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Dental primer and adhesive containing a new antibacterial quaternary ammonium monomer dimethylaminododecyl methacrylate

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ABSTRACT

Objectives: The main reason for restoration failure is secondary caries caused by biofilm acids. Replacing the failed restorations accounts for 50–70% of all operative work. The objectives of this study were to incorporate a new quaternary ammonium monomer (dimethylaminododecyl methacrylate, DMADDM) and nanoparticles of silver (NAg) into a primer and an adhesive, and to investigate their effects on antibacterial and dentin bonding properties.

Methods: Scotchbond Multi-Purpose (SBMP) served as control. DMADDM was synthesized and incorporated with NAg into primer/adhesive. A dental plaque microcosm biofilm model with human saliva was used to investigate metabolic activity, colony-forming units (CFU), and lactic acid. Dentin shear bond strengths were measured.

Results: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the new DMADDM were orders of magnitude lower than those of a previous quaternary ammonium dimethacrylate (QADM). Uncured primer with DMADDM had much larger inhibition zones than QADM ($p < 0.05$). Cured primer/adhesive with DMADDM-NAg greatly reduced biofilm metabolic activity ($p < 0.05$). Combining DMADDM with NAg in primer/adhesive resulted in less CFU than DMADDM alone ($p < 0.05$). Lactic acid production by biofilms was reduced by 20-fold via DMADDM-NAg, compared to control. Incorporation of DMADDM and NAg into primer/adhesive did not adversely affect dentin bond strength.

Conclusions: A new antibacterial monomer DMADDM was synthesized and incorporated into primer/adhesive for the first time. The bonding agents are promising to combat residual bacteria in tooth cavity and invading bacteria at tooth-restoration margins to inhibit caries. DMADDM and NAg are promising for use into a wide range of dental adhesive systems and restoratives.

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1. Introduction

Half of all dental restorations fail within 10 years, mainly due to secondary caries and fracture.[1–5] Replacing the failed restorations accounts for 50–70% of all restorations performed.[6–8] This is costly, considering that the annual cost for tooth cavity restorations in the U.S. was $46 billion in 2005.[9] Furthermore, the need is rapidly increasing with the baby boomers entering into retirement, and with increases in life expectancy and in tooth retention in seniors.[10] Composites are the principal material for cavity restorations.[4,5,11,12] Advances in polymers and fillers have significantly enhanced the composite properties.[13–18] However, one major drawback is that composites tend to accumulate more biofilms/plaques in vivo than other restorative materials.[19,20] Biofilms with the exposure to fermentable carbohydrates are responsible for tooth caries.[21,22] Hence, efforts were made to develop antibacterial resins, and novel quaternary ammonium methacrylates (QAMs) were synthesized.[23–30] Previous studies made 12-methacryloyloxydodecyl-pyridinium bromide (MDPB) and other antibacterial monomers to hinder bacteria and biofilm growth.[23–30]

Besides composites, it is also important to develop antibacterial adhesives, because composite restorations are bonded to the tooth structure via adhesives.[31–35] Efforts have been made in previous studies to increase the dentin bond strength and determine the mechanisms of tooth-restoration adhesion.[36–40] It is beneficial for the adhesive to be antibacterial to reduce biofilm acids and caries at the margins.[23,25,41] Besides residual bacteria in the prepared tooth cavity, marginal leakage would allow bacteria to invade the tooth-restoration interface. Therefore, antibacterial adhesives are being developed to help inhibit the residual as well as the invading bacteria.[42,43] Previous studies showed that MDPB-containing adhesives inhibited *Streptococcus mutans* (*S. mutans*) growth.[24,44] Another study developed an antibacterial adhesive containing methacryloxyl ethyl cetyl dimethyl ammonium chloride (DMAE-CB).[25] Besides the adhesive, it is also useful for the primer to be antibacterial, because the primer directly contacts the tooth structure. A primer containing MDPB was reported to possess strong antibacterial functions.[44,45] In another study, chlorhexidine particles were mixed into a primer to obtain antibacterial properties.[46] Recently, a quaternary ammonium dimethacrylate (QADM) was synthesized and incorporated into a nanocomposite and a primer.[28,47,48] In our preliminary study, a new quaternary ammonium monomer, dimethylaminododecyl methacrylate (DMADDM), was synthesized. DMADDM was much more strongly antibacterial than QADM as shown in preliminary results. However, DMADDM has not been tested in primer and adhesive.

The objectives of this study were to incorporate the new DMADDM into primer and adhesive, and to investigate the effects on antibacterial and dentin bonding properties for the first time. It was hypothesized that: (1) the new DMADDM will possess much stronger antibacterial potency than the previously-synthesized QADM in minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests; (2) primer and adhesive containing DMADDM will inhibit microcosm biofilm growth, metabolic activity, and lactic acid production; (3) combining DMADDM with nanoparticles of silver (NAg) in primer and adhesive will further increase the anti-biofilm potency, without compromising dentin bond strength, compared to commercial non-antibacterial primer and adhesive control.

2. Materials and methods

2.1. Developing new antibacterial monomers

Two new antibacterial monomers were synthesized: dimethylaminohexyl methacrylate (DMAHM) with an alkyl chain length of 6, and dimethylaminododecyl methacrylate (DMADDM) with an alkyl chain length of 12. A modified Menschutkin reaction method was employed, which used a tertiary amine group to react with an organo-halide, following previous studies.[28,47,48] To synthesize DMAHM, 2-bromoethyl methacrylate (BEMA) served as the organo halide, and N,N-dimethylaminohexane (DMAH) served as the tertiary amine. Ten mmol of DMAH (Tokyo Chemical Industry, Tokyo, Japan), 10 mmol of BEMA (Monomer-Polymer and Dajac Labs, Trevose, PA), and 3 g of ethanol were added to a 20 mL scintillation vial with a magnetic stir bar. The vial was capped and stirred at 70 °C for 24 h. After the reaction was complete, the ethanol solvent was removed via evaporation, yielding DMAHM as a clear, colorless, and viscous liquid. To synthesize the second new monomer DMADDM, BEMA was the organo halide, and 1-(dimethylamino)dodecane (DMAD) was the tertiary amine. Ten mmol of DMAD (Tokyo Chemical Industry) and 10 mmol of BEMA were added in a 20 mL vial, while otherwise following the same procedures as for DMAHM. Fourier transform infrared (FTIR) spectroscopy (Nicolet 6700, Thermo Scientific, Waltham, MA) spectra of the starting materials and the products were collected between two KBr windows in the 4000–400 cm^{-1} region. ^1H NMR spectra (GSX 270, JEOL) were taken in deuterated chloroform at a concentration of about 3%.[28] The reactions and products of DMAHM and DMADDM were all verified in preliminary studies.

2.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The use of *S. mutans* (ATCC 700610, American Type Culture, Manassas, VA) was approved by the University of Maryland. *S. mutans* is a cariogenic, aerotolerant anaerobic bacterium and the primary causative agent of dental caries.[22] MIC and MBC were determined via serial microdilution assays.[45,49] Unpolymerized DMAHM or DMADDM monomer was dissolved in brain heart infusion (BHI) broth (BD, Franklin Lakes, NJ) to a concentration of 200 mg/mL. From these starting solutions, serial two fold dilutions were made into 1 mL volumes of BHI broth. Fifteen μL of stock *S. mutans* was added to 15 mL of BHI broth with 0.2% sucrose and incubated at 37 °C with 5% CO_2. Overnight cultures of *S. mutans* were adjusted to 2×10^6 CFU/mL with BHI, and 50 μL of inoculum was added to each well of a 96-well plate containing 50 μL of a series of antibacterial monomer dilution broths. BHI with 1×10^6 CFU/mL bacteria suspension without antibacterial agent served as negative