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Protein-repelling adhesive resin containing calcium phosphate nanoparticles with repeated ion-recharge and re-releases

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ABSTRACT

Objectives: The objectives were to develop a calcium (Ca) and phosphate (P) ion-rechargeable and protein-repellent adhesive containing nanoparticles of amorphous calcium phosphate (NACP) and 2-methacryloyloxyethyl phosphorylcholine (MPC), and investigate the MPC effects on ion recharge and re-releases for the first time.

Methods: Pyromellitic glycerol dimethacrylate and ethoxylated bisphenol-A dimethacrylate were used to fabricate adhesive PEHB. Six adhesives were tested: (1) Scotchbond (SBMP); (2) PEHB, (3) PEHB + 20%NACP; (4) PEHB + 30%NACP; (5) PEHB + 20%NACP + 3%MPC; (6) PEHB + 30%NACP + 3%MPC. Dentin shear bond strength, Ca/P ion release, recharge and re-release, and protein adsorption were measured. A microcosm biofilm model was tested for lactic-acid production and colony-forming units (CFU).

Results: Adding NACP + MPC did not negatively affect dentin bond strength ($p > 0.1$). With increasing the number of recharge/re-release cycles, the Ca/P ion re-release reached similarly higher levels ($p > 0.1$), indicating long-term remineralization capability. One recharge enabled the adhesives to have continued re-releases for 21 days. Incorporation of 3% MPC yielded 10-fold decrease in protein adsorption, and 1–2 log decrease in biofilm CFU.

Conclusions: The new rechargeable adhesive with MPC + 30%NACP greatly reduced protein adsorption, biofilm growth and lactic acid. Incorporation of MPC did not compromise the excellent Ca/P ion release, rechargeability, and dentin bond strength.

Clinical significance: Novel bioactive adhesive containing MPC + NACP is promising to repel proteins and bacteria, and inhibit secondary caries at the restoration margins. The method of NACP + MPC to combine CaP-recharge and protein-repellency is applicable to the development of a new generation of materials including composites and cements to suppress oral biofilms and plaque formation and protect tooth structures.

1. Introduction

Secondary caries is defined as a caries lesion at the margin of the restoration and is a predominant reason for restoration failure [1–3]. Hydroxyapatite is the main component of tooth enamel that could decompose into Ca and PO_4 during the formation and growth of oral biofilm, leading to caries formation [4]. This process often occurs at the margins that are secluded and in shortage of oxygen and mechanical disturbance, such as the gingival margins of class II restorations [5]. The presence of micro-gaps at the bonded interface further complicates biofilm removal, leading to bacterial invasion along the adhesive-tooth interface, demineralization, and eventually secondary caries formation [6,7].

Dental composite is the material of choice for direct anterior

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restorations and is increasingly implemented in posterior restorations [8–10]. Composite failure due to secondary caries has been attributed to some factors such as increase in surface roughness due to deterioration, decrease in hardness, and the polymerization shrinkage leading to microgap formation and microleakage [11]. Advances in fillers and polymers have produced composites with improved surface properties and lower shrinkage [12,13].

Adhesive systems aim to bond the composite restoration to tooth structures. Extensive studies have been performed to improve and characterize enamel and dentin bonding since a strong and durable adhesion is a key factor in the success of the restoration [14,15]. Bonded composite allows practitioners to avoid removing healthy tooth structure to achieve retention and resistance form, and enables the procedure to be minimally invasive [16]. The traditional approach of complete caries removal had carious dentin left in 72% of cavities [17]; therefore, by saving more affected tissues, carious lesions inadvertently will harbor more residual bacteria [18]. Adhesive systems with bactericidal properties enable the suppression of residual bacteria and improve the survival of the restored tooth [19]. Several components have been incorporated into the adhesives to reduce bacterial growth, to inhibit the metabolic activity of cariogenic microorganisms and to disinfect cavities from residual bacteria [19–22]. These antibacterial adhesives are of particular importance to target biofilms at the margins to reduce secondary caries and improve longevity of composite restorations.

The formation of a biofilm on tooth surfaces is initiated by selective adsorption of salivary proteins to form a pellicle within a few minutes after tooth cleaning [23]. As bacteria approach the pellicle layer, weak physicochemical forces are generated, which may shortly become irreversible due to adhesins on the microbial cell surfaces [24]. Bacterial colonization starts with the adhesion of early colonizers, such as *Streptococcus mutans* [25]. Then, other oral bacteria may co-aggregate to produce a biofilm on the tooth surface. Oral bacteria then metabolize nutrients in the oral cavity to gene1rate organic acids that cause hydroxyapatite decomposition leading to caries [26]. Therefore, it is expected that the initiation of secondary caries could be minimized by precluding protein adsorption and the subsequent bacterial adhesion on restorative margins. 2-methacryloyloxyethyl phosphorylcholine (MPC) is a biocompatible polymer that has a phospholipid polar group, imitating the structure of biomembranes [27]. MPC has been found to reduce protein adsorption and bacterial adhesion, and inhibit cell attachment in medical devices [28,29]. When incorporated into a dental adhesive, MPC greatly reduced the protein adsorption and bacterial adhesion [30].

The incorporation of nanoparticles of amorphous calcium phosphate (NACP) in adhesives provided releases of calcium (Ca) and phosphate (P) ions [31], resulting in the precipitation and deposition of minerals into tooth structures [32]. NACP could neutralize bacterial acids and increase the local pH from 4 to nearly 6, which could avoid caries formation [33,34]. More recently, a novel Ca and P ion recharge technology was developed to produce long-term ion release [35]. The NACP resins could be repeatedly recharged with Ca and P ions, via solutions such as mouthwashes, to have long-term remineralization effects [35,36]. However, the previous rechargeable NACP-containing adhesive had no protein-repellent activity [35]. A recent study added MPC into an adhesive which was shown to greatly reduce biofilm attachment [37]. However, that study did not investigate the influence of adding MPC on the ion recharge and re-release efficacy, and there was no demonstration whether the protein-repellent adhesive was Ca and P ion-rechargeable or not [37].

Accordingly, the objectives of this study were to develop a novel Ca and P ion-rechargeable adhesive with protein-repellent function, and to evaluate the addition of MPC on Ca and P recharge and re-release properties for the first time. The following hypotheses were tested: (1) Incorporating NACP and MPC into adhesive would not compromise dentin bond strength, compared to that without NACP and MPC; (2) the rechargeable adhesive with NACP and MPC would have strong protein-repelling properties; and (3) the rechargeable and protein-repelling adhesive would have excellent Ca and P ion recharge and long-term ion re-release, which would not decrease with increasing number of recharge and re-release cycles.

2. Materials and methods

2.1. Fabrication of CaP-rechargeable and protein-repellent adhesive

To fabricate the primer, pyromellitic glycerol dimethacrylate (PMGDM) and 2-hydroxyethyl methacrylate (HEMA) (Esstech, Essington, PA) were mixed at a mass ratio 3.3/1, with 50% acetone solvent (all mass fractions) [38]. The adhesive consisted of 44.5% PMGDM, 39.5% ethoxylated bisphenol A dimethacrylate (EBPADMA) (Sigma-Aldrich, St, Louis, MO), 10% HEMA, and 5% bisphenol A glycidyl dimethacrylate (BisGMA) [36]. Phenylbis (2,4,6-trimethylbenzoyl) phosphine oxide (Esstech) was added as a photo-initiator at 1% mass fraction [36]. This adhesive is termed PEHB.

NACP [$Ca_3(PO_4)_2$] was synthesized via a spray-drying technique as previously described [31,39]. Briefly, calcium carbonate and dicalcium phosphate anhydrous were dissolved into an acetic acid solution. The concentrations of Ca and P ion concentrations were 8 mmol/L and 5.333 mmol/L, respectively, yielding a Ca/P molar ratio of 1.5. The solution was sprayed into a heated chamber to evaporate the water and volatile acid. The dried NACP powder was collected by an electrostatic precipitator, which yielded NACP with mean particle size of approximately 116 nm [31,39]. NACP were mixed into PEHB adhesive at mass fractions of 20% and 30%, which were previously shown to produce high levels of Ca and P ion release with no adverse effect on the dentin shear bond strength [36].

The protein-repellent monomer, 2-methacryloyloxyethyl phosphorylcholine (MPC), was synthesized via a method reported by Ishihara et al. [40] and is commercially available (Sigma-Aldrich). The MPC powder was mixed with PEHB at mass percentage of 3%, because it was shown in our preliminary study that 3% MPC yielded a strong protein-repellency without compromising the dentin shear bond strength.

Scotchbond Multi-Purpose bonding system (SBMP, 3 M, St. Paul, MN) served as the commercial control. According to the manufacturer, SBMP primer contained 35–45% HEMA, 10–20% copolymer of acrylic and itaconic acids, and 40–50% water. SBMP adhesive contained 60–70% BisGMA and 30–40% HEMA. Five systems were investigated:

[1] SBMP commercial control (referred to as SBMP control);
[2] PEHB control;
[3] PEHB + 20% NACP;
[4] PEHB + 30% NACP;
[5] PEHB + 20% NACP + 3% MPC
[6] PEHB + 30% NACP + 3% MPC

2.2. Dentin shear bond strength testing

Extracted human third molars were stored in 0.01% thymol solution at 4 °C. The use of teeth was approved by the University of Maryland Baltimore Institutional Review Board. Teeth were sectioned perpendicular to their long axis with a diamond saw under water coolant (Isomet, Buehler, Lake Bluff, IL) to expose the mid-coronal dentin, which was then polished with 600-grit SiC paper. The dentin surface was etched with 37% phosphoric acid (ScotchBond Etchant, 3 M, St. Paul, MN) for 15 s (s), then rinsed with water for 15 s. Primer was applied with a brush-tip applicator, followed by a gentle air blow for 5 s. An adhesive was then applied and light-cured for 20 s with Optilux curing unit (VCL 401, Demeron Kerr, Danbury, CT). A stainless-steel cylindrical mold (inner diameter = 4 mm, thickness = 1.5 mm) was placed on the adhesive-treated dentin surface [38]. A composite (TPH, Dentsply/Sirona, Milford, DE) was placed in the mold and light-cured for 60 s. The bonded specimens were stored in distilled water at 37 °C