Modelling Cystic Fibrosis Chronic Infection Over An Engineered Mucus-like Hydrogel

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INTRODUCTION

Airway mucus:

- Protects airways by trapping and clearing harmful pathogens
- Biological hydrogel, primarily composed of water
- Main solid component is large glycoproteins called mucins

In cystic fibrosis (CF), a genetic disease which negatively impacts the lungs, airway mucus has many altered properties, including increased **viscoelasticity** [1] and increased **solids and mucin protein concentration** [2], [3]. These altered properties reduce the ability for trapped pathogens to be cleared, leading to chronic bacterial airway infections. Chronic bacterial infections of CF airways are known to show increased resistance to antibiotics and to be associated with increased mortality in CF patients. This emphasizes the importance of a system which can be used to study bacterial colonization of these infections *in vitro*.

This project aims to develop healthy and CF mucus-like hydrogels which are compatible with both mammalian cells and relevant CF pathogens, with the final goal being to combine these elements to model chronic CF airway infections *in vitro.*

	10 mg/ml Alginate			15 mg/ml Alginate		
	0 mg/ml Mucins	10 mg/ml Mucins	40 mg/ml Mucins	0 mg/ml Mucins	10 mg/ml Mucins	40 mg/ml Mucins
0.5 mg/ml $CaCl_2$	Gel-A	Gel-B	Gel-C	Gel-D	Gel-E	Gel-F
0.6 mg/ml $CaCl_2$	Gel-G	Gel-H	Gel-I	Gel-J	Gel-K	Gel-L

OBJECTIVES

1. Develop healthy and CF mucus-like hydrogels with differing **compositions** and **viscoelastic properties**.
2. Deposit multiple species of bacteria, using an aqueous two-phase system (ATPS), onto the mucus-like hydrogels.

RESULTS

OBJECTIVE 1: Hydrogel development and characterization

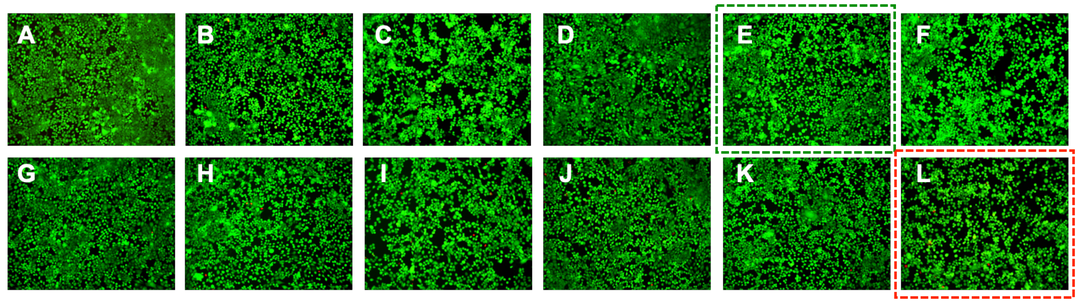


FIGURE 1: All potential hydrogel formulations resulted in acceptable cell viability. Live/dead assay was used to evaluate cell viability of a human bronchial epithelial cell (16-HBE) monolayer after incubation beneath the hydrogel layer for 48 hours. Live/dead assay consisted of Calcein AM (live) and Ethidium Homodimer-1 (dead). **A, D, G, J:** Hydrogel formulations with 0% mucins. **B, E, H, K:** Hydrogel formulations with 1% mucins. **C, F, I, L:** Hydrogel formulations with 5% mucins. 10X magnification.

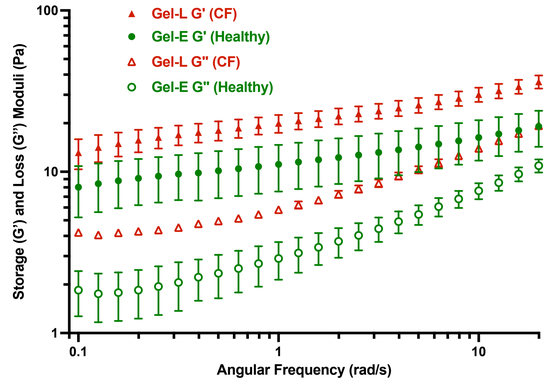


FIGURE 2: Chosen hydrogel formulations for CF and healthy model showed significantly different viscoelastic properties and both exhibited elastic-dominant viscoelastic behavior. Storage (G') and loss (G'') moduli were determined for hydrogel formulations at multiple angular frequencies using TA Instruments AR-2000 rheometer.

OBJECTIVE 2: Microbial species growth

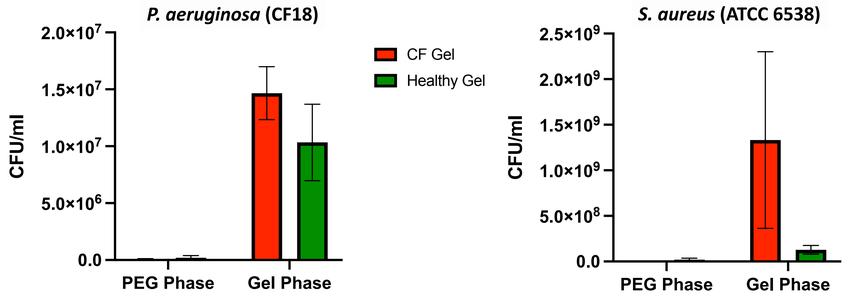


FIGURE 3: Relevant CF pathogens preferred growth within the hydrogel phase of the airway models. *P. aeruginosa* and *S. aureus* were grown in short term (5 hour) co-culture over top of the CF and healthy hydrogel formulations. Microbes were grown within an ATPS to encourage confined growth on top of and within the hydrogel. Microbial species growth in the hydrogel phase and in the liquid polyethylene glycol (PEG) phase was determined using colony forming unit (CFU) enumeration.

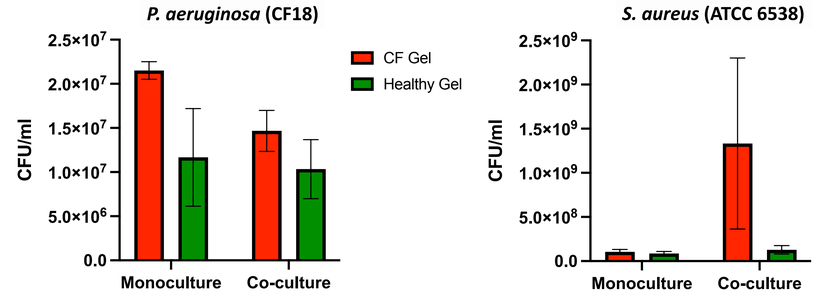
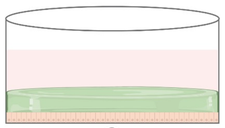


FIGURE 4: *S. aureus* (ATCC 6538) showed synergistic growth in microbial co-culture while *P. aeruginosa* (CF18) growth was relatively unchanged in microbial co-culture. Microbial growth of *P. aeruginosa* and *S. aureus* in short term (5 hour) co-culture with the hydrogels was compared to when the species were grown in monoculture with the hydrogels. *S. aureus* appeared to have increased growth in co-culture with *P. aeruginosa* and the CF hydrogel.

METHODS

Hydrogel development, characterization, and cytocompatibility testing



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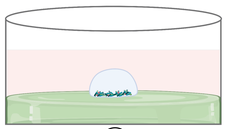
Growth of relevant CF pathogens on and within chosen hydrogels



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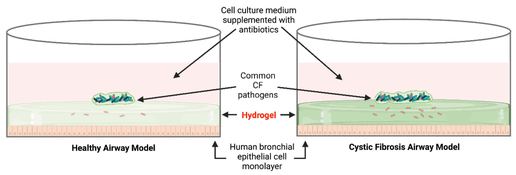
Final system of airway models



CONCLUSION

- The inclusion of a CF mucus-like hydrogel allows for a more realistic in vitro model to study host-microbe and microbe-microbe interactions in CF airway infection
- The addition of a healthy airway model to the system can provide a better understanding of how mucus physical properties and composition impact bacteria growth in CF airway infections

Future direction:

- The final system of airway models (a combination of Objectives 1 and 2) will be exposed to relevant CF antibiotics and the growth and antibiotic susceptibility of the microbes within the models will be examined

References

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Acknowledgements

We acknowledge funding support from Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant program (RGPIN-2018-05742), the Canada Foundation for Innovation – John R. Evan Leaders Fund (project# 36032) and Research Nova Scotia - Scotia Scholar Award (CO).

Special thanks to the Cheng Lab (Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia) for providing training and access to *P. aeruginosa* (CF18).