Exploring Barriers to Mass Transfer at the Intestinal Mucosa: The Mucus Layer

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The Mucus Layer - 1

- Secreted by goblet cells and absorptive enterocytes found around the villi (see photo).
- Largely known for its protective role of the epithelial surface and a likely significant contributor to mass transfer barrier known as the UWL.
- Composed of ~ 95% water and ~5% mucins (a glycoprotein that is a product of MUC genes).
- A semi-permeable gel-like membrane.

Microscopic view of the cellular composition of the apex of a villus (Science Photo Library 2011). Goblet cells are stained pink while absorptive enterocytes are stained green.
Previous macro- (Cone 2010) and micro-rheological (Sellers et al. 1991) characterization of mucus was conducted on post mortem samples scraped from animal intestinal mucosa.

This procedure disrupts regional variation (Sawaguchi et al. 2002) and may alter mucus physical characteristics as it detaches the basal layer from the apical membranes of enterocytes.

The current work characterizes the physical and micro-rheological properties of villus mucus in situ which would provide much needed information that will enhance our understanding of the role of small intestinal mucus layer.

This is needed before the dynamics of mass transfer of nutrients and pharmaceuticals across the mucus layer can be realistically modeled.
The apparatus used to study living intestinal villi

- Design of an experimental apparatus that allowed living ileal intestinal villi to be maintained in the visual field of a laser micro-rheometer.
- Development of a system of micro-adjustment enabling the focal plane to traverse the length of the villus.
Summary of methods

> The experimental apparatus allows clear visualization of individual living villus.
> Application of mucin stain (Dextran Alexa Fluor 488, Life Technologies) allows mucus coat to be visualized.
> Micro-rheological analysis is performed in situ and is related to villus width and distance from the tip.
Micro-rheometry of villus mucus

> Fluorescent naked or amine coated polystyrene beads applied to the villi.

> Naked beads adhere to mucus and allow its physical limits and intrinsic elasticity to be determined by applying force with laser tweezers.

> Amine coated beads float in the mucus layer and allow local apparent viscosity and rheology to be determined.

> The Brownian motion of beads at various points along the villi (and its mucous environment) are recorded as videos.

> Relative movements of the beads are tracked and their movements analyzed.
Mean Squared Displacement (MSD) plots of ensembles surrounding the apices of villi.

MSD obtained from our experimental work (for a similar sized bead) done in situ of the mucus in its villus environment from small intestinal tissue.

MSD obtained by Dawson et al (2003) for in vitro micro-rheological analysis of mucus in sputum of patients with cystic fibrosis.

Mean Squared Displacement (MSD) plots were comparable with those reported by other researchers of sputum mucins.
> At lower frequencies of shear, mucus behaves as a viscous fluid (seen with the higher magnitude plot of $G''$ over $G'$) while at higher frequencies of shear, the mucus exhibits elastic gel-like properties (seen with the higher magnitude plot of $G'$ over $G''$ after the crossover point).

> Our mucus samples thus demonstrates ‘shear-hardening’ attributes as postulated by Taylor et al. (2003).
Special mucus for the small intestine?

- There have been no previous reports on the rheology of mucus in situ or on villus mucus - our study shows it exhibits ‘shear-hardening’
- This differs from the ‘shear-thinning’ reported in sputum and mucus harvested post mortem (Dawson et al. 2003, Ceilli et al. 2007).
- This indicates, either that the rheology of mucus in situ differs from that in bulked samples, or that the behaviour of villus mucus differs radically from that in other regions.
- Villus mucus is known to be broadly similar in chemical composition to others in the GIT but to differ in the degree of glucosylation and sialation.
General resistance to mechanical abrasion?

- Gut mucus may protect the delicate lining of the gut from mechanical injury

- Shear rates are normally low as digesta moves slowly through the gut. Mucus are less viscoelastic at low shear rates and thus may lubricate and facilitate flow

- Shear rates will be higher where a sharp edge projects from a bolus and is driven against the mucosa. Local stress hardening will help to protect the mucosa from damage.
Where do we go from here?

1. **Is mucus evenly deposited around the villus or is there more at the tip?** (The latter would hinder mass transfer at the site of active absorption and incapacitate villus vascular countercurrent systems).
   > *Assess the depth of mucus at various points around the villi using a larger bead.*

2. **Does mucus only protect the tip of the villus from mechanical abrasion?**
   > *Assess the micro-rheological properties of the mucus layer at various distances along the length and away from a villus.*
   > *Assess the mucus tensile breaking strength of mucus around the villi using strongly adherent polystyrene beads.*

3. **Are these properties more evident in sites where shear forces are greater e.g. the colon?**
   > *Compare mucus properties at various locations along the GIT i.e. the small intestine and colon.*
References


Thank you for your time!!

Any Questions??