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Effect of salivary pellicle on antibacterial activity of novel antibacterial dental adhesives using a dental plaque microcosm biofilm model

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ABSTRACT

Objectives. Antibacterial primer and adhesive are promising to inhibit biofilms and caries. Since restorations in vivo are exposed to saliva, one concern is the attenuation of antibacterial activity due to salivary pellicles. The objective of this study was to investigate the effects of salivary pellicles on bonding agents containing a new monomer dimethylaminododecyl methacrylate (DMADDM) or nanoparticles of silver (NAg) against biofilms for the first time.
Methods. DMADDM and NAg were synthesized and incorporated into Scotchbond Multi-Purpose adhesive and primer. Specimens were either coated or not coated with salivary pellicles. A microcosm biofilm model was used with mixed saliva from ten donors. Two types of culture medium were used: an artificial saliva medium (McBain), and Brain Heart Infusion (BHI) medium without salivary proteins. Metabolic activity, colony-forming units (CFU), and lactic acid production of plaque microcosm biofilms were measured ($n = 6$).
Results. Bonding agents containing DMADDM and NAg greatly inhibited biofilm activities, even with salivary pellicles. When using BHI, the pre-coating of salivary pellicles on resin surfaces significantly decreased the antibacterial effect ($p < 0.05$). When using artificial saliva medium, pre-coating of salivary pellicles on resin did not decrease the antibacterial effect. These results suggest that artificial saliva yielded medium-derived pellicles on resin surfaces, which provided attenuating effects on biofilms similar to salivary pellicles. Compared with the commercial control, the DMADDM-containing bonding agent reduced biofilm CFU by about two orders of magnitude.
Significance. Novel DMADDM- and NAg-containing bonding agents substantially reduced biofilm growth even with salivary pellicle coating on surfaces, indicating a promising usage in saliva-rich environment. DMADDM and NAg may be useful in a wide range of primers, adhesives and other restoratives to achieve antibacterial and anti-caries capabilities.

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1. Introduction

Composites are popular dental filling materials because of their esthetics and improved handling and load-bearing properties [1–6]. After bonded into a tooth cavity with an adhesive, the composite restoration is expected to perform oral functions durably [7–10]. However, nearly half of all restorations fail within 10 years, and replacing them accounts for 50–70% of all restorative dentistry [11,12]. One main problem is that composites tend to accumulate more biofilms than other restorative materials in vivo [13,14]. Furthermore, gap formation can be observed between the adhesive and the primed dentin, or between the adhesive and the hybrid layer [15,16]. Hence, microleakage can occur and biofilms at the restoration margins can penetrate into the bonded interface, producing acids and causing secondary caries, which is the main reason for restoration failure [17,18]. Therefore, antibacterial composites and adhesives are needed to combat biofilms and caries.

Novel polymers containing quaternary ammonium salts (QAS) were developed [19–25]. Monomers such as 12-methacryloyloxydodecylpyridinium bromide (MDPB) and other antibacterial monomers could copolymerize with dental resins to form antibacterial polymer matrices that can effectively reduce bacteria growth [19,20,23,25–28]. Adhesives bond the composite restoration to the tooth structure [29–35]. Hence antibacterial adhesives containing MDPB were developed to inhibit bacteria at the tooth-restoration margins [20,36]. In addition, a methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB) adhesive also inhibited biofilm growth [37]. These polymerizable cationic monomers covalently bonded within the polymer matrix and killed bacteria upon contact. In addition to QAS, silver particles were also used to provide antibacterial activity to resinous materials [38,39]. Nanoparticles of silver (NAg) were well dispersed in the resinous matrix to exert antibacterial activity for adhesives and composites [24,40,41].

The human oral cavity supports a diverse microbial consortium comprising of hundreds of bacterial species [42,43]. Saliva in the oral cavity can be absorbed onto dental restoration surfaces to provide anchor points for bacteria [44] and may block certain functional groups of material surfaces. Several publications suggested that proteins adsorbed from physiological fluids, such as saliva-derived protein films, are able to attenuate the antibacterial properties of the underlying surfaces significantly [45–47].

In our previous studies, antibacterial resins containing a quaternary ammonium dimethacrylate (QADM) were developed [24,28,40]. More recently, a new quaternary ammonium monomer, dimethylaminododecyl methacrylate (DMADDM), was synthesized, which showed much stronger antibacterial activity than the previously-used QADM in adhesives [48]. However, the effects of salivary pellicle covering the resin containing DMADDM on its antibacterial potency have not been reported.

Therefore, the objectives of this study were to develop antibacterial adhesive and primer containing DMADDM and NAg, and to investigate their effects on microcosm biofilm properties with or without human salivary pellicle coverage. Microcosm biofilms were inoculated with mixed saliva from ten human donors. Human saliva without bacteria provided the salivary pellicles. Two types of culture medium were tested: (1) BHI medium which contained no salivary proteins, to compare human salivary pellicle-covered resin surfaces with non-pellicle surfaces; and (2) McBain medium which contained proteins to mimic saliva, to compare human salivary pellicle-covered resin surfaces with medium-derived pellicle surfaces. The following hypotheses were investigated: (1) human salivary pellicle coating will significantly reduce the antibacterial efficacy of DMADDM and NAg containing adhesive with biofilms cultured in BHI medium; (2) human salivary pellicle coating will not decrease the antibacterial efficacy of DMADDM and NAg adhesive with biofilms cultured in McBain medium; (3) adhesives containing DMADDM and NAg will be strongly antibacterial even when the resin surface was covered with human salivary pellicles.

2. Materials and methods

2.1. *Antibacterial adhesive system containing DMADDM*

Scotchbond Multi-Purpose bonding system (3 M, St. Paul, MN) was used as the parent bonding system and referred as "SBMP". According to the manufacturer, SBMP etchant contained 37% phosphoric acid. SBMP primer single bottle contained 35–45% 2-hydroxyethylmethacrylate (HEMA), 10–20% copolymer of acrylic and itaconic acids, and 40–50% water. SBMP adhesive contained 60–70% BisGMA and 30–40% HEMA.

DMADDM was a quaternary ammonium methacrylate, and was recently synthesized and incorporated into composites [48]. The synthesis used a modified Menschutkin reaction, where a tertiary amine group was reacted with an organohalide [23,24]. A benefit of this reaction is that the products are generated at virtually quantitative amounts and require minimal purification. Ten mmol of 1-(dimethylamino)docecane (Sigma, St. Louis, MO) and 10 mM of 2-bromoethyl methacrylate (BEMA, Monomer-Polymer and Dajec Labs, Trevose, PA) were combined with 3 g of ethanol in a 20 mL scintillation vial. The vial was stirred at 70 °C for 24 h. The solvent was then removed via evaporation, yielding DMADDM as a clear, colorless, and viscous liquid. DMADDM was mixed with SBMP primer at a DMADDM/(SBMP primer + DMADDM) mass fraction of 5%, following previous studies [36,48]. The same 5% mass fraction of DMADDM was incorporated into SBMP adhesive.

2.2. *Antibacterial adhesive system containing NAg*

Silver 2-ethylhexanoate powder (Strem, New Buryport, MA) was dissolved in 2-(tert-butylamino)ethyl methacrylate (TBAEMA, Sigma) at 0.08 g of silver salt per 1 g of TBAEMA [39]. TBAEMA was used because it improves the solubility by forming Ag—N coordination bonds with Ag ions, thereby facilitating the Ag salt to dissolve in the resin solution. TBAEMA contains reactive methacrylate groups and therefore can be chemically incorporated into a dental resin upon photopolymerization [39,40]. This method produced NAg with a mean silver particle