Journal of the mechanical behavior of biomedical materials 71 (2017) 216–222

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

A foam model highlights the differences of the macro- and microrheology of respiratory horse mucus

Andreas Gross[a], Afra Torge[b], Ulrich F. Schaefer[b], Marc Schneider[b], Claus-Michael Lehr[b,c], Christian Wagner[a,d,*]

[a] Experimental Physics, Saarland University, Campus, 66123 Saarbrücken, Germany
[b] Biopharmaceutics and Pharmaceutical Technology, Department of Pharmacy, Saarland University, Campus, 66123 Saarbrücken, Germany
[c] Department of Drug Delivery, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), 66123 Saarbrücken, Germany
[d] Physics and Materials Science Research Unit, University of Luxembourg, 162a avenue de la Faencerie, L-1511 Luxembourg, Luxembourg

ARTICLE INFO

Keywords:
Mucus
Respiratory mucus
Horse
Microrheology
SAOS
LAOS

ABSTRACT

Native horse mucus is characterized with micro- and macrorheology and compared to hydroxyethylcellulose (HEC) gel as a model. Both systems show comparable viscoelastic properties on the microscale and for the HEC the macrorheology is in good agreement with the microrheology. For the mucus, the viscoelastic moduli on the macroscale are several orders of magnitude larger than on the microscale. Large amplitude oscillatory shear experiments show that the mucus responds nonlinearly at much smaller deformations than HEC. This behavior fosters the assumption that the mucus has a foam like structure on the microscale compared to the typical mesh like structure of the HEC, a model that is supported by cryogenic-scanning-electron-microscopy (CSEM) images. These images allow also to determine the relative amount of volume that is occupied by the pores and the scaffold. Consequently, we can estimate the elastic modulus of the scaffold. We conclude that this particular foam like microstructure should be considered as a key factor for the transport of particulate matter which plays a central role in mucus function with respect to particle penetration.

1. Introduction

Respiratory mucus is found in the conducting airways covering the ciliated epithelium. The mucus is typically split into two layers, the periciliary layer between the cilia and the top layer forming a viscoelastic gel (Button et al., 2012). The mucus layer protects the epithelium from inhaled particles and foreign materials due to its sticky nature. Accumulation of these materials is avoided as a result of the coordinated beating of the cilia the so-called mucociliary clearance. The mucus together with the mucociliary escalator of the conducting airways is a very efficient clearance mechanism also preventing efficient drug delivery across this barrier.

This respiratory mucus, composed from mucin macromolecules, carbohydrates, proteins, and sulphate bound to oligosaccharide side chains (Fuloria and Rubin, 2000; Henning et al., 2008) forms a biological gel with unique properties (Schuster et al., 2013). The interaction of all kind of inhaled drugs and drug carriers with this layer and the penetration potential in and through the mucus is of outmost importance for possible therapeutic approaches.

Clearly, for drug delivery purposes the biochemistry of penetrating objects plays an important role but also the rheological behavior of the mucus layer. The rheological properties of mucus have been already investigated in many studies, most of them focusing on human tracheal mucus (King and Macklem, 1977; Jeanneret-Grosjean et al., 1988; Rubin et al., 1990; Zayas et al., 1990; Gerber et al., 2000) but they also include the examination of cystic fibrosis sputum (Dawson et al., 2003; Forier et al., 2013, 2014), cervicovaginal mucus (Lai et al., 2009), gastropod pedal mucus (Ewoldt et al., 2007), as well as pig intestinal mucus (Macierzanka et al., 2011). An excellent overview on the rheological studies is given by Lai et al. (2009). Since typically only small amounts of mucus are available for experiments, microscopic methods like magnetic microrheometry with test beads of the size of 50 μm to 150 μm were already applied in the 1970′s (King and Macklem, 1977). Multiple particle tracking (MPT) has evolved to one of the most favored methods in context with the microrheological characterization of biological fluids in general and of mucus in particular (Oelschlaeger et al., 2008). Still, the number of microrheological studies where the viscoelastic moduli are determined from the

* Corresponding author at: Experimental Physics, Saarland University, Campus, 66123 Saarbrücken, Germany.
E-mail address: c.wagner@mx.uni-saarland.de (C. Wagner).

http://dx.doi.org/10.1016/j.jmbbm.2017.03.009
Received 19 December 2016; Received in revised form 4 March 2017; Accepted 9 March 2017
Available online 16 March 2017
1751-6161/

Brownian fluctuations spectrum of colloidal probes remain limited (Lai et al., 2009). One important observation in this particular study of Lai et al. was that the viscosity observed using a 1 μm sized colloidal probe is much smaller than the results obtained on the macroscale. The results were interpreted with a model that assumes that the colloidal probe used can diffuse almost freely through the polymeric mucin network. In consequence, the influence of a variety of particle coatings has been examined extensively during the past decade with the goal to optimize particle transport through this natural barrier (Dawson et al., 2003; Lai et al., 2007, 2009; Macierzanka et al., 2011; Yang et al., 2011; Fröhlich and Roblegg, 2014). Only recently, it was shown by use of active microrheology and cryogenic-scanning-electron-microscopy (CSEM) (Kirch et al., 2012) that mucus should have a porous structure on the micron scale. The active manipulation of immersed particles offers a deeper insight into the material properties of mucus, especially into the strength of its scaffold. A further step was to demonstrate, that passive immersed particles show a very heterogenous diffusion behavior, ranging from particles firmly sticking to the supposed scaffold and particles moving almost freely in an viscous environment (Murgia et al., 2016). However, so far, studies utilizing optically trapped microparticles have been scarce although they are able to greatly enhance our understanding of material properties. They enable the mapping of pore sizes and, by taking the local mobility of particles into account, allow to distinguish in an unambiguous way between a weak and a strong confinement. By utilizing strong optical traps, the rigidity of the mucus mesh can be probed in order to determine which forces the material is able to resist to.

In this study, we will first use a sophisticated linear response theory based on the Kramers-Kronig relation in order to obtain the microscopic complex loss and storage modulus. Due to the heterogeneity of the mucus, these values show a significant scattering, especially if compared to our model gel, a hydroxyethylcellulose gel (HEC). Both the mucins in the mucus form the gel network by non-covalent interchain interactions, and the HEC is a classical hydrogel without any covalent interchain interactions. Therefore one might expect certain differences, but an explanation for the cause of the large heterogeneity of the mucus is still missing. Additionally we compare our microscopic data to results obtained by macroscopic oscillatory shear rheometry. The results from the microscopic and macroscopic measurements are in perfect agreement for the HEC gel, while there is a huge difference for the mucus that seems to be much stiffer on the macroscopic scale. The CSEM images allow to hypothesize a foam-like structure for the mucus with a comparable rigid scaffold and pores with ”walls” that are filled with a solution of low viscosity and elasticity, compared to the meshlike structure of HEC. By evaluating the volume percentage of the pores compared to the scaffold we can estimate its elastic module by use of a foam-model. Clearly, the biochemistry of penetrating objects plays an important role in the diffusional properties of the mucus but we will show that it has also unique viscoelastic properties that differ strongly from synthetic gels. We postulate that both aspects need to be considered for drug delivery to the airways using particulate carriers.

2. Materials and methods

2.1. Sample gels

All our experiments on mucus were performed with native respiratory horse mucus. It was obtained during bronchoscopy from the distal region of four healthy horses and stored at 193 K until use. According to earlier studies, such storage conditions are not known to influence the material properties (Gastaldi et al., 2000). As a synthetic model gel for comparison, a 1% (w/w) hydroxyethylcellulose gel (HEC; Natrosol 250 HHX Pharm, Ashland Aqualon Functional Ingredients) was chosen because it had similar viscoelastic moduli on the microscale. For the microrheology two kinds of particles were used, polymethylmethacrylate (PMMA) beads with a size of 4 μm and melamin resin beads with a size of 5 μm (Sigma-Aldrich). A Gene Frame (art.-no. AB-0576, ABgene, Epsom, United Kingdom) was used in microrheology as a sample cell to handle the low sample volume of 25 μl.

In preparation of the experiments, HEC was dissolved in water and shaken gently for 24 hours. For the microrheology, approximately 2–4 μl of each particle suspension (solid content: 10%) were mixed with 100 μl of sample resulting in particle concentrations of less than 1%. Thus, hydrodynamic interactions between multiple particles are negligible. These samples were vortexed for about 5 minutes before use to make sure that the beads were distributed homogeneously. Afterwards, a Gene Frame was filled with the respective amount of sample and sealed airtight using a coverslip. No additional preparation of the samples was necessary for experiments in the cone and plate rheometer. All experiments in both setups were performed at room temperature.

2.2. Macrorheology

A rotational Mars II (Thermo Scientific GmbH, Karlsruhe, Germany) was used to perform the small and large amplitude oscillatory shear (SAOS and LAOS) experiments. With SAOS experiments the linear response of the material is tested, whilst LAOS experiments are used to characterize the nonlinear properties. First strain amplitude sweeps were performed in order to determine the region of linear response and the nonlinear properties of both materials and then a frequency sweep in the linear range was performed. The rheometer was equipped with a cone and plate geometry with a cone angle of 0.5° for the measurements on mucus and a second geometry with an angle of 2° in case of the HEC gel. In case of mucus, this enabled us to perform measurements on volumes as small as 500 μl with an acceptable signal-to-noise ratio. In case of HEC, bigger sample volumes were available so using the more sensitive 2° geometry was a feasible option.

2.3. Microrheology

The optical tweezers setup described in Ref. Ziehl et al. (2009); Kirch et al. (2012) was used to perform passive microrheology. Particle positions in the focus of the laser beam were recorded with a high speed camera (HiSpec 2G; Fastec Imaging) at a frame rate of 16 kHz. The recorded picture series were analyzed using a particle tracking algorithm based on the cross-correlation of successive images (Ziehl et al., 2009). The complex shear modulus G^* was then determined by applying a method proposed by Schnurr (Schnurr et al., 1997). For this purpose, the Langevin equation describing the interaction of the confined bead with its surroundings is recast in frequency-space in such a way that particle displacements $\tilde{x}$ and the Brownian random force $\widetilde{F_r}$ are linked by the susceptibility or compliance $\tilde{\alpha}$

$$\tilde{x}(\omega) = \tilde{\alpha}^*(\omega)\widetilde{F_r}(\omega), \tag{1}$$

where

$$\tilde{\alpha}^*(\omega) = \frac{1}{k - i\omega\tilde{\zeta}(\omega)}. \tag{2}$$

The susceptibility is a function of the trap stiffness k and the frequency-dependent friction coefficient ζ. It is a complex quantity whose imaginary part is related to the power spectral density of particle displacements $<|\tilde{x}(\omega)|^2>$ by the fluctuation-dissipation-theorem (Landau et al., 1966)

$$<|\tilde{x}(\omega)|^2> = \frac{2k_BT}{\omega}\alpha''(\omega) \tag{3}$$

with Boltzmann's constant k_B and the temperature T. The Kramers-Kronig-relations allow the determination of the real part of the compliance by computing the principal value integral