Electronic Supporting Information (ESI)

Rheological Characterization of Mammalian Lung Mucus

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ESI 1.Experimental Section: Mucus Collection and Separation.

Mucus was collected via postmortem lavage method on a 14 year old, 446 kg quarter horse gelding humanely euthanized by intravenous barbiturate overdose. The respiratory tract of the horse was deemed normal based on physical examination and pulmonary auscultation antemortem, as well as postmortem gross necropsy and histopathology findings. Briefly, within 10 minutes of death, with the horse in dorsal recumbency, a 26mm diameter cuffed endotracheal tube was passed via the oral cavity into the proximal trachea and the cuff was inflated. The head was elevated, a tube was passed into the endotracheal tube, and approximately 4 liters of physiologic saline solution (0.9 % NaCl) was passed into distal trachea and lungs. Saline was retained within the lungs for 45-60 seconds during which time repeated cycles of external compression were applied to the thorax. The horse's head was then lowered and hind legs were elevated 1.5 meters by use of a hoist. Fluid was allowed to drain freely from the endotracheal tube into a sterile stainless steel container. Approximately 2 liters of fluid was retrieved and stored at ~4 °C overnight before rheological characterization. Lung lavage solution was centrifuged for 1hr at 4180 rcf and mucus was collected from the walls of the centrifuge tubes. The saline residue was discarded, and additional lung lavage fluid was added to the same centrifuge tubes. This process was repeated to attain sufficient mucus from the walls of the centrifuge tube for mechanical characterization.

ESI 2.Drying Effects on Lung Mucus Morphology.

'As-collected' mucus in the presence of physiologic saline solution was placed on a glass slide and left to dry inside a ventilated hood. Optical microscopy images were then collected using an Olympus BX51 polarized light microscope. As observed, different globular fiber-like structures were obtained after the mucus was dried, matching the morphological structure obtained at pH 5.5 (Fig. 1E). Despite the distinct similarities of this elongated branch structure, future studies should address the mucin aggregation and interactions of mucin chains with more powerful imaging techniques such as atomic force microscopy, as discussed elsewhere.[1-4]

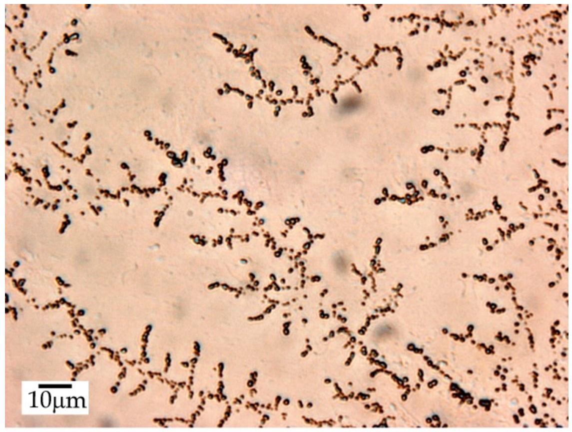

Figure S1. Dried mucus structure for the 'as-collected' sample showed branch-like structures

ESI 3.ThermogravimetricAnalysis of Horse Lung Mucus Samples

Experimental Conditions. For the thermogravimetric analysis (TGA) of mucus samples, 7-8 mg samples were placed directly in an alumina cup using a microsyringe. Samples were heated from ambient temperature (22 °C) to 500 °C in a SDT Q600 (TA instruments), under a nitrogen atmosphere at 10 °C/min rate. These measurements were done to investigate the solid and water contents in these mammalian lung mucus samples. An average solids content of 2.16 ± 0.22 % was measured.

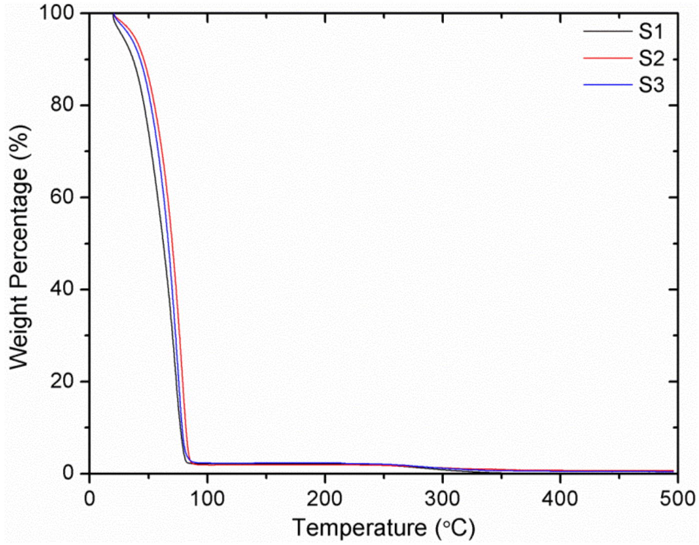

Figure S2. Thermogravimetric analysis for three different horse lung mucus samples showed an approximate 2.2 % solids content.

ESI 4.Rheological Characterization of Horse Lung Mucus Samples

Equipment: A Discovery Hybrid HR-2 rheometer (TA-instruments) was used for rheological characterization purposes.

Rheological measurements: Flow sweep and amplitude sweep experiments were conducted at 22 °C, from 0.01 to 100 s^{-1} shear rate, and 5 points per decade were collected. Amplitude sweep tests were performed at 1 rad/s, from 0.1 to 500 % strain, collecting 5 points per decade, under continuous oscillations (direct strain) mode, and using transient data acquisition mode. Frequency sweep tests were conducted at 2% strain, from 0.1 to 10 rad/s, collecting 5 points per decade. For all experiments, a thin-layer of oil was used at the periphery to avoid sample desiccation.

SAOS Measurements: Frequency sweep results demonstrate a soft-solid (gel-like) behavior for mucus samples as shown in Figure S3. These results are due to possible crosslinking in mucus samples or by the presence of mucin fiber-like structures observed in the morphological characterization of the samples (Fig. 1).

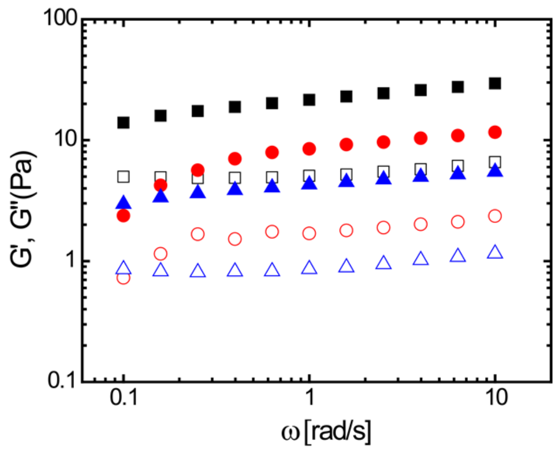

Figure S3. Storage modulus, G' (filled symbols), and loss modulus, G" (open symbols), for three horse lung mucus samples measured at a constant strain (γ_o) of 2%.

LAOS Measurements: Amplitude sweep results for sine strain data was analyzed using MITlaos version 2.1 for Matlab.[5]

LAOS: Elastic properties

The minimum-strain modulus (G'_m) and the large-strain modulus (G'_L) provides information about the nonlinearity behavior of a particular sample.[6] At low strain values, it was observed that $G'_m = G'_L = G_1' = G'$ which is an indicator of linear viscoelastic regime. At large strain-values, slight variations on the strain moduli were obtained; thus, a non-linear viscoelastic regime is expected as discussed in the main manuscript. Results for the different strain moduli obtained for three different lung mucus samples are shown in Figure S4.

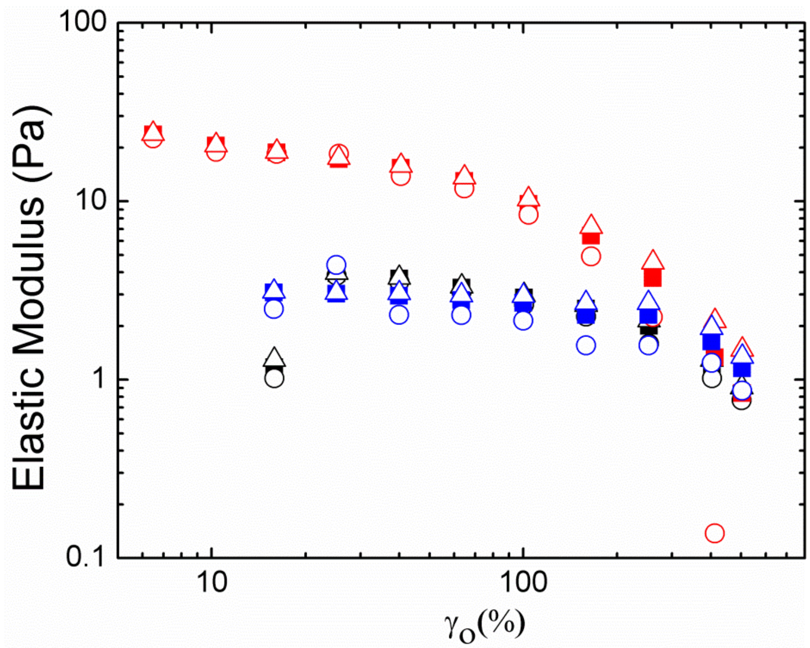

Figure S4. Different viscoelastic moduli for G_1' (squares), G_m'(circles), and G_L'(triangles) for three separate samples (black, red, and blue**)**

References

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