Critical Reviews in Microbiology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/imby20

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To cite this article: Simón Álvarez, Camila Leiva-Sabadini, Christina M. A. P. Schuh & Sebastian Aguayo (2022) Bacterial adhesion to collagens: implications for biofilm formation and disease progression in the oral cavity, Critical Reviews in Microbiology, 48:1, 83-95, DOI: 10.1080/1040841X.2021.1944054

To link to this article: https://doi.org/10.1080/1040841X.2021.1944054

Published online: 16 Jul 2021.

CRITICAL REVIEWS IN MICROBIOLOGY
2022, VOL. 48, NO. 1, 83–95
https://doi.org/10.1080/1040841X.2021.1944054

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REVIEW ARTICLE

Bacterial adhesion to collagens: implications for biofilm formation and disease progression in the oral cavity

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ABSTRACT

Collagen is the most abundant structural protein in the body and the main component of the extracellular matrix of most tissues, including dentine and periodontal tissues. Despite the well-characterized role of collagen and specifically type-I collagen, as a ligand for host cells, its role as a substrate for bacterial adhesion and biofilm formation is less explored. Therefore, the purpose of this review is to discuss recent findings regarding the adhesion of oral bacteria to collagen surfaces and its role in the progression and severity of oral and systemic diseases. Initial oral colonizers such as streptococci have evolved collagen-binding proteins (cbp) that are important for the colonization of dentine and periodontal tissues. Also, periodontal pathogens such as *Porphyromonas gingivalis* and *Tannerella forsythia* utilise cbps for tissue sensing and subsequent invasion. The implications of bacteria-collagen coupling in the context of collagen biomaterials and regenerative dentistry approaches are also addressed. Furthermore, the importance of interdisciplinary techniques such as atomic force microscopy for the nanocharacterization of bacteria-collagen interactions is also considered. Overall, understanding the process of oral bacterial adhesion onto collagen is important for developing future therapeutic approaches against oral and systemic diseases, by modulating the early stages of biofilm formation.

ARTICLE HISTORY
Received 8 March 2021
Accepted 2 June 2021
Published online 14 July 2021

KEYWORDS
Biofilms; atomic force microscopy; dental caries; periodontal disease; bacterial adhesion

1. Introduction

Collagen is the most abundant structural protein in the body and the main component of the basal structures of connective tissue (Hulmes 2002; Orgel et al. 2011). Furthermore, it is a major component of the extracellular matrix (ECM) and can be found in most tissues across the body (Kadler et al. 2007; Hernández Ríos et al. 2009; Gordon and Hahn 2010; Varma et al. 2016). As such, it serves as a ligand for a diverse array of cell receptors including integrins and thus is an important substrate for cellular anchoring (Boraschi-Diaz et al. 2017).

Currently, the collagen family is comprised of 28 different types (collagen I – collagen XXVIII) which are encoded by at least 45 different genes (Ricard-Blum 2011; Biochemistry of Collagens 2014). Collagen can also be characterized according to its structure in fibril-forming collagens (types I, II, III, V, XI, XXIV, XXVII) (Myllyharju and Kivirikko 2001; Heino 2007), fibril-associated collagens with interruptions in triple helix or FACITs (types IX, XII, XIV, XVI, XIX, XX, XXI, XXII, XXVI) (Myllyharju and Kivirikko 2001; Ricard-Blum and Ruggiero 2005; Heino 2007; Gordon and Hahn 2010; Ricard-Blum 2011), and basal membrane collagen (type IV) (Myllyharju and Kivirikko 2001; Heino 2007). Furthermore, transmembrane collagens (types XIII, XVII, XXIII, XXV) participate in the formation of hemidesmosomes and focal contacts (Myllyharju and Kivirikko 2001; Ricard-Blum and Ruggiero 2005; Heino 2007; Gordon and Hahn 2010). There are also other collagens involved in the formation of hexagonal networks (types VIII and X) (Myllyharju and Kivirikko 2001; Ricard-Blum and Ruggiero 2005; Heino 2007; Gordon and Hahn 2010), anchoring fibrils (types VII and XXVIII) (Myllyharju and Kivirikko 2001; Ricard-Blum and Ruggiero 2005; Heino 2007) and multiplexins (collagen XV and XVIII), many of which also participate in the stabilization of the basal membrane (Myllyharju and Kivirikko 2001; Ricard-Blum and Ruggiero 2005; Heino 2007). Most importantly, these different types of collagen play fundamental roles depending on the tissue where they are found (Myllyharju and Kivirikko 2001) and are

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distributed amongst a wide range of tissues including bone, cartilage, skin (Ricard-Blum 2011; Gursoy et al. 2013; Avilés-Reyes et al. 2017), basal membrane (Haapasalo et al. 1991; Ahuja et al. 2012; Yost and Duran-Pinedo 2018), and in the extracellular matrix where it is associated with other proteins such as laminin, elastin, and fibronectin, among others (Beg et al. 2002; Madani et al. 2017). In the oral cavity, collagen is present in clinically important tissues such as the periodontal ligament (types I, III, IV, V, VI and XII) (Ahuja et al. 2012; Yost and Duran-Pinedo 2018) and dentine (types I and III) (Ahuja et al. 2012; Yost and Duran-Pinedo 2018). Amongst these collagens, type-I fibrillar collagen is the most abundant type as it plays an important role in the structural integrity and support of tissues (Kadler et al. 2007; Gordon and Hahn 2010; Varma et al. 2016).

Type-I collagen is hierarchical fibrillar collagen synthesized by a variety of connective tissue cells such as fibroblasts, chondrocytes and osteoblasts (Malone et al. 2005; Biochemistry of Collagens 2014). Within the oral cavity, many soft and hard biological tissues include type-I collagen as an important part of their organic matrix (Ricard-Blum and Ruggiero 2005; Zhang et al. 2011; Bregou Bourgeois et al. 2016; Ibrahim et al. 2019;). Type-I collagen plays crucial role in the anchoring of teeth to the alveolar bone (i.e. periodontal ligament) as well as acting as a scaffold for guiding the mineralization of dentine during tooth formation (Goldberg 2011). In dentine, the presence of this collagen matrix is crucial for the structural stability and elasticity of the tooth (Butler and Ritchie 1995; Bertinetti et al. 2015). The most common amino acid sequence for collagen is glycine-X-Y, where X and Y are most frequently represented by proline and hydroxyproline (Brodsky and Persikov 2005; Malone et al. 2005; Kadler et al. 2007). An important characteristic of type-I collagen is its arrangement in a triple helix formation by the arrangement of two $\alpha1$ chains and one $\alpha2$ chain (Ricard-Blum and Ruggiero 2005; Bou-Gharios et al. 2020). The triple helix creates a fibrillar structure and its organization determines the location and accessibility of active sites for interaction with relevant ligands. During cellular synthesis, type-I collagen molecules undergo several intracellular modifications such as hydroxylations (Matthew 2009); glycosylation and addition of extension peptides (Di Lullo et al. 2002; Role of Glycosyltransferase 25 domain 1 in Type I Collagen Glycosylation and Molecular Phenotypes 2019). Subsequently, molecules are secreted into the extracellular space where they undergo multiple steps of post-translational modification such as enzymatic removal of the carboxyl and amine groups (Kadler et al. 2007), and subsequent molecule crosslinking which promotes organization into micro-fibrils, fibrils and finally mature collagen (Brodsky and Persikov 2005).

2. Collagens as important substrates for cell anchoring

As collagen is highly present in the ECM, it is no surprise that cells possess multiple specialized receptors for collagen binding. Within these, many subtypes of integrins are known to be important mediators for cell-collagen coupling (Heino 2007), such as the $\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$ and $\alpha11\beta1$ integrins (Jokinen et al. 2004; White et al. 2004). Overall, it is believed that the interaction between integrins and collagen is crucial in physiological and pathological processes such as inflammation, tissue regeneration, wound healing, and tumour growth and metastasis (Zeltz and Gullberg 2016). Besides integrins, cells also express other important collagen-binding receptors such as discoidin domain receptors, platelet glycoprotein VI, leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1), and GPR56 receptors (reviewed in (Zeltz and Gullberg 2016) and (Heino 2007)). Osteoclast-associated receptors (OSCAR) are also known to bind type-I and type-III fibrillar collagen and are expressed by osteoclasts, vascular endothelial cells, macrophages, neutrophils, monocytes, and dendritic cells (Haywood et al. 2016). Besides type-I and type-III collagen, an important number of collagen types have been described as substrates for cell sensing and adhesion within tissues, including basal type-IV collagen (Heino 2007). More recently, it has been found that type-XII collagen is also an important substrate for bone formation, as it modulates osteoblast polarity and cell-cell communications (Izu et al. 2011), although the specific receptors involved in this interaction are not yet well known (Chiquet et al. 2014).

More specifically, in the oral cavity, the attachment of cellular components to collagen is crucial for tissue homeostasis. Collagens are an important matrix constituent in the dental pulp (Linde 1985), dentine (Goldberg 2011), periodontal ligament (Yamauchi et al. 1986), alveolar bone (Shah et al. 2019), and oral mucosa (Nguyen et al. 2019), among others. The periodontal ligament is notorious for its fibrillar and non-fibrillar collagenous components, as it is known to possess types I, III, IV, V, VI, XII and XIV collagen within its matrix (reviewed by (Kaku and Yamauchi 2014)). Among other functions, these collagens provide an important substrate for the anchoring of diverse cells. For example, the $\alpha2\beta1$ integrin has been found in primate

periodontal ligament fibroblasts and is believed to be important for cell anchoring to the matrix (Steffensen et al. 1992). Furthermore, the α11β1 integrin expressed by periodontal ligament fibroblasts is believed to play an important role in generating sufficient force for tooth eruption, by binding to type-I collagen (Popova et al. 2007). Work by Berendsen et al. has shown that β1-containing integrins in periodontal ligament fibroblasts bind type-I and type-III collagen and that the αvβ3 integrin binds type-V collagen, which promotes tissue contraction during wound healing (Berendsen et al. 2006). In dentine and dental pulp, in-vitro studies on odontoblast-like cells have shown their ability to bind type-I and type-IV collagen potentially due to α2, α6 and β1 integrins (Baldión et al. 2018). Also, work by Ozeki et al. demonstrated that odontoblast-like cell adhesion to collagen is primarily mediated by α1 integrins whereas the mobility of these cells on type-I collagen was mostly dependent on α1β1 integrins, which are believed to promote function and cell positioning of these cells during odontogenic differentiation (Ozeki et al. 2014). In-vitro investigations by Bae et al. have also shown that human dental pulp cells express α2 and β1 when co-cultured with type-I collagen, which mediate their adhesion to collagen surfaces (Bae et al. 2012).

However, host cells are not the only ones utilizing collagen as an anchoring substrate in the human body. A wide range of resident bacteria, including microorganisms that are part of the oral microbiome, have evolved towards utilizing collagens as crucial substrates for attachment and subsequent biofilm formation.

3. Oral biofilms: from initial surface attachment to disease modulation

Bacteria are ubiquitously found in nature, and thus it is no wonder that the oral cavity is populated by hundreds of different species of microbes (Jakubovics and Shi 2020). Many oral microbes have developed a unique survival strategy through the formation of biofilms, that arise from the planktonic state and associate together to form surface-bound "communities" (previously known as *dental plaque)* (Berger et al. 2018). These microbial communities also produce a matrix of extracellular polymeric substances (EPS) consisting of protein, polysaccharides, and nucleic acids, which promotes bacterial adhesion and generates protection from external factors such as antibiotics and salivary flow (Guo et al. 2019). Up to 40% of the dry weight of oral biofilms is composed of polysaccharides, which are mainly glucans synthesized by the action of microbial glucosyltransferases (Koo et al. 2010). Despite the historical negative connotation of oral biofilms within the dentistry field, it is now known that the sole presence of biofilm on oral surfaces is not a causative factor for the development of oral diseases. In fact, it is the ecological dysregulation (i.e. dysbiosis) of these biofilms that is implicated in dental caries and periodontal disease (Lamont et al. 2018; Farkash et al. 2019; Funahashi et al. 2019; Curtis et al. 2020).

The process of biofilm formation can be divided into several steps, which initiates with reversible and irreversible attachment to surfaces and is continued by cell proliferation, EPS production, and maturation into a complex 3D architecture (He et al. 2019). Thus, bacterial adhesion to surfaces is considered the crucial initial stage for the formation of a biofilm. During irreversible adhesion, bacterial surface molecules (i.e. adhesins) come into contact with substrates and promote attachment to both biological and artificial surfaces (Aguayo and Bozec 2016). In the oral setting, bacteria have evolved to adhere to a wide array of substrates including fibrinogen, fibrin, salivary pellicle components, and collagen, amongst others (Katharios-Lanwermeyer et al. 2014). For example, primary colonizers capable of recognizing salivary glycoproteins such as gp-340 include *Streptococcus sanguinis* (Loimaranta et al. 2005), *Streptococcus gordonii* (Love et al. 2000; Bensing et al. 2004; Loimaranta et al. 2005; Kerrigan et al. 2007; Nobbs et al. 2007; Jakubovics et al. 2009; Moses et al. 2013), *Streptococcus mitis* and *Streptococcus oralis* (Loimaranta et al. 2005). Once these bacteria are irreversibly attached to oral surfaces, they promote the arrival of late colonizers such *as Fusobacterium nucleatum*, *Treponema denticola*, *Tannerella forsythia*, and *Porphyromonas gingivalis*, which co-aggregate to existing micro-colonies and increase species diversity and biomass (Katharios-Lanwermeyer et al. 2014).

3.1. Oral biofilm dysbiosis and disease

Once a mature biofilm is established on the surface of the tooth, certain ecological shifts such as the presence of fermentable carbohydrates (dental caries) or altered immune responses (periodontal disease) are able to generate changes in the number and proportions of species within the oral biofilm (Silva et al. 2008; Miller et al. 2015; Avilés-Reyes et al. 2017; Jakubovics and Shi 2020). In the case of dental caries, an increase in the abundance of acidogenic strains such as *Streptococcus mutans* and lactobacilli compared to non-acidogenic strains (such as *S. sanguinis*) occurs. This predominance of acidogenic strains causes a reduction of the local pH

which results in demineralization of the tooth surface (Liu et al. 2018), and over time, can promote the development of caries lesions (Pitts et al. 2017).

For periodontal disease, an increase in the abundance of keystone species such as *P. gingivalis* is associated with changes in local microbiota which in turn modulate the host immune response towards the destruction of periodontal tissues (Hajishengallis et al. 2012). In this disease, the eubiotic subgingival microbiome dominated by Actinomyces spp., Streptococcus spp., Neisseria spp., *F. nucleatum*, and Veillonella spp. (Loimaranta et al. 2005; Abusleme et al. 2013; Lamont et al. 2018; Mosaddad et al. 2019) shifts towards the increase of periodontopathic bacteria such as *P. gingivalis*, *T. forsythia*, *T. denticola* and *Aggregatibacter actinomycetemcomitans*, among others (Haapasalo et al. 1991; Jakubovics et al. 2005; Kumagai et al. 2005; Loimaranta et al. 2005; Abusleme et al. 2013; Vieira Colombo et al. 2016; Kinane et al. 2017; Sanz et al. 2017; Yost and Duran-Pinedo 2018; Lamont et al. 2018; Mosaddad et al. 2019; Ng et al. 2019). This local dysbiosis is believed to be a result of alterations of the local environment such as lack of hygiene, host immune response (Curtis et al. 2020) and even genetic components (Zhang et al. 2020), and the resulting exacerbated inflammatory response leads to the destruction of the supporting periodontal tissue and eventual tooth loss (Vieira Colombo et al. 2016; Curtis et al. 2020; Zhang et al. 2020).

As collagen is an important component of tissues in the oral cavity, it represents a crucial substrate for cellular adhesion (Boraschi-Diaz et al. 2017) as well as for bacterial attachment and biofilm formation. Thus, this review will now discuss the importance of collagens as substrates for biofilm formation and its implication in the development of relevant oral diseases.

4. Collagen as an important substrate for oral biofilm formation: role of early-colonizing streptococcus species

As previously discussed, biofilm formation on oral surfaces is a clinically relevant process involved in the pathogenesis of dental caries and periodontal disease. Thus, oral streptococci have adapted towards optimizing their attachment to oral tissues and salivary pellicle by means of the expression of specific bacterial-surface proteins, also known as *adhesins*. Most adhesins are cell-wall or membrane-bound receptors with the ability to sense and binding specific matrix and/or salivary molecules. For example, bacteria such as streptococci express adhesins for important salivary proteins such as mucin, agglutinin, and amylase, which facilitate their adhesion to pellicle-coated surfaces.

Naturally, as collagen is such an abundant component of the matrix of soft and hard oral surfaces, streptococci have evolved to express specific collagen-binding adhesins, also known as collagen-binding proteins (cbp) (Figure 1). As, the dentinal collagen matrix is exposed during demineralisation (Deyhle et al. 2011; Takahashi and Nyvad 2016,), dentinal type-I collagen is also believed to be an important substrate for oral streptococci to attach via cbps (Romanos and Bernimoulin 1990; Switalski et al. 1993). One of the most studied species regarding cbp is *S. mutans*, mostly due to its importance in the pathogenesis of dental caries. It is known that *S. mutans* strains express a range of collagen-binding adhesins for type-I collagen such as Cnm, Cbm, WapA and CnaB (Sullan et al. 2015; Avilés-Reyes et al. 2017; Esberg et al. 2017), which are believed to play an active role in both surface attachment and systemic invasion into the bloodstream and other tissues. Both Cbm and Cnm have been mostly linked to serotype *k* strains of *S. mutans* (Nomura et al. 2012), which are associated with higher 5-year caries increments (Esberg et al. 2017) and virulence in systemic diseases such as infective endocarditis and colitis (Kojima et al. 2012; Nomura et al. 2014). Furthermore, Nomura et al. have observed that children carrying *S. mutans* strains positive for Cnm had higher numbers of clinical parameters indicative of dental caries (Nomura et al. 2009), and Miller et al. showed that Cnm contributes to *S. mutans* epithelial cell invasion, collagen adhesion and caries severity (Miller et al. 2015). Recent work by Araújo Alves and collaborators demonstrated that Cnm in *S. mutans* is regulated by the CovR and VicRKX regulatory systems for virulence gene expression, which is conserved among other streptocccal species (Araújo Alves et al. 2018).

Furthermore, other early oral colonizers such as *S. gordonii* express relevant cbps. Surface proteins SspA (172 kDa) and SspB (164 kDa) (Moses et al., 2013), members of the antigen I/II family, are able to recognize several ligands including salivary agglutinin glycoprotein (gp-340), type-I collagen and β1 integrins (Kerrigan et al., 2007). Furthermore, SspA and SspB also play an important role in the coaggregation of *S. gordonii* with other relevant oral microorganisms such as *Candida albicans, Porphyromonas gingivalis* and *Actinomyces naeslundii* (Holmes et al. 1996; Love et al. 2000). Collagen binding has thus been suggested as an important factor for streptococcal adhesion to dentine as well as for the formation of biofilms and microcolonies within tubules and root canal wall (Moses et al. 2013).

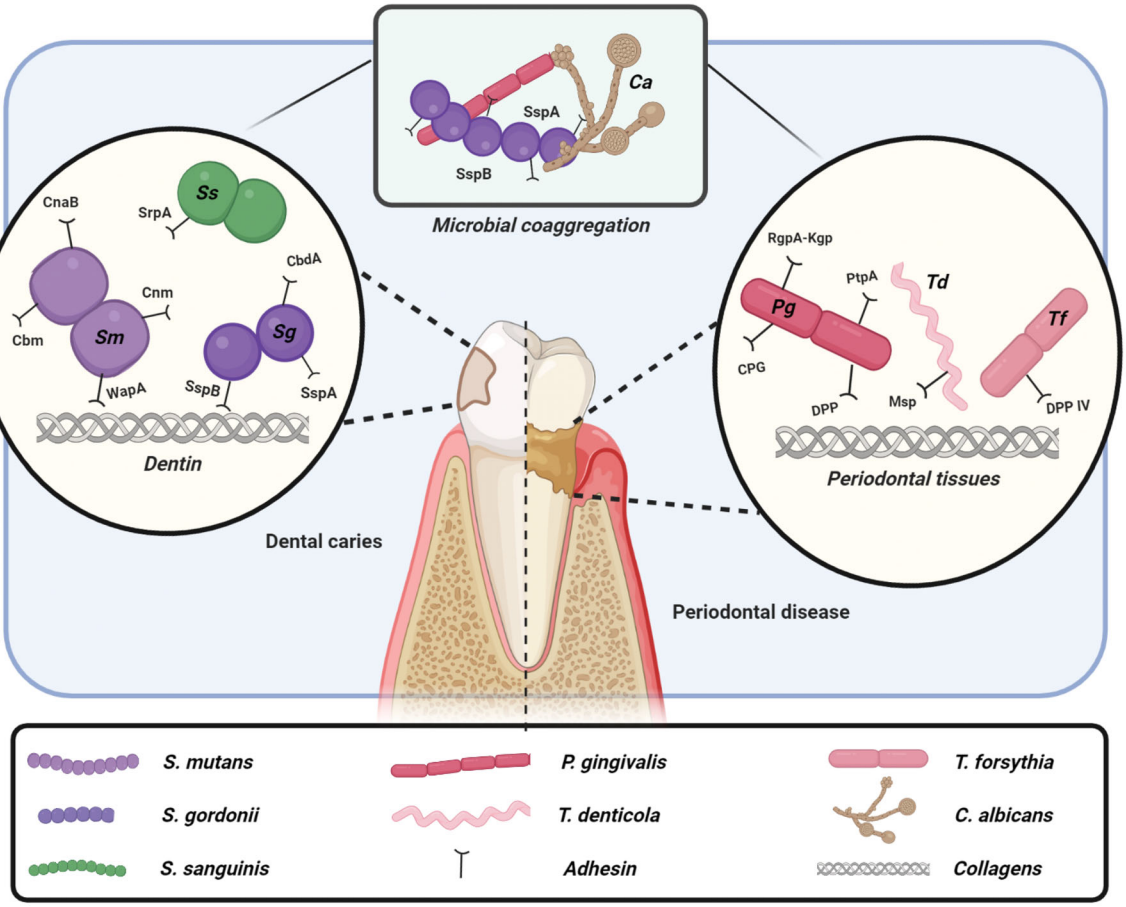

Figure 1. Overview of the main collagen-binding proteins (cbp) present in oral bacterial colonizers, and their association with relevant surfaces and diseases in the mouth. In the case of dental caries, the ability to bind collagen by colonizers such as *S. mutans, S. gordonii* and *S. sanguinis* allow the establishment of biofilms within dentin and other collagenous tissues. *S. mutans* is notorious in its capacity to express an array of cbps that are considered an important virulence factor for caries disease. Cbps also play important roles in periodontal disease, not only for the establishment of subgingival biofilms but also for tissue invasion and destruction of the extracellular matrix. Additionally, cbps have been found to mediate bacteria-bacteria and bacteria-fungi co-aggregation within the oral microbiome, suggesting their relevance in *C. albicans* associated oral pathologies.

Other primary colonizers including *Streptococcus salivarius*, *S. sanguinis*, *S. oralis*, and *S. mitis*, also express adhesins believed to have collagen-binding properties. *S. salivarius* adhesins SrpA, SrpB and Srpc were found to mediate attachment of the strain to diverse matrix components including type-I and type-IV collagen, and are believed to play a major role in host colonization (Couvigny et al. 2017). *S. sanguinis,* considered one of the first colonizers of dental surfaces (Yamaguchi et al. 2006), also expresses SrpA adhesins for surface sensing and attachment (Loimaranta et al. 2005). The ability of *S. mitis* to adhere to glycoproteins in saliva by amylase binding protein (Abp) is critical for biofilm formation (Elliott et al. 2003) and *S. oralis* can bind gp-340 and possibly fibronectin via a CshA-like protein (Nobbs et al. 2009).

Interestingly, although not normally considered an oral initial colonizer, *Staphylococcus aureus* can be found as part of the flora in the peri-implant. *S. aureus* has been found to attach to titanium surfaces and associated with suppuration in peri-implant sites (Aguayo et al., 2015; Harris et al., 2006; Renvert et al., 2008). This strain also is known to express collagen-binding proteins such as cna, part of the MSCRAMMs family (microbial surface component recognizing adhesive matrix molecules) (Madani et al. 2017). This protein has two regions (A and B), the ligand-binding domain of this protein is in the N- terminal A region, and the B region is necessary to activate the adhesive function of the protein. Herman-Bausier et al. have shown cna protein and collagen form very strong bond *in vivo* (Herman-Bausier et al. 2016). Cna can enhance the virulence of *S. aureus* by binding to complement protein C1q, preventing the classical pathway of complement fixation (Valotteau et al. 2017). Madani A. et al. found that cna binds to disorganised collagen fibres stronger than to

"stretched" fibres, potentially explaining why *S. aureus* is usually found in sites where injuries or inflammation are present (Madani et al. 2017).

5. Collagen binding for soft tissue invasion: role of collagen-binding proteins in the pathogenesis of periodontal disease

Despite the importance of collagen as a substrate to promote biofilm formation, some oral bacteria have also developed collagen-binding proteins for the purpose of attaching to the surface for invasion and/or degradation. Both tissue invasion and collagen degradation are important phases in the progression of periodontal disease, and thus it is no surprise that periodontal bacteria have evolved the ability of binding collagen.

Various adhesins have been identified in secondary oral colonizers, allowing adhesion to matrix components such as laminin, fibronectin, and various types of collagen, among others. It is believed that this ability to attach to collagen surfaces is an important virulence factor for these strains and is a crucial factor for the development and progression of subgingival biofilms and periodontal disease. Bacteria such as *P. gingivalis* have been shown to be able to bind type I collagen through DPP IV, PtpA and DPP-7 or CPG-70 (Kumagai et al. 2005; Yost and Duran-Pinedo 2018). In addition, *P. gingivalis* produces an extracellular complex of proteases and adhesins called RgpA-Kgp that is important in the process of attachment to various connective tissue proteins such as type-V collagen (O'Brien-Simpson et al. 2009). Furthermore, fimbriae present in *P. gingivalis* are known to bind type-I collagen through the FimA, C, D and E adhesins (Singh et al. 2012). On the other hand, *T. forsythia* is known to bind type-I and type-III collagen, which is important in the process of periodontal tissue invasion (Yost and Duran-Pinedo 2018). Type-I collagen-binding during periodontal invasion is also present in other periodontopathic bacteria such as *A. actinomycetemcomitans* via the EmaA adhesin (Yu et al. 2009; Nobbs et al. 2011), and in *T. denticola* via the Msp adhesin (Edwards et al. 2005).

Once these bacteria have successfully adhered to collagen and established a biofilm, they are able to progress with the destruction of the matrix of supporting periodontal tissues via enzymatic activity. It is known that *P. gingivalis* possesses different types of collagenases (Kumagai et al. 2005), and strains such as *T. forsythia* also have the ability to degrade type-I collagen (Yost and Duran-Pinedo 2018). Also, strains such as *T. forsythia* produce methylglyoxal (MGO), an important by-product of glucose metabolism that can covalently modify collagen by the generation of advanced glycation end-products (AGEs) (Retamal et al. 2016; Settem et al. 2018). Additionally, *T. denticola* can also bind denatured type-I collagen as well as to other relevant matrix proteins such as fibrinogen (Haapasalo et al. 1991), and utilize this ability to further invade the periodontal tissues during infection.

6. Involvement of collagen binding in the pathogenesis of oral microbiome-derived systemic diseases

Interestingly, in recent years it has become increasingly evident that components of the oral microbiome are not only involved in local disease, but are also implied in a wide range of systemic pathologies including diabetes mellitus, cardiovascular disease, and Alzheimer's disease (AD) (Bui et al. 2019; González-Sanmiguel et al. 2020). This is believed to be due to a combination of direct microbial invasion into the bloodstream (including bacterial toxins and byproducts) and a systemic inflammatory response. Within this context, collagen-binding by oral bacteria is a crucial initial step in allowing their progression into tissues and bloodstream, and thus a potentially important target against the development of certain systemic diseases. For example, Abranches et al. observed that cnm is necessary for *S. mutans* invasion of human coronary artery endothelial cells (Abranches et al. 2011) and thus suggests its involvement in cardiovascular pathology. Additionally, Nakano et al. observed that cbp expression by *S. mutans* is associated with a higher risk of developing haemorrhagic stroke (Nakano et al. 2011), and further studies have shown the involvement of cbp-possitive *S. mutans* in infective endocarditis, ulcerative colitis, and non-alcoholic fatty liver disease (Kojima et al. 2012; Nomura et al. 2013; Naka et al. 2014). It is believed that the virulence behind cbp expression is a combination of the ability to bind collagen in oral tissues for tissue penetration into the bloodstream, and the subsequent capacity of binding exposed collagen in injured tissues such as the endothelium (Nakano et al. 2011).

Also, as described above, certain periodontopathic strains have the ability of binding collagen during periodontal disease, conferring the ability to invade tissues and eventually entering the bloodstream. *P. gingivalis* is notorious as an invading species, and its ability to bind to collagens and other components of the matrix is a crucial virulence factor for this behaviour (reviewed in (Singh et al. 2012)). Furthermore, *P. gingivalis* proteases such as the RgpA gingipain, have also been found to

favour collagen adhesion (Tokuda et al. 1998). These properties are highly relevant in the context of AD, as both *P. gingivalis* cells and gingipains are found in the brains of AD patients and are believed to play an important role in neuroinflammation and neurodegeneration (Dominy et al. 2019; González-Sanmiguel et al. 2020).

7. Advanced nanoscale techniques to explore bacterial adhesion onto collagen surfaces: atomic force microscopy (AFM) and single-cell force spectroscopy (SCFS)

Currently, much interest has been placed on understanding bacterial-surface interactions within the context of health and disease. Until recently, techniques for studying bacterial adhesion were limited to the microscopic assessment of biofilm formation and UFC counts, amongst others. However, with the advent of atomic force microscopy (AFM) researchers are now able to probe into bacterial adhesion in real-time at both cellular and sub-cellular levels (Garcia 2020). By functionalizing AFM tips with bacterial cells or molecules, nanoscale techniques such as single-cell force spectroscopy (SCFS) and single-molecule force spectroscopy (SMFS) allow experiments to be performed with living cells in buffer conditions, which yield important information on bacterial nanomechanics and their interaction with biological and artificial substrates (Aguayo et al. 2015). These in-vitro techniques have provided much insight on the mechanics of collagen-binding by many relevant oral colonizers, and their implications in health and disease.

Overall, AFM experiments have confirmed that a wide range of microorganisms have the ability to bind collagen at the nanoscale. Herman-Bausier et al. utilized a combination of SCFS and SMFS to explore the attachment of the CWA protein serine-aspartate repeat protein F of *Staphylococcus epidermidis* onto type-I collagen and found the formation of both weak and strong bonds between the bacterial protein and collagen (Herman-Bausier and Dufrêne 2016). Herman-Bausier et al. also explored the interaction between staphylococcal collagen-binding protein Cna and collagen substrates, finding strong adhesion forces that were inhibited by the use of monoclonal antibodies targeting this interaction (Herman-Bausier et al. 2016). Furthermore, El-Kirat-Chatel et al. have found that *Burkholderia cepacian*, a bacterium involved in cystic fibrosis, can bind collagen via the BCAM0224 adhesin, which may prove to be important in the process of host colonization (El-Kirat-Chatel et al. 2013). In another study Becke et al. demonstrated that the pilus protein RrgB of *Streptococcus pneumoniae* binds to type-I collagen in a force-dependent manner, confirming the importance of pili in the attachment of bacteria onto host substrates (Becke et al. 2019).

Some recent research has also investigated bacteria-collagen interactions within the context of oral biofilm formation utilizing AFM-based experiments. Soell et al. explored adhesion between the *S. mutans* antigen I/II and type-I collagen surfaces and found that this interaction was weaker than antigen I/II-fibronectin coupling (Soell et al., 2010). Further work on *S. mutans* by Sullan et al. demonstrated that the P1 adhesin is able to bind multiple substrates, including type-I collagen, utilizing a combination of SCFS and SMFS approaches (Sullan et al. 2015). Schuh et al. observed that *S. sanguinis* and *S. mutans* show different binding profiles to native and crosslinked type-I collagen surfaces, confirming that the binding strength between oral streptococci and collagen substrates is time-dependent and strain-specific, and observed that MGO-modified collagen alters the attachment of these strains to collagen surfaces in a differential manner (Schuh et al. 2021). AFM observations were paired with biofilm structure analysis and extracellular matrix characterization to show that in-vitro, collagen crosslinking alters not only initial bacterial adhesion but also early biofilm formation by oral streptococci (Schuh et al. 2021). Overall, future work and explorations into the biophysics of bacteria-collagen interactions with AFM are promising avenues to further understand the initial determinants behind oral biofilm-mediated diseases.

8. Implications of bacterial collagen-binding for collagen-based tissue engineering approaches

Regenerative medicine approaches, as well as tissue engineering, have become increasingly popular in dentistry in the past decades, having found recognition in many clinically proficient techniques such as guided tissue regeneration (GTR) or guided bone regeneration (GBR). These approaches are usually centred on the functional replacement of lost dental pulp, alveolar bone, or gingival tissue; and the use of barrier membranes in periodontal regeneration or bone augmentation has gained notorious popularity in the fields of dental implantology and periodontology, due to its high predictability and great clinical results (Lee and Kim 2014).

Within this context, type-I collagen is one of the most widely used biomaterials in clinics due to its

excellent biocompatibility and versatility and can be found in the shape of membranes, gels, and/or sponges (Sbricoli et al. 2020). As discussed throughout this review, several bacterial strains have demonstrated collagen-binding proteins as well as proteases capable of degrading collagen and other matrix proteins. Concerns on the clinical implications of using collagen as a standard treatment have been raised in several studies throughout the past decades. More specifically, it has been shown that pathological bacterial strains like *T. denticola*, *S. mutans, A. actinomycetemcomitans* and *P. gingivalis* demonstrate affinity to collagen scaffolds (Edelmayer et al. 2020). Thus, newer studies have been acknowledging these shortcomings of collagen scaffolds, recognizing the need to potentially develop new collagen-based materials with antibacterial or antibiofilm properties (Edelmayer et al., 2020). This is especially important considering the recent advent of antimicrobial resistance and treatment-resistant biomaterial-related infections. Thus, several groups have been integrating antimicrobial agents into collagen membranes including silver nanoparticles, chitosan, and other antibiotics (Chen et al. 2018; Edelmayer et al. 2020), in hopes of decreasing infection risks of the biomaterial, especially in cases where collagen membranes become exposed to the oral environment after surgery. Also, future strategies for the design of novel collagen-based biomaterials in the oral cavity could potentially include the modulation of collagen-binding sites, in order to inhibit the attachment of pathogenic strains.

9. Future work and perspectives

As discussed throughout this review, collagens including type-I collagen are not only an important scaffold providing structural support for cells and tissues but also an important and relevant substrate for bacterial attachment and subsequent biofilm formation. Thus, it is no surprise that both supragingival and subgingival oral strains possess many specialized adhesins for collagen binding. *S. mutans* is particularly interesting, as it expresses a wide range of cbps that have most likely evolved to potentiate adhesion onto dentinal collagen (Table 1). However, the biological implication of

Table 1. Summary of relevant oral bacterial species and their collagen-binding adhesins.

Genus	Species	Adhesin	Binding to ECM	References
Streptococcaceae	*S. mutans*	a. Cnm	a. Collagen I, II, III, IV, laminin	Loimaranta et al. 2005; Sato et al. 2004; Abranches et al. 2011; Avilés-Reyes et al. 2017
		b. SpaP (P1)	b. gp-340, Collagen I, beta-1 integrin, laminin, fibronectin	Loimaranta et al. 2005; Sciotti et al. 2020; Avilés-Reyes et al. 2017
		c. WapA	c. Collagen	Avilés-Reyes et al. 2017; Han et al. 2006
		d. Gbp	d. Glucan	Avilés-Reyes et al. 2017
		e. Cbm	e. Collagen	Nomura et al. 2012; Avilés-Reyes et al. 2017; Han et al. 2006
Sthaphylococcaceae	*S. aureus*	a. Cna	a. Collagen, C1q, fibronectin	Herman-Bausier et al. 2016; Madani et al. 2017; Mirzaee et al. 2015
Streptococcaceae	*S. gordonii*	a. GspB	a. gp-340, GPIb alfa	Loimaranta et al. 2005; Kerrigan et al. 2007
		b. Has	b. gp-340, GPIb alfa	Bensing et al. 2004; Loimaranta et al. 2005; Kerrigan et al. 2007; Jakubovics et al. 2009
		c. SspA y SspB	c. gp-340, collagen I, beta-1 integrin	Love et al. 2000; Kerrigan et al. 2007; Nobbs et al. 2007; Jakubovics et al. 2009; Moses et al. 2013
		d. CshA /CshB	d. Fibronectin	Loimaranta et al. 2005; Jakubovics et al. 2009
		e. CbdA	e. Collagen I	Nobbs et al. 2007; Moses et al. 2013; Avilés-Reyes et al. 2017
Streptococcaceae	*S. sanguinis*	a. SrpA	a. gp-340	Loimaranta et al. 2005
Streptococcaceae	*S. oralis*	a. SoaA	a. gp-340	Loimaranta et al. 2005; Love et al. 2000
		b. CshA-like protein	b. Fibronectin	Elliott et al. 2003
Streptococcaceae	*S. mitis*	a. Abp	a.a-amylase	Elliott et al. 2003
Porphyromonadaceae	*P. gingivalis*	a. DPPIV	a. Collagen I (proline specific), fibronectin	Kumagai et al. 2005; Yost and Duran-Pinedo 2018
		b. PtpA RgpA-Kgp	b. Collagen I	Kumagai et al. 2005; Yost and Duran-Pinedo 2018
		c. DPP-7, CPG-70	c. Collagen I	Kumagai et al. 2005; Yost and Duran-Pinedo 2018
Spirochaetaceae	*T. denticola*	a. Msp	a. Laminin, fibronectin, collagen I, IV, gelatine, fibrinogen, RGD peptide	Edwards et al. 2005; Haapasalo et al. 1991
Porphyromonadaceae	*T. forsythia*	a. DPPIV	a. Collagen I, III,	Yost and Duran-Pinedo 2018
		b. BspA	b. Fibrinogen, fibronectin	Yost and Duran-Pinedo 2018

collagen-binding by *S. mutans* needs to be further explored in coming years.

Furthermore, the effect of collagen modifications by genetic or environmental factors on bacterial adhesion is also a topic of interest. Recent work by Schuh et al. has observed that type-I collagen crosslinking impacts adhesion and biofilm formation of *S. mutans* and *S. sanguinis* in an in-vitro model, and that strong crosslinking of the collagen matrix results in a significant reduction of biofilm formation for both initial colonizers (Schuh et al. 2021). It remains highly likely that modifications of collagen by genetic diseases (Ibrahim et al. 2019), UV exposure (Jariashvili et al. 2012), and radiation therapy (GonzÁlez-Arriagada et al. 2019; Muñoz et al. 2020), amongst others, will also impact bacterial adhesion and biofilm formation. However, further research is necessary to explore the impact of these factors in diseases such as dental caries and periodontal disease.

Overall, current research is seeking out novel ways to inhibit or modulate the initial attachment between oral colonizers and surfaces, in hopes of treating diseases such as dental caries before they become established. The disease burden of dental caries and periodontal disease remains very high, and the low effectiveness of local antibiotic treatments paired with increasing reports of antimicrobial resistance has created the need to look for alternative approaches against oral biofilm-mediated diseases. Within this field, understanding the interaction between oral colonizers and collagen surfaces present in dentine and periodontal tissues may prove crucial for developing novel therapies to modulate the local biofilm microenvironment to prevent and/or reverse dysbiosis.

10. Conclusion

Overall, collagen functions as an important ligand for both host cells and components of the oral microbiome; thus, the ability of oral bacteria to attach to collagens is an important virulence factor implicated in the development of important oral diseases such as dental caries and periodontal disease. Both early and late colonizers can attach to dentinal and periodontal collagens via cbps, which allow them to establish biofilms on surfaces and subsequently invade surrounding tissues. Furthermore, attachment of bacteria to collagen also has important implications in the use of collagen-based biomaterials for regeneration, explaining the need to develop novel approaches in order to reduce infection of these materials and further treatment complications. Current biophysics-based techniques such as AFM allow us to characterize bacteria-collagen interactions at nanometric and single-cellular levels as never before. Finally, understanding the specifics of oral bacterial adhesion onto collagen is key for developing novel therapeutic approaches against oral biofilm-mediated diseases, by modulating the early stages of attachment and biofilm formation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the CONICYT FONDECYT Iniciacion Grant [#11180101]. Figures were created with Biorender.com.

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