



Synthesis and characterization of silver phosphate/calcium phosphate mixed particles capable of silver nanoparticle formation by photoreduction



Livia C. Natale^a, Yvette Alania^a, Marcela C. Rodrigues^a, Alyne Simões^a, Douglas N. de Souza^a, Erick de Lima^a, Victor E. Arana-Chavez^a, Thiago L.R. Hewer^b, Rochelle Hiers^d, Fernando L. Esteban-Florez^d, Giancarlo E.S. Brito^c, Sharukh Khajotia^d, Roberto R. Braga^{a,*}

^a Department of Biomaterials and Oral Biology, School of Dentistry, University of São Paulo School of Dentistry, São Paulo, Brazil

^b Department of Chemical Engineering, Polytechnic School, University of São Paulo, São Paulo, Brazil

^c Department of Applied Physics, Institute of Physics, University of São Paulo, São Paulo, Brazil

^d Department of Dental Materials, University of Oklahoma Health Sciences Center, Oklahoma City, USA

ARTICLE INFO

Article history:

Received 11 November 2016

Accepted 12 March 2017

Available online 15 March 2017

Keywords:

Calcium phosphate

Silver phosphate

X-ray diffraction

Ion release

Antibacterial

ABSTRACT

Silver phosphate is a semi-conductor sensitive to UV-Vis radiation (<530 nm). Exposure to radiation removes electrons from the oxygen valence shell, which are scavenged by silver cations (Ag^+), forming metallic silver (Ag^0) nanoparticles. The possibility of silver nanoparticle formation *in situ* by a photoreduction process was the basis for the application of mixed calcium phosphate/silver phosphate particles as remineralizing and antibacterial fillers in resin-based dental materials. Mixed phosphate particles were synthesized, characterized and added to a dimethacrylate resin in 20% or 30% mass fractions to investigate their efficacy as ion-releasing fillers for dental remineralization and antibacterial activity. The formation of metallic silver nanoparticles after exposure to visible radiation from a dental curing unit (peak emission: 470 nm) was demonstrated by particle X-ray diffraction and scanning electron microscopy analysis of the composite fractured surface. Calcium and phosphate release from materials containing the mixed particles were similar to those containing pure CaP particles, whereas *Streptococcus mutans* colonies were reduced by three orders of magnitude in relation to the control, which can be attributed to silver release. As expected, the optical properties of the materials containing mixed phosphate particles were compromised by the presence of silver. Nevertheless, materials containing mixed phosphate particles presented higher fracture strength and elastic modulus than those with pure CaP particles.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Dental caries is a multi-factorial infectious disease that leads to irreversible loss of the mineralized tooth structure. Its treatment includes the removal of affected tooth structure and subsequent restoration of cavitated lesions to arrest their progression and re-establish both the original tooth anatomy and its masticatory function. In some cases, it is also important that the restorative material mimics the optical behavior of the surrounding tooth structure for aesthetic reasons. Recent epidemiological data shows that untreated caries lesions in permanent teeth represent the most prevalent health condition worldwide, affecting 2.4 billion people [1].

Dimethacrylate-based composites have become the material of choice for cavity restorations due to their handling characteristics, mechanical properties and optical behavior. However, these materials have a higher tendency to accumulate biofilm in comparison to natural enamel, ceramics and silver amalgam [2,3], which increases the risk of new caries lesions at the tooth/restoration interface and, consequently, restoration failure [4–6]. In order to reduce the incidence of secondary caries around composite restorations and extend their service life, several research groups have investigated ways to introduce remineralizing and/or antibacterial capabilities into resin-based composites [7–13].

Calcium orthophosphate particles used as bioactive fillers in resin-based composites were shown to remineralize non-cavitated enamel lesions [8,14,15] and demineralized dentin [16–18], and postpone the development of caries lesions in the presence of biofilm [19]. Unfortunately, the incorporation of calcium orthophosphate particles in resin matrices has drawbacks. First, the resulting materials have poor

* Corresponding author at: Departamento de Biomateriais e Biologia Oral da FOUUSP, Av. Prof. Lineu Prestes, 2227, São Paulo SP 05508-000, Brazil.
E-mail address: rrbraga@usp.br (R.R. Braga).

mechanical strength and fracture toughness due to the lack of chemical interaction between both phases. As the particles are not bonded to the matrix, they behave as inclusions (defects) in the material, which act as stress raisers and facilitate crack propagation [20,21]. Second, some calcium orthophosphates present refractive index incompatible with that of the resin matrix, compromising the material's translucency [22].

Composites containing antibacterial agents such as quaternary ammonium compounds (QAMs) [23], chlorhexidine [24], and silver nanoparticles [7,10] were shown to reduce both planktonic bacteria and cariogenic biofilm formation. Among them, silver nanoparticles have been extensively studied due to their low toxicity, effectiveness in low concentrations, and long-term action [25]. The incorporation of silver nanoparticles within the resin matrix can occur by fostering their formation *in situ* through the chemical reduction of silver salts, such as silver nitrate [26,27], silver benzoate [10] or silver 2-ethylhexanoate [9, 28], with antibacterial effect in concentrations ranging between 0.03% and 0.50% (by mass). A critical aspect of silver-containing composites is the compromise between silver content and optical properties, as silver oxidation makes the material dark and reduces light transmission, which may impair photoactivation, compromising polymer degree of conversion and overall performance [10,25]. Dimethacrylate-based materials containing both calcium orthophosphate fillers and silver nanoparticles were evaluated for antibacterial activity against cariogenic microorganisms, with good results [28,29].

In the present study, an alternative approach for the development of dimethacrylate-based materials with combined remineralizing and antibacterial activities is proposed, based on the incorporation of mixed particles containing calcium phosphate and silver phosphate (Ag_3PO_4) domains. Silver phosphate is a semiconductor studied in environmental chemistry due to its photocatalytic abilities [30]. When excited by radiation in the UV-Vis range (<530 nm), electrons from the valence band of oxygen atoms are displaced, creating positively-charged holes (*i.e.*, sites from a full valence band with one electron absent) where organic contaminants can be degraded. As a secondary effect, the silver cations scavenge the excited electrons and are reduced to metallic silver [31]. Dental composites polymerized by visible light typically employ camphorquinone as photoinitiator, which has a maximum absorption peak of 470 nm wavelength [32]. Therefore, it can be hypothesized that radiation emitted by dental light curing units could effectively promote the formation of metallic silver nanoparticles *in situ*, by the reduction of Ag^+ from Ag_3PO_4 .

Based on the above, the purpose of the present study was to describe the synthesis and characterization of a mixed calcium phosphate/silver phosphate particle and evaluate its potential as a calcium-, phosphate-, and silver-releasing filler when incorporated in a dimethacrylate-based resin matrix. Materials containing different particle mass fractions were evaluated regarding their degree of conversion, mechanical properties, optical properties, ion release and antimicrobial activity. As controls, particles composed only of calcium orthophosphate as well as the unfilled resin were tested. The null hypotheses were: 1) particle mass fraction does not affect the evaluated properties in comparison to the unfilled resin; 2) the type of particle does not influence the evaluated properties.

2. Methods

2.1. Synthesis of particles

Nominally pure calcium phosphate and the mixed silver/calcium phosphate were prepared by the conventional co-precipitation method. The pure calcium phosphate and mixed silver/calcium phosphates started from diammonium phosphate [$(\text{NH}_4)_2\text{HPO}_4$], calcium nitrate [$\text{Ca}(\text{NO}_3)_2$] and silver nitrate (AgNO_3 , all precursors from Sigma-Aldrich, St. Louis, MO, USA). For the pure calcium phosphate, $\text{Ca}(\text{NO}_3)_2$ and $(\text{NH}_4)_2\text{HPO}_4$ aqueous solutions were initially prepared in concentrations of $0.20 \text{ mol}\cdot\text{L}^{-1}$ and $0.24 \text{ mol}\cdot\text{L}^{-1}$, respectively. Using a

peristaltic pump and flow rate of $1.8 \text{ mL}\cdot\text{min}^{-1}$, 60 mL of $(\text{NH}_4)_2\text{HPO}_4$ solution was pumped into 60 mL of $\text{Ca}(\text{NO}_3)_2$ solution. After complete transfer, the mixture was stirred for 5 h at room temperature. The mixed silver/calcium phosphates were prepared by a similar process as described above with some minor modifications. The appropriate amount of AgNO_3 was dissolved in water and mixed with 60 mL of $\text{Ca}(\text{NO}_3)_2$ aqueous solution ($\text{Ag}/\text{Ca} = 5\%$ by weight). The $(\text{NH}_4)_2\text{HPO}_4$ solution was then added drop-wise ($1.8 \text{ mL}\cdot\text{min}^{-1}$) under vigorous stirring to the silver/calcium nitrate solution. After complete transfer, the mixture was kept under constant stirring for 5 h at room temperature. The pure calcium phosphate and mixed silver/calcium phosphates were dialyzed in a 2500 kDa semi-permeable cellulose membrane. Finally, the material was filtered and dried at 80°C in an oven. All products were kept in desiccators under CaCl_2 prior to use.

2.2. Particle characterization

X-ray diffraction (XRD) was used to identify the crystalline phases present in the particles. Diffractograms were obtained using Nickel-filtered $\text{CuK}\alpha$ radiation at 40 kV and 30 mA, utilizing an equipment with geometry $\theta/2\theta$ and continuous readings from 10° to 60° at 0.05° intervals, 10 s per interval (Multiflex, Rigaku Corp., Tokyo, Japan). Silver and calcium contents in the particles were determined by inductively coupled plasma – optical emission spectroscopy (Arcos ICP-OES, Spectro Analytical Instruments GmbH, Kleve, Germany). A volumetric adsorption analyzer (Quantachrome, model Nova 1200e, Boynton Beach, FL, USA) was used to obtain Nitrogen adsorption isotherms from which the particles' surface areas were calculated according to the Brunauer, Emmet and Teller (BET) method. Particle size distribution was obtained by low-angle laser scattering (Mastersizer 2000, Malvern Ltd., Malvern, United Kingdom).

2.3. Composite formulation

A resin mixture containing equimolar amounts of BisGMA (bisphenol-A glycidyl dimethacrylate) and TEGDMA (triethylene glycol dimethacrylate) was prepared. Photoinitiators camphorquinone and EDMAB (ethyl-4-dimethylaminoethyl benzoate; all chemicals from Sigma-Aldrich) were added at mass fractions of 0.5% each. Four experimental composites were prepared by incorporating 20% or 30% (by mass) of either calcium phosphate or calcium phosphate/silver phosphate particles to the resin. Preliminary results with materials containing 10% filler showed that calcium and phosphate were released in concentrations too low to promote remineralization and had no detectable silver release. Composites were mechanically mixed under vacuum (Speedmixer DAC 150.1 FVZ-K, Flacktec Inc., Landrum, SC, USA) and kept under refrigeration until 2 h prior to use.

2.4. Degree of conversion

The uncured composite was inserted in a silicone mold (diameter = 7 mm, thickness = 1 mm, $n = 3/\text{group}$) and pressed between two microscopy glass slides. The vibrational spectrum of the uncured paste in the near-infrared range was obtained by the co-addition of 32 scans between the wavelengths of 4000 and $10,000 \text{ cm}^{-1}$, with a resolution of 4 cm^{-1} (Vertex 70, Bruker Optik GmbH, Ettlingen, Germany). The composite was kept between the glass slides during photoactivation ($1200 \text{ mW}/\text{cm}^2$ for 20 s) followed by dry-storage for 24 h at 37°C . A new spectrum was then obtained and the degree of conversion (DC) was calculated as the percent reduction in the area of the band located at 6165 cm^{-1} (corresponding to the $=\text{C}-\text{H}_2$ group) using the following equation:

$$\text{DC} = \left(1 - \frac{\text{polymerized}}{\text{non-polymerized}}\right) \times 100$$

Download English Version:

<https://daneshyari.com/en/article/5435139>

Download Persian Version:

<https://daneshyari.com/article/5435139>

[Daneshyari.com](https://daneshyari.com)