REVIEW ARTICLE

Salivary mucins in host defense and disease prevention

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Mucus forms a protective coating on wet epithelial surfaces throughout the body that houses the microbiota and plays a key role in host defense. Mucins, the primary structural components of mucus that creates its viscoelastic properties, are critical components of the gel layer that protect against invading pathogens. Altered mucin production has been implicated in diseases such as ulcerative colitis, asthma, and cystic fibrosis, which highlights the importance of mucins in maintaining homeostasis. Different types of mucins exist throughout the body in various locations such as the gastrointestinal tract, lungs, and female genital tract, but this review will focus on mucins in the oral cavity. Salivary mucin structure, localization within the oral cavity, and defense mechanisms will be discussed. These concepts will then be applied to present what is known about the protective function of mucins in oral diseases such as HIV/AIDS, oral candidiasis, and dental caries.

Keywords: *saliva*; *mucin*; *oral health*; *MUC5B*; *MUC7*

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Mucins, the primary gel-forming components of mucus, provide a critical layer of protection on wet epithelial surfaces in the body including the gastrointestinal tract, female genital tract, and respiratory tract. Unregulated mucin production can greatly affect the health of the host. For example, mice spontaneously develop ulcerative colitis when intestinal mucin is artificially downregulated (1). In a separate study where lung mucin was downregulated in mice, there were significantly more bacteria in their lungs, which greatly reduced long-term survival (2). In the oral cavity, decreased salivary flow is linked to the increased incidence of candidiasis and dental caries, which could be caused by reduced levels of salivary mucins (3–6). These findings highlight the importance of regulated mucin production, but our understanding of the precise mechanisms by which mucins provide protection in the oral cavity is continually being revised. One of the primary questions that still remains unanswered is how the mucus layer protects the oral cavity from pathogenic microbes and harmful substances while also providing a home for the beneficial oral microbiota. This review will provide an overview of what is known about the structural features of salivary mucins, potential mechanisms by which salivary mucins protect the oral cavity without widespread bacterial killing, and how they play a role in the prevention of specific oral diseases.

Introduction to salivary mucins

There are at least 20 identified mucins throughout the human body that cover wet epithelial surfaces such as the gastrointestinal tract, respiratory tract, and eyes. A summary of areas where mucins can be found in the body is given in Fig. 1a. Each of these mucins has a unique structure that can influence its localization and function. This section will address structural aspects of the mucins found in the oral cavity, MUC5B, MUC7, MUC19, MUC1, and MUC4 (7).

Mucins in the oral cavity

Each of the salivary mucins MUC5B, MUC7, MUC19, MUC1, and MUC4 are composed of a unique domain structure that influences the mucins' physical properties and localization in the oral cavity (Fig. 1b). MUC5B is the primary gel-forming mucin in the mouth that is secreted by mucous cells in the submandibular, sublingual, palatine, and labial salivary glands (8, 9). Transcripts and glycoproteins of MUC19, another gel-forming salivary mucin, have been identified, but MUC5B is still thought to be the predominate gel-forming mucin in the oral cavity (10–12).

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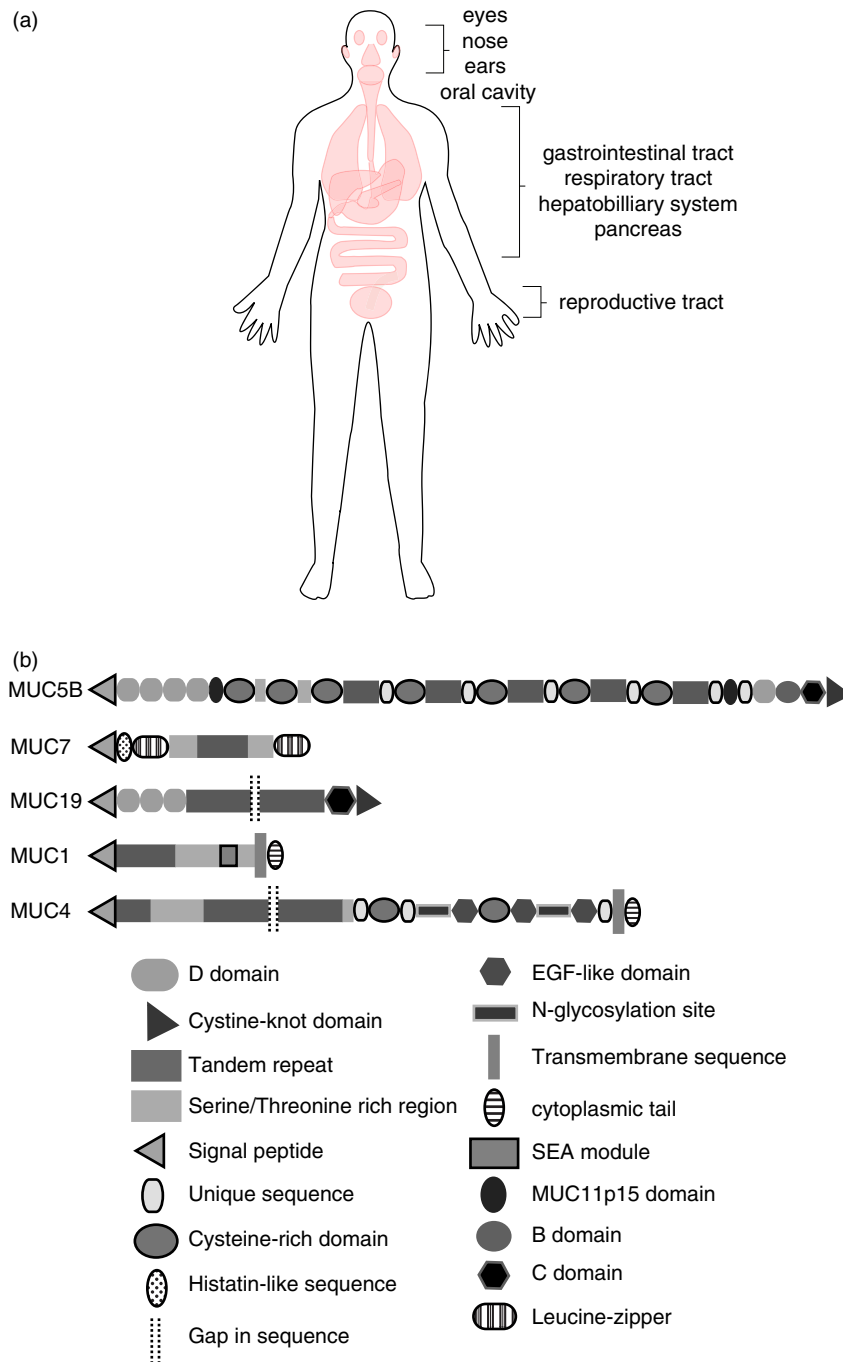
Fig. 1. Introduction to mucins. (a) Epithelial surfaces where mucins can be found in the body. (b) Predicted domain structures of the salivary mucins MUC5B (20, 21), MUC7 (21, 22), MUC19 (12), MUC1 (21, 23, 24), and MUC4 (25). Note that predicted domain sequences can vary based on detection method.

MUC7 is also a secreted mucin that exists primarily as monomers or dimers and lacks gel-forming properties. These monomers and dimers are able to self-associate, however, to form higher order assemblies that could be important for bacterial aggregation (13). MUC7 localization within salivary glands varies between individuals; it has been identified in mucous cells of submandibular and sublingual glands, but the presence of MUC7 in serous cells of these glands is variable (14). MUC1 and MUC4 are membrane-associated mucins that line the ducts of parotid, submandibular, and minor salivary glands (15, 16). These mucins may play a role in cell signal transduction and could form scaffolds for secreted mucins to bind (15, 17–19). Although several salivary mucins have been

introduced, the following sections will focus specifically on MUC7 and MUC5B structure and function because these are the primary mucins found in saliva.

Salivary mucin structure and secretion

MUC7 and MUC5B have several unique aspects of their primary sequences that determine their ability to form gels and higher order structures, but they also share several commonalities. Both MUC7 and MUC5B are composed of a protein backbone with glycan chains radiating outward to form a 'bottle-brush' structure. There have been several excellent papers that outline the composition of their glycan chains, which can be referred to for more detailed descriptions (26–29).

The MUC5B backbone is composed of approximately 5,700 amino acids and is broadly organized into the N-terminus, central glycosylated region, and C-terminus (30–32). The exact number of amino acids in the backbone varies among studies most likely because of variations in the tandem repeat region. MUC5B's central glycosylated region contains repeating units of 29 amino acids that are rich in serine and threonine (30). The C-terminal domain participates in disulfide bond formation, which links individual MUC5B monomers into dimers, and then polymer chains form through disulfide bond formation at the N-terminus (33, 34). Several excellent reviews detail the formation of mucin polymers and packaging within the cell (35–37). Once the packaged mucin granule is secreted, divalent calcium ions, which stabilize the folded mucin polymer within the secretory granule, are exchanged for monovalent sodium ions (31, 38). The increased osmotic pressure leads to hydration, which drives expansion of the polymers and formation of a gel (31). The expanded polymers cross-link via entanglement of glycoprotein polymer chains and/or non-covalent bonds formed by hydrophobic or carbohydrate–carbohydrate interactions (39–43). Calcium may also mediate cross-linking of MUC5B to form higher order structures (44). The resulting hydrogel coats the oral epithelium as part of the protective pellicle layer and houses a vast number of oral microbes (45, 46).

MUC7 has a 357-amino-acid backbone with a central region of repeating units composed of 23 amino acids (47). MUC7 lacks a terminal cysteine rich domain; therefore, MUC7 mucins are unable to form polymers and exist mainly as monomers (22). There are several excellent reviews that further detail the structure of these mucins (29, 36, 37, 48–52). The differences in MUC7 and MUC5B structure and physical location in the oral cavity impact the ways in which they provide protection, which is addressed in the following section.

Mechanisms of protection by salivary mucins

Mucins protect the oral cavity through several different mechanisms that are influenced by their unique polymer structures. First, mucins can interact with salivary proteins to alter their localization and retention, which could provide increased protection for the oral cavity. In addition, MUC7 and MUC5B can interact with oral microbes to facilitate their removal and/or reduce their pathogenicity.

Interactions between mucins and salivary proteins

One way MUC7 and MUC5B protect the oral cavity is by binding to antibacterial salivary proteins, which can influence the proteins' localization in the oral cavity, increase their retention time, and alter their biological activity. When a library of submandibular gland proteins was screened for interaction with MUC7, acidic and basic proline-rich proteins, statherins and histatin 1 were found to bind the N-terminal domain on the MUC7 polypeptide backbone (53). These proteins all have antimicrobial properties; therefore, increasing their availability in saliva could be beneficial to oral health. Western blotting revealed that MUC5B also forms heterotypic complexes with the same salivary proteins as MUC7 (54). In some cases, salivary mucins have been shown to be involved in sIgA binding to the mucosal pellicle, which would enhance sIgA concentration near the oral epithelium (55). MUC5B and MUC7 binding to this select group of salivary proteins indicates that the formation of these complexes is protein specific (54). To better understand the nature of these complexes, Iontcheva et al. (54) show that the interaction between MUC5B and proline-rich proteins, statherins, and histatins can be dissociated using denaturing conditions, indicating that these proteins bind through hydrophobic or ionic interactions, hydrogen bonding, or van der Waals forces. In some cases, proline-rich proteins and statherins were also able to form bonds with MUC5B that were resistant to denaturing conditions, suggesting that covalent interactions may be involved in some types of complexes (54). Collectively, these studies indicate that salivary mucins may serve as carriers for antibacterial salivary proteins to transport them throughout the oral cavity, increase their retention in the dental pellicle, and/or protect proteins from proteolytic degradation through the formation of complexes. Further studies are needed, however, to better understand the effect of these complexes on the biological activity of each component.

Mucins binding microbes

In addition to forming complexes with salivary proteins, early studies show that submandibular/sublingual gland saliva and salivary mucins aggregate specific strains of suspended bacteria and induce bacterial attachment to mucin-coated surfaces (56–59). These studies primarily focus on interactions between salivary mucins and oral streptococci. Because mucins induce aggregation or surface attachment of certain bacterial species, this indicates that the bacteria recognize and bind specific glycans on the mucins,

such as sialic acid and blood-group antigens (59–61). According to these observations, salivary mucins protect the oral cavity in two ways: 1) by aggregating bacteria suspended in saliva, which facilitates their removal from the oral cavity during swallowing and 2) by glycan-specific interactions with bacteria that lead to their dispersal and selective removal. Importantly, many of these early studies do not distinguish between MUC5B and MUC7 salivary mucins. When these two mucins are electrophor-etically separated, however, radiolabeled *Streptococcus sanguinis*, *S. sobrinus*, and *S. oralis* bind to MUC7 but not MUC5B (62). Another similar study also shows that MUC7 aggregates *S. gordonii* and promotes its adherence to surfaces whereas MUC5B has no effect on aggregation or binding (63). These findings indicate that MUC7 and MUC5B exert their protective effects through different mechanisms.

Once MUC7 was found to be the primary mucin directly interacting with various oral bacterial species, researchers began probing the mechanism of MUC7 binding to such a wide variety of bacterial species. Sialic acid residues on MUC7 glycans were found to play a role in binding several *S. gordonii* and *S. sanguinis* strains; when the sialic acid was removed using neuraminidase, binding of these strains was significantly reduced (64, 65). The surface glycoproteins GspB and Hsa on *S. gordonii* strains were shown to mediate binding to sialic acid residues on the mucin surface (66). Because several bacterial species bind MUC7 in a sialic acid-dependent manner, these receptors may be conserved among different streptococcal species that recognize sialic acid glycans, but further research is needed to verify this. Although several *S. gordonii* and *S. sanguinis* strains bind MUC7 via sialic acid, other *S. gordonii* strains were found to bind MUC7 through alternate surface proteins. When surface proteins from *S. gordonii* PK488 were overlaid with MUC7, the proteins alpha-enolase, EF-G, oligopeptide-binding lipoprotein, and EF-Tu, were found to bind MUC7 (67). Several of these proteins are classically thought to be intracellular; however, the authors provide evidence that they can also be found on the cell surface (67). In addition to the numerous oral streptococcal species studied, several non-streptococcal species, such as *Escherichia coli* and *Staphylococcus aureus*, have also been shown to bind MUC7 (68, 69). Taken together, these studies illustrate that MUC7 protects the oral cavity from a wide array of oral bacteria, and the protective mechanism generally relies on direct contact with microbes. MUC7 could exert its protective effects in saliva or in the mucosal pellicle, since it has been found in both locations (70, 71). The exact mechanism of interaction between MUC7 and each microbe is complex and is generally species and strain specific. Further research is needed to better characterize MUC7's interactions with different types of oral microbes and to understand how the interaction differs between each species.

Mucin mediated reduction in microbial pathogenicity

In contrast to MUC7, MUC5B appears to bind only a limited number of oral pathogens despite its hetero-geneous glycan chains. Murray et al. tested the binding of 16 *Streptococcus* species to MUC5B, but none of the tested strains bound this mucin (62). One explanation is that the heterogeneous glycan chains found on MUC5B would prevent binding due to the inability of bacteria to form multiple bonds or attachment points. In line with this hypothesis, *Haemophilus parainfluenzae* was shown to bind MUC5B, but this bacterium interacts with the naked peptide backbone as opposed to the glycan chains (72). *Helicobacter pylori* is another bacterium that binds MUC5B through a neutrophil-activating protein on its surface that mediates binding to sulfated glycans (73). Although few studies have shown bacteria binding to MUC5B, there could be other oral microbes that do interact directly with MUC5B, but their interactions have not yet been characterized. The limited number of bacteria known to bind MUC5B compared to MUC7 highlights the point that this mucin protects the oral cavity in a unique way that is not yet fully understood. A recent study shows that solutions of MUC5B prevent *Streptococcus mutans* attachment to surfaces by keeping the bacterium in the planktonic state, indicating that MUC5B may protect the oral epithelium by repelling bacteria from its surface (74). MUC5B's ability to form a gel layer that guards against pathogenic microbes but does not cause bacterial killing is a unique property that contrasts with other defense proteins in saliva, such as antimicrobial peptides.

Salivary mucins in disease prevention

The mechanisms through which salivary mucins protect the oral cavity are diverse and differ between MUC7 and MUC5B, but both mucins play a role in protecting the oral cavity from an array of diseases. Salivary mucins are able to limit viral infection of T cells in the case of HIV/AIDS, fungal infection in candidiasis, and surface attachment of cavity-forming bacteria. Salivary mucins' ability to interact with this striking array of oral microbes points to its unique role in the oral cavity.

HIV/AIDS

Although HIV has been detected in the oral cavity of individuals affected by HIV/AIDS, there is little evidence of HIV being transmitted through oral secretions (75, 76). Early studies show that whole saliva and, more speci-fically, saliva from the submandibular and sublingual glands inhibit HIV-1 infection (77–80). These studies indicate that mucins are likely implicated in the inhibition of HIV infection because filtering submandibular/sublin-gual gland saliva abolishes the secretions' protective effects and electron micrographs reveal that the virus is being

aggregated, which can be a characteristic of mucins. When purified MUC5B and MUC7 are mixed with HIV-1 and then put in contact with T cells, all T cells remain healthy and uninfected (81). T cells remained uninfected in the presence of very low concentrations of purified mucins, at time points up to 3 h of incubation with HIV (81). One of the latest studies indicates that the protective effects of purified MUC5B and MUC7 mucins do not change when these mucins are purified from HIV-1 positive or negative individuals; however, an earlier study did not find this to be true (82, 83). Although the protective effects of MUC5B and MUC7 mucins from HIV-1 positive individuals appear to be maintained, once an individual is infected with HIV-1, the concentration of MUC5B in whole saliva is significantly decreased compared with non-infected individuals, which could make MUC5B an easily accessible diagnostic marker of HIV-1 infection (84).

Candidiasis

Several studies have shown that salivary mucins induce phenotypic changes in *Candida albicans*. *C. albicans* is the primary microbe responsible for oral candidiasis, an overgrowth of the fungus on oral tissues. The opportunistic fungus exists as part of the normal oral flora in many individuals but can become pathogenic in immune-compromised individuals and lead to life-threatening systemic infection if left unchecked (85, 86). MUC5B salivary mucins and bovine submaxillary mucin repress *C. albicans* virulence by reducing the formation of hyphae, which are associated with host cell invasion (87–90). MUC5B's ability to reduce *C. albicans* virulence without killing the fungus could explain how this opportunistic pathogen can exist as part of a healthy oral microbiota without the development of overt candidiasis. The phenotypic changes induced by mucins are a result of changes at the level of mRNA transcription, which downregulate genes necessary for hyphal development along with other general virulence factors (87).

MUC7, on the other hand, protects the oral cavity from *C. albicans* through physical binding (91). The synthesis of 12-144mer peptides from the N-terminal region of the MUC7 peptide backbone, has led to pivotal insights into the interaction between MUC7 and *C. albicans*. All of these peptides have candidacidal activity that rivals other antimicrobials in the oral cavity, such as histatin-5 (92–95). When used at physiological concentrations, these peptides have very low cytotoxicity to human cell lines and are not rapidly degraded in whole saliva (94). The peptide acts by accumulating on *C. albicans*' cell surface and then, when it reaches a critical concentration, it disrupts the outer membrane, which ultimately leads to cell death (96). Although intact MUC7 does not exhibit the same candidacidal activity as these peptides, MUC7 can be degraded in whole saliva to yield truncated peptide sequences that could exhibit the observed candidacidal

effects (95). These studies, which break-down the complex structure of MUC7, enhance our basic understanding of this mucin's mechanism of action and illustrate how this knowledge could be harnessed to develop antifungal therapies to treat candidiasis.

Dental cavities

Both MUC5B and MUC7 salivary mucins are implicated in the prevention of dental cavity formation. Suspensions of purified human MUC5B at physiological concentrations reduce the attachment and biofilm formation of *Streptococcus mutans*, one of the primary bacterium known to cause cavities (74). MUC5B reduces *S. mutans* attachment and biofilm formation on various surfaces by keeping bacteria in the planktonic state, as opposed to reducing the number of viable cells by bacterial killing (74). This mechanism of protection is similar to the one described for *C. albicans* in the previous section; MUC5B reduces bacterial virulence, which allows the microbe to exist as part of the oral microbiota without harming the host. An epidemiological study shows that adolescents who have increased numbers of cavities actually have more MUC5B and MUC1 in their saliva compared with children who have fewer cavities; the authors postulate that MUC1, a membrane-bound mucin, acts as a scaffold for MUC5B. If the MUC1 scaffold is shed into saliva then MUC5B will also accumulate in saliva as opposed to remaining in the mucosal pellicle (19). The reduced levels of MUC5B retained in the pellicle leave teeth vulnerable to *S. mutans* attachment and subsequent cavity formation.

The importance of MUC7 in cavity prevention has also been demonstrated in elderly populations who naturally have reduced levels of this mucin in their saliva. The study shows that elderly individuals with lower MUC7 concentrations have increased *S. mutans* titers in their saliva compared with those who have higher MUC7 concentrations (97). MUC7 is known to protect the oral cavity from dental cavity formation by directly binding *S. mutans* through the bacterium's alpha-enolase surface protein, which could explain why reduced MUC7 concentrations lead to increased *S. mutans* titers (98). These studies indicate that MUC5B and MUC7 are important in the prevention of cavity formation. MUC5B reduces *S. mutans* surface colonization and MUC7 binds the cariogenic bacterium to facilitate its removal.

Future directions and unanswered questions

There have been large strides made over the past several decades that identified salivary mucins as key defense components in the oral cavity. Researchers have primarily begun to understand the way mucins protect the oral cavity by studying their interaction with specific pathogenic oral microbes, but, when these studies are viewed as a whole, trends begin to emerge that hint at a general underlying mechanism of protection. MUC7 usually
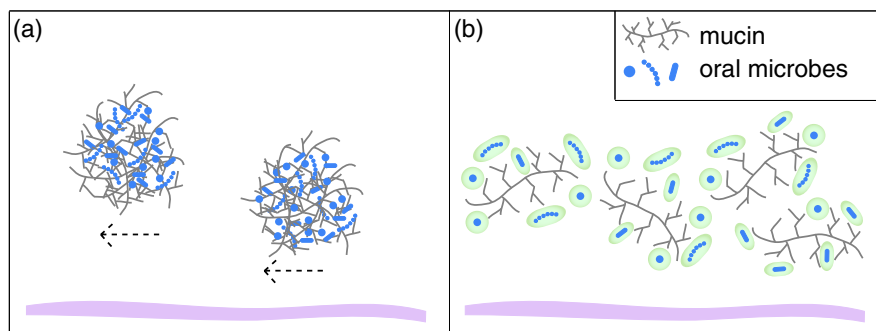
Fig. 2. Potential ways salivary mucins could protect the oral cavity from microbial colonization. (a) Salivary mucins could agglutinate microbes, which would facilitate their removal during swallowing. (b) Salivary mucins could also disperse bacteria through glycan-specific interactions. Selective interactions between mucins and microbes may have downstream effects on genetic regulation that reduce virulence and suppress biofilm formation. Changes in microbial physiology are indicated by green shading.

interacts directly with microbes to bind them, which facilitates their removal. MUC5B, on the other hand, physically interacts with only a limited number of microbes; it protects by reducing microbial virulence without killing the organism. MUC5B's ability to allow opportunistic pathogens to exist and live within the oral microbiota as non-pathogenic residents is unprecedented by other antimicrobial proteins in saliva. Further research is needed to better understand how MUC5B is able to create a gel that houses millions of oral bacteria while coaxing potentially harmful microbes into passive existence. Furthermore, the physiological changes that MUC5B induces in bacteria could alter interactions between resident microbes, which would affect the development of the oral microbiota. Understanding how mucins protect the body could open the doors to an entirely new set of therapeutic tools that aim to prevent microbes from transitioning into a pathogenic state as opposed to antibiotics, which treat the microbe once it is already virulent and can lead to antibiotic resistance.

Conclusions

Mucins play a complex and important protective role in the human body that is vital to maintain health. In the oral cavity, the physical characteristics of salivary mucins are fairly well characterized, but our understanding of how they exert their protective properties is continually being revised and reevaluated. The interaction between salivary mucins and oral bacteria depends on several factors including the type of salivary mucin, bacterial species, and strain. There are two primary mechanisms through which salivary mucins can interact with microbes to provide protection: 1) salivary mucins can agglutinate microbes, which would facilitate bulk removal (Fig. 2a), and 2) specific glycans on salivary mucins can selectively interact with microbes so that pathogens remain dispersed (Fig. 2b). Downstream effects of specific glycan interactions could then lead to changes in genetic regulation that reduce microbial virulence. The concept that mucins pro-

tect the body by reducing microbial virulence is highlighted in several oral disease models including HIV/AIDS, oral candidiasis, and dental caries. For example, in the presence of salivary mucins, HIV-1 infection of T cells is reduced, hyphal formation in *C. albicans* is limited, and *S. mutans* biofilm formation is decreased. In all of these cases, mucins hinder key steps that are necessary for the microbe to transition into a virulent state. More research is needed, however, to better understand how salivary mucins interact with such a vast array of oral microbes to suppress their pathogenicity. Once this is better understood, salivary mucins or engineered mimetics could potentially be used as therapeutic tools to prevent or treat diseases in novel ways.

Conflict of interest and funding

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