HHS Public Access

Author manuscript

Cell Immunol. Author manuscript; available in PMC 2019 November 01.

Published in final edited form as:

Cell Immunol. 2018 November ; 333: 19–33. doi:10.1016/j.cellimm.2018.08.008.

Glycan Recognition at the Saliva – Oral Microbiome Interface

Benjamin W. Cross and **Stefan Ruhl***

Department of Oral Biology, University at Buffalo, Buffalo, NY

Abstract

The mouth is a first critical interface where most potentially harmful substances or pathogens contact the host environment. Adaptive and innate immune defense mechanisms are established there to inactivate or eliminate pathogenic microbes that traverse the oral environment on the way to their target organs and tissues. Protein and glycoprotein components of saliva play a particularly important role in modulating the oral microbiota and helping with the clearance of pathogens. It has long been acknowledged that glycobiological and glycoimmunological aspects play a pivotal role in oral host-microbe, microbe-host, and microbe-microbe interactions in the mouth. In this review, we aim to delineate how glycan-mediated host defense mechanisms in the oral cavity support human health. We will describe the role of glycans attached to large molecular size salivary glycoproteins which act as a first line of primordial host defense in the human mouth. We will further discuss how glycan recognition contributes to both colonization and clearance of oral microbes.

Keywords

Oral biology; glycobiology; microbiology; host defense; saliva; salivary proteins; mucins; microbial adhesins; glycans; evolution

1. Introduction.

The mouth is the natural port of entry to both the gastrointestinal and respiratory tracts. As such, it is the first critical interface where most potentially harmful substances or pathogens contact the host environment. Adaptive and innate immune defense mechanisms are established in the oral cavity to inactivate or eliminate pathogenic microbes that traverse the oral environment on the way to their target organs and tissues. Starting at birth, the host defense systems of the oral cavity become exposed to microbes. Infants habitually put anything within reach into their mouths. Such early exposure to a wide variety of microbes derived from caretakers and the environment impacts the developing immune system and shapes the developing oral and gut microbiomes. All interactions of microbes with host surfaces in the oral cavity take place in a saliva-soaked environment. Protein and

* *Corresponding author*. Stefan Ruhl, Department of Oral Biology, School of Dental Medicine, University at Buffalo, The State University of New York, 213A Foster Hall, 3435 Main Street, Buffalo, NY 14214., Phone: +1-716-829-6073,. shruhl@buffalo.edu.

glycoprotein components of saliva play a particularly important role in modulating the oral microbiota. On the one hand they foster and maintain host colonization by a beneficial microflora, and on the other hand they aid in the clearance of pathogens. Over the years, scientists in different disciplines have studied the microbiota of the oral cavity and the oral host molecules that interact with the microbes. It has long been acknowledged that glycobiological and glycoimmunological aspects play a pivotal role in oral host-microbe, microbe-host, and microbe-microbe interactions in the mouth. Historically, some basic, widely applicable insights in glycan-mediated interactions were gained from investigations in the confined area of oral biology, and the ease of retrieving samples from the mouth certainly facilitated that progress.

In this review, we aim to delineate why knowledge of oral glycobiology is of relevance even for immunologists and microbiologists who may not directly be interested in the biology of the oral cavity, but still may want to know how glycan-mediated host defense mechanisms in the oral cavity support human health. We will not cover those immunological and innate host defense mechanisms in which glycans don't or have not yet been found to play a role. Those interactions, although important for oral health, are in many instances not unique to the oral cavity and are not principally different from host defense mechanisms elsewhere in the body. The immunological host defense mechanisms in the oral cavity have recently been reviewed [1]. We will also not cover the interesting and very relevant ways systemic immune deficiency and salivary gland dysfunction cause dysbiosis in the oral ecosystem and beyond. These topics have also been thoroughly reviewed elsewhere [2–4]. Here, we will discuss the role of glycans attached to mostly large molecular size salivary glycoproteins which act as a first line of primordial host defense in the human mouth. We will describe the various types of proteinglycan interactions between salivary glycoproteins, bacteria, fungi, and viruses that contribute to both colonization and clearance of oral microbes. We will further detail glycan-mediated interactions among different microbes that lead to the formation of multi-species biofilms in the oral cavity. Finally, we discuss the few existing clinical, translational applications of glycans and point to promising future avenues for "glyco-therapy" in the oral cavity.

2. Historical aspects of glycobiology in the oral cavity.

Some of the earliest discoveries in glycoscience were made studying salivary components. Salivary gland-derived mucins were among the first glycoproteins to be chemically analyzed. Eichwald, a physician from Russia working in Scherer's laboratory in Germany was the first in 1865 to describe the presence of mucins in salivary glands and found that they were composed of protein and sugar [5]. Obolenski in Hoppe-Seyler's laboratory isolated mucin from bovine submaxillary glands and described it in 1877 as a reducing substance with acidic properties [6]. In 1888, Hammarsten finally demonstrated that carbohydrates were a major component of mucins [7].The German chemist Hoppe-Seyler described mucins isolated from the nests of the Asian swiftlet [8]. These nests are mainly composed of the bird's dried salivary secretion and are still up-to-today a valuable ingredient in traditional Chinese cuisine for preparing bird's nest soup.

In 1936 the Swedish scientist Gunnar Blix characterized a particularly acidic carbohydrate as an important component of bovine submaxillary mucin [9]. He named this carbohydrate *sialic acid* after the Greek word for saliva, το σιαλον [10]. Sialic acid was obtained in crystalline form from bovine submaxillary mucin in 1954 [11]. In 1958 it was discovered that sialic acid was identical to a sugar isolated from the glycolipids of neural tissue in 1941 by Ernst Klenk [12–14] which he named *neuraminic acid* after the Greek word νεῦρον for nerve.

Ward Pigman discovered that the sialic acid containing glycans of bovine submaxillary mucin are attached to the protein backbone at serine and threonine residues [15]. The term s*ialic acid* actually describes a family of compounds made of neuraminic acid with various substitutions [16]. The first crystallized form was *N*-acetylneuraminic acid (Neu5Ac). Another widely distributed form is *N*-glycolylneuraminic acid (Neu5Gc). Most mammals produce both Neu5Ac and Neu5Gc, but humans have lost the ability to synthesize Neu5Gc [17]. Salivary mucins of different mammalian species carry different ratios of Neu5Ac and Neu5Gc. Mucins were shown to exhibit species specificity, tissue specificity, and interindividual differences. Modifications are introduced by substitutions with *O*-acetyl groups on various positions of the sialic acid molecule. *O*-acetylation of salivary mucins also differ between different mammalian species. This was shown in studies analyzing mucins derived from bovine, porcine, ovine, and equine submandibular glands [18–23]. As described further below, the glycans on salivary mucins play important roles in interacting with oral bacteria, fungi, and viruses (Fig. 1). They are believed to be important players in maintaining oral and systemic health. Sialic acids are particularly important for interactions with the oral microbiota as they are prominently exposed as the outermost glycan termini on most mammalian glycans and, as such, are critical determinants of the glycan motifs first encountered by approaching microorganisms. Notably from a historical standpoint, virus binding to sialic acids on salivary mucins was demonstrated as early as 1960 by Gottschalk and coworkers who showed the ability of mucins to inhibit viral hemagglutination [24]. Another important discovery was that blood group antigens are present in salivary secretions [25, 26]. Oral biologists also realized early that sialic acid and other glycans on salivary mucins serve as recognition motifs and binding sites for certain commensal strains of bacteria in the oral cavity [27–29].

3. Host-derived innate and immune-related compounds in human saliva.

Saliva plays an important role as a biofluid typical for the oral cavity. Besides its function in preprocessing of food for efficient mastication, and lubrication of the food bolus for swallowing, saliva contributes significantly to protection of the mineralized surface of teeth and the integrity of the epithelial integuments lining the oral cavity. How important saliva is becomes evident when salivary flow is compromised in patients with salivary gland dysfunctions that cause a dry mouth. Inadequate salivary flow causes diffulties masticating food, swallowing, and speaking, interferes with taste perception and digestion, and increases the risk of tooth demineralization, dental caries, oral mucositis, and fungal infections [2, 3, 30]. Many of the beneficial functions of saliva for maintenance of a healthy oral cavity are attributed to the proteins and glycoproteins in saliva. Whole mouth saliva is mainly composed of the secretions of the major and minor salivary glands. Blood plasma tissue

exudate mostly derived from gingival crevicular fluid also contributes significantly to whole mouth saliva (Fig. 1F). Salivary gland secretions also contain glycolipids [31]. Several biological and pathological functions have been associated with glycolipids in saliva, but substantial research in this area has not been continued in recent years [32, 33].

Of interest to the immunologist are the numerous antimicrobial, antifungal, and antiviral components of saliva [30, 34–36]. These include most prominently lysozyme, lactoferrin, salivary peroxidase, histatins, cystatins, and defensins. These important components of saliva are reviewed elsewhere [37–40]. The antimicrobial activity of salivary proteins may even extend beyond the oropharynx into the proximal digestive tract, there exerting specific antimicrobial activity against enteric pathogens [41]. Besides antimicrobial components, saliva also contains growth factors and cytokines that likely play a role in wound healing in the oral cavity, oropharynx, and esophagus [42, 43]. All these components will not be covered in this review because glycan-mediated interactions thus far have not been found to play a major role in their function.

The salivary proteome contains more than a thousand proteins and peptides [44]. Yet, only about a dozen of major protein species occur in saliva abundantly enough to be visualized by gel electrophoresis [45, 46]. The majority of salivary protein content is composed of a few major protein families: basic and acidic proline rich proteins, salivary amylase, mucins, salivary agglutinin (gp340/DMBT1), salivary cystatins, histatins, and statherin. By weight, most salivary proteins are glycosylated [47]. Heavily glycosylated salivary proteins include salivary agglutinin (gp340/DMBT1), mucins, proline-rich glycoprotein, and secretory immunoglobulin A. Table 1 provides a more comprehensive list of glycosylated salivary proteins. Other common post-translational modifications of salivary proteins include phosphorylation, sulfation, ubiquitination, and postsecretory proteolytic processing as described in excellent recent reviews [3, 47, 48]. The rich and diverse glycan landscape in the oral environment is shaped by large-molecular-weight, mostly mucous glycoproteins in saliva [49, 50] (Fig. 2). Some of the most abundant and important salivary proteins are listed in Table 1.

3.1 Salivary mucins.

Salivary mucins are the most densely glycosylated proteins in saliva and play an important role in oral host defense [51, 52] (Fig. 2). They are mostly decorated with O-glycans linked to serine and threonine residues in the protein backbone, but also contain some N-glycans linked to asparagine. The dense O-glycosylation confers a bottle-brush like structure to the molecule [53, 54] (Fig. 2A). Salivary mucins from different mammalian species vary widely in the types of sialoglycans attached to the mucin backbone. Variations include different types of terminal sialic acids, their modifications by *O*-acetylation, and the linkage to their subterminal glycan chains [22, 55]. Bacteria, fungi, and viruses have evolved many types of lectin-like adhesins that facilitate attachment to the many types of glycan moieties on salivary mucins. These interactions are described in more detail further below. The predominant mucins in human saliva are the high-molecular-weight mucin MUC5B and the lower molecular-weight mucin MUC7 [54]. MUC5B is a gel-forming mucin [56, 57] and much larger than the soluble and evolutionary younger mucin MUC7 [58, 59]. Recently, the

presence of MUC19, another largemolecular-weight mucin in human saliva has been described [60]. The glycans attached to MUC5B and MUC7 have been identified, and their glycosylation patterns were found to be different [49, 50].

The glycans attached to the mucin backbone, particularly the negatively charged terminal sialic acid residues, confer viscoelastic properties to saliva. This aids in food bolus formation and lubrication thereby facilitating the swallowing of food [54]. Salivary mucins, along with other salivary proteins, adsorb to the tooth enamel, there forming a thin proteinaceous film, called the acquired enamel pellicle [61, 62] (Fig. 1). This film protects the mineralized surface of teeth from erosion by acids in the diet or acids produced by oral bacteria, the latter being a first step in the initiation of dental caries [63–65]. The lubricating effect of glycosylated mucins also protects the integrity of the tooth occlusal surfaces from attrition through antagonistic teeth and excessive wear through abrasive food components. As described further below, many oral bacteria and systemic pathogens possess adhesins that bind to sialic acids on salivary mucins and other sialoglycoproteins in saliva.

Both MUC5B and MUC7 are the major carriers of blood group glycan motifs in saliva [49, 66–68]. Blood group glycans on salivary mucins likely evolved to capture microbial pathogens, and thus play a role in host defense [57]. It has been shown for instance that the gastric pathogen *Helicobacter pylori* binds to salivary mucin MUC5B and salivary agglutinin through recognition of Lewis b glycan motifs [69–73], but the biological outcome of that interaction on host defense is open to debate. Agglutination of microbes in the mouth normally leads to clearance and destruction of these organisms by the stomach environment. However, in the case of *H. pylori*, “clearance” of this pathogen from the oral cavity deposits *H. pylori* in its preferred environment, the stomach. *Helicobacter* has also been detected in dental plaque, but in most studies oral colonization did not correlate with stomach colonization or gastric inflammation [71, 74]. One of the main receptors for *H. pylori* on the gastric epithelium is the mucin MUC5AC, which carries Lewis b glycan motifs [75, 76] just like the salivary mucin MUC5B [69, 73]. If salivary MUC5B was bound by *H. pylori* in the mouth and still remained bound once in the stomach, it could possibly prevent binding to MUC5AC and, thus, inhibit stomach colonization. Salivary mucin MUC5B bound to the surface of *Helicobacter* may also serve the pathogen as a molecular camouflage to prevent its detection by the immune system [70] (Fig. 1E).

3.2 Salivary agglutinins.

Particular components of saliva, called agglutinins which include the above-mentioned mucins, can clump bacteria, viruses, and fungi as long as these organisms are suspended in the fluid, i.e. in planktonic phase, leading to their clearance from the oral environment through swallowing [77]. A number of such agglutinins have been identified by *in vitro* studies. Among them are the salivary mucins MUC5B [50] and MUC7 [49, 59, 78] (Fig. 2A), salivary agglutinin gp340/DMBT1 [72, 79, 80] (Fig. 2B), secretory immunoglobulin A [81–84], and free secretory component [85]. Salivary secretory IgA not only plays an important role in mucosal immune defense in the mouth [86, 87], but is also recognized by glycan-binding adhesins of oral actinomyces and streptococci [88]. Only a few and sometimes contradictory studies have been performed in the past to investigate the *in-vivo*

significance of salivary agglutinins for oral and systemic health. There is evidence that salivary agglutination of *S. gordonii* enhances its phagocytosis by neutrophils [89]. It remains still a matter of dispute whether and how salivary agglutinins participate in clearance of oral bacteria causing dental caries, such as *Streptococcus mutans* and *S. sobrinus* [90–92], or in agglutination of systemic pathogens such as *Staphylococcus aureus* [93] and *Helicobacter pylori* [70] (Fig. 1D). Conversely, agglutinins have been described to facilitate bacterial attachment to the tooth surface thereby promoting colonization [72, 94–96]. Agglutinins bound to bacteria may potentially also lead to interspecies attachment during the establishment of oral biofilms [77]. The role of glycoprotein agglutinins in biofilm formation is discussed in more detail in section **8**.

4. Evolution & coevolution of host glycans and microbes in the oral cavity.

The wide variety of mutual interactions between host glycoproteins and oral microorganisms have been shaped by coevolution of the microorganisms with their host [97]. This coevolution has led to an ecosystem that generally benefits both the host and its indigenous microbiota. In the oral cavity, glycoproteins in saliva can serve as both substrates for attachment and as agents of bacterial clearance. Salivary glycoproteins on the tooth surface or the oral epithelium serve as anchors for bacterial adhesins, allowing the bacteria to take a foothold in the oral cavity and multiply. This can eventually lead to the formation of dental and oral biofilms, a necessary adaptation to avoid being flushed away by the constant salivary flow in the oral environment [30]. Conversely, salivary agglutinins are believed to encourage clearance of bacteria by facilitating the swallowing of bacteria along with saliva [77]. Colonization by beneficial bacteria is tolerated because it creates an environment that likely prevents colonization and infection by more pathogenic microbes. Human and bacterial coevolution has thus developed mechanisms to encourage attachment and colonization of commensal bacteria while discouraging the growth of pathogens. Oral bacterial adhesins have presumably gone through millions of years of rapid evolution to maximize binding to specific aspects of the glycome or sialoglycome in their ecological niche or host species [97]. It is also likely that bacterial adhesins have evolved the means to recognize glycans produced by other microbes. These may include common nonulosonic acids and “exotic” non-mammalian glycans. This bacteria-bacteria binding may occur between members of the same species or with other members of the biofilm community.

The fact that salivary glycoproteins, in particular the salivary mucins, are decorated with an incredible variety of different glycans can be explained by the so-called *Red Queen* effect. Microbes evolved to express novel glycan-binding adhesins to colonize host surfaces, and the host in turn evolved novel glycan motifs to which pathogenic microbes cannot bind. According to the Red Queen effect, this race continues by microbes again evolving novel or modified adhesins that are able to recognize those new host glycan motifs, and so on [98]. One important and recently evolved glycan motif in humans is sialic acid. The rich variety of structural subtypes of sialic acids on salivary mucins and their differences in different mammalian species are likely the result of microbial host coevolution [99, 100]. Also, the loss of Neu5Gc in the human lineage about 2 million years ago could have been the result of selective pressure by an unknown pathogen on our hominin ancestors [101]. Microbial-host coevolution may not only affect the structure of glycans, but also their numbers, densities,

and sterical presentations on their protein or lipid backbone, and perhaps in a larger sense on any cell or biological surface [102]. An example for that effect may be the observed variations in the number of densly O-glycosylated PTS repeat domains in salivary agglutinins [103] and mucins [104, 105] which are known to interact with microbes through glycan-mediated binding (sections **3.1** and **3.2**). For example, variations in subexonic PTS copy number repeats in salivary mucin MUC7 underwent rapid evolution in the primate lineage and among other mammalian species [59], and similar mechanisms involving possible pathogenic pressure may explain the observable variations in MUC7 among geographically distinct human populations [58].

5. Oral microbial glycan-binding molecules.

Attachment to glycans on host tissues is a common first step toward colonization, and specific glycan structures sometimes contribute to the characteristic tropism to particular sites of infection observed in many microbes (Fig. 1A and C). Many oral bacteria have evolved an astonishing tropism for the oral cavity and even for specific sites within that space, e.g. the tooth surface, the interdental approximal space, the periodontal pocket, the surface of the tongue, or the oral mucosal epithelium. Mutans streptococci in particular are extremely specialized for their ecological habitat on the surface of the tooth, and are rarely found in the oral cavity before tooth eruption [106–108].

Initial attraction of oral bacteria to surfaces in the oral cavity is mediated by physicochemical forces that lead to a primary association of the bacteria with the surface [109]. For more permanent adhesion to take place, bacteria express highly specialized proteins, called adhesins, which bind to specific cognate motifs on host molecules. These specific binding interactions are mediated by stereochemical and electrostatic forces, and can be based on proteinprotein or protein-glycan binding. While the recognition of amino acid motifs by oral bacterial adhesins certainly plays an important role for binding to a variety of biomolecules [110–113], this review focuses on the recognition of glycan motifs mediated by glycan-binding adhesins expressed by oral bacteria. Glycan-binding proteins are often highly specific, but have low affinity [114–116]. Low affinity interactions can become biologically relevant because microbial cells often express many copies of an adhesin protein on their surface, and glycoproteins, particularly mucins, often carry clustered glycan receptors. The effect is not simply additive, because the initial interaction increases the chances of further interactions, thus increasing avidity of microbial binding. In most of the examples presented below, the bacterial adhesins do not function as independent points of attachment between bacteria and host, but instead behave in a concerted fashion. Multiple weak adhesin-glycan links work together to form a lasting bond that can be competitively inhibited only by very high concentrations of soluble glycans, an effective mechanism that microbes may have evolved to strengthen their attachment to host surfaces.

Bacterial adhesins are frequently associated with large, non-flagellar surface structures, called pili or fimbriae, that extend away from the bacterial surface. Due to their length, these pili are likely the first bacterial proteins to come into contact with host surfaces [117]. Bacterial adhesins are themselves often glycosylated. The purpose of this glycosylation is not firmly established, but it may help maintain the physical shape of the adhesin, preventing

folding and extending the protein away from the bacterial cell surface [118–120]. Oral microbes encounter a rich diversity of glycan structures in the human mouth. This diversity comes from the variety of mucous glycoproteins present in saliva [49, 50] (Table 1), food intake by the host, and the innumerable glycan motifs expressed by other members of the oral biofilm community [121, 122]. Considering this diversity of potential binding targets, the range of oral bacterial glycanbinding adhesins is likely infinitely greater than what is currently known.

A selection of well-characterized microbial glycan-binding adhesins is presented in Table 2. Below, we will provide a more detailed description of several representative, well characterized adhesin families including the serine rich repeat (SRR) protein adhesins found on oral streptococci (subsection **5.1**) and the type 2 fimbrial lectin-like adhesins of oral actinomyces (subsection **5.2**). For both of these adhesin families, ample and detailed structural knowledge exists for both the glycan-binding bacterial adhesins and the glycan motifs recognized by the corresponding adhesin glycan-binding pocket.

5.1 Streptococcal serine-rich repeat protein adhesins.

Viridans streptococci are prevalent among the earliest colonizers of the tooth surface [123]. Oral streptococci express multiple adhesins [124] which allow them to recognize a wide spectrum of adhesion substrates, including salivary glycoproteins present in the acquired enamel pellicle on the tooth surface. [125, 126]. The existence of a streptococcal lectin that binds host sialic acids on salivary mucins, was first discovered in the late 1970's [28, 29, 127]. Later, a lectin-like adhesin expressed by *S. gordonii* DL1, named Hsa, was isolated based on its ability to bind sialic acid on red blood cells, and was found to be a high molecular weight glycoprotein associated with fibrillar structures on the bacterial surface [128]. This adhesin was cloned by Takahashi and coworkers and determined to be a streptococcal serine-rich repeat (SRR) protein [129]. An especially detailed description of the serine rich repeat family of adhesins is included in the following to illustrate the variation in glycan binding specificity possible within such a closely-related group of adhesins.

The SRR glycoproteins comprise a unique family of bacterial adhesins that bind a wide range of ligands, and have a significant impact on biofilm formation and virulence [130–136]. The overall domain organization of the SRR glycoproteins is conserved, with an atypical Nterminal signal peptide followed by a short SRR domain, a ligand binding region, a long SRR domain, and a C-terminal LPXTG cell wall anchoring motif. The long SRR domain is highly glycosylated. These cell wall-associated glycoproteins most likely facilitate the early colonization of the oropharynx by streptococci, and are associated with pathogenicity in the setting of an infective endocarditis model [130]. Although the ligands for the SRR glycoproteins are not known for most species, it is clear that they include both glycans and proteins. The binding regions vary considerably between species, which likely accounts for the range of ligands bound by this family of adhesins.

Through binding of whole bacteria and of their recombinant ligand binding domains to a sialoglycan array, it was found that each unique sialic acid binding SRR protein adhesin has a distinctive binding profile that is influenced by both the subtype of sialic acid, its linkage,

and the underlying glycan chain [137–139]. For example, Hsa of *S. gordonii* DL1 showed broad specificity for a sialic acids α2–3-lined to a wide range of subterminal glycans, but GspB of *S. gordonii* M99 showed a narrower specificity, binding primarily sialyl-T antigen (Neu5Acα2–3Galβ1–3GalNAc) terminating glycans [137]. The binding patterns of purified SRR ligand binding domains closely matched the binding patterns of whole cells, suggesting that the SRR proteins are sufficient to explain streptococcal sialic acid binding. Furthermore, isogenic mutants lacking SRR genes did not bind to any sialoglycans on the array [137]. The binding regions of SRR proteins are stable modules that can be efficiently expressed in a standard bacterial host such as *E. coli*. This stability, combined with the selectivity for specific underlying glycan structures, makes SRR adhesins potentially useful tools for studying glycobiology, which will be discussed in the last section of this review (section **9**). Interestingly, none of the SRR protein adhesins bound to α2–6-linked sialic acids, even though this sialic acid configuration is quite prevalent in the oral cavity and on salivary glycoproteins [49, 50, 72, 73, 140].

Among the best structurally characterized of the SRR glycoproteins is *gordonii* surface protein B (GspB) of *Streptococcus gordonii* strain M99. GspB mediates the binding of streptococci to human salivary glycoproteins and surface glycoproteins on platelets [141, 142]. Recent crystallographic studies have revealed that the binding region of GspB is comprised of three independently folded domains, with the second domain exhibiting the same topology as eukaryotic Siglecs [143]. Domain deletion studies indicate that only two of the domains, the Siglec and Unique domains, are required for sialic acid binding. The purpose of the third domain, CnaA, is unclear, but its structure resembles MSCRAMM-related peptide-binding domains, first identified within the *S. aureus* CNA protein adhesin [131, 138]. SrpA of *S. sanguinis* SK36 is another well-characterized, lectin-like SRR adhesins that binds sialic acids [129, 141, 142, 144–146]. Interestingly, Hsa, GspB, and SrpA mediate shear-enhanced binding to their cognate receptor glycans on salivary glycoproteins and on platelets [147, 148]. In that respect it may be of relevance that CnaA domains have been shown to be efficient dissipaters of mechanical forces [149].

5.2 Other bacterial glycan-binding proteins.

Similar to viridans streptococci, oral actinomyces are among the earliest colonizers of the tooth surface [150]. Unlike streptococci, oral actinomyces have also been associated with initiation of periodontal disease [151, 152]. Two types of fimbrial adhesin have been characterized in actinomyces. Type-1 fimbriae were found to be responsible for binding to salivary proline-rich proteins through recognition of an amino acid motif [112, 113]. Type-2 fimbriae were shown to carry a galactose-specific lectin [150] that mediates bacterial coaggregation with certain strains of streptococci [153, 154]. The receptor glycan motif for type-2 fimbriae on streptococcal partner cells lies within the so-called streptococcal receptor polysaccharides (see section **7**) [121]. Further investigation into the glycan-binding specificity of the type-2 fimbriae showed a preference for Galβ1–3GalNAc, GalNAcβ1–3Gal, and to a lesser extent other glycans with a terminal Galβ1–3 linkage [155–158]. The glycan motif Galβ13GalNAc, also called the T antigen, is one of the most common O-glycan cores, but in human tissues it is typically cryptic, meaning other saccharides are attached to the terminal end of the glycan chain [159]. Type-2 fimbriated actinomyces

cannot bind to sialyl-T antigen unless terminal sialic acid is removed by the action of sialidase [160, 161]. Notably, actinomyces express a sialidase [162] to gain access to their preferred glycan receptor (further discussed in sections **7** and **8**). Type-2 fimbriated actinomyces bind to eukaryotic cell surfaces by recognizing cognate Gal/GalNAc-containing glycan motifs on epithelial cells [163] and phagocytes [164, 165]. Salivary glycoproteins including salivary mucin MUC7 [166] and secretory immunoglobulin A [88] are also bound by type-2 fimbriae. Molecular cloning of the fimbrial subunits revealed the fimbrial tip as the adhesin protein responsible for binding Gal/GalNAc-containing glycans, and was named coaggregation factor A (CafA) [167].

Streptococcus mutans is strongly implicated in the development of dental caries [168]. In consequence, the adhesins that allow it to attach to oral surfaces have been intensely studied for many years. Initial attachment of *S. mutans* to the tooth surface is most likely initiated by the SpaP protein, an adhesin of the antigen I/II family that has been shown to bind salivary agglutinin [96]. Similar to the SRR family, antigen I/II family adhesins are fibrillar proteins found in many species of oral streptococci that display a range of binding substrates including proteins and glycans [169–171]. After initial attachment, *S. mutans* glucosyltransferase (Gtf) enzymes produce an extracellular matrix of glucan polysaccharides that help establish a permanent biofilm [172]. *S. mutans* attaches to this glucan matrix via lectin-like glucan-binding proteins (Gbp) [170].

5.3 Viral and fungal glycan-binding proteins.

Oral viruses and fungi also express lectin-like adhesins that aid their attachment to host tissues. Viruses that enter though the oral cavity often bind heparan sulfate, a polysaccharide commonly found in all animal tissues. Viral proteins that attach to heparan sulfate include herpes simplex glycoproteins (gB, gC, and gD), enterovirus capsid proteins, and HIV gp120 [173] [174, 175] [176]. The viruses likely use heparan sulfate as part of a co-receptor system in which initial contact is made with heparan sulfate, followed by adhesion to a more specific cellular receptor. The hemagglutinin (HA) protein of the influenza virus is possibly the most widely known sialic acid binding protein [177, 178]. A shift in binding preference from α2–3-linked sialic acid to α2–6-linked sialic acid is believed to facilitate the transition from avian to human hosts [179]. The binding of HA to sialic acid on host cell surfaces is a crucial step in the influenza lifecycle, but salivary proteins decorated with sialic acid can intercept HA binding, leading to agglutination and clearance of the viral particles [180, 181]. The salivary mucin MUC5B in particular has been shown to have antiviral effects against influenza virus [182]. As many more pathogenic viruses use the oral cavity as a port of entry [183], likely a high number of viral glycan-binding interactions with salivary glycoproteins are yet to be discovered.

Candida albicans is a commensal yeast species that is a common part of the human oral flora but can cause oral candidiasis in immunocompromised patients or in individuals with a dry mouth [184]. It expresses agglutinin like sequence (Als) proteins that bind to a broad range of mammalian proteins, and likely also interact with bacteria. Als1 binds to fucose-containing glycans, and has been shown to bind fibronectin and laminin glycoproteins *in vitro* [185]. Binding of the N-terminal domain of the Als1 protein (Als1p-N) was tested on a

glycan array. Als1p-N bound most readily to Fucα1–2Galβ1–4GlcNAc, but it also bound to many glycans with a terminal fucose, and even to fucose alone [185]. *Candida albicans* also binds to salivary mucin MUC7 [186] and salivary glycans inhibit binding of *C. albicans* to oral epithelial cells [187]. Thus, additional glycan-binding adhesins expressed by *C. albicans* likely play a role in binding to salivary glycoproteins. *Candida glabrata* is less common than *C. albicans*, but has been shown to also cause oral candidiasis. This species expresses several epithelial adhesin (Epa) proteins that bind to glycans [188]. Epa1 appears to be the main mediator of adherence to human epithelial cells. It binds to most glycans with a terminal galactose β-linked to galactose or glucose [188]. Deletion of Epa1 substantially reduced *Candida* adherence to epithelial cells [189].

6. Bacterial surface glycans.

In conjunction with their glycan binding, oral bacteria produce a wide variety of extracellular glycans of their own [121, 190]. This combination of glycan production and glycan attachment results in elaborate, interconnected patterns of interspecies coadhesion and interaction (Fig. 1B) [125]. For example, oral streptococci produce long extracellular glycans with a repeating pattern, called streptococcal receptor polysaccharides (RPS). There are six common variations in RPS structure, and all of them are bound by actinomyces type-2 fimbrial adhesins. Only four of the RPS structures are also bound by oral streptococci [121, 191]. Additional variations in streptococcal polysaccharide structure have been discovered that differ from the six common RPS types [190, 192, 193]. Based on analogy with the RPS glycan motifs, it can be predicted that unknown partner bacteria might exist in oral biofilms that express hitherto undiscovered adhesins recognizing these rare glycan motifs to engage in bacterial coadhesion.

Many bacterial genera present in oral biofilms, including *Fusobacterium*, *Neisseria*, *Porphyromonas, Streptococcus,* and *Tannerella*, express sialic acids or other nonulosonic acids on their surface [194–196]. Bacterial sialic acid may be synthesized *de novo*, scavenged from host glycans, or produced from host-derived intermediates [197, 198]. Since sialic acid is a very common human glycan structure, its presence on the bacterial surface may prevent attack by the immune system by serving as a type of molecular camouflage. For instance, *H. influenzae* gains a survival advantage in human serum from presenting sialic acid on lipopolysaccharide (LPS) [199]. Sialylation of *Porphyromonas gingivalis* LPS appeared to have no effect on its inflammatory potential [196]. The effects of bacterial sialylation may not always be protective when considering the variety of glycan structures underlying the sialic acid and the variety of sialic acid-binding Siglec receptors on eukaryotic cells [200].

7. Bacterial foraging on oral host glycans.

Scavenging glycans from host glycoproteins appears to be a common strategy of oral and intestinal bacteria [197, 201]. Strains of *S. gordonii*, *S. oralis*, *S. mitis,* and *S. sanguinis* are able to survive using saliva as a sole carbon source [202]. These strains show a preference for Nglycans linked to the proline-rich glycoprotein (PRG) [202]. For *S. gordonii*, this specificity for PRG is due to the sequential action of three glycoside hydrolases that are able

to break down the most common glycan structure on PRG [203]. The ability to scavenge host sialic acids has also been observed in *Haemophilus influenzae*, *Tannerella forsythia,* and *Vibrio cholerae*. These species carry an orthologous *N*-acetylneuraminic acid lyase (*nanA*) gene, which is necessary in the first step of sialic acid catabolism [198, 204, 205]. The *nanA* gene has been found in other bacterial genera including *Streptomyces*, *Streptococcus*, *Staphylococcus*, *Clostridium*, *Lactobacillus*, *Escherichia*, *Salmonella*, and many more, suggesting that they may also be able to scavenge host sialic acids from their environment [204].

Bacteria that can metabolize sialic acid often express sialidase enzymes in order to liberate terminal sialic acids from glycans. NanH is an interesting sialidase expressed by *T. forsythia* and several species of *Actinomyces* [160, 161, 205]. The NanH enzyme is believed to play a role in bacterial attachment to human epithelial cells. It first acts as a lectin, anchoring the bacteria to the host cell, then it removes sialic acid from the host glycoprotein, providing a useful carbon source [205]. The removal of sialic acid from O-linked glycans may reveal the T antigen (Galβ1–3GalNAc) or other beta-galactoside structures for bacterial access [206]. This, for instance, allows binding of the actinomyces type-2 fimbrial adhesins to their receptor glycan motifs as described in section **5.2**. Sialidase-dependent attachment of *Actinomyces naeslundii* and *Actinomyces viscosus* to human erythrocytes [207] and polymorphonuclear leukocytes [164, 206] has been described (Fig. 1F). Similar sialidase-dependent attachment has been observed for *S. gordonii*, *S. oralis*, and *S. pneumoniae*, but these species rely on different adhesins, which bind to β1–4-linked galactose [208–211]. *Streptococcus gordonii* does not produce its own sialidase, but instead relies on sialidases produced by other vicinal bacterial species in the biofilm environment [208]. Depending on their host species and specific site of colonization, oral bacteria should be expected to express sialidases that match the types of sialic acids prevalent in their surroundings. In this context, a sialidase was characterized from oral *S. sanguinis* that can release *O*-acetylated sialic acids which are resistant to cleavage by commonly used microbial sialidases [212]. The ability of bacteria to modify glycans in their environment, combined with their ability to bind the underlying, previously cryptic glycans, adds yet another level of complexity to interspecies coadhesion and interaction [125].

8. Role of glycans and glycan binding in oral biofilm formation.

Bacteria normally do not adhere directly to the mineralized tooth surface. In the mouth, tooth enamel is coated with the acquired enamel pellicle, a thin film of adsorbed salivary proteins and glycoproteins [61]. The interactions between lectin-like adhesins on bacteria and complementary glycan motifs on glycoproteins in the salivary pellicle play central roles in initial bacterial colonization [213] (Fig. 1A). Several human salivary proteins have been identified as counter-receptors for oral bacteria and their glycan-binding adhesins [88, 140, 166, 214, 215]. Major salivary glycoproteins such as the salivary mucin MUC7 serve as prominent targets for bacterial binding [166, 215]. Also systemic respiratory or gastrointestinal pathogens were found to bind to specific glycan motifs on MUC7 and on other salivary glycoproteins [69, 70, 73, 93, 216].

Lectin-glycan binding is also important for bacterial coadhesion and coaggregation, which lead to the formation of multispecies microbial biofilms that have the potential to become pathogenic communities causing dental, oral, and perhaps also systemic disease. Bacterial c*oaggregation* is generally defined as the formation of bacterial aggregates due to bacterial cellcell recognition of genetically distinct partner strains [217]. Bacterial *coadhesion* is defined as the binding of a planktonic bacterium to an already surface-attached partner strain, as in the formation of early multispecies dental plaque biofilms [218] (Fig. 1B).

The spatial organization of oral biofilms plays an important role in their development and survival [219, 220]. Species with mutualistic symbiotic relationships benefit from close proximity, and beneficial interspecies coaggregation and coadhesion is often mediated by lectinglycan binding [154, 221, 222] (Fig. 1B). The enormous diversity of species commonly found in the oral environment [223–225], combined with the potentially infinite number of possible glycan motifs, [121, 122] results in an extremely complex ecosystem that is constantly being destroyed by chewing or brushing, and rebuilt by microbial coadhesion and coaggregation.

Despite the overwhelming number of species and interactions in the oral environment, progress is being made in investigating which microbial species are associated with oral health or disease, and in determining their mechanisms of attachment [226]. Also, numerous laboratory strains and clinical isolates have been tested for inter-bacterial binding *in vitro* by the conventional bacterial coaggregation assay [154, 227]. Thus far however, the mechanisms of inter-bacterial adhesion down to the molecular level have been studied for only a few of these interactions [121, 167]. The traditional in-suspension coaggregation assay has provided a wealth of information for interbacterial adhesion, but is not well suited to handle the multitude of microbial species present in oral biofilms. A method was recently developed that enables investigators to screen multi-species biofilms for coadhesion partners with the option to isolate and identify them [228]. This coadhesion assay, based on immobilization of one bacterial partner strain [229], allows the use of well-known reference strains as probes to efficiently screen mixed bacterial cultures for unknown co-adhering partner organisms that express complementary adhesins or glycan receptors [228]. Clearly, a better understanding is warranted of how oral biofilm bacteria act together *in vivo*. Imaging and functional approaches used in more recent *insitu* studies will likely provide further insight in glycan-mediated mechanisms of interbacterial interactions [230].

Dysbiosis in oral microbial biofilms can lead to the onset of dental caries and periodontal disease [4, 231]. If bacteria get dispersed in the blood circulation, they can even become causative agents of systemic disease [226, 232]. One example that is clearly based on glycan-recognition is infective endocarditis caused by streptococci of the oral viridans group that are normally harmless commensal inhabitants of the oral cavity. As mentioned further above, they are often among the first bacteria to attach to the saliva-coated surfaces of teeth [125, 233]. However, when oral streptococci gain access to the blood circulation through accidental microlesions in the oral epithelia or through invasive dental treatment, these organisms can become causative agents of infective endocarditis and sepsis [108, 234–236]. The binding of bacteria to platelets has been recognized as a central process in the pathogenesis of infective endocarditis [237, 238], and that binding is partially mediated by

lectin-like bacterial adhesins. The Sigleclike binding regions of streptococcal SRR adhesins selectively target GPIbα on human platelets [137], where they adhere specifically to O-linked glycans on the mucin-like stalk of GPIbα [130, 131, 137, 141, 214]. This binding may cause a tropism of oral streptococci to platelets on injured heart valves. An alternative mechanism is suggested by a recent study which found that streptococci adhere preferentially to platelets in the context of whole blood, and may thus be carried by circulating platelets to the damaged endocardial surfaces [137]. The contribution of Siglec-like adhesins in the development of endocarditis has been demonstrated *in vivo* using a rat model [130, 239]. Recently, streptococcal recognition of sialoglycans on plasma glycoproteins was investigated and suggested to modulate the propensity of streptococci to establish endocardial infections [240].

9. Perspectives for use of glycans or glycan-binding molecules in prevention or diagnosis of oral disease.

Since initial attachment is a crucial first step in the development of pathogenic oral biofilms, many research groups have attempted to identify treatments that inhibit the binding ability of bacterial lectin-like adhesins. A long-sought potential solution to these problems is immunization against the bacterial adhesins [241, 242]. Specifically, the glucosyltransferase (Gtf) and glycan-binding protein B (GbpB) of *S. mutans* have been the target of vaccine development for many years [243]. Rodent studies have demonstrated that vaccines containing the Gtf or GbpB protein result in detectable levels of specific salivary IgA and serum IgG. Importantly, this immunization results in significantly less total caries [244–246]. It is believed that IgA and IgG present in saliva bind to Gtf or GbpB, thereby preventing bacterial adhesion to extracellular bacterial polysaccharides in dental plaque and leading to agglutination and clearance of the *S. mutans* organisms [246]. Immunization with dead or attenuated *S. mutans* cells was not pursued because antibodies against *S. mutans* whole cells showed crossreactions with human heart tissue [247–249].

Several human trials have tested vaccines based on Gtf, GbpB, and other *S. mutans* surface proteins. Immunization consistently resulted in detectable salivary IgA antibodies that target the desired antigen [250, 251]. Small-scale human trials suggest that vaccination using Gtf reduces the proportion of *S. mutans* in the oral cavity [252, 253]. Regrettably, no studies on the effectiveness of these vaccines in preventing dental caries have been published, and no large scale clinical trials have been initiated. Future attempts will have to consider the existence of multiple other cariogenic species besides *S. mutans*, the maintenance of protective levels of antibodies in saliva, and the public reluctance to yet another vaccine for pediatric use [254]. Passive immunization against *S. mutans* may circumvent some of these concerns, and has been tested in several animal models. Topically applied antibodies to *S. mutans* surface proteins, including GbpB, significantly reduced caries formation in rats and macaques [255, 256]. There is also evidence that passive immunization in humans can reduce the bacterial burden of *S. mutans* in the oral cavity [257–259], but thus far it was not proven that passive immunization can reduce the incidence or severity of dental caries in humans. The lack of clinical data showing efficacy of these vaccines in preventing dental caries is unfortunate, but could present a major opportunity for future dental research.

As mentioned before, the number of well-characterized bacterial lectins represents likely only a miniscule fraction of the total potential lectin diversity in the oral cavity. Further investigating the unidentified bacterial glycan-binding molecules will allow to better understand the role of glycans in microbe-host and microbe-microbe interactions in the human mouth and, ultimately, to study their relevance for oral disease, including the two major diseases in the oral cavity, dental caries and periodontal disease. Once the molecular basis of these glycan-mediated bacteria-host interactions is revealed, it might be possible to refine the use of custom-designed glycan or lectin analogues, and specifically target the adhesins of pathogenic microbes in the oral cavity. Similar *glyco-therapeutic* approaches are already under investigation for other pathogens [260–262].

Besides their role in human health, the extreme variety of lectins in the oral microbial community represents an untapped source of highly specific, affordable, and easy-to-use molecular glycan probes. The majority of the currently available glycan-binding molecules, i.e. the lectins, were traditionally discovered by their ability to agglutinate red blood cells. Because of this selection, there is a bias towards host glycans present on erythrocytes, which represent only a limited portion of the diversity of glycans found in nature. This approach has likely missed many existing natural lectins that bind to other kinds of glycan motifs, e.g. the polysaccharide motifs expressed on bacteria [121, 122]. Considering (a) the multitude of bacterial species identified in oral biofilms, (b) the rich diversity of species-typical sialoglycans found on salivary glycoproteins, and (c) the innumerable glycan motifs expressed on the surface of other bacteria, it is likely that many more unidentified lectin-like bacterial adhesins exist in the oral microbiome, beyond the few ones currently known. It seems only logical and timely to harvest these unidentified glycan-binding proteins already honed by evolution, and shape them into tools for glycan recognition.

The creation of a toolkit of novel, highly specific bacteria-derived glycan-binding probes will be groundbreaking not only for identification and study of glycans in oral biology, but also for use as glycan-tracking reagents for salivary diagnostics pertaining to oral and systemic disease. In a broader perspective, novel bacterial glycan-binding proteins will become useful tools to probe the glycomes of all types of cells and tissues, allowing for simple glycan analysis in most fields of vertebrate biology. Such glycan-binding probes, derived from oral bacteria and engineered to suit experimental needs, might be used with any medical or biological sample in a number of assays including array technology, flow cytometry, lectin histochemistry, lectin blotting, and lectin ELISA. Many more uses for lectin probes will likely become obvious as we continue to discover new classes of lectins and new examples of lectin-glycan interactions in nature.

ACKNOWLEDGEMENTS

Funding: This work was supported by the National Institutes of Health [R01 DE019807, R21 DE025826, and U01 CA221244]. BC was supported by NIDCR training grant T32DE023526.

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HIGHLIGHTS

- Saliva plays an important role for host defense in the oral cavity.
- Glycoproteins in saliva create a diverse glycan landscape in the mouth.
- Oral bacterial adhesins bind to glycan motifs on salivary glycoproteins.
- Oral bacterial glycan binding contributes to biofilm formation.
- The majority of glycan-binding molecules in the mouth still remains unexplored.

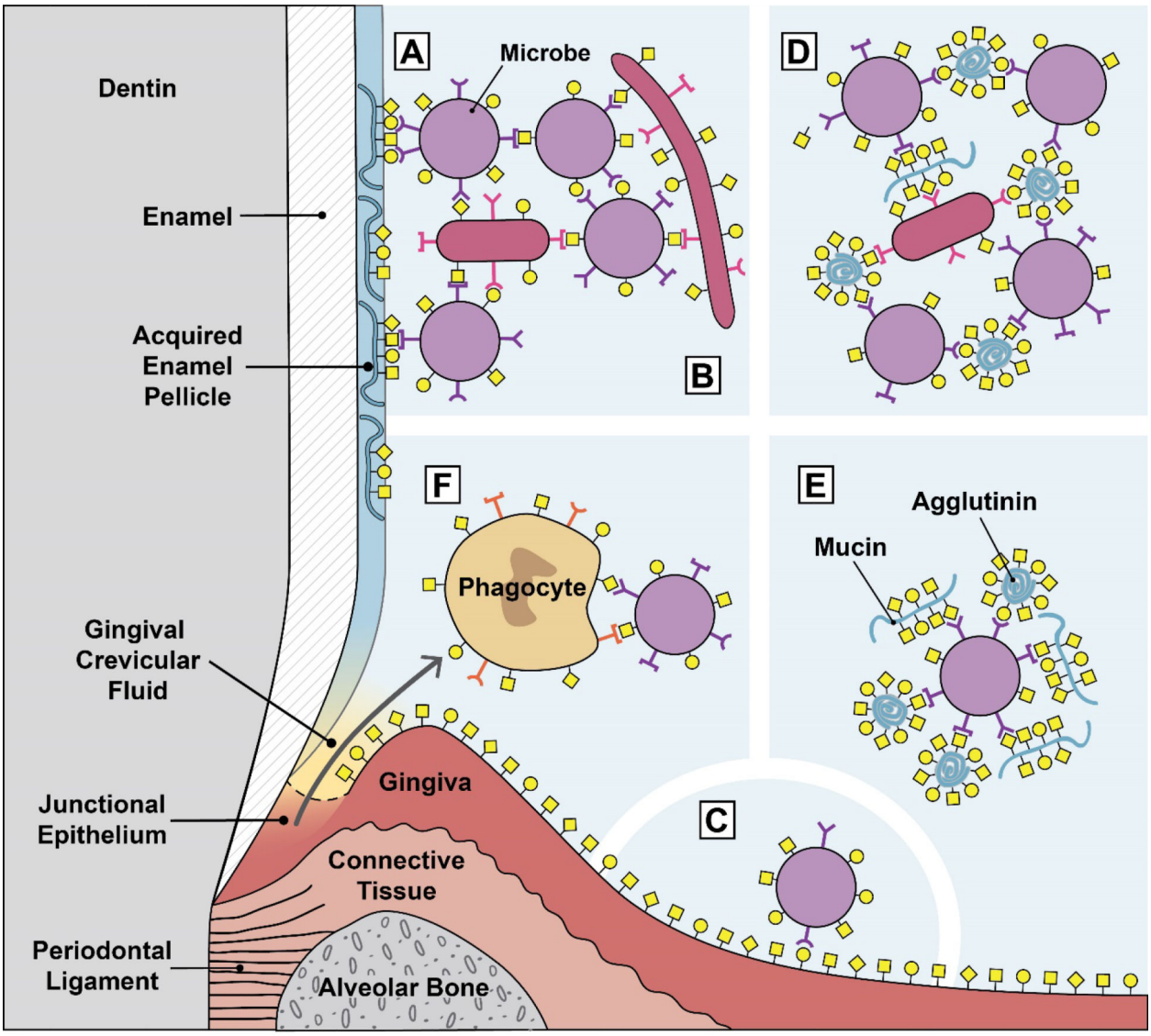

Fig. 1.

Glycan-mediated interactions in the oral environment. **A.** Microbial attachment to the tooth surface. Microbial adhesins attach to mostly salivary glycoproteins adsorbed to the tooth surface as part of the acquired enamel pellicle. This is typically the first step in oral biofilm formation. **B.** Microbial adhesins attach to glycans on other microbes. This example shows microbial *coadhesion*, the process of attaching to microbes that are part of a biofilm. If this microbe-microbe attachment occurs in suspension, i.e. in the planktonic phase, it is called microbial *coaggregation.* **C.** Microbial adhesins attach to glycans on oral epithelia. This interaction normally does not result in long-term colonization because the oral epithelium is a shedding surface. **D.** Salivary glycoproteins agglutinate microbes. Natural salivary flow causes these aggregates to be swallowed, resulting in clearance of the microbes from the oral cavity. **E.** Salivary glycoproteins can serve as molecular camouflage. A microbial cells is covered by multiple bound salivary agglutinins and mucins. This might protect the microbe from immune surveillance by masking the underlying microbial cell surface. **F.** Lectin-

mediated phagocytosis. Phagocyte lectins bind to microbial glycans, and microbial adhesins bind to phagocyte glycans. These interactions can both potentially lead to phagocyte activation and phagocytosis of the bacteria.

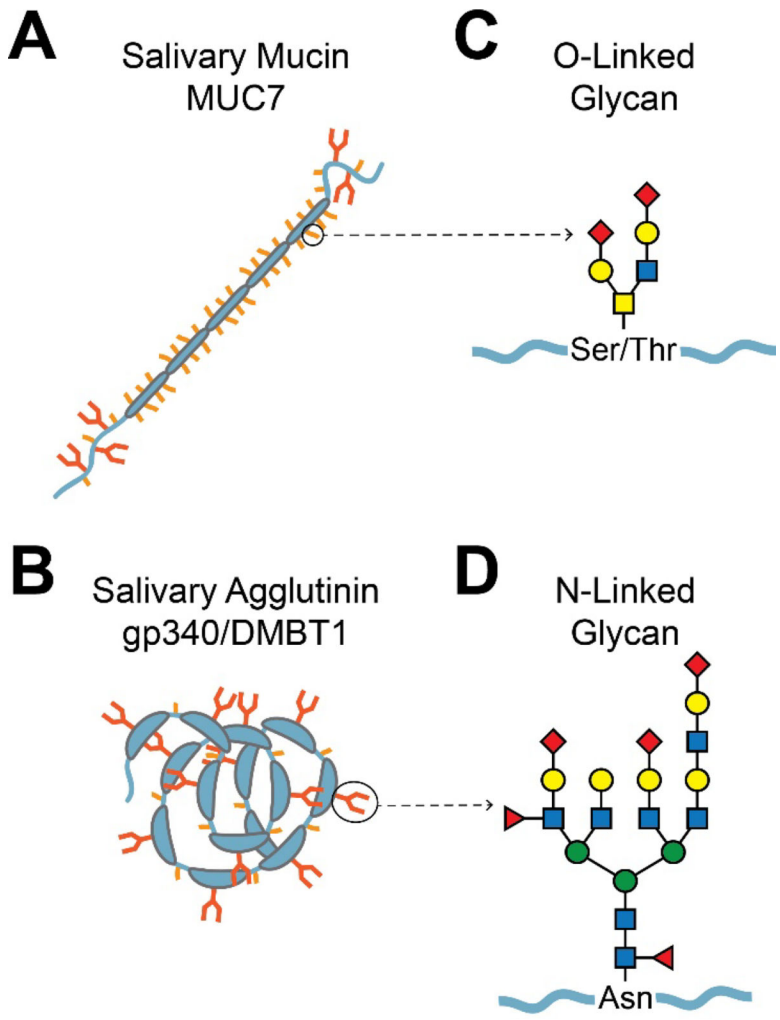

Fig. 2.

Typical salivary glycoproteins and their glycans. **A.** Mucins. Shown is a schematic diagram of the salivary mucin MUC7. The mucin protein backbone (apomucin) contains tandem repeats of proline, serine, and threonine-rich (PTS) domains, which are densely O-glycosylated. Non-repeat regions at the N- and C-terminals can be both O- and N-glycosylated. Dense O-glycosylation is believed to prevent typical protein folding, resulting in a bottle-brush-like shape. The larger gel-forming mucins such as MUC5B (not shown) are composed of several covalently linked mucin subunits. **B.** Agglutinins. Shown is a schematic diagram of the salivary agglutinin gp340/DMBT1. This protein contains several scavenger receptor cysteine-rich (SRCR) domains, which constitute the majority of the protein [263]. The SRCR domains often mediate ligand binding or protein-protein interactions with microbes. N-glycosylation occurs within the SRCR domains, and O-glycosylation occurs between the SRCR domains in serine-threonine-rich motifs that are approximately 20 amino acids in length [264]. **C.** Representation of a typical O-glycan chain. The base of an O-glycan is typically comprised of GalNAc α-linked to the hydroxyl group of serine or threonine. Other specific saccharide subunits and their attachments vary, but there are often two major branches with a small number of subunits. **D.** Representation of a typical

Nglycan. N-glycans are attached to asparagine. All of the characterized eukaryotic N-glycans begin with a GlcNAcβ1-Asn linkage, but other saccharide linkages have been observed in prokaryotes. N-glycans are generally larger than O-glycans, containing more saccharide subunits and two to four major branches.

Table 1.

Glycosylated major salivary proteins

Protein Name	Functions	Glycosylation	Predicted Molecular Mass [kDa]	Apparent Molecular Mass Including Glycosylation [kDa]
Mucin-5B (MUC5B, MG1)	Lubrication, tissue protection, microbial agglutination [52]	O-linked >> N- linked [67, 265]	596	>1000 [266]
IgA secretory chain (polymeric immunoglobulin receptor, PIGR)	Microbial agglutination, antigen binding [88, 267, 268]	N-linked and O- linked [81, 85, 269]	83	~390 (whole SIgA) [266, 270]
Salivary Agglutinin, (SAG, parotid agglutinin, gp-340, DMBT1)	Microbial agglutination [79]	N-linked > O- linked [72, 79, 80, 271]	261	340 [80]
Mucin-7 (MUC7, MG2)	Microbial adhesion, microbial agglutination, lubrication [52, 216]	O-linked >> N- linked [49, 265, 272]	39	200–250 [266]
Salivary peroxidase (SPO, lactoperoxidase, LPO)	Antimicrobial [38, 40]	N-linked	80	70–100 [266, 273]
Lactotransferrin (LTF, lactoferrin, LF)	Iron binding, bacteriostatic [274]	N-linked [275]	78	77 [266]
Immunoglobulin alpha heavy chain (IGHA1/2, IgA)	Antigen binding, microbial binding [88, 276]	N-linked (IgA1 carries O- glycans) [81–84, 268, 277]	38	~64 [268]
Alpha-amylase 1, salivary amylase (AMY1A)	Starch digestion, bacterial adhesion and agglutination [278–280]	N-linked [281]	58	55–60 [266]
Carbonic anhydrase 6 (CA6)	Buffering, reversible hydration of carbon dioxide [269, 282, 283]	O-linked and N- linked [266]	35	42–56 [266]
Immunoglobulin gamma chain (IGHG1/2, IgG)	Antigen binding [269]	N-linked	36	~51 [284, 285]
Zinc-a2-glyco-protein (AZGP1)	Lipid degradation [286]	N-linked	34	41–42 [287]
Glandular kallikreins (KLK1, KLK2, etc.)	Serine proteases	O-linked and N- linked	27–30	38–40 [288]
Proline-rich glycoprotein (PRG, GI)	Lubrication, microbial agglutination [140]	N-linked [140, 289]	39	3 9 [266]
BPI fold-containing family A member 2 (BPIFA2, parotid secretory protein, PSP, SPLUNC2)	Host defense, binds bacterial lipopolysaccharide [290]	N-linked [291, 292]	27	27 [292, 293]
Prolactin-inducible protein (PIP)	Aspartic-type proteinase	N-linked	17	17–20 [294, 295]
Beta-2-microglobulin	Similar to MHC class 1, bacterial agglutination	N-linked	14 precursor	12 [296]
Basic salivary proline- rich proteins 1–4 (PRB1/2/3/4)	Dietary tannin precipitation [297]	O-linked and N- linked (some members) [298]	31–41 proteolytically cleaved into smaller peptides	5–11 [299–301]

Table 2.

Oral microbial glycan-binding proteins

Microbial Protein Adhesin[1]	Microbial Species	Host or Microbial Glycans Recognized
	Bacteria	
Serine-rich repeat protein (SRR) adhesins (e.g. Hsa, GspB, SrpA)	*Streptococcus gordonii, Streptococcus sanguinis*	Sialic acid, Neu5Ac/Neu5Gcα2–3~ [137]
Glycan-binding protein A, GbpA/B/C/D	*Streptococcus mutans*	Glucose (dextran) [302]
Antigen I/II family, SpaP, SSP-5, SspA/B	*Streptococcus gordonii, Streptococcus mutans, Streptococcus sanguinis*	Sialic acid [303], fucose, lactose [304], N-acetylgalactosamine [305]
Type-2 fimbriae coaggregation factor A (CafA)	*Actinomyces oris, Actinomyces naeslundii*	Galß1–3GalNAc, GalNAcβ1–3Gal [156, 167]
NanH	*Tannerella forsythia*	Sialic acid [205, 306]
	Fungi	
Als1	*Candida albicans*	Fuca1–2Galß1–4GlcNAc, Fucose [185]
Epa1/6/7	*Candida glabrata*	Galß1–3~, Galβ1–4~ [188, 189]
	Viruses	
Capsid proteins	Hand-foot-and-mouth disease virus (FMDV)	Heparan sulfate [173]
Hemagglutinin (HA)	Influenza A and B	Neu5Aca2–3Gal, Neu5Acα2–6Gal [177, 178]
gB/C/D	Herpes simplex virus (HSV)	3-O-sulfated heparan sulfate [174, 175]
Gp120	Human immunodeficiency viurs (HIV)	Heparan sulfate [176]

[1]Table 2 lists only a select number of oral microbial glycan-binding proteins for which both the adhesin and the cognate glycan receptors have been identified and well characterized. Many more glycan-binding activities of oral microorganisms have been described or postulated but could not be listed here due to space limitations.