Understanding Mucus: Components, Carriage and Conduct

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Introduction

Mucus is found in the eye, the dermis, and the cervico-vaginal, gastrointestinal and respiratory tracts. It plays a vital role in protecting cells and preventing bacterial infection.
Mucin, a glycoprotein (multiple sugars attached to a protein), is the gel-forming component of mucus and the major non-water constituent.

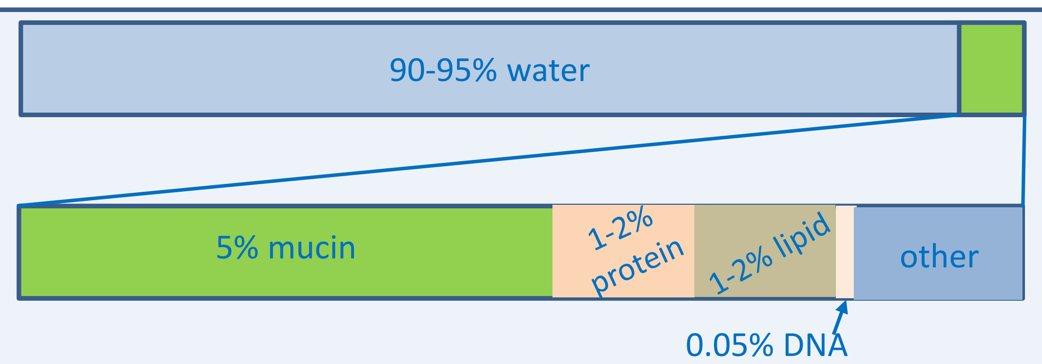

Figure 1. The constituents of mucus.

Mucin forms a network within the mucus creating small holes, or pores, in the gel for substances to penetrate, or permeate it, allowing them to be absorbed into the body.
Mucus currently used in absorptive research can be toxic to cell cultures, posing a huge problem. Commercial supplies of mucus and mucin may not be pure, so using it would not mimic reality.

Aims

- To understand the composition and behaviour of small intestinal mucus.
- To formulate a recipe using purified mucus constituents to make a non-toxic mucus-like gel to be used in experimental research, to develop drug-delivery systems through the mucus barrier.

Results

PAS and Bradford assay

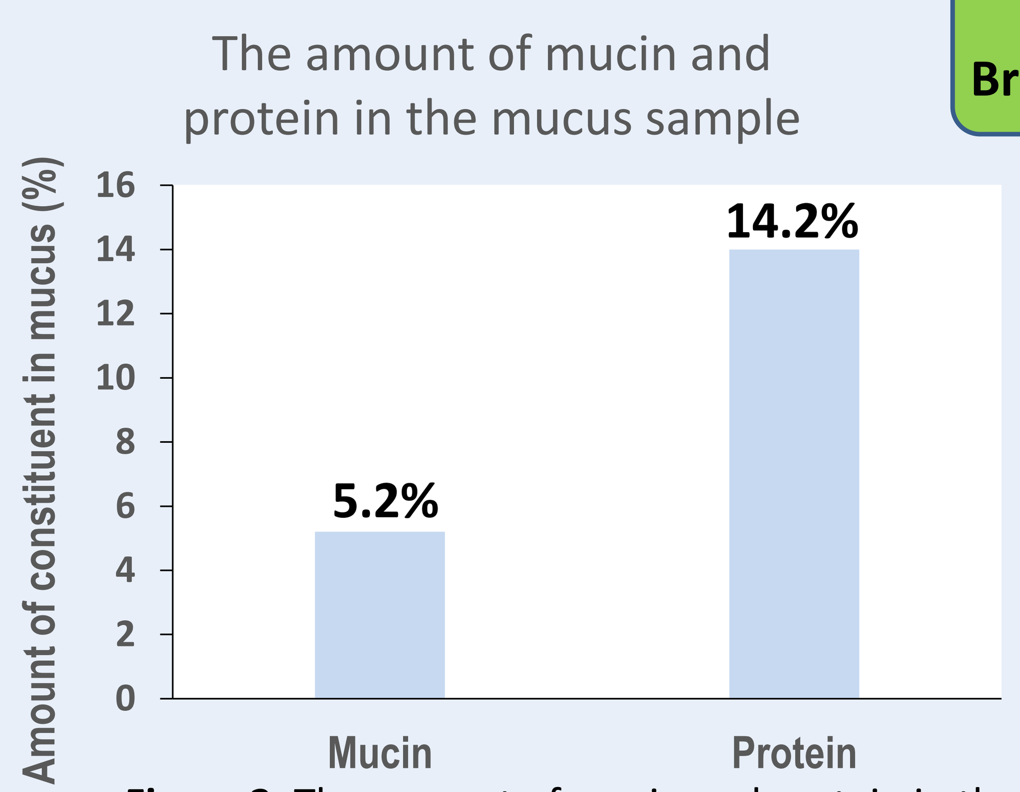

Figure 2. The amount of mucin and protein in the sample of small intestinal mucus.

Rheology: the study of the deformation and flow of matter

Breakdown point: the point at which a gel becomes a liquid

Viscometry: the study of the flow of a liquid

Rheology

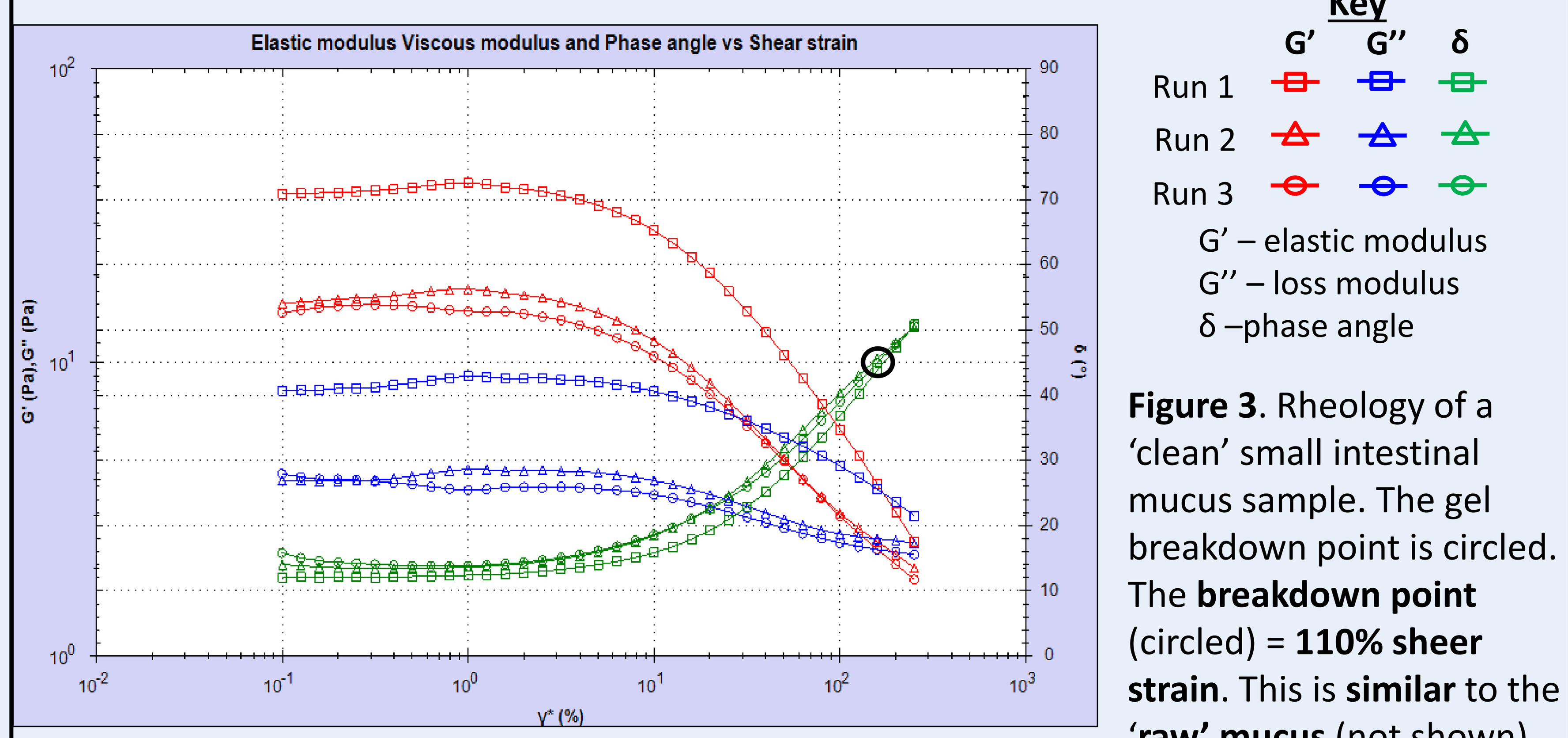

Figure 3. Rheology of a 'clean' small intestinal mucus sample. The gel breakdown point is circled. The **breakdown point** (circled) = **110% sheer strain**. This is **similar** to the **'raw' mucus** (not shown).

Permeation assay

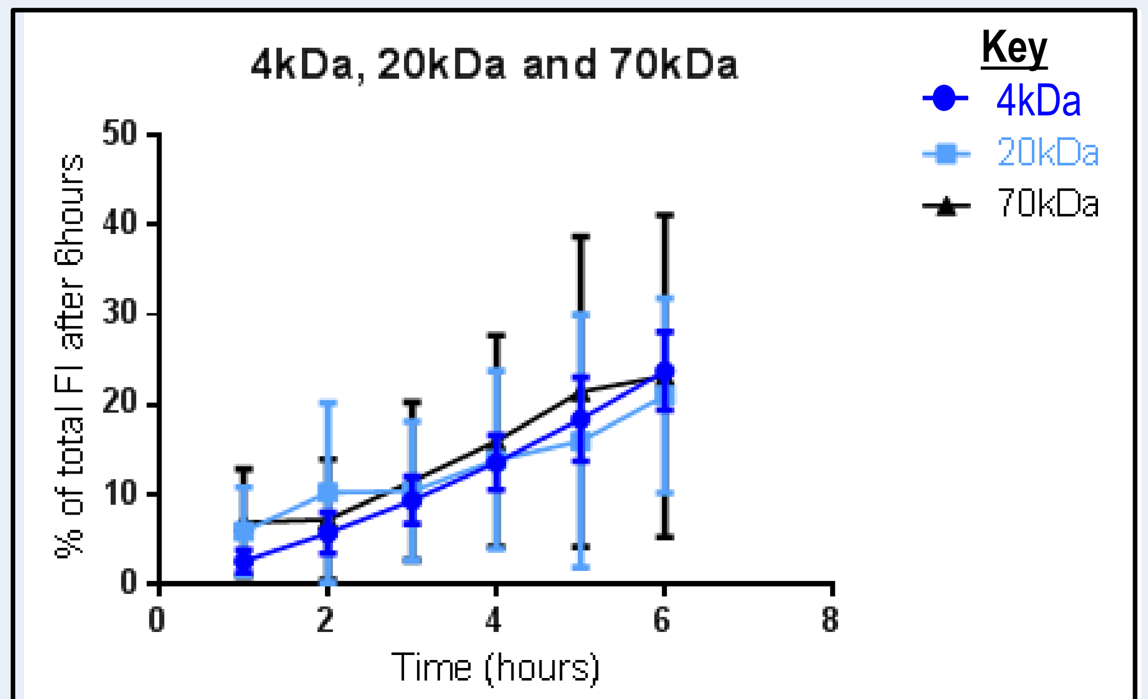

Figure 4. Permeation assay through small intestinal mucus samples using 3 different sized particles.

Viscometry

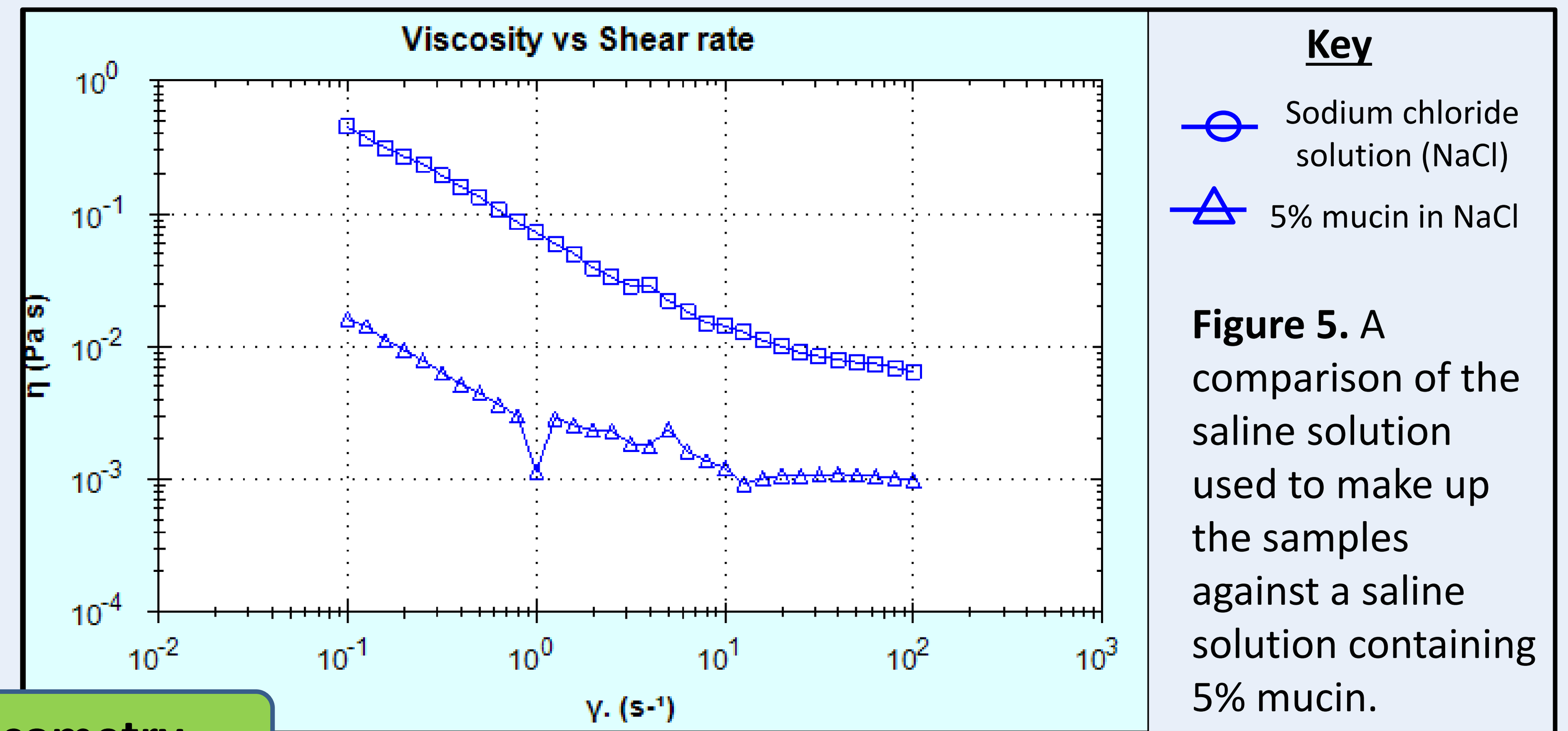

Figure 5. A comparison of the saline solution used to make up the samples against a saline solution containing 5% mucin.

Methodology

Raw mucus → Clean mucus

Periodic Acid Schiffs Assay to determine the amount of glycoprotein in mucus, i.e. mucin.

Bradford assay to determine the amount of protein in mucus.

Rheology to study the solid and liquid properties of the mucus gel, e.g. the breakdown point.

Permeation assay to test how size can affect travel though mucus. 3 fluorescent molecules of varying sizes were compared.

Raw mucus → Pure mucin

Vacuum dialysis to concentrate the mucus-like solution to a gel.

Viscometry to study the liquid properties of the mucus-like solution.

Discussion

The determined concentration of mucin in mucus supported the literature, validating the chosen assay. The concentration of protein did not coincide with the literature, suggesting that the assay could be detecting other proteins, e.g. the protein component of the mucin, or protein from an overly vigorous mucus extraction process which sheds too many cells. A different assay, such as the Lowry assay, could be used instead.
There was no significant difference in permeation between different sized particles used, hence further analysis is required. Different types of particles could also be used.
The rheology and viscometry results provide standards to which different combinations of mucus constituents can be compared in the future.

Conclusions

- Further analysis of protein levels in mucus are required, ideally using other methods.
- The pores in mucus are large enough to allow the passage of all 3 fluorescent molecules through mucus.
- Different approaches should be considered to create a mucus substitute. This will enable more rheological tests to be carried out to improve the mucus-like gel recipe.

Acknowledgements
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Reference
Pearson JP, Chater PI, Wilcox MD. The properties of the mucus barrier, a unique gel--how can nanoparticles cross it? Therapeutic delivery. 2016;7:229-44.

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