Modeling Airway Dysfunction in Asthma Using Synthetic Mucus Biomaterials

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Statement of Purpose: As asthma worsens, occlusion of airways with mucus significantly contributes to airflow obstruction and reduced lung function. Recent evidence from clinical studies has shown mucus obtained from adults and children with asthma possesses altered mucin composition, specifically a shift from mucin 5B (MUC5B) to mucin AC (MUC5AC) as the predominant secreted mucin.[1] Mucins are large (~MDa) glycosylated proteins that form the mucus gel through entanglements and disulfide crosslinks, resulting in a mesh-like network that acts as a selectively permeable barrier and gives rise to the viscoelastic properties, providing the lungs with a defense mechanism against infection from respiratory viruses. However, how mucin composition alters the functional properties of the mucus gel is not yet fully understood. To study this, we have engineered a highly tunable synthetic mucus biomaterial to mimic the properties of native mucus to understand the transport and barrier function of native mucus in health and disease.

Methods: Mucus hydrogels were prepared using two types of commercially available crude mucins: porcine gastric mucin (PGM) containing MUC5AC, and mucin from bovine submaxillary glands (BSM), containing MUC5B. Mucins were mixed with a cross-linking reagent, 4-arm PEG-thiol to mediate cross-linking between mucin biopolymers.[2] To mimic disease progression, mucins were mixed at varying ratios of MUC5B:MUC5AC (75:25 – healthy, 50:50 – mild asthma, and 25:75 – asthma in exacerbation). Bulk rheology and particle tracking microrheology (PTM) was used to determine the biophysical properties of mucus biomaterials with varying mucin ratios. Functional properties of the mucus hydrogels were assessed by transplanting the biomaterial on to human airway epithelial (HAE) cultures, which can recapitulate *in vivo* airway features, such as coordinated ciliary beating. Transport was assessed by tracking apically applied microspheres and measuring their velocity. Barrier properties against respiratory virus were assessed by directly measuring the diffusion of fluorescent influenza (IAV) within synthetic mucus gels using PTM and determining the extent of infection in synthetic mucus-coated HAE tissue cultures by staining for IAV nucleoprotein after 2 hours of infection.

Results: We found by systematically varying mucin composition that mucus gel viscoelasticity is enhanced when predominantly composed of MUC5AC, resulting in a significant reduction in transport rates on HAE cultures (**Fig 1. A, B**). We measured the diffusion rate of fluorescently labeled IAV in synthetic mucus with different MUC5B:MUC5AC ratios and found IAV had a higher average diffusion rate in MUC5AC-rich synthetic mucus compared to MUC5B-rich gels (**Fig 1. C**). We further tested if synthetic mucus with variable mucin content were more

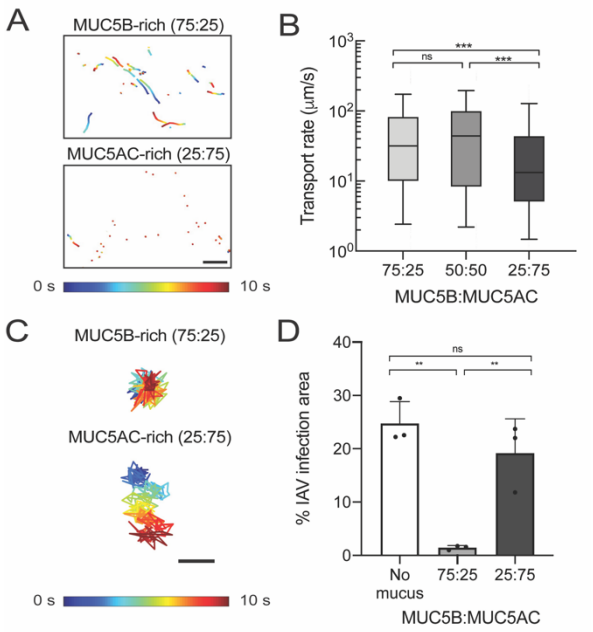

Figure 1. (A) Trajectories of microspheres on synthetic mucus gels transplanted onto HAE cultures. Color scale: 10 seconds. Scale bar = 100 μm. **(B)** Box-and-whisker plots of microsphere transport rate on HAE cultures with hydrogels with varying MUC5B:MUC5AC ratio. **(C)** Trajectories of IAV diffusion using PTM. Color scale: 10 seconds. Scale bar = 200 nm. **(D)** Percentage of infection area as determined by IAV nucleoprotein staining for uncoated and synthetic mucus coated HAE cultures.

or less effective at blocking IAV infection in HAE cultures. In these studies, HAE cultures coated with MUC5AC-rich synthetic mucus resulted in similar frequency of infected cells to uncoated controls, based on IAV nucleoprotein staining, while MUC5B-rich gel-coated cultures were highly protected and resulted in a significant reduction in infection (**Fig 1. D**).[3]

Conclusion: Using bioengineered synthetic mucus, we have found that the changes observed in mucus composition in asthma can have a major impact on gel viscoelasticity which in turn reduces its capacity to be effectively cleared. We also observed asthma-like mucus biomaterials were less capable of protecting the airway surface from infection by IAV. The model established herein could also be applied to other lung diseases to understand airway dysfunction and uncover disease mechanisms that provide the basis for new therapeutic interventions.

References: (1) Lachowicz-Scroggins, M. E. *AJRCCM*. 2016 **(2)** Joyner, K. *Soft Matter*. 2019 (**3**) Song, ACSBSE. 2021