Available online at www.sciencedirect.com

ScienceDirect

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

Bifunctional dentifrice: Amorphous polyphosphate a regeneratively active sealant with potent anti-*Streptococcus mutans* activity

Werner E.G. Müller[a,*], *Meik Neufurth*[a], *Maximilian Ackermann*[b], *Emad Tolba*[a], *Michael Korzhev*[a], *Shunfeng Wang*[a], *Qingling Feng*[c], *Heinz C. Schröder*[a], *Xiaohong Wang*[a,*]

[a] *ERC Advanced Investigator Grant Research Group at the Institute for Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Duesbergweg 6, D-55128 Mainz, Germany*

[b] *Institute of Functional and Clinical Anatomy, University Medical Center of the Johannes Gutenberg University, Johann Joachim Becher Weg 13, D-55099 Mainz, Germany*

[c] *Laboratory of Advanced Materials of Ministry of Education of China, School of Materials Science and Engineering, Tsinghua University, Beijing 100084, China*

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form
17 March 2017
Accepted 11 April 2017

Keywords:
Inorganic polyphosphate
Microparticles
Dentifrice
Alkaline phosphatase
Antibacterial activity
Anti-biofilm activity
Enamel/dentin damage repair
Streptococcus mutans
Caries

ABSTRACT

Objective. In this study we demonstrate that inorganic polyphosphate (polyP) exhibits a dual protective effect on teeth: it elicits a strong antibacterial effect against the cariogenic bacterium *Streptococcus mutans* and, in form of amorphous calcium polyP microparticles (size of 100–400 nm), it efficiently reseals cracks/fissures in the tooth enamel and dentin.

Methods. Three different formulations of amorphous polyP microparticles (Ca-polyP, Zn-polyP and Sr-polyP) were prepared.

Results. Among the different polyP microparticles tested, the Ca-polyP microparticles, as a component of a newly developed formulation of a dentifrice, turned out to be most effective in inhibiting growth of *S. mutans*. Further studies have shown that it is mainly the soluble polyP, which has a strong antibacterial activity, either given as sodium salt of polyP or formed by partial disintegration of the microparticles *via* the alkaline phosphatase present in the oropharyngeal cavity. In addition, we demonstrate that the developed toothpaste containing incorporated amorphous polyP microparticles, efficiently reduces dental biofilm formation.

Significance. From our results we conclude that polyP microparticles, if added to toothpaste in an amorphous state, might be beneficial not only for restoring tooth damages but also because they provide a suitable depot of functionally/antibacterially active soluble polyP.

1. Introduction

The development of tooth is an intricate process and the result of a cumulative and reiterative molecular signaling between dental epithelium and dental mesenchyme [1]. While dental mesenchyme differentiates into the pulp and the dentin segment of the complex tooth organ, the epithelial tissues give rise to the enamel layer producing the dental enamel [2]. Both inorganic matrices, enamel and dentin,

* *Corresponding authors.* Fax: +49 6131 39 25243.
E-mail addresses: wmueller@uni-mainz.de (W.E.G. Müller), wang013@uni-mainz.de (X. Wang).
http://dx.doi.org/10.1016/j.dental.2017.04.009

are composed primarily of calcium and phosphate linked together by physico-chemical interactions [3]. The overall chemical composition of tooth basically consists of hydroxyapatite (HA) crystals, $Ca_{10}(PO_4)_6(OH)_2$, supplemented with minor components, *e.g.*, carbon, magnesium, sodium, and fluoride. These latter constituents are biologically important [4]. While enamel is built from ribbon-like carbonatoapatite crystals, measuring 60–70 nm in width and 25–30 nm in thickness, dentin is formed of plate-like crystallites, with 2–5 nm in thickness and 60 nm in length [5]. Mature enamel is an acellular zone that does not regenerate by itself, in contrast to other biomineralized tissues such as bone and dentin [6].

Consequently, the enamel is a biologically inert biomaterial, while dentin, likewise a biomaterial, is regeneratively active. However, appositional Ca-phosphate deposits can be formed on both components of teeth if suitable externally supplied precursors are provided. It is proven that phosphate and fluoride ions can be integrated into living enamel and dentin [7]. In addition, amorphous, non-crystalline, but not (or only slightly) crystalline solids can be integrated into the crystalline bone or tooth matrix [8,9]. Learning from nature and asking for the source of phosphate, required for the synthesis of bone and tooth, it became overt that the substrate of choice is inorganic polyphosphate (polyP), a bio-polymer synthesized by cells in humans, in particular blood platelets [10–12]. PolyP is a polymer of three to hundreds of orthophosphate residues that are linked together by high-energy phosphoanhydride bonds, like in ATP [13]. Like prokaryotes, eukaryotes, including humans, store polyP together with Ca^{2+} in 100–200 nm large acidocalcisomes, where the polymer surely remains in the amorphous state [14,15]. Recently, we succeeded to fabricate amorphous polyP microparticles, as well as nanoparticles by adding Ca^{2+} in an over-stoichiometric ratio to soluble Na-polyP (based on phosphate units) [16]. The amorphous calcium polyP (Ca-polyP) particles formed were found to be capable of stimulating bone formation both *in vitro* and *in vivo* [8,17]. PolyP is a decisive inducer of the gene encoding the alkaline phosphatase (ALP) [17]. In addition, this polymer, as Na-polyP or as polyP particles, is prone to enzymatic hydrolysis by ALP [18]. Experimental evidence suggests that in mammalian cells polyP is taken up, as microparticles or nanoparticles, by clathrin-mediated/receptor-mediated endocytosis [11,19].

The ALP has been identified in dental pellicle, a protein film that is formed on the surface enamel by binding of glycoproteins present in the saliva [20]. Dental pellicle is formed in seconds after tooth cleaning and by that protects the tooth from metabolic acids, produced by oral microorganisms, as well as from extrinsic stains [21–23]. Among the powerful agents, displacing stain from teeth, are Na-pyrophosphate and Na-tripolyphosphate, which act both as dentifrice abrasives [24] and as antibacterial agents against *Streptococcus mutans* [25]. *S. mutans* have a central role in the etiology of dental caries since they are strong acid producers; by that, these bacteria cause an acidic environment with the (potential) consequence of creating cavities. The synthesis of polyP by bacteria in the dental pellicle has been experimentally demonstrated and reported to be associated with the appearance of electron-lucent "holes", resembling acidocalcisomes [26].

Very recently we demonstrated that dentifrice, supplemented with polyP microparticles, can sustainably reseal cracks in both tooth enamel and dentin [9]. In the present study we highlight the dual effect of polyP, *first* to elicit strong antibacterial effect against the caries- and cavity-inducing bacterium *S. mutans*, and, *second* to reseal dental cracks/fissures. In addition, we present a formulation of a dentifrice containing polyP compounds/particles in a functionally active amorphous state.

2. Materials and methods

2.1. Materials

Sodium polyphosphate (Na-polyP) with an average chain length of ≈40 phosphate units was obtained from Chemische Fabrik Budenheim (Budenheim, Germany).

2.2. Preparation of amorphous Ca-polyP microparticles

Based on our previously outlined strategy [16] we formulated solid salts between Ca^{2+} and polyP (Ca-polyP) that are amorphous. The standard Ca-polyP particles have a size range between 100 nm and 400 nm [16,19] and can be produced with a definite size range by selecting a tuned alteration of the Ca:P starting molar ratio. A higher ratio of Ca:P (>2) will result in a smaller size of the particles, compared to larger particles that are formed at lower Ca:P ratios (<2).

In the present study Ca-polyP microparticles (Ca-polyP-MP) were prepared following the standard procedure [16,19]. In this procedure the weight concentration ratio between Ca and P was set to ≈2. For this formulation, 2.8 g of $CaCl_2{\cdot}2H_2O$ (#223506; Sigma–Aldrich, Taufkirchen; Germany) were dissolved in 50 ml ethanol solution (96%) and added drop-wise to 1 g of Na-polyP, dissolved in 50 ml distilled water at room temperature. The suspension formed was kept at pH 6 (using 1 N NaOH) and stirred for 5 h. The microparticles formed were collected by filtration (Nalgene Filter Units [pore size 0.45 μm]; Cole-Parmer, Kehl/Rhein; Germany) and washed three times with ethanol. Subsequently, the particles were dried at 60 °C and sieved through a sieve shaker AS 200 (mesh size 100 μm; Retsch GmbH, Haan; Germany). The resulting microparticles are termed "Ca-polyP-MP". The particles, for which Ca^{2+} has been replaced by zinc (Zn^{2+}) ["Zn-polyP-MP"] or strontium (Sr^{2+}) cations ["Sr-polyP-MP"], were prepared accordingly, by replacing $CaCl_2{\cdot}2H_2O$ with 2.74 g of $ZnCl_2$ (anhydrous zinc chloride, #793523; Sigma) or 3.19 g of $SrCl_2$ (hexahydrate; #107865, Merck, Darmstadt; Germany), respectively.

2.3. Tooth samples

We used molar and premolar human teeth as sample material for treatment with the experimental dentifrice. They were provided by the Institute of Functional and Clinical Anatomy, University Medical Center of the Johannes Gutenberg University, Mainz, Germany, following the ethical guidelines of the University Medical Center Mainz. Prior to the experiments, the specimens were cleaned from organic material by incubation in 4% sodium hypochlorite solution for 4 h. Subsequently, the samples were thoroughly rinsed with distilled water then air dried.