Novel Dimethacrylates with Quaternary Ammonium Functionalities for Reduced Bacteria Adhesion

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Statement of Purpose: Biofilm growth and secondary caries have resulted in the failure of many dental composite restorations. One approach to address this issue is the modification of dental polymeric materials to reduce bacterial adhesion and subsequent biofilm growth. Quaternary ammonium salts, commonly used as disinfectants with anti-microbial properties,[1] have potential as anti-bacterial agents in the oral environment. Our objective was to synthesize and characterize an ionic dimethacrylate (IDMA) containing a quaternary ammonium group for reducing bacterial growth while retaining desirable physical properties, including miscibility with common dental monomers.

Methods: Bis(2-methacryloyloxy-ethyl) dimethyl-ammonium bromide (IDMA-1) was fabricated from 2-(N,N-dimethylamino)ethyl methacrylate (DMAEMA) and 2-bromoethyl methacrylate (BEMA) using the Menschutkin reaction. The resultant product (Fig. 1) was characterized via Fourier transform infrared spectroscopy (FTIR) and ^{1}H nuclear magnetic resonance (^{1}H NMR). IDMA-1 was then added to a 50:50 (by mass) mixture of bisphenol A glycerolate dimethacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA) at 10 %, 20 %, and 30 % IDMA-1 by mass. Controls contained no IDMA-1. Viscosity of activated resins and water contact angle of polymerized resins were quantified using rheology and goniometry, respectively. Initial attachment was assessed by inoculating copolymers with *Streptococcus mutans* UA159 for 4 h in phosphate buffered saline.[2] For biofilm studies, copolymers containing 0 % or 20 % IDMA-1 were cultured with *S. mutans* in brain heart infusion broth with 1 % (by mass) sucrose for 4 h. All samples were fixed, stained, and imaged using confocal microscopy.[2] To assess cytotoxicity, RAW 264.7 murine macrophages were cultured on the copolymers for 24 h. Cell density (microscopy), viability (live/dead staining), and enzymatic activity (tetrazolium reduction) were assessed. Images were analyzed using ImagePro Plus. One-way analysis of variance with post-hoc tests (95 % confidence) was used to identify statistical differences.

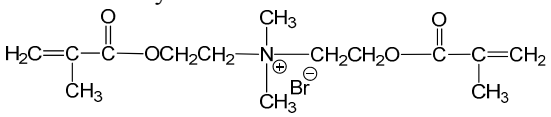

Figure 1. Chemical structure of IDMA-1.

Results: FTIR and ^{1}H NMR confirmed the successful synthesis of IDMA-1 in high yields. Unlike other methacrylates that contain pendant quaternary ammonium salts and have limited solubility in common dimethacrylate monomers, IDMA-1 was miscible with common dental monomers over a large composition range and had only minimal effects on resin viscosity and polymer hydrophobicity. *S. mutans* attachment on polymers was significantly reduced with as little as 10 % IDMA-1 (Fig. 2). Additional IDMA-1 had no further effect. Differences in *S. mutans* morphology were evident, with clustering present on 20 % and 30 % IDMA-1 copolymers. Initial biofilm studies revealed that polymers with 20 % IDMA-1 resulted in denser biofilm structures relative to looser biofilms on control polymers. Microcolony formation on 20 % and 30 % IDMA-1 copolymers (Fig. 2) and denser biofilms due to 20 % IDMA-1 may indicate an unfavorable environment for *S. mutans* as IDMA-1 concentration increases. Effects on mammalian cells were evident, with ≥ 10 % IDMA-1 significantly reducing macrophage density. However, cell viability and enzymatic activity were only reduced for with ≥ 20 % IDMA-1.

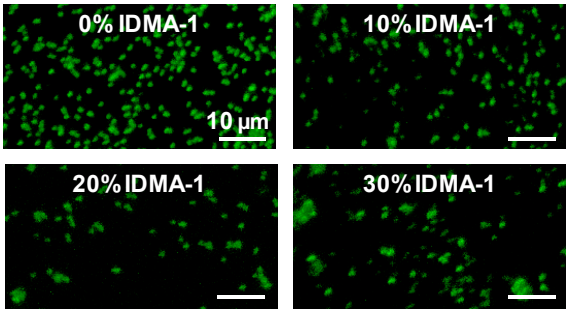

Figure 2. Initial attachment of *S. mutans* (SYTOX green stain).

Conclusions: Using the Menschutkin reaction, a low viscosity, highly miscible dimethacrylate containing a quaternary ammonium group was easily synthesized. Incorporation of IDMA-1 into common dimethacrylate polymers reduced initial bacterial adhesion and affected early biofilm structure. However, ≥ 20 % IDMA-1 (by mass) was cytotoxic to mammalian cells. Therefore, ≤ 10% IDMA-1 is recommended for cell-contacting applications to minimize toxicity to mammalian cells while still reducing initial bacterial growth. Additional studies are needed to further investigate the nature of bacterial growth on IDMA-1 copolymers, including viability of microcolonies and biofilms as well as differences in biofilm growth and structure.

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References:

[1] Worley SD, Sun G. Trends Polymer Sci. 2009;4:364.
[2] Zeiger DN *et al*. Langmuir. 2009; PMID:19839634.