NOTE: Different macro- and micro-rheological properties of native porcine respiratory and intestinal mucus

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ABSTRACT: *Aim of this study was to investigate the similarities and differences at macro- and microscale in the viscoelastic properties of mucus that covers the epithelia of the intestinal and respiratory tract. Natural mucus was collected from pulmonary and intestinal regions of healthy pigs. Macro-rheological investigations were carried out through conventional plate-plate rheometry. Microrheology was investigated using optical tweezers. Our data revealed significant differences both in macro- and micro-rheological properties between respiratory and intestinal mucus.*

Mucus is a biological hydrogel with a highly inhomogeneous and complex structure[1,2] with unique viscoelastic properties[3-6]. As a consequence mucus represents also an important biological barrier that prevents immediate access to the surface of mucosal epithelial cells which holds for both molecules as well as particles. However, in the same way as mucus function may differ from organ to organ (e.g. lubrification, protection, etc.), its viscoelastic properties are also likely to be different according to the respective biological needs. In order to address this hypothesis, we have chosen to compare mucus from the intestinal and the respiratory tract as mucus from either organ has to perform rather different functions. The pig was chosen as mucus from this source can be obtained relatively easily, also considering ethical aspects.

Mucus-extraction: Mucus was collected from pigs used for experimental surgery studies, which had to be killed afterwards. Therefore, no animals had to suffer or to be sacrificed to obtain this biological material, in line with the 3R-concept. Care was taken that the preceding surgery experiments had no influence on the organs relevant to our studies. Mucus was collected within 30 minutes after euthanasia. The trachea was cut into halves[4] and mucus was obtained by carefully scratching. Intestinal mucus was harvested from the ileum and duodenum of the same animal after a short rinse with water[5].

Hydroxyethyl cellulose (HEC) gel preparation: For comparison, HEC gels of 1 and 2 % (w/v) were prepared using NatrosolHHX 250 (Hercules Aqualon, Düsseldorf, Germany).

Macro-rheological studies: Macro-rheological studies of storage and loss module (G' & G'') were performed with a 25 mm plate-plate geometry in a MARS II rheometer (ThermoHaake, Karlsruhe, Germany) as used in previous studies[5,6]. The volume of the mucus samples, put in the rheometer, was between 20 and 30 µL, resulting in a gap distance between 40 and 60 µm. Measurements at 1 Hz were used for comparison.

Micro-rheological studies: Passive and active microbead rheology with optical tweezers provides a promising laser-based method for investigating the Brownian motion and induced movement of microparticles in a laser trap[7,8]. A Tweez250i system from Aresis (Ljubljana, Slovenia) was utilized. Melamine resin beads of 2.86 and 6 µm diameter were purchased from Microparticles GmbH (Berlin, Germany).

Sample preparation was performed for both mucus and HEC gels as described in previous experimental studies[7].

Passive microbead rheology was studied by recording the restricted Brownian motion of microparticles at a frame rate of ~670 fps. Based on the experimental conditions, mainly the viscosity of the interstitial fluid in the mucus-pores is measured by this method. For more details see supplementary material S1.

Active microbead rheology was performed by applying a sinewave (Frequency = 0.1 Hz & Amplitude = 1 µm) to microparticles. The amplitude of particle movement (output displacement response) to an input displacement of 1 µm is measured from the displacement of the particle over time. As the amplitude of this active particle movement is at least 10times larger than the movement in passive microbead rheology measurements, additional macro-rheological effects cannot be excluded.

Statistics: OriginPro 2015 (Northampton, USA) and One-way ANOVA were used for Box-and-whisker plots as well as for statistical calculations. Moreover the Scheffé`s method as post hoc analysis was applied. The level of statistical significance is indicated by the corresponding p-value.

Macrorheology: Viscoelastic properties of porcine mucus from ileum, duodenum and trachea are depicted in Figure 1A and 1B. One-way ANOVA reveals statistically significant ($p<0.05$) differences between intestinal and respiratory mucus, but there is no significant difference between mucus from ileum and duodenum.

Figure 1: Macro-rheological A: elastic (G') and B: viscous moduli (G'') of mucus preparations at 1Hz. At least 20 measurements were performed for each region.

Passive microbead rheology: Figure 2 shows the results of the elastic moduli G' from passive microbead rheological studies by means of optical tweezers for 2.86 and 6 µm particles (for more details see supplementary material S1). In contrast to the macro-rheological investigations, no statistically significant ($p>0.05$) differences are found between intestinal and respiratory mucus for both 2.86 and 6 µm beads. It can be concluded that there are no micro-rheological differences between intestinal and respiratory mucus obtained from the same mammalian species (pig).

Figure 2: Micro-rheological elastic moduli (G') with optical tweezers for (A) 2.86 µm and (B) 6 µm beads. At least 20 measurements were performed for each region.

Active microbead rheology: Figure 3 reveals that microparticle movement in HEC gels (A, B; dashed line) has similar pattern to particle movement in water (solid line) but not in either respiratory or intestinal (ileal) mucus (C, D). Here, 6 µm particle movement is shown as an instance of the motion of (micro)particles in complex structures and the same holds true for 2.86 µm particles. These findings correlate with previous studies on equine respiratory tract mucus preparations with optical tweezers[7].

Figure 3: Optical trap displacement in µm of a 6 µm particle in (A) HEC-1%, (B) HEC-2%, (C) respiratory, and (D) ileal mucus (dashed line). Displacement of particles in water is shown as a solid line in each graph for comparison. At least 20 measurements were performed for each region.

Figure 4: Output displacement response in µm of mucus preparations for (A) 2.86 µm and (B) 6 µm beads. At least 20 measurements were performed for each region.

Active microbead rheology (output displacement response) measures the force of the beads against single mucin-fibres and the resistance to the bead movement. One-Way ANOVA reveals statistically significant differences ($p<0.05$) between such data for pulmonary and intestinal mucus, whereas no statistically significant differences were found between ileal and duodenal mucus (Figure 4). The higher output displacement response for 2.86 µm and 6 µm beads, as observed for pulmonary mucus in comparison to intestinal mucus, can be explained by either a lower rigidity of pulmonary mucin fibres or a larger mesh size for the pulmonary mucus network. This finding points to differences in the mucus barrier function between the investigated organs, i.e. intestine and lung, and route of administration.

CONCLUSIONS: Macro-rheological investigations (plate-plate viscosimetry) on mucus obtained from the respiratory (trachea) and intestinal tract (ileum and duodenum) of the same species (pig) revealed significant differences in viscoelastic properties for these two organs. Passive microbead rheological measurements (particle tracking of Brownian dynamics), taking place only in the interstitial fluid of the mucus-pores, are not able to detect these differences. However, active microbead rheology measurements (induced movement by optical tweezers) again revealed significant differences between respiratory and intestinal mucus, pointing to biomechanical differences in the constituting mucin fibres or structural differences in the resulting hydrogel networks.

AUTHOR CONTRIBUTIONS

Harish Bokkasam, Ulrich F. Schaefer, Claus-Michael Lehr conceived and designed the research. Harish Bokkasam performed the experiments and Harish Bokkasam, Matthias Ernst analysed the data. Matthias Ernst, Marco Guenther contributed numerical analysis tools. Harish Bokkasam, Matthias Ernst, Ulrich F. Schaefer, Claus-Michael Lehr wrote the manuscript.

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