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Evaluation of antibacterial and remineralizing nanocomposite and adhesive in rat tooth cavity model

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ABSTRACT

Antibacterial and remineralizing dental composites and adhesives were recently developed to inhibit biofilm acids and combat secondary caries. It is not clear what effect these materials will have on dental pulps in vivo. The objectives of this study were to investigate the antibacterial and remineralizing restorations in a rat tooth cavity model, and determine pulpal inflammatory response and tertiary dentin formation. Nanoparticles of amorphous calcium phosphate (NACP) and antibacterial dimethylaminododecyl methacrylate (DMADDM) were synthesized and incorporated into a composite and an adhesive. Occlusal cavities were prepared in the first molars of rats and restored with four types of restoration: control composite and adhesive; control plus DMADDM; control plus NACP; and control plus both DMADDM and NACP. At 8 or 30 days, rat molars were harvested for histological analysis. For inflammatory cell response, regardless of time periods, the NACP group and the DMADDM + NACP group showed lower scores (better biocompatibility) than the control group ($p = 0.014$ for 8 days, $p = 0.018$ for 30 days). For tissue disorganization, NACP and DMADDM + NACP had better scores than the control ($p = 0.027$) at 30 days. At 8 days, restorations containing NACP had a tertiary dentin thickness (TDT) that was five- to six-fold that of the control. At 30 days, restorations containing NACP had a TDT that was four- to six-fold that of the control. In conclusion, novel antibacterial and remineralizing restorations were tested in rat teeth in vivo for the first time. Composite and adhesive containing NACP and DMADDM exhibited milder pulpal inflammation and much greater tertiary dentin formation than the control adhesive and composite. Therefore, the novel composite and adhesive containing NACP and DMADDM are promising as a new therapeutic restorative system to not only combat oral pathogens and biofilm acids as shown previously, but also facilitate the healing of the dentin–pulp complex.

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1. Introduction

Dental resin composites are continuously being improved in terms of physical and load-bearing properties and are increasingly being used clinically [1–3]. After being bonded to dental tissues with adhesives [4], the restorations are intended to function in the oral cavities durably. However, secondary caries at the tooth–restoration margins compromises the longevity [5–7]. Nearly half of all restorations fail within 10 years, and replacing them accounts for 50–70% of all restorations performed [8,9]. Dental caries, a dietary carbohydrate-modified bacterial infectious disease, is a common infection in humans [10–12]. The basic mechanism of caries is demineralization of enamel and dentin via acid generated by a bacterial biofilm [13–15]. Composites cannot hinder bacteria colonization or combat demineralization. To address this issue, efforts are underway to develop antibacterial resins to reduce caries [16–21]. Novel polymers containing quaternary ammonium methacrylates (QAMs) were developed [17–23]. 12-Methacryloyloxydodecylpyridinium bromide (MDPB) and methacryloxylethylcetyl dimethyl ammonium chloride (DMAE-CB) could copolymerize with other dental monomers to form antibacterial polymer matrices to reduce bacterial growth [17,18,22]. Recently, dimethylaminododecyl methacrylate (DMADDM) was synthesized and exhibited a stronger antibacterial efficacy [24]. A bonding agent containing DMADDM showed no decrease in antibacterial activity after 6 months of water aging, compared with that at 1 day [25]. However, the in vivo properties of DMADDM resins need to be tested.

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New restorative materials need to be compatible with the dentin–pulp complex. It is important to investigate the influence of antibacterial monomers on pulpal healing after cavity restoration. MDPB exhibited a low level of toxicity to human pulpal cells similar to triethyleneglycol dimethacrylate (TEGDMA) [26]. The incorporation of MDPB into a primer did not increase the toxicity on pulpal cells [27]. The inhibitory effects of MDPB on the proliferation and mineralization of odontoblast-like MDPC-23 mouse cells were lower than those of bisphenol A glycidyl methacrylate (BisGMA), indicating that MDPB had better biocompatibility than BisGMA [28]. For the new DMADDM, its median lethal concentration was between 20 and 40 $\mu g\ ml^{-1}$, 20-fold higher than that of the BisGMA control, indicating a much lower cytotoxicity than BisGMA [29].

Most previous studies on biocompatibility of antibacterial monomers were in vitro. Animal models could provide an alternative to human models for the assessment of restoratives. Previous animal models used monkeys [30], dogs [31], ferrets [32] and rats [33]. Ethical and cost considerations have favored rat models by many researchers. Several studies showed that the healing of rat molar pulps after pulp-capping was histologically similar to humans and other animal species [34]. Rat molars could be considered anatomically, biologically and physiologically similar to human molars [34]. Previous studies used maxillary molars in rats and class I [35–37] and class V preparations [38] were tested. However, there has been no report on investigating antibacterial QAM-containing adhesives and composites in a rat tooth model. It remains to be investigated whether the QAM-incorporating materials in deep cavity restorations would exert adverse influence on the dentin–pulp complex in vivo.

Another approach to combat caries is to impart remineralizing capability to restorations [39–45]. Previous studies incorporated calcium phosphate (CaP) particles of ~1–55 μm in size into resins [39,40]. These traditional CaP composites released calcium (Ca) and phosphate (P) ions and remineralized tooth lesions in vitro [39,40]. CaP nanoparticles of ~100 nm in size were also filled into resins [41,42]. Composites containing nanoparticles of amorphous calcium phosphate (NACP) with a high surface area released high levels of Ca and inorganic phosphate (Pi) ions while possessing flexural strength nearly twofold that of traditional CaP composites [41,42]. However, to date, there has been no report on in vivo pulpal response to composites and adhesives containing antibacterial monomer and CaP nanoparticles.

The objectives of this study were to investigate novel antibacterial and remineralizing restoratives in a rat tooth model, and examine pulpal inflammation and tertiary dentin formation using nanocomposite and adhesive containing NACP and DMADDM. It was hypothesized that: (i) the antibacterial and remineralizing nanocomposite and adhesive will be more biocompatible with less pulpal inflammation than traditional composite and adhesive control; (ii) adding DMADDM into the composite and adhesive with antibacterial activity will not adversely affect pulpal response, compared with the control without DMADDM; and (iii) adding NACP into the composite and adhesive will reduce pulpal inflammation and greatly increase tertiary dentin formation.

2. Materials and methods

2.1. Synthesis of NACP and DMADDM

NACP were synthesized via a spray-drying technique as previously described [41,42,44]. Briefly, a solution was prepared by dissolving calcium carbonate ($CaCO_3$, Fisher, Fair Lawn, NJ) and dicalcium phosphate anhydrous ($CaHPO_4$) (J.T. Baker, Phillipsburg, NJ) into an acetic acid solution. This solution was sprayed through a nozzle into a heated chamber. The water and volatile acid were evaporated and expelled into an exhaust hood [41,42,44]. The dried particles were collected by an electrostatic precipitator [44]. A previous study determined that the NACP surface area was 17.76 $m^2\ g^{-1}$ and mean particle size was 116 nm [44].

The synthesis of DMADDM was recently described [24,25]. Briefly, a modified Menschutkin reaction was used where a tertiary amine group was reacted with an organo-halide [23]. A benefit of this reaction is that the reaction products are generated in quantitative amounts and require minimal purification. Aliquots of 10 mmol of 1-(dimethylamino)docecane (Tokyo Chemical Industry, Japan) and 10 mmol of 2-bromoethyl methacrylate (Monomer-Polymer and Dajec, Trevose, PA) 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 evaporated in the air, yielding DMADDM as a clear, colorless and viscous liquid [24,25]. The reaction and product of DMADDM were verified via Fourier transform infrared spectroscopy as described in a recent study [24].

2.2. Fabrication of composite and bonding agent

BisGMA and TEGDMA (Esstech, Essington, PA) at a mass ratio of 1:1 were rendered light-curable with 0.2% camphorquinone and 0.8% ethyl 4-*N*,*N*-dimethylaminobenzoate (all mass fractions) [44]. DMADDM was mixed with the photo-activated BisGMA–TEGDMA resin at 5% by mass: DMADDM/(BisGMA–TEGDMA + DMADDM) = 5%. This resin is referred to as BisGMA–TEGDMA–DMADDM. Barium boroaluminosilicate glass with a median particle size of 1.4 μm (Caulk/Dentsply, Milford, DE) was silanized with 4% 3-methacryloxypropyltrimethoxysilane and 2% n-propylamine [44]. NACP and glass particles were mixed into resin to fabricate the following composites:

(1) Control composite: 30% BisGMA–TEGDMA resin + 70% glass.
(2) DMADDM composite: 30% BisGMA–TEGDMA–DMADDM resin + 70% glass.
(3) NACP composite: 30% BisGMA–TEGDMA + 35% glass + 30% NACP.
(4) DMADDM + NACP composite: 30% BisGMA–TEGDMA–DMADDM + 35% glass + 30% NACP.

The parent adhesive system was Scotchbond multi-purpose bonding system (3 M, St Paul, MN), referred as "SBMP". According to the manufacturer, SBMP adhesive contained 60–70% of BisGMA, 30–40% of 2-hydroxyethyl methacrylate (HEMA), tertiary amines and photo-initiator. SBMP primer contained 35–45% of HEMA, 10–20% of a copolymer of acrylic and itaconic acids and 40–50% water. Four bonding agents were tested:

(1) Control bonding agent: unmodified SBMP primer and adhesive.
(2) DMADDM bonding agent: SBMP primer + 5% DMADDM, SBMP adhesive + 5% DMADDM.
(3) NACP bonding agent: unmodified SBMP primer, SBMP adhesive + 30% NACP.
(4) DMADDM + NACP bonding agent: SBMP primer + 5% DMADDM, SBMP adhesive + 5% DMADDM + 30% NACP.

The 5% quaternary ammonium was used following previous studies which showed strong antibacterial activity without negatively affecting dentin bond strength [25]. The 30% NACP in resin was shown to result in high levels of Ca and Pi ion release [44,46].