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Novel hydroxyapatite nanorods improve anti-caries efficacy of enamel infiltrants

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ABSTRACT

Objectives. Enamel resin infiltrants are biomaterials able to treat enamel caries at early stages. Nevertheless, they cannot prevent further demineralization of mineral-depleted enamel. Therefore, the aim of this work was to synthesize and incorporate specific hydroxyapatite nanoparticles (HAps) into the resin infiltrant to overcome this issue.

Methods. HAps were prepared using a hydrothermal method (0 h, 2 h and 5 h). The crystallinity, crystallite size and morphology of the nanoparticles were characterized through XRD, FT-IR and TEM. HAps were then incorporated (10 wt%) into a light-curing co-monomer resin blend (control) to create different resin-based enamel infiltrants (HAp-0 h, HAp-2 h and HAp-5 h), whose degree of conversion (DC) was assessed by FT-IR. Enamel caries lesions were first artificially created in extracted human molars and infiltrated using the tested resin infiltrants. Specimens were submitted to pH-cycling to simulate recurrent caries. Knoop microhardness of resin-infiltrated underlying and surrounding enamel was analyzed before and after pH challenge.

Results. Whilst HAp-0 h resulted amorphous, HAp-2 h and HAp-5 h presented nanorod morphology and higher crystallinity. Resin infiltration doped with HAp-2 h and HAp-5 h caused higher enamel resistance against demineralization compared to control HAp-free and HAp-0 h infiltration. The inclusion of more crystalline HAp nanorods (HAp-2 h and HAp-5 h) increased significantly ($p < 0.05$) the DC.

Significance. Incorporation of more crystalline HAp nanorods into enamel resin infiltrants may be a feasible method to improve the overall performance in the prevention of recurrent demineralization (e.g. caries lesion) in resin-infiltrated enamel.

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1. Introduction

Hydroxyapatite [HAp = $Ca_{10}(PO_4)_6(OH)_2$] is the most abundant calcium-phosphate mineral present in bone and teeth [1–3]. HAp can be synthesized as bioceramic and used as solid or porous coating for implants' surfaces or as filler in bio-composites [4–6]. HAp possesses interesting properties of biocompatibility, osteoconductivity and bioactivity, which make this material an excellent candidate for therapeutic applications in biomedical science [7–9] and bioactive therapeutic dentistry [10]. Several methods are currently employed to synthesize HAp crystals [5], such as reactions in the solid state [7], co-precipitation [8], hydrothermal reaction [9,10], sol–gel [11], micro-emulsions [12] and mechanic-synthesis [13]. Each of these syntheses may generate HAp particles with a wide range of particle sizes as well as different chemico-physical characteristics and properties. However, over the last ten years, innovative methods have been used to generate nanoparticles to be incorporated in biomaterials in order to obtain superior physicochemical properties and greater functionalities [14]. Indeed, nanotechnology has the potential to benefit the development of HAp applied in medicine and dentistry [15]. Nano-crystalline HAp can exhibit enhanced bioreactivity [16,17]. This superior performance of nano-HAp is due to their similarity to natural HAp found in human hard tissues. Furthermore, nanoscale HAps exhibit higher sintering, densification and tensile strength [10,18]. However, the bioactivity, biocompatibility and mechanical properties of HAps are determined by their morphology, crystallite size and degree of purity [19]. Therefore, it is highly desirable to synthesize HAps with a controlled-method to achieve precise morphology, crystallinity and size [5]. The hydrothermal synthesis appears to be a feasible alternative to accomplish these targets [10]. Indeed, the outstanding advantage of this route is the capability of inducing the 1D growth, leading to the formation of nanorods, which represents the morphology of HAp in bone and teeth [5,18].

Dental caries remains one of the most predominant health disorders in modern society. Caries progression has its threshold generally influenced by the adherence of a specific and complex biofilm onto enamel surface [20]. A cariogenic biofilm utilizes carbohydrates such as sugars as energy source, which are then digested and transformed in catabolic acids (i.e. mainly lactic and acetic acids), which firstly demineralize enamel and subsequently underlying dentin [21]. Likewise most human diseases, dental caries may be easily and more accurately controlled in its initial stages. To date, biomaterials able to arrest caries progression at very early stages of enamel demineralization are resin infiltrants [22]. These materials consist of very low viscosity dimethacrylate-based monomers capable of infiltrating demineralized enamel and paralyzing caries progression. Nevertheless, such materials are unable to prevent further recurrent caries and to remineralize the infiltrated treated enamel [23].

In dental biomaterials, nano-HAp has shown to be an adequate filler for adhesive resins [24–30] to improve their adhesion to dental hard tissues and preserve mechanical properties after water aging [25]. However, there is no information so far regarding the use of resin-based enamel infiltrants doped with different types of nano-HAp and on their potential in inhibiting recurrent enamel demineralization.

Thus, the aims of this study were (1) to synthesize and characterize nano-hydroxyapatite (HAp) by using co-precipitation and hydrothermal method in order to regulate crystallinity, crystallite size and morphology of the particles, and (2) to assess their effects on the degree of conversion (DC) and on the protective role of enamel infiltrants containing 10 wt% HAp nanoparticles against recurrent demineralization. The hypotheses tested were: (1) the hydrothermal synthesis creates nanoparticles of hydroxyapatite with resembling shape and size of the enamel HAp; (2) the presence of the HAp nanoparticles in the infiltrant resin would induce difference in the polymerization (DC); (3) the addition of HAp nanoparticles improves the enamel resistance against recurrent demineralization at surrounding, underlying and infiltrated area.

2. Materials and methods

2.1. Synthesis of HAp nanoparticles

A solution of phosphoric acid (0.3 mol L^{-1} H_3PO_4) was added to a 0.5 mol L^{-1} $CaCl_2{\cdot}H_2O$ (99.67% purity, Quimex, Dinamica, São Paulo, Brazil) solution (molar ratio Ca/P = 1.67) under continuous stirring at room temperature. A white precipitate was obtained by the addition of 30% NH_4OH (99.5% purity, Vetec, São Paulo, Brazil) solution up to reach pH 9 [24]. The white precipitate was washed with distilled water and vacuum filtered. A part of this precipitate represented the specimen HAp0 h. Thereafter, the powder was dispersed in NH_4Cl 0.1 mol L^{-1} solution (99.5% purity, Vetec) with pH 9; the weight ratio between the precipitate and the solution was 1:10. The suspensions were placed in a Teflon autoclave covered with stainless steel to receive the hydrothermal treatment at 150 °C for 2 h (HAp2 h) or 5 h (HAp5 h). Finally, the material was vacuum filtered, washed and dried at 80 °C for 4 h and stored in the desiccator [10]. The synthesis followed the equation:

$$10CaCl_2 + 6H_3PO_4 + 2NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18HCl + 2NH_4Cl \quad (1)$$

2.2. X-ray diffraction

The XRD assay was performed using a X-ray powder diffractometer X'Pert MPD (PANalytical, Westborough, USA) equipped with Co Kα tube (λ = 1.7890 nm), 40 kV voltage and a 30 mA current in a range of scanning $2\theta = 20–80°$. The diffraction patterns were obtained using Bragg–Brentano geometry in continuous mode with speed of 0.5° min^{-1} and step size of 0.02° (2θ). The Rietveld structure refinement was used for interpreting and analyzing the diffraction data using the program Topas Academic [26]. The crystallite size was refined considering an anisotropic macro [32,33]. For this particular hexagonal case, the Lorentzian and Gaussian components were constrained to be equal for the group planes ($h\,0\,0$) = ($0\,k\,0$), and ($h\,0\,l$) = ($0\,k\,l$). All others were refined independently. The background was adjusted by 5 parameters using a Chebyschev