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Long-term dentin remineralization by poly(amido amine) and rechargeable calcium phosphate nanocomposite after fluid challenges

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ABSTRACT

Objective. Previous studies investigated short-term dentin remineralization; studies on long-term dentin remineralization after fluid challenges mimicking fluids in oral environment are lacking. The objective of this study was to develop a long-term remineralization method to via poly(amido amine) (PAMAM) and rechargeable composite containing nanoparticles of amorphous calcium phosphate (NACP) after fluid challenges for the first time.

Methods. NACP composite was immersed at pH 4 to exhaust its calcium (Ca) and phosphate (P) ions, and then recharged with Ca and P ions, to test the remineralization of the exhausted and recharged NACP composite. Dentin was acid-etched with 37% phosphoric acid. Four groups were prepared: (1) dentin control, (2) dentin with PAMAM, (3) dentin with the recharged NACP composite, and (4) dentin with PAMAM plus recharged NACP composite. PAMAM-coated dentin was immersed in phosphate-buffered saline with shaking for 72 days, because there is fluid flow in the mouth which could potentially detach the PAMAM from dentin. Specimens were treated with a cyclic artificial saliva/lactic acid regimen for 35 days.

Results. After 72 days of immersion plus shaking, the PAMAM still successfully fulfilled its mineralization nucleation. The recharged NACP composite still provided acid-neutralization and ion re-release, which did not decrease with increasing the number of recharge cycles. The immersed-PAMAM plus NACP achieved complete dentin remineralization and restored the hardness to that of healthy dentin.

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Significance. In conclusion, superior long-term remineralization of the PAMAM plus NACP method was demonstrated for the first time. The immersed-PAMAM plus recharged NACP completely remineralized the pre-demineralized dentin, even after prolonged fluid-challenge similar to that in oral environment. The novel PAMAM plus NACP composite method is promising to provide long-term tooth protection and caries inhibition.

1. Introduction

Tooth cavity restorations costs about $46 billion annually in the USA, and the replacement of failed restorations accounts for 50–70% of all restorations placed [1]. Secondary caries is a main reason for composite restoration failures [2–6]. Natural remineralization via saliva is too weak to reverse the caries process when bacterial acid challenge is severe [7–10]. Hence, several methods were applied to enhance tooth remineralization. For example, nucleation template materials were applied on teeth to help attract calcium (Ca) and phosphorus (P) ions for remineralization [11–17]. Poly(amido amine) (PAMAM) dendrimers are highly-branched polymers with internal cavities and abundant ending functional groups [18]. They are promising for tooth remineralization by providing nucleation templates. Indeed, hydroxyl-terminated PAMAM (PAMAM-OH) induced remineralization and deep dentinal tubule occlusion [19]. Carboxylic-terminated PAMAM (PAMAM-COOH) could absorb Ca and P ions in collagen fibrils to regenerate intrafibrillar minerals [20]. Amine-terminated PAMAM (PAMAM-NH_2) facilitated mineral deposit in pre-demineralized dentin [21]. Phosphate-terminated PAMAM (PAMAM-PO_3H_2) remineralized the demineralized dentin in the oral cavity of mice [22].

Another important approach for remineralization was to use resins containing CaP [23–25]. For example, resins containing nanoparticles of amorphous calcium phosphate (NACP) released high levels of Ca and P ions [26] and rapidly neutralized acid challenges [27]. NACP composite remineralized enamel lesions in vitro [28], and inhibited caries formation at the tooth-composite interface in a human in situ model [29].

In our previous study, the PAMAM plus NACP composite combination method (PAMAM + NACP composite) achieved triple benefits: providing the nucleation templates, acid-neutralization, and ion release, thereby remineralizing the pre-demineralized dentin [30]. However, the results also showed that the ion release of NACP composite diminished with increasing immersion time. In addition, the fluids in the oral cavity may detach and remove the PAMAM macromolecules from dentin, thus removing the nucleation templates. Therefore, there is a need to develop a PAMAM + NACP composite method that is effective for dentin remineralization even with fluid shaking and immersion. To date, there has been no report on the remineralization effect of PAMAM + NACP composite after long periods of fluid immersion and fluid shaking challenges.

Calcium phosphate composites released Ca and P ions lasting for only several weeks and then the ion release was diminished. Recently, a new NACP composite was developed using a resin of ethoxylated bisphenol A dimethacrylate (EBPADMA) and pyromellitic glycerol dimethacrylate (PMGDM) (referred to as EBPM); this composite could be repeatedly recharged for long-term Ca and P ion release [31]. Therefore, it would be promising to combine PAMAM with the rechargeable NACP composite to achieve long-term dentin remineralization with fluid challenges.

Accordingly, the objectives of this study were to: (1) develop a novel remineralization method that is effective even after long periods of fluid challenges; and (2) investigate the effects of PAMAM after fluid immersion and shaking, plus the ion-exhausted and then recharged NACP composite, on dentin remineralization for the first time. It was hypothesized that: (1) PAMAM could still fulfill its nucleation template function even after prolonged fluid challenge of immersion and shaking; (2) the exhausted and recharged NACP composite could still provide acid-neutralization and ion re-release capability; (3) the fluid challenged-PAMAM plus the recharged NACP composite would achieve complete remineralization of pre-demineralized dentin and restore its hardness to that of healthy dentin.

2. Materials and methods

2.1. PAMAM synthesis and rechargeable NACP composite fabrication

PAMAM dendrimers were synthesized as previously described [32], and the third generation of PAMAM-NH_2 (G3-PAMAM-NH_2) was obtained from Chenyuan Dendrimer Company (Weihai, China). In this article, the term "PAMAM" refers to G3-PAMAM-NH_2. PAMAM solution (1 mg/mL) was prepared by dissolving 10 mg of PAMAM powder in 10 mL of deionized water [21].

A spray-drying technique was used to synthesize NACP following a previous study, which yielded NACP with a mean particle size of 116 nm [26]. EBPM resin consisted of PMGDM and EBPADMA at a mass ratio of 1:1, which was then light-activated with 0.2% (all mass %) of camphorquinone and 0.8% of ethyl 4-*N*,*N*-dimethylaminobenzoate [31]. Barium-boroaluminosilicate glass particles (median diameter = 1.4 μm, Caulk/Dentsply, Milford, DE, USA) were silanized with 4% 3-methacryloxypropyltrimethoxysilane and 2% *n*-propylamine. The EBPM-NACP composite had 40% EBPM, 40% NACP and 20% glass particles. The composite paste was placed into molds of 2 × 2 × 12 mm and photo-cured (Triad 2000, Dentsply, York, PA) for 60 s on each open side of the mold.

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