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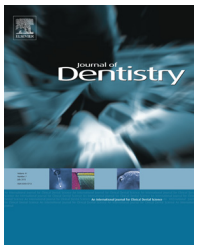

Time-kill behaviour against eight bacterial species and cytotoxicity of antibacterial monomers

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ABSTRACT

Objectives: The objectives of this study were to investigate: (1) the antibacterial activity of two antibacterial monomers, dimethylaminododecyl methacrylate (DMADDM) and dimethylammoniumethyl dimethacrylate (DMAEDM), against eight different species of oral pathogens for the first time; (2) the cytotoxicity of DMAEDM and DMADDM.

Methods: DMAEDM and DMADDM were synthesized by reacting a tertiary amine group with an organo-halide. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against eight species of bacteria were tested. Time-kill determinations were performed to examine the bactericidal kinetics. Cytotoxicity of monomers on human gingival fibroblasts (HGF) was assessed using a methyl thiazolyltetrazolium assay and live/dead viability assay.

Results: DMADDM showed strong bactericidal activity against all bacteria, with MIC of 1.2–9.8 μg/mL. DMAEDM had MIC of 20–80 mg/mL. Time-kill determinations indicated that DMADDM and DMAEDM had rapid killing effects against eight species of bacteria, and eliminated all bacteria in 30 min at the concentration of 4-fold MBC. Median lethal concentration for DMADDM and DMAEDM was between 20 and 40 μg/mL, which was 20-fold higher than 1–2 μg/mL for BisGMA control.

Conclusions: DMAEDM and DMADDM were tested in time-kill assay against eight species of oral bacteria for the first time. Both were effective in bacteria-inhibition, but DMADDM had a higher potency than DMAEDM. Different killing efficacy was found against different bacteria species. DMAEDM and DMADDM had much lower cytotoxicity than BisGMA. Therefore, DMADDM and DMAEDM are promising for use in bonding agents and other restorative/preventive materials to combat a variety of oral pathogens.

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1. Introduction

Composites are popular dental filling materials because of their aesthetics and improved handling and load-bearing properties.[1–3] After being bonded to dental tissue with adhesives,[4] it is desirable for the restorations to function in the oral cavities durably. However, nearly half of all dental restorations fail within 10 years, and replacing them accounts for 50–70% of all restorations performed.[5,6] One main problem

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is that resin composites tend to accumulate more biofilms and plaques than other restorative materials in vivo.[7,8] In addition, microgap formation can be observed between the adhesive resin and the primed dentine, or between the adhesive resin and the hybrid layer.[9,10] Biofilms at the restoration margins could penetrate into the bonded interface to produce acids and cause secondary caries, which was considered as one of the primary reasons for restoration failure.[11,12]

Therefore, efforts have been made to develop antibacterial dental composites and adhesive systems.[13–18] Novel polymers containing quaternary ammonium methacrylates (QAMs) were developed.[14–21] Monomers such as 12-methacryloyloxydodecylpyridinium bromide (MDPB) could copolymerize with other dental monomers to form antibacterial polymer matrices that can effectively reduce bacteria growth.[14,19] Previous studies showed that adhesives containing MDPB substantially reduced the growth of *Streptococcus mutans*.[19,22] An adhesive with methacryloxylethylcetyl dimethyl ammonium chloride (DMAE-CB) also reduced biofilm growth.[15] These polymerizable cationic monomers were covalently bonded within the polymer matrix and could kill bacteria upon contact without releasing compounds that might be toxic to mammalian cells. This was supported by the fact that the antibacterial capability of the resins was long-lasting.[14–16,19,23]

Recently, two QAMs were synthesized: dimethylaminododecyl methacrylate (DMADDM), and bis(2-methacryloyloxyethyl) dimethylammonium bromide (a quaternary ammonium dimethacrylate termed "QADM").[24–28] QADM is dimethylammoniumethyl dimethacrylate, which is referred to as DMAEDM in this article, to follow the same abbreviation pattern as DMADDM based on the chemical structure name of the compound. Primers and adhesives containing DMAEDM inhibited a dental plaque microcosm biofilm growth and lactic acid production.[24,25] DMAEDM-containing resins suppressed the glucosyltransferases (*gtf*) gene expressions of *S. mutans*, which were important for the synthesis of extracellular glucans and for bacterial cell adhesion and biofilm formation.[26] DMADDM exhibited a stronger antibacterial efficacy than DMAEDM.[27] A bonding agent containing DMADDM showed no decrease in antibacterial activity after 6 months of water-ageing, while the dentine bond strength after 6 months was higher for DMADDM-containing bonding agent than a commercial control.[28] However, the antibacterial activity of DMAEDM and DMADDM against different species of oral bacteria and the cytotoxicity of DMAEDM and DMADDM remain to be investigated.

Accordingly, the objectives of this study were to investigate: (1) the antibacterial activity of DMAEDM and DMADDM against eight different species of oral pathogens; (2) cytotoxicity of DMAEDM and DMADDM. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured. Time-kill behaviour was determined to examine the kinetics of DMADDM and DMAEDM against eight species of bacteria. Cytotoxicity was assessed using human gingival fibroblasts. It was hypothesized that: (1) DMADDM and DMAEDM have potent antibacterial functions against all eight species of bacteria; (2) there are significant differences in the monomers' antibacterial efficacy against the different bacterial species; (3) both DMADDM and DMAEDM have minimal cytotoxicity towards human gingival fibroblasts.

2. Materials and methods

2.1. Synthesis of antibacterial quaternary ammonium methacrylates

The synthesis of DMAEDM and DMADDM were recently described.[20,24,27,28] Briefly, a modified Menschutkin reaction was employed, where a tertiary amine group was reacted with an organo-halide. To synthesize DMAEDM, 10 mmol of 2-(N,N-dimethylamino)ethyl methacrylate (DMAEMA, Sigma–Aldrich, St. Louis, MO) and 10 mmol 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 60 °C for 24 h to complete the reaction. Then the solvent was removed by evaporation, yielding DMAEDM as a clear, colourless, and viscous liquid.[20,24] To synthesize DMADDM, 10 mmol of 1-(dimethylamino)dodecane (DMAD) (Tokyo Chemical Industry, Tokyo, Japan) and 10 mmol of BEMA 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, yielding DMADDM as a clear, colourless, and viscous liquid.[27,28] The structures of DMAEDM and DMADDM are shown in Fig. 1.

2.2. Culture of eight different species of oral bacteria

The eight species of oral and perioral bacteria are listed in Table 1. *S. mutans* UA159, *Actinomyces viscosus* ATCC15987, *Streptococcus sanguinis* ATCC6715 and *Enterococcus faecalis* ATCC29212 (American Type Culture, Manassas, VA) were cultured in Brain Heart Infusion broth (BHI, Becton Dickinson, Sparks, MD). *Lactobacillus acidophilus* ATCC393 (American Type Culture) were cultured in Lactobacillus MRS broth (Research Product, Mount Prospect, IL). *Staphylococcus aureus* ATCC29213

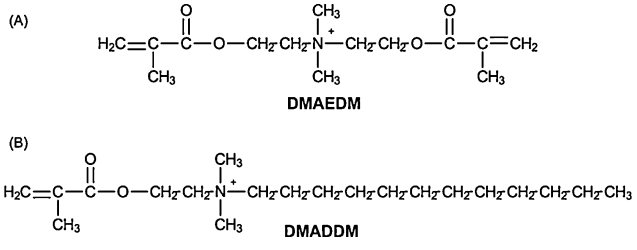

Fig. 1 – Chemical structures of the synthesized QAMs. (A) DMAEDM contains two methacrylate groups. It has a short alkyl chain length of 2. (B) DMADDM contains a single methacrylate group and a long alkyl chain with a chain length of 12.