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Evaluation of antibiofilm and mechanical properties of new nanocomposites based on acrylic resins and silver vanadate nanoparticles

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ABSTRACT

Objective: The purpose of this study was evaluate, for the first time, the impact of incorporation of nanostructured silver vanadate (β-$AgVO_3$) in antibiofilm and mechanical properties of dental acrylic resins (poly(methyl methacrylate), PMMA).
Design: The β-$AgVO_3$ was synthesized and characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy, and microanalysis (SEM/EDS). Resins specimens were prepared with 0–10% wt.% β-$AgVO_3$ and characterized by SEM, XRD and optical microscopy. The antibiofim activity of the samples against *Candida albicans* and *Streptococcus mutans* was investigated by XTT reduction test, colony-forming units (CFUs), and confocal laser scanning microscopy (CLSM). The flexural strength, hardness, and surface roughness of the samples containing β-$AgVO_3$ were compared with the pure PMMA matrix.
Results: The incorporation of 10% β-$AgVO_3$ significantly reduced the metabolic activity of *C. albicans* and *S. mutans* ($p < 0.05$). There was a reduction in microbial load (CFU/mL) of microorganisms for the different concentrations used ($p < 0.05$), which was confirmed by confocal microscopy. The addition of β-$AgVO_3$ did not change the mechanical properties of hardness and surface roughness of the resins ($p > 0.05$). However, flexural strength decreased with the addition of amounts greater than 1% ($p < 0.05$).
Conclusions: β-$AgVO_3$ additions in dental acrylic resin may have an impact on inhibition of biofilm of main microorganisms associated with dental prostheses. However, the viability of clinical use should be evaluated in function of changed promoted in some mechanical properties.

1. Introduction

The formation of biofilm on the surfaces of provisional crowns and fixed/removable prostheses plays an important role in the development of caries, periodontal disease and mucositis (Queiroz et al., 2013; Rupf et al., 2012). These factors limit the longevity of rehabilitative treatment and constitute a risk of opportunistic infections, reducing quality of life and generating additional costs to patients (Larazin et al., 2012). The colonization process on the surface of dental prostheses is characterized by various steps (Rupf et al., 2012) and occurs because acrylic resins have porosity, an absence of ionic charge on the surface, roughness, and a capacity to absorb fluids, all of which lead to the accumulation of microorganisms (Santos, Pithon, Carvalho, Ramos, & Romanos, 2013; Sivakumar et al., 2014). The development of dental acrylic resins capable of inhibiting biofilm formation is therefore critical in the control of oral disease (Wang, Shen, & Haapasalo, 2014).

In an effort to add antimicrobial activity to these materials, studies have used additives such as nystatin, miconazole, chlorhexidine, modified monomers, zirconium dioxide nanoparticles and aluminum borate (Alcântara et al., 2012; Han et al., 2015; Redding et al., 2009). The antimicrobial properties of silver (Ag) dates to 3000 years ago, and the mechanism is based on the interaction of silver with thiol groups of enzymes involved in bacterial cell metabolism thus causing cell death (Kwakye-

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Awuah, Williams, Kenward, & Radecka, 2008). Due to this property, silver ions and silver nanoparticles (AgNPs) have also been introduced in dental materials (Bürgers et al., 2009; Yang, Zhang, Yu, Zhen, & Huang, 2014). For AgNPs these properties appear to be even better in function to their nanoscale dimensions, interact extensively with microorganisms (Fan et al., 2011; Holtz, Lima, Souza Filho, Brocchi, & Alves, 2012; Kumar, Jolivalt, Pulpytel, Jafari, & Arefi-Khonsari, 2013; Hojati et al., 2013). However, in addition to great aesthetic inconvenience (color), there is the difficulty of stabilizing nanoparticles when used alone (Holtz et al., 2012). AgNPs tend to cluster, which may negatively influence in their antimicrobial effect by reducing the surface contact area (Shameli et al., 2011).

A possible alternative to overcome this problem was recently proposed and is to support AgNPs on Ag nanowires vanadate (β-$AgVO_3$) (Holtz et al., 2010). This nanomaterial has antimicrobial property as it the silver and the vanadium elements act synergistically and interact with microorganisms' cell membranes. In addition, this compound has a high dispersion of silver nanoparticles on silver vanadate nanowires, maintaining high surface contact with the microorganisms (Holtz et al., 2012, 2010). Previous studies have shown the possibility of using this hybrid nanomaterial as an antimicrobial additive for water-based paint for domestic or hospital environment in order to improve sanitary conditions and reduce the number of hospital infections once it exhibits antibacterial activity 30 times larger than that of Oxacillin (Holtz et al., 2010) and as an additive for acrylic resin for using in dental applications, as it functionalized samples with this nanomaterial showed inhibition zone against the growth of the main microorganisms in the oral cavity demonstrating the need for studies on biofilm models as well as the mechanical properties (de Castro et al., 2014).

The objective of this study was therefore to evaluate the antibiofilm activity of two acrylic resins containing β-$AgVO_3$ against *Candida albicans* and *Streptococcus* mutans, the main microorganisms associated with dental prostheses. The hardness, surface roughness, and flexural strength of the resins were also evaluated, as these mechanical properties, together with antibiofilm action, are factors that are directly related to the effectiveness of the material in the oral cavity.

2. Materials and methods

2.1. Synthesis of nanostructured silver vanadate

Nanostructured silver vanadate (β-$AgVO_3$) was synthesized through a precipitation reaction between silver nitrate ($AgNO_3$, Merck 99.8%) and ammonium metavanadate (NH_4VO_3, Merck 99%) according to the methodology described by Holtz et al. (2010). Initially, 1.3569 g of $AgNO_3$ and 0.9736 g of NH_4VO_3 were each solubilized in 200 mL of distilled water. The solutions were stirred separately on a 65 °C heated surface for 10 min. Next, the silver nitrate solution was added dropwise using a burette into the ammonium metavanadate solution under constant stirring at 65 °C. The precipitate obtained was washed with distilled water and absolute ethanol several times, filtered and then dried in a vacuum line for 10 h.

2.2. Characterization of β-$AgVO_3$

The silver vanadate was characterized to ensure standardization of the resulting materials. For this purpose, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy, and microanalysis (SEM/EDS) were used. XRD analysis was performed at room temperature using a Shimadzu XRD-7000 diffractometer operating with Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 30 mA. Data were collected continuously from $10° < 2\theta < 70°$ with a velocity of 1°/min to identify the crystal structure. The crystallinity index (IC) of the sample was determined (Krimm & Tobolsky, 1951). The IC (%) was calculated from the ratio between the crystalline diffracted area (AC) and the total area of diffraction (AT = crystalline + amorphous), as shown in Eq. (1):

$$IC = \left(\frac{AC}{AT}\right) \times 100 \quad (1)$$

Infrared spectra with Fourier transform were collected on an FTLA 2000-102 spectrometer using transmittance mode in the region of 4000–400 cm^{-1} to monitor β-$AgVO_3$ synthesis. Finally, the morphological analysis of β-$AgVO_3$ was performed by scanning electron microscopy (SEM) on a Magellan 400L FEI microscope coupled with an energy dispersive X-ray spectrometer (EDS), which allowed for qualitative chemical analysis.

2.3. Preparation of functionalized resins with β-$AgVO_3$

To prepare the specimens, Dencor Lay autopolymerizable (SC) and **Clássico** thermopolymerizable (TR) (Clássico Artigos Odontológicos®) acrylic resins were used. Six experimental groups functionalized with β-$AgVO_3$ at concentrations of 0.5, 1, 2.5, 5 and 10% (wt.%) and a control group without additive were formed. The β-$AgVO_3$ was added to the polymer particle, and after homogenization, the monomer was added at a ratio of three parts powder (polymer) to one part liquid (monomer) according to the manufacturer's recommendations. During the plastic phase, the resin was placed in holes prepared in metal muffles with appropriate dimensions for each test and was cured according to the manufacturer's recommendations. For the antibiofilm tests, surface roughness was standardized at 0.2 μm using a Surftest SJ-201P (Mitutoyo Corporation).

2.4. Characterization of functionalized resins

The characterization of the samples with β-$AgVO_3$ was performed in comparison with control samples without nanomaterial by XRD and SEM order to identify the changes introduced by the incorporation and the pattern of dispersion of the particles. These techniques were performed as described previously for the characterization of β-$AgVO_3$. The morphology of resins was also analyzed by optical microscopy using a Leica MZ7.5 stereomicroscope coupled with a DFC-490 camera.

2.5. Antibiofilm experiment

Two standard strains were used in the study: *C. albicans* (ATCC 10231) and *S. mutans* (ATCC 25175). For inoculum preparation, the microorganisms were seeded in Petri dishes with specific culture media (Sabouraud Dextrose Agar for yeast and modified SB-20 Agar for bacteria). After incubation at 37 °C for 48 h, standardized *S. mutans* (10^8 CFU/mL) and *C. albicans* (10^6 CFU/mL) microbial inocula were obtained in 0.85% saline using a PCB 687 spectrophotometer (BYK Gardner). The biofilm was formed in 24-well polystyrene plates. Sterile resin samples were placed individually in each well, and 1000 μL of the inoculated culture medium was transferred to each hole. The plates were incubated at 37 °C for 1 h 30 min with stirring at 750 rpm in a bacteriological incubator (Incubator Shaker Mod.—EC-320, Cienlab) for adherence of the biofilm. After the adhesion period, the specimens were rinsed with 2000 μL of 0.85% saline to remove non-adherent cells, to buffer the medium, and to remove metaboliteNext, 1000 s. Next, 1000 μL of sterile culture medium was added to each well to