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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.% BisGMA, 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

with 10% bovine foetal serum, penicillin (100 U/mL) and streptomycin (100 U/mL) at 37 °C in a humid atmosphere containing 5% CO_2. The culture medium was replaced every three days, and the cells were split whenever they reached 70% confluence. Cell viability was evaluated using the Trypan Blue exclusion method, and the cells were seeded onto 96-well culture plates to perform the colorimetric MTT assay. Octuplicates containing 0.2 mL per well of NIH-3T3 at a concentration of 6×10^6 cells were cultured in contact with hydroxyapatite and culture medium alone as a negative control for cytotoxicity in a 5% CO_2. The hydroxyapatite powder was tested at 1 mg/mL (100×) and 0.5 mg/mL (200×). As a positive control for cytotoxicity, 100% copper sulfate was used. The culture media was removed, and 10% of an MTT solution (5 mg/mL in PBS) was added to each well. The cultures were incubated at 37 °C and sheltered from light until the presence of formazan violet crystals was evaluated. To solubilize the formazan crystals, 100 μL of dimethylsulfoxide (DMSO) were added to each well, and the absorbance was measured at an 570 nm in an ELISA plate reader (Bio-Rad Microplate Reader Benchmark, Inc., USA). The evaluations were conducted for periods of 24, 48 and 72 h.

2.4. Radiopacity

The radiopacity of model adhesive resins was evaluated based on an ISO 4049[19] standard, except for specimens' dimensions. Five specimens per group ($n = 35$) that were 5.0 mm (± 0.5 mm) in diameter and 1.0 mm (±0.2 mm) thick were evaluated. X-ray images were obtained using a digital system with phosphorous plates (VistaScan, Dürr Dental GmbH & Co. KG, Bietigheim-Bissingen, Germany) at 70 kV and 8 mA, with 0.2 s of exposure time and a focus-film distance of 400 mm. One specimen from each group with the same concentration of HA_{nano} was positioned in each film for a total of five films per concentration. An aluminium step-wedge was simultaneously exposed with the specimens in all images. The aluminium step-wedge thickness ranged from 0.5 mm to 5.0 mm in increments of 0.5 mm. The images were saved in the TIFF format for less compressed files. Digital images were processed using Photoshop software (Adobe Systems Incorporated, CA, USA). The means and standard deviations of the grey levels (pixel density) of the aluminium step-wedge and the specimens were obtained in a standardized area.

2.5. Degree of conversion

The degree of conversion of the experimental adhesive resins was evaluated using real-time Fourier Transform Infrared Spectroscopy (RT-FTIR) with a Shimadzu Prestige21 (Shimadzu, Japan) spectrometer equipped with an attenuated total reflectance device. This device was composed of a horizontal ZnSe crystal with a mirror angle of 45° (PIKE Technologies, USA). A support was coupled to the spectrometer to fix the light-curing unit and standardize the distance between the fibre tip and sample at 5 mm. The analyses were performed at a controlled room temperature of 23 ± 2 °C and 60 ± 5% relative humidity. The temperature of the attenuated total reflectance crystal surface was approximately 25 °C. The sample (3 μL) was directly dispensed onto the ZnSe crystal and light-activated for 20 s ($n = 1$).[20,21] The degree of conversion was calculated as described in a previous study, considering the intensity of the carbon–carbon double bond stretching vibration (peak height) at 1635 cm^{-1}, and using the symmetric ring stretching at 1610 cm^{-1} from the polymerized and unpolymerized samples as an internal standard.[22]

2.6. Flexural strength

The model adhesive resin specimens designated for the flexural tests were fabricated in customized stainless steel moulds according to ISO 4049 specifications, except for the dimensions (12 mm in length, 2 mm in width, and 2 mm in height).[23] The dental adhesives were placed into the mould, which was positioned on top of an acetate strip. The top and bottom surfaces of the specimens were then light-polymerized with two irradiations of 20 s on each side. After polymerization, the specimens were removed from the mould and stored in distilled water at 37 ± 1 °C for 24 h. Ten specimens were produced for each group ($n = 10$); in total, 70 specimens were submitted to flexural strength testing. The flexural strength tests were performed with a universal testing machine at a crosshead speed of 1.0 mm/min. The flexural strength (σ) of each specimen was calculated in megapascals (MPa) according to the equation:

$$\sigma = \frac{3LF}{2BH^2} \quad (1)$$

where, F is the maximum load in Newtons, L is the distance in millimetres between the supports, B is the width in millimetres of the specimen measured immediately prior to testing, and H is the height in millimetres.

2.7. Softening in ethanol

To determine the degradation in solvent, the specimens produced for the radiopacity evaluation were used. Five specimens for each experimental adhesive ($n = 5$) were embedded in an acrylic resin and polished in a polisher (Model 3v, Arotec, Cotia, SP, Brazil) with a felt disc that was saturated with an alumina suspension (Alumina, 10 μm, Arotec, Cotia, SP, Brazil) after the specimens were stored and dried at 37 °C for 24 h. The specimens were subjected to a microhardness test in which three indentations (25 g/15 s), 100 μm apart from each other, were assessed using a digital microhardness tester (HMV 2, Shimadzu, Tokyo, Japan). The calculation of the hardness value was carried out by Eq. (2):

$$\text{Knoop microhardness} = \frac{14228 \cdot c}{d^2} \quad (2)$$

In which 14,228 is a constant, c is the load in grams, and d is the length of the larger diagonal in μm.

The initial Knoop Hardness Number (KHN_1) was registered, and the specimens were then subjected to softening in absolute ethanol for 4 h at 37 °C after the hardness test was repeated, and the post-conditioning hardness value was measured (KHN_2). The percentage difference of KHN_1 and KHN_2 was calculated.

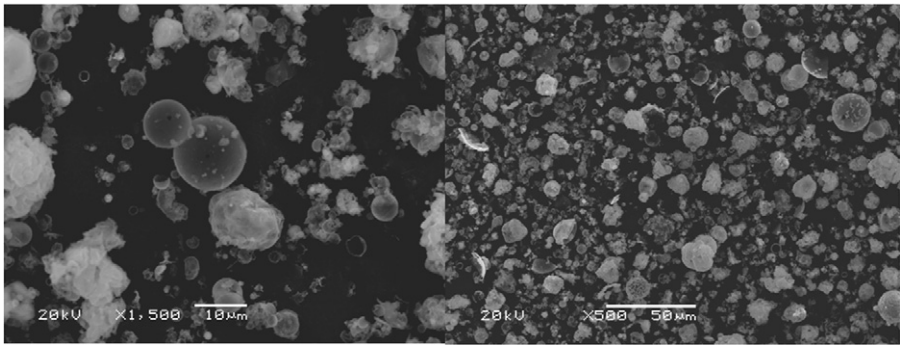

Fig. 1 – Representative scanning electron microscopy of hydroxyapatite powder showing spherical primary particles and secondary aggregates (500× and 1500×).

2.8. Microshear bond strength test

Seventy bovine lower incisor teeth that had been stored in 4 °C distilled water for no more than 3 months were used in this study. The teeth were embedded in acrylic resin, and the labial enamel was ground down to expose the superficial dentine. The dentine was ground with 600-grit SiC paper for 30 s in running water to produce a standardized smear layer. The teeth were divided into seven groups according to the HA_{nano} concentration in the experimental adhesive resins. The dentine was acid etched for 15 s with 37% phosphoric acid gel and washed for the same amount of time. The water was gently removed with an absorbent paper. A commercial primer (Primer Scotch Bond Multi Purpose, 3 M ESPE, St. Paul, MN, USA) was applied to the dentine and dried for 10 s. Then, the model adhesive resins were applied and photoactivated for 20 s. In each tooth, 6 cylindrical composite restorations (Z350, 3 M ESPE, St. Paul, MN, USA) with 0.88 (±0.03) mm^2 of adhesive area were made and photoactivated for 40 s, and the teeth were stored in distilled water at 37 °C. The cylindrical restorations were produced using a polyvinylsiloxane matrix, with 2 mm distance between them. The specimens were mounted in a universal testing machine (DL-2000, EMIC, São José dos Campos, Brazil), and a shear force was applied at a cross-head speed of 1 mm/min using a steel wire (Ø 0.4 mm). The wire was positioned at the bond interface, and the cylinder was pulled. The microshear bond strengths are expressed in MPa.

2.9. Statistical analysis

The normality of data was evaluated using the Kolmogorov-Smirnov test. The statistical analysis was performed using one-way ANOVA (HA_{nano} concentration) and Tukey's post hoc test at the 0.05 level of significance for all tests except the cytotoxicity assays and softening in ethanol. Cytotoxicity was analyzed by two-way ANOVA and the Bonferroni post hoc test. For the analysis of softening in ethanol, a paired Student's *t*-test (KHN1 and KHN2) and a one-way ANOVA for ΔKHN% were used.

3. Results

The morphology of the HA powder is shown in the SEM micrographs presented in Fig. 1. It was possible to observe that the powder was composed of primary particles and secondary aggregates. However, some unshaped particles could also be seen. Based on a detailed analysis of the SEM micrographs, we found that the primary particles had diameters between 1 and 10 μm. The specific surface area of the HA_{nano} powder prepared by the method described in this work was 15.096 m^2/g, and according to our granulometric analysis. The crystallite mean size of the HA obtained presented a normal distribution and was 26.7 nm. Typical chemical groups were identified by micro Raman analysis (Fig. 2), that could be identified as phosphate (υ1 PO_4 ~960 cm^{-1}) and carbonate (CO_3 ~1070 cm^{-1}).

The results of the cytotoxicity assays are shown in Table 3. The two-way ANOVA presented a statistically significant interaction between the factors ($p < 0.001$). At 24 h, HA powder showed higher values than the positive control ($p < 0.01$). At 48 h, no significant difference was detected ($p > 0.05$) and, at 72 h, the HA powder presented significantly lower values than 100% copper sulfate for concentrations of 1 mg/mL and 0.5 mg/mL ($p < 0.01$). All groups presented higher citotoxicity with the increase of time ($p < 0.05$).

The radiodensity values of the model dental adhesives are presented in Fig. 3. The density of pixels varied between 44.04 and 53.45, showing no significantly difference compared with 1 mm aluminium (55.09).

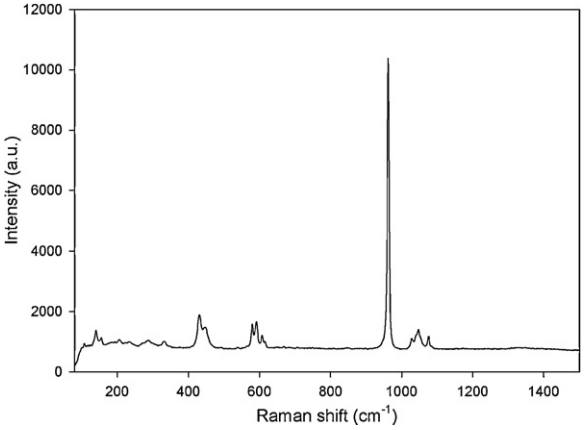

Fig. 2 – Typical Raman shift of nanostructured hydroxyapatite.

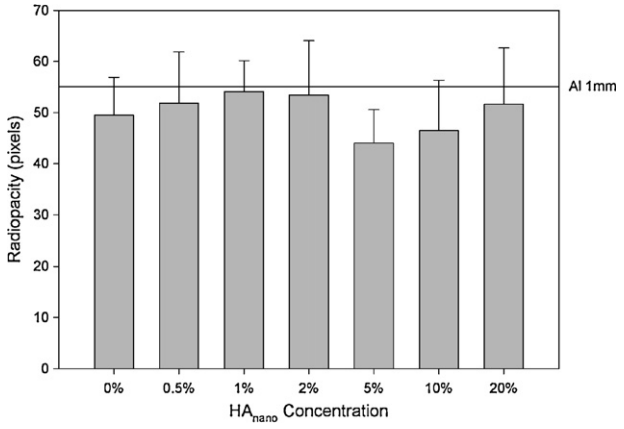

Fig. 3 – Means and standard deviations of radiopacity values, in pixels, for model dental adhesive resins. The reference line shows the radiopacity, in pixels, for 1 mm Al. No significant difference was found with the addition of varying concentrations of HA_{nano}.

All groups showed a degree of conversion higher than 50% with 20 s of photo-activation. The higher value was 63.84% and was presented by the group with 1% HA by weight. The values of the degree of conversion are show in Table 1.

The flexural strength values of the model dental adhesives are presented in Table 1. There were no significant differences between the experimental groups and the control group ($HA_{0\%}$).

The Knoop microhardness values before and after solvent immersions are shown in Table 2. Increased concentrations of HA_{nano} lead to increased microhardness values before and after solvent immersion. After the immersion in solvent, all groups showed decreased values of microhardness. The percentage of reduction (Table 2) was lower as the HA_{nano} concentration increased: 32.35; 18.70; 16.73; 19.55; 13.99; 13.27; 9.62, respectively for the groups $HA_{0\%}$; $HA_{0.5\%}$; $HA_{1\%}$; $HA_{2\%}$; $HA_{5\%}$; $HA_{10\%}$ and $HA_{20\%}$.

The results for the microshear bond strength are shown in Table 1. The addition of 2% of nanostructured hydroxyapatite resulted in a higher microshear bond strength than the control group (without HA_{nano}) ($p < 0.05$).

Table 1 – Values of degree of conversion (%) and mean values (standard deviation) of flexural strength and microshear bond strength (μSBS), in MPa, of model adhesive resins.

Groups	Degree of conversion (%)	Flexural strength (MPa)	μSBS (MPa)
HA 0%	57.9	137.54 (24.1) ^{A}	26.59 (±2.32) ^{BCD}
HA 0.5%	58.8	143.40 (22.1) ^{A}	27.32 (±3.90) ^{BC}
HA 1%	63.8	136.40 (18.4) ^{A}	26.80 (±5.41) ^{BCD}
HA 2%	59.8	142.10 (16.9) ^{A}	33.28 (±5.09) ^{A}
HA 5%	61.8	139.10 (15.2) ^{A}	31.99 (±5.34) ^{AB}
HA 10%	63.6	128.60 (18.4) ^{A}	21.27 (±4.41) ^{D}
HA 20%	52.2	123.30 (23.4) ^{A}	21.62 (±2.95) ^{CD}

Same letter indicates no statistical difference in same column ($p > 0.05$).

Table 2 – Microhardness values of the model adhesives before (KHN1) and after the immersion in solvent (KHN2) and the variation of microhardness values (Δ%).

Groups	KHN1	KHN2	ΔKHN%
HA 0%	22.6 (0.6) ^{Ba}	15.3 (2.4) ^{b}	32.4 (9.6) ^{C}
HA 0.5%	22.8 (1.9) ^{Ba}	18.5 (0.8) ^{b}	18.7 (7.0) ^{B}
HA 1%	22.7 (0.9) ^{Ba}	18.9 (0.7) ^{b}	16.7 (2.9) ^{B}
HA 2%	23.2 (0.3) ^{Ba}	18.6 (1.0) ^{b}	19.6 (4.7) ^{B}
HA 5%	23.4 (0.4) ^{Ba}	20.2 (0.6) ^{b}	13.9 (1.9) ^{B}
HA 10%	23.9 (0.2) ^{Aa}	20.7 (0.3) ^{b}	13.3 (1.1) ^{B}
HA 20%	25.1 (0.8) ^{Aa}	22.7 (0.8) ^{b}	9.6 (4.7) ^{A}

Different capital letter indicates statistical difference in same column ($p < 0.05$). Different small letter indicates statistical difference in same row ($p < 0.05$).

Table 3 – Cytotoxicity of nanostructured hydroxyapatite at different times (24 h, 48 h and 72 h) and at different dissolutions (100× and 200×), in absorbance at 570 nm.

	24 h	48 h	72 h
Control	0.84 (±0.22) ^{B,c}	2.24 (±0.16) ^{A,b}	2.89 (±0.29) ^{A,a}
HA100	1.23 (±0.16) ^{A,b}	2.31 (±0.22) ^{A,a}	2.52 (±0.24) ^{B,a}
HA200	1.07(±0.32) ^{AB,b}	2.50 (±0.23) ^{A,a}	2.58 (±0.21) ^{B,a}

Different capital letter indicates statistical difference in the same column ($p < 0.05$). Different small letter indicates statistical difference in the same line ($p < 0.05$)

4. Discussion

The addition of filler to adhesive resin may increase the adhesion to a tooth substrate[5] and decrease the degradation of the material over time.[6] In this study, a nanostructure hydroxyapatite was successfully produced using a flame-based process. At the nanoscale, the particles (mean size of 26.7 nm) could be observed alone and in small aggregates. The incorporation of nanostructured hydroxyapatite increased the microhardness, microshear bond strength and degradation resistance. No changes were observed in the flexural strength or degree of conversion and, even at the nanoscale, the hydroxyapatite presented no cytotoxic effects. The nanostructured hydroxyapatite that was produced in the present study presented favourable results for adhesive resin incorporation, and group with 2% of nanostrucured hydroxyapatite showed the most favourable results. However, the null hypothesis can be rejected because some adhesive resin properties were influenced by the addition of HA_{nano}.

The longevity of dental restorations is related to the quality of the polymer formed.[24] Several efforts have been made to produce adhesive systems that are less prone to degradation. The reduction of the hydrophilicity of the adhesive resin,[22] the inhibition of collagen degradation by enzymes[25–27] and the addition of filler to the adhesive resin[5,7] have been proposed to increase restoration longevity. The addition of filler decreases the relative amount of polymeric matrix, decreasing the stress of contraction[28] and hydrolytic degradation.[29] Inorganic fillers are less prone to degradation than polymeric matrix[6] and lead to a more mechanically resistant material.[30] In the present study, the addition of

nanostructured hydroxyapatite produced an adhesive resin with less degradation in absolute ethanol. The control group (without filler addition) presented a greater than 32% reduction in microhardness after 4 h of immersion in solvent. On the other hand, the group with 20% of nanostructured hydroxyapatite presents only a 9.62% reduction in the microhardness values. Moreover, the addition of HA_{nano} increases the microhardness before immersion in ethanol. The diffusion of solvent through the polymer chains results in elution of components and the plasticization of the composite.[24] This process initially affects the surface properties, such as the hardness and wear resistance, and therefore the longevity of the restorative treatment.[31] In the present study, changes in the surface properties were evident based on the microhardness and were less pronounced in the samples with higher amounts of HA_{nano}. However, a homogeneous distribution of particles in the bulk of the polymer seemed to be achieved because there was no influence on the flexural strength values with the increased addition of HA_{nano}.

A reduced level of degradation of adhesive resin could lead to a more stable and durable restorative treatment.[5] In this study, the immediate bond strength was evaluated, and the groups with 2% and 5% HA_{nano} presented more favourable results than groups with 10% and 20%. The addition of filler to the hybrid layer could improve the mechanical properties of this layer and increase the bond strength. Although, higher amounts of filler – especially at the nanoscale – increase the viscosity of the polymer, resulting in difficult diffusion into demineralized dentine and a lower bond strength.[32] The groups with 10% and 20% HA_{nano} showed bond strengths with no significant difference compared to the control group ($HA_{0\%}$) and were therefore equivalent to a commercial adhesive resin without filler. Most of the commercial adhesive systems present acceptable immediate bond strength values. However, long-term analysis shows a degradation of bond strength leading to a weak hybrid layer that is more prone to failure. Several reasons that could affect hybrid layer degradation, including collagen degradation[25] and polymer degradation,[33] and polymers with a lower degree of conversion are more prone to plasticization.[29] Non-polymerized monomers or oligomers could be leached from the bulk of the polymer, leading to degradation of the hybrid layer.[24] In this study, the addition of HA_{nano} did not influence the degree of conversion when compared to other model adhesive resins and commercial systems.[34,35] Considering that the groups with HA_{nano} addition presented statistically less degradation than the control group, the longitudinal bond strength may be affected.[36]

A clinical implication of the addition of fillers to adhesive resins could be the radiopacity that is produced. The radiopacity could decrease the occurrence of failure to diagnose recurrent caries and restoration overhangs.[37] Adhesive systems with no radiopaque characteristics could promote a false-positive diagnosis of demineralized tissue under restoration.[38] The addition of HA_{nano} was not able to increase the radiopacity of the adhesive resins. However, an association with another compound[20] with higher radiopacity could increase this property without compromising the other characteristics, such as biocompatibility, degradation and bond strength. The addition of some kind of fillers could prevent the hybrid layer degradation and to preserve its bonding efficacy overtime, inhibiting the collagen degradation.[39] Nanostructured hydroxyapatite could be leached from adhesive resin and be deposited around denuded collagen.

Because adhesive resin degrades over time and the fillers can be leached, the absence of cytotoxic effects is desirable. However, nanoscale particles could present cytotoxic effects.[40] The mechanism of cell damage could be related to the generation of radicals through catalytic processes, oxidative stress, the induction of apoptotic and necrotic cell death and a decrease in proliferation.[40] Furthermore, a biological-like particle (synthetic nanostructured hydroxyapatite) seems to be a promising filler for adhesive resins because the adhesive resin can be in close proximity to the pulp tissue. In the present study, we produced nanostructured hydroxyapatite and incorporated this material into an experimental adhesive resin.

5. Conclusions

The incorporation of 2% of nanostructured hydroxyapatite into an adhesive resin presented the best results and may be a promising filler for adhesive resin.

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