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Novel dental adhesive resin with crack self-healing, antimicrobial and remineralization properties

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ABSTRACT

Objectives: Secondary caries at the tooth-restoration margins is a primary reason for restoration failure. Cracks at the margins lead to leakage which can trap bacteria, producing acids to cause caries. To date, there has been no report on developing an adhesive resin that has self-healing, antibacterial and remineralizing capabilities. The objectives of this study were to: (1) develop the first self-healing adhesive with antimicrobial and remineralizing capabilities, and (2) investigate the effects of incorporating microcapsules, dimethylaminohexadecyl methacrylate (DMAHDM) and nanoparticles of amorphous calcium phosphate (NACP) for the first time.

Methods: Self-healing microcapsules were synthesized with poly(urea-formaldehyde) (PUF) shells containing triethylene glycol dimethacrylate (TEGDMA) as the healing liquid. The new adhesive contained 7.5% microcapsules, 10% DMAHDM and 20% NACP. A single edge V-notched beam (SEVNB) method was used to measure the fracture toughness K_{IC} and the autonomous crack-healing efficiency. An oral plaque microcosm biofilm model was tested.

Results: The new self-healing, antimicrobial and remineralizing dental adhesive matched the dentin bond strength of a commercial control ($p > 0.1$). The new adhesive achieved successful crack-healing, with an excellent K_{IC} recovery of 67%. The new adhesive had strong antimicrobial activity, reducing biofilm colony-forming units by four orders of magnitude, and reducing biofilm acid production to 1/100th that of biofilms on the commercial control resin.

Conclusions: A self-healing adhesive with antibacterial and remineralizing capabilities was developed for the first time. Excellent dentin bond strength, autonomous crack-healing and K_{IC} recovery, and strong anti-biofilm properties were achieved for the new adhesive resin.

Clinical significance: The novel method of using triple agents (self-healing microcapsules + DMAHDM + NACP) is promising for applications in dental adhesives, cements, sealants and composites to combat the two main challenges: fracture and secondary caries.

1. Introduction

Composites are popular esthetic alternatives to dental amalgams [1–3]. Advances in polymer chemistry and fillers have enhanced the performance of composites [4–9]. However, one main drawback is that composites collect more biofilms than other restorative materials [10]. Oral biofilms produce acids which can cause tooth caries [11]. Secondary caries at the bonded tooth-composite margins is one of the primary reasons for restoration failures [12,13]. Therefore, efforts have been undertaken to enhance the tooth-restoration bond strength [14]. Since secondary caries often occurs at the margins, it is high desirable to develop antimicrobial adhesives to inhibit bacteria and combat

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caries at the margins [15]. Previous studies incorporated quaternary ammonium methacrylates (QAMs) into dental resins [15]. Adhesives containing 12-methacryloyloxydodecyl-pyridinium bromide (MDPB) displayed a strong inhibition on bacterial growth [16]. Several studies also reported resins with quaternary ammonium polyethylenimine (PEI) nanoparticles [17], an adhesive containing methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB) [18], glass ionomer cements having antibacterial functions [19], and resins containing quaternary ammonium dimethacrylate and nano-silver [20,21]. A new QAM with an alkyl chain length of 16 was recently synthesized (dimethylaminohexadecyl methacrylate, DMAHDM) which showed a potent anti-biofilm activity [22,23]. Recent studies also developed adhesives containing nanoparticles of amorphous calcium phosphate (NACP) which could remineralize tooth lesions and neutralize acids [24,25]. Resins containing NACP released supersaturating levels of calcium (Ca) and phosphate (P) ions to cause remineralization [26].

At the restoration-tooth bonded interface, the bond longevity is mainly affected by the degradation of the hybrid layer [27] as well as micro-cracks at the margins induced by polymerization shrinkage, cyclic loading, and thermal and mechanical fatigue [28]. Efforts to improve the durability of the resin-dentin bond have included the use of inhibitors of matrix metalloproteinases (MMPs) which degrade the hybrid layer [29], and new bonding techniques such as “ethanol-wet bonding” to create a hydrophobic hybrid layer [30]. Since the micro-cracks could result in micro-leakage and bacteria invasion [31], it would be highly beneficial to develop an adhesive having autonomous crack-healing ability to self-heal these micro-cracks.

Previous studies developed autonomous crack-healing (or self-healing) polymers with microcapsules which had a shell encapsulating a healing liquid [32]. A propagating crack in the polymer matrix would rupture the microcapsules, releasing the healing liquid into the crack planes. The healing liquid contacts the catalyst in the matrix, which triggers the polymerization of the healing liquid and heals the crack [33]. One study used dicyclopentadiene (DCPD) encapsulated in a poly (urea-formaldehyde) (PUF) shell to form microcapsules [34]. These microcapsules were added into epoxy with a transition metal catalyst (named Grubb’s catalyst), and a healing efficiency of 75% was achieved [34]. This autonomous crack-healing system was then applied to a dental composite, which achieved a 57% healing efficiency [35]. The DCPD-containing microcapsules were added into a resin without impairing the original mechanical properties of the matrix [36]. Another study developed polyurethane (PU) shell-based triethylene glycol dimethacrylate (TEGDMA) containing nanocapsules; however, that study did not mention the use of a catalyst and did not demonstrate autonomous crack-healing [37]. To date, there has been no further report on the use of DCPD and Grubb’s catalyst in dental materials, likely due to the toxicity of DCPD [38], Grubb’s catalyst toxicity, and the high cost [39]. Recently, novel crack-healing poly(urea-formaldehyde) (PUF) microcapsules containing TEGDMA and *N,N*-dihydroxyethyl-*p*-toluidine (DHEPT) were synthesized [40]. They were incorporated into a composite containing NACP to obtain crack-healing, antibacterial, and remineralization capabilities [41]. The long-term crack-healing of this composite was also demonstrated [42]. However, to date, there has been no report on the development of an adhesive resin with triple benefits of autonomous crack-healing, antimicrobial and remineralizing capabilities.

Therefore, the objectives of this study were to develop the first self-healing, antimicrobial and remineralizing dental adhesive, and determine the effects on dentin bond strength, self-healing efficiency, and the suppression of oral plaque microcosm biofilms for the first time. The following hypotheses were tested: (1) Incorporation of self-healing microcapsules, antibacterial DMAHDM and remineralizing NACP into the adhesive would not reduce the dentin bond strength; (2) Incorporation of the microcapsules would impart autonomous crack-healing to the adhesive; (3) This autonomous crack-healing adhesive containing DMAHDM and NACP would exhibit strong antimicrobial properties against oral plaque microcosm biofilms.

2. Materials and methods

2.1. Development of autonomous-healing microcapsules (MC)

Autonomous crack-healing microcapsules were prepared via *in situ* polymerization of formaldehyde and urea, following a previous study [40,43]. DHEPT (Sigma-Aldrich, St. Louis, MO) at 1% mass fraction was added to TEGDMA monomer (Esstech, Essington, PA). Fifty mL of water and 13 mL of a 2.5% aqueous solution of ethylene-maleic anhydride (EMA) copolymer (Sigma-Aldrich) were mixed in a 250 mL round bottom glass flask. The flask was suspended in a water bath on a hot-plate (Isotemp, Fisher Scientific, Pittsburg, PA). The EMA solution was used as a surfactant to form an “oil-in-water” emulsion (“oil” being TEGDMA-DHEPT). Under agitation by a magnetic stir bar (diameter = 7.8 mm, length = 50 mm, Fisher Scientific) at 300 rpm, the shell-forming material urea (1.25 g), ammonium chloride (0.125 g) and resorcinol (0.125 g) (Sigma-Aldrich) were added into the solution. The resorcinol was added in the reaction of shell formation to enhance the rigidity of the shells [40]. The pH was adjusted to 3.5 via drop-wise addition of 1 M sodium hydroxide solution. Then, the agitation rate was increased to 400 rpm, and 30 mL of the TEGDMA-DHEPT liquid was added into the flask. A stabilized emulsion of fine TEGDMA-DHEPT droplets was formed after 10 min of agitation. Then, 3.15 g of a 37% aqueous solution of formaldehyde (Sigma-Aldrich) was added, and the flask was sealed with aluminum foil to prevent evaporation. The temperature of the water bath was raised to 55 °C and the shell material was isothermally polymerized for 4 h (h) under continuous agitation. In this process, ammonium chloride catalyzed the reaction of urea with formaldehyde to form PUF at the oil-water interface to develop the shell [40]. The microcapsules thus obtained were rinsed with water and acetone, vacuum-filtered, and air-dried for 24 h. The microcapsules were examined with scanning electronic microscopy (SEM, Quanta 200, FEI, Hillsboro, OR). The microcapsules had a mean diameter of 70 μm as shown in a previous study [40]. The ability of the encapsulated TEGDMA to polymerize was verified by near-infrared (NIR) spectroscopy (Nicolet 6700, Thermo Scientific, Waltham, MA) in a previous study [40].

2.2. Synthesis of NACP and DMAHDM

NACP were prepared using a spray-drying technique [44]. Briefly, calcium carbonate ($CaCO_3$, Fisher Scientific) and dicalcium phosphate anhydrous ($CaHPO_4$, Baker Chemical, Phillipsburg, NJ) were dissolved into an acetic acid solution to obtain Ca and P ionic concentrations of 8 mmol/L and 5.333 mmol/L, respectively, yielding a Ca/P molar ratio of 1.5. Then the solution was sprayed into a heated chamber, and an electrostatic precipitator (AirQuality, Minneapolis, MN) was used to collect the dried particles. This produced NACP with a mean particle dimension of 116 nm [26]. The purpose of incorporating NACP into dental resins was for Ca and P ion release which could suppress demineralization and promote remineralization [21,26].

The process of synthesis of DMAHDM was recently reported [22]. Briefly, 10 mmol of 2-(dimethy-lamino) ethyl methacrylate (DMAEMA, Sigma-Aldrich) and 10 mmol of 1-bromohexadecane (BHD, TCI America, Port-land, OR) were dissolved in 3 g of ethanol and allowed to react at 70 °C for 24 h under agitation for the reaction to complete. The solvent was then removed via evaporation, yielding DMAHDM as a clear, colorless, and viscous liquid [22].

2.3. Fabrication of autonomous-healing, antimicrobial and remineralizing adhesive

The experimental primer contained pyromellitic glycerol dimethacrylate (PMGDM) (Hampford, Stratford, CT) and 2-hydroxyethyl