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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 (Lai et al., 2009; Sigurdsson et al., 2013) with unique viscoelastic properties (Allen et al., 1987; Kilcoyne et al., 2012; Taylor et al., 2005; Taylor et al., 2004). 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.

1. 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 min after euthanasia. The trachea was cut into halves (Kilcoyne et al., 2012) 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 (Taylor et al., 2005).

2. Hydroxyethyl cellulose (HEC) gel preparation

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

3. 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 (Taylor et al., 2005, 2004). 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.

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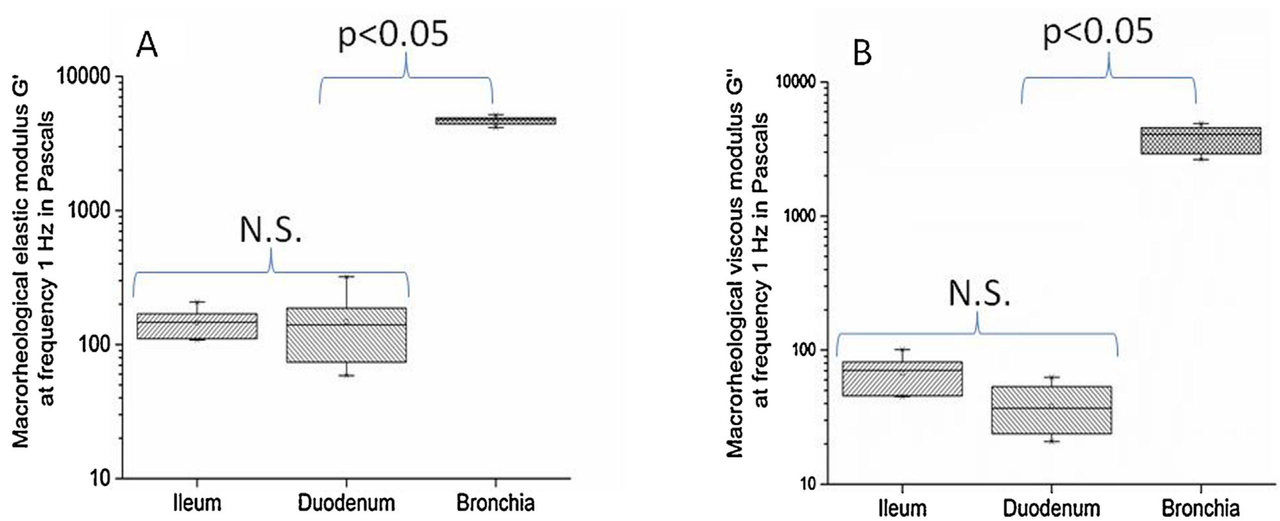

Fig. 1. Macro-rheological A: elastic (G′) and B: viscous moduli (G″) of mucus preparations at 1 Hz. At least 20 measurements were performed for each region.

4. 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 (Kirch et al., 2012; Capitanio and Pavone, 2013). 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 (Kirch et al., 2012).

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.

5. 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.

6. Macrorheology

Viscoelastic properties of porcine mucus from ileum, duodenum and trachea are depicted in Fig. 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.

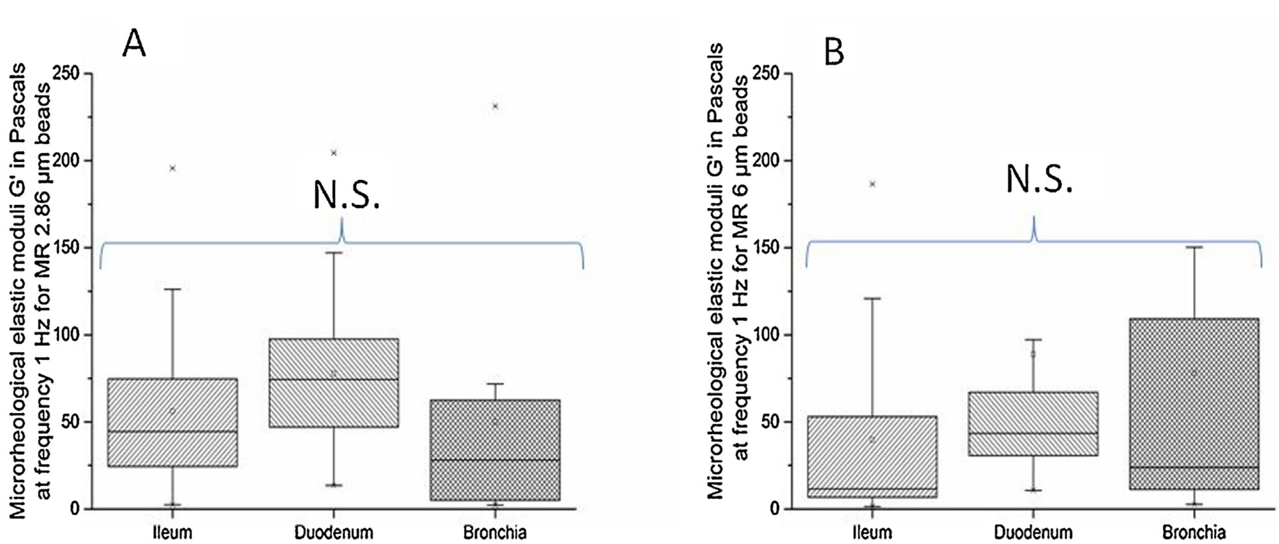

Fig. 2. Micro-rheological elastic moduli (G′) with optical tweezers for (A) 2.86 μm and (B) 6 μm beads. At least 20 measurements were performed for each region.