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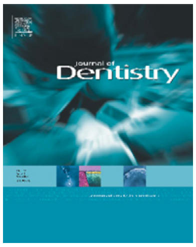

The addition of nanostructured hydroxyapatite to an experimental adhesive resin

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ABSTRACT

Objectives: Was produced nanostructured hydroxyapatite (HA_{nano}) and evaluated the influence of its incorporation in an adhesive resin.
Methods: HA_{nano} was produced by a flame-based process and was characterized by scanning electron microscopy. The surface area, particle size, micro-Raman and cytotoxicity were evaluated. The organic phase was formulated by mixing 50 wt.% Bis-GMA, 25 wt.% TEGDMA, and 25 wt.% HEMA. HA_{nano} was added at seven different concentrations: 0; 0.5; 1; 2; 5; 10 and 20 wt.%. Adhesive resins with hydroxyapatite incorporation were evaluated for their radiopacity, degree of conversion, flexural strength, softening in solvent and microshear bond strength. The data were analyzed by one-way ANOVA and Tukey's post hoc test ($\alpha = 0.05$), except for softening in solvent (paired *t*-test) and cytotoxicity (two-way ANOVA and Bonferroni).
Results: HA_{nano} presented 15.096 m^2/g of specific surface area and a mean size of 26.7 nm. The radiopacity values were not different from those of 1-mm aluminium. The degree of conversion ranged from 52.2 to 63.8%. The incorporation of HA_{nano} did not influence the flexural strength, which ranged from 123.3 to 143.4 MPa. The percentage of reduction of the microhardness after immersion in the solvent became lower as the HA_{nano} concentration increased. The addition of 2% nanostructured hydroxyapatite resulted in a higher value of microshear bond strength than the control group ($p < 0.05$).
Conclusions: The incorporation of 2% of nanostructured hydroxyapatite into an adhesive resin presented the best results.
Clinical significance: The incorporation of nanostructured hydroxyapatite increases the adhesive properties and may be a promising filler for adhesive resin.

1. Introduction

The addition of fillers to (di)methacrylate polymers to produce restorative resin composites represents a paradigm change in restorative dentistry.[1] Several inorganic[2,3] and organic[4] fillers were proposed to increase the composite resin properties, such as microhardness, wear resistance, reduced water sorption and solubility.[4,5] Adhesive resins are produced with similar organic matrices of composite resin, but usually without filler addition.[5] Some studies and commercial products have incorporated fillers into adhesive systems[3] with the goal of reducing the water sorption and solubility[6]

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and improving the mechanical properties of the adhesive and hybrid layers.[5,7]

Adhesion to a tooth substrate is directly related to the quality of the formed polymer.[8] In an attempt to improve the mechanical properties of the material and, consequently, the mechanical properties of the hybrid layer,[5,7] nanoparticles could be added to adhesive resins. Nanoparticles have been widely used for restorative composite resins[9] and, recently, for adhesive resins.[5,7] Reduction of the particle size from the micrometre scale to the nanometer scale could change the mechanical, physical and chemical properties of the materials.[10] The hardness, active surface area, chemical reactivity and biological activity of fillers are altered by the reduction of the particles to the nanometer scale.[10] However, the addition of nanofillers to an adhesive resin could reduce the wetting of the resin in the dentine substrate[11] and the availability of light into the bulk of resin, reducing the degree of conversion, since the refractive index of fillers are often different of the organic matrix.[12,13] The light could be reflected and absorbed by fillers, reducing it availability for camphoroquinone excitation.[13]

Hydroxyapatite has been proposed as a filler for composite materials[14] and was recently introduced into adhesives[15] and root canal sealers[16] at the nanometer scale, improving several of the material properties. However, to the best of our knowledge, there are no reports on the use of nanostructured hydroxyapatite in adhesive resins. The purpose of this study was to produce nanostructured hydroxyapatite and evaluate the influence of its incorporation in an adhesive resin. The null hypothesis is that the addition of nanostructured hydroxyapatite will not influence the experimental adhesive resin properties.

2. Materials and methods

The monomers bisphenol A glycol dimethacrylate (BisGMA), triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), camphorquinone (CQ) and ethyl 4-dimethylaminobenzoate (EDAB) were used in this study (Esstech Inc., Essington, PA, USA). These materials were used without further processing. Nanostructured hydroxyapatite (HA_{nano}) was obtained using a flame as the source of energy.[17] The organic phase was formulated by mixing 50 wt.% Bis-GMA, 25 wt.% TEGDMA and 25 wt.% HEMA. CQ and EDAB were added at 1 mol% for all groups according to the moles of monomer used. HA_{nano} was added at seven different concentrations: 0; 0.5; 1; 2; 5; 10 and 20 wt.%. No radical scavenger was added. The nanostructured hydroxyapatite was submitted to the silanization process to improve the adhesion interface between charged particles and the matrix with 5% silane and 95% solvent (acetone) by weight.[18] The specimens were stored for 24 h at 37 °C to allow for complete solvent evaporation. All formulations were weighed with an analytical balance (AG 200, Gehaka, Brazil), mixed and ultrasonicated (CBU 100/1 LDG, Plana, Brazil) for 1 h. To perform monomer photo-activation, a light emitting diode unit (Radii, SDI, Australia) was used. An irradiation value of 1200 mW/cm^2 was confirmed with a digital power metre (Ophir Optronics, USA) and the photo-activation was performed 5 mm apart of the sample for all tests.

2.1. *Synthesis of nanostructured hydroxyapatite*

The flame-based equipment developed in our laboratory to produce and collect hydroxyapatite powders was composed of three components: the atomization device, the pilot and main flame and the powder collector system.[17] Two perpendicular needles comprised the major component of the atomization device, where the precursor solution, composed of 14.6 g of calcium acetate ($Ca(CH_3COO)\cdot 2H_2O$) and 6.6 g of ammonium phosphate ($(NH_4)2HPO_4$), both supplied by Synth®, was processed after its preparation. Thus, a spray was formed in the atomization device, which also directed the spray to the flame. The precursor solution flows through the inner needle (0.6 mm diameter), the atomization gas (compressed air at 2 L/min) flows through the larger one (1.5 mm diameter). To control the precursor solution flux (2 mL/min) delivered to the atomization device, a Cole-Parmer (Masterflex model – Brazil) peristaltic pump was employed.

The spray was then directed to the pilot flame where a Bunsen–Meker burner was used with propane and butane as the combustible gas. Once in contact with the pilot flame, the precursor solution was burned, and another flame was produced and designated as the main flame. This secondary flame promoted the chemical reactions that led to the formation of the powders. The particles were further collected in the powder collector system, which was composed of a stainless steel chamber where a metallic mesh with a 25-μm opening was coupled perpendicularly to the gas stream, retaining the powder in the mesh. When the metallic mesh was saturated with the particles, the equipment was turned off to remove the powder. Then, the powder was calcined at 600 °C for 2 h using an electric furnace to remove byproducts or radicals that are typically present in hydrocarbon flames and consequently in the powder.

2.2. *Characterization of particle size and surface area*

Scanning electronic microscopy (SEM, JSM 5800, Jeol, Akishima, Tokyo, Japan) was used to evaluate the morphology of the HA_{nano} powders. For conductive reasons, a thin layer of gold was deposited on the samples before the analysis. The sputter coating was applied for 80 s, producing a layer with about 15 nm of thickness.

The typical chemical groups of hydroxyapatite were identified by micro Raman spectroscopy (SENTERRA, Bruker Optics, Ettlingen, Germany) device. The range of the analysis was 80–2700 cm^{-1}.

The specific surface area of the HA_{nano} powder was determined through the Brunauer–Emmett–Teller (BET) method (Autosorb Automated Gas Sorption System, Quantachrome, Boynton Beach, Florida, USA). Before the analysis, the sample was outgassed at 300 °C for 3 h. The particle size distribution was assessed using a laser diffraction particle size analyzer (CILAS 1180, Orleans, France).

2.3. *Cytotoxicity*

The NIH-3T3 fibroblasts cell line (American Type Culture Collection – ATCC n° CRL-1658™ Rockville, MD) was cultured with DMEM (Sigma Chemical Co. St. Louis, MO) supplemented