The Effects of Cell Mechanical Property Alterations on the Survival of Lung Epithelial Cells Under Shear Stress

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BACKGROUND
- Acute Respiratory Distress Syndrome (ARDS) is a clinical condition where edema fluid is accumulated in lung due to inflammation. The accumulated fluid results in a disruption for the process of gas exchange inside the lung.
- The ARDS patients can not inflate their lungs normally and therefore they would need to be mechanically ventilated.
- Mechanical ventilation further damage the delicate epithelium lining the walls of the small airways and alveoli. This medical condition is known as Ventilator-Induced Lung Injury (VILI)

GOALS & OBJECTIVES
To enhance the survival of Epithelial Cells (EpCs) in the distal lung during mechanical ventilation treatment of ARDS through the alteration of cells’ structure and elasticity by treating the cells with steroid drugs; Dexamethasone (DEX) and Trans-Dehydroandrosterone (DHEA).

METHODOLOGY
We utilized an in-vitro model developed previously in our lab to expose lung EpCs to conditions like the ones associated with VILI. The general experimental methodology is as following:
1. Cell Culture and Optimum Concentration for drugs: We utilized 1.2 rat lung cells. Cells were harvested with 0.125% trypsin, counted, and seeded onto 40-mm-diameter coverslips. These coverslips fit to the bottom of the flow chamber. The coverslips were coated with collagen solution prior to cell seeding, to prevent cellular detachment during bubble exposure. The optimum concentrations for the drugs that do not induce cell death at static cultures were for DEX and DHEA were 200μg/mL, and 2.5 μg/mL respectively.
2. Generation of airway reopening conditions: We model, we expose EpCs to shear stresses associated with airway reopening in ARDS (Fig 2A). Therefore, reopening conditions are generated using a parallel plate flow chamber (Fig 2B). Silicone gasket with thickness of 1 mm was used in this study to represent the flow in distal airways having about 1mm diameter (Fig 2C). A propagation of a single, long air bubble generated over the surface of the EpCs (Fig 2D).

RESULTS

Viability Results

Fig 3. Assessment of cell injuries for bubble experiments: A. Representative fluorescent labelled assay pictures. Static cultures (upper pictures) did lead to minor cell injury. Bubble flow resulted in significant cell death, and DEX, DHEA and LAT then flow exposure decreased cell injury significantly (lower pictures). Scale bars is 10 μm.

Cytoskeletal Staining:

Fig 4. Cell morphology for treated and untreated cells via AFM. While non-treated cells show a rounded morphology, all treated cells show a smaller more elongated morphology.

CONCLUSIONS
To conclude, we tested two anti-inflammatory steroid agents, DEX, DHEA to investigate their effects in changing actin cytoskeleton in EPCs and hence cell mechanics which is expected to alter their survival under shear stress. An in vitro flow system to mimic airway reopening was used in the project. According to our results, pre-exposure of either DEX or DHEA to cultured cells significantly decreased cellular injuries associated with VILI.

SIGNIFICANCE
Our results provided evidence for potential beneficial effects of anti-inflammatory agents DEX or DHEAs against VILI for ARDS treatment. Results from this study are critical for VILI and be readily applicable to future clinical studies for VILI.

POST PROJECT RECOMMENDATIONS & PLANS
- The role of cell’s stiffness on survival doesn’t seem to be clearly understood. It seems that there are two ways that can result less mortality rates 1) increasing stiffness 2) Decreasing Stiffness
- Advance techniques such as Optical Tweezers can be utilized in further studies for this project
- We have found in the literature more steroidal anti-inflammatory drugs like the ones that we are used that could have similar effects on cells. These drugs could be applicable for more studies in our approach to alter cell mechanics for VILI prevention. In addition, non-steroidal drugs have been found as well. In the future, both will be incorporated for similar studies
- We aim to study in the future the specific role of drugs in cytoskeleton reorganizations from a chemical perspective