Abstract

Emphysema is a pulmonary disease characterized by progressive destruction of lung tissue, leading to enlarged air spaces and increased compliance. Small animal models of emphysema are often used to study both macroscopic and microscopic changes in disease progression. In this study, we aim to (1) establish an elastase-induced model of emphysema, and (2) optimize techniques for preparing and stretching lung slices. In Project 1, mice were treated with porcine pancreatic elastase (PPE) to simulate emphysema progression in vivo. Histological and biochemical analyses were performed to quantify alterations in alveolar structure and function. In Project 2, we used a vibrating mini-stretcher to prepare precision-cut lung slices (PCLS) for use in ex vivo experiments. This platform allowed us to perform precise and controlled experiments on lung tissue, providing a valuable tool for studying emphysema.

Methods

Project 1

• Four (N=4) C57BL/6J male mice were treated with PPE through oropharyngeal instillation at 4.11, 6.85, 8.22, and 8.22 international units (IU)
• Control (N=2) mice underwent the same procedures as mice treated with PPE. Mice were anesthetized with ketamine/xylazine, and sacrificed at two weeks. The lungs were excised, flushed with saline, and sliced into rectangular strips
• Tissue strips from the control and PPE-treated mice were imaged using a confocal laser microscope during unstretched and stretched state
• Changes in alveolar morphology were analyzed using custom-written MATLAB (R2016a, MathWorks, Natick, MA) scripts.
• We calculated the total alveolar volume fraction, orientation, area, eccentricity, and minor/major axes ratios for individual alveoli in each image.

Project 2

• Lungs from male Sprague-Dawley rats were excised, filled with 2% agarose solution at 37°C, and chilled on ice to ensure gelling
• Lung tissue was trimmed and placed to a stage, a sleeve was then placed around the tissue/stage and filled with agarose for support during slicing
• A vibrating mini-stretcher (Capea Instruments Ltd, United Kingdom) was used to prepare precision-cut lung slices (PCLS) with thicknesses 250μm, a 4mm biopsy punch was then used to divide the slices into symmetric, circular tissue samples. See Figure 1
• Slices were placed incubated for 12 hours in media supplemented with CSE (concentrations ranging from 0 to 2 cigarettes/100ml media).
• 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was used to determine cell viability and CSE-dose response curve for the incubated tissue slices.
• We designed and calibrated a 3D-printed, 6-well, reusable “flexcell” insert with a silicone membrane for use in a multi-well stretcher device.
• Acrylic dots were added to the deformable silicone membrane, and twenty-nine images across a range of displacements were captured for each of the six wells
• Changes in the area of the circular region was used to relate membrane strain area to vertical displacement of the traveling stage.

Discussion

• We demonstrated that intratracheal PPE instillation in mice can be used to model tissue alterations in patients with emphysema.
• We optimized a novel ex vivo platform for studying the pathophysiological effects of smoking in stretched PCLS.
• Future work will investigate the relationship between stretch pattern and cell inflammatory response using the calibrated multi-well stretcher.

Conclusions


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References