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Novel dental adhesive with triple benefits of calcium phosphate recharge, protein-repellent and antibacterial functions

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ABSTRACT

Objective. A new adhesive containing nanoparticles of amorphous calcium phosphate (NACP) with calcium (Ca) and phosphate (P) ion rechargeability was recently developed; however, it was not antibacterial. The objectives of this study were to: (1) develop a novel adhesive with triple benefits of Ca and P ion recharge, protein-repellent and antibacterial functions via dimethylaminohexadecyl methacrylate (DMAHDM) and 2-methacryloyloxyethyl phosphorylcholine (MPC); and (2) investigate dentin bond strength, protein adsorption, Ca and P ion concentration, microcosm biofilm response and pH properties.

Methods. MPC, DMAHDM and NACP were mixed into a resin consisting of ethoxylated bisphenol A dimethacrylate (EBPADMA), pyromellitic glycerol dimethacrylate (PMGDM), 2-hydroxyethyl methacrylate (HEMA) and bisphenol A glycidyl dimethacrylate (BisGMA). Protein adsorption was measured using a micro bicinchoninic acid method. A human saliva microcosm biofilm model was tested on resins. Colony-forming units (CFU), live/dead assay, metabolic activity, Ca and P ion concentration and biofilm culture medium pH were determined.

Results. The adhesive with 5% MPC + 5% DMAHDM + 30% NACP inhibited biofilm growth, reducing biofilm CFU by 4 log, compared to control ($p < 0.05$). Dentin shear bond strengths were similar ($p > 0.1$). Biofilm medium became a Ca and P ion reservoir having ion concentration increasing with NACP filler level. The adhesive with 5% MPC + 5% DMAHDM + 30% NACP maintained a safe pH > 6, while commercial adhesive had a cariogenic pH of 4.

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Significance. The new adhesive with triple benefits of Ca and P ion recharge, protein-repellent and antibacterial functions substantially reduced biofilm growth, reducing biofilm CFU by 4 orders of magnitude, and yielding a much higher pH than commercial adhesive. This novel adhesive is promising to protect tooth structures from biofilm acids. The method of using NACP, MPC and DMAHDM is promising for application to other dental materials to combat caries.

1. Introduction

Because of their esthetics and direct-filling capability, composites and adhesives are widely used to restore tooth cavities [1–4]. However, dental resins were shown to accumulate more biofilms and plaques than amalgams and glass ionomer restorations [5]. The acid production by biofilms can decrease the local pH to a cariogenic range of 5–4, which could lead to tooth structure demineralization and secondary caries formation [6]. Recurrent caries is the main reason for restoration failures, and replacement of the failed restorations accounts for 50–70% of all restorations performed [7]. The tooth-restoration bonded interface has been identified as the weak link [7,8].

A strong and durable adhesion to dental hard tissues is a key factor in the success of the restoration [9–12]. The mechanism of dentin bonding involves the infiltration of adhesive monomers into a demineralized dentin collagen matrix and the formation of the hybrid layer (HL) [9–12]. The adhesive is not only a connection between the tooth structure and the restorative composite, it also serves as a barrier to protect the demineralized collagen scaffold from the acidic and enzymatic attacks of the oral bacteria, enzymes and fluids [10,13]. Clinically, residual bacteria could exist in the prepared tooth cavity. In addition, microleakage could allow bacteria to invade the tooth-restoration interfaces. Therefore, it is desirable for the adhesive to be antibacterial to inhibit recurrent caries at the margins [14–17]. For this purpose, quaternary ammonium methacrylates (QAMs) were incorporated into dental resins to achieve antibacterial activities to combat biofilm growth and acid production [14,18]. Resins containing 12-methacryloyloxydodecylpyridinium bromide (MDPB) had a potent antibacterial function [14]. Recently, a new dimethylaminohexadecyl methacrylate (DMAHDM) was synthesized and incorporated into composites and bonding agents, achieving strong inhibition against oral biofilms [19]. In addition, oral bacteria attach to dental resins through a layer of adsorbed salivary proteins on the resin surface, which is a prerequisite for bacterial adhesion and biofilm growth [20]. Therefore, rendering the resin protein-repellent would help to repel bacteria attachment. Indeed, studies showed that a protein-repellent agent 2-methacryloyloxyethyl phosphorylcholine (MPC) could be incorporated into resins to repel proteins and bacteria [21].

Another approach for caries-inhibition is to incorporate calcium phosphate (CaP) particles into resins to promote remineralization and suppress demineralization [22–24]. Adhesives containing CaP particles could remineralize the remnants of tooth lesions in the cavity as well as the acid-etched dentin, and hence are promising to improve the longevity of the restorations [25,26]. Recently, bonding agents containing nanoparticles of amorphous calcium phosphate (NACP) were developed [27,28]. These bonding agents could release high levels of Ca and P ions to induce remineralization and combat caries [27,28]. The addition of NACP did not negatively affect the dentin bond strength [27,28]. Due to their small particle sizes, the NACP readily flowed with bonding agent into dentinal tubules to form resin tags [27]. The NACP adhesive was "smart" because it could substantially increase the Ca and P ion release at a low cariogenic pH when these ions would be most needed to combat caries [28]. For both total-etch and self-etch bonding systems, the bonding stability is limited by the degradation of the HL [13,29,30]. The Ca and P ion release from adhesive may be highly beneficial and can serve as seed crystals to facilitate remineralization in HL and at the tooth-restoration margins [13,25]. Thus, the CaP adhesive may protect the exposed collagen within the bonded interface and improve the bonding stability and durability [13,31]. Therefore, the NACP-containing adhesive with Ca and P ion release could be meritorious in protecting the weak link of the tooth restoration. However, the Ca and P ion release from CaP resins lasted for only a couple of months and then diminished over time [22,32,33]. Recently, a rechargeable CaP resin was developed with long-term Ca and P ion release for the first time [34]. Its Ca and P ion recharge and re-release were sustained, showing no decrease in ion release with increasing the number of recharge/re-release cycles. However, while NACP resins have remineralization and acid neutralizing capabilities, they are not antibacterial [27]. To date, there has been no report on the incorporation of DMAHDM and MPC into the rechargeable NACP adhesive to achieve both protein-repellent, antibacterial and long-term CaP ion recharge and re-release capabilities.

Therefore, the objectives of this study were to develop a novel bioactive adhesive for caries-inhibition by incorporating DMAHDM and MPC into a rechargeable NACP adhesive, and to investigate the dentin bond strength, protein adsorption, biofilm response and pH properties. It was hypothesized that: (1) Incorporating MPC, DMAHDM and NACP into the adhesive would yield dentin bond strength similar to a commercial control adhesive; (2) Incorporating MPC, DMAHDM into the rechargeable NACP adhesive would greatly decrease protein-adsorption, biofilm growth and viability; (3) Increasing NACP filler level in the resin would increase the pH and Ca and P ion concentrations in the biofilm culture medium.