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Micro- and macrorheology of mucus ☆

Samuel K. Lai [a], Ying-Ying Wang [c], Denis Wirtz [a,b], Justin Hanes [a,b,c,d,*]

[a] Department of Chemical & Biomolecular Engineering (JH Primary Appointment), Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, USA
[b] Institute for NanoBioTechnology, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, USA
[c] Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[d] Department of Oncology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

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ABSTRACT

Mucus is a complex biological material that lubricates and protects the human lungs, gastrointestinal (GI) tract, vagina, eyes, and other moist mucosal surfaces. Mucus serves as a physical barrier against foreign particles, including toxins, pathogens, and environmental ultrafine particles, while allowing rapid passage of selected gases, ions, nutrients, and many proteins. Its selective barrier properties are precisely regulated at the biochemical level across vastly different length scales. At the macroscale, mucus behaves as a non-Newtonian gel, distinguished from classical solids and liquids by its response to shear rate and shear stress, while, at the nanoscale, it behaves as a low viscosity fluid. Advances in the rheological characterization of mucus from the macroscopic to nanoscopic levels have contributed critical understanding to mucus physiology, disease pathology, and the development of drug delivery systems designed for use at mucosal surfaces. This article reviews the biochemistry that governs mucus rheology, the macro- and microrheology of human and laboratory animal mucus, rheological techniques applied to mucus, and the importance of an improved understanding of the physical properties of mucus to advancing the field of drug and gene delivery.

Contents

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* Corresponding author. Department of Chemical & Biomolecular Engineering (JH Primary Appointment), Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, USA.
E-mail address: hanes@jhu.edu (J. Hanes).

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1. Introduction

Mucus is a thick substance that lines the luminal surface of the gastrointestinal (GI), respiratory, urogenital, and eye tissues, as well as the peritoneal surface of intra-abdominal organs in humans and most animals. The function of mucus varies between different organs. At exposed surfaces, mucus acts as the outermost line of protection against foreign pathogens [1–3], toxins [4], and environmental ultrafine particles [2,5]. In the GI tract, mucus also aids the transport of chyme from the gut to the colon by serving as a lubricant during the peristaltic process while allowing rapid entry and exit of nutrients and waste [6,7]. At the surfaces of internal organs, mucus serves as a lubricant to minimize friction between organs. In performing its numerous functions, mucus is continuously secreted, shed, and finally digested, recycled, or discarded.

At the chemical level, mucus is an integrated structure of biopolymers. Its physical behavior is complex (non-Newtonian), with highly variable properties that are between those of a viscous liquid and an elastic solid. Rheological measurements, including viscosity (resistance to flow) and elasticity (stiffness), are often used together to describe the consistency of mucus. The rheological properties of mucus vary as a function of shear stress, time scale (rate) of shearing, and length scale. Changes in the rheological properties of mucus may greatly affect its ability to function as a lubricant, selective barrier, and the body's first line of defense against infection [8–10]. If mucus becomes too thick, for example in severe bronchitis [11] or cystic fibrosis [12–14] where the sputum viscosity can be more than 100,000 times that of water, patients experience great difficulty in mucus clearance, resulting in bacterial overgrowth. On the other hand, in women with bacterial vaginosis, the viscosity of vaginal fluids is significantly lower than in those with normal flora, which may be responsible for the increased risk of infection by HIV and *Neisseria gonorrhoeae*, as well as other adverse gynecological conditions [15].

We first discuss the distinction between macro- and microrheology of mucus and provide important background on the biochemistry of mucus, with an emphasis on the regulatory mechanisms that control its viscoelastic properties. We then discuss the microrheology of mucus, focusing on the rheology of mucus as encountered by micro- and nanoscopic entities, such as viruses, proteins, bacteria, and drug delivery particles. We specifically address the importance of understanding mucus microrheology to the design of therapeutic nanoparticle systems targeted to mucosal tissues. We then examine the macrorheological behavior of mucus and the impact of macrorheology on improved understanding of human physiology, disease pathology, and therapeutic strategies. In the last section, we review rheological techniques used to characterize mucus across vastly different length scales.

2. Macrorheology vs. microrheology

Characterization of the physical properties of mucus largely focuses on two properties: (i) viscous or loss modulus (G''), which is the extent to which the gel resists the tendency to flow, and (ii) elastic or storage modulus (G'), which measures the tendency for the gel to recover its original shape following stress-induced deformation. Together, these properties describe the rheology of complex biological fluids. An illustration of the steady state viscosity of a purely viscous fluid, an elastic solid, and a viscoelastic gel is shown in Fig. 1. The phase angle or loss tangent value δ, calculated from the inverse tangent of G''/G', is also a common parameter for characterizing mucus (δ=0° for a Hookean solid; δ=90° for a viscous liquid; δ<45° for a viscoelastic solid and δ>45° for a viscoelastic liquid). A number of additional physical properties are also used to describe mucus. For example, creep, measured by applying a constant stress and measuring the strain or deformation created as a function of time, quantifies the tendency of a gel to deform permanently. Spinnability (or *spinnbarkeit*), which measures the capacity of fluids to be drawn into threads, represents an indirect measurement of the adhesive and elastic properties of mucus.

At the macro (bulk fluid) scale, mucus is commonly referred to as a viscoelastic gel because it possesses both flow (viscosity) and deformation (elasticity) properties. In particular, the bulk rheology of mucus is characterized by a non-Newtonian viscosity that is non-linear with shear rate, posing strong resistance to deformation at low shear rates and weak resistance at high shear rates. The bulk rheology is critical for the proper macroscale function of mucus, including mucus clearance and lubrication. However, the bulk viscosity of typical mucus secretions (~2000-fold or more viscous than water at low shear) would seemingly preclude the diffusion of particles or even small proteins at appreciable rates. Since mucus affords rapid passage of select proteins and particles, bulk-fluid macrorheological characterization is inadequate for understanding the barrier properties of mucus, especially at length scales relevant to pathogens, toxins, and foreign particles.

The term microrheology has been used to describe techniques that measure the macroviscoelasticity of minute volumes of mucus. Another common definition of microrheology, and the one we adopt, is related to the characterization of the viscoelasticity that is encountered by micro- and nanoscale entities. In contrast to bulk rheology, which provides averaged measurements of physical properties, microrheology can measure heterogeneity in a sample's physical properties with high spatial resolution. In essence, microrheology affords detailed characterization of the viscosity and elasticity of biological fluids, accounting for both contributions from the fluid within the biopolymer network as well as the network mesh itself. Thus, microrheological studies are important for characterizing the local mechanical properties of biological fluids that are overlooked by bulk rheological techniques.

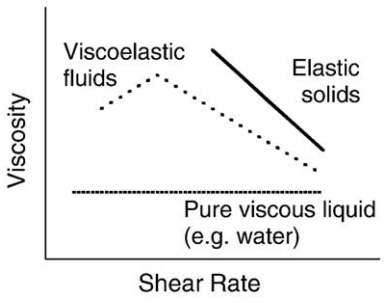

Fig. 1. Illustration of the steady state viscosity vs. shear rate profiles of liquids, solids, and viscoelastic substances. The viscosity of a liquid is constant, while the viscosity of a yielding solid decreases with shear rate. However, the viscosity of a viscoelastic material is more complex. In the above example of a thixotropic fluid, the steady state viscosity first increases at low shear rates (shear thickening), then progressively decreases at larger shear rates (shear thinning).