Undergraduate Students- Population, Health & Wellness

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

Haya Kordi[1], Amena Al-Sadi[1], Fatiha Benslimane[2], Huseyin Cagatay Yalcin[2]
[1]Biomedical Science Department, College of Health Sciences, Qatar University, Doha, Qatar
[2]Biomedical Research Center, Qatar University, Doha, Qatar

Background

Acute respiratory distress syndrome (ARDS) is an infectious clinical condition in which gas exchange inside 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 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.

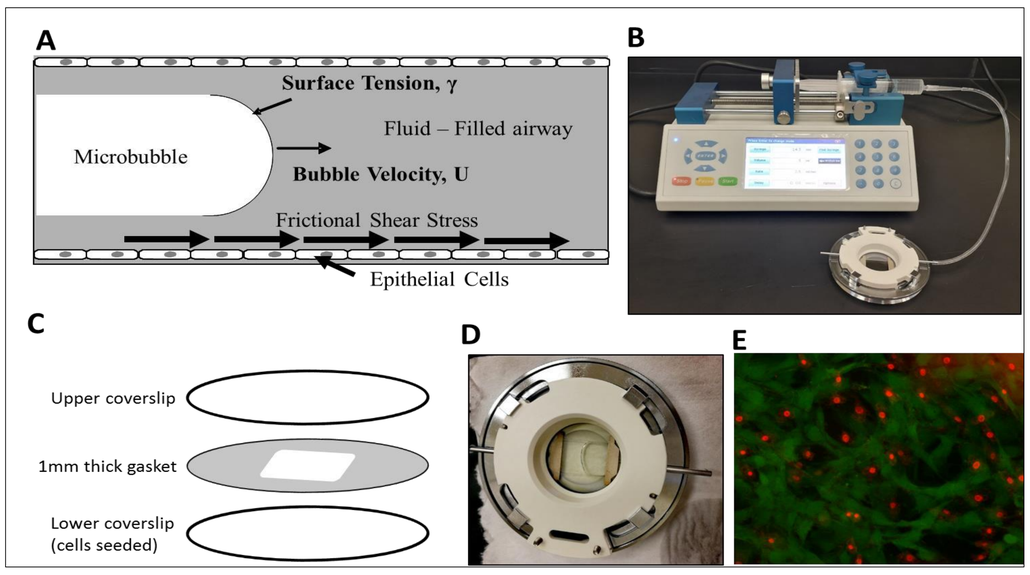

Figure 1: Experimental setup. A. We expose epithelial cells to frictional shear stress relevant to airway reopening in our setup. **B.** A single bubble is propagated over cell monolayer by filling the chamber and then retracting fluid over the cells. **C.** Parallel plate flow chamber is exposed of an upper cover slip, cell seeded lower coverslip, and membrane sandwiched between two coverslips. **D.** A propogating bubble is seen. **E.** Fluorescent live/dead stain method used to assess viability. Here, Green=calcein stained live cells, Red=ethidium stained dead cells.

Cell culture:
L2 (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 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

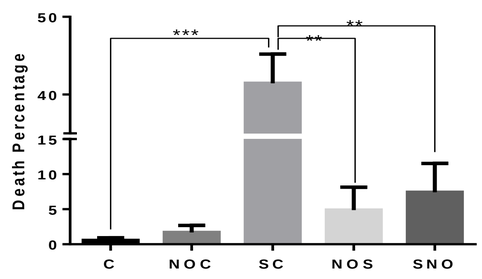

Figure 2: Cells survival rate. Lung epithelial cells were treated with media as control (C), nitric oxide (NOC), exposed to shear (SC), nitric oxide then shear (NOS) or shear then nitric oxide (SNO).There was a significant decrease in the death percentage with the treatment with nitric oxide pre and post sheer exposure.

AFM

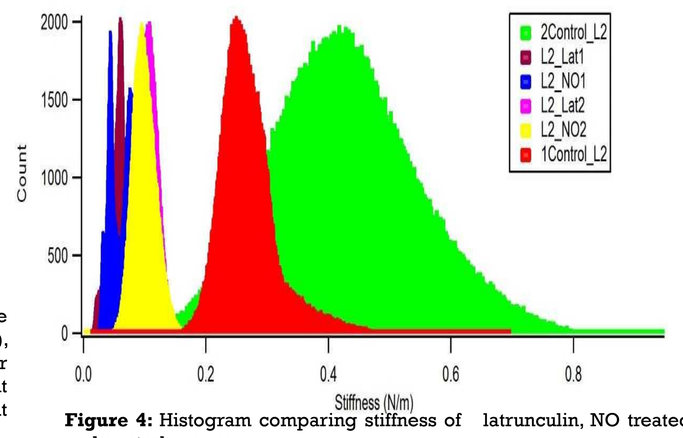

Figure 4: Histogram comparing stiffness of latrunculin, NO treated and control groups.

Viability

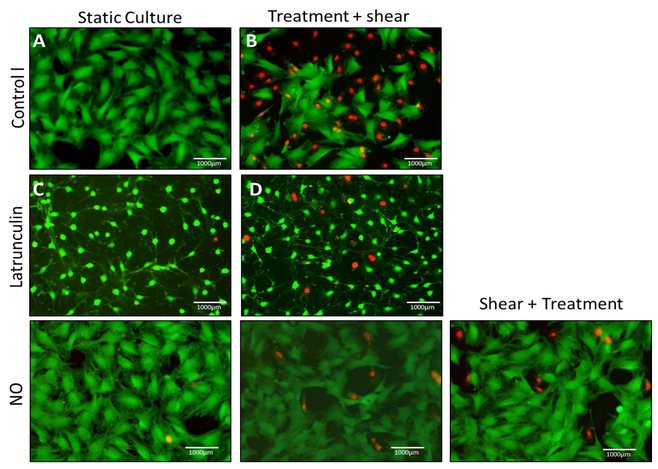

Figure 3: Live/dead staining results for the nanoparticles tested on L2 (ATCC® CCL149™) lung epithelial cells.

Cytoskeletal staining

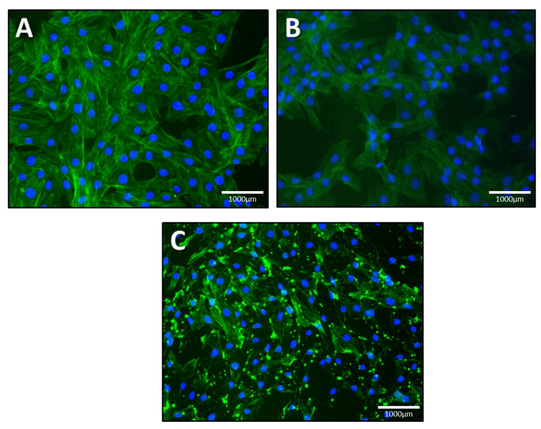

Figure 5: cytoskeletal staining of lung epithelial cells A: control, **B**: NO treated cells and **C:** latrunculin treated cells. Green= stained actin, Blue= DAPI labeled nuclei.

RT-PCR

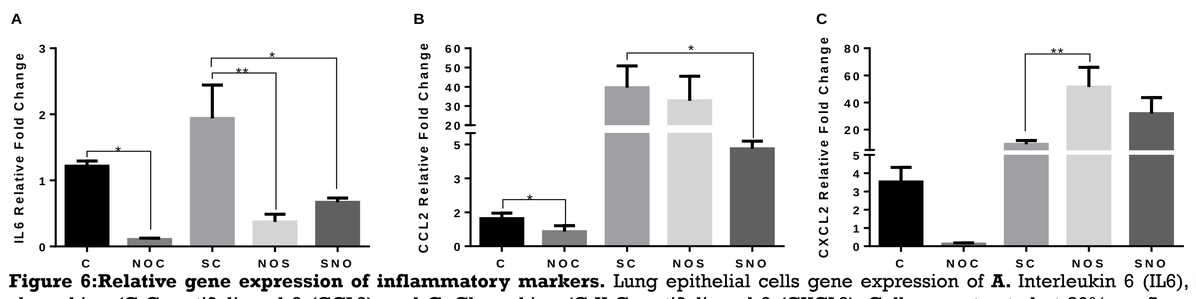

Figure 6:Relative gene expression of inflammatory markers. Lung epithelial cells gene expression of **A.** Interleukin 6 (IL6), **B.** chemokine (C-C motif) ligand 2 (CCL2), and **C.** Chemokine (C-X-C motif) ligand 2 (CXCL2). Cells were treated at 80% confluency. There was a significant decrease in IL6 and CCL2 when cells were treated NO pre and post sheer and a significant increase in CXCL2 between SC and NOS

ELISA

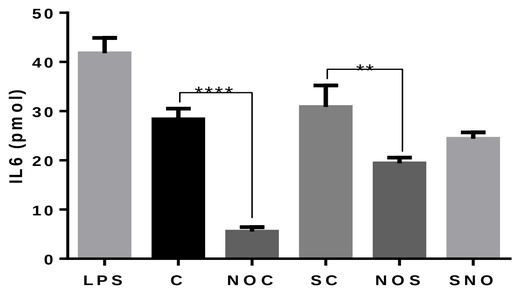

Figure 7: Interleukin 6 (IL6) protein levels. Lung epithelial cells IL6 expression was quantified via ELISA and depressed values of IL6 were noticed in NOS and SNO compared to SC.

Results

- NO administration into EpCs (pre or post shear stress flow), enhances cells survival against shear stress in our *in vitro* chamber.
- Further assessment revealed, this is due to softening of cell cytoskeleton as well as decrease in expression of inflammatory markers in response to NO

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

- Akmal, A. H., & Hasan, M. (2008). Role of nitric oxide in management of acute respiratory distress syndrome. *Annals of thoracic medicine, 3*(3), 100-3.
- Biehl, M., Kashiouris, M. G., & Gajic, O. (2013). Ventilator-Induced Lung Injury: Minimizing Its Impact in Patients with or at Risk for ARDS. *Respiratory Care, 58*(6), 927–937.
- Chiumello, D., Pristine, G., & Slutsky, A. S. (1999). Mechanical ventilation affects local and systemic cytokines in an animal model of acute respiratory distress syndrome. *Am J Respir Crit Care Med, 160*(1), 109-116.
- Yalcin, H. C., Hallow, K. M., Wang, J., Wei, M. T., Ou-Yang, H. D., & Ghadiali, S. N. (2009). Influence of cytoskeletal structure and mechanics on epithelial cell injury during cyclic airway reopening. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19700641.