meeting, 2014; Wu et al, Nuc Acid Delivery, 2013)
  - Untargeted or Surfactant-C targeted (type II pneumocytes).
  - Nasal or Aerosol Delivery in C57BL/6 mice → high biodistribution to the lung
Nanotechnology Based Drug Delivery

• Delivery of Cy5 loaded lipoplex → ~90% delivery to ATII cells

• Delivery of test microRNA (miR-486) → ↑↑ expression in ATII cells

• Ongoing studies: delivery miR-146a → effect on mechanically induced lung inflammation.
Current Activities: Ex-vivo Translation

- Ex-vivo Model of Ventilation Induced Lung Injury

- Current projects:
  - miR-delivery to lungs ex-vivo
  - Scale up to human lungs (Dr. Bryan Whitson, MD, PhD, Surgery).

![Graph showing IL-1B Concentration (pg/mL) over time with annotations for 0 min and 60 min.](image)
• Engineering Approach → Changes in cell mechanics can be used to prevent physical injury to the lung – Simvastatin in clinical trials

• Mechanotransduction & microRNAs → miR146a is mechano-sensitive and can regulate mechanically-induced inflammation

• Nanotechnology → Delivery of microRNAs to specific cells in the lung (epithelial)

• Ongoing projects:
  • use miR146a to regulate ventilation induced lung inflammation without compromising innate immunity
  • Use ex-vivo models of VILI to test larger scale delivery and treatment of lung inflammation (porcine and human)
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Questions?