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Protein-repellent and antibacterial functions of a calcium phosphate rechargeable nanocomposite

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ABSTRACT

Objectives: We recently developed a new rechargeable composite with nanoparticles of amorphous calcium phosphate (NACP) having long-term calcium (Ca) and phosphate (P) ion release; however, this composite was not antibacterial. The objectives of this study were to: (1) incorporate dimethylaminohexadecyl methacrylate (DMAHDM) and 2-methacryloyloxyethyl phosphorylcholine (MPC) into rechargeable NACP composite, and (2) investigate mechanical properties, protein adsorption and biofilm response of composite, and the pH of biofilm medium.

Methods: MPC, DMAHDM and NACP were mixed into a resin of ethoxylated bisphenol A dimethacrylate (EBPADMA) and pyromellitic glycerol dimethacrylate (PMGDM). Protein adsorption was measured using a micro bicinchoninic acid method. A human saliva microcosm biofilm model was used to grow biofilms on composites. Colony-forming units (CFU), live/dead assay, metabolic activity, and biofilm culture medium pH were determined. The tests used $n = 6$.

Results: The composite with 3% MPC had protein adsorption an order of magnitude less than that of a commercial composite ($p < 0.05$). Control composites were fully covered by live bacteria. Live bacteria were reduced via MPC; 3% MPC + 3% DMAHDM had the least live bacteria ($p < 0.05$). The composite with 3% MPC + 3% DMAHDM inhibited biofilm growth and viability, reducing biofilm CFU by 3 log compared to commercial control composite ($p < 0.05$), while having a flexural strength similar to that of the commercial composite ($p > 0.1$). The composite containing 3% MPC + 3% DMAHDM with biofilm culture maintained a pH above 6.5, while the commercial composite had a cariogenic pH of 4.2 in biofilm culture medium.

Conclusions: The new protein-repellent and antibacterial NACP rechargeable composite substantially reduced biofilm growth, yielding a much higher pH than a commercial composite.

Clinical significance: This novel bioactive nanocomposite is promising to protect tooth structures from biofilm acids and caries. The method of using NACP, MPC and DMAHDM may be applicable to other dental materials to reduce plaque buildup and secondary caries.

1. Introductions

Tooth caries is a common biofilm-related oral disease [1]. Tooth cavity restorations cost the United States annually $46 billion in 2005 [2]. Because of their esthetics and direct-filling capability, resin composites are widely used to restore tooth cavities with bonding agents [3–6]. Extensive efforts have improved the resin compositions, curing efficacy, and mechanical and physical

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properties of the composites [7–15]. However, secondary caries still limits the longevity of composite restorations [5,16–18]. More than half of all restorations fail in 10 years [19], and the replacement of failed restorations accounts for 50% to 70% of all restorations performed [19]. Dental composites generally do not inhibit bacterial adhesion. On the contrary, previous studies showed that composites accumulated more biofilms and plaque than other restorative materials [20–22]. Biofilm acids can decrease the local pH to a cariogenic range of 5.5–4, which could lead to tooth structure demineralization and secondary caries formation [23].

To combat caries, calcium phosphate (CaP) composites were developed that could release calcium (Ca) and phosphate (P) ions to remineralize tooth lesions [24,25]. Re-incorporation of minerals into demineralized dentin could serve as sites for nucleation and remineralization [26]. Composites with nanoparticles of amorphous calcium phosphate (NACP) were developed [23,27,28]. NACP nanocomposite released Ca and P ions similar to traditional CaP composites [24,25], but with a 2-fold increase in strength for load-bearing restorations [23]. NACP composite was "smart" and rapidly neutralized acids to protect tooth structures [27]. In a human *in situ* model, NACP composite inhibited caries at the enamel-composite margins [29].

However, the Ca and P ion release from previous CaP composites lasted for only a couple of months and then diminished over time [23–25,28]. Such short-term ion release is not suitable for dental applications where the restoration should last for many years. Recently, a rechargeable CaP composite was developed with long-term Ca and P ion release for the first time [30,31]. Its Ca and P ion recharge and re-release were sustained, showing no decrease with increasing the recharge/re-release cycles [30,31]. However, while CaP composites have remineralization and acid neutralizing capabilities, they do not have significant antibacterial activity.

Quaternary ammonium methacrylates (QAMs) were incorporated into dental resins to achieve antibacterial activities to combat biofilm growth and acid production [32–34]. Resins containing 12-methacryloyloxydodecylpyridinium bromide (MDPB) had a potent antibacterial function [32,33]. A quaternary ammonium dimethacrylate (QADM) was incorporated into a composite, yielding a strong antibiofilm effect [31]. Recently, a new dimethylaminohexadecyl methacrylate (DMAHDM) was synthesized and incorporated into dental resin, which showed the strongest antibacterial effect among the antibacterial monomers tested [35]. In addition, 2-metha-cryloyloxyethyl phosphorylcholine (MPC) was incorporated into resins to repel protein and bacterial attachment [36]. However, to date, literature and patent searches revealed no report on rechargeable CaP dental composite that is also antibacterial.

Therefore, the objectives of this study were to develop the first rechargeable CaP dental composite with protein-repellent and antibacterial functions, and to investigate the effects of DMAHDM and MPC on mechanical properties, protein adsorption, dental plaque microcosm biofilm response and pH. It was hypothesized that: (1) Incorporating MPC and DMAHDM into the rechargeable NACP composite would still retain mechanical properties matching those of a commercial control composite; (2) Incorporating MPC and DMAHDM into rechargeable NACP composite would greatly decrease biofilm growth, viability and CFU; (3) Incorporating MPC and DMAHDM into rechargeable NACP composite would yield biofilm culture medium pH much higher than that of control.

2. Materials and methods

2.1. Fabrication of rechargeable NACP nanocomposite

Ethoxylated bisphenol A dimethacrylate (EBPADMA) (Sigma-Aldrich, St, Louis, MO) and pyromellitic glycerol dimethacrylate (PMGDM) (Esstech, Essington, PA) were mixed at a mass ratio of 1:1 to form the resin matrix [31]. PMGDM was selected because it is an acidic adhesive monomer and can chelate with calcium and phosphate ions from the recharge solution to achieve recharge capability, follow recent studies on rechargeable CaP composite [30,31,37]. This resin was rendered light-curable with 0.2% camphorquinone and 0.8% ethyl 4-*N*,*N*-dimethylaminobenzoate [37], and is referred to as the EBPM resin.

NACP [$Ca_3(PO_4)_2$] were synthesized via a spry-drying technique as previously described [23,28]. Briefly, calcium carbonate and dicalcium phosphate anhydrous were dissolved into an acetic acid solution. The concentrations of Ca and P ions were 8 mmol/L and 5.333 mmol/L, respectively. The solution was sprayed into a heated chamber to evaporate the water and volatile acid. The dried NACP powders were collected using an electrostatic precipitator. This yielded NACP with a mean particle size of 116 nm [28]. As a co-filler for mechanical reinforcement, barium boroaluminosilicate glass particles with a median size of 1.4 μm (Caulk/Dentsply, Milford, DE) were silanized with 4% 3-methacryloxypropyltrimethoxysilane and 2% *n*-propylamine (mass fractions) [23]. Mass fractions of 30% NACP and 35% glass particles were mixed with the EBPM resin to form a cohesive composite paste. The composite with EBPM resin containing NACP was shown previously to have long-term Ca and P ion release with recharge and re-release capability [31,37].

2.2. Development of protein-repellent, antibacterial and rechargeable NACP nanocomposite

The protein-repellent MPC was obtained commercially (Sigma-Aldrich) which was synthesized using a method reported previously [38]. MPC is a methacrylate with a phospholipid polar group in the side chain [38]. Antibacterial monomer DMAHDM was synthesized using a modified Menschutkin reaction where a tertiary amine group was reacted with an organo-halide [39]. Briefly, 10 mmol of 2-(dimethylamino)ethyl methacrylate (DMAEMA, Sigma–Aldrich) 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, colorless and viscous liquid [35].

MPC and DMAHDM were mixed with the EBPM resin, which was further mixed with NACP and glass fillers, at mass fractions listed in the following groups:

(1) **Rechargeable NACP composite control:** 35% EBPM resin matrix + 30% NACP and 35% glass fillers (referred to as "EBPM + NACP control");
(2) **Rechargeable NACP composite with MPC:** 32% EBPM + 3% MPC + 30% NACP + 35% glass fillers (referred to as "EBPM + NACP + MPC");
(3) **Rechargeable NACP composite with MPC and 1.5% DMAHDM:** 30.5% EBPM + 3% MPC + 1.5% DMAHDM + 30% NACP + 35% glass fillers (referred to as "EBPM + NACP + MPC + 1.5DMAHDM");
(4) **Rechargeable NACP composite with MPC and 3% DMAHDM:** 29% EBPM + 3% MPC + 3% DMAHDM + 30% NACP + 35% glass fillers (referred to as "EBPM + NACP + MPC + 3DMAHDM").

MPC mass fraction in the composite was 3% because a previous study showed that this yielded a strong protein-repellent property without compromising mechanical properties [36]. The DMAHDM mass fractions in the composite were 0%, 1.5% and 3%, respectively, following previous studies [36]. A commercial composite (Heliomolar, Ivoclar, Amherst, NY) also served as a control. Heliomolar contained 66.7% of nanofillers of 40–200 nm of silica and