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The role of mucosal barriers in disease progression and transmission

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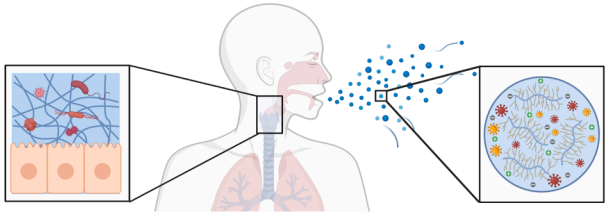
GRAPHICAL ABSTRACT



Mucins regulate pathogen transport and virulence **within hosts** via:
- Acting as size-based and biochemical barriers
- Binding to and sequestering pathogens
- Regulating microbial communication

Mucins alter pathogen survival and stability **at the point of transmission** via:
- Altering emitted droplet size distribution
- Altering droplet evaporation kinetics
- Influencing protein stability in evaporating droplets

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ABSTRACT

Mucus is a biological hydrogel that coats and protects all non-keratinized wet epithelial surfaces. Mucins, the primary structural components of mucus, are critical components of the gel layer that protect against invading pathogens. For communicable diseases, pathogen-mucin interactions contribute to the pathogen's fate and the potential for disease progression in-host, as well as the potential for onward transmission. We begin by reviewing in-host mucus filtering mechanisms, including size filtering and interaction filtering, which regulate the permeability of mucus barriers to all molecules including pathogens. Next, we discuss the role of mucins in communicable diseases at the point of transmission (i.e. how the encapsulation of pathogens in emitted mucosal droplets externally to hosts may modulate pathogen infectivity and viability). Overall, mucosal barriers modulate both host susceptibility as well as the dynamics of population-level disease transmission. The study of mucins and their use in models and experimental systems are therefore crucial for understanding the mechanistic biophysical principles underlying disease transmission and the early stages of host infection.

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1. Introduction

Mucus is a biological hydrogel that lubricates every wet epithelial surface of the body, including the respiratory tract, gastrointestinal tract (GI[1]), and reproductive tract. This lubricious characteristic is essential in protecting epithelia against mechanical damage from shear-induced forces involved in digestion, blinking, and exhalation [1–3]. Mucus serves as a dynamic physicochemical semipermeable barrier that permits the transport and exchange of select molecules (i.e., nutrients, water, gases, odorants, hormones) while trapping and immobilizing foreign and harmful substances (i.e., toxins, heavy metals, or biological substances such as pathogenic bacteria, viruses, or parasites) [4,5].

Humans continuously secrete mucus, amounting to approximately 10 L per day [6]. Mucus layers are subsequently shed, discarded, or digested and renewed by the continued mucus secretion of underlying epithelial cells. The lifetime or "clearance time" of mucus is short, often observed between minutes and hours, with the fastest turnover typically observed in the thinnest mucus layers (i.e., nasal tract) [7]. Thus, biological or synthetic particles must penetrate mucus faster than the natural turnover to reach their target sites.

The role of mucus and mucin, its primary structural component, in disease progression within individual hosts and in host-to-host transmission processes is increasingly being recognized. Mucins play a vital role in protective and defensive mechanisms against pathogens. Within hosts, the mesh network of mucin polymers in mucus acts as a size and biochemical filter to trap pathogens before they can reach target epithelial cells. However, some pathogens have adapted ways to avoid entrapment. Even large macromolecules are not always filtered by their size [8]; instead, a cascade of signals and interactions can alter the mucus environment and facilitate the transport of large molecules that would otherwise become immobilized and eventually cleared from the mucus layer.

At the point of transmission of infectious diseases such as influenza, mucin interactions outside of the host are equally as important as those within the host for continued survival of the pathogen as it travels from individual to individual. The in-host and ambient environments are vastly different in terms of temperature, humidity, pH, sunlight exposure, and other factors. Mucosalivary droplets ejected from infected hosts transport the pathogen to surfaces or ventilation systems or keep them suspended in the air prior to being introduced to the mucosa of another susceptible host. In the ambient environment, subsequent drying or evaporation of the water contents of these droplets leads to increased concentrations of other components such as salts, which may prove toxic to the pathogen and result in its inactivation [9,10].

Recently, pathogen–mucin interactions within hosts and at the point of transmission have been recognized as key research areas and have been integrated into models for within-host disease progression and population-level disease transmission [11–16]. While these two classes of models (i.e., within-host and population-level) are useful for simulating distinct phenomena, for a given disease the dynamics of both types of models are intimately related. It remains a challenge to bridge these models across different time and length scales; yet doing so is key for understanding the progression from within-host infection to host-to-host transmission [11]. Importantly, incorporating mucosal barriers will be critical for the development of first-principles and predictive models. Additionally, a better understanding of how mucins bind and sequester pathogens will be invaluable for guiding the development of mucin-mimetic biomaterials, including coatings that may prevent or immobilize the transfer of bacteria or viruses that elicit infection and disease.

In this review, we explore the role of mucus and mucins in disease progression within hosts and transmission between hosts. In Section 2, we describe the detailed biochemistry of mucosal barriers. In Section 3, we cover experimental protocols for working with mucus in laboratory settings, particularly via the purification of native mucins. In Section 4, we discuss the within-host protective role of the mucin network in terms of selective permeability in the context of both viruses and bacteria. In Section 5, we explore the role of mucus and mucins during transmission events, particularly in the context of viruses. Finally, we offer concluding remarks in Section 6.

2. Mucus biochemistry

Native mucus is primarily water (95%), with the remaining 5% comprised of salts (0.5%–1%), lipids (1%–2%), and proteins [17]. Mucins are large glycoproteins that contribute primarily to the viscoelastic and gel-like properties of mucus. Mucin is present at varying concentrations throughout the body: 1%–5% in the GI tract [8], up to 2% in the airways [18], and at lower concentrations in tear fluid (<0.02%) [19] and salivary fluid (~0.3%) [20].

The 21 mucin-type glycoproteins that belong to the MUC gene family and are found in humans (https://www.genenames.org) can be divided into two families: secreted and membrane-bound [21]. Membrane-bound mucins are relatively short compared with secreted mucins and are on the order of hundreds of nanometers in length, whereas secreted mucins can span several microns [7]. Within secreted mucins, there exist gel-forming mucins (MUC2, MUC5AC, MUC5B, MUC6, MUC19) as well as two nonpolymeric glycoproteins (MUC7 and MUC8) [22]. Moreover, in the airway, it has been suggested that membrane-spanning mucins form a brush-like structure within a periciliary layer immediately adjacent to epithelial cells, which is covered by a separate secreted mucus layer [23]. Indeed, different mucosal surfaces throughout the body produce different types of mucins [21]. For example, in the GI tract, MUC2 and MUC5AC are the most abundantly secreted mucins compared with the low amounts of MUC5B, MUC6, and MUC7 that are also present [8,22,24]. While MUC2 is almost entirely absent from other regions of the body, MUC5AC and MUC5B are more broadly expressed. MUC5AC is a major mucin component of gastric mucus [22], tear fluid [19], airways [25], and the female reproductive tract [26]. MUC5B features importantly in the airways and female reproductive tract [25,26], as well as in the salivary glands along with MUC7, which is exclusively found in salivary fluid [27].

Mucins typically have molecular weights in the range of 0.5–40 MDa, formed from the linking of a number of mucin monomers [28], each approximately 0.3–0.5 MDa [29]. Up to 80% of the mucin mass is attributed to its heavy glycosylation while the remaining mass represents the protein backbone [17,30]. Mucins contain variable-number tandem repeats (VNTRs) that are rich in proline, threonine, and/or serine (PTS domains) along with cysteine-rich regions at the amino and carboxy terminals and distributed between the PTS domains [21]. Mucins contain a number of PTS sequences along their protein backbone, where oligosaccharide chains, or glycans, are anchored onto the serine and threonine residues via O-linked glycosylation [17]. The glycosylation of serine and threonine residues results in a "bottle-brush" arrangement of glycans along the protein core [17,30]. Other carbohydrates that can be glycosylated to mucin include fucose, mannose, sulfate, and sialic acid [30]. The high sialic acid and sulfate content of mucins gives them an overall negative charge, which results in intramolecular repulsion under aqueous conditions [31]. Although the different mucin types contain similar structures, individual mucins have specialized functions and roles in the regions where they are expressed. These different roles arise from variability in their PTS-repeated domains, particularly their unique glycosylation signatures, sequences, and VNTRs [8].

[1] GI: gastrointestinal tract; HA: hemagglutinin; HIV: human immunodeficiency virus; IAV: influenza A virus; NA: neuraminidase; Neu5AC: N-glycolylneuraminic acid; Neu5Gc: N-glycolylneuraminic acid; PSM: porcine submaxillary mucin; PTS: proline, threonine, and/or serine; RH: relative humidity; RSV: respiratory syncytial virus; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; Sialic acids: Sias; SPT: single particle tracking; VNTR: variable-number tandem repeat.

Beyond biochemical differences between mucins, the thickness of mucus layers varies for different mucosal niches; in the gut, mucus layers are thick and adhere to the epithelium, but in the airway, mucus layers are thin and mobile. For example, salivary film has an estimated thickness of 70–100 μm [32], whereas the mucus layer along the respiratory tract is relatively thin (nasal cavity: 5–15 μm [33]; trachea: 10–30 μm [34]; bronchi: 2–5 μm [34]). In contrast, the thickness of the mucus layer in the GI tract varies along its length, being thinnest in the small intestine (150–300 μm), followed by the stomach (300 μm), and thickest in the large intestine (700 μm) [35,36]. Although natural processes such as digestion, violent exhalations, or blinking mechanically deform mucus, mucosal layers restore themselves through the regular secretion of mucus by epithelial cells and through rapid self-healing to retain their biophysical and viscoelastic properties [21].

In aqueous solutions, mucin molecules form polymeric networks maintained by physical entanglements and covalent and noncovalent interactions [8,37]. While noncovalent binding is relatively weaker than covalent binding, the cumulative effect of van der Waals, hydrophobic, ionic, hydrogen bonding, and other binding interactions can result in strong, long-lived mucin–mucin interactions [8,37]. Mucus gel structure, the strength of interactions within its network, and its bulk properties (e.g., macrorheological properties) can be regulated by various environmental modifications including the density of physical and chemical cross-links, changes to mucin conformation through variations in pH or ionic strength, and modifications to hydration via changes in mucin glycan density or identity [21].

Hydration is attributed not only to the high capacity of glycan chains to retain water [38] but also to variations in ionic composition and concentration. For example, hydrogen ions can shield glycosylated regions of mucin, affecting their electrostatic charge [39]. Other ions common to most mucus secretions include sodium chloride, potassium chloride, sodium bicarbonate, phosphate, magnesium, and calcium ions [6,22]. Highly acidic environments are believed to promote mucin aggregation (or phase separation), which increases mucus bulk viscoelasticity. This increased mucus viscoelasticity results in a stiffer mucin gel lining in the stomach, serving as a protective barrier for the epithelial lining against acidic gastric juices. While increased viscoelasticity may have a protective effect in certain areas, such as the GI tract, it can have negative effects in the respiratory tract, where increased viscosity reduces effective mucociliary clearance. Generally, lung mucus, nasal mucus, and saliva have a neutral pH while eye mucus is slightly basic (pH ~ 7.8) [7]. In contrast, gastric mucus has a wide pH range across the layer's thickness; the pH increases from acidic (pH ~ 1–2) to neutral between the luminal and epithelial surfaces [7].

The maintenance of mucus layers relies on a tight regulation of mucins, water, and ions [40] to produce different mechanical and biochemical properties needed for physiological function in different regions of the body. Dysregulation of any of these components can alter the mechanical properties of mucus and can provide ripe conditions for the proliferation of microbes and the progressive infiltration of pathogens.

3. Mucus harvesting and mucin purification

Although the *in vivo* composition and structure of mucus are preserved in native harvested mucus, the heterogeneity of mucus and the extensive variation in composition between individuals, and even within an individual, can make it difficult to interpret and compare experiments with native mucus [21]. As such, gels reconstituted from purified mucin molecules are an accepted experimental model for mucus that mimics selected properties of mucus and is relatively more homogenous than native samples because of the removal of other mucus components. Reconstituted mucin gels not only have a well-defined composition, but produce well-controlled, reproducible environments for assessing the influence of select factors.

Researchers can isolate mucins from mucosal tissues by either extracting mucus layers [41] or homogenizing whole tissues [42]. Pigs and cows have served as the primary sources of mucus due to their wide availability and the large amounts of mucus they contain relative to other sources. Depending on the source, researchers apply different techniques to animal tissues, such as mucus scraping, to extract mucin-containing material [43]. Purification is achieved by making use of mucin's unique physical and chemical characteristics, including their solubility, large size, and strong negative charge. Importantly, mucins are not completely resistant to degradation: the glycosylated fractions of mucins are relatively better protected against proteolytic degradation, while the unglycosylated portions are more vulnerable. Hence, researchers must take care both during mucin purification and when working with native mucus samples to mitigate mucin degradation or they must account for such processes in any physicochemical readouts of mucin gel properties [44].

Human mucin sources [43] may be more difficult to access and less abundant than animal tissue sources. Because of the limited availability of human mucins, research has relied heavily on commercial sources of mucins, specifically the porcine gastric mucin MUC5AC and the bovine submaxillary mucin MUC5B, which are the most relevant mucin models to humans. The two most widely used commercial purified mucins come in powdered form and are produced by Sigma Aldrich: "mucin from porcine stomach, Type II" and "mucin from porcine stomach, Type III" [45].

The harsh treatment processes during commercial mucin purification have been associated with altered mucin structures [21], causing changes to the physicochemical properties of gels reconstituted from these materials [46,47]. In fact, industrially purified mucins have been found to have a lower capacity for forming gels [45,48], and the resulting gels are less lubricious [45,49] than native mucin purified in-lab. A growing number of studies have used gels reconstituted from lab-purified mucins, which retain physicochemical properties relative to native mucus, enabling researchers to interrogate structure–function relationships of mucin glycoproteins [50–54]. Thus, the development of protocols to purify commercial mucins both at scale and while preserving their native structure is an essential area for future work.

4. Mucin networks as within-host semipermeable barriers

4.1. Overview of biopolymer network filtering methods

4.1.1. Size filtering

Mucins form a selectively permeable physical barrier capable of restricting or permitting the passage of certain molecules. The polymer mesh formed by mucin molecules can be characterized by the distance between junctions in the network, known as the pore size (or mesh size). The pore size of mucin gels spans tens of nanometers to thousands of nanometers (~20–1800 nm) [8]. This pore size varies with respect to not only its location in the body, but health status as well. For instance, the typical pore size for respiratory mucus is approximately 500 nm; however, the pore size decreases to approximately 150 nm in patients with cystic fibrosis, a chronic lung condition distinguished by mucus dehydration and ion-channel dysregulation [55].

On a macroscopic level, this polymer network increases the bulk viscosity of mucin gels by several orders of magnitude (1,000–10,000 times greater than the viscosity of water) [7]. In these networks, classical application of the Stokes–Einstein equation would predict displacements much smaller than the typical thickness of mucus layers over timescales relevant for mucus clearance for viruses or hydrophilic macromolecules. Yet, various studies have observed a decrease in particle mobility through mucus with increasing particle size that is inconsistent with the theoretical prediction arising from the background viscosity and Stokes–Einstein relationship [56–59]. This discrepancy suggests that particles smaller than the average pore size of mucus are capable of diffusing (assuming no biochemical interaction with mucin components) through low-viscosity pores within the mucus viscoelastic

matrix. This behavior indicates a size filtering mechanism that allows molecules and particles that are smaller than the pore size to cross between mucin molecules, while larger particles are trapped and confined (illustrated in Fig. 1) [8]. However, evidence has shown that certain macromolecules larger than the mucin network pores are capable of rapidly diffusing through mucus [60,61], suggesting that other methods of filtration apart from size filtration control mucus permeability.

4.1.2. Interaction filtering

Particles are not strictly prevented from penetrating through mucus by their size, but also by the classes of network interactions discussed in Section 2 with mucin molecules (illustrated in Fig. 1). These interactions allow for particle filtration on the basis of particle surface properties. Some particles, even those smaller than the characteristic mucus pore size, may interact frequently or strongly with mucus components and become confined or completely immobile, while others can exhibit a combination of weak, lower-frequency interactions, allowing them to diffuse freely. Moreover, particles or certain mucus treatments can alter the pore size, enabling larger particles to penetrate. For example, the diffusion of nanoparticles [62] and influenza virus [57] in mucus treated with mucolytic agents was greater than that observed in untreated mucus. In contrast, in the presence of emulsifiers (i.e., carboxyl methylcellulose), researchers observed a lower mucus pore size and lower diffusion rates of *Escherichia coli* [63]. Similarly, modified nanoparticles coated with mucolytic proteases show enhanced transport through mucus as a result of their ability to degrade mucin polymers [64].

Apart from the particle's surface chemistry, the number of particle binding sites with an affinity for mucus can impact its degree of interaction with mucins. For instance, small, relatively hydrophobic molecules show enhanced diffusivity through mucus relative to larger, biochemically similar molecules because they form only a few low-affinity, short-lived bonds with mucin polymers. In contrast, the negatively charged glycan domains on mucins are sites where small cationic molecules and polyvalent cations can attach strongly [65]. Although a higher positive charge is associated with stronger binding between particles and mucus, overall surface charge is not an exact predictor for the strength of binding and resulting transport. This finding is supported by work demonstrating that the geometric arrangement of positive and negative charges for an equivalent overall surface charge can influence transport [66].

The dense carbohydrate chains on mucins serve as binding sites for nanoparticles and various pathogens. Although mucin's sugar chains provide anti-proteolytic properties, mucins are not completely resistant to degradation by bacterial species or other changes to their structure by factors such as pH, ionic strength, and exposure to ambient air, temperature, or light. Bacterial enzymes can degrade mucins through proteolytic or polysaccharide cleavage, which enhances bacterial permeability through mucus and accommodates microbial growth [67]. Microbial degradation of mucin is also influenced by glycosylation patterns which are unique to each mucin protein [68] as bacteria can have glycan-binding specificity [69,70]. It has been hypothesized that colonic mucus is less susceptible than gastric mucus to degradation by *Clostridium* and *Bacteriodes* species, potentially due to the different amounts of sulfated and fucosylated sugars in these mucus types [68,71]. For example, MUC2, which is found in the intestine, has a high degree of sialylation and sulfation [72] while nearly half of the O-glycans of MUC5AC, which is secreted in the stomach [22], have low sialylation and fucosylation [72].

Mucin can protect underlying epithelial cells by presenting "decoy" glycans for bacteria to bind, thus preventing the bacteria from reaching their target cells [73]. It is believed that the diversity of glycans on mucins allows mucins to bind and trap a broad spectrum of bacteria that can eventually be removed by the natural turnover of mucus [73]. Thus, the diverse glycan signatures expressed on the mucins of an individual play a significant role in determining an individual's susceptibility to infection [73].

4.2. Within-host mucin/virus interactions

The host-to-host transmission patterns of viral respiratory infectious diseases such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza, and respiratory syncytial virus (RSV) are closely tied to the biophysical processes that occur within hosts and external to hosts at the point of transmission. Mucosal barriers are key components that influence disease pathogenesis and transmission via physiochemical interactions with viruses, which can alter infection dynamics within hosts and the viability of viruses emitted from an infected individual in the form of mucosalivary droplets.

In a host, the mucus layer lining the respiratory tract serves as the "first line of defense" against inhaled pathogens [74]. Viruses are generally 20–200 nm in diameter, which allows them to penetrate the pores of mucin gels [6]. However, adhesive interactions with mucus may slow this diffusion depending on the surface properties of the virus [74]. Instead of secreting mucin-degrading enzymes as bacterial species do, viruses have evolved surface chemistries that favor minimal biochemical interactions with the components of mucus barriers [75,76]. Non-enveloped viruses, such as human papilloma virus and norovirus, are believed to be minimally adhesive to mucin due to the mixture of positive and negative surface charges that result in an overall neutral surface charge [77]. In addition to their net charge, non-enveloped viruses

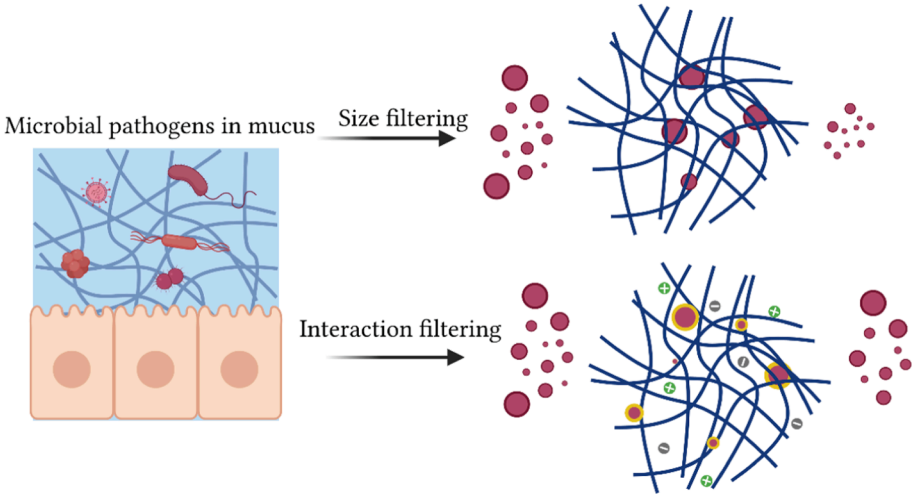

Fig. 1. Filtering mechanisms regulating mucus permeability: size filtering and interaction filtering. Size filtering allows molecules and particles smaller than the mucin network mesh size to cross, while larger molecules are rejected. Interaction filtering allows particles to be selected according to their surface properties and binding interactions with the mucin network. Some particles interact strongly with mucus and are trapped (particles with thick yellow–orange edges), whereas other particles exhibit only weak interactions and pass through the network (particles with thin back edges).

may not interact with mucin via hydrophobic interactions because they have few hydrophobic regions [68].

Recent evidence suggests that viruses may more effectively spread and infect target cells as an aggregate of infectious units [78]. Variations in pH and salt concentration have been shown to produce viral aggregates in saliva [79]. However, the benefits of forming these larger virion aggregates in terms of greater infection potential can be expected to be offset by enhanced steric or adhesive interactions with the mucin network, illustrating a mechanism by which mucus may display antiviral properties.

Early researchers determined that influenza A viruses (IAVs) have an affinity for mucus [80]. During transmission, the virus initially encounters respiratory tract mucus in the nasal cavity or oral cavity and must overcome this barrier to reach its target epithelial cells. Among other purified salivary proteins, MUC5B from human whole saliva has been show to inhibit IAVs at physiologically relevant concentrations [81]. It has been long hypothesized that mucus may act as a barrier against IAV infection by imitating cell surface receptors [82]. Mucins are rich in terminal sialic acids (Sias), which are thought to act as "decoy receptors" that can trap IAVs in the mucus layer and then clear viruses by the natural turnover of mucus [29,82,83]. In the human respiratory tract, the distribution of terminal Sias alpha2,6 and alpha2,3, which are also expressed in the porcine respiratory tract [74], varies along the respiratory tract and with aging [84]. Specific sialic acid types are more abundant in certain hosts and in particular physiological locations. For example, alpha2,3-linked Sias are more abundant in the GI tract of avian hosts, while alpha2,6-linked Sias are more abundant in the human upper respiratory tract [74,84]. Viruses also have a Sias binding preference: human influenza viruses preferentially bind alpha2,6-linked Sias, while avian and equine influenza viruses preferentially bind to alpha2,3-linked Sias[85,86]. Thus, host restriction (i.e., virus receptor specificity vs. host receptor) and susceptibility may be significantly influenced by factors such as structural variations in sialic acid linkages, spatial distribution of linkages in hosts, and Sias binding preferences [87].

Two surface proteins of IAVs, hemagglutinin (HA) and neuraminidase (NA), have specialized functions that initiate infection. HA binds to sialic acid receptors on the surface of cells and induces membrane fusion [88]. NA is responsible for releasing the virus into cells by cleaving the receptors [89]. While mucus is protective against IAVs, NA potentially circumvents entrapment of the virus by cleaving mucin's "decoy receptor" and enabling the virus to transport across the mucus barrier to infect the epithelium. In an *in vitro* investigation in which influenza viruses were added to a layer of porcine respiratory mucus [83], the degree of penetration of the viruses in the mucus layer was shown to be enhanced by the addition of NA, while the addition of oseltamivir, an NA inhibitor, demonstrated reduced penetration of the viruses [83]. Similarly, in another *in vitro* study with swine- and human-origin viruses, purified sialylated human salivary mucins competitively inhibited NA cleavage in a dose-dependent manner, whereas porcine submaxillary mucin (PSM) could not prevent infection of underlying Madin–Darby canine kidney cells [82]. Although PSM also contains sialic acids, the presentation of sialic acid differs between PSM and human salivary mucin. Human influenza viruses bind alpha2,6-linked N-glycolylneuraminic acid (Neu5Ac), while PSM and many other animal models express N-glycolylneuraminic acid (Neu5Gc) [90]. This aspect is especially important to note in the selection of animal mucus models because the studied virus may not interact with receptors encountered in the native mucus environment.

The importance of the mucosal barriers in determining the fate of pathogens in hosts is becoming increasingly recognized. Recently, theoretical and computational models of within-host disease spread have incorporated physiological characteristics of mucosal layers and biophysical properties of viruses [12–16]. In particular, two studies investigated the spread of infection by SARS-CoV-2 [12] and influenza [13] virions throughout the respiratory tract. These studies incorporated not only the thickness of the mucosal layer but the advection of the layer by underlying cilia, along with pathogen diffusion and cell infection. In brief, mucus can be characterized by rheological measurements to obtain information about bulk gel properties and the mucin network; this is done by either rheometers (macroscopic measurements) or by single particle tracking methods (SPT, microscopic measurements) [91]. In SPT methods, charged fluorescent micrometer-sized probes are dispersed in the gel, imaged with a microscope, and tracked using SPT software. The same method is often used to measure the transport behavior of biological or synthetic particles.

As models further develop and distinct properties of mucosal barriers can be incorporated, it will be crucial to determine which factors are the key drivers of different phases of disease transmission (i.e., clearance, infection, progression). Presently, the viral diffusivities used in models are the combined effect of steric and binding interactions; more work is needed to separate these two effects to not only understand the mechanisms by which pathogens move through mucus but also to more effectively target pathogens. These models serve as platforms for exploring disease outcomes and can also be leveraged to identify effective treatments against viral infection, develop methods to strengthen the mucus barrier (e.g. tighten mucus pores, increase strength/frequency of pathogen-mucin binding), and understand mechanisms by which viruses become immobilized and inactivated in mucus. For example, earlier modeling explored the capacity of virus-specific antibodies for blocking human immunodeficiency virus (HIV) infections *in vivo* [15,16] and investigated antibody characteristics to maximize their pathogen-trapping capabilities [14,92].

It is important to note that models often rely on properties that have been measured in experiments either *in vivo* or *in vitro*. Recent work has demonstrated varying levels of agreement in the transport of synthetic particles in native mucus and experimental model systems simulating native mucus (e.g., gels reconstituted from mucin, commercial mucins, or other commercial polymers) [93]. Therefore, it will be important to consider how environmental conditions (e.g. pH, temperature, ion and polymer type/ concentration) and instrumental methods (refer to [21,94] for experimental techniques for characterizing transport through mucus) affect predictions for estimates of drug or virion mobility in mucosal layers.

4.3. Within-host mucin/bacteria interactions

While one of mucus' primary roles is to serve as a selective and protective barrier to underlying epithelial cells, it also serves as a nutrient source on which bacteria can proliferate and thrive. Indeed, a number of diverse bacterial communities thrive in the mucus environment [95], even with its high resistance to microbial proteases. The degradation of mucin can indirectly benefit certain bacteria, including pathogenic bacteria, that lack specific enzymes by providing a nutrient source of mucus-derived sugars [96]. At the same time, mucus-degrading species can promote the selection of commensal microbes and support a beneficial microbiota. Apart from mucins serving as a nutrient source, the molecules harbored in the network of mucins or mucins themselves may trigger changes in the expression of bacterial species [97]. This behavior emphasizes the crucial role of mucus in cases where certain bacterial species would otherwise compete [52] or where a bacterial species (i.e., opportunistic pathogen) would otherwise present with virulence features (i.e., biofilm growth) [98,99]. In addition, mucins, similarly to their interactions with certain viruses, can behave as non-productive decoys that prevent the interaction of bacterial adhesins with epithelial surfaces [100].

While viruses such as RSV can change the composition of mucus by increasing the production of mucus-secreting cells [101], their effect on mucus rheology has not yet been studied for common infectious viral diseases. During infection with certain viruses such as SARS-CoV-2 [102] and in chronic diseases such as cystic fibrosis, asthma, and chronic obstructive pulmonary disease [55], the dysregulation of water

and ion concentrations in mucus layers can strongly impact mucus hydration. Dysregulated hydration can result in a thickened mucus layer that is less easily cleared, which can further impact disease progression. Interestingly, in the case of viral infections, these effects generally appear to initiate after the onset of infection. In contrast, other pathogens can alter the properties of mucus to enhance their transport. Studies have shown that the pathogenic bacterium *Helicobacter pylori*, which is responsible for gastric ulcers, achieves motility in the mucus layer by modifying the layer's rheological properties [50,103]. *H. pylori* is able to colonize in the harsh acidic environment of the human stomach by producing urease, which catalyzes urea hydrolysis to yield ammonia, resulting in an elevated pH [50]. This increased environmental pH reduces the mucus viscoelasticity and increases motility across the GI mucus layer [50].

It is possible that viruses and bacteria may be mutually beneficial to one another. Bacteria may aid viruses in overcoming the mucus barrier. In particular, mucin-degrading species may break down mucin sugars, facilitating a path for viruses to penetrate. Once viruses reach their target cells and shut down the body's immune defenses, bacteria have the potential to initiate their own infection. This behavior can be seen in viral infections that result in secondary bacterial infections due to altered immune function or altered dynamics of inter-microbial interactions [104].

5. Role of mucus in infectious disease transmission

When a virus is emitted, whether through coughing, sneezing, talking, or breathing, it is enveloped in respiratory tract fluid, and its successful onward transmission depends on it remaining viable until its transfer to a new host. Real-time reverse transcription PCR detection results for throat, nasal, saliva, and sputum specimens from individuals with respiratory infections (i.e., influenza and SARS-CoV-2) have shown that exhalation emissions originating from different regions of the respiratory tract can exhibit a range of viral loads. Air samples in areas with nearby infected individuals not only contain viral RNA but also live, culturable viruses, supporting the route of aerosol transmission. Respiratory droplets traveling in the air will be entrained and advected in ambient air flows or the cloud of moist buoyant air emitted by the individual [105,106]. Larger droplets may settle quickly to the ground and contribute to infection via fomites, whereas smaller aerosolized droplets may remain suspended in the air [13,113]. As previously discussed, mucin polymers contribute to the viscoelastic and biochemical properties of mucosal sources within the body. Additionally, the presence of polymers shifts the size distributions of droplets generated when solutions are sprayed, as occurs during sneezing and coughing [107,108]. Under different ambient temperature and humidity conditions, droplets will undergo differential degrees of evaporation, which induce a variety of physicochemical transformations to the droplet, thus determining the duration of pathogen viability. Finally, we note that there may be important differences in the "quality" of the exhaled aerosol (e.g. droplet size and spatial dispersion) between individuals [109].

Researchers have extensively studied the effect of external climate factors or ambient conditions, such as temperature and humidity (particularly relative humidity [RH]), on virus viability [10,110–115]. Among two early studies on this subject, only one found increased virus viability at lower temperatures [111], but both concluded varied effects of RH for the types of viruses tested [110,111]. More recent studies have found IAV viability in droplets to be highest at low RH [114], or highest at low and high RH and lowest in intermediate RH ranges [116]. The latter finding, including decreased viability with increasing temperature, was also observed in work combining experimental data for SARS-CoV-2 and other human coronaviruses [113]. The interplay among ionic strength, pH, and RH in the droplet complicates the identification of physical mechanisms for pathogen inactivation and survival. As the droplet evaporates and shrinks, the concentrations of salts, proteins, and other components increase by nearly an order of magnitude due to water loss by evaporation [9], which can alter the pH of the droplet environment [10]. Moreover, apart from evolving concentrations, the presence of solutes in the water broadly alters evaporation parameters, including droplet lifetime, evolution of the droplet morphology, and final residue or nucleus size.

The effect of the presence of proteins, particularly mucins, on the viability of viruses in droplets remains unresolved. Early work found that the addition of bovine serum albumin to Langat virus droplets increased survival across a range of RH values [117]. A more recent study showed that the presence of bovine serum albumin protected both bacteriophage MS2, a non-enveloped virus, and bacteriophage φ6, an enveloped virus, from inactivation in droplets [112]. At intermediate RHs, the viability of IAV decreased in saline solutions, yet increased dramatically in the presence of salt and mucus [10]. However, protein-rich media alone with salt did not significantly alter the viability, highlighting a potentially unique effect of mucins in mitigating adverse effects of elevated salt concentrations on virus survival [10]. In recent work, the remains or dried residue of saline droplets versus salt–mucin droplets evaporating on superhydrophobic substrates emulating the drying of aerosol droplets were found to be distinct [118]. In the saline droplets, a single crystal shape remained while in the salt-mucin droplets, a "bone-like" structure remained, indicating a disruption in crystallization by the presence of the proteins (Fig. 2) [118]. Similarly, on more wetting surfaces, modified crystallization patterns arose in the presence of mucins (Fig. 2) [119].

During evaporation, droplets with solutes including viruses, bacteria, proteins, and salts form dried precipitates with patterns resulting from the agglomeration of salt, proteins, and other materials. Generally, these patterns arise from capillary or Marangoni flows inside the droplets. Capillary flows within droplets lead to the deposition of solute particles near the pinned contact line, causing the formation of a so-called "coffee ring" pattern upon drying. In contrast, Marangoni flows in droplets arise from variations or gradients in surface tension, temperature, or solute concentration at the liquid interface of the droplet. This gradient will determine whether solutes are directed toward or away from the droplet's contact line [119]. The evaporation-induced solute concentration gradient near the droplet surface not only rearranges the deposition of solutes but also slows the drying or evaporation process and leads to the formation of a crust or shell. Depending on the type of solute, the resulting crust may be dry (i.e., salty droplets) or a "gel-like wet skin" composed of a combination of polymers, proteins, and other suspended particles [120]. As evaporation continues, the crust becomes thicker, which further reduces the evaporation rate. In the case of a wet gel-like crust, water will continue to evaporate through its pores via diffusion. This behavior is corroborated by recent work demonstrating changes in the transparency of droplets with porcine gastric mucin and salt (RH $<$ 80%), suggesting the onset of gelation [9]. Higher ionic strength may promote gelation by screening electrostatic attractions within and between mucins, which may also promote the aggregation of mucin molecules into a more concentrated layer [46,121,122].

Separate from salt effects, the pH of droplets varies during evaporation. This process is sensitive to the surrounding environmental temperature and RH [123,124]. Due to the loss of water during evaporation, the concentration of free H^+ ions in a droplet increases, reducing the droplet pH. Similarly, the enrichment of ions such as H_3O^+ and OH^- at the droplet interface may create pH gradients inside the droplets [10,125]. Both non-enveloped and enveloped viruses are generally more susceptible to inactivation in acidic and basic solutions than in pH-neutral solutions [126]. At extreme pH values, virus structures are destroyed, and the virus is inactivated [127,128]. Mucins also undergo conformational changes in response to pH changes in their environments. At near neutral pH levels, mucins exist as random coils [30], while under acidic conditions near pH = 2, carboxylate salt bridges on the mucins break. The breaking of carboxylate bridges causes the mucins to unfold and expose hydrophobic regions, which then cross-link to form a gel [30,129,130]. Thus, pathogens may become embedded within the

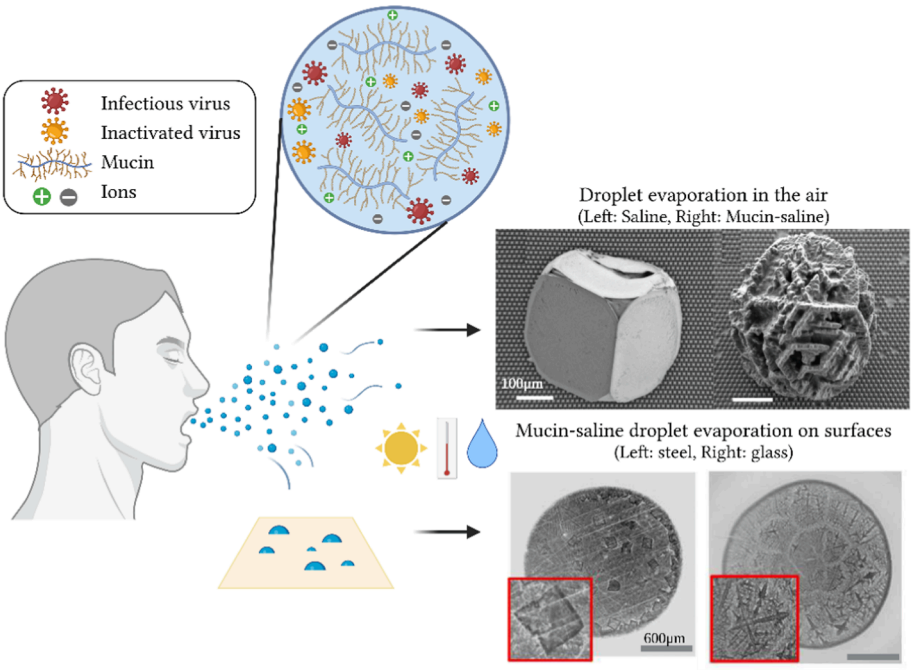

Fig. 2. The surrounding environment (sunlight, temperature, moisture) and composition of respiratory droplets affect their evaporation behavior, as well as the ionic concentration/strength, and pH of the droplet environment over time. The evaporation of droplets on superhydrophobic surfaces (to simulate evaporation in the air) of saline and mucin–saline results in distinct final residues upon drying (image reproduced with permission from [118] in the top right panel). Similarly, evaporation on surfaces results in flat residues with distinctive morphologies depending on the surface properties and droplet contents (image reproduced from [119] in the bottom right panel). Altogether, the temporal evolution of droplet composition and resulting deposition patterns modulate the infectivity and viability of pathogens encapsulated in mucosal droplets.

gel-like residue crust. Pathogens may benefit from being blanketed by this crust by obtaining protection from the harsh non-native conditions of the surrounding environment.

The presence of salts and their elevated levels in evaporating droplets can have deleterious effects on enveloped viruses. Researchers have studied various effects of salts on viruses, including osmotic damage and ion-induced structural changes to lipid bilayers. Salts in solution, such as sodium chloride, challenge the survival of enveloped viruses such as φ6, influenza, or coronaviruses, due to the osmotic pressure difference across the lipid membrane. While most microorganisms, as well as human/animal/plant cells, can maintain an osmotic pressure balance, the enveloped virus experiences increased osmotic stress during the drying process of a droplet. Without the ability to transport water across the virus lipid membrane due to a lack of water regulatory channels, enveloped viruses are vulnerable to osmotic damage [118]. A previous study evidenced enhanced inactivation of viruses by salts at specific pH levels [128], leading to alterations in membrane structure; however, the exact mechanism has not been identified [10]. While salts appear to be toxic to enveloped viruses, salts improve the viability of non-enveloped viruses, possibly because they are less susceptible to structural damage than enveloped viruses. Ultimately, once the exterior of an enveloped virus is damaged, the virus is compromised and loses its infectivity, in part due to a loss of critical envelope proteins needed for binding to host cell receptors. Yet, non-enveloped viruses contain these proteins responsible for cell attachment on their capsids and are reportedly more resistant to inactivation [131]. Studies have demonstrated that viruses tend to aggregate in solutions with high salt, which may increase their stability in such environments [132]. Virus aggregation may be enhanced in evaporating droplets as salt concentrations increase concurrently with droplet shrinkage, and hence, aggregation may enhance the viability of non-enveloped viruses even under conditions of complete desiccation.

While mucins in the body function as potential site receptor decoys to pathogens or as physical barriers to pathogen entry, outside of the body, they are potentially advantageous to pathogens in terms of promoting viability. Often, models and experiments on the transport and viability of airborne viruses assume that the projected fluid can be modeled as water. However, this oversimplification ignores the complex composition and interactions that occur between respiratory tract fluids and pathogens. Even experimental studies that do incorporate the effect of mucins largely use commercial porcine gastric mucins in mixtures to model mucosalivary droplets. As discussed in Section 3, commercial, industrially purified mucins such as porcine gastric mucin do not form gels and exhibit dramatically lower anti-viral and anti-bacterial activity [47,133], as well as inferior lubricity [134]. Hence, to further explore the effects of mucin in pathogen transmission, the use of lab-purified mucins will be crucial to preserve these complex physicochemical interactions.

6. Conclusion

A vast array of research has demonstrated unique characteristics of mucins that can be potentially advantageous or deleterious to pathogens by promoting binding and sequestration within hosts. Yet, many questions remain in our understanding of the mechanistic details by which mucus, particularly mucins, interacts with pathogens and modulates disease progression and transmission both within and external to hosts. Careful experimental studies assessing pathogen transport through mucin gels and viability external to hosts are necessary to begin to answer these questions. We note that the model systems chosen to study these problems, in terms of both mucins and pathogens, will be critical. Indeed, while the limited availability of native mucus and physiologically intact lab-purified mucins has prompted the use of commercial mucin molecules, significant work remains to demonstrate whether these polymers are physicochemically comparable to native products. Insight from such studies will enable the effect of mucosal barriers to be incorporated into models for disease transmission from first-principles, improving their mechanistic basis and predictive ability, with important implications for designing disease mitigation strategies and guiding public health policy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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