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Antibacterial activity and ion release of bonding agent containing amorphous calcium phosphate nanoparticles☆

Chen Chen[a,b], Michael D. Weir[a], Lei Cheng[b], Nancy J. Lin[c], Sheng Lin-Gibson[c], Laurence C. Chow[d], Xuedong Zhou[b,**], Hockin H.K. Xu[a,e,f,*]

[a] Department of Endodontics, Prosthodontics and Operative Dentistry, University of Maryland Dental School, 650 West Baltimore Street, Baltimore, MD 21201, USA
[b] State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, China
[c] Biomaterials Group, Biosystems and Biomaterials Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA
[d] Dr. Anthony Volper Research Center, American Dental Association Foundation, Gaithersburg, MD 20899, USA
[e] Center for Stem Cell Biology & Regenerative Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[f] Department of Mechanical Engineering, University of Maryland, Baltimore County, MD 21250, USA

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ABSTRACT

Objective. Recurrent caries at the margins is a primary reason for restoration failure. The objectives of this study were to develop bonding agent with the double benefits of antibacterial and remineralizing capabilities, to investigate the effects of NACP filler level and solution pH on Ca and P ion release from adhesive, and to examine the antibacterial and dentin bond properties.

Methods. Nanoparticles of amorphous calcium phosphate (NACP) and a quaternary ammonium monomer (dimethylaminododecyl methacrylate, DMADDM) were synthesized. Scotchbond Multi-Purpose (SBMP) primer and adhesive served as control. DMADDM was incorporated into primer and adhesive at 5% by mass. NACP was incorporated into adhesive at filler mass fractions of 10%, 20%, 30% and 40%. A dental plaque microcosm biofilm model was used to test the antibacterial bonding agents. Calcium (Ca) and phosphate (P) ion releases from the cured adhesive samples were measured vs. filler level and solution pH of 7, 5.5 and 4.

Results. Adding 5% DMADDM and 10–40% NACP into bonding agent, and water-aging for 28 days, did not affect dentin bond strength, compared to SBMP control at 1 day ($p > 0.1$). Adding DMADDM into bonding agent substantially decreased the biofilm metabolic activity and lactic acid production. Total microorganisms, total streptococci, and mutans streptococci were greatly reduced for bonding agents containing DMADDM. Increasing NACP filler

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* *Corresponding author at*: Biomaterials & Tissue Engineering Division, Department of Endodontics, University of Maryland Dental School, Baltimore, MD 21201, USA. Tel.: +1 410 7067047; fax: +1 410 7063028.
** *Corresponding author.*
E-mail addresses: zhouxd@scu.edu.cn (X. Zhou), hxu@umaryland.edu (H.H.K. Xu).

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level from 10% to 40% in adhesive increased the Ca and P ion release by an order of magnitude. Decreasing solution pH from 7 to 4 increased the ion release from adhesive by 6–10 folds.

Significance. Bonding agents containing antibacterial DMADDM and remineralizer NACP were formulated to have Ca and P ion release, which increased with NACP filler level from 10% to 40% in adhesive. NACP adhesive was "smart" and dramatically increased the ion release at cariogenic pH 4, when these ions would be most-needed to inhibit caries. Therefore, bonding agent containing DMADDM and NACP may be promising to inhibit biofilms and remineralize tooth lesions thereby increasing the restoration longevity.

1. Introduction

Resin composites are the principal material for tooth cavity restorations due to their esthetics and direct-filling capability [1–4]. Advances in polymers and fillers have significantly enhanced the composite properties and clinical performance [5–11]. However, a serious drawback is that composites tend to accumulate more biofilms/plaques in vivo than tooth enamel and other restorative materials [12,13]. Biofilms with exposure to fermentable carbohydrates produce acids and are responsible for tooth caries [14,15]. Furthermore, composite restorations are bonded to the tooth structure via adhesives [16–19]. The bonded interface is considered the weak link between the restoration and the tooth structure [16]. Hence extensive studies were performed to increase the bond strength and determine the mechanisms of tooth-restoration adhesion [20–24]. One approach was to develop antibacterial adhesives to reduce biofilms and caries at the margins [25–28]. The rationale was that, after tooth cavity preparation, there are residual bacteria in the dentinal tubules. In addition, marginal leakage during service could also allow new bacteria to invade into the tooth-restoration interfaces. Therefore, antibacterial bonding agents would be highly beneficial to combat bacteria and caries [29,30].

Accordingly, efforts were made to synthesize quaternary ammonium methacrylates (QAMs) and develop antibacterial resins [25,31–35]. The 12-methacryloyloxydodecyl-pyridinium bromide (MDPB)-containing adhesive effectively inhibited oral bacteria growth [26,29]. Antibacterial adhesive containing methacryloxyl ethyl cetyl dimethyl ammonium chloride (DMAE-CB) was also synthesized [27]. Other studies developed a quaternary ammonium dimethacrylate (QADM) for incorporation into resins [33,36,37]. Recently, a new quaternary ammonium monomer, dimethylaminododecyl methacrylate (DMADDM), was synthesized and imparted a potent anti-biofilm activity to bonding agent [38].

Another approach to combat caries was to incorporate calcium phosphate (CaP) particles to achieve remineralization capability [39–42]. Composites were filled with amorphous calcium phosphate (ACP) in which the ACP particles had diameters of several μm to 55 μm [43]. ACP composites released supersaturating levels of calcium (Ca) and phosphate (P) ions and remineralized enamel lesions in vitro [43]. One drawback of traditional CaP composites is that they are mechanically weak and cannot be used as bulk restoratives [39,40]. More recently, nanoparticles of ACP (NACP) were synthesized and incorporated into resins [44,45]. NACP nanocomposite released high levels of Ca and P ions while possessing mechanical properties similar to commercial composite control [44]. NACP nanocomposite neutralized acid challenges [45], remineralized enamel lesions in vitro [46], and inhibited caries in vivo [47]. Due to the small particle size and high surface area, NACP in resin would have three main advantages: (1) improved mechanical properties [44], (2) high levels of ion release [44], and (3) being able to flow with bonding agent into small dentinal tubules [48]. However, the Ca and P ion release from bonding agent with various amounts of NACP has not been measured in previous studies.

The objectives of this study were to develop bonding agent with double benefits of antibacterial and remineralizing capabilities, to investigate the effect of NACP filler level and solution pH on Ca and P ion release from the adhesive, and to examine the antibacterial and dentin bonding properties. It was hypothesized that: (1) Incorporation of DMADDM and NACP and water-aging for 1 month will not negatively affect the dentin bond strength; (2) DMADDM will impart a strong antibacterial activity to bonding agent, while NACP will have no effect on antibacterial activity; (3) Ca and P ion release will be directly proportional to NACP filler level in adhesive: (4) NACP adhesive will be "smart" to increase the ion release at acidic cariogenic pH, when these ions would be most-needed to inhibit caries.

2. Materials and methods

2.1. *Synthesis of NACP and DMADDM*

Nanoparticles of ACP ($Ca_3[PO_4]_2$), referred to as NACP, were synthesized using a spray-drying technique as described previously [44,45]. Briefly, calcium carbonate ($CaCO_3$, Fisher, Fair Lawn, NJ) and dicalcium phosphate anhydrous ($CaHPO_4$, Baker Chemical, Phillipsburg, NJ) were dissolved into an acetic acid solution to obtain final Ca and P ionic concentrations of 8 mmol/L and 5.333 mmol/L, respectively. The Ca/P molar ratio for the solution was 1.5, the same as that for ACP. The solution was sprayed into the heated chamber of the spray-drying apparatus. The dried particles were collected via an electrostatic precipitator (AirQuality, Minneapolis, MN), yielding NACP with a mean particle size of 116 nm [44].