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Mucins and Their Role in Shaping the Functions of Mucus Barriers

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Keywords

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Abstract

We review what is currently understood about how the structure of the primary solid component of mucus, the glycoprotein mucin, gives rise to the mechanical and biochemical properties of mucus that are required for it to perform its diverse physiological roles. Macroscale processes such as lubrication require mucus of a certain stiffness and spinnability, which are set by structural features of the mucin network, including the identity and density of cross-links and the degree of glycosylation. At the microscale, these same features affect the mechanical environment experienced by small particles and play a crucial role in establishing an interaction-based filter. Finally, mucin glycans are critical for regulating microbial interactions, serving as receptor binding sites for adhesion, as nutrient sources, and as environmental signals. We conclude by discussing how these structural principles can be used in the design of synthetic mucin-mimetic materials and provide suggestions for directions of future work in this field.

Contents

1. INTRODUCTION

Mucus is a biological hydrogel that coats every wet epithelial surface of the body, including the eyes, lungs, and stomach (**Figure 1**). Mucus serves as a lubricant to protect epithelia against shear-induced damage from mechanical forces associated with processes such as digestion and blinking (Argüeso & Gipson 2001, Nordgård & Draget 2015, Sellers et al. 1988) and acts as a selective physicochemical barrier by excluding foreign or harmful molecules while permitting the passage of desirable agents such as nutrients (Bansil & Turner 2006, Linden et al. 2008).

The primary structural component of mucus is the glycoprotein mucin (**Figure 2**), and to date 21 mucin-type glycoproteins, which collectively belong to the *MUC* gene family, have been identified in the human body and are recognized by the Human Genome Organization gene nomenclature committee (**http://www.genenames.org**). The protein backbone of the mucin molecule is composed of a variable number of tandem repeats rich in proline, threonine, and/or serine (PTS domains), as well as cysteine-rich regions at the amino terminus, at the carboxy terminus, and interspersed between the PTS domains (Bansil & Turner 2006, Dekker et al. 2002). These PTS domains are characterized by dense O-linked glycosylation and structurally resemble bottlebrushes, with branched oligosaccharide chains, or glycans, arranged radially from the protein core (Bansil & Turner 2006). Approximately 80% of the mass of these molecules is derived from carbohydrates, with the protein backbone making up the remaining 20% (Bansil & Turner 2006). Within the mucin family, there are two distinct subgroups: (*a*) secreted mucins and (*b*) tethered, cell surface–associated mucins. Within the secreted mucins, there exist five oligomeric, gel-forming mucins (MUC2, MUC5AC, MUC5B, MUC6, and MUC19) (Thornton et al. 2008), as well as two nonpolymeric glycoproteins (MUC7 and MUC8) (Thornton et al. 2008). The gel-forming mucins (**Figure 2*a***) reside outside the epithelial cell layer (Hattrup & Gendler 2008), and the individual mucin subunits polymerize via end-to-end disulfide bonds to form even larger macromonomer chains that are arranged in a linear fashion (Kesimer et al. 2010, Taylor et al. 2005), although some studies have suggested the possibility of branched, trimer structures for specific mucins (Ambort et al. 2012, Godl et al. 2002, Nilsson et al. 2014). In contrast, the 11 tethered mucins (MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20,

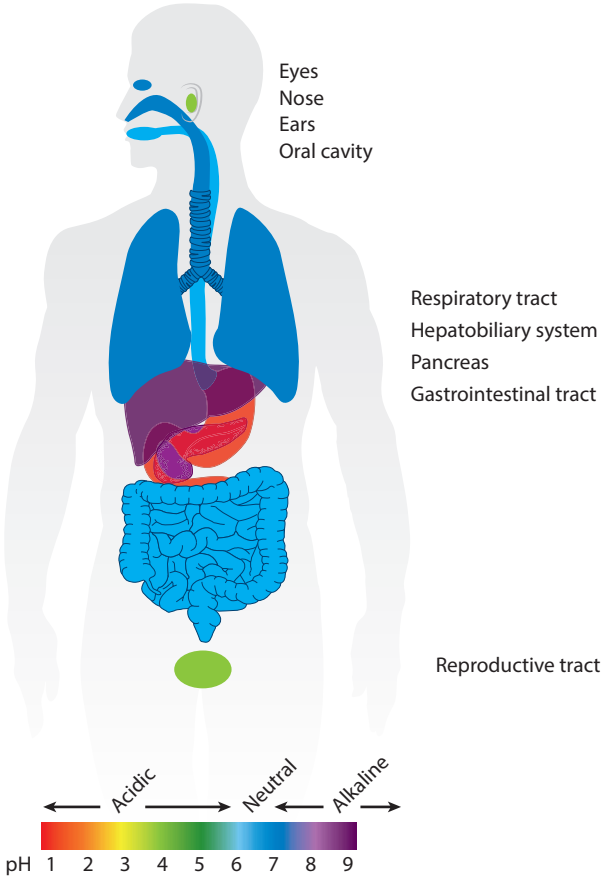

Figure 1

Epithelial surfaces of the body coated by mucus, with characteristic pH levels. Figure adapted from Frenkel & Ribbeck (2015a).

and MUC21) have short cytoplasmic domains that reside inside of the cell, as well as extensive extracellular domains (Hattrup & Gendler 2008) (**Figure 2*b***). The three remaining mucins are oviductal glycoprotein 1 (previously known as MUC9), endomucin (also known as MUC14), and MUC22. Mucins are generated in and secreted from goblet cells, which are specialized cells in the surface epithelium, and from mucous cells in submucosal glands (Hattrup & Gendler 2008, Thornton et al. 2008). Here, we focus on secreted mucins and note that detailed reviews on the structures of both types of mucins can be found in prior literature (Hattrup & Gendler 2008, Thornton et al. 2008).

As depicted in **Figure 2*c***, in aqueous solution, the high-molecular-weight mucin molecules form a network mediated by a complex series of reversible physical bonds including hydrophobic interactions and chain entanglements and stabilized by electrostatic repulsion between the negatively charged polysaccharide side chains (Schipper et al. 2007). Mucin molecule configuration and network interaction strength are sensitive to variations in pH as well as to concentration of ions and small molecules across the organ systems (Wagner et al. 2017) (**Figure 1**). Consequently, mucus layers possess different mechanical and biochemical properties, depending on their location and intended physiological function. For instance, the eyes have a thin, watery mucin solution

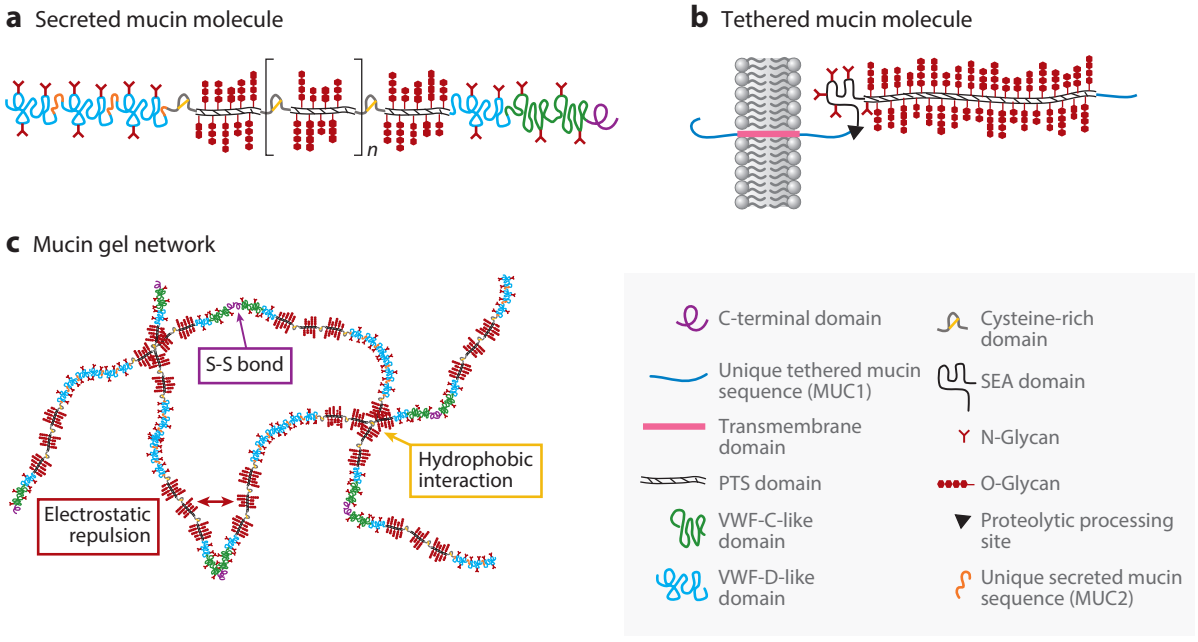

Figure 2

(*a*,*b*) Schematics of the general structure of (*a*) secreted and (*b*) tethered mucin molecules. (*c*) An illustration of the network established by the gel-forming, secreted mucins. Individual mucin subunits associate via end-to-end disulfide bonds to form even larger macromonomer chains. The network is formed from reversible associations such as hydrophobic interactions between the nonglycosylated, cysteine-rich regions of the molecules and is stabilized by electrostatic repulsion between the charged sugar side chains. Abbreviation: PTS domain, proline, threonine, and/or serine domain. Panels *a* and *b* adapted from Bansil & Turner (2006), Dekker et al. (2002), and McGuckin et al. (2011) with permission from Elsevier and Springer Nature. Panel *c* adapted with permission from Wagner et al. (2017). Copyright 2017, American Chemical Society.

on the surface for hydration and lubrication (Argüeso & Gipson 2001). In contrast, the mucus lining the stomach is a stiffer mucin gel that protects the epithelial lining against acidic (pH ≈ 1–2) gastric juices (Celli et al. 2007). Altered mechanical and biochemical properties of mucus can also indicate disease or pathological manipulation; one example is the increased bacterial load and thick mucus associated with cystic fibrosis (CF) (Lai et al. 2009a).

We begin this review by considering the relationship between the mechanical properties of mucus and the structure of mucin molecules in the context of health and disease. We next explore how the structure of mucin molecules affects the biochemical properties of mucus by analyzing its role as a selective barrier as well as its interactions with microbes. Finally, we consider an important engineering application of these concepts—the design of synthetic mucin-mimetic polymers—and provide our outlook for future research directions in this field.

2. INFLUENCE OF MUCIN BIOCHEMISTRY ON THE MECHANICAL PROPERTIES OF MUCUS AND MUCIN GELS

Native mucus is composed primarily of water (~95%), mucins, lipids, salts, and proteins involved in defense such as immunoglobulins (Bansil & Turner 2006). The mechanical properties of mucus are primarily due to the presence of mucins (Bansil & Turner 2006). Consequently, gels reconstituted from purified mucin molecules are an established experimental system for

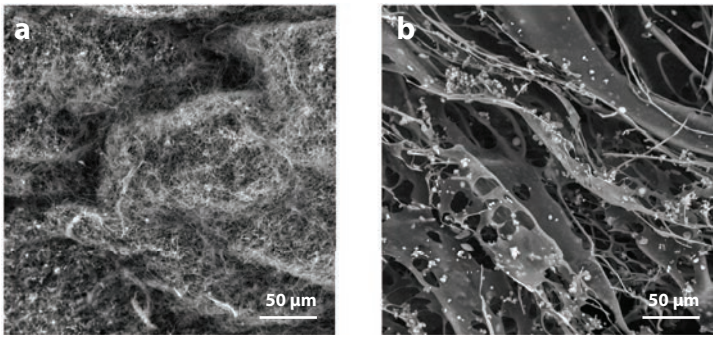

Figure 3

Scanning electron microscopy (SEM) reveals structural changes to mucins associated with different purification processes. (*a*) MUC5AC mucin purified from pig gastric mucus by the Ribbeck lab. (*b*) Industrially purified porcine gastric mucin supplied by Sigma-Aldrich.

studying the physicochemical properties of mucus (Celli et al. 2007, 2009; Georgiades et al. 2014). Reconstituted mucin gels generally exhibit less heterogeneity between samples due to eliminated mucus components and, unlike native mucus, can be prepared to specified conditions. However, further analysis is required to determine whether the mechanical properties of reconstituted mucin gels are representative of those of native mucus. For instance, Sellers et al. (1988) found that gels of purified gastric, colonic, and duodenal mucin reconstituted to physiological concentrations had the same form of mechanical spectra as did their corresponding native mucus secretion, while Raynal et al. (2002) found that the diffusion of microspheres and mucin components in saliva was significantly different from that in gels reconstituted from MUC5B purified using 6 M guanidinium chloride (GdmCl). By studying the diffusion of drug molecules and 200-nm polystyrene particles with different surface chemistries, respectively, Larhed et al. (1997) and Crater & Carrier (2010) determined that gels reconstituted from commercially available purified porcine gastric mucin (PGM; Sigma-Aldrich) were not an accurate model for native porcine intestinal mucus. As a result of proteolytic digestion during purification, commercial preparations of mucin have been reported to not form pH-dependent gels (Bansil & Turner 2006, Bansil et al. 2013). Indeed, significant structural differences are apparent in the scanning electron microscopy (SEM) images of native mucin purified by the Ribbeck lab (**Figure 3*a***) and Sigma-Aldrich PGM (**Figure 3*b***). In addition, Raynal et al. (2003) have shown that, unlike native mucins, MUC5B mucins purified with 6 M GdmCl cannot bind calcium, which highlights the sensitivity of mucin structure to the purification process. Finally, the properties of native mucus are also sensitive to enzymatic degradation (Wagner & McKinley 2017) and to mechanical manipulation such as freeze-thaw cycles (Smith-Dupont et al. 2017), demonstrating the importance of handling procedures for faithful characterization of mucus.

The complex macromolecular network contained within mucus is capable of stretching and reorienting in response to external force or deformation. Thus, mucus is a viscoelastic material, meaning that its mechanical response to an imposed deformation lies somewhere between that of a pure solid (Hooke's law of elasticity) and that of a pure liquid (Newton's law of viscosity) (Bird et al. 1987, pp. 255–84). The microstructure of native mucus is highly heterogeneous, with a distribution of pore sizes ranging between 50 nm and 1 µm having been measured in cervicovaginal mucus across several pH values (Wang et al. 2013). Consequently, the mechanical properties of mucus are sensitive to the length scale over which they are measured. Here, we review how the structure of mucin molecules influences both the macroscopic rheological properties (specifically the stiffness and spinnability) and the microscopic viscoelastic response of mucus and mucin gels. We also discuss how these properties are related to physiological function in health and disease.

2.1. Stiffness

The stiffness of mucus gels varies throughout the body, depending on the physiological role of the mucus layer. Furthermore, mucus is able to rapidly self-heal and recover its initial stiffness following mechanical deformations associated with processes such as coughing. In this section, we review the experimental work and measurement techniques that have explored the biochemical mechanisms by which mucus stiffness is regulated in vivo. These mechanisms include modifications to the degree of physical and chemical cross-linking in the mucin network, modifications to the conformation of the mucins themselves through variations in the pH level or salt concentration, and modifications to the extent of gel swelling through manipulation of the identity and density of the mucin glycans.

As a result of complex structural features such as network cross-links and potentially branched macromonomer aggregates (Godl et al. 2002), it is impossible to assign a single length scale to the mucin network illustrated schematically in **Figure 2*c*** (Jaishankar 2014). Since the mucin network is cross-linked via both weak, physical bonds and strong, chemical bonds, the same holds true for defining a single characteristic timescale (Jaishankar 2014). Complex fluids with multiscale microstructures possess wide ranges of length scales and timescales (Jaishankar 2014), which generally manifest as broad, power law–like spectra during experiments such as stress relaxation or small-amplitude oscillatory shear (SAOS) (Jaishankar & McKinley 2014). For mucus and mucin gels, such experiments are typically performed by modulating the displacement profile of the top fixture of small-diameter cone-and-plate or parallel-plate setups to minimize sample volume requirements. The response of native mucus [including porcine gastric, intestinal, duodenal, and colonic mucus (Nordgård & Draget 2015; Philippe et al. 2017; Sellers et al. 1983, 1988; Taylor et al. 2003); equine respiratory mucus (Gross et al. 2017); human saliva (Stokes & Davies 2007, Wagner & McKinley 2017); cervical mucus (Critchfield et al. 2013); and cervicovaginal mucus (Wang et al. 2013)] under SAOS is predominantly solid like [storage or elastic modulus (G') > loss or viscous modulus (G'')], with slowly varying dependencies of both G' and G'' on the oscillation frequency. Similar broad-spectrum responses were also observed during creep compliance measurements performed on Sigma-Aldrich mucin gels (Caicedo & Perilla 2015) and during stress relaxation experiments in native porcine gastric mucus (Philippe et al. 2017).

For entangled solutions of flexible polymer chains, the plateau shear modulus is inversely proportional to the cube of the mesh size of the network (Broedersz & MacKintosh 2014). Although mucin gels do not exhibit a well-defined plateau modulus (and hence mesh size) as a result of their multiscale microstructure (Wagner & McKinley 2017), their shear moduli can be altered by modifying the number density of cross-links in the network. For instance, decreasing the number of strong disulfide bonds in the mucin network through the addition of reducing agents such as tris(2-carboxyethyl)phosphine (Wang et al. 2013), mercaptoethanol (Sellers et al. 1983), and the mucolytic agent *N*-acetyl cysteine (Vukosavljevic et al. 2017) decreased the viscoelastic moduli of native mucus (Sellers et al. 1983, Vukosavljevic et al. 2017) and increased its mesh size (Wang et al. 2013). Yuan et al. (2015) demonstrated that the viscoelastic moduli of porcine gastric mucus can be increased through exposure to an oxidizing agent (dimethyl sulfoxide) and the subsequent creation of additional disulfide bonds. They proposed that oxidative stress from neutrophilic inflammation may contribute to the increased stiffness of CF mucus (Yuan et al. 2015). In addition to these examples of manipulating the strong covalent bonds of the mucin network, Wagner et al. (2017) reduced the viscoelastic moduli of reconstituted MUC5AC gels by eliminating weak hydrophobic associations using the surfactant 1,2-hexanediol. Lastly, various physiological factors, including trefoil factor peptide 3 (Bastholm et al. 2017) and inflammatory mediators in CF sputum (Serisier et al. 2009), also appear to influence the structure and modulus of mucus, although the mechanisms in these cases remain unresolved.

Another approach to microstructural rearrangement involves modifying the conformation of the individual mucin molecules through pH modulation (Bhaskar et al. 1991, Celli et al. 2007, Wagner et al. 2017) or through changes to the salt concentration (Celli et al. 2007, Wagner et al. 2017). Decreasing the pH from neutral to acidic (pH $\approx$ 1–2) increases the viscosity and viscoelastic moduli of MUC5AC gels by up to 2–3 orders of magnitude (Bhaskar et al. 1991, Celli et al. 2007, Wagner et al. 2017); this mechanism is believed to be an important part of how the mucus lining in the stomach limits the diffusion of hydrogen ions to the underlying epithelium, thus protecting it from acid digestion (Bhaskar et al. 1991, Celli et al. 2009). From these results, Celli et al. (2007) proposed that the ulcer-causing bacterium *Helicobacter pylori* enhances its motility in the mucus lining of the stomach by locally hydrolyzing urea, which raises the pH of the gel and reduces its storage and loss moduli (Bansil et al. 2013, 2015; Celli et al. 2009). Interestingly, the viscoelastic moduli of native mucus have been found to be only slightly responsive to pH changes (Sellers et al. 1983, Wang et al. 2013).

Another physicochemical feature of mucins that impacts the modulus of mucus is their dense degree of glycosylation. The grafting of sugar side chains to the amino acid backbone of synthetic mucin-like molecules reduces their flexibility (Kramer et al. 2015), which may alter the structure of an entangled network of these molecules. Furthermore, the high concentration of negative fixed charges associated with the sugar side chains of the mucin molecules results in the partitioning of counterions into the gel matrix to preserve electroneutrality, and the extent of this partitioning depends on the pH and ionic strength of the surrounding environment (Tam & Verdugo 1981). The resulting osmotic pressure difference leads to the redistribution of water and ions between the gel and the aqueous medium, which affects the degree of swelling of the mucus and hence its rheological properties (Tam & Verdugo 1981). Experimentally, samples of luteal cow cervical mucus separated from an aqueous bath by a Nucleopore filter swelled in response to changes in both the pH and salt concentration of the bath, while samples obtained during the estrous cycle did not undergo swelling (Tam & Verdugo 1981). These findings suggest an important role for the Donnan equilibrium process and mucus hydration in regulating the rheological properties and function of cervical mucus over the course of the menstrual cycle. In addition, Crouzier et al. (2015) showed that the hydration and lubricity of mucin-coated surfaces are significantly reduced when deglycosylated mucins, which possess diminished quantities of negative fixed charges, are used in place of the intact native molecule.

Finally, we note that weak cross-linking interactions in the mucin network also allow mucus to self-heal following the large, nonlinear deformations associated with physiological events such as coughing or the passage of food through the digestive tract (Taylor et al. 2005). Taylor et al. (2003) demonstrated this ability experimentally by performing repeated stress increase/decrease sweeps on porcine gastric mucus at different frequencies. They observed that, apart from a large initial decrease in G' and G'' following the first sweep, the viscoelastic moduli of the gels recovered completely during the unloading portion of each stress cycle (Taylor et al. 2003). The details of the dynamical processes that regulate this self-healing remain an open and intriguing area of research.

2.2. Spinnability

The spinnability of mucus, or its propensity to form filaments when stretched, is an important determinant of its ability to perform its physiological functions. In this section, we review experimental studies of the biochemical mechanisms that influence spinnability, which include the extent of cross-linking in the mucin network as well as the length of the mucin molecules and the identity and density of the mucin glycans. Additionally, for saliva in particular, studies have shown that spinnability is strongly dependent on the stimulation method used to procure the sample, as well as on how the sample is stored and handled.

The unique structure of the mucin network imparts an extensional character to mucus, which is essential for proper function during physiological processes such as mucociliary clearance (Tabatabaei et al. 2015), bolus formation (Burbidge & Le Révérend 2016), and swallowing (Burbidge & Le Révérend 2016). Experimentally, extensional flows are commonly established by stretching a sample between two vertically aligned plates. Depending on the experimental setup, the final height at breakup of the stretched sample may be measured [using tools such as the filament stretching extensional rheometer, developed by the Cambridge Polymer Group (McKinley & Sridhar 2002), and the Filancemeter, developed by Zahm et al. (1986)], or the radius of the thinning filament may be monitored to extract a relaxation time associated with the material [using the Capillary Breakup Extensional Rheometer (CaBER) developed by the Cambridge Polymer Group (Anna & McKinley 2001)].

As in the case of shear deformations, the organization of the mucin network plays a critical role in the extensional rheological response of mucus and mucin gels. Wagner & McKinley (2017) showed that the extensional properties of saliva, as measured by its relaxation time and the time to breakup of a stretched filament, are highly sensitive to the age of the saliva sample. They attributed this observation to a reduction in the molecular weight and hence the extensibility of the mucin molecules as a result of proteolytic degradation, which is consistent with previous measurements and observations of salivary protein temporal stability (Aggazzotti 1922, Esser et al. 2008). The extensional properties of saliva (Zahm et al. 1986) [as well as its shear rheological response (Stokes & Davies 2007)] are highly sensitive to how the sample is collected as well as to whether or not the donor has fasted. For instance, Vijay et al. (2015) recently demonstrated that salivary spinnability is closely correlated with bicarbonate concentration and pH, which vary significantly between samples procured using different stimulation methods. Mechanistically, they suggest that, in addition to directly altering the pH of the sample, bicarbonate ions may interact with free or bound calcium to modify the structure and cross-linking of the mucin network and hence the spinnability of the sample (Vijay et al. 2015). There is initial experimental and numerical evidence that CaBER measurements of sputum from patients with chronic obstructive pulmonary disease (COPD) may be sensitive enough to serve as biomarkers for stages of active infection (Tabatabaei et al. 2015), and Zussman et al. (2007) have proposed that the altered extensional rheological properties of saliva measured with CaBER between teenagers and the elderly may be related to the prevalence of different oral and dental conditions within each age group.

Finally, we conjecture that the glycosylation patterns of mucin molecules may also play a role in the extensional properties of mucus. For example, studies of the O-glycosylation of mucins obtained from cervical mucus have found a relative increase in the abundance of neutral glycans versus acidic ones (such as those containing sialic acid) during ovulation relative to periods in the menstrual cycle prior or subsequent to this event (Andersch-Björkman et al. 2007). Although earlier studies did not find significant differences in mucin glycosylation over the course of the menstrual cycle, they did find that G' of cervical mucus, and hence the cross-linking density of the gel (Broedersz & MacKintosh 2014), is correlated with the ratio of sialic acid to fucose residues in the sample (Wolf et al. 1980). In terms of extensional rheology, studies by Chretien et al. (1979) showed that the spinnability of cervical mucus over the course of the menstrual cycle is highest during the ovulatory phase. A reduction in sialic acid content may then be associated with a reduction in the degree of cross-linking (and hence G') of the gel and with an increase in the flexibility of the mucin chain (Kramer et al. 2015), both of which may promote increased sample spinnability. Cervical mucus samples from pregnant women at high risk for preterm birth have also been found to be significantly more spinnable (and less viscoelastic) than those from their low-risk counterparts (Critchfield et al. 2013), and one mechanism for altered mucoadhesivity in

the etiology of preterm birth may be pathogenic cleavage of negatively charged sialic acid residues from the mucin molecules (Smith-Dupont et al. 2017).

Altered glycosylation profiles have been reported in other mucin-related diseases such as Sjögren's syndrome (Chaudhury et al. 2016, Hall et al. 2017), dry eye disease (Stephens & McNamara 2015), and dry mouth (Chaudhury et al. 2015). In contrast to the changes in cervical mucus previously described, Chaudhury et al. measured a reduction in both the spinnability of saliva samples and the degree of mucin glycosylation (sialic acid residues in particular) in patients with Sjögren's syndrome (Chaudhury et al. 2016) and dry mouth (Chaudhury et al. 2015). They proposed that a reduction in the overall negative charge of the mucin molecules may promote reduced water retention and dehydration in saliva, as well as modifications to its rheological behavior (Chaudhury et al. 2015, 2016). Therefore, although mucin glycosylation appears to play a role in determining the extensional rheology of mucus and saliva, the exact mechanisms by which this occurs are not entirely clear.

2.3. Microscopic Viscoelastic Response

Microrheological techniques involve measuring the mechanical response of materials by using embedded colloidal probes that can be driven either actively, for instance, by the use of optical or magnetic tweezers, or passively by thermal fluctuations of the material (Squires & Mason 2010). These techniques provide highly sensitive measures of spatial heterogeneity and gelation kinetics (Larsen & Furst 2008), and the very limited sample volume (microliters) that they require (Squires & Mason 2010) makes them particularly attractive for characterizing sample-limited materials such as mucus.

The mean squared displacement (MSD) of passive colloidal beads can be directly related to the linear viscoelasticity of the samples that they are embedded in through the generalized Stokes-Einstein relationship (Mason & Weitz 1995). Certain assumptions must be met, including that the embedded probes be significantly larger than the characteristic length scale of heterogeneity within the gels and that the probes not interact with the gel components (Squires & Mason 2010). In general, these assumptions do not hold for complex biological gels such as mucus, and consequently microscopic and macroscopic measurements of viscoelasticity frequently disagree (Bansil et al. 2013, Bokkasam et al. 2016, Wagner et al. 2017). The range of pore sizes in mucus and mucin gels results in probe-size dependent effects, including a heterogeneous population of freely diffusing probes in the void regions of the gel as well as immobile particles confined by the polymer mesh (Lieleg et al. 2010, Murgia et al. 2016, Wagner et al. 2017). The biochemical structure of mucin molecules also permits interactions with probes of diverse surface chemistries (Lieleg et al. 2010). Therefore, it is advised that (*a*) the information obtained from microrheological measurements be used in complement with that obtained from macrorheology for mucus and mucin gels (Lai et al. 2009a) and that (*b*) caution be taken if such information is to be used as a stand-alone predictor of the bulk viscoelastic moduli of the samples.

2.3.1. Passive microrheology. Passive microrheological techniques such as fluorescence recovery after photobleaching (Lai et al. 2009a), fluorescence imaging of concentration profiles, and photoactivatable nanoparticle diffusion assays (Schuster et al. 2017) are performed at equilibrium and hence can probe only linear material responses (Squires & Mason 2010). The most commonly used passive microrheological technique in the study of mucus and mucin gels is single-particle tracking (SPT), in which the spatial location of colloidal particles is recorded over time at set intervals. The ensemble average MSD of all the tracked particles is typically extracted from these data and plotted as a function of the delay time $\Delta\tau$. In general, the MSD is expressed in

power-law form, represented in one dimension as MSD $= 2D_\alpha \Delta\tau^\alpha$, where D_α is a generalized diffusion coefficient (Metzler & Klafter 2000). When $\alpha < 1$, the motion of the particle is subdiffusive, and when $\alpha > 1$, the motion is superdiffusive (Metzler et al. 2014). For normal diffusive motion, the MSD is expected to scale linearly with the lag time ($\alpha = 1$) (Metzler et al. 2014).

The MSD of micrometer-sized tracer particles is correlated with mucus solids concentration ranging from healthy levels to those typically associated with respiratory diseases such as COPD and CF and compromised mucus barrier function (Hill et al. 2014). Georgiades et al. (2014) studied the creep compliance of 500-nm probes in reconstituted MUC2 and MUC5AC gels and used a Maxwell model to extract the dependence of the gel viscosity on the mucin concentration at pH 7. For both types of mucin, these authors observed a transition from diffusive to subdiffusive probe motion at concentrations near 25–30 mg/mL, as well as a significant decrease in α and probe mobility when the pH of 1-wt% solutions was lowered to pH 1. In contrast, Wagner et al. (2017) reported increased mobility of larger 1-μm probes in 1-wt% MUC5AC gels as the pH was lowered from pH 7 to pH 2. They attribute this increased mobility to high degrees of heterogeneity within the mucin gel, as reflected in a bimodal distribution of mobile, freely diffusing particles and more confined ones undergoing subdiffusive motion (Wagner et al. 2017). These distinct particle motions are consistent with previous observations of phase separation within MUC5AC gels into mucin-rich and mucin-poor domains near pH 2 (Bansil & Turner 2006, Bhaskar et al. 1991, Maleki et al. 2008). Mechanistically, such phase separation has been attributed to the mucin molecules unfolding in response to the destruction of salt bridges, which may expose previously hidden hydrophobic cross-linking sites along the protein backbone and allow for increased levels of mucin association and aggregation (Bansil & Turner 2006).

SPT also allows investigators to measure the effect of additives on the microstructure of the mucin network. For example, Dawson et al. (2003) observed increased trajectory homogeneity within 200-nm carboxylated particles upon addition of recombinant human deoxyribonuclease to CF sputum, whose pathologically elevated viscoelasticity levels are due in part to the presence of large quantities of DNA (Lai et al. 2009a). Dawson et al. (2003) hypothesized that the disappearance of the slowest trajectories may have been due to the release of particles from DNA/mucin aggregates as large DNA segments were hydrolyzed into smaller fragments and that the migration of small fragments into the pores may have increased the local viscoelasticity of the pores and eliminated the possibility of fast, diffusive particle motion. SPT has also been used to investigate the effects of surfactant addition (Wagner et al. 2017, Wang et al. 2013) and reducing agents (Georgiades et al. 2014, Wang et al. 2013) on the mechanical properties of both native mucus and reconstituted MUC5AC gels; the effects of chaotropic agents (Georgiades et al. 2014), urea (Georgiades et al. 2014), salt (Wagner et al. 2017), and a plant-derived polyphenol (Georgiades et al. 2014) on reconstituted mucin gels; and the effects of calcium chelators (Wang et al. 2013) on native mucus.

2.3.2. Active microrheology. To probe the nonlinear microscopic response of mucus and mucin gels, one must use active microrheological techniques in which the particles are driven within the material by an external force (Squires & Mason 2010). Weigand et al. (2017) used optically trapped probes to investigate the rheological response of mucus from the *Chaetopterus* marine worm and found that the viscoelastic moduli were probe size dependent for particles up to 10 μm in diameter, with elastic effects becoming increasingly important as the bead size increased. Kirch et al. (2012) investigated the microrheological response, pore size, and rigidity of native respiratory mucus using optical tweezers. Consistent with the heterogeneity previously measured in native mucus using passive techniques (Murgia et al. 2016), these authors observed that the majority of particles could not be moved appreciably, likely due to the stiffness of the polymer scaffold, while other

particles, possibly located within larger pores or void regions of the sample, could be displaced much more easily (Kirch et al. 2012).

In summary, both active and passive microrheological measurements of mucus can be performed with very limited sample volume and permit a detailed spatial characterization of the material microstructure that is inaccessible to macroscopic tools. Generally speaking, micro- and macrorheological measurements of mucus are not in agreement due to sample heterogeneity and probe-mucin biochemical interactions. Nevertheless, characterization of mechanical properties at the microscopic length scale is critical for understanding the environment faced by small molecules and bioengineered nanoparticles.

3. INFLUENCE OF MUCIN BIOCHEMISTRY ON THE PERMEABILITY OF MUCUS AND MUCIN GELS

In addition to determining the mechanical properties of mucus, the cross-linked network of mucin polymers also establishes a selectively sticky, weblike filter that, together with other mucosal components, allows mucus to regulate the diffusion of both particles and small molecules. As a first mechanism, the mucin network filters particles on the basis of size by excluding those larger than the distance between the cross-links of the mesh. Additionally, the abundance of charged and hydrophobic residues on the mucin polymers allows mucus to selectively control the passage of molecules smaller than the mesh size through physical binding interactions. Indeed, identically sized particles [10 nm (Smith-Dupont et al. 2017) to 1 μm diameter (Lieleg et al. 2010)] with different surface properties exhibit different diffusion profiles in both reconstituted mucin gels (Crater & Carrier 2010, Lieleg et al. 2010) and native mucus (Crater & Carrier 2010, Dawson et al. 2003, Ensign et al. 2012b, Schuster et al. 2013, Smith-Dupont et al. 2017, Wang et al. 2013).

The specific molecular properties that promote the adhesion of a molecule to a mucus barrier, and specifically to the mucin polymers, remain poorly understood (Carlson et al. 2018, Newby et al. 2018, Witten et al. 2018). For example, more positively charged molecules bind more strongly to net negatively charged mucin (Witten et al. 2018). However, Li et al. (2013) reported that with alternating blocks of positive and negative charge, small peptides interact more strongly with mucins than do peptides of the same overall charge but with singly alternating charges, highlighting the importance of both charge magnitude and spatial arrangement. Furthermore, the presence of hydrophobic moieties on a molecule increases its degree of binding to mucus, but it is believed that spatial arrangements of proximal charge and other chemical parameters on the molecule in question are also important (Carlson et al. 2018, Witten et al. 2018). An improved understanding of the mechanisms that determine adhesiveness to the mucus barrier will therefore be critical for the design of particles with specific transport rates through mucus, including in the context of optimizing drug delivery. For the interested reader, we refer to a series of excellent reviews that have been published in the last year and that give a comprehensive overview of strategies to overcome the mucus barrier in mucosal drug delivery (Araújo et al. 2018, Bansil & Turner 2018, García-Díaz et al. 2018, Huckaby & Lai 2018, Khutoryanskiy 2018, Lock et al. 2018, Menzel & Bernkop-Schnürch 2018, Murgia et al. 2018, Taherali et al. 2018, Wu et al. 2018).

When retention within the mucus layer is desired, interactions with mucin and other mucus components may be beneficial (Witten & Ribbeck 2017). For most drug delivery applications, however, particularly in sites of rapid mucus turnover, strategies that discourage mucus binding have been more effective (Ensign et al. 2012b, Lai et al. 2009b, Newby et al. 2018, Witten et al. 2018), which has driven efforts to design muco-inert surface coatings such as polyethylene glycol corona (Schuster et al. 2013; Suk et al. 2009, 2016). These coatings have improved the transport rate of particles smaller than the mesh size of the mucus gel (Schuster et al. 2013), but the passage

of particles larger than this threshold size is impeded by steric interactions regardless of the particle surface chemistry. The role of mucus in drug delivery has been previously reviewed and is not discussed extensively here (Boegh & Nielsen 2015, Duncan et al. 2016, Ensign et al. 2012a, Witten et al. 2018).

In several diseases, compromised function of the mucosal layer as a selective barrier results in serious physiological consequences. For example, drug delivery to the CF lung is challenging due to the decreased permeability of CF mucus (Witten et al. 2018), which occurs through a combination of a hyperconcentration of mucus components (Button et al. 2016), increased disulfide cross-linking (Yuan et al. 2015), and calcium-mediated compaction owing to impaired bicarbonate secretion (Chen et al. 2010). The cervical mucus of women at high risk for preterm birth is more permeable to peptide probes than are samples from their low-risk pregnant counterparts (Smith-Dupont et al. 2017). As preterm birth is frequently associated with intra-amniotic infection arising from microbial ascension across the cervical mucus plug, these findings suggest increased permeability of the mucosal barrier to the passage of pathogens (Smith-Dupont et al. 2017). Lastly, infection with the bacterium *H. pylori* and the onset of chronic active gastritis and duodenal ulcer disease may be associated due to degradation of the gastric mucus barrier by toxins produced by this pathogen (Lichtenberger 1995). Together, these examples suggest that mucosal permeability is a promising biomarker for a wide range of diseases. Manifestations of global alterations to mucus integrity (Gustafsson et al. 2012, Lira-Junior & Figueredo 2016) may further increase the usefulness of mucus permeability as a diagnostic indicator, particularly in situations in which the affected mucosal barrier is less accessible for biopsy. In this way, the ability to regularly infer diagnostic information from readily available mucus sources such as saliva may permit more dynamic monitoring of patient health without necessarily needing to rely on in vivo measurements or invasive biopsy procedures.

4. INFLUENCE OF MUCIN BIOCHEMISTRY ON MICROBIAL PHYSIOLOGY

The human body is colonized by trillions of microbes, many of which reside inside the mucus barrier. While many of these microbes are generally beneficial to the host, mucus is also capable of accommodating potentially problematic microbes like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. In health, mucus appears to play an important role in suppressing virulence of these opportunistic pathogens and in promoting their coexistence as part of a stable microbiota (Swidsinski et al. 2005). Defects in mucin expression and mucus production are associated not only with pathologies like CF (Henke et al. 2004, 2007; Lai et al. 2009a), inflammatory bowel disease (IBD) (Johansson et al. 2008), and Sjögren's syndrome (Chaudhury et al. 2016, Hall et al. 2017) but also with the proliferation of opportunistic pathogens. These diseases are often associated with the formation of biofilms (Cunha-Cruz et al. 2013, Singh et al. 2000, Swidsinski et al. 2005), which are cellular communities of microbes that secrete and surround themselves with a sticky extracellular matrix that can act as a protective barrier against environmental stresses such as antibiotic therapies and the immune system. Biofilms are also associated with infections of nonmucosal surfaces such as those occurring on indwelling devices and burn wounds (Parsek & Singh 2003). **Figure 4*a*** schematically shows the relationship between microbes and diseased versus healthy mucus.

In this section, we begin by discussing the role of mucins in attenuating virulence in opportunistic microbes. We then review our current knowledge of the mechanisms through which these and other mucin-microbe interactions occur. Finally, we summarize these concepts in the form of a model for how mucins and mucus may influence microbial behavior and community assembly.

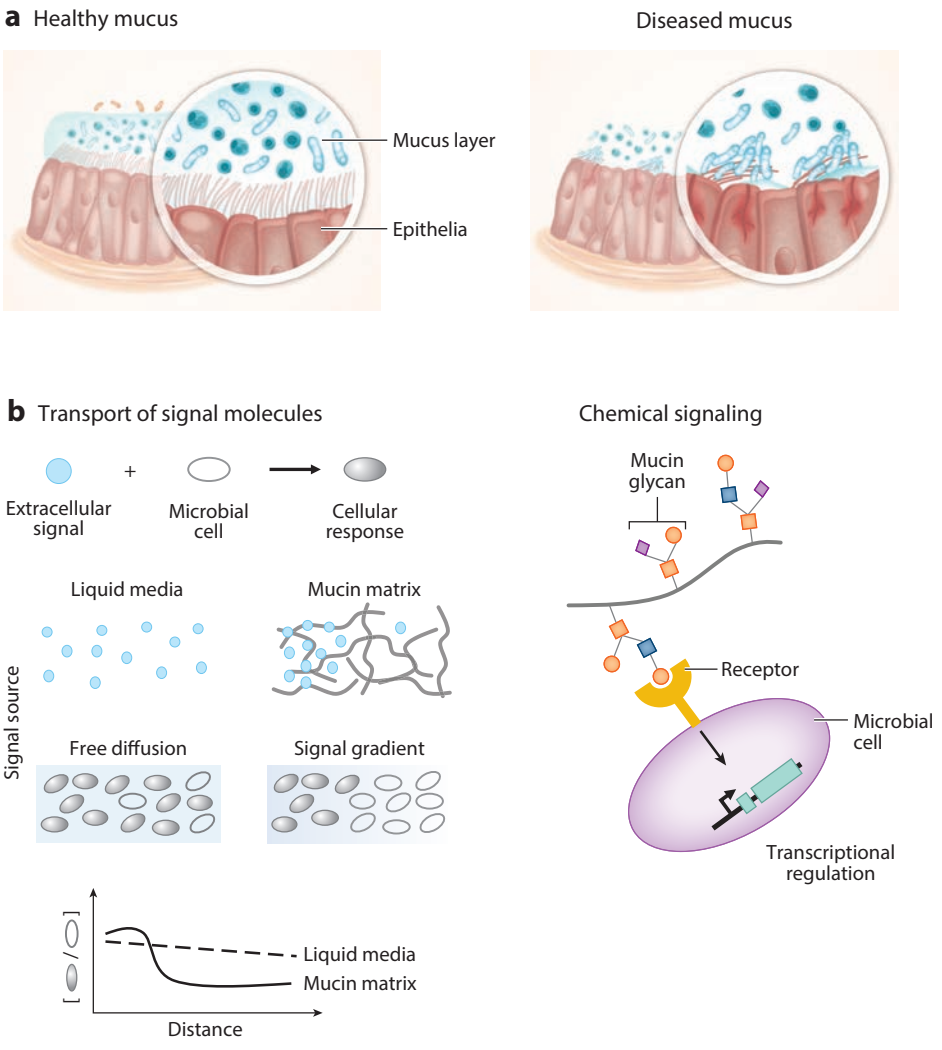

Figure 4

Microbial interactions with the mucus environment influence pathogenicity. (*a*) Mucus accommodates a great diversity of microbes and protect against epithelial adhesion and cytotoxicity by pathogenic microbes. Dysregulation of mucus or changes in mucus properties are associated with dysbiosis and susceptibility to infection. (*b*) Key structural properties of mucins are hypothesized to shape microbiological behavior within mucus and the three-dimensional mucin network. For example, the barrier properties of mucus can limit the diffusion of microbial signaling molecules or toxins through an environment. This can result in signal gradients that promote or interfere with intercellular communications, which may ultimately influence community-level behaviors or facilitate interactions not possible in an unstructured environment. In addition, mucin-associated glycans may serve as chemical signals that attenuate microbial virulence.

4.1. Mucins Suppress Virulence of Opportunistic Microbes

Mucin expression is often differentially regulated in response to infection, suggesting a function in managing infection. For example, middle ear epithelia enhance expression of the genes encoding the gel-forming mucins MUC2, MUC5AC, and MUC5B in response to three bacteria associated with otitis media: *Streptococcus pneumoniae*, *Haemophilus influenza*, and *Moraxella catarrhalis*

(Kerschner et al. 2014). Similarly, in lung epithelia, enhanced expression of *MUC5AC* and *MUC2* is observed upon exposure to a wide variety of gram-negative and gram-positive bacteria, including *P. aeruginosa* and *S. aureus* (Dohrman et al. 1998, Li et al. 1997, McNamara et al. 2001).

A number of important in vivo studies support a function of mucins in controlling virulence. For example, the lower airways of the healthy lung have traditionally been viewed as effectively sterile, owing to the efficiency of the mucus barrier in trapping and removing particulates and pathogens by mucociliary clearance. MUC5B in airway mucus has a role in mucociliary clearance, controlling respiratory infections, and maintaining immune homeostasis in mice (Roy et al. 2014). *Muc5b* knockout mice had an accumulation of and chronic infection by multiple bacterial species that are normally absent in MUC5B-rich mucus, including the pneumonia-causing pathogen *S. aureus*. Similarly, in the epithelial lining of the stomach, secreted MUC5AC and surface-associated MUC1 protect against microbes like *H. pylori*, which can bind to these mucins via fucosylated and sialylated glycans (Mahdavi et al. 2002). In the rhesus monkey, mucins that efficiently bind *H. pylori* can aid in the removal of bacteria from the gastric niche (Lindén et al. 2008). MUC1 limits the density of *H. pylori* in mice and limits adhesion to the cell surface in humans (Lindén et al. 2009). MUC1 can also protect the epithelium by acting as a physical barrier to other non-MUC1 binding bacteria (Lindén et al. 2009), further illustrating the capability of this polymer to protect the host from a broad range of bacteria.

In vitro experiments using purified mucins have begun to shed light on how these biopolymers influence the physiology of microbes and could control pathogenicity. The key innovations of the following selected in vitro experiments are the native purification of mucins and their presentation as a three-dimensional network, which allow for the study of both direct mucin-microbe interactions and the phenotypic consequences of the physicochemical properties of the surrounding matrix on the inhabiting cells. For example, purified mucin MUC5B isolated from human saliva protects surfaces from biofilm formation by the pathogen *Streptococcus mutans* (Frenkel & Ribbeck 2015b), which is one of the causative agents of the disease dental caries. Similarly, while MUC5AC was not found to be essential for mucociliary clearance (Roy et al. 2014), in vitro studies with natively purified mucins have implicated this polymer in suppressing *P. aeruginosa* biofilm formation by promoting a more disperse planktonic lifestyle (Caldara et al. 2012). Since bacterial growth was not inhibited in either of these studies, we conclude that mucin-mediated defense against microbes results from modulation of host and/or pathogen behavior rather than from direct antimicrobial interactions.

Notably, the virulence-attenuating capacity of mucins extends beyond bacterial pathogens. *C. albicans* is an important opportunistic fungal pathogen that is typically part of the healthy microbiota but can cause infections like oral thrush and vaginitis. *C. albicans* pathogenicity is associated with virulence traits such as surface adhesion, biofilm formation, filamentation, and the secretion of proteases. The secreted gel-forming mucins MUC5AC, MUC5B, and MUC2 suppress filamentation, and MUC5AC suppresses expression of virulence-associated genes and prevents *C. albicans* from forming biofilms on synthetic surfaces and human epithelial cells (Kavanaugh et al. 2014). This ability to manipulate *C. albicans* physiology implicates mucins as key host signals for retaining *C. albicans* and other microbial pathogens in a host-compatible state.

These in vitro systems with purified mucins can also be used to study how mucosal environments influence multispecies interactions to support stable microbial communities. In the oral cavity, for example, saliva protects the underlying mucosa and structures an oral microbial community comprising >500 microbial species (Siqueira & Rôças 2017, Verma et al. 2018). This community includes *S. mutans* (Kroes et al. 1999, Siqueira & Rôças 2017, Verma et al. 2018), which is often considered to be antagonistic to other bacterial species in coculture because it generates large amounts of lactic acid. While *S. mutans* will generally outcompete the commensal bacteria

Streptococcus sanguinis in standard growth media, it appears that solutions containing the native salivary mucin MUC5B can support the prolonged coexistence of these two bacteria (Frenkel & Ribbeck 2017). This result suggests that mucins may play a specific role in suppressing interspecific competition and promoting stable assembly of multispecies communities in the oral cavity and likely also in other mucosal environments. Collectively, these studies indicate that healthy mucin production and function limit the virulence of microbial pathogens and prevent disease progression. Furthermore, these studies highlight how simple native mucin networks are capable of reconstituting important microbial interactions that cannot be captured with other in vitro models, such as growing microbes in media alone, or with currently available commercial mucins that have lost part of their native configuration (**Figure 3*b***).

4.2. Mechanisms of Mucin-Microbe Interactions

The mechanisms that permit mucins to suppress virulence traits in opportunistic pathogens and promote stability of microbial communities are numerous. In a first instance, mucins can regulate microbial interactions and promote a stable microbiota by serving as a source of nutrients for certain microbes. While most microbes lack the complete machinery required to degrade and use host-derived substrates, growth of certain commensal bacteria is linked to the metabolism of host-derived limiting nutrients. For example, in the oral cavity and gut, consortia of bacteria, including *Akkermansia muciniphila*, *Bifidobacterium bifidum*, and *Streptococcus mitior*, are capable of metabolizing mucin-derived glycans as a primary carbon source (Bradshaw et al. 1994, Png et al. 2010, Sonnenburg et al. 2005, Tailford et al. 2015, van der Hoeven et al. 1990, Wickström & Svensäter 2008). This metabolic advantage of utilizing host-derived nutrients by commensal bacteria can prevent the establishment of enteric pathogens by competitive exclusion (Bergstrom & Xia 2013, Buffie & Pamer 2013, Malago 2015) and can promote stable community assembly. The current understanding of the molecular mechanisms employed by mucin-degrading bacteria to use host glycans and adapt to the mucosal environment has been previously reviewed (Tailford et al. 2015), but this area of research remains largely open.

In addition to serving as a nutrient source, mucus can also influence microbial behavior and community structure by forming a three-dimensional matrix that acts as a physical barrier between microbes. As an example, in the stomach and large intestine, there is evidence that mucus provides the spatial structure necessary to protect the underlying epithelia from microbial damage and accommodate a community of beneficial microorganisms (Johansson et al. 2011). The three-dimensional meshwork of mucus may spatially segregate microbes to some extent, affecting their ability to compete with each other and for nutrient sources. Mucins may also create heterogeneity by binding and enriching for nutrients, which can lead to spatial niche partitioning, as can be observed along the gastrointestinal tract (Lu et al. 2014). Furthermore, by influencing the selective transport of small signaling molecules, mucins also have the potential to interfere with intercellular communication and regulate the assembly of microbial communities. Many microbes rely on the production of signaling molecules to control traits that affect community structure, such as biofilm formation, toxin and antibiotic production, DNA transfer, and various forms of motility. We hypothesize that through their rich biochemistry, mucin polymers selectively bind and reduce the diffusion of certain signaling molecules and toxins, promoting the formation of discrete spatial gradients and microenvironments capable of supporting the coexistence of otherwise antagonistic species. The interaction between mucins and secreted microbial factors and the influence of mucins on community assembly and protection against invading pathogens are relatively new concepts that will likely require a multidisciplinary approach to be systematically evaluated.

Mucins also may protect against epithelial adhesion and cytotoxicity of pathogenic microbes by interacting directly with microbes. Specifically, the mucin backbone is grafted with more than

200 unique glycan structures (Jin et al. 2017) that could serve as potential interaction sites for microbes. Many pathogens, including the malaria parasite *Plasmodium falciparum* (Beeson et al. 2000) and the bacterium *H. pylori* (Simon et al. 1997), initiate infection by binding host cell glycans. Cell surface glycans can also act as specific receptors for viral hemagglutinins, such as in the cases of influenza virus (Kastner et al. 2017, Varki 1993) and human immunodeficiency virus (HIV) (Bode et al. 2012), as well as microbial cytotoxins, such as cholera toxin (Fishman & Atikkan 1980, Varki 1993). The glycans of secreted glycoconjugates like mucin are postulated to act as decoys for such pathogens, diverting the binding of the pathogen or toxin away from target cell surfaces (Varki 1993, 2006) or agglutinating pathogens for mucus clearance, thereby protecting the underlying epithelial cells. Mucins isolated from porcine gastric mucus prevent the infection of epithelial cells by a broad range of small mucosotropic viruses such as human papilloma virus type 16, Merkel cell polyoma virus, and a strain of influenza A virus by trapping the viruses in the biopolymer matrix (Lieleg et al. 2012). Similarly, submandibular-sublingual mucin agglutinates the oral bacteria *S. sanguinis* and *S. mutans* (Levine et al. 1978). Levine et al. (1978) also found that removing terminal sialic acid residues from salivary mucin glycoproteins prevented their ability to agglutinate *S. sanguinis*, but not *S. mutans*, suggesting that mucin-bacteria interactions involve specific, distinct carbohydrate moieties.

Lastly, mucins, and in particular their associated glycans, may also influence the behavior and composition of the microbiota by presenting biochemical signals that influence gene expression and cell physiology. Metabolic cues help pathogens coordinate the expression of virulence factors (Görke & Stülke 2008, Le Bouguénec & Schouler 2011, Lopez & Skaar 2018, Rohmer et al. 2011, Stulke & Hillen 1999), and the great structural diversity of mucin-associated glycans makes them well suited to encode a wealth of biological information. In fact, oligosaccharides serve many biological functions in other contexts. Dietary prebiotic oligosaccharides can promote microbiome stability (Preidis & Versalovic 2009) and can protect against virulence by enteric pathogens (Macfarlane et al. 2008). Cell surface glycans are important for cell-cell signaling (Kobata 1992, Varki 1993), and in plants, glycan signals regulate important processes such as host cell defense, growth, and development, even at low concentrations (John et al. 1997). Similarly, glycosylation is important in signal transduction for mammalian systems. For example, deglycosylation of glycoprotein hormones such as human β chorionic gonadotrophin prevents intracellular signaling (Varki 1993). Covalently linked sugar chains on the surface of mammalian cells help in communication and recognition through cell-cell adhesion (Fukuda et al. 1999, Varki 1993). In immune cell function, the selectin family of receptor proteins mediates the adhesion of leukocytes to endothelial cells via recognition of sialylated, fucosylated, sulfated glycans (Fukuda et al. 1999). It is not yet clear which specific receptors or signaling pathways in microbial cells are regulated by glycans; however, promising candidates for mucin glycan sensing include two-component systems or second-messenger systems, many of which have sugar binding sites (reviewed in Mascher et al. 2006, Postma et al. 1993).

The large diversity of mucin glycans provides many potential recognition sites for microbes, each of which may have a distinct signaling potential and role in regulating microbial behavior, but assessing their individual bioactivities has been an intractable problem. A class of structurally similar sugars found in human breast milk, known as human milk glycans (HMO), may provide important insights into the function of complex mucin glycans. HMO have garnered significant attention due to their ability to regulate the infant microbiota (Bode et al. 2012, Pudlo et al. 2015, Tailford et al. 2015, Yu et al. 2013). These glycans modulate immune responses (Eiwegger et al. 2004), specifically global transcriptional profiles of commensal microorganisms (Garrido et al. 2015), and protect against virulence of opportunistic pathogens. For example, α1-2-fucosylated HMO prevented epithelial adhesion of the pathogen *Campylobacter jejuni* in

vitro (Ruiz-Palacios et al. 2003), and pooled HMO blocked *Entamoeba histolytica* attachment to the surface of human intestinal epithelial cells, protecting host cells from cytotoxicity (Jantscher-Krenn et al. 2012). We anticipate that systematic analysis of mucins and their associated glycans as decoy binding sites and as signaling molecules will reveal specific protective functions not dissimilar to those recently observed with HMO (Yu et al. 2012, 2014).

When mucus structure and properties are compromised, the protective capacity of mucus can be significantly diminished. As discussed in Section 3, alterations to the permeability of the mucosal layer can result in serious physiological consequences such as intra-amniotic infection and chronic gastritis due to infection with the bacterium *H. pylori*. Similarly, changes in the physical and biochemical properties of mucins that can be observed in certain pathologies like IBD, CF, and Sjögren's syndrome have the potential to influence susceptibility to opportunistic infection (**Figure 4*a***). For example, the diseased mucus in the CF lung does not provide the same protection against microbes such as *P. aeruginosa* as does mucus in the healthy lung (Singh et al. 2000). Such diminished protection could be due to changes in mucin gene expression and production (Henke et al. 2004, 2007); mucin degradation (Flynn et al. 2016); dehydration of the mucus layer (Lai et al. 2009a), which could impact the diffusion of immunological factors and secreted microbial products; and/or changes to mucin glycosylation patterns (Schulz et al. 2007) that could influence the signaling potential of mucin.

Together, mucin-microbe interactions are multifaceted, with both physicochemical properties and glycosylation patterns playing important roles. **Figure 4*b*** presents two important, but understudied, mechanisms for how the mucin gel may interact with microbes to influence community assembly and behavior. Specifically, the ability of mucins to impose geometric and diffusive constraints and the ability of mucins to display instructive signals via associated glycans have strong potential to regulate microbial behavior and virulence. It will be important to continue characterizing the therapeutic nature of mucin and how specific mucin moieties modulate the behavior, pathogenicity, and community assembly of host-associated microbes. By better understanding how alterations to the biochemistry of mucins and mucus impact their protective capacity, it may be possible to repair these barriers in disease and to restore healthy mucosal function. The role of mucins and specifically their associated glycans in maintaining a healthy microbiota and preventing infections makes these molecules an appealing alternative to traditional antibiotic therapies.

5. DESIGN OF SYNTHETIC MUCIN-MIMETIC MATERIALS

The development of mucus-mimetic materials derived from synthetic sources remains highly valuable. For example, the assessment of binding (mucoadhesion) of an engineered drug delivery vehicle or, alternatively, its ability to penetrate through a mucosal layer is typically performed using ex vivo animal tissue (Cook et al. 2015). However, such tissues may be difficult to source and generally require animal sacrifice (Authimoolam & Dziubla 2016, Cook et al. 2015). Furthermore, high degrees of variability within tissue samples from a given animal donor and between tissues from different animal donors may lower the reproducibility and interpretability of the tests performed (Cook et al. 2015, Hall et al. 2011, Hamed & Fiegel 2014). Cook et al. (2015) showed that the mucoadhesion of chitosan and pectin to porcine gastric mucosa and a synthetic glass-bound hydrogel containing 20 mol% of the glycomonomer *N*-acryloyl-D-glucosamine copolymerized with 2-hydroxyethylmethacrylate is similar when washed with phosphate-buffered saline or simulated gastric juice. Synthetic mucus-mimetic materials are being developed to have similar binding behavior toward pharmaceutical tablets as native mucosal tissues (Hall et al. 2011, Khutoryanskaya et al. 2010); however, to our knowledge, successful clinical use of such materials has yet to be shown.

The development of synthetic mucins also has applications for the treatment of diseases related to mucus barrier dysfunction. Mahalingam et al. (2011) developed a biocompatible, pH-responsive,

mucin-like polymer constructed from phenylboronic acid and salicylhydroxamic acid individually copolymerized with a 2-hydroxypropyl methacrylamide backbone capable of nearly immobilizing free and cell-bound HIV virions when in hydrogel form. These authors proposed applying this material to the vaginal lumen prior to intercourse to serve as a barrier (in addition to cervical mucus) to the passage of sexually transmitted virions (Mahalingam et al. 2011). Intriguingly, these materials also self-heal following high shear rate deformations (Mahalingam et al. 2011), presumably due to their weakly cross-linked, transient network structure. Indeed, the ability to self-heal will undoubtedly prove to be an essential feature of any successful synthetic mucin-like material to faithfully recreate the ability of mucus to maintain an effective and dynamic physicochemical barrier. On the basis of previous findings from women at high risk for preterm birth (Smith-Dupont et al. 2017), we suspect that the application of these or similar hydrogels may also be an effective strategy for reducing the incidence of intra-amniotic infection during pregnancy. In severe cases of salivary gland dysfunction, replacement of saliva with a synthetic substitute is necessary for regular physiological function (Ferguson & Barker 1994). Commercial substitutes include xanthan gum– and carboxymethyl cellulose–based formulations, although their rheological properties are substantially different from those of native saliva (Preetha & Banerjee 2005). Generally speaking, we anticipate mucus replacements to be effective in facilitating the regeneration of damaged mucosal epithelia in cases of inflammation or ulcers and in reestablishing a healthy microbiota following dysbiosis.

The intricacy of mucin molecules complicates the incorporation of every functional moiety into the design of a synthetic polymer. One challenge is to determine the necessary parameters and biochemical groups that are required for native mucin functionality. To do so, we propose using the structural features of mucins highlighted as contributors to specific physicochemical properties in Sections 2–4 to inform the design of simplified polymers with specific chemical functional groups (**Figure 5**).

Efficient strategies for tuning the stiffness of a gel resulting from an aqueous solution of synthetic mucin molecules (beyond, of course, increasing polymer concentration) may include modifying the density (*i*) and identity (*ii*) of the functional groups on the synthetic molecules (**Figure 5*a***). Such modifications could alter the mesh size of the polymer network and in certain cases could prompt spinodal decomposition, which could mimic the pH-induced phase separation associated with the increased stiffness of mucin gels at acidic pH levels (Wagner et al. 2017). In addition, the conformation and net charge of the polymer backbone (*iii*) may play an important role in determining the stiffness of the gel. For instance, at pH 2 mucin molecules are nearly neutrally charged and adopt a stiff, elongated conformation by breaking salt bridges that are capable of concealing hydrophobic binding sites at pH 7 (Bansil & Turner 2006). One aspect that remains unknown, however, is whether gels formed from molecules with backbones derived from synthetic polymers such as polyethylene glycol will be capable of possessing similar physicochemical properties to mucus or whether the use of amino acid–based backbones will be necessary. Finally, the degree of stiffness and lubricity (Crouzier et al. 2015) of the gel can also be controlled by modifying the grafting density of charged sugar side chains (*iv*), which was recently achieved by Kramer et al. (2015) in a dual-end functionalized α-GalNAc-Ser α-amino acid *N*-carboxyanhydride system.

In a similar way, altering the length of the synthetic polymers (*v*), the density of the interaction moieties (*i*), and grafted sugar side chain identity (*vi*) and density (*iv*) may be important design strategies for tuning the spinnability of the resulting gel (**Figure 5*b***). On the basis of the findings in Section 2.3, design strategies *i* through *iii* are also likely to be useful for tuning the microscopic viscoelastic response and microstructure of the gel (**Figure 5*c***). To alter the permeability of the gel (**Figure 5*d***), the literature presented in Section 3 suggests that modifications to the mesh size and biochemical composition of the polymer network are useful strategies for tuning the degrees

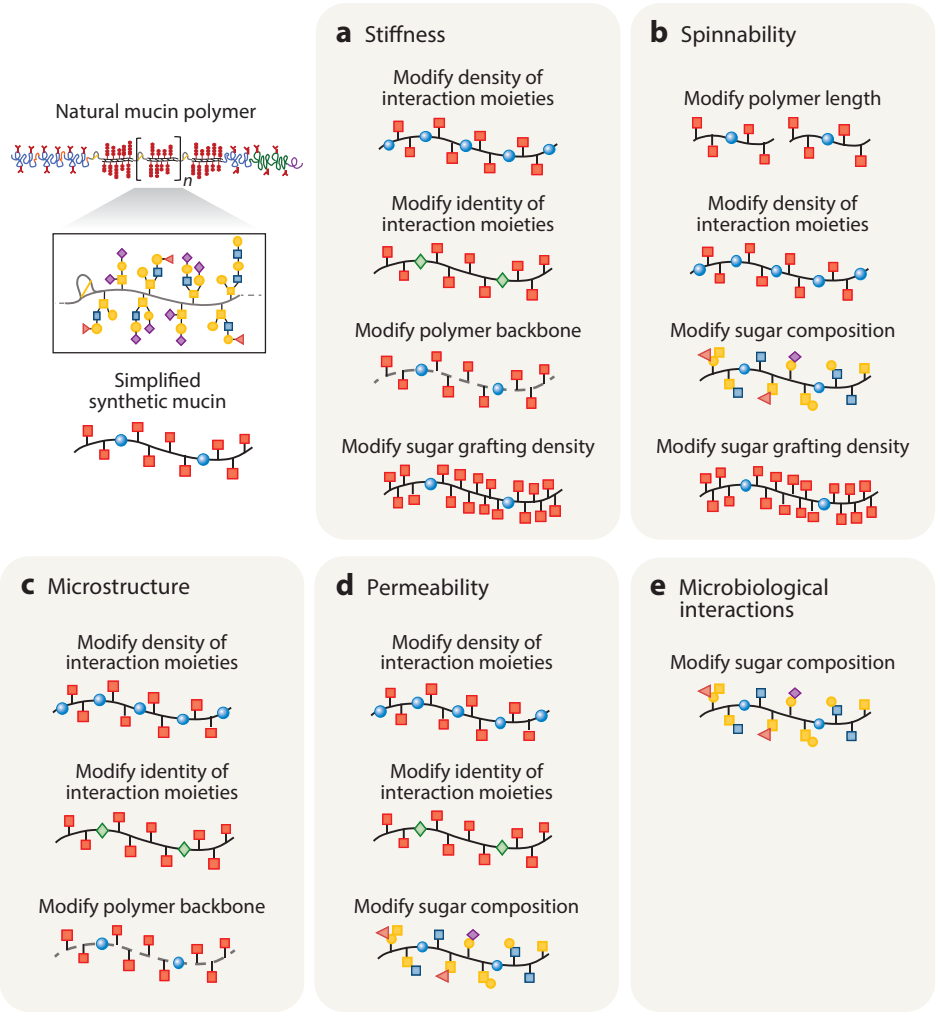

Figure 5

Proposed variations in the structure of synthetic mucin molecules to achieve specific functionalities in the contexts of (*a*) stiffness, (*b*) spinnability, (*c*) microscopic viscoelastic response and microstructure, (*d*) permeability, and (*e*) microbiological interactions. These variations will further elucidate the relationship between mucin structure and function and the specificity of glycan substructure. Studies of synthetic mucins may focus on how variations in the geometry of presenting a given glycan (e.g., polymer length and branching; grafting density) influence functionality. Studies with varied complex glycan compositions may reveal substructural portions of glycans required for activity. Glycan symbol nomenclature: yellow square, GalNAC; blue square, GlcNAc; yellow circle, galactose; purple triangle, fucose; purple diamond, Neu5Ac; red square, generic mucin sugar.

of steric and interaction-based filtering, respectively. From a design perspective, the former can be achieved by modifying the density of interaction moieties on the polymer backbone (*i*), while changing their identity (*ii*) and modifying the biochemical composition of the grafted sugar side chains (*vi*) are likely effective strategies for controlling interaction-based filtering. Finally, to engineer specific microbiological or immunological functionality (**Figure 5*e***), the literature in Section 4 suggests that modification to the biochemical composition of the grafted sugar side chains (*vi*) may be an effective experimental strategy.

Ultimately, we suspect that close collaboration between chemists and biologists will be required to create chemically complex synthetic polymers that also possess the biologically relevant properties of native mucins that are important for drug delivery and the treatment of mucosal diseases.

6. CONCLUSION

The physicochemical properties of mucus are largely due to the presence of its primary structural component, mucin polymers. In aqueous solution, mucins associate to form physical networks, and the rheological properties of the resulting hydrogels are essential for proper lubrication and protection from mechanical damage throughout the body. Mucin polymers also interact biochemically with microbes and foreign molecules, allowing mucus to serve as a protective barrier and as an ecological niche for the microbiome. One theme that is not addressed in this review is the interaction of mucins with the immune system. Nevertheless, we anticipate that the potential for mucins to interfere with immunological factors (such as antibodies and cytokines) as well as to regulate signaling via the mucin glycans will reveal important roles for mucins in shaping the immune system.

Because of the critical physiological roles that mucus plays, there is a growing need to understand the mechanistic details by which mucus and, in particular, mucins contribute to a healthy barrier. There is thus also an increased need for mucus model systems for the study of microbial behavior and the immune system, as well as the design and testing of drugs for the treatment of mucosal diseases. The inability of native mucus supplies to satisfy this demand has prompted the development of synthetic mucin molecules and mucosal tissue–mimetic polymers, but significant work remains to be done for these synthetic substitutes to achieve the functionality of native mucus. We suspect that the incorporation of simplified structural moieties into the synthetic molecule will help achieve this goal on the basis of biochemical features that contribute to a given mucosal physicochemical property.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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