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CLINICAL REVIEW

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Proteins, Pathogens, and Failure at the Composite-Tooth Interface

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ABSTRACT

In the United States, composites accounted for nearly 70% of the 173.2 million composite and amalgam restorations placed in 2006 (Kingman *et al.*, 2012), and it is likely that the use of composite will continue to increase as dentists phase out dental amalgam. This trend is not, however, without consequences. The failure rate of composite restorations is double that of amalgam (Ferracane, 2013). Composite restorations accumulate more biofilm, experience more secondary decay, and require more frequent replacement. *In vivo* biodegradation of the adhesive bond at the composite-tooth interface is a major contributor to the cascade of events leading to restoration failure. Binding by proteins, particularly gp340, from the salivary pellicle leads to biofilm attachment, which accelerates degradation of the interfacial bond and demineralization of the tooth by recruiting the pioneer bacterium *Streptococcus mutans* to the surface. Bacterial production of lactic acid lowers the pH of the oral microenvironment, erodes hydroxyapatite in enamel and dentin, and promotes hydrolysis of the adhesive. Secreted esterases further hydrolyze the adhesive polymer, exposing the soft underlying collagenous dentinal matrix and allowing further infiltration by the pathogenic biofilm. Manifold approaches are being pursued to increase the longevity of composite dental restorations based on the major contributing factors responsible for degradation. The key material and biological components and the interactions involved in the destructive processes, including recent advances in understanding the structural and molecular basis of biofilm recruitment, are described in this review. Innovative strategies to mitigate these pathogenic effects and slow deterioration are discussed.

KEY WORDS: dentin bonding agents, methacrylate, gp340, *Streptococcus mutans*, esterases, biofilm.

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INTRODUCTION

In 2006, 173.2 million composite and amalgam restorations were placed in the United States (Kingman *et al.*, 2012), and the results from clinical studies suggest that more than half were replacements for failed restorations (Murray *et al.*, 2002). Replacement of failed restorations consumes 60% of the average dentist's practice time (National Institute of Dental and Craniofacial Research, 2009). This emphasis on replacement therapy will increase as dentists discontinue their use of dental amalgam (Krifka *et al.*, 2013). Dental amalgam is being discontinued in response to global concerns about mercury in the environment.

Resin composite is the most common alternative to dental amalgam, but moderate to large composite restorations have higher failure rates, more recurrent decay, and increased frequency of replacement as compared with amalgam (Simecek *et al.*, 2009). For example, the need for additional restorative care was 50% greater in children treated with composite as compared with amalgam (DeRouen *et al.*, 2006). In comparison to amalgam, caries is the most frequent reason for failure of composite (Opdam *et al.*, 2010). *In vivo* biodegradation of the bond between the composite and tooth (*i.e.*, the composite restoration's adhesive bond layer) is considered a particularly critical contributor to secondary loss of adhesion, microleakage, and decay (Donmez *et al.*, 2005).

This review article focuses on key biological and physicochemical interactions involved in the failure of composite restorations. While it is likely that degradation of the restoration starts with the tooth surface, the interface between the synthetic material and biological tissue plays a vital role in shifting the microbial ecology from a state of health to a disease-associated state. Recent advances in biofilm recruitment and innovative strategies to mitigate its pathogenic effects at the composite-tooth interface are discussed.

RECURRENT DECAY

Clinically, 80% to 90% of recurrent decay is located at the gingival margin of class II and V restorations (Figure 1; Li *et al.*, 2014). Recurrent decay is linked to failure of the bond between the tooth and composite and increased levels of the cariogenic bacterium, *Streptococcus mutans*, localized around the perimeter of these materials (Leinfelder, 2000). The composite is too viscous to bond directly to the tooth—a low viscosity adhesive must be used to bond the composite to the tooth. Clinicians frequently find very little enamel available for bonding at the gingival margin; thus, the bond at this margin

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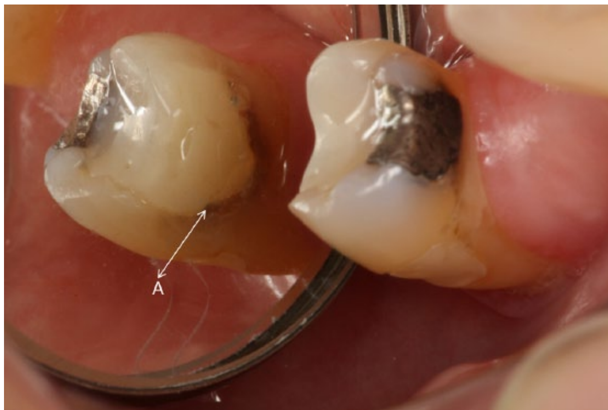

Figure 1. Clinical photo of a composite restoration showing decay along the gingival margin. The letter A marks the gingival margin.

depends on the integrity of the adhesive seal formed with dentin. At the vulnerable gingival margin, the adhesive is the barrier between the prepared tooth and the surrounding environment.

A failed adhesive means that there are gaps between the tooth and composite: gaps that can be penetrated by oral fluids, bacterial enzymes, and bacteria. The infiltration of these noxious agents into the gaps between the tooth and composite will lead to recurrent decay, hypersensitivity, pulpal inflammation, and restoration failure (Murray *et al.*, 2002). The lack of durable and effective dentin adhesives is considered one of the major problems with the use of composites in direct restorative dentistry (Spencer *et al.*, 2010).

ADHESIVE BOND

The fundamental processes involved in bonding total-etch (etch-and-rinse) adhesives to dentin include removal of the mineral phase from the dentin substrate without altering the collagen matrix and filling the voids left by the mineral with adhesive that undergoes complete *in situ* polymerization (*i.e.*, the hybrid layer). In self-etch adhesive systems, the etching and priming/bonding are combined in 1 step (Liu *et al.*, 2011). The adhesive formulations include hydrophilic and acidic monomers to achieve the task of etching and priming simultaneously (Moszner *et al.*, 2005).

The ideal hybrid layer is characterized as a collagen network infused and reinforced by polymer (Singh *et al.*, 2014). Ideally, this polymer-collagen composite will provide a durable and continuous link between the bulk adhesive and dentin. Numerous studies indicate that this ideal is not achieved, and the major mechanisms involved in deterioration of the hybrid layer are degradation of water-rich, resin-sparse collagen fibrils and hydrolysis of the adhesive (Hashimoto, 2010; Perdigão *et al.*, 2013).

Discrepancy between the depth of dentin demineralization and adhesive infiltration means that there is exposed collagen within the hybrid layer (Spencer and Swafford, 1999; Kermanshahi *et al.*, 2010). Degradation of the exposed collagen matrix follows a cascade of events that begins with acid etching of the dentin. Acid etching disrupts the tooth structure—creating the porosity that is critical to adhesive infiltration, but acid etching also stimulates proteolytic enzymes (*e.g.*, matrix metalloproteinases [MMPs]), which can degrade the exposed collagen (Pashley *et al.*, 2004). It is hypothesized that this type of degradation is most important acutely in the period following adhesive application. The predominant methods to address this acute attack on the hybrid layer have been MMP inhibitors (*e.g.*, chlorhexidine) or MMP inhibitor–conjugated resin monomers (Brackett *et al.*, 2011). These methods have shown promise in terms of slowing collagen degradation and shifting the major site of failure elsewhere (Tjäderhane *et al.*, 2013). The method is not, however, without its limitations. For example, the addition of 2 wt% chlorhexidine to a 70/30 mol% 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane (BisGMA)/triethylene glycol dimethacrylate (TEGDMA) resin system led to a statistically significant decrease in degree of conversion (Pallan *et al.*, 2012). The degree of polymerization of urethane dimethacrylate (UDMA)/TEGDMA resin system was reduced by the addition of chlorhexidine (Anusavice *et al.*, 2006). Biomimetic remineralization (Tay and Pashley, 2008) impedes MMP attack of the exposed collagen layer, but this technique does not prevent water sorption and hydrolysis of the adhesive components (Brackett *et al.*, 2011).

Chronic deterioration of the hybrid layer involves hydrolysis and leaching of the adhesive that has infiltrated the collagen matrix. Leaching is facilitated by water ingress into the loosely cross-linked or hydrophilic domains of the adhesive (Parthasarathy *et al.*, 2012). Water may also be trapped within the adhesive during photopolymerization. Water promotes the chemical hydrolysis of ester bonds in methacrylate materials, and although this reaction is expected to be relatively slow at neutral pH, excursions in pH may lead to transient acid or base catalysis. With time, local domains of the methacrylate network become sufficiently degraded to permit access by esterases (Finer *et al.*, 2004; Bourbia *et al.*, 2013), which greatly accelerate ester bond hydrolysis. Esterase activities of *S. mutans* are also at levels sufficient to hydrolyze the ester bonds in methacrylate adhesives (Bourbia *et al.*, 2013).

Molecular and mechanical modeling in conjunction with synthesis of new methacrylate monomers and multiscale adhesive/dentin (a/d) interfacial characterization has been used as a path to the development of new water-compatible, esterase-resistant adhesives (Spencer *et al.*, 2010). Methacrylate side chains are selectively modified so that they are both water compatible and esterase resistant (Park *et al.*, 2008; Park *et al.*, 2009a; Park *et al.*, 2009b). For example, adhesive formulations containing BisGMA, 2-hydroxyethyl methacrylate (HEMA), and a new multifunctional methacrylate with a branched side chain (*i.e.*, trimethylolpropane mono allyl ether dimethacrylate) showed significantly greater esterase resistance than BisGMA/HEMA adhesives when both formulations are photopolymerized in the presence of water (Park *et al.*, 2009a). Adhesive formulations containing BisGMA, HEMA, and a new trimethacrylate monomer with urethane-linked groups—that is, 1,1,1-tri-[4-(methacryloxyethylamino-carbonyloxy)-phenyl] ethane (MPE)—showed greater esterase resistance than BisGMA/HEMA formulations when both are photopolymerized in 16 wt% water (Park *et al.*, 2008).

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COMPOSITE FAILURE

After nearly 6 decades of research, dental composites continue to show limited clinical service as a result of decay or fracture (Drummond, 2008; Opdam *et al.*, 2010). Recurrent decay at the composite-tooth interface has consistently been the primary reason for replacement of composite restorations (Ferracane, 2013), and *S. mutans* is a primary pathogenic bacterium responsible for recurrent decay (Nyvad and Kilian, 1987). Adhesion of *S. mutans* to the tooth surface changes the local environment to support the subsequent colonization of the surface by other bacterial species, ultimately forming a microecosystem known as a biofilm. In addition to its role as a "pioneer" organism in biofilm formation, *S. mutans* increases the acidity of the environment by producing lactic acid, which damages the tooth, adhesive, and composite.

Composites accumulate more biofilm than other restorative materials (Beyth *et al.*, 2008). Cavity preparations are infected with residual *S. mutans* (Li *et al.*, 2009). Degradation of methacrylate ester groups in adhesives and composites produces carboxylic acids—the same functional group that is the culprit in lactic acid–induced demineralization. The breaking of covalent bonds by addition of water to ester bonds is considered one of the main reasons for resin degradation within the hybrid layer (Brackett *et al.*, 2011). The failure of the adhesive bond layer provides crevices that are readily penetrated by pathogens. Adhesive failure in the presence of bacteria, esterases (Bourbia *et al.*, 2013), and dental plaque biofilm (Busscher *et al.*, 2010) provokes a cascade of events leading to deterioration of the interfacial bond and failure of the composite restoration.

BIOFILMS, DENTAL PLAQUE, AND COMPOSITE

The direct cause of decay at the margins of composite restorations is cariogenic plaque (Filoche *et al.*, 2010). The cariogenic plaques result when the low populations of acidogenic and aciduric bacteria increase following high-frequency sugars and other fermentable carbohydrate exposure (Marsh, 2003). The extended acidification of plaque ($pH < 5$) is a result of the metabolic activity of the microbiota. The acid demineralizes the tooth and causes severe damage to the surface of the composite restoration, prompting further biofilm-microbial attachment and deterioration of the restoration (Beyth *et al.*, 2008).

While there is extensive evidence supporting the role of pathogenic bacteria in the destruction of dental restorations, the extent to which the restoration itself provokes a microenvironment that contributes to the progression of pathogen-based destruction of the tooth is still being defined, particularly at the molecular level. Composite restorative systems permit attachment of salivary proteins and pioneer microorganisms involved in decay. The metabolism of these bacteria is altered in the presence of the composite restorative system, enabling promotion of the pathogenic effects (Delaviz *et al.*, 2014). As discussed above, the interface between the restoration and tooth (*i.e.*, the composite restoration's adhesive bond layer) is the most vulnerable site (Donmez *et al.*, 2005). This interface has greater surface roughness, and the polymer is more heterogeneous and porous than enamel, providing increased opportunity for attachment of proteins, primarily gp340, and microbes that lead to subsequent degradation of the repaired tooth (Beyth *et al.*, 2008). This degradation is particularly evident at the gingival margin where it is difficult to clean (Spencer *et al.*, 2010).

Binding studies to dental materials and methacrylate polymers demonstrate that modulating the chemical properties of the polymer affects protein adhesion and microbial attachment (Weerkamp *et al.*, 1988; Olsson *et al.*, 1992; Murata *et al.*, 2007), but insufficient data are available to define the molecular basis of attachment. These studies have examined resin systems in which the chemical composition has been modified by substituting functional groups on the monomers, altering the cross-linker and/or changing the formulation components. While studies have reported that, in the oral cavity, less biofilm is recovered from supragingival hydrophobic surfaces (Busscher *et al.*, 2010), it has been very difficult to provide unequivocal evidence of systematic differences in salivary protein binding as a function of material composition and surface charge. Chemical changes to individual monomers not only introduce new moieties onto the surface of the polymer (Weerkamp *et al.*, 1988; Murata *et al.*, 2007) but also affect its physical properties, including surface roughness and porosity, which are known to influence adhesion to surfaces and infiltration (Busscher *et al.*, 2010). Charged moieties promote hydration of the polymer, which can increase swelling and encourage degradation (Delaviz *et al.*, 2014). As such, balancing the various advantageous and destructive processes that affect a formulation is required to optimize longevity. While the individual studies are certainly correct, the limited data set includes seemingly contradictory results and emphasizes the need for a thorough, systematic characterization of all relevant parameters.

Modification of the resin composition has been examined in an effort to prevent attachment of bacteria to the polymer surface. Quaternary ammonium compounds have demonstrated antibacterial properties and have been incorporated into methacrylate polymers for this purpose (Li *et al.*, 2014), where it does appear that the antibacterial properties of an individual monomer depend on the surface density of that modified monomer (Murata *et al.*, 2007). The free monomers, however, have proven to be significantly more potent at preventing growth of pathogenic microbes in solution than through contact killing at the polymer surface once incorporated (Murata *et al.*, 2007). In addition, monomers specifically containing quaternary amines have been shown to inhibit MMP activity (Tezvergil-Mutluay *et al.*, 2011). An interesting adaptation used to improve surface properties is the subsequent physical or chemical modification of the polymer. Physical modification includes polishing the surface of the polymer to reduce surface roughness. Methacrylate polymer surfaces may be chemically modified to make them zwitterionic to prevent fouling (Xiang *et al.*, 2014). Our group recently applied this approach to dentin adhesive formulations and demonstrated that grafting the metal abstraction peptide, a novel inhibitor of MMP-8, via a synthetic linker to the surface of an amine-containing methacrylate polymer completely abrogated catalysis by this enzyme (Dixit *et al.*, 2014).

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COMPOSITION OF THE PATHOGENIC BIOFILM

Dental plaque is a complex biofilm composed of proteins and microorganisms. The main players identified in cariogenic pathogenicity are a salivary agglutinin (SAG) glycoprotein (gp340) and the pioneer bacterium *S. mutans*, although others may contribute. The normal function of gp340, a component of the salivary pellicle, is to bind to and clear microbes from the oral cavity by inducing their aggregation. In the presence of a composite restoration, gp340 binds bacteria and adheres to the nonnative surface to support the attachment of the pathogenic biofilm at the vulnerable composite-adhesive-tooth interfaces (Gibbons and Hay, 1989). The gp340 protein binds to the partner protein AgI/II on the surface of *S. mutans* and its homologues on other bacteria, thereby promoting aggregation of the microbes and enabling their recruitment and retention at the restoration-tooth interface. Following adhesion, when bacteria become exposed to sugars that fuel the metabolic production and extracellular release of lactic acid, the tooth is exposed to low pH, which promotes demineralization. Because the acid is produced in the immediate proximity of residual healthy tooth, loss of exposed enamel and dentin occurs.

gp340 also adheres to the crystalline hydroxylapatite matrix of the tooth's enamel and supports subsequent attachment of *S. mutans* and other microbes to this surface. Based on investigations with sintered hydroxylapatite, which provides surface characteristics similar to human enamel, attachment to enamel is inhibited by the small salivary phosphoproteins statherin and histatin 1 (Shimotoyodome *et al.*, 2006). It has been demonstrated that soluble, synthetic phospho-containing compounds also inhibit binding to enamel. Methacryloyloxydecyl phosphate–PEG competes effectively for binding to the enamel surface and successfully prevents adherence by salivary proteins and microbes to this highly regular surface (Shimotoyodome *et al.*, 2007). Reversal by methacryloyloxydecyl phosphate–PEG after salivary protein adhesion is less effective and does not prevent subsequent microbial attachment (Shimotoyodome *et al.*, 2007). This result suggests that it is more difficult to displace microbes than to prevent attachment of the proteins responsible for adhesion of the biofilm. The development of strategies to prevent adhesion to the tooth and polymeric restorative materials is important to preserving the integrity of both the healthy tooth and the repair. Under *in vivo* conditions, successful application of these strategies may be confounded by variables such as wear and shear forces.

gp340 STRUCTURE AND INTERACTIONS

gp340 is a ~360-kDa multidomain glycosylated protein. The protein consists of 17 folded domains: 14 scavenger receptor cysteine-rich (SRCR) repeat domains, the last of which is sandwiched between 2 CUB (C1r/C1s Uef Bmp1) domains, followed by a C-terminal zona pellucida domain to cap the polypeptide chain. All gp340 domains have architectures that typically function in binding interactions, especially SRCR. Each SRCR domain, named for the order in which it appears beginning with SRCR1, is 100-110 residues, and there is a short flexible intervening segment between each, except the fourth and fifth domains (Purushotham and Deivanayagam, 2013). These connector segments are referred to as SIDs, and this is where the covalent attachment of O-glycans, which contribute to bacterial binding, occurs (Purushotham and Deivanayagam, 2014). The SRCR domains in gp340 interact with microorganisms and can either facilitate clearance or mediate infection (Stoddard *et al.*, 2007). The first repeat domain (SRCR1) binds to the capsid proteins of HIV, whereas SRCR2 aggregates bacteria (Chu *et al.*, 2013). These interactions are specific, but individual domains can interact with multiple partners (Loimaranta *et al.*, 2005). The sequence motif VEVLXXXXW within the SRCR domains was identified as a critical feature involved in aggregation of bacteria (Bikker *et al.*, 2004). Importantly, calcium binding to the SRCR domains of gp340 was shown to greatly enhance binding to AgI/II (Purushotham and Deivanayagam, 2014).

No high-resolution structure information is available for any of the domains of gp340, but a crystal structure of the SRCR fold was solved for a homologous protein domain derived from a macrophage receptor called MARCO (Ojala *et al.*, 2007), which also can aggregate bacteria. This structure shows a clear patch of negative charge on the surface, for which calcium ion binding was observed. A region of positive charge is also apparent, and this feature was shown to participate in binding to the ligands found on pathogens. Coordination of calcium to the acidic residues appears to enhance aggregation by promoting ligand binding in a cooperative manner. The SRCR domain from MARCO is not embedded within a string of like domains, as is the case for gp340, and it is interesting that this protein had to form a trimer for ligand binding to occur. This parallels the increased aggregation observed for the first 3 SRCR domains of gp340 over SRCR1 (Purushotham and Deivanayagam, 2014). Structure comparison suggests that similar electrostatic features exist on gp340 SRCR domains, which likely accomplish calcium binding (Figures 2, 3). This interaction is particularly interesting because calcium is required under flow conditions for high-affinity binding between gp340 and AgI/II (Hajishengallis *et al.*, 1994). These findings indicate that calcium plays an important role in modulating adhesion and aggregation *via* SRCR domains.

A high-resolution crystal structure of a portion of the AgI/II protein from *S. mutans* (A_3VP_1) was solved independently (Larson *et al.*, 2010). The bacterial antigen has 3 regions (P, V, and A; named for the proline-rich, variable, and alanine-rich sequences, respectively), which arrange to form a fibrillar structure (Figure 4) based on a unique interaction between α-helices in the A region and polyproline type II helices in the P region. Here, only 1 P and A unit each are present, but 3 repeats exist in the full-length protein, which also has a C-terminal domain that anchors AgI/II to the bacterial cell wall. The P and A regions associate to form a long stalk that positions the intervening globular V domain at the far end, approximately 50 nm away from the bacterial surface. A stable interaction is achieved through 2 chemical means: van der Waals contact between the side chains of proline (within PXXP motifs from the P region) and tyrosine (from the A region) and also a repeating pattern of

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Figure 2. Sequence alignment of scavenger receptor cysteine-rich (SRCR) domains from gp340 and MARCO performed with ClustalW. Secondary structure elements shown below the sequence reflect the crystal structure of MARCO (PDB: oy1a), and those above were predicted *via* Protein Predict for SRCR1. Beta strands are shown as cyan arrows and alpha helices as orange cylinders. The acidic residues involved in calcium binding are shown in red. The region with low similarity is shown in gray. The sequence identified by Bikker *et al.* (2004) involved in aggregation is underlined.

hydrogen bonds between the backbone of the P region residues and the side chain moieties from asparagine residues in the helical A region. Binding to SAG was also evaluated in this study, and 2 distinct regions of the bacterial antigen were shown to participate. SAG is a complex that contains gp340, sIgA, and a yet unidentified 80-kDa protein. The isolated V domain bound to SAG but had reduced affinity compared to the larger A_3VP_1 fragment, which was comparable to full-length AgI/II. Despite being located at the opposite end of the fibrillar structure, the C-terminal domain also bound to SAG. Based on these combined data sets, a model of the direct interaction between AgI/II and SRCR was proposed in which both ends of the 50-nm long filbrillar structure interact with SAG to accomplish aggregation (Figure 4). Some conformational flexibility was observed in the AgI/II bacterial antigen protein (Larson *et al.*, 2010), and as such, it is possible that the structure folds up on itself to bind gp340 at a single high-affinity site. It also is highly feasible that different domains of the long gp340 protein (and/or other components of the SAG complex) bind selectively to each of these 2 distinct regions and tether the bacteria to assemble the larger aggregate.

The recent availability of this high-resolution structure information is already enabling better understanding of how native components may facilitate pathogenic outcomes, and it has provided a basis for further hypothesis development. Careful structural analysis of the AgI/II structure shows parallels to the collagen triple helix and suggests that the similarities may support direct binding interactions with the collagen matrix in the dentin (Larson *et al.*, 2010). Erosion of the protective enamel and demineralization of the underlying dentin adjacent to a restoration exposes the collagenous substrate and permits infiltration of proteins and microbes. This creates opportunity for microbial attachment via the A and P regions of AgI/II on *S. mutans* to the softer tooth structure. Once this type of extended interaction occurs, competition for binding by SAG would become less effective and would inhibit the ability of SAG to clear pathogenic bacteria. This activity leads to a negative spiral of events that degrades the composite restoration and culminates in secondary caries and pulp pathology (Beyth *et al.*, 2008;

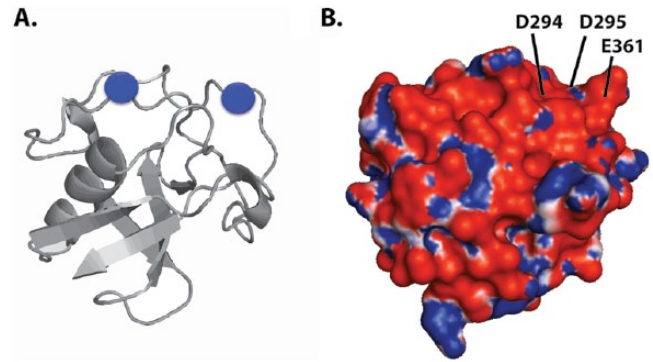

Figure 3. Homology model of SRCR3 domain from gp340 based on the crystal structure of the scavenger receptor cysteine-rich (SRCR) domain from MARCO (PDB: o1y3). (**A**) Ribbon diagram of the backbone fold is shown in gray with the 2 Ca^{2+} ions (blue circles) positioned in the same acidic regions as in MARCO. (**B**) Electrostatic surface map of gp340 SRCR domain (same orientation), in which red coloration reflects acidic and blue indicates basic regions. The acidic residues corresponding to the analogous positions in MARCO involved in calcium binding are labeled.

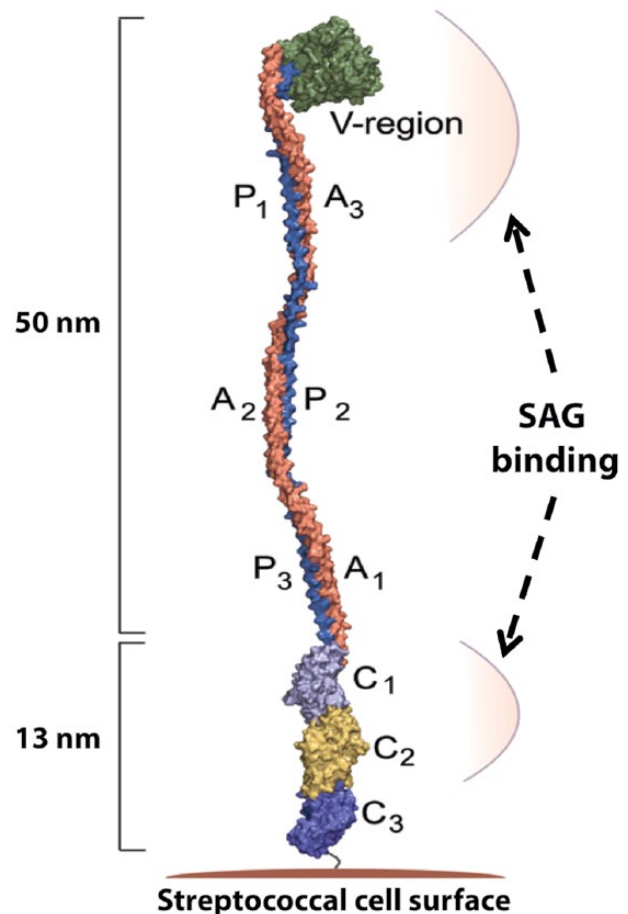

Figure 4. Space-filling model of the structure of the A_3VP_1 fragment from the AgI/II protein. The V- and C-terminal regions of AgI/II involved in attachment of *Streptococcus mutans* to salivary agglutinin (SAG) are indicated by arrows. Figure is provided by C. Deivanayagam at University of Alabama at Birmingham.

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Busscher *et al.*, 2010). This negative spiral of events is apparent at the gingival margin of the composite restoration (Spencer *et al.*, 2010).

There are frequently gaps between the tooth and composite at the gingival margin of the composite restoration (Spencer *et al.*, 2010). Oral fluids, salivary enzymes, and bacteria infiltrate the marginal gaps and crevices between the tooth and composite. Once *S. mutans* is bound to the collagenous dentin matrix, the metabolic production of acid would accelerate demineralization because of close proximity and persistence of a low pH environment (Delaviz *et al.*, 2014). Because the substrate is in a crevice that is more protected from fluid flow, it is also largely isolated from saliva and the neutralization and pH equilibration provided by saliva (Marsh, 2003; Beyth *et al.*, 2008). In light of these pathogenic mechanisms, effective approaches to increasing longevity of composite restorations would be to prevent gp340-based attachment of microbes, particularly *S. mutans*, and to slow acid erosion of the adjacent mineralized native tooth structure by introducing a buffering agent into the adhesive (Laurence *et al.*, 2013).

SUMMARY

Acidification of the oral microenvironment caused by metabolic activity of the microbiota promotes demineralization of tooth structure at the margin of composite restorations. Demineralization creates additional opportunity for adhesion by the biofilm—for example, salivary protein gp340 and the pioneer pathogenic bacterium *S. mutans*—thereby accelerating the degradation process of the composite restoration.

With a failure rate nearly double that of amalgam (Ferracane, 2013), the increasing trend to replace amalgam with composite could be detrimental for patients. Removal of these restorations leads to loss of sound tooth structure with concomitant weakening of the tooth and possible pulpal injury (Hunter *et al.*, 1995). Over the lifetime of the patient, the repeated loss of sound tooth structure will translate to the need for more complex restorations and, eventually, total tooth loss. The higher failure rate and need for frequent replacement could translate to a significant reduction in the quality of life for the nearly 32% of the U.S. population with natural dentition who do not receive regular dental treatment (Health US, 2007). Similarly, the higher total colony-forming units of *S. mutans* at the margins of composite as compared to comparable amalgam restorations could translate to an increase in untreated dental disease and a significant reduction in the quality of life for the 4 million U.S. children who do not receive regular dental care (Palmer, 2013).

It is unlikely that dental plaque biofilm can be eliminated. It may, however, be possible to reduce the pathogenic impact of the biofilm by engineering novel anticariogenic dentin adhesives or by engineering resin matrices that shift the microbial ecology at the composite-tooth interface from a disease-associated state to a "healthy" state.

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