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CAMILA PROVENZI

EFEITO DA INCORPORAÇÃO DE DIÓXIDO DE ZIRCÔNIO NANOESTRUTURADO EM UMA RESINA ADESIVA EXPERIMENTAL

Porto Alegre
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Trabalho de Conclusão de Curso apresentado ao Curso de Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Cirurgião-Dentista.

Orientador: Vicente Castelo Branco Leitune

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"A verdadeira viagem de descobrimento não consiste em procurar novas paisagens, mas em ter novos olhos".

(Marcel Proust)

RESUMO

PROVENZI, Camila. **Efeito da incorporação de dióxido de zircônio nanoestruturado em uma resina adesiva experimental**. 2014. 47 f. Trabalho de Conclusão de Curso (Graduação) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2014.

O objetivo deste estudo foi avaliar a influência da incorporação de dióxido de zircônio (ZrO_2) em uma resina adesiva experimental. As partículas de ZrO_2 foram caracterizadas por Difração de Raio-X, espectroscopia Micro Raman e o método Brunauer-Emmett-Teller (BET). Uma resina base foi formulada com 50% BisGMA, 25% TEGDMA e 25% HEMA e um sistema fotoiniciador. O ZrO_2 foi incorporado nas concentrações 0%, 0,5%, 1%, 4,8% e 9,1% peso. Três amostras (n=3) de 3µl cada foram avaliadas quanto ao grau de conversão utilizando espectroscopia de infravermelho por transformada de Fourier (FTIR) com um dispositivo de reflectância total atenuada. A radiopacidade (n=5) foi avaliada de acordo com a ISO 4049, com um sistema digital com placas de fósforo. A degradação em solvente (n=5) foi analisada por meio da microdureza Knoop (15g por 10s) dos espécimes antes e depois da imersão em etanol absoluto por 4h. A resistência de união à microtração foi avaliada em 24h e 1 ano em uma máquina universal de ensaios (EMIC). Doze dentes por grupo (n=12) foram avaliados. Para a avaliação da interface por espectroscopia Raman foram confeccionadas restaurações em dentina bovina, e depois realizada a análise da camada híbrida. A deposição mineral na interface adesiva foi avaliada por espectroscopia Raman antes e após 14 dias de imersão em SBF. A difração de raio-X mostrou as fases monoclínica e tetragonal no pó de ZrO_2. A área de superfície foi 37,41 m^2/g e o espectro Raman mostrou grupos típicos de ZrO_2. O acréscimo de ZrO_2 não influenciou a radiopacidade. As amostras com 4,8% e 9,1% tiveram aumento na dureza inicial e maior degradação em solvente ($p<0,05$). O grau de conversão aumentou significativamente no grupo com adição de 1% ZrO_2 ($p<0,05$). Na análise da interface foi possível verificar a penetração da resina adesiva e da zircônia na camada híbrida. A resistência de união não foi influenciada em 24h e 1 ano para os grupos avaliados. Após 1 ano, houve redução nos valores de resistência de união ($p<0,05$). Os grupos com adição de 4,8% e 9,1% ZrO_2 promoveram deposição mineral na interface adesiva. Conclui-se que a adição de ZrO_2 em até 1% melhorou as propriedades de uma resina adesiva experimental.

Palavras-Chave: Materiais dentários. Adesivos dentinários. Polímeros

ABSTRACT

PROVENZI, Camila. **Effect of incorporation of nanostructured zirconium dioxide in an experimental adhesive resin.** 2014. 47 f. Final Paper (Graduation in Dentistry) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2014.

The aim of this study was to evaluate the influence of the incorporation of zirconium dioxide (ZrO_2) in an experimental adhesive resin. ZrO_2 particles were characterized by X-Ray diffraction (DRX), Micro-Raman spectroscopy and Brunauer–Emmett–Teller (BET) method**.** A base resin was formulated with 50% BisGMA, 25% TEGDMA, 25% HEMA and a photoinitiator system. The ZrO_2 was incorporated at concentrations of 0%, 0,5%, 1%, 4,8% and 9,1% by weight. Three samples (n = 3) of 3µl each one were assessed for degree of conversion using Fourier Transform Infrared Spectroscopy (FTIR) with an attenuated total reflectance device. The radiopacity (n = 5) was evaluated according to ISO 4049 with a digital system using phosphor plates. The degradation in solvent (n = 5) was analyzed measuring knoop microhardness (15 g for 10 sec) of the specimens before and after immersion for 4 hours in absolute ethanol. Microtensile bond strength was evaluated in 24 hours and 1 year in a testing machine. Twelve teeth per group (n = 12) were evaluated. For the analysis of the interface by Raman spectroscopy restaurations in bovine dentin were made, and the hybrid layer was assessed. Mineral deposition at the adhesive interface was evaluated by Raman spectroscopy both before and after 14 days of immersion in SBF. DRX showed monoclinic and tetragonal phases of $ZrO_{2,}$ the surface area was 37.41 m^2/g and Raman spectrum showed typical groups of $ZrO_{2.}$ The addition of ZrO_2 did not influenced radiopacity. Groups with 4,8% and 9,1% ZrO_2 showed higher initial hardness and increased solvent degradation ($p<0.05$). The degree of conversion was significantly increased in the group with addition of 1% ZrO_2 ($p<0.05$). In the analysis of the interface was possible to verify the penetration of adhesive resin in the hybrid layer. The bond strength was not affected in 24 hours and 1 year for the evaluated groups. After 1 year, there was a reduction in microtensile bond strength values ($p <0.05$). Groups with addition of 4,8% and 9,1% ZrO_2 promoted mineral deposition at the adhesive interface. It can be concluded that the addition of ZrO_2 can improve the properties of an experimental adhesive resin.

Keywords: Dental materials. Zirconium oxide. Adhesives.

SUMÁRIO

1 INTRODUÇÃO

O desenvolvimento de sistemas adesivos tem sido estudado ao longo do tempo, e hoje se tem um entendimento maior acerca dos fenômenos que ocorrem durante o processo de adesão, tendo já sido descritos na literatura o condicionamento ácido do esmalte (BUONOCORE, 1955), da dentina (FUSAYAMA, 1979), até a caracterização da camada híbrida (NAKABAYASHI, 1982) e o papel de seus constituintes no processo (VAN LANDUYT, 2007). Contudo aprimoramentos nos sistemas adesivos vêm sendo buscados ao longo do tempo, tais como maior radiopacidade (COLLARES, 2010), maior resistência à degradação hidrolítica (FERRACANE, 2006) e maior capacidade de interação do material com as estruturas biológicas (TAY; PASHLEY, 2009).

A adesão dentinária tem peculiaridades em relação à adesão ao esmalte, sendo mais complexa que esta. Na dentina, o conteúdo mineral é reduzido proporcionalmente (NAKABAYASHI; PASHLEY, 1998) e, após a realização do condicionamento ácido, as fibras de colágeno da matriz dentinária são expostas possibilitando a infiltração de monômeros do adesivo entre elas e a formação de retenções micromecânicas (VAN MEERBEK, 1992). No entanto, apesar do avanço nos estudos, ainda são encontrados problemas como a degradação hidrolítica da camada híbrida ao longo do tempo, nanoinfiltração e, clinicamente, a redução da longevidade dos materiais em boca (DE MUNCK, 2010; SANO, 1995a; SANO, 1995b; ZHANG; KERN, 2009).

A adição de partículas inorgânicas como componente de carga à matriz polimérica visa aprimorar as propriedades mecânicas dos materiais, e já foi realizada sob diversas formas em materiais de uso odontológico à base de metacrilato, como resinas compostas (SKOVGAARD, 2001), cimentos endodônticos (COLLARES, 2010) e inclusive adesivos dentinários (LEITUNE, 2013a,b). Incorporando-se componentes inorgânicos, há uma redução na quantidade proporcional de matriz. Assim, problemas decorrentes dela são reduzidos, por exemplo, contração de polimerização, desgaste e degradação ao longo do tempo, bem como ausência de radiopacidade, principalmente em materiais odontológicos (AMIROUCHE-KORICHI; MOUZALI; WATTS, 2009; COLLARES, 2010; DURNER, 2012; LEITUNE, 2013a,b; MALACARNE, 2006; SKOVGAARD, 2001).

As partículas de carga, cujo índice de refração é maior que o apresentado pelos monômeros, podem alterar a disponibilidade de energia luminosa no interior do polímero, alterando o seu grau de conversão e tornando-o mais opaco (ANDRZEJEWSKA, 2001; SHORTALL, 2008). Além disso, alterações na reologia do material podem influenciar no molhamento e na penetração no substrato dentário, influenciando diretamente na adesão (BRESCHI, 2008). No entanto, partículas de carga também são menos propensas à degradação (KLAPDOHR; MOSZNER, 2005). Em adesivos odontológicos, podem aumentar a estabilidade da camada híbrida e, consequentemente, a longevidade do tratamento restaurador. Conforme o tamanho das partículas é reduzido, a sua área de superfície, reatividade e poder terapêutico são aumentados, bem como a fração volumétrica que se pode incorporar sem que haja prejuízo nas propriedades do material (HAMOUDA, 2012; LANONE; BOCZKOWSKI, 2006).

Zircônia (ZrO_2) é um óxido metálico amplamente usado como biomaterial em dispositivos protéticos médicos (MANICONE; IOMMETTI; RAFFAELLI, 2007; SARKAR, 2013) e também está presente na odontologia tanto sob a forma de cerâmica pura, em coroas cerâmicas, ou como partícula de carga em compósitos odontológicos comerciais (DENRY; KELLY, 2008; HOLLAND, 2008; REGES, 2002), por possuir como propriedades principalmente a biocompatibilidade e resistência mecânica (MANICONE; ROSSI; RAFFAELLI, 2007; SARKAR, 2013). Entretanto, a utilização de partículas nanoestruturadas de zircônia (ZrO_2) ainda não foi completamente compreendida e avaliada, especialmente sob o aspecto longitudinal.

2 OBJETIVOS

Com base no exposto, os objetivos do presente estudo serão:

a) Caracterizar a partícula do dióxido de zircônio (ZrO_2);
b) Formular uma resina adesiva à base de metacrilato com incorporação de ZrO_2 em diferentes concentrações e, posteriormente, caracterizar as propriedades das resinas adesivas obtidas.

3 ARTIGO CIENTÍFICO

Este trabalho de conclusão de curso se apresenta na forma de artigo científico, escrito na língua inglesa e segue as normas referentes ao periódico Journal of Dentistry (ISSN: 0300-5712) para o qual será submetido.

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Effect of nanostructured zirconium dioxide incorporation in an experimental adhesive resin

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Keywords: Nanostructures, Zirconium Dioxide, Dentin Bonding Agents.

3.1 Abstract

Objectives**:** The aim of this study was to evaluate the influence of nanostructured zirconium dioxide incorporation in an experimental methacrylate-based adhesive resin.

Methods**:** ZrO_2 particles were characterized by X-Ray diffraction (DRX), micro-Raman spectroscopy and Brunauer–Emmett–Teller (BET) method**.** An experimental adhesive resin was formulated with 0, 0.5, 1, 4.8 and 9.1wt% ZrO_2. Formulated adhesives were evaluated based on degree of conversion (DC), radiopacity, softening in solvent (SS), microtensile bond strength (µTBS) immediately and after 1-year aging, and hybrid layer was assessed with Micro-Raman spectroscopy at baseline and after 14 days immersed in SBF to evaluate mineral deposition.

Results**:** DRX showed monoclinic and tetragonal phases of $ZrO_{2,}$ the surface area was 37.41 m^2/g and Raman spectrum showed typical chemical groups of $ZrO_{2.}$ The addition of ZrO_2 did not influenced radiopacity. Groups with 4.8% and 9.1% ZrO_2 showed higher initial hardness and increased SS ($p<0.05$). DC was significantly increased in the group with addition of 1% ZrO_2 ($p<0.05$). In the analysis of the interface was possible to verify the penetration of adhesive resin and ZrO_2 particles in the hybrid layer. The µTBS was not affected in 24 hours and 1 year for the evaluated groups. After 1 year, there was a reduction in µTBS values ($p<0.05$). Groups with addition of 4.8% and 9.1% ZrO_2 promoted mineral deposition at the interface.

Conclusions: The incorporation of nanostructured zirconium dioxide (ZrO_2) increases methacrylate-based adhesive resins properties and may be promising for developing new dental adhesive systems with enhanced properties.

Clinical Significance: The incorporation of zirconium dioxide (ZrO_2) promoted mineral deposition on the adhesive interface and, if µTBS is longitudinally evaluated after immersion in SBF, it may lead to better outcomes for long-term adhesion.

3.2 Introduction

In the last decade the role of constituents of adhesive systems in the adhesion process has been studied (1-3). However, some issues such as the hybrid layer degradation over time, nanoleakage and, clinically, reduction of the longevity of materials into the oral environment remain a concern to dentin adhesion (4-7). The development of polymeric materials with better clinical behavior has been subject of several studies, and also interested in the scientific community and industry (8-11).

The addition of inorganic particles as filler components into an adhesive resin aims to improve its properties, by reducing the problems related to the organic matrix, such as polymerization shrinkage (12), sorption and solubility (13). Several studies have incorporated fillers, such as hydroxyapatite (14), niobium (15) and tantalum oxide (16) into the adhesive resin matrix and improved mechanical properties were observed. Whereas filler particles, besides are less prone to degradation (17), may reduce the composite structural defects that could lead to catastrophic failures, its incorporation in adhesives can also lead to a more long-term stable hybrid layer and, therefore, to a more durable restorative treatment (18).

Moreover, by reducing the particle size down to nanoscale, there is an increase in the surface area and it becomes more reactive (19). Due to it, chemical and biological activity can be enhanced, besides mechanical properties arising to the increase in the volume ratio of the fillers that can be incorporated into matrix (20).

Zirconia (ZrO_2) is a metallic oxide widely used as a biomaterial in medical devices, especially orthopedic as hip prosthesis, due to its proven high compressive strength and biocompatibility (21, 22). In the chemical industry, its monoclinic crystalline phase is widely used due to its ability of photocatalysis. (23, 24) In dentistry, zirconia is commonly stabilized in its tetragonal phase and applied as the main constituent of ceramic dental crowns in posterior teeth, once its color is very similar to the tooth, besides it has properties that are close to those of metals (21, 25-27). In addition, particles of ZrO_2 are present as reinforcing elements in composites for dental restorative procedures with the aim of improving its mechanical properties and reducing polymerization shrinkage (10). Its incorporation in adhesives enhanced radiopacity and microhardness (28). Zirconia has also shown bioactive properties both in monoclinic and tetragonal phases (22, 30).

Therefore, it appears to be an alternative to the development of dental adhesives. No studies to date, however, assessed its influence on adhesives bond strength over time (29). Furthermore, zirconia particles, when used in its pure form, undergo a volumetric expansion of ~4.5% caused by the martensitic transformation (t → m) (26), which could lead, in a dentine adhesive, to an increase on its imbrication with the collagen fibers in the adhesive interface.

Despite the widespread knowledge about zirconia applications in different areas of dentistry, the use of nanostructured non-stabilized particles has not been completely lengthwise assessed and understood yet. The purpose of this study was to develop an adhesive resin, with zirconium dioxide, and evaluate the properties of the obtained composite. The null hypothesis was that the addition of ZrO_2 has no influence on adhesive resin properties.

3.2 Materials and Methods

The reagents that composed the formulation of the adhesive resin were Bisphenol A Glycerolate Dimethacrylate (BisGMA), Triethylene Glycol Dimethacrylate (TEGDMA) and 2-hydroxyethyl methacrylate (HEMA). Camphoroquinone (CQ), ethyl 4-dimethylaminobenzoate (EDAB) and Diphenyliodonium Hexafluorphospate (DPIHFP) composed the initiator system. Zirconium dioxide (ZrO_2) was provided by Evonik (Essen, North Rhine-Westphalia, Germany), and was submitted to a silanisation process (31) using 5% of ɣ-MTBS (ɣ-methacryloxypropyltrimethoxysilane, Aldrich) and 95% of acetone as solvent, in weight, stored for 24h at 37°C to solvent evaporation.

To produce the organic matrix of adhesive resin 50 wt% Bis-GMA, 25 wt% TEGDMA and 25 wt% HEMA were proportioned. CQ, EDAB and DPIFHP were added at 1 mol% and 0.01 wt% BHT to all groups, . ZrO_2 were incorporated to the blend at concentrations of 0.5%, 1%, 4.8% and 9.1%, in weight. Moreover, a control group was made for each test without ZrO_2 incorporation.

To photo-activate the monomers a light-emitting diode (Radii Cal, SDI Ltd., Bayswater, Victoria, Australia) was used, with light intensity of $1200 mW/cm^2$.

Characterization of ZrO_2

X-ray diffraction

To identify the crystalline phases in the powder, a Phillips diffractometer (X'Pert MPD Model) was used, operating at an acceleration voltage of 40kV and electrical current of 40mA, using CuKα radiation. The scanning rate was 0.05°/min, and the time-step was 1s.

Micro-Raman Spectroscopy

The groups of ZrO_2 compounds were identified by micro-Raman spectroscopy (SENTERRA model, Bruker Optics, Ettlingen, Germany) equipment. A laser with 785nm wavelength was used, with 100mW of intensity, for 5 seconds and 2 co-additions. The range of analysis was $400\text{-}1200 cm^{-1}$.

Surface area

The specific surface area of the ZrO_2 powder was determined through Brunauer-Emmett-Teller (BET) method, using a Quantachrome NOVA1000 Autosorb Automated Gas Sorption System (Boynton Beach, FL, USA).

Degree of Conversion

The degree of conversion of the experimental adhesive resins was evaluated using Fourier Transform Infrared Spectroscopy (FTIR) with an infrared spectrometer (Vertex 70 model, Bruker Optics, Ettlingen, Baden-Württemberg, Germany) equipped with an attenuated total reflectance (ATR) device, and consisting of a horizontal diamond crystal with a mirror angle of 45°.Three samples (n=3) of 3μL were directly dispensed on the diamond crystal and the measurement was performed before and immediately after the polymerization (light-activation for 20s) (32). The percentage of unreacted double carbon-carbon bonds (%C=C) was determined by the ratio of the absorbance intensity between aliphatic carbon double bonds, considering it 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 (3). The degree of conversion (DC) was determined by subtracting the %C=C from 100%. To obtain the degree of conversion it was used equation 1:

$$(1) \qquad DC = 1 - \left(\frac{\text{absorbance(1635 cm}-1\text{) / absorbance (1610 cm}-1\text{) polymer}}{\text{absorbance (1635 cm}-1\text{) / absorbance(1610 cm}-1\text{) monomer}}\right) x\ 100$$

Radiopacity

The radiopacity evaluation of the experimental adhesives was performed according to the ISO 4049:2009 (33) standards. Five samples of 10 mm (± 0.1 mm) in diameter and 1.0 mm (± 0.01 mm) in thickness were produced per group. The radiographic images were obtained using a X-Ray source (model Spectro 70X, Dabi Atlante, Ribeirão Preto, SP, Brazil) and a digital system with phosporous plates (VistaScan; Durr Dental GmbH & Co. KG, Bietigheim-Bissingen, Alemanha) operating at 70kV and 8 mA. Each X-Ray image was taken with a focus-film distance of 400mm and exposure time of 0.4s. A specimen from each group was placed on each plate for a total of five samples per plate. An aluminum step-wedge was exposed simultaneously with the specimens in all images. The thickness of the aluminum scale ranged from 0.5 to 5.0 mm in 0.5 mm increments. The images were saved in TIFF format and assessed using ImageJ (National Institutes of Health, Bethesda, Maryland, USA)), by calculating the density of pixels in grayscale, using the aluminium step-wedge for comparison.

Softening in Solvent

To determine the softening in solvent, the specimens made for radiopacity assessment were used, 24h after polymerization. Five specimens for each experimental adhesive (n = 5) were embedded in chemically activated acrylic resin and polished in a polisher (Model 3v, Arotec, Cotia, SP, Brazil) with silicon carbide sandpaper with grain 600, 1200 and 2000 under constant irrigation. The specimens were submitted to a Knoop microhardness test in which three indentations (15g for 10s) were made on the surface of each specimen and assessed using an automatic microhardness tester HMV-2 (Shimadzu, Tokyo, Japan). The calculation of the hardness value was given by equation 2:

$$KHN = \frac{14228\ x\ c}{d^2} \tag{2}$$

in which 14228 is a constant, c is the load in grams and d is the length of the longest diagonal of the rhombus formed in µm. The initial knoop microhardness number (KHN_1) was registered as the arithmetic average of five measurements.

Once obtained KHN_1 results for each group, the specimens were immersed in absolute ethanol for a period of 4 hours. Immediately after the immersion the microhardness test was repeated in each specimen, obtaining KHN_2. The softening in solvent value was obtained by calculating the percentage difference between KHN_1 and KHN_2.

Microtensile Bond Strength

One hundred and twenty bovine lower incisors were used after cleaned from organic debris and stored in distilled water at 4°C for less than three months. The teeth had the labial enamel removed to expose the superficial dentin, which was polished with silicon carbide sandpaper with grain 600 for 30 seconds under constant irrigation to produce a standardized smear layer. The teeth were divided into five groups. In each tooth, on the exposed dentin, after surface etching with 37% phosphoric acid for 15 seconds, a commercial primer (Primer, Scotchbond Multi-use, 3M ESPE, St Paul, MN, USA) was applied for 20 seconds, and the solvent was evaporated with an air spray. Immediately after it, the adhesive was applied according to the experimental groups and photocured for 20s using a LED curing light (Radii Cal, SDI, Australia). Two increments of composite resin (Z350, 3M ESPE, St Paul, MN, USA) were placed on the cured adhesive to cover the dentin surface completely. Both increments were photocured for 40s each and the teeth were stored for 24 hours in distilled water in a light-proof container at 37°C. After, the samples were cut perpendicular to the flat adhesive-dentine surface with a diamond disc at low speed under constant irrigation (Isomet, Buehler Ltd, Lake Bluff, IL, USA), producing four to six sticks for each tooth, with an adhesive interface area of approximately 0.5mm². These sticks have their extremities fixed to a device for microtensile testing with cyanoacrylate adhesive. The microtensile assays were performed in a universal testing machine EMIC DL-2000 (EMIC, São José dos Pinhais, PR, Brazil) at a speed of 1 mm/min. The values of bond strength were calculated in MPa. Tooth was considered the sample unit. The microtensile bond strenght test was performed in half of the samples of each experimental group 24h after photocuring adhesives and, one year later, in the other half which was stored in distilled water at 37°C.

Interface Characterization by Micro Raman

The interface between adhesive and underlying dentin was analyzed by Micro Raman vibrational microscopy. A 100mW diode laser with 785nm wavelength and spectral resolution of ~3.5cm^{-1} was used in a SENTERRA Raman spectrometer (Bruker Optics, Ettlingen, Germany). Sticks produced as described for microtensile evaluation were used, immediately before the immediate microtensile bond strength test. One-dimensional mapping was performed over 100µm line across the adhesive–dentine interface at 1µm intervals. One mapping was performed per group at arbitrary sites. Post-processing was performed in Opus 6.5 software and consisted of analysis with modeling which allowed distinguishing spectral components of the adhesive and dentine. One correspondent peak of each substance was used for integration: phosphate at 960cm^{-1} (34), methacrylate at 1610cm^{-1} (3) and zirconia at 476 cm^{-1} (35).

Mineral Deposition

Restorations were made on bovine dentin and subsequently interfaces were produced by sectioning perpendicular to dentin-adhesive interface, with a diamond disc at high speed under constant irrigation. The interfaces were analyzed by Micro Raman spectroscopy using the same parameters described above. A two-dimensional mapping was performed by group, at arbitrary sites, over a 50x20µm area, at 1µm intervals. The analyzes were performed 24h after photocuring, then immersed in 20 mL of Simulated Body Fluid (SBF) (36), and re-evaluated on the same side of the sample after 14 days of immersion. The previously known hydroxyapatite peak (910cm^{-1}) (34) was used for integration and evaluation of mineral deposition at the interface area.

Statistical Analysis

The normality of the obtained data was tested using the Shapiro-Wilk test. Statistical analysis was performed for radiopacity, softening in solvent and DC data using one-way analysis of variance - ANOVA (ZrO_2 concentration) and Tukey's *post hoc* multiple comparisons test. KHN_1 and KHN_2 were assessed through paired Student t test. µTBS data were analyzed through two-way ANOVA (ZrO_2 concentration and time) and Tukey. All tests were performed at 0.05 level of significance.

3.4 RESULTS

The X-Ray diffractogram of the analysed sample is shown in Figure 1. It was possible to identify two polymorphic phases of ZrO_2. The combined JCPDS pattern of baddeleyite or primitive monoclinic ZrO_2 (24-1165) and ICSD pattern of tetragonal ZrO_2 (068781) match with the observed XRD peaks. The specific surface area value was 37.41 m^2/g. Raman analysis is presented in Figure 2 and showed typical chemical groups of ZrO_2 compounds, with 476cm^{-1} peak.

The Degree of Conversion (DC) values are presented in Table 1 and ranged from 71.7 (± 0.3) in the control group to 76.7 (± 0.3) at 1%. Only the group with addition of 1% ZrO_2 significantly increased GC compared to the group without ZrO_2 addition. Knoop microhardness values before and after immersion in solvent, and the degradation percentages are shown in Table 1. The group with 4.8% ZrO_2 presented higher initial mean values of microhardness Knoop (KHN_1) than the control group ($p<0.05$). After immersion in solvent, all groups presented reduction in hardness values (KHN_2). The percentage of hardness reduction was higher as increased the concentration of ZrO_2 incorporated. The groups with addition of 0.5% and 1% ZrO_2 had no difference to the control group ($p>0.05$). The radiopacity values are illustrated in Figure 3 and presented in pixel density. There was no difference between groups with ZrO_2 incorporation and the control group ($p>0.05$).

Mean and standard deviation values of µTBS tested after 24h and 1 year of water aging are shown in Table 2. There was no statistical difference between all groups in 24h or in 1 year. In 24h test the values ranged from 45.23 MPa to 55.81 MPa. After 1 year the values ranged from 30.92 to 52.94 MPa. Analyzing the variable time there was a significant reduction in the values from 24h to 1 year ($p<0.05$), independently of ZrO_2 concentration. No statistical significant interaction was verified ($p>0.05$).

Representative images of interfaces characterized by Micro-Raman spectroscopy are shown in Figure 4. The right-located color scale represent the amount, through the assessed area, of the elements which corresponding peaks were used for integration. It was possible to observe the presence of adhesive across the hybrid layer. It was also possible to observe the penetration of nanostructured zirconia at almost the same extension of dentin demineralization.

Representative images of interfaces evatuated by Micro-Raman for mineral deposition at the baseline (0 days) and 14 days after immersion in simulated body fluid (SBF) are shown in Figure 5. After 14 days, the groups with 4.8% and 9.1% addition of ZrO_2 promoted mineral deposition compared to control group.

3.5 DISCUSSION

The addition of inorganic particles as filler component in resinous matrix aims at improving the properties of the developed material, because as the volume fraction of inorganic content increases, the fraction of organic matrix decreases. Thereby, problems related to polymerization shrinkage (10, 12), sorption and solubility (13) are reduced, preventing, thus, degradation of material (18). However, this incorporation should happen without a decrease in its original properties and consequent injury to other physicochemical characteristics. In the present study, the null hypothesis was rejected, since the addition of ZrO_2 had influence on adhesive resin changing properties.

In this study, adhesive resins were formulated with incorporation of zirconia nanofillers. The addition of 1% ZrO_2 had a significant effect on increasing degree of conversion, and all presented values were higher than 70%. The particles of the oxide presented two crystalline phases, a favorable superficial area, which allows it to present greater reactivity, and a penetration into the hybrid layer. Moreover, the addition of 4.8% and 9.1% of ZrO_2 promoted mineral deposition over the adhesive-dentin interface.

Particles which present small size and, therefore, a large surface area, can be added into resin matrix in small volume fractions. As this amount increases its dispersion into polymer matrix is hampered, beyond jeopardizing absolute light transmission and conversion (37, 38). Furthermore, adhesive resins require low viscosity to enable efficient monomers infiltration into the collagen layer exposed after etching (1, 39). Therefore, the amount of filler to be incorporated is limited. Due to it, in the present study zirconia nanoparticles were added into resin matrix only at up to 9.1wt%.

Zirconium dioxide is widely used in dentistry. Furthermore, it was selected in this study due to presenting mainly availability and high purity. An increasing number of studies has been directed to the development of composites which filler particles act not only as reinforcing elements, but also interact with tooth substrate promoting mineral deposition over it (22, 29).

At room temperature and ambient pressure, the thermodynamically stable phase of pure zirconia is monoclinic and metastable tetragonal phase easily transforms into monoclinic phase. This transformation (t → m) is followed by a volumetric expansion (26, 40). Zirconia in powder form is very sensitive to steam and exposure to moisture from the environment can cause a martensitic phase transformation, besides the shearing stress caused in the comminution (41-43) The XRD peaks, showing the oxide as a blend of monoclinic and tetragonal forms, corroborates it.

A dental adhesive should ideally present enough radiopacity to allow differentiation between dental tissues and the material and, thus, propitiate the differential diagnosis of secondary caries, which can also behave as a radiolucent line underlying the restoration (44-46). This property is mainly related to the atomic number (Z) and material thickness (47). Although Zirconium (Z=40) presents considerable radiopacity when composing dental ceramic systems, the minimum application thickness of 2 mm is always respected (21), while in adhesive resins this measure must be thinner. Furthermore, current commercial composites containing $ZrO_{2,}$ which are also sufficiently radiopaque, present up to 80wt% of filler content (48), higher values than those used in this study. In other study, zirconia particles incorporated in a commercial adhesive enhanced radiopacity values, but only over 15% of filler incorporation (28). ISO 4049 recommends minimum value for radiopacity must be 1mmAl (33). Although ZrO_2 addition was not able to increase the radiopacity of experimental adhesive resins, falling below ISO requirement, the values obtained in this study are comparable with most adhesives currently available on market (46). Therefore the association with another material with higher radiopacity (49) could promote an increase in this property, while conserving the advantageous characteristics of zirconia.

Degree of conversion is shown as the percentage of consumption of aliphatic bonds during the polymerization and is associated not only with increased mechanical properties (18, 38) but also with polymer stability. Low degree of conversion values can lead to a more soluble polymer, consequently more susceptible to degradation (18, 32), since it contains a higher amount of unreacted monomers in the polymer matrix. Using the same methods of measurement and conventional irradiation conditions, a degree of conversion between 55-75% is acceptable for dental comonomeric blends (50). Considering filler particles, due to their high refraction index, disperse light, reducing its incidence and avaliability within the polymer and turn it more opaque (37, 38), one would expect that addition of ZrO_2 particles, which refractive index is approximately 2.00 (51, 52), could adversely affect the degree of conversion. However, it was observed that the addition of zirconia showed no difference from control group, except in the group with 1% ZrO_2, which significantly increased degree of conversion ($p < 0.05$), and all groups showed values higher than 55%. It could be explained by the photocatalytic activity exhibited by zirconia and already demonstrated in previous studies (23, 24), which present a great light absorption band close to that obtained by LED (24). Photocatalysis is a property of monoclinic, triclinic, and cubic phases and consists in chemical photodegradation by metal catalysts or their more stable oxides (53, 54). However, its influence on the used co-monomer blend may be confirmed in further studies. Furthermore, in the groups with addition of 4.8 and 9.1% ZrO_2 there was an increase in the opacity of the polymer resulting from the addition of fillers, which could have impaired the increase in the degree of conversion values in these groups.

Softening in solvent parameters also influence susceptibility to degradation polymers and inorganic particles are less prone to degradation. In this study, knoop microhardness measured before immersion in ethanol (KHN_1) increased with higher amounts of ZrO_2 incorporated, presenting a significant increase in the group with addition of 4.8% of ZrO_2 ($p<0.05$). A study which incorporated zirconia in a commercial adhesive observed enhancement in knoop microhardness only in groups with at least 24.8% of filler incorporation (28). Evaluating composites with polymeric matrix and inorganic fillers, however, the increase in hardness does not occur directly proportional to the increase of load amount. During the indentation, hard particles are pressed into the softer matrix rather than being plastically deformed under the applied load. Because of it, they cannot display their inherent hardness. With the increase of filler content, surrounding matrix comply less. It may explain why the increse on microhardness cannot be detected in the small concentrations, but only in the group with 4.8% ZrO_2 (55). In the group with 9.1% ZrO_2, the increase in hardness was probably not significant because the homogenization of the filler particles in the matrix may have been impaired. Numerically, even without significant differences, the values of degree of conversion have also been slightly smaller than the group with 5%.

In this study, hardness after immersion in ethanol for 4 h (KHN_2) decreased in all tested groups. The degradation percentage (Δ%) was higher in the groups with addition of 4.8% and 9.1% ($p<0.05$). Degradation happens due to the solvent penetration and its diffusion into the composite structure, leading to elution of its components. Polymers with higher amounts of cross-linking are less prone to degradation considering the limited solvent diffusion (18). In this study the higher degradation percentages in groups with filler addition can be explained by the absence of an effective bond between the polymer matrix and zirconia particles. Although there is evidence that silane, which was used in this study, may improve adhesion by increasing wetting and consequently promoting greater contact between methacrylate and the surface of zirconia (56-58), this agent finds no means of getting any molecular link due to the high crystalline content and limited presence of glassy phase in this ceramics (59).

Filled adhesive resins behavior when exposed to a solvent can be correlated with its stability in the oral environment (2, 18) and its ability to withstand hydrolytic degradation (1). In the present study, Raman analysis showed the presence of adhesive across the hybrid layer. It was also possible to observe the penetration of nanostructured zirconia at almost the same extension of dentin demineralization, allowing its interaction with dentin tissue. The addition of filler to the hybrid layer could improve its mechanical properties and enhance its stability. Once zirconia is known to be bioactive when assuming tetragonal and/or monoclinic structure (22, 29), it could improve bond strength durability by forming Zr-OH groups when in contact with the tubular fluid. These groups could promote effective apatite nucleation and its precipitation onto the interface (60). The bioactivity of an artificial material can be evaluated by examining the formation of apatite on its surface in simulated body fluid (36). After 14 days of immersion in SBF, Raman analysis was performed and groups with 4.8% and 9.1% addition of ZrO_2 promoted mineral deposition in the hybrid layer. However, in this study, the bond strength was evaluated by microtensile test immediately and after aging in distilled water for 1 year, and there was no difference between the filled and the control groups in 24h or in 1 year. It must be considered, therefore, evaluating the longitudinal bond strength of restorations stored in an environment that enables the mineral deposition, like SBF.

One study incorporated zirconia nanoparticles at similar concentrations in a commercial adhesive, which formulation is similar to the organic matrix used in this study (29). However, in that study, there was a significant increase, with no difference between the concentrations, in the immediate bond strength to dentin (30). However, in contrast to this study, the particles used were stabilized by yttria

(Y_2O_3), and may have reduced the martensitic transformation (t → m) presented by zirconia particles (26, 40, 41, 42) which leads to an alteration of its phase integrity and increases its susceptibility to aging (61, 62). This transformation could have influenced the bond strength in this study because it reduces the resistance of ZrO_2 to crack propagation. When zirconia is stabilized, the crack, during its propagation within a polymer network is unlikely to have sufficient energy to penetrate the filler particles. Crack path from the bottom of the hybrid layer onwards faced a tougher adhesive layer, acting as a barrier to its extension (30). However, this effect could be limited at concentrations as low as those used in this study.

In this study, still in the µTBS test, analyzing the variable time, there was a significant reduction in the values from 24h to 1 year ($p<0.05$). Most studies report significant decreases in bond strength, even after short storage periods in water, supposedly caused by hydrolitic degradation of interface components (1). Furthermore, the absence of bond between particles and matrix may have favored particles leaching from the polymer matrix.

The presence of zirconia in the hybrid layer could not prevent this degradation, but it must be considered that, in this study, specimens were stored in distilled water, which can have, in addition to increased particles susceptibility to aging, inhibited mineral deposition on the surface of the adhesive interface. Therefore, storing restored teeth in SBF during the aging period to allow mineral deposition at hybrid layer could promote a as a reinforcement in it and lead to better outcomes for long-term adhesion.

3.6 CONCLUSION

In the present study, an adhesive resin with ZrO_2 incorporation was formulated without a decrease in its original properties with up to 1% ZrO_2. The incorporation of 1% ZrO_2 increased significantly the degree of conversion of an experimental adhesive resin. The addition of 4.8% and 9.1% ZrO_2 promoted mineral deposition at interface. Thus, the addition of nanostructured non stabilized ZrO_2 could improve properties to methacrylate-based adhesive resins.

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3.9 TABLES

Table 1. Mean and standard deviation values of degree of conversion (DC), hardness of the adhesive before (KHN_1) and after immersion in solvent (KHN_2), and the variation of hardness values (Δ%).

Groups (%)	DC	KHN_1	KHN_2	Δ%
0 %	71.7(±0.3) ^{B}	21.4 (±1.0) ^{BC,a}	16.8 (±2.0) ^{b}	-21.3 (±7.0) ^{A}
0.5 %	74.0(±0.4) ^{AB}	21.4 (±1.8) ^{BC,a}	17.6 (±1.3) ^{b}	- 17.1 (±9.1) ^{A}
1 %	76.7(±0.3) ^{A}	20.6 (±2.7) ^{C,a}	13.8 (±3.6) ^{b}	-33.2 (±13.3) ^{AB}
4.8 %	73.0(±3.1) ^{AB}	26.2 (±2.0) ^{A,a}	15.4 (±1.4) ^{b}	-40.7 (±8.1) ^{B}
9.1 %	72.0(±1.1) ^{B}	24.6 (±1.5) ^{AB,a}	13.1 (±1.9) ^{b}	-46.3 (±10.1) ^{B}

Different capital letters indicate statistically significant difference in the same column ($p<0.05$). Different small letters indicate statistically significant difference in the line ($p<0.05$)

Table 2. Mean and standard deviation values of the µTBS test in 24h and after 1 year of water aging.

Groups (%)	µTBS 24h	µTBS 1 year
0 %	55.81 (±21.41)	41.72 (±12.96)
0.5 %	52.23 (±18.00)	52.94 (±24.23)
1 %	55.57 (±18.73)	45.80 (±9.21)
4.8%	45.23 (±17.97)	42.04 (±11.35)
9.1%	52.25 (±9.42)	30.92 (±13.29)

* Interaction p >0,05, time p<0,05, concentration p>0,05.

3.10 FIGURES

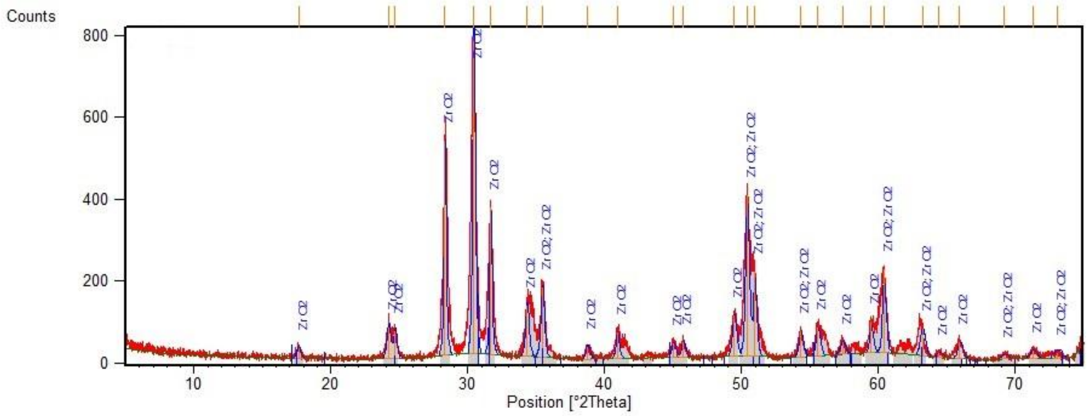

Figure 1. XRD pattern of the zirconium dioxide powder.

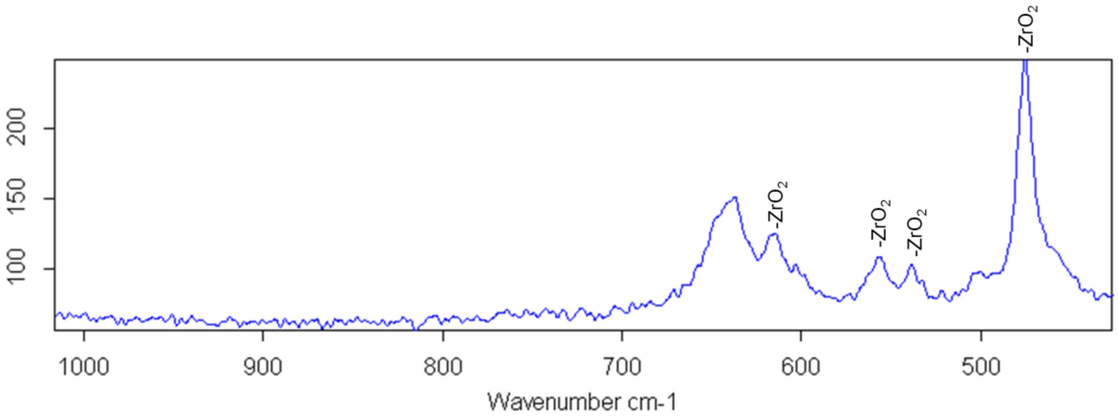

Figure 2. Micro-Raman spectrum of the zirconium dioxide powder.

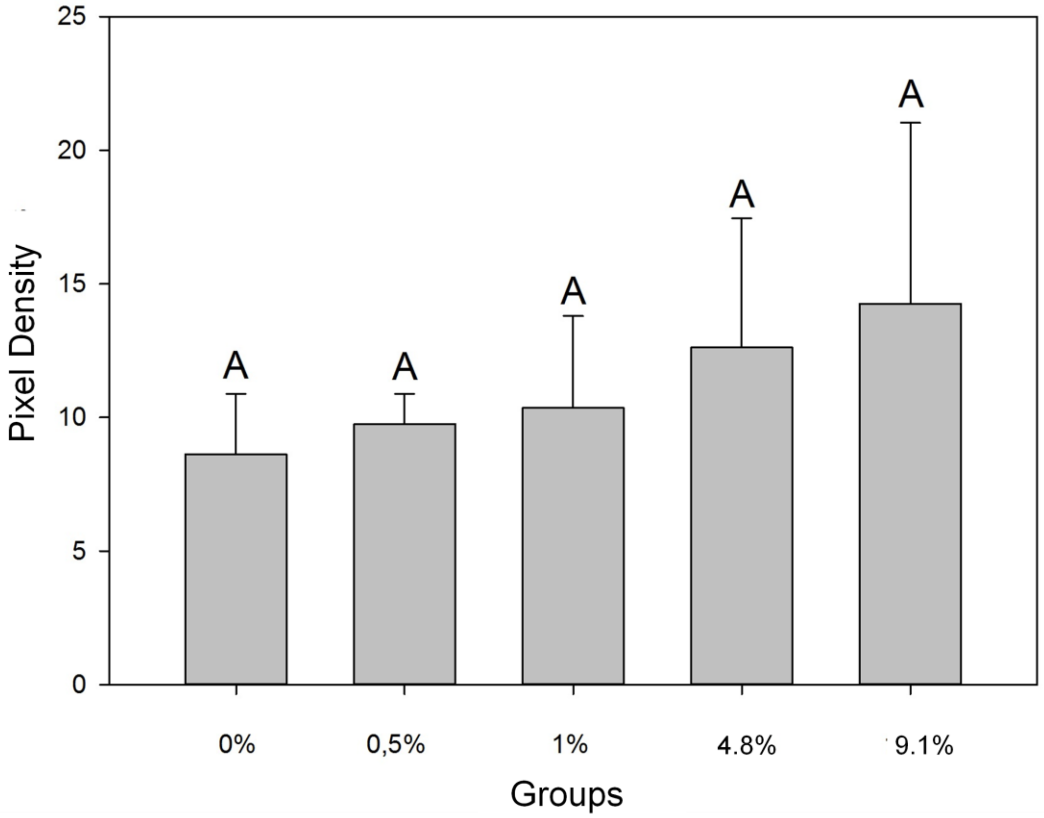

Figure 3. Mean and standard deviation values for radiopacity of adhesive resin. Equal capital letters indicate no statistical difference ($p < 0.05$).

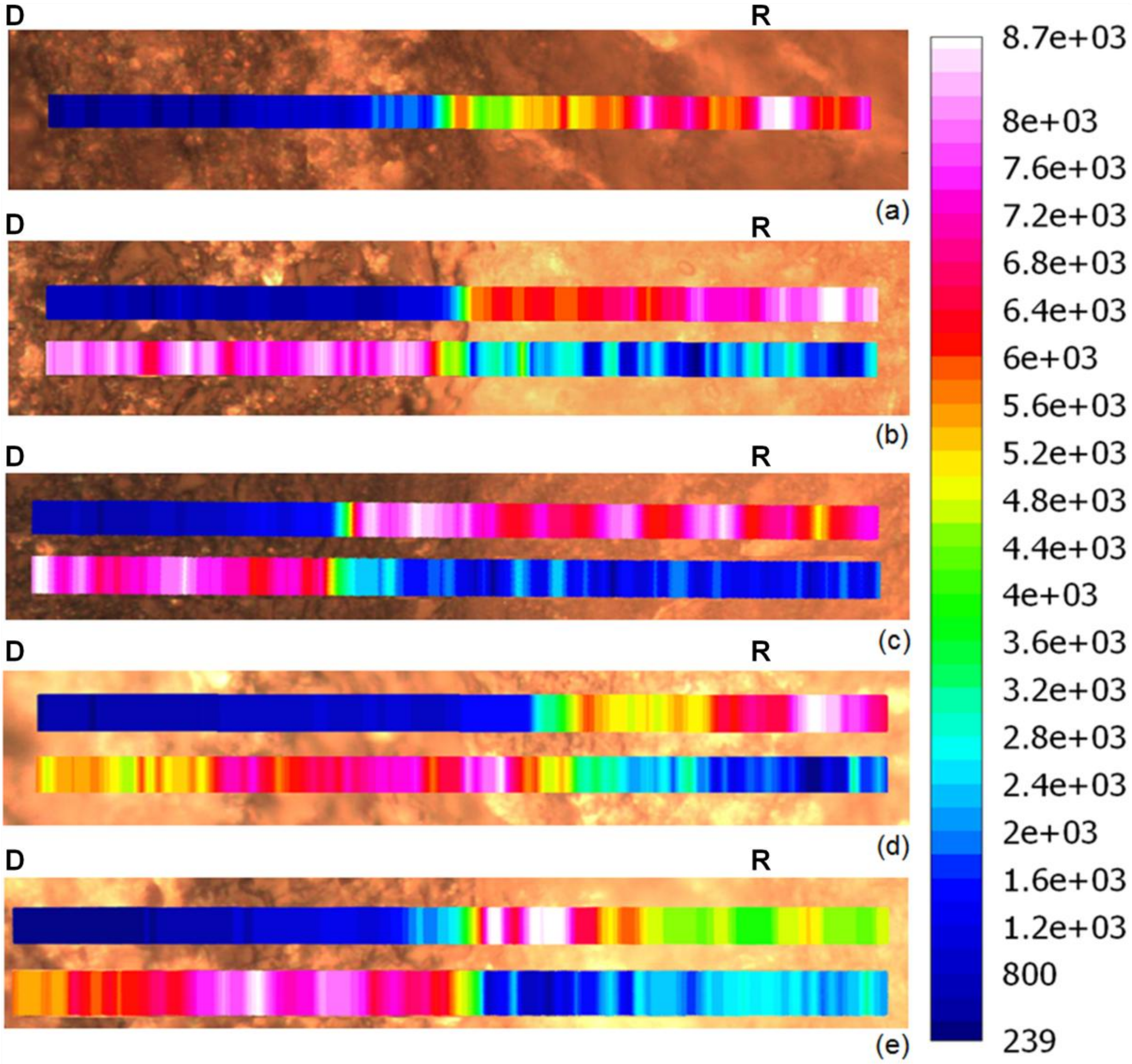

Figure 4. Dentin-adhesive interfaces characterization and representative color scale (right-located). D letters represent the part correspondent to dentin, and R letters represent the part correspondent to resin at the hybrid layer. (a) Adhesive without ZrO_2 integrated by methacrylate peak (1610 cm^{-1}). (b) Interface integrated by phosphate peak (910cm^{-1}) above and zirconia peak (476cm^{-1}) below, when assessed the group with 0.5% ZrO_2 addition. (c) Adhesive with 1% ZrO_2 addition. (d) Adhesive with 4.8% ZrO_2 addition. (e) Adhesive with 9.1% ZrO_2 addition.

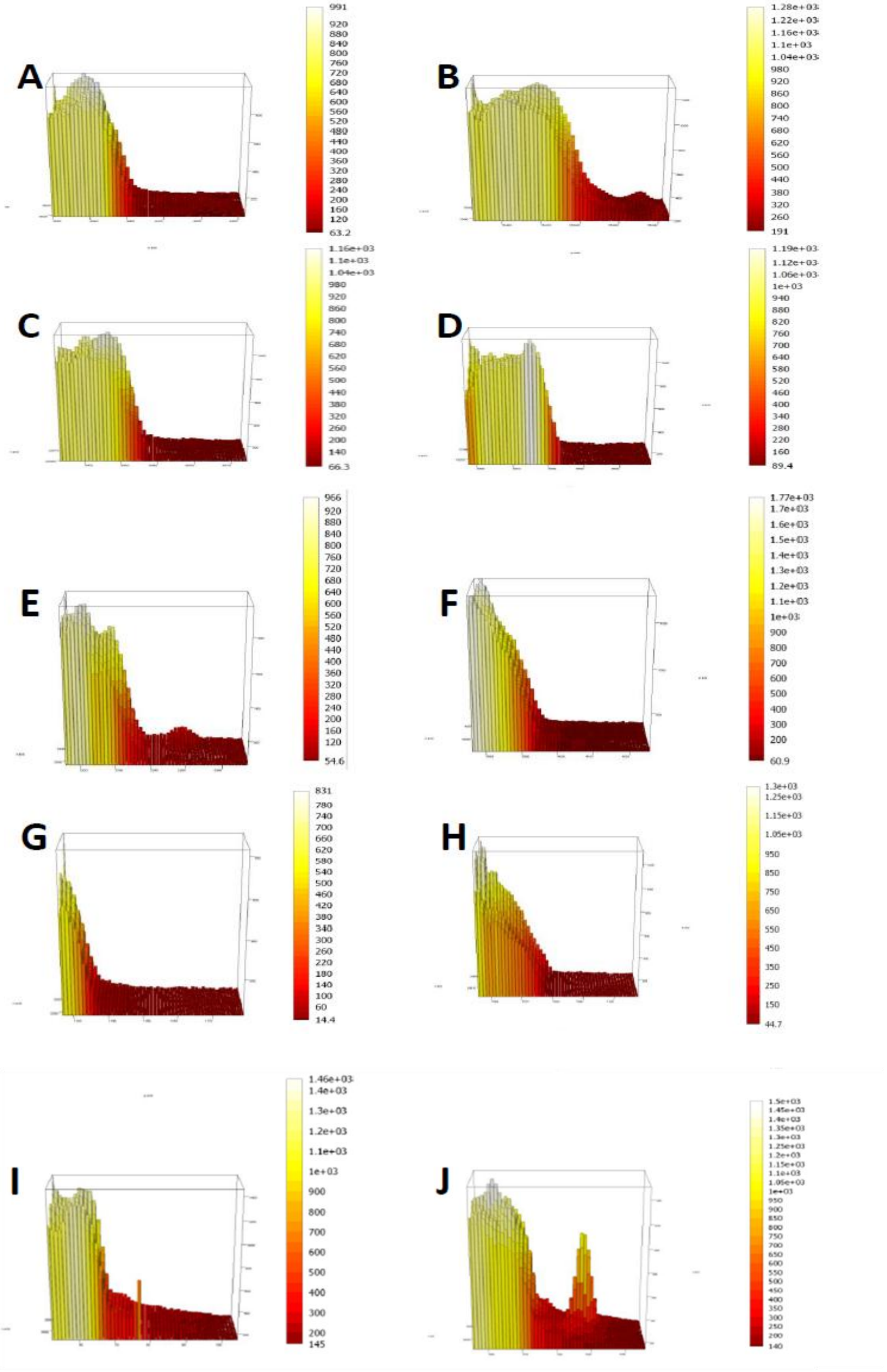

Figure 5. Representative 3D images of dentin-adhesive interfaces characterization integrated by phosphate peak (960 cm^{-1}) at baseline (0 days) and after 14 days immersed in SBF. A, C, E, G and I represent experimental groups with 0, 0.5, 1, 4.8 and 9.1 wt% of filler, respectively, at baseline (0

days). B, D, F, H and J represent the same groups, respectively, 0, 0.5, 1, 4.8 and 9.1 wt% of filler, after 14 days of immersion in Simulated Body fluid (SBF).

4 CONSIDERAÇÕES FINAIS

A busca pelo aprimoramento das propriedades dos materiais para uso odontológico tem levado à realização de diversos estudos. Dentre as modificações realizadas, destaca-se a incorporação de cargas inorgânicas à matriz polimérica desses materiais (COLLARES, 2010; LEITUNE, 2013a; LEITUNE, 2013b). No presente estudo foi possível desenvolver uma resina adesiva odontológica à base de metacrilato com a incorporação de partículas de dióxido de zircônio nanoestruturado (ZrO_2).

A avaliação *in vitro* das diversas propriedades do material resultante, associada com a caracterização da partícula, é indispensável para que se tenha uma maior compreensão acerca da interação dos componentes do material entre si, bem como do seu provável comportamento no ambiente oral.

A zircônia apresenta diversas formas polimórficas (DENRY; KELLY, 2008; HEUER, 1986). A avaliação do pó de ZrO_2 utilizado neste estudo mostrou que ele se caracteriza por uma mistura das fases monoclínica e tetragonal. Quando ambas ocorrem sob condições ambientes de temperatura e pressão pode-se inferir a ocorrência da transformação martensítica, em que a fase tetragonal, metaestável, transforma-se em monoclínica (GARVIE, 1975; HEUER, 1986).

Como resultado, foram obtidos adesivos experimentais com desempenho semelhante ao grupo sem adição de carga em relação à radiopacidade e à degradação em solvente até 1% de incorporação, a primeira devido às baixas concentrações utilizadas. Associando-se com outro agente radiopacificante (CARREÑO, 2012), no entanto, poder-se-ia melhorar essa propriedade, essencial para o diagnóstico de cáries secundárias pelo cirurgião-dentista (COLLARES, 2010; SCHULZ, 2008). As concentrações 0,5% e 1% ZrO_2 mantiveram níveis de amolecimento comparáveis ao controle após a imersão em etanol, mesmo com a ausência de uma união efetiva entre as partículas de carga e a matriz (PASSOS, 2010) que poderia facilitar a penetração do solvente e aumentar a degradação do polímero (FERRACANE, 2006).

Além disso, todos os grupos obtiveram grau de conversão entre 55-75%, parâmetros aceitáveis para monômeros de uso odontológico (STANSBURY; DICKENS, 2001). A adição de 1% ZrO_2 aumentou o grau de conversão, possivelmente devido à atividade fotocatalítica apresentada pela zircônia (KHAN; KIM, 2009; MISHRA, 2007).

Os resultados da análise por espectroscopia Raman indicaram a penetração das partículas de zircônia no interior do substrato dentário, permitindo a sua interação com o tecido dentinário. A presença de carga ao longo da camada híbrida pode aumentar a sua estabilidade a longo prazo. Considerando o potencial bioativo das partículas de zircônia, visto que os grupos com adição de 4,8% e 9,1% ZrO_2 promoveram deposição mineral ao longo da interface quando comparados ao controle após a imersão em SBF, pode-se esperar que essa resistência seja potencializada.

No entanto, devido á ocorrência da transformação martensítica nas partículas de zircônia, tanto a resistência à propagação de trincas diminui como a sua susceptibilidade ao envelhecimento em água aumenta (DEVILLE; CHEVALIER; GREMILLARD, 2006). Isto pode explicar a ausência de diferença encontrada entre os grupos com adição de ZrO_2 e o controle na avaliação da resistência de união à microtração, tanto imediata quanto após 1 ano. Além disso, também corrobora a redução nos valores após o período de envelhecimento em água. A incorporação de 10-MDP na formulação do adesivo provavelmente possa aumentar a união da matriz resinosa às partículas de ZrO_2 incorporadas (DE SOUZA, 2014), aumentando a sua estabilidade na matriz.

Dessa forma, pode-se concluir que, no presente estudo, a incorporação de ZrO_2 em uma resina adesiva experimental à base de metacrilato ocorreu sem um decréscimo em suas propriedades originais, e ainda melhorou o grau de conversão, foi capaz de penetrar no substrato dentário e promover deposição mineral na interface.

Para que se obtenha melhores resultados de adesão a longo prazo sugere-se que as partículas de zircônia sejam estabilizadas por óxidos que permitam a manutenção da fase cristalina tetragonal em temperatura ambiente, evitando assim a transformação martensítica e aumentando sua resistência à propagação de trincas (HANNINK; KELLY; MUDDLE, 2006). Também se propõe que os espécimes restaurados sejam armazenados em SBF durante o período de envelhecimento para permitir a deposição mineral e reforço efetivo na camada híbrida, prevenindo a sua degradação.

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