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Protein-repellent and antibacterial dental composite to inhibit biofilms and caries

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ABSTRACT

Objectives: Biofilm acids contribute to secondary caries, which is a main reason for dental restoration failures. The objectives of this study were to: (1) develop a protein-repellent and antibacterial composite, and (2) investigate the effects of combining 2-methacryloyloxyethyl phosphorylcholine (MPC) with quaternary ammonium dimethylaminohexadecyl methacrylate (DMAHDM) on composite mechanical properties and biofilm response for the first time.
Methods: MPC, DMAHDM and glass particles were mixed into a dental resin composite. Mechanical properties were measured in three-point flexure. Protein adsorption onto the composites was measured by a micro bicinchoninic acid method. A human saliva microcosm model was used to grow biofilms on composites. Colony-forming unit (CFU) counts, live/dead assay, metabolic activity, and lactic acid production of biofilms were determined.
Results: Incorporation of 3% MPC and 1.5% DMAHDM into composite achieved protein-repellent and antibacterial capabilities without compromising the mechanical properties. Composite with 3% MPC + 1.5% DMAHDM had protein adsorption that was 1/10 that of a commercial composite ($p < 0.05$). The composite with 3% MPC + 1.5% DMAHDM had much greater reduction in biofilm growth than using MPC or DMAHDM alone ($p < 0.05$). Biofilm CFU counts on composite with 3% MPC + 1.5% DMAHDM were more than three orders of magnitude lower than that of commercial control.
Conclusions: Dental composite with a combination of strong protein-repellent and antibacterial capabilities was developed for the first time. Composite containing MPC and DMAHDM greatly reduced biofilm growth and lactic acid production, without compromising mechanical properties of the composite.

Clinical significance: Novel composite with MPC and DMAHDM greatly reduced biofilm activity and is promising to inhibit secondary caries. The dual agents of MPC plus DMAHDM may have wide applicability to other dental materials.

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1. Introduction

Dental caries remains the most common and widespread biofilm-dependent oral disease.[1,2] Because of their aesthetics and direct-filling capability, resin composites are widely used to restore tooth cavities.[3,4] Extensive efforts have improved the resin compositions and cure conditions, and reduced the polymerization shrinkage.[5–12] Nonetheless, secondary caries still limits the lifetime of composite restorations.[13,14] More than half of the restorations placed annually are replacements of failed restorations,[15] and the annual cost for tooth cavity restorations was approximately $46 billion in 2005 in the United States.[16] Dental composites generally do not inhibit bacterial adhesion and biofilm formation. On the contrary, previous studies have shown that composites tend to accumulate more biofilms and plaques *in vivo* than other restorative materials.[17,18]

Efforts have been made to incorporate antibacterial agents into composites. One class of such composites involved the use of quaternary ammonium methacrylates (QAMs).[19–23] Composites containing 12-methacryloyloxydodecylpyridinium bromide (MDPB) were effective in reducing bacterial viability.[19,20] Other antibacterial composites used agents including methacryloxylethyl cetyl dimethyl ammonium chloride and cetylpyridinium chloride.[21–23] Recently, a quaternary ammonium dimethacrylate (QADM) was synthesized and incorporated into composite, achieving strong antibacterial effects.[24–27] The antibacterial potency of quaternary ammonium compounds was shown to increase with increasing the alkyl chain length (CL) of the ammonium groups.[28] A series of new QAMs with CL varying from 3 to 18 were synthesized and incorporated into composites and bonding agents.[27,29] The results showed that a new dimethylamino-hexadecyl methacrylate (DMAHDM) with CL of 16 had the strongest antibacterial activity.[29]

Other efforts were made to develop surfaces with bacteria-repellent capability by coating the surface with layers of highly hydrophilic material.[30] Hydrophilic material surfaces can repel protein adsorption and bacterial adhesion.[31,32] 2-Methacryloyloxyethyl phosphorylcholine (MPC) is a methacrylate with a phospholipid polar group in the side chain, and is one of the most common biocompatible and hydrophilic biomedical polymers.[33] Highly hydrophilic surface coatings using MPC polymers are well known to reduce protein adsorption and bacterial adhesion.[34–37] However, there has been no report on the development of protein-repellent dental composite. Furthermore, there has been no report on dental composite that incorporates both MPC and DMAHDM to possess double benefits of protein-repellent and antibacterial capabilities.

One drawback of QAM-containing composites is that the adsorption of salivary proteins on composite surfaces could decrease the efficacy of "contact-inhibition", thereby reducing the antibacterial potency.[21,22] Therefore, a composite containing both MPC and QAM may protect the antibacterial potency of the composite by repelling protein adsorption, thereby increasing the composite surface-bacteria contact and hence the contact-killing efficacy. Hence, it would be highly desirable to combine MPC with DMAHDM to achieve double benefits of protein-repellent and antibacterial activities for dental composites.

Accordingly, the objectives of this study were to: (1) develop a novel protein-repellent and antibacterial composite, and (2) investigate the combined effects of MPC and DMAHDM on protein adsorption, dental plaque microcosm biofilm response, and mechanical properties of the composite for the first time. It was hypothesized that: (1) the composite containing MPC and DMAHDM would have good mechanical properties matching those with 0% MPC and 0% DMAHDM, and those of a commercial control composite; (2) composite containing MPC and DMAHDM would have much less protein adsorption than the controls; (3) incorporating MPC or DMAHDM individually into composite would yield substantial decreases in biofilm growth on composite; and (4) incorporating both MPC and DMAHDM into composite would achieve much greater biofilm-inhibition than using MPC or DMAHDM alone.

2. Materials and methods

2.1. *Preparation of composites containing MPC and DMAHDM*

MPC was obtained commercially (Sigma–Aldrich, St. Louis, MO) which was synthesized *via* a method reported by Ishihara et al.[33] BisGMA (bisphenol A glycidyl dimethacrylate) and TEGDMA (triethylene glycol dimethacrylate) (Esstech, Essington, PA) were mixed at a mass ratio = 1:1, and rendered light-curable with 0.2% camphorquinone and 0.8% ethyl 4-N,N-dimethylaminobenzoate (mass fractions). The MPC powder was mixed with the photo-activated BisGMA-TEGDMA resin (referred to as BT) at a MPC/(BT + MPC) mass fraction of 10%. Preliminary study on a series of mass fractions indicated that this mass fraction yielded a strong protein-repellent property without compromising mechanical properties of the resin.

DMAHDM with an alkyl chain length of 16 was synthesized using a modified Menschutkin reaction where a tertiary amine group was reacted with an organo-halide.[29,38,39] A benefit of this reaction is that the reaction products are generated at virtually quantitative amounts and require minimal purification. Briefly, 10 mmol of 2-(dimethylamino)ethyl methacrylate (DMAEMA, Sigma–Aldrich, St. Louis, MO) and 10 mmol of 1-bromohexadecane (BHD, TCI America, Portland, OR) 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 DMAHDM as a clear, colourless, and viscous liquid.[27,29] DMAHDM was incorporated into the BisGMA-TEGDMA resin at DMAHDM/(BT + DMAHDM) mass fractions of 0%, 5%, 7.5%, and 10%. The 10% DMAHDM was used following previous studies.[27,29] The 5% and 7.5% DMAHDM were used because 10% DMAHDM appeared to lower the composite strength when combined with MPC.

Each resin was filled with glass particles (barium boroaluminosilicate, mean size = 1.4 μm, Caulk/Dentsply, Milford, DE) silanized with 4% 3-methacryloxypropyltrimethoxysilane and 2% n-propylamine.[40] A filler mass fraction of 70% was used to yield a cohesive paste. Since the resin mass fraction in the composite was 30%, the MPC mass fraction in the final composite was 3%. The DMAHDM mass fractions in the composite were 0%, 1.5%, 2.25%, and 3%, respectively. The