Testing a novel 3D-bioprint design of airway smooth muscle (ASM)

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Introduction

- Asthma is the leading cause of hospitalization in Canadian children[1]
- The disease is characterized by airway wall remodeling and excessive contraction of ASM, altering lung tissue stiffness[2]
- Conventional 2D cell models fail to accurately replicate the microenvironment changes that occur in stiffened tissues
- However, *3D bioprinting technology* aims to create functional tissue constructs that mimic the increased wall stiffening in the human body

- **Objective of this study**:
  - To test our novel "spiderweb" design and its ability in holding up to contractions

Figure 1. Visual depiction of the spiderweb design and printed construct. ASM ring constrained between 6 layers of acellular ink above and below.

Methods

1. Pre-Bioprinting
- Create a 3D design
- Prepare an alginate-based bioink with natural matrix proteins and ASM cells

2. Bioprinting
- Bioprint ASM constructs:
  - ASM ring within acellular frame of varying alginate concentrations: 0.75%, 1.00%, 1.25%
  - A printed plastic holder is used to prevent muscle collapsing

3. Post-Bioprinting
- Conduct experiments to test muscle shortening and strength
- Measure and analyze luminal area

Results

Contractile Experiment

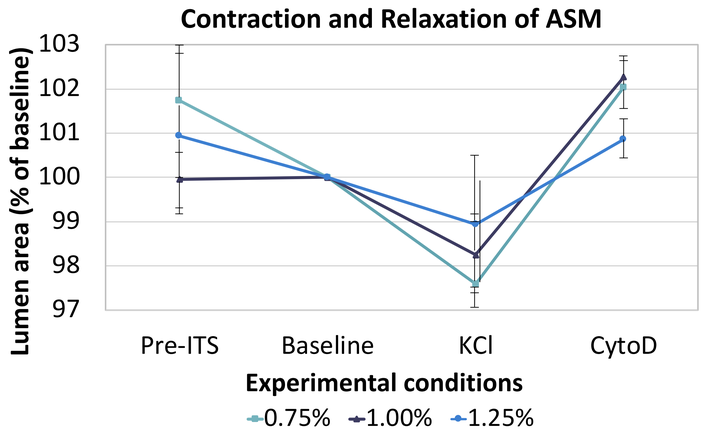

Figure 2. Graphical results of the contractile experiment from each condition with reductions in area representing contractions. ASM minimally constricted during serum withdrawal, KCl caused a modest contraction (<5% lumen area reduction), and Cytochalasin-D notably reversed the contraction The effect of acellular stiffness on contraction and relaxation was not significant (p=0.7883).

Citrate Experiment

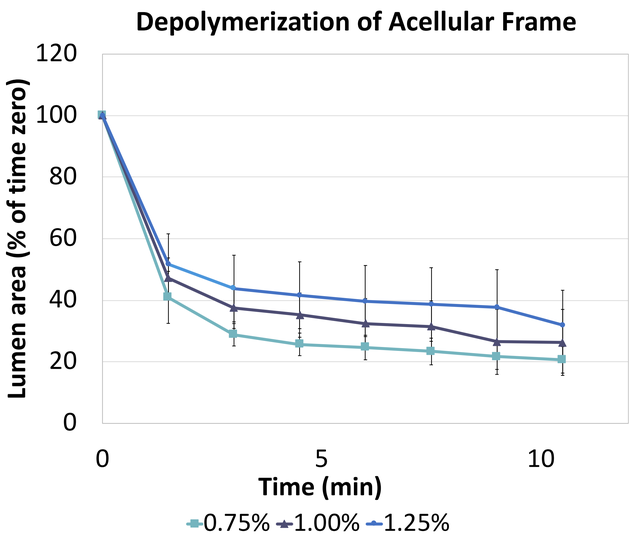

Figure 3. Graphical results of the citrate experiment over a period of ten minutes. High concentrations of citrate and EDTA resulted in a rapid depolymerization (<1 minute) and a large reduction in luminal area (>50%). Reductions in the lumen area were significant, with 0.75% acellular frame being greater (p=0.0126).

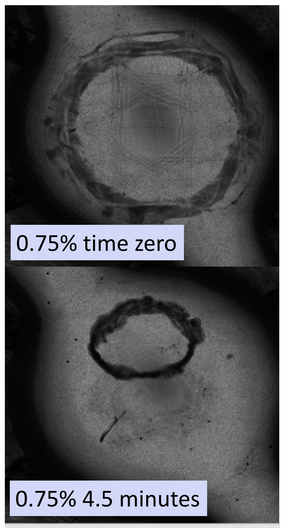

Figure 4. Results of the depolymerization of 0.75% acellular frame, demonstrating a dramatic collapse when the load is removed

Discussion

- **Contractile experiment** demonstrates the muscle ring's highly elastic structure
  - KCl acts as a potassium depolarizing solution, allowing calcium dependent contractions
  - CytoD acts as a filament disruptor, disrupting the ability for the cell to maintain contractile force

- **Citrate experiment** indicates the acellular structure is able to provide a strong load against contractions
  - Citrate pulls calcium out of solution to depolymerize and dissolve alginate

Conclusion

- Our novel "spiderweb" design allow tissue constructs to create a large amount of force
- The acellular load does an excellent job at holding the muscle, thus has the ability to hold well to contractions

- Future directions:
  - Characterize and experiment on how ASM responds to varying stiffness
  - Provide potential insight into the role of tissue stiffening in asthma

References:

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(2) Doeing, D.C., & Solway, J. (2013). Airway smooth muscle in the pathophysiology and treatment of asthma. *J. Appl. Physiol, 114*(7): 834-843. doi: 10.1152/japplphysiol.00950.2012

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