Recovering functionalities of deficient mucus with a polyethylene glycol-lectin conjugate

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Statement of Purpose: Mucus is a fascinating biomaterial located at the interface of our wet epithelium and the external environment. This hydrogel protects our body from external threats such as dehydration and infectious pathogens. However with ageing and in some pathological circumstances like inflammatory bowel disease, the mucus barrier is altered and leads to dehydration and increase risk of infection. It would be beneficial to restore mucus functionalities in these situations, however the literature is scarce in strategies to complement functionally-defective mucus. In this study, we show that one key aspect of mucus function, hydration, can be achieved by binding to the compromised mucosa a synthetic and highly hydrated polymer. The polymer is conjugated with a carbohydrate-binding lectin to maximize its interaction with mucins (the main component of mucus) and cell surface.

Methods: We generated a mucus complement composed of 1) a high molecular weight and highly hydrated polyethylene glycol polymer (PEG, 40 kDa) that recapitulates certain properties of mucins and 2) the wheat germ agglutinin lectin protein (WGA), that efficiently binds glycans located on the cell surfaces and in mucus. The two molecules were conjugated by reacting succinimidyl carboxymethyl ester modified PEG with the free amine groups on the WGA molecules. The WGA-PEG conjugate was tested for its capacity to hydrate and prevent bacteria attachment using a mucus models, made of purified mucin molecules adsorbed on polystyrene surfaces. A model for an altered mucus layer was also developed by chemically removing the mucin glycan's. The hydration level of mucins or treated mucins was obtained by combining quartz crystal microbalance with dissipation monitoring (QCM-D) and ellipsometry measurements. The ability of the Escherichia coli bacteria to bind mucins, altered mucin or mucin treated with the mucus complement was tested using both the surface adsorbed mucus model and the HT29-MTX colonic cell line that is able to secrete a mucus layer. The mucus layer could be altered by treatment with a mucus-dissolving agent (N-acetylcysteine).

Results: The synthesis resulted in a range of WGA-PEG conjugates with one or more PEG molecules per WGA monomer. The mucus layer model composed of adsorbed native mucins was highly hydrated, with ~95% water. On the other hand, the altered mucus layer model, yielded thinner, less hydrated monolayers, containing ~40% water. The WGA lectin retained its capacity to bind native mucins after being conjugated to PEG molecules and when added to dehydrated mucin coating, the muco-complement was able to fully restore the hydration of a model dehydrated mucin coatings (see Figure 1B).

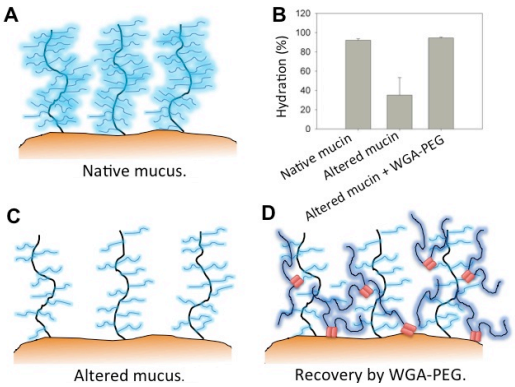

Figure 1. Schematic of a healthy mucus layer (A), loosing its hydration and barrier properties in pathological cercumstances (B), and recovered fucntionalities like hydration (B) by WGA-PEG (D).

Mucin monolayers were able to reduce Escherichia coli adhesion. Mucins altered by deglycosylation could not prevent adhesion, however when treated with WGA-PEG, the repelling effect was recovered. The ability of WGA-PEG to recover mucin's natural ability to reduce E. coli adhesion was confirmed using a mucus-secreting cell line. The conjugate coated the cells and their mucus-secreted layer. The addition of WGA-PEG decreased E. coli adhesion both with and without the presence of a mucus layer. Thus the conjugate acted as an efficient antifouling agent for living surfaces. A preliminary toxicity study showed that the conjugate is not cytotoxic both for bacteria and cells.

Conclusions: The treatment of the wet epithelium with WGA-PEG is a promising strategy to treat pathologies caused by an altered mucus layer. The treatment complements surfaces depleted in mucus by restoring hydration and reducing risk of infection. This strategy could also be applied as a preventive treatment against common respiratory bacterial infections by reinforcing our natural barrier. Further studies will investigate how the addition of these polymers affects the rheology and the commensal microbes hosted in the three-dimensional mucus gel.