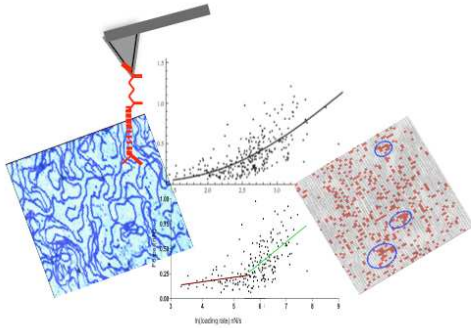
University of BRISTOL

MUCUS GELS MODULATE BACTERIAL ADHESION

MONICA BERRY

NSQI, School of Physics, University of Bristol, Bristol, UK



Mucins are highly glycosylated linear polymers whose gels line, for example, the eye and the respiratory and gastrointestinal tracts. Commensal bacteria play an important role in mucin gel turnover, binding to and degrading mucins, while organisms that overcome the gel defences may cause infection and disease. Microorganisms have an exquisite selectivity for binding partners: a sialic acid might be adhesive or not depending on its linkage to the preceding sugar in the chain. Using atomic force microscopy (AFM) we characterised the distribution of sialic acids and mucin peptide core epitopes to understand the packaging of mucins in ocular surface gels and assess the balance of pro- and anti-adhesive epitopes at the gel surface.

Here, we addressed purified human ocular mucins, whose architecture and glycosylation have been previously studied (1, 2) and preocular gels (3). AFM tips were functionalised with lectins specific to two sialic acid linkages and antibodies to sequences within the mucin peptide core. The frequency and distribution of each of the epitopes was analysed from topographic and adhesion maps obtained using a Dimension AFM with a Nanoscope IV controller (Veeco, USA) in Force Volume mode, in liquid. For each bioprobe, molecular bond characteristics (rate of dissociation, and potential width) were compared between purified mucins and mucus gels to evaluate any effects of the molecular environment. Clustering of epitopes was established by comparison with randomly-generated distributions.

Irrespective of mucin molecules being deposited on mica or part of a gel, lectins unbinding from sialic acids best fit a double energy barrier model (4). Similar behaviour has been described for other lectins, and correlates with extended and bent conformations in P and L lectins (5). The ratio of α2,3– to α2,6–linked sialic acids is reversed at the surface of the preocular gel compared with purified mucins. Far fewer clusters of α2,3-linked sialic acids are seen on the gel surface than on mucin molecules, while the opposite is true for α2,6 – linked sialic acids, which are more numerous and have more neighbouring interactions on the gel surface than in purified mucins. Unbinding forces between antibodies and the mucin peptide core show an exponential relationship with the loading rate, rather than discrete transition states. When pulled through, or out of, the network, gel forming mucins extend further than from purified networks adherent to mica, suggesting that they are either highly coiled, or very mobile in the ocular and buccal gels.

Mucin molecules are not fully exposed at the surface of the preocular gel, a configuration that could protect from proteolytic cleavage by external organisms. Furthermore, for the ocular surface, the surface is largely anti-adhesive for *Pseudomonas aeruginosa*. For this pathogen, isolated pro-adhesive epitopes at the gel surface are unlikely to promote adhesion, while the few clusters might promote the wrapping of bacteria by mucins and subsequent elimination from the mucosal surface.

1. McMaster TJ, Berry M, Corfield AP, & Miles MJ (1999) Atomic force microscopy of the submolecular architecture of hydrated ocular mucins. Biophys J 77(1):533-541.
2. Royle L, et al. (2008) Glycan structures of ocular surface mucins in man, rabbit and dog display species differences. Glycoconj J 25:763–773
3. Berry M, Brayshaw D, & McMaster TJ (2004) Dynamic molecular resolution imaging of preocular fluid impressions. Br J Ophthalmol 88(11):1460-1466.
4. Baos SC, Phillips DB, L Wildling, McMaster TJ, & Berry M *in the press* Distribution of Sialic Acids on Mucins and Gels: A Defense Mechanism. Biophys J. doi:10.1016/j.bpj.2011.08.058
5. Phan UT, Waldron TT, & Springer TA (2006) Remodeling of the lectin-EGF-like domain interface in P- and L-selectin increases adhesiveness and shear resistance under hydrodynamic force. Nat. Immunol. 7:883–889.