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Dental plaque microcosm response to bonding agents containing quaternary ammonium methacrylates with different chain lengths and charge densities

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ABSTRACT

Objectives: Antibacterial bonding agents are promising to combat bacteria and caries at tooth-restoration margins. The objectives of this study were to incorporate new quaternary ammonium methacrylates (QAMs) to bonding agent and determine the effects of alkyl chain length (CL) and quaternary amine charge density on dental plaque microcosm bacteria response for the first time.

Methods: Six QAMs were synthesized with CL = 3, 6, 9, 12, 16, 18. Each QAM was incorporated into Scotchbond multi-purpose (SBMP). To determine the charge density effect, dimethylaminododecyl methacrylate (DMAHDM, CL = 16) was mixed into SBMP at mass fraction = 0%, 2.5%, 5%, 7.5%, 10%. Charge density was measured using a fluorescein dye method. Dental plaque microcosm using saliva from ten donors was tested. Bacteria were inoculated on resins. Early-attachment was tested at 4 h. Biofilm colony-forming units (CFU) were measured at 2 days.

Results: Incorporating QAMs into SBMP reduced bacteria early-attachment. Microcosm biofilm CFU for CL = 16 was 4 log lower than SBMP control. Charge density of bonding agent increased with DMAHDM content. Bacteria early-attachment decreased with increasing charge density. Biofilm CFU at 10% DMAHDM was reduced by 4 log. The killing effect was similarly-strong against total microorganisms, total streptococci, and mutans streptococci.

Conclusions: Increasing alkyl chain length and charge density of bonding agent was shown for the first time to decrease microcosm bacteria attachment and reduce biofilm CFU by 4 orders of magnitude. Novel antibacterial resins with tailored chain length and charge density are promising for wide applications in bonding, cements, sealants and composites to inhibit biofilms and caries.

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1. Introduction

Approximately 200 million dental restorations are performed in the United States each year.[1] Composites are increasingly popular due to their excellent aesthetics and direct-filling ability.[2–6] Extensive studies have resulted in substantial improvements in composite fillers, polymer compositions, and handling and polymerization properties.[2–6] Dental caries is a dietary carbohydrate-modified bacterial infectious disease, which is one of the most common bacterial infections in humans.[7] Tooth demineralization is caused by acid generated by bacterial biofilms (dental plaque) in the presence of fermentable carbohydrates.[8] Previous studies showed that composites in vivo had more biofilms and plaques than other restorative materials.[9,10] These plaques could lead to secondary caries at the tooth-restoration margins, which has been suggested as a primary reason for restoration failure.[6,11] The replacement of failed restorations accounts for more than half of all the restorations performed.[12]

Efforts were made to synthesize quaternary ammonium methacrylates (QAMs) for use in antibacterial dental resins.[13–17] Through co-polymerization, 12-methacryloyloxydodecylpyridinium bromide (MDPB) was covalently bonded in the resin matrix, thus becoming immobilized, achieving durable contact-killing capability against oral bacteria.[18,19] Other antibacterial formulations were also prepared, including a methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB)-containing adhesive,[20] antibacterial glass ionomer cements,[21] and antibacterial nanocomposites and bonding agents using a quaternary ammonium dimethacrylate (QADM).[22–24] Bonding agents are important in adhering the restorations to tooth structures.[25,26] Bonding methods and procedures have been improved and the tooth-restoration bond strength has been enhanced.[27–31] Antibacterial bonding agents are considered to be beneficial to reduce caries at the tooth-restoration margins.[18–20] They can kill the residual bacteria in the prepared tooth cavity, and inhibit new bacteria at the tooth-restoration interface due to marginal leakage.[13,18,19] For these reasons, efforts have been devoted to developing antibacterial primers and adhesive containing QAMs.[13,18–20,23,24,32,33]

Quaternary ammonium salts (QAS) can cause bacteria lysis by binding to cell membrane to cause cytoplasmic leakage.[34,35] When the negatively charged bacteria contact the positive quaternary amine charge (N^+), the electric balance is disturbed and the bacterium could explode under its own osmotic pressure.[34,35] Long cationic polymers can penetrate bacterial cells to disrupt membranes, like a needle bursting a balloon.[36,37] Indeed, previous studies revealed that the antibacterial potency of quaternary ammonium compounds increased with an increase in the alkyl chain length (CL) for the ammonium groups.[21,38–40] One study showed that the antibacterial activity of quaternary ammonium monomers which were synthesized from dimethylaminoethyl methacrylate (DMAEMA) against *Escherichia coli* and *Staphylococcus aureus* increased with the number of carbon atoms in the alkyl chain.[38] Another study reported that increasing the CL increased the biocidal activity of poly(quaternary ammonium salt) (PQAS)-containing glass-ionomer cement against *Streptococcus mutans*.[21] Another investigation found that the antibacterial potency of quaternary ammonium thiol derivatives increased with CL, reached a maximal efficacy and then decreased with further increasing the CL.[39] This is consistent with a separate study showing that the antibacterial activity of methacrylate monomers containing quaternary ammonium salt increased when CL was increased from 5 to 16, and then decreased when CL was further increased to 18.[40] Therefore, CL and quaternary amine charge density of QAMs are two important factors. However, to date, there has been no report on the effects of CL and charge density of bonding agents on dental plaque microcosm bacterial behaviour.

In the present study, a series of new QAMs with CL varying from 3 to 18 were synthesized and incorporated into bonding agent, and the antibacterial properties were measured using a dental plaque microcosm model with human saliva as inoculum. The objectives of this study were to investigate for the first time: (1) the CL effect on microcosm early-attachment to bonding agent resin as well as biofilm colony-forming units (CFU); (2) the charge density effect on microcosm early-attachment and biofilm CFU, by varying QAM mass fraction in the bonding agent. It was hypothesized that: (1) Microcosm attachment and biofilm CFU will be inversely proportional to the CL of QAM in bonding agent; (2) bacteria attachment and biofilm CFU will be inversely proportional to bonding agent charge density; (3) dental plaque microcosm biofilm CFU can be reduced by several orders of magnitude via the new antibacterial bonding agent.

2. Materials and methods

2.1. *Antibacterial bonding agents with QAMs of different CL*

The synthesis of QAMs was recently described, which employed a Menschutkin reaction via the addition reaction of tertiary amines with organo-halides.[15,22] The 2-(dimethylamino) ethyl methacrylate (DMAEMA, Sigma–Aldrich, St. Louis MO) was the methacrylate-containing tertiary amine. The following is an example to make dimethylaminododecyl methacrylate (DMADDM) with CL of 12. Ten mmol of DMAEMA, 10 mmol of 1-bromododecane (BDD) (TCI America, Portland, OR), and 3 g of ethanol as solvent were added to a vial. The vial was capped and stirred at 70 °C for 24 h for the reaction to proceed.[41] After the reaction was completed the ethanol solvent was removed via evaporation. This process yielded DMADDM as a clear viscous liquid, and the reaction product was verified in a pilot study via Fourier transform infrared spectroscopy.[41] The same method was used to synthesize six QAMs with a series of CL. Namely, DMAEMA was reacted with 1-bromopropane (BP) to form dimethylaminopropyl methacrylate (DMAPM, CL = 3). DMAEMA was reacted with 1-bromohexane (BH) to form dimethylaminohexyl methacrylate (DMAHM, CL = 6). DMAEMA was reacted with 1-bromononane (BN) to form dimethylaminononyl methacrylate (DMANM, CL = 9). DMAEMA was reacted with 1-bromododecane (BDD) to form dimethylaminododecyl methacrylate (DMADDM, CL = 12). DMAEMA was reacted with 1-bromohexadecane (BHD) to form dimethylaminohexadecyl