DENTAL MATERIALS 33 (2017) 818–829

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Poly(amido amine) and calcium phosphate nanocomposite remineralization of dentin in acidic solution without calcium phosphate ions

Kunneng Liang[a,b], Han Zhou[a,b], Michael D. Weir[b], Chongyun Bao[a,b], Mark A. Reynolds[b], Xuedong Zhou[a], Jiyao Li[a,**], Hockin H.K. Xu[b,c,d,*]

[a] State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, China

[b] Department of Endodontics, Periodontics and Prosthodontics, University of Maryland School of Dentistry, Baltimore, MD 21201, USA

[c] Center for Stem Cell Biology & Regenerative Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA

[d] Department of Mechanical Engineering, University of Maryland Baltimore County, Baltimore County, MD 21250, USA

ARTICLE INFO

Article history:
Received 28 November 2016
Received in revised form
19 April 2017
Accepted 21 April 2017

Keywords:
Dentin remineralization
Poly(amido amine) dendrimer
Calcium phosphate nanocomposite
Dry mouth
Lactic acid
Caries inhibition

ABSTRACT

Objective. Patients with dry mouth often have an acidic oral environment lacking saliva that provides calcium (Ca) and phosphate (P) ions. However, there has been no study on dentin remineralization by placing samples in an acidic solution without Ca and P ions. Previous studies used saliva-like solutions with neutral pH and Ca and P ions. Therefore, the objective of this study was to investigate a novel method of combining poly(amido amine) (PAMAM) with a composite of nanoparticles of amorphous calcium phosphate (NACP) on dentin remineralization in an acidic solution without Ca and P ions for the first time.

Methods. Demineralized dentin specimens were tested into four groups: (1) dentin control, (2) dentin coated with PAMAM, (3) dentin with NACP nanocomposite, (4) dentin with PAMAM plus NACP composite. Specimens were treated with lactic acid at pH 4 without initial Ca and P ions for 21 days. Acid neutralization and Ca and P ion concentrations were measured. Dentin specimens were examined by scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and hardness testing vs. remineralization efficacy.

Results. NACP composite had mechanical properties similar to commercial control composites ($p > 0.1$). NACP composite neutralized acid and released Ca and P ions. PAMAM alone failed to induce dentin remineralization. NACP alone achieved mild remineralization and slightly increased dentin hardness at 21 days ($p > 0.1$). In contrast, the PAMAM + NACP nanocomposite method in acid solution without initial Ca and P ions greatly remineralized the pre-demineralized dentin, restoring its hardness to approach that of healthy dentin ($p > 0.1$).

Significance. Dentin remineralization via PAMAM + NACP in pH 4 acid without initial Ca and P ions was demonstrated for the first time, when conventional methods such as PAMAM did

* *Corresponding author at*: Department of Endodontics, Periodontics and Prosthodontics, University of Maryland Dental School, Baltimore, MD 21201, USA.
** *Corresponding author*.
E-mail addresses: jiyaoliscu@163.com (J. Li), hxu@umaryland.edu (H.H.K. Xu).
http://dx.doi.org/10.1016/j.dental.2017.04.016

not work. The novel PAMAM + NACP nanocomposite method is promising to protect tooth structures, especially for patients with reduced saliva to inhibit caries.

1. Introduction

In the United States, 166 million tooth cavity restorations are placed annually, costing $46 billion per year [1]. Composites are popular due to their esthetics, direct-filling ability and enhanced performance [2,3]. However, secondary caries is a frequent reason for failure [4–6], and replacement of failed restorations accounts for 50–70% of all restorations placed [4–6]. Among dental patients, individuals with a higher caries risk often have a higher occurrence of initial and secondary caries [7,8]. Saliva plays an important role in the inhibition of dental caries [9–12]. In healthy individuals, the saliva fluid covers the dental hard tissues at all times. The constant flow of saliva helps remove food debris and bacteria by swallowing [9–12]. Saliva has a strong buffer capacity due to its components of bicarbonates, phosphates, and urea [9–12]. With frequent flows, saliva can penetrate into bacteria biofilms, neutralize the acids produced by cariogenic bacteria, and increase the local pH [9–12]. In addition, saliva contains high concentrations of calcium (Ca) and phosphate (P) ions, which help to decrease the solubility of hydroxyapatite, the major component of teeth. The large amounts of Ca and P ions in saliva also favor and promote tooth remineralization [9–12].

However, many people suffer from reduced salivary gland functions; indeed, about 30% of the population reports dry mouth [10]. Many factors can cause xerostomia or hyposalivation, such as excessive intake of alcohol, certain medicines, nutrition deficiency, certain systemic diseases, salivary dysfunction, Sjögren's syndrome, etc. [13]. Saliva flow can drop to only 15%–33% of its normal level due to hyposalivation [12,13]. This means minimal saliva penetration into biofilm to neutralize acids and supply Ca and P ions [12]. Low salivary flow occurs often in seniors [9]. Root caries in the United States increased with aging, from 7% among young people, to 56% in seniors of $\geq$75 years of age [14]. In addition, patients with head and neck cancers taking radiation therapy can have extreme saliva reduction [15–18], causing rampant radiation caries. To date, there is no effective method for people with dry mouth to inhibit caries. While saliva substitutes are used to relieve the sensation of dry mouth, they offer little to protect the teeth [19].

In addition, natural remineralization via saliva can only overcome a relatively low level of caries challenges. When bacterial acid challenge is severe, natural remineralization is insufficient to halt the caries process [20]. Therefore, nucleation templates were applied to tooth lesion surfaces to promote remineralization [21–30]. This strategy is somewhat effective for healthy individuals. However, traditional remineralization agents are ineffective for patients with severe saliva reduction [9,10,12]. For example, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) did not reduce radiation caries progression [31]. There is a need to develop a new remineralization method that is effective in an acidic and Ca and P ion-deficient environment to protect teeth, especially for patients with reduced saliva.

Poly(amido amine) (PAMAM) dendrimers are highly-branched polymers with a central core and a large amount of reactive functional groups [32]. They can serve as nucleation templates to induce remineralization [33–39]. For example, amine-terminated PAMAM (PAMAM-NH_2) regenerated minerals in demineralized dentin and collagen fibrils [36,38]. Polyhydroxy-terminated PAMAM (PAMAM-OH) induced dentinal tubule occlusion [34]. Carboxylic-terminated PAMAM (PAMAM-COOH) could absorb Ca and P ions in collagen fibrils to form intrafibrillar minerals [33]. Phosphate-terminated PAMAM (PAMAM-PO_3H_2) remineralized the demineralized dentin in an animal model [39].

Another approach for remineralization is via calcium phosphate (CaP) composites [40,41]. Resins containing nanoparticles of amorphous calcium phosphate (NACP) released high levels of Ca and P ions [42–46]. NACP nanocomposite rapidly neutralized acids [43], and remineralized enamel lesions in vitro [45]. In a human in situ model, NACP nanocomposite inhibited caries at the enamel-restoration margins [46]. It would be interesting to combine PAMAM with NACP. PAMAM can absorb Ca and P ions for remineralization [33–39]; however, it relies on the supply of Ca and P ions which may be deficient in cases of dry mouth. To date, there has been no report on combining PAMAM with NACP composite, except our pilot study which used a cyclic artificial saliva/lactic acid regimen, where remineralization relied on the artificial saliva containing Ca and P ions [47]. It did not investigate dentin remineralization in an acidic environment without Ca and P ions.

The objectives of this study were to develop a novel remineralization method that is effective even in an acidic solution without any initial Ca and P ions, and to investigate the effects of combining PAMAM with NACP nanocomposite on dentin remineralization, acid neutralization, and dentin hardness. It was hypothesized that: (1) PAMAM alone could not remineralize dentin in a lactic acid solution; (2) NACP nanocomposite could neutralize the acid, release Ca and P ions and promote dentin remineralization in lactic acid; (3) the novel PAMAM + NACP composite combined method would achieve the greatest remineralization in dentin, and effectively restore the hardness for pre-demineralized dentin.

2. Materials and methods

2.1. PAMAM synthesis

PAMAM dendrimers were synthesized following a previous study [48]. Briefly, the divergent synthesis of PAMAM dendrimers included a two-step interactive sequence to produce