Bacteria-mucus interactions & their role in chronic lung infections

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Introduction

Mucus is a biological hydrogel, comprised of biopolymers called mucins (MUC5B and MUC5AC), that lubricates and protects epithelial cells throughout the body as well as helps to control the microenvironment surrounding the cells. In patients with obstructive lung diseases, such as Cystic Fibrosis, Chronic Obstructive Pulmonary Disease and Asthma, due to defects in ion transport proteins, mucus accumulates along the airway resulting in impaired muco-ciliary clearance, leaving the epithelial cells more susceptible to bacterial infection.

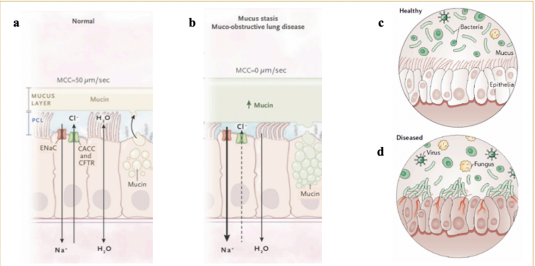

Fig. 1. Healthy vs unhealthy airway. **a** A normal/healthy airway has proper ion transport into and out of the epithelial cells and has a muco-ciliary clearance rate (MCC) of 50 μm/sec. **b** In an unhealthy airway there is mucus accumulation and a MCC of 0 μm/sec.[1] **c** Healthy airways have micro-organisms dispersed throughout the mucus and separated from the epithelia. **d** Unhealthy airways leave the epithelia susceptible to bacterial infection due to impaired muco-ciliary clearance.[2]

In this work, we explored how P. aeruginosa affects mucus gel assembly and how gel properties influence bacterial growth by tracking nanoparticles in mucin-based hydrogels with and without P. aeruginosa present in them. Using a previously established mucus model we can understand mucus-gel assembly and the influence of P. aeruginosa on mucus.

Methods

Pseudomonas aeruginosa

Strain: PAO1
- Fluorescent

Culture Conditions:
- Plated on Pseudomonas isolation agar

Media:
- Brain Heart Infusion (BHI) broth media

Mucin-based hydrogels

Mucins:
- Porcine gastric mucin (PGM) → primarily MUC5AC mucin
- Bovine submaxillary mucin (BSM) → primarily MUC5B mucin

Crosslinking Agent: 4-arm PEG-thiol (PEG-4SH)
- This reagent helps to initiate cross-linking of mucins and hydrogel formation

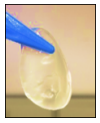

Fig. 2. Hydrogel[3]

Mucins + Crosslinking Agent = Hydrogel	(BHI media + Mucins) + Crosslinking Agent = BHI media hydrogel

Particle tracking microrheology

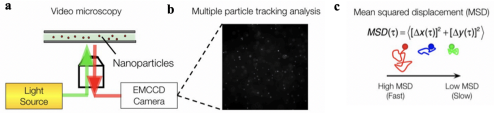

Fig. 3. Process of particle tracking microrheology. **a** Fluorescent video microscopy is used to track the diffusion of nanoparticles throughout hydrogels. **b** Image of a video taken during fluorescent video microscopy. **c** Computer analysis of the videos outputs values for the mean square displacement of each nanoparticle. This is used to determine rheological properties of the hydrogel such as pore size, viscosity, and elasticity

Preparation of BSM hydrogels

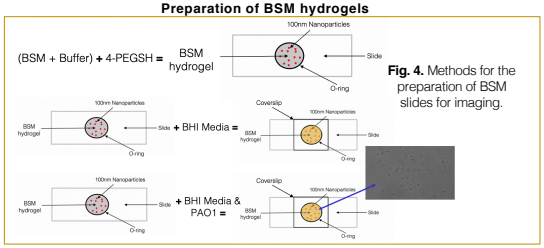

Fig. 4. Methods for the preparation of BSM slides for imaging.

Previous Work

Multiple particle tracking of nanoparticles in PGM hydrogels

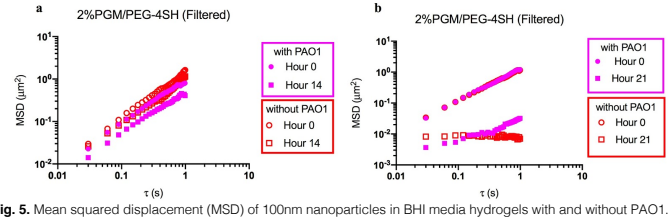

Fig. 5. Mean squared displacement (MSD) of 100nm nanoparticles in BHI media hydrogels with and without PAO1. **a** MSD over a period of one second at hours 0 and 14. **b** MSD over a period of one second at hours 0 and 21.

Previous experiments demonstrated that the introduction of PAO1 during gelation drastically altered hydrogel assembly (Fig 5). To decouple the effects of the addition of PAO1 during assembly compared to effects on the final structure, a topical addition of PAO1 implemented into the experimental set up (Fig 4).

Results

Multiple particle tracking of nanoparticles in BSM hydrogels

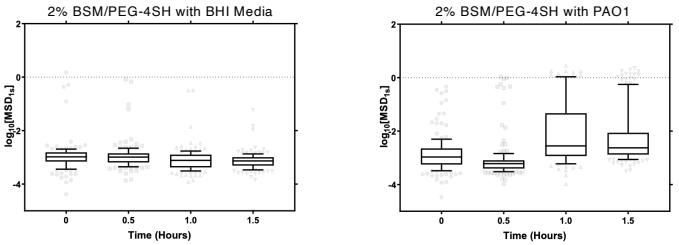

Fig. 6. Mean squared displacement (MSD) of 100nm nanoparticles in BSM hydrogels over a period of 1.5 hours. **a** MSD over a period of one second in a 2% w/v BSM/4 arm-PEG-thiol hydrogel with BHI media on top. **b** MSD over a period of one second in a 2% w/v BSM/4 arm-PEG-thiol hydrogel with PAO1 in BHI media on top.

Embedded 100 nm particles showed no change in mean squared displacement (MSD) after 1.5 hours of exposure to BHI media (Fig.5a), demonstrating a tightly crossed liked hydrogel network. However, after 1 hour of exposure to PAO1 a sharp increase in MSD is observed indication a disruption of the mucin hydrogel network.

Confocal microscopy of PAO1 in mucin hydrogels

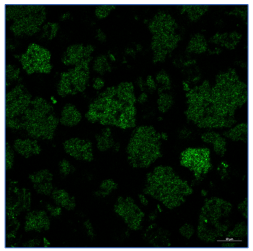

Fig. 7. PAO1 on top of a 2% BSM gel. Imaged using a Zeiss LSM 800 microscope.

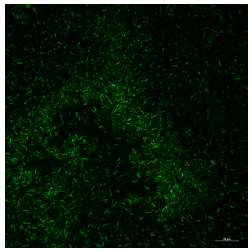

Fig. 9. PAO1 in BHI Media Imaged using a Zeiss LSM 800 microscope.

Confocal images reveal mucin hydrogels disrupt the dispersion of PAO1 (Fig. 6) compared to BHI media (Fig. 7). This disruptive effect was also previously observed in natively purified mucins.[5] This results highlights the ability of mucin hydrogels to model physiological conditions.

Conclusions

- PAO1 increases the gelation time of BHI media hydrogels
- PAO1 disrupts the BSM hydrogel network, demonstrated the by the increase in 100 nm particle displacement over 1.5 hours.
- PAO1 dispersion is altered by BSM hydrogels compared to media suspension
- This work provides a physical relevant model to systematically study the interactions between *pseudomonas aeruginosa* and mucus.

Future Directions

1. Work with Prof. Amanda Oglesby-Sherrouse's Lab at the University of Maryland Baltimore (UMB) to study gene regulation in PAO1 and bacterial behavior beyond growth in BHI media hydrogels
2. Study a different type of bacteria known to infect individuals with Cystic Fibrosis

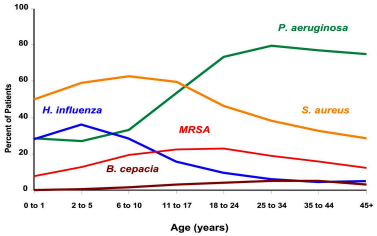

Fig. 9. Types of bacteria that can infect an individual with Cystic Fibrosis and the percent of people who get those infections based on age.[4]

Acknowledgements

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References

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