



# Poly (amido amine) and nano-calcium phosphate bonding agent to remineralize tooth dentin in cyclic artificial saliva/lactic acid



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## ARTICLE INFO

### Article history:

Received 14 July 2016

Received in revised form 12 October 2016

Accepted 7 November 2016

Available online 9 November 2016

### Keywords:

Dentin

Remineralization

Poly (amido amine) dendrimer

Bonding agent

Acid challenge

Calcium phosphate nanoparticles

## ABSTRACT

The objectives of this study were to develop a novel method to remineralize dentin lesions, and investigate the remineralization effects of poly (amido amine) (PAMAM) dendrimer plus a bonding agent with nanoparticles of amorphous calcium phosphate (NACP) in a cyclic artificial saliva/lactic acid environment for the first time. Dentin lesions were produced via phosphoric acid. Four groups were tested: (1) dentin control, (2) dentin with PAMAM, (3) dentin with NACP bonding agent, and (4) dentin with PAMAM plus NACP bonding agent. Specimens were treated with cyclic artificial saliva/lactic acid. The remineralized dentin was examined using scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), hardness and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). NACP bonding agent yielded a dentin shear bond strength similar to commercial controls (Prime & Bond NT, Dentsply; Scotchbond Multi-purpose, 3M) ( $p > 0.1$ ). Increasing NACP in bonding agent from 0 to 40% did not affect bond strength. NACP bonding agent neutralized the acid and released Ca ions with concentrations of 4 to 20 mmol/L, and P ions of 2 to 9 mmol/L. PAMAM or NACP bonding agent alone achieved slight remineralization. The PAMAM + NACP group achieved the greatest dentin remineralization ( $p < 0.05$ ). At 20 days, PAMAM + NACP increased the hardness of pre-demineralized dentin to reach the normal dentin hardness ( $p > 0.1$ ). In conclusion, superior remineralization of PAMAM + NACP bonding agent was demonstrated for the first time. PAMAM + NACP bonding agent induced dentin remineralization under acid challenge, when conventional remineralization methods such as PAMAM alone did not work well. The novel PAMAM + NACP bonding agent method is promising to improve the longevity of resin-dentin bonds, inhibit caries, and protect teeth.

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## 1. Introduction

Composites are popular for tooth restorations due to their esthetics and direct-filling capability [1,2]. Composites and bonding agent have been improved through materials compositions and placement technologies [3,4]. A strong and durable adhesion to dental hard tissues is a key factor in the success of the restoration [3–6]. However, the oral environment poses severe challenges such as chewing forces and biofilm acids which limit the longevity of the restoration. The resin-tooth bonded interface represents the weak link in the restoration [6], and secondary (recurrent) caries at the margins is a chief limitation to the restoration's service life [7,8]. Therefore, there is an increasing need for the development of dentin bond protection and caries-inhibition strategies.

Dentin bonding involves the infiltration of bonding agent into demineralized dentin collagen to form a hybrid layer (HL) [3–6]. The bonding agent is not only a connection between the tooth structure and the composite, but also a barrier to protect the demineralized collagen scaffold from the acidic and enzymatic attacks of the oral bacteria, enzymes and fluids [6,9,10]. The bond stability is limited by the HL degradation [9]. Hence, improving the long-term stability of HL is key to prolonging the longevity of resin-dentin bonds. Since minerals can protect external risk factors from invading HL, remineralization of the denuded HL appears to be an important strategy for strengthening the resin-dentin bond [11–13].

The process of dental caries is now well-understood [14]. Acid-producing bacteria feed on fermentable carbohydrates and produce organic acids, which dissolve hydroxyapatite minerals leading to caries lesions [15,16]. Prior to cavitation, a subsurface lesion with partial demineralization is present, and this lesion can be remineralized under appropriate conditions [17]. However, natural remineralization can only overcome a low level of caries challenge. When bacterial acid challenge is severe,

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natural remineralization is insufficient [17]. Therefore, the development of effective remineralization approaches is needed to combat secondary caries at the margins and strengthen and protect the tooth structures.

Efforts were made to apply remineralization coatings onto tooth lesions [11–13,18–21]. One such method involved the use of poly (amide amine) (PAMAM) dendrimers [22–29]. PAMAM dendrimers are highly branched polymers and characterized by the presence of internal cavities, a large number of reactive end groups with well-defined sizes and shapes [22]. Recently, several types of PAMAM dendrimers were used as nucleation templates to induce tooth remineralization [23–29]. Amine-terminated PAMAM (PAMAM-NH<sub>2</sub>) regenerated mineral on collagen fibrils [25,27,29]. Polyhydroxy-terminated PAMAM-OH remineralized and induced dentinal tubule occlusion [24]. Carboxylic-terminated PAMAM (PAMAM-COOH) could absorb calcium (Ca) and phosphate (P) ions in collagen fibrils to form intrafibrillar minerals [23]. Furthermore, phosphate-terminated PAMAM (PAMAM-PO<sub>3</sub>H<sub>2</sub>) remineralized dentin in an animal model [28].

Another approach is to incorporate calcium phosphate (CaP) particles into dental resins [30–33]. Bonding agent containing CaP particles could remineralize the remnants of tooth lesions in the cavity and the acid-etched dentin [11]. The Ca and P ion release from bonding agent may serve as seed crystals to facilitate remineralization in HL and at the tooth-restoration margins [9,11]. Thus, CaP bonding agent may protect the exposed collagen in the bonded interface and improve its durability [9,13]. Recently, bonding agents containing nanoparticles of amorphous calcium phosphate (NACP) were developed [34–37]. They could release high levels of Ca and P ions to induce remineralization [34–37]. Due to their small particle sizes, NACP readily flowed with bonding agent into dentinal tubules to form resin tags [34,35]. NACP was “smart” and could substantially increase the Ca and P ion release at a cariogenic pH, when these ions would be most needed to combat caries [36]. In addition, NACP nanocomposite rapidly neutralized a pH 4 solution and increased the pH to a safe level of above 5.5 [38]. NACP nanocomposite remineralized enamel lesions *in vitro*, achieving a remineralization that was 4-fold that of a commercial fluoride-releasing composite [32]. In a human *in situ* model, the NACP nanocomposite inhibited caries at the enamel-composite margins, reducing enamel mineral loss to 1/3 that of a control composite [39].

However, literature search revealed no report on dentin remineralization properties of NACP bonding agent. Furthermore, there has been no report on the effect of combining PAMAM with NACP bonding agent on remineralization. In addition, previous remineralizations induced by PAMAM were achieved in a neutral pH environment [23–28]. There has been no report on remineralization of PAMAM with NACP bonding agent in an acid-challenge environment, even though acid challenges are often encountered orally.

Because acids dissolve hydroxyapatite in teeth [15–17], remineralization in an acid challenge is difficult to achieve, but is more realistic. PAMAM is an excellent nucleation template and can rapidly absorb Ca and P ions to cause remineralization [23–29]; however, it cannot neutralize acids. On the other hand, NACP can neutralize acids and release Ca and P ions [38,40] to decrease the damage caused by acids [32]. However, the lack of nucleation template on the demineralized dentin could limit its remineralization effect [18]. Therefore, it would be highly desirable to combine NACP bonding agent with PAMAM to achieve the double benefits of remineralization-promotion and demineralization-prevention.

Accordingly, the objective of this study was to investigate the effects of PAMAM, NACP bonding agent, and PAMAM + NACP bonding agent, on dentin remineralization in a cyclic artificial saliva/acid challenge environment for the first time. It was hypothesized that: (1) PAMAM treatment would effectively remineralize the demineralized dentin in the cyclic artificial saliva/acid regimen; (2) NACP bonding agent would increase Ca and P ion concentrations, neutralize the acid and promote dentin remineralization in the cyclic artificial saliva/acid treatment; and (3) the combined PAMAM plus NACP bonding agent

method would achieve much greater remineralization in demineralized dentin than PAMAM or NACP alone.

## 2. Materials and methods

### 2.1. PAMAM synthesis

PAMAM dendrimers were synthesized as described previously [41, 42]. Briefly, the divergent synthesis of PAMAM dendrimers included a two-step interactive sequence to produce amine-terminated structures. Iterative sequencing involved alkylation with methyl acrylate (MA), followed by amidation with excess 1,2-ethylenediamine (EDA). The alkylation step produced ester-terminated intermediates that were called “half-generations”. The second step involved amidation of the ester-terminated intermediates with a large excess of EDA to produce amine terminated intermediates, which were called “full-generations”. The third generation PAMAM dendrimers were synthesized for further carboxyl modification. 2 g of dry third generation PAMAM was dissolved in 25 mL dimethyl sulfoxide (DMSO) in a round-bottom flask. Then 15 mL of DMSO solution containing 5.15 g succinic anhydride (molar ratio of SAH/—NH<sub>2</sub> = 5:1) was added under vigorous stirring and was reacted for 24 h without oxygen. The DMSO solution was dialyzed against water to remove the excess succinic anhydride as well as the organic solvent. The aqueous retentate was filtered using a 0.45 mm diameter membrane, and then was lyophilized. The first and second generations are linear molecules, while the third generation is a sphere molecule with more functional groups to absorb more Ca and P ions during remineralization. Previous studies showed that PAMAM-COOH effectively remineralized the demineralized human tooth [23,42]. The present study used the third generation PAMAM-COOH (G3-PAMAM-COOH) from Chenyuan Dendrimer Tech. (Weihai, China). In this article, the term “PAMAM” refers to G3-PAMAM-COOH. PAMAM solution was prepared by dissolving 500 mg of PAMAM powder in 50 mL of distilled water to achieve a concentration of 10 mg/mL [23,42].

### 2.2. NACP bonding agent fabrication

NACP [Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>] were synthesized via a spray-drying technique [32, 40]. Briefly, calcium carbonate and dicalcium phosphate were dissolved into an acetic acid solution. The Ca and P ion concentrations were 8 and 5.333 mmol/L, respectively. The solution was sprayed into a heated chamber to evaporate the water and volatile acid. The dried particles were collected by an electrostatic precipitator. This yielded NACP with a mean particle size of 116 nm [32,40]. Barium boroaluminosilicate glass particles (1.4 μm median size, Caulk/Dentsply, Milford, DE) were silanized with 4% 3-methacryloxypropyltrimethoxysilane and 2% n-propylamine as previously described [32,40].

A pyromellitic glycerol dimethacrylate (PMGDM)-containing primer, previously reported to yield good dentin bonding properties [35, 37], was used. It contained PMGDM (Esstech, Essington, PA) and 2-hydroxyethyl methacrylate (HEMA) (Esstech, Essington, PA) at a mass ratio 3.3/1, with 50% acetone solvent (all mass fractions) [35,37].

To fabricate the bonding agent, PMGDM and ethoxylated bisphenol A dimethacrylate (EBPADMA) (Sigma-Aldrich, St. Louis, MO) were mixed at a 1:1 mass ratio [37]. It was rendered light-curable with 1% phenylbis (2,4,6-trimethylbenzoyl) phosphine oxide (Sigma-Aldrich) [37]. Then, 10% HEMA and 5% bisphenol A glycidyl dimethacrylate (BisGMA) (Esstech) were incorporated into the PMGDM-EBPADMA resin. This PMGDM-EBPADMA-HEMA-BisGMA bonding agent is designated PEHB. Our preliminary study showed a high level of Ca and P ion release and recharge using the PEHB resin. PMGDM is an acidic adhesive monomer [43], and can chelate with calcium ions from the recharging solution to achieve the recharging capability. Previous studies showed that HEMA could improve flowability and hydrophilicity, and a small amount of BisGMA could improve the cross-linkage of monomers and the bonding strength of the bonding agent [43].

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