Development and in Vitro Testing of a Nitric Oxide Nanoparticle Carrier for Acute Lung Injury

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Background
Acute respiratory distress syndrome (ARDS) is an infectious clinical condition in which gas exchange in the airways and alveoli are disturbed. Fluid-filled lungs need to be mechanically ventilated for airway reopening. Ventilation might further damage delicate lung tissue and lead to edema, a phenomenon known as ventilator-induced lung injury (VILI). This is a result of propagation of small air bubbles in gas exchange sites, injuring epithelial cells due to shear stress. Potential rescue of epithelial cells (EPCs) under injurious stresses is possible by altering their mechanical properties and hence deformation amount under stress (decreased stiffness, decreased deformation). This is possible by altering the cytoskeleton. Nitric oxide (NO) inhalation therapy for ARDS enhances oxygenation. Also, NO secretion was shown to decrease stiffness in various tissue types which can aid as a treatment of conditions like ARDS. One issue with using NO is that the life-time is too short so the treatment is not very effective. We have used nanoparticles which secretes NO in aqueous environment. We hypothesize that Administration of NO through releasing polymers will soften lung cells and suppress inflammatory markers which enhance survival of lung cells against shear stress.

Objectives
- Establishing an in-vitro model for exposing lung cells to shear stress.
- Testing the effect of NO releasing nanoparticles in cell stiffness and expression of inflammatory markers.
- Testing the effect of shear stress on lung cells.
- Testing the protective role of NO releasing particles on reducing cell injury that is induced by shear stress in the in vitro model of airway reopening.

Methodology
- We have developed an in-vitro model of airway reopening to expose lung EPCs to injurious stresses associated with mechanical ventilation using parallel-plate flow chamber.

Cell culture: L9 (ATCC® CCL149™) lung epithelial cells were grown on circular coverslips until confluence.

Study group categorization:
- Control 1: No chemicals or stress (ve control)
- Control 2: Cells subjected to stress by the flow chamber only (Bubble control)
- Control 3: Cells subjected to nitric oxide (NO) nanoparticles only (NO control)
- Experimental group 1: Cells subjected to stress by the flow chamber and then treated with NO nanoparticles.
- Experimental group 2: Cells treated with NO nanoparticles and then subjected to shear stress.

NO Solution preparation: (NO-8 particles were used).
A concentration of 5mg/ml of NO beads was prepared and 2 ml of solution was added to cell cultures and incubated for one hour for maximum NO release.

- Actin polymerization/depolymerization assessment
- AFM for stiffness measurement
- RT-PCR for inflammatory markers
- Rat IL-6 immunoassay (ELISA) quantification

Results & Discussion

![Viability](image)

**Viability**

![AFM](image)

**AFM**

Figure 2: Cella survival rate. Lung epithelial cells were treated with media as control (C), nitric oxide (NO), exposed to shear (S), nitric oxide then shear (NO/S) or shear then nitric oxide (S/NO). There was a significant decrease in cell death percentage with the treatment with nitric oxide pre and post shear exposure.

![Cytoskeletal staining](image)

**Cytoskeletal staining**

Figure 3: Live/dead staining results for the nanoparticles tested on L9 (ATCC® CCL149™) lung epithelial cells.

![RT-PCR](image)

**RT-PCR**

Figure 4: Relative quantification of inflammatory markers. Lung epithelial cells gene expression were quantified using RT-PCR. Cells were treated with NO, CCL2 or in combination with NO and CCL2. The mRNA was quantified using RT-PCR and the results compared to the control (SC).

![ELISA](image)

**ELISA**

Figure 5: Interleukins 6 (IL-6) protein levels. Lung epithelial cell IL-6 expression was quantified via ELISA and determined values of IL-6 were noticed in NO and N0/S compared to SC.

Conclusion & Future work

- Delivering NO particles with the aid of biotechnology might be an effective treatment for ARDS considering the positive effects of increasing softness and reducing inflammatory markers.
- Future studies: will include a broader inflammatory panel analysis (TNF-a and IL-1β) as it would give a broader insight on the effect of NO-RPs on the inflammatory statues of the cells. In addition to including another cell line such as lung alveolar cells would emphasize the effectiveness of the treatment. Finally, our collaborators produced a number of nanoparticles, future studies will include a comparison between these nanoparticles to determine the most effective particles to be tested later in animal models.

References