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ÓXIDO DE ZINCO NANOESTRUTURADO EM CIMENTOS ENDODÔNTICOS A BASE DE METACRILATO

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: Fabrício Mezzomo Collares

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RESUMO

KLEIN, Mariana. **Óxido de zinco nanoestruturado em cimentos endodônticos a base de metacrilato**. 2013. 39 f. Trabalho de Conclusão de Curso (Graduação em Odontologia) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2013.

O objetivo desse estudo foi caracterizar partículas de óxido de zinco nanoestruturado (ZnO_{nano}) e avaliar a influência de sua incorporação em um cimento endodôntico a base de metacrilato. Partículas de ZnO_{nano} produzidas pelo processo de evaporação térmica foram caracterizadas por microscopia eletrônica de varredura e método Brunauer-Emmett-Teller (BET). Um cimento experimental foi formulado usando monômeros de metacrilato e iniciadores e ZnO_{nano} foi adicionado nas concentrações 0, 20, 30 e 40%, em peso. Os cimentos experimentais com incorporação de ZnO_{nano} foram avaliados quanto a radiopacidade, escoamento, espessura de película, conforme a ISO 6876:2001, grau de conversão (GC) imediatamente após a fotoativação e após 7 e 14 dias a 37ºC, sorção de água, solubilidade, conforme a ISO 4049:2009, e o pH da água onde os espécimes ficaram imersos foi mensurado. A atividade antibacteriana dos cimentos em *Enterococcus faecalis* foi avaliada por teste de contato direto e a interface cimento/dentina foi avaliada por espectroscopia Raman (micro-Raman). O diâmetro médio das partículas de ZnO_{nano} foi de 40 nm, e a área superficial de 16 m^2/g. A radiopacidade do grupo ZnO_{nano} 40% não mostrou diferença estatística para 1mmAl. A adição de ZnO_{nano} aumentou estatisticamente o escoamento e a espessura de película dos cimentos e diminuiu o pH da água, quando comparados ao ZnO_{nano} 0%. O GC dos grupos experimentais diminuiu em relação ao ZnO_{nano} 0% após 7 e 14 dias. Não houve diferença estatística entre os valores de sorção e solubilidade dos grupos. A adição de ZnO_{nano} reduziu estatisticamente a contagem microbiana após 24 h comparado com o

grupo ZnO_{nano}0%. Na análise Raman, foi possível observar a presença de ZnO_{nano} no interior do substrato dentário. ZnO_{nano} mostrou características promissoras como carga no desenvolvimento de novos cimentos endodônticos.

Palavras-chave: Análise espectral Raman. Espectroscopia Infravermelho Transformada de Fourier. Nanoestruturas. Óxido de zinco.

ABSTRACT

KLEIN, Mariana. **Nanostructured zinc oxide on methacrylate-based endodontic sealers**. 2013. 39 f. Final Paper (Graduation in Dentistry) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2013.

The aim of this study was to characterize nanostructured zinc oxide (ZnO_{nano}) particles and to evaluate the influence of its incorporation in a methacrylate-based endodontic sealer. ZnO_{nano} particles produced by thermal evaporation process were characterized by scanning electron microscopy and Brunauer–Emmett–Teller (BET) method**.** An experimental sealer was formulated using methacrylate monomers and initiators, and ZnO_{nano} was added at concentrations of 0, 20, 30 and 40 wt%. Formulated sealers with ZnO_{nano} incorporation were evaluated based on radiopacity, flow, film thickness, according to ISO 6826:2001, degree of conversion (DC) immediately after photo-activation and after 7 and 14 days at 37ºC, water sorption (WS) and solubility (SL), according to ISO 4049:2009 and pH of the water where specimens were immersed was measured. Antibacterial activity on *Enterococcus faecalis* was evaluated by direct contact test and dentin-sealer interface was characterized by Raman spectroscopy (micro-Raman). ZnO_{nano} mean particle size obtained was 40 nm with 16 m^2/g of surface area. Radiopacity of ZnO_{nano} 40% group showed no statistically significant difference to 1 mmAl. ZnO_{nano} addition significantly increased flow and film thickness compared to ZnO_{nano} 0% and decreased water's pH. DC of experimental groups decreased compared to ZnO_{nano} 0% after 7 and 14 days. There was no statistical difference between groups considering WS and SL values. ZnO_{nano} addition statistically reduced bacterial count after 24 h when compared to group with ZnO_{nano}0%. At Raman analysis it was possible to detect

ZnO_{nano} inside dental substrate. ZnO_{nano} showed promising characteristics as a filler for endodontic root canal sealers.

Keywords: Raman spectrum analysis. Fourier Transform Infrared Spectroscopy. Nanostructures. Zinc oxide.

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1 INTRODUÇÃO

A completa obturação do sistema de canais radiculares é um passo determinante para o sucesso da terapia endodôntica. Mesmo após a correta desinfecção, falhas no preenchimento radicular podem determinar necessidade de retratamento[1-3]. Os cimentos endodônticos que apresentam melhores propriedades são os de base resinosa, por serem menos solúveis[4,5] aos tecidos apicais e por possuírem melhores propriedades mecânicas[6]. No entanto, os materiais disponíveis atualmente apresentam limitações quanto à ausência de atividade antimicrobiana significativa[7,8] e quanto à capacidade de selamento do sistema de canais[9].

Materiais poliméricos com base metacrilato apresentam-se como boas matrizes para a incorporação de carga, sendo frequentemente utilizados em materiais restauradores como resina composta[10], sistemas adesivos[11] e cimentos resinosos como agentes de cimentação[12]. Além disso, materiais de base epóxica e de metacrilato tem sido apresentados no mercado como cimentos obturadores de canais radiculares[13, 14].

O desenvolvimento de materiais poliméricos com características clínicas melhores vem sendo objeto de diversos estudos[10-12]. A busca por materiais capazes de determinar aumento da longevidade do tratamento vem sendo alvo de interesse da comunidade científica e da indústria[11, 14, 15]. O acréscimo de substâncias inorgânicas à matriz orgânica visa melhorar as propriedades mecânicas, químicas e físicas do material - aumentar a radiopacidade, diminuir a contração de polimerização, diminuir o desgaste e diminuir a degradação ao longo do tempo[9, 14-16]. Contudo, essa incorporação deve acontecer sem que haja um decréscimo em suas propriedades originais, não prejudicando as demais características físico-químicas.

As propriedades do material polimérico dependem da qualidade da rede polimérica formada[17-21], sendo demonstrada a influência do grau de conversão do polímero no seu desempenho mecânico[18, 19]. Com a adição de carga, a alteração do índice de refração dos

materiais fotoativados pode alterar a disponibilidade de energia luminosa no interior do polímero formado[17] e dificultar a mobilidade das cadeias durante a propagação da polimerização[18]. Quanto menor o número de ligações duplas alifáticas entre carbonos restantes depois de polimerizados, melhores são as propriedades mecânicas do polímero[19,22] e menor a solubilidade do material[23]. Além disso, alterações na reologia do material podem influenciar no molhamento e na penetração no substrato dentário[24, 25]. Por outro lado, estudos mostram que propriedades como sorção e radiopacidade do material são melhoradas com o aumento de volume de carga na matriz resinosa[16, 21, 26]. A influência na degradação hidrolítica[21] pode determinar aumento da longevidade do tratamento e a radiopacidade adequada permite distinguir o cimento obturador da estrutura dental normal, possibilitando a avaliação da qualidade do preenchimento radicular[16].

Diversos estudos tem sido realizados com objetivo de se buscar novas cargas que promovam o desenvolvimento de materiais com melhores propriedades[11, 14-16]. E, com esta mesma finalidade, a utilização de nanopartículas vem se desenvolvendo em ampla escala, inclusive em composições odontológicas[27]. A nanotecnologia permite a obtenção de materiais com diferentes propriedades, dependendo do tamanho e tipo de partícula. O tamanho nanométrico amplia a área superficial da partícula e sua reatividade, resultando na alteração das características do material. Além disso, o aumento da área superficial permite maior interação dessas partículas com os tecidos, aumentando o efeito terapêutico deste material[28]. As nanopartículas se destacam também quanto à sua atividade antimicrobiana, sendo esta mais expressiva quanto menor a dimensão das partículas[29]. Sendo assim, a possibilidade de se desenvolver materiais com propriedades físicas, químicas e mecânicas melhoradas, com maiores atividades terapêutica e antimicrobiana estimula a utilização das nanopartículas para o desenvolvimento de novas composições odontológicas[27]. Diversos materiais podem ser transformados em nanopartículas, dentre eles o óxido de zinco[30].

O óxido de zinco (ZnO) é um material inorgânico de amplo uso na Odontologia, e tem seu uso consagrado principalmente em cimentos endodônticos. Isso porque possui propriedades como radiopacidade, biocompatibilidade[31], estabilidade, além de apresentar potencial antiinflamatório e antimicrobiano[7]. Cimentos a base de óxido de zinco são amplamente conhecidos e foram utilizados como padrão de comparação para novos cimentos desenvolvidos[7]. O ZnO é amplamente utilizado em composições odontológicas (p.ex., cimentos e materiais restauradores) na forma de micro e macro partículas. Entretanto, a utilização de ZnO nanoestruturado (ZnO_{nano}) ainda não foi amplamente avaliada em materiais odontológicos.

2 OBJETIVOS

O objetivo do presente estudo foi desenvolver um cimento endodôntico de matriz resinosa com a incorporação de óxido de zinco nanoestruturado.

Objetivos específicos:

- Caracterizar o óxido de zinco nanoestruturado quanto à composição, ao tamanho de partícula e à área de superfície;

- Desenvolver um cimento a base de metacrilato com a incorporação de óxido de zinco nanoestruturado em diferentes concentrações;

- Avaliar as propriedades físicas, químicas e antimicrobianas dos novos cimentos endodônticos desenvolvidos.

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.

Nanostructured zinc oxide as a filler of resin-based root canal sealers

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Keywords: Raman Spectrum Analysis, Fourier Transform Infrared Spectroscopy, Nanostructures, Zinc Oxide.

3.1 Abstract

***Objectives*:** The aim of this study was to characterize nanostructured zinc oxide (ZnO_{nano}) particles and to evaluate the influence of its incorporation in an experimental methacrylate-based root canal sealer.

***Methods*:** ZnO_{nano} particles produced by thermal evaporation process were characterized by scanning electron microscopy and Brunauer–Emmett–Teller (BET) method**.** An experimental sealer was formulated using methacrylate monomers and initiators, and ZnO_{nano} was added at concentrations of 0, 20, 30 and 40 wt%. Formulated sealers with ZnO_{nano} incorporation were evaluated based on radiopacity, flow, film thickness, according to ISO 6826:2001, degree of conversion (DC) immediately after photo-activation and after 7 and 14 days at 37ºC, water sorption (WS) and solubility (SL), according to ISO 4049:2009, and pH of the water where specimens were immersed was measured. Antibacterial activity on *Enterococcus faecalis* was evaluated by direct contact inhibition (n=3) and dentin-sealer interface was characterized by Raman spectroscopy (micro-Raman).

***Results*:** ZnO_{nano} mean particle size obtained was 40 nm with 16 m^2/g of surface area. Radiopacity of ZnO_{nano} 40% group showed no statistically significant difference to 1mmAl. ZnO_{nano} addition significantly increased flow and film thickness compared to ZnO_{nano} 0% and decreased water's pH. DC of experimental sealers decreased compared to ZnO_{nano} after 7 and 14 days. There was no statistical difference between groups considering WS and SL values. ZnO_{nano} addition statistically reduced bacterial count after 24 h compared to ZnO_{nano} 0%. At Raman analysis it was possible to detect ZnO_{nano} inside dental substrate.

Conclusions: In the present study, the incorporation of 20%wt of ZnO_{nano} at an experimental methacrylate-based resin promoted satisfactory characteristics as radiopacity, flow, film thickness, degree of conversion, water sorption, solubility, presenting antimicrobial potential

and the ability to infiltrate through the dentin tubules. Thus, the addition of ZnO_{nano} could add interesting properties to methacrylate-based endodontic sealers.

Clinical Significance: The incorporation of nanostructured zinc oxide (ZnO_{nano}) increases endododontic root canal sealer properties and may be promising for developing new dental materials with enhanced properties.

3.2 Introduction

Zinc oxide (ZnO) is a metallic oxide which has been widely explored in multiple areas of science due to properties like semiconducting, piezoelectrics, and pyroelectrics (1). In Dentistry, ZnO is added to a several types of material due to its physical and chemical properties (2-4). The conventional endodontic sealer based on ZnO is one of the most used in Dentistry with a well known clinical behavior and tissue reaction. However, this conventional water based sealers present high solubility and could decrease the long term success of endodontic therapy (5, 6). Newly developed resin-based sealers promote more stable filling to the root canal and is the current focus on sealers development and research with a great variety of particles addition (7, 8).

The decreased size of fillers promotes modifications in characteristics, physical and chemical properties of materials (9, 10). Particles with nanometer size presents high surface area and increased surface reactivity due to a higher percentage of atoms on material's surface (10). A great variety of nanostructures could be achieved with ZnO such as nanocombs, nanorings and nanohelixes/nanosprings depending on the growth conditions (1). Recognized antibacterial properties are also encouraging a variety of applications which increases its efficacy with decreased particle size of ZnO (11). ZnO nanoparticles could be used as cosmetics (12), drug carriers and fillings in biomedical materials (13) due to its biocompatibility allowing it to be incorporated into dental materials such composite resins (2, 4) and adhesives (3, 14).

Zinc oxide macro and microstructures are already widely used in dentistry. However, its incorporation in nanoscale form in methacrylate-based sealers has not already been studied. The purpose of this study was to characterize nanostructured zinc oxide (ZnO_{nano}) particles and to evaluate the influence of its incorporation in an experimental methacrylate-

based root canal sealer. The null hypothesis was that the addition of ZnO_{nano} has no influence on metacrylate-based root canal sealer properties.

3.3 Materials and Methods

The monomers used in this study to produce an experimental root canal sealer were urethane dimethacrylate (UDMA), glycerol-1,3-dimethacrylate (GDMA), ethoxylated bisphenol A glycol dimethacrylate (BISEMA6), camphorquinone (CQ), N,N-dihydroxyethyl-para–toluidine (DHEPT) and benzoil-peroxyde (BP). These materials were used without further processing. Nanostructured zinc oxide (ZnO_{nano}) particles were obtained by thermal evaporation process (15-17). The filler was silanised using 5% of silane (g-methacryloxypropyltrimethoxysilane, Aldrich Chemical Co., Milwaukee, WI, USA) and 95% of solvent (acetone), in weight (18). The mixture was stored for 24 h at 37 °C to ensure complete solvent evaporation. Components were weighed using an analytical balance (AUW220D, Shimadzu, Tokyo, Japan).

Filler particles were added to the methacrylate-based resin in three different weight ratios (20, 30 and 40%) and a control with no ZnO_{nano} addition. The mixture (resin/particles) was hand-mixed for 30 s, sonicated for 90 s and hand-mixed again for 30 s. To perform sealer photoactivation, a light-emitting diode activation unit (Radiical; SDI, Bayswater, Australia) was used. An irradiation value of 1200 mW/cm^2 was confirmed with a digital power meter. Particle size, superficial area and particle structure were analyzed by scanning electron microscopy (SEM) using Hitachi (TM3000 model) and using a Quantachrome NOVA1000 Autosorb Automated Gas Sorption System (Boynton Beach, FL, USA), the specific surface area of the ZnO_{nano} powder was determined through the Brunauer–Emmett–Teller (BET) method (15).

Flow test

The flow test was conducted in accordance with ISO 6876:2001 (19). A total of 0.5 ml of each experimental sealer was placed on a glass plate (40.0 x 40.0 x 5.0 mm) with a graduated syringe. At 180 ± 5 s after the start of mixing, another plate with a mass of 20 ± 2 g and a load of 100 g was applied on top of the material. Ten minutes after the start of mixing, the load was removed, and the major and minor diameters of the compressed material were measured using a digital caliper. If both measurements were consistent to within 1.0 mm, the results were recorded. If the discs were not uniformly circular or the major and minor diameter did not match within 1 mm, the test was repeated. For each experimental group, the test was conducted three times and the mean value was taken.

Film thickness

The film thickness evaluation was conducted in accordance with the ISO 6876:2001 (19). Two glass plates (5.0 mm thick and 40.0 mm side) were placed together, and their combined thickness was measured. A drop of 0.5 ml of experimental sealer was placed at the centre of one of the plates, and a second plate was placed on top of the material. At 180 ± 5 s after the start of mixing, a load of 150 N was applied vertically onto the top glass plate. Ten minutes after the mixing, the thickness of the two glass plates and the interposed sealer film was measured using a digital caliper. The difference in the thickness of the two glass plates, with and without sealer, was the film thickness of the experimental sealer material. The mean value of three measurements for each sealer was taken as the film thickness of the material.

Water Sorption and Solubility

Water sorption and solubility tests were performed according to ISO 4049:2009 (20), except for the specimen's dimensions (10.0 ± 0.1 mm diameter, 1.0 ± 0.1 mm thickness). The specimens (n = 5) were stored in a desiccator at 37°C containing silica gel freshly dried and maintained at 37ºC. Each specimen was weighed to an accuracy of 0.01 mg at repeated intervals of 24 hours in an analytical balance (AUW220D, Shimadzu, Tokyo, Japan) until a constant mass (m_1) was obtained (i.e., until the mass loss of each specimen was not more than 0.1 mg in a 24 hours period). After final drying, two measurements of the diameter, at right angles to each other were taken with a digital caliper and the mean diameter was calculated. The thickness of the specimen was measured at the centre of the specimen and at four equally spaced points on the circumference. The area was calculated, in mm^2, from the mean diameter and then, using the mean thickness, the volume was calculated in mm^3. Thereafter, the specimens were stored in water at 37°C for 7 days, the volume of water for immersion being at least 10 ml *per* specimen. Then, the specimens were removed from the liquids, which were stored in a light-free container. Each specimen was weighed after being dry slightly to blot away surface water and weight was obtained (m_2) i.e., the mass of the hydrated specimens. After weighted, the specimens were returned to the first desiccator and the cycle was repeated and the constant mass was recorded as m_3. Water sorption (WS) and solubility (SL) in micrograms per cubic millimeter were calculated according previous study (21).

pH

The pH test was performed using the liquid stored during the sorption and solubility tests (n = 5) on a digital pHmeter (pH 21, Hanna Instruments, São Paulo, Brazil), totaling five measurements per group.

Radiopacity

The radiopacity of the experimental sealers was performed according to ISO 6876:2001 (19). Five specimens per group were produced with 10.0 mm ± 0.1 mm in diameter and 1.0 mm ± 0.1 mm thick. Radiographic images were obtained using a phosphor plate digital system (VistaScan; Dürr Dental GmbH & Co. KG, Bietigheim-Bissingen, Germany) at 70 kV and 8 mA, with 0.4 s of exposure time and a focus-film distance of 400 mm. For each film, one specimen from each concentration was positioned, for a total of four specimens per film. An aluminium step-wedge was exposed simultaneously with the specimens in all images. The aluminium step-wedge thickness ranged from 0.5 to 9.0 mm in increments of 0.5 mm. The aluminium alloy used was Al 99.12, Fe 0.47, Mg 0.41 and with <0.1 of Cu (mass %). The images were saved in TIFF format and analyzed using Photoshop software (Adobe Systems Incorporated, San Jose, 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 of 2 mm^2 (21).

Degree of conversion

The degree of conversion (DC) was measured by Fourier Transform Infrared Spectroscopy (FTIR) with a spectrometer (Vetrex 70, Bruker Optics, Ettingen, Germany) equipped with an attenuated total reflectance device, composed of a horizontal diamond crystal with a mirror angle of 45 degrees. A LED curing unit (Radiical; SDI, Bayswater, Australia) with irradiance of 1200 mW/cm^2 was fixed on a support to standardize the distance between the tip and the sample at 5 mm. The sample (uncured composite) was directly dispensed (3 µL) on the top of the diamond crystal and photoactivated for 60 seconds (n = 3). Data was evaluated with the Opus software (Bruker Optics, Ettlingen, Germany), with Blackman-Harris 3-Term apodization in a range of 4000 to 400 cm^{-1} and a resolution of 4 cm^{-1}. The DC was calculated considering the intensity of 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 (21). The analyses were performed with the same specimens after 7 and 14 days stored at 37ºC, at a dry place protected from light.

Direct Contact Inhibition

The antibacterial activities of the endodontic sealer were tested by Direct Contact Inhibition. A collection strain of *Enterococcus faecalis* (ATCC 29212; American Type Culture Collection, Rockville, MD) has been used in this study. Bacteria from frozen stock cultures were grown aerobically in Brain Heart Infusion (BHI) broth (HiMedia Laboratories Pvt.Ltd, Mumbai, India) at 37ºC. Cells were harvested by centrifugation and resuspended in fresh medium. Inocula were prepared by adjusting the cell suspension to a predetermined

optical density (OD) of 0.02 at 600 nm. For Direct Contact Inhibition (DCT), three specimens per group were produced with 3 mm (± 0.1 mm) in diameter and 1.0 mm (± 0.1 mm) thick. The specimens were sterilized in hydrogen peroxide plasma. Using a 96-well flat bottom plate, each specimen was placed on a well containing 300 µl BHI broth (HiMedia Laboratories Pvt. Ltd, Mumbai, India). Then, each well was inoculated with 20 µL of the *Enterococcus faecalis* suspension. The negative control consisted of three sets of wells containing uninoculated fresh medium (300 µl). Immediately after the placement of inoculums and after a 24 hours period, 90 µl of each well content were diluted in a saline to 10^{-8}. The 10^{-1}, 10^{-3}, 10^{-6} and 10^{-8} dilutions were plated in BHI Agar (HiMedia Laboratories Pvt.Ltd, Mumbai, India), using 25 µl aliquots of each dilution in duplicate. Plates were incubated at 37ºC, under anaerobic conditions. After 24 hours, colony were counted visually, scaled by dilution factors and then transformed into colony forming units (CFUs) per milliliters. The number obtained was transformed into base 10 logarithm ($\log_{10}$). The groups were statistically compared to each other. The experiment was carried out under aseptic conditions.

Interface characterization

Four lower incisor human teeth were cleaned of organic debris and stored in distilled water at 4°C for one week. The roots were sectioned below the enamel cement junction to obtain roots with a final length of 15 mm. The root canals were chemical-mechanically prepared with first series Kerr files until 40 file, in an ascending sequence, using a step back technique, associated with sodium hypochlorite 1% irrigation. The canals were prepared 1 mm below the final strength. After the chemical-mechanical prepare, the canals were irrigated with 3 ml EDTA for 1 minute and then washed with 3ml of sodium hypochlorite and 3 ml of

distilled water. The canals were dried with absorbent paper cones. The roots were then randomly divided and filled with the experimental sealer and gutta-percha cones as core material. The sealer was photoactivated for 40 seconds from the top of the root canal cervical portion and stored at 37±1ºC for seven days. After this period the roots were sectioned at low speed under constant irrigation (Low Speed Saw, Buehler, Lake Bluff, IL, USA) perpendicular to the long axis of the root in three sections, each one with approximately 5 mm. Micro-Raman spectroscopy was performed using a SENTERRA Raman Microscope (Bruker Optics, Ettlingen, Germany). It was used a 785 nm laser for 5 s with 2 co-additions, totalling 10 s with 100mW of laser power, resolution of 3 - 5 cm^{-1} and spectra were analyzed between 427 and 1800 cm^{-1}. A scan in mapping mode was performed in a defined line with 150 µm of length, including sealer and dentin so it was possible to characterize the interface and analyzed the sealer penetration into dentin.

Statistical Analysis

Data of radiopacity, flow, film thickness, water sorption, solubility and pH tests were analyzed using one-way ANOVA (ZnO_{nano} concentration) and Tukey. Direct contact inhibition test data was analyzed two-way ANOVA (ZnOnano concentration and time) and Student Newman Keuls test. Degree of conversion data was analyzed by repeated-measures ANOVA and Tukey. All tests were performed at 0.05 level of significance.

3.4. Results

ZnO_{nano} obtained is presented in Figure 1. Particles presented acicular shape also named as nanorods, tetrapods, tetra-needle-like, tetraleg ZnO nanostructures (15). The mean particle size obtained was 40 nm with 16 m^2/g of superficial area.

The flow, film thickness, water sorption, solubility, pH and radiopacity values are shown in Table 1. The flow of experimental sealers decreased as the concentration of ZnO_{nano} increased and ranged from 19.77 to 17.20 mm from 20 to 40%, respectively. The flow of the control group - with no ZnO_{nano} addition - was not possible to be measured because it exceeded the plate limits. The ZnO_{nano} addition significantly increased the film thickness, and that was no statistical difference between 20, 30 and 40% groups. In water sorption and solubility tests no significant difference was observed among groups. The pH of the water where specimens were immersed ranged from 6.75 to 6.14 from 0% to 40%, respectively. The radiopacity of experimental sealers ranged from 66.8 to 123.2 pixels, increasing with the concentration of ZnO_{nano} addition. The ZnO_{nano} 40% group showed no statistical difference from 1mmAl.

The DC values are shown in Figure 2. The DC of all groups showed statistical difference to control group after 7 and 14 days stored at 37ºC. Immediately after photoactivation only 20% group showed no difference to the group without ZnO_{nano} addition. But even the group with 40% of ZnO_{nano} addition, that showed the lowest degree of conversion value after photoactivation (2.43%), increased with time, showing 25.88% of conversion after 14 days at 37ºC.

Figure 3 shows the Raman spectrum of experimental endodontic sealer and root canal dentin. At Raman analyses it was possible to observe the ZnO_{nano} (582 cm^{-1}) penetration into dentin as function of the decreased phosphate peak (910 cm^{-1}) high.

Direct contact inhibition results are shown in Table 2. Immediately after the inoculums placement, it was not possible to detect any difference at the bacteria colonization between the groups. Otherwise, after 24 hours the groups with ZnO_{nano} addition showed a reduced number of CFU when compared with the group without any filler addition ($p<0.05$).

3.5 Discussion

The addition of inorganic particles to a polymeric matrix should improve its chemical and physical properties (22), as viscosity (23), radiopacity (8), water sorption and solubility (24). Zinc oxide particles present overbroad utilization in dentistry due to its long term clinical knowledge and biocompatibility (6). Furthermore, zinc oxide particles present antimicrobial properties that increase with decrease in particle size (1, 11). In this study, ZnO_{nano} particles were characterized and its addition to an experimental methacrylate-based resin increased its radiopacity and promoted satisfactory flow, film thickness, degree of conversion, water sorption and solubility and antimicrobial properties. Therefore, the null hypothesis was rejected.

The success of the endodontic therapy depends on a complete root canal filling to promote an adequate sealing (25). The sealer should present sufficiently flow to penetrate into irregularities and ramifications of the root canal system to promote a complete obliteration. Flow and film thickness are influenced by the filler size. The reduction of particles size to nanosized increases the surface area (e.g., 16 m^2/g of surface area in this study) decreasing the volume fraction that could be added to polymeric matrix. In the present study, nanosized particles (mean particle size of 40 nm) of zinc oxide were produced and used for sealer development. However, even with the addition of 40% wt of ZnO_{nano}, the flow and film thickness did not present statistical differences from an addition of 20%. The values found for flow and film thickness were close to the ones established at ISO 6876:2001 (19) (more than 20 mm and less than 50 µm, respectively). Despite the fact that some values were not in accordance to the ISO standard, the standard is for conventional aqueous based endodontic sealer and methacrylate-based materials tends to behave differently regarding rheological properties (26). One could think that a sealer should present a low viscosity, however a sealer

with higher flow could overflow beyond the apex leading to biocompatibility concerns. Furthermore, the ZnO_{nano} addition adjusted the viscosity of the sealer, considering that the group without nanoparticles addition showed a flow that could not be measured (i.e., material flowed beyond the glass plate). The ZnO_{nano} addition promoted reliable handling characteristics to be used as an endodontic sealer.

Considering the hydrophilic behavior of dentin, the wet environment could interfere in the polymer network formation leading to a resin more prone to long term degradation (24). It is well known that methacrylate-based materials present hydrolytic degradation over time in oral environment (27). The water sorption and solubility of polymer could lead to a variety of chemical and physical processes that may result in deleterious effects on the structure and function of dental materials. Polymerized methacrylate-based material in a wet environment could suffer a swelling process. After swelling, leaching of unreacted monomers could occur, affecting stability of material over time (28). Solubilization of components could lead to gap formation or fluid infiltration and leaching of monomers to the periapical region producing cytotoxicity reactions (29). In this study, water sorption and solubility values showed no statistical difference among groups. Lack of solubility has also been stated as an ideal characteristic for root canal filling materials (30) to prevent inflammatory process at apical region. The pH of residual solubility test water showed decreased values as the ZnO_{nano} concentration increased. However, all groups presented a pH near neutrality. In this study, water sorption and solubility test was performed following ISO 4049:2009 (20) because methacrylate-based root canal sealers do not present standardization, considering that ISO used for endodontic sealers only approaches aqueous based cements.

The water sorption and solubility properties present a correlation with degree of conversion (31). High degree of conversion may lead to low solubility, decreasing the amount of unreacted monomer into polymeric matrix. Thus, increased DC is related to increased

mechanical properties (21, 32, 33). The groups with 20 and 30% of ZnO_{nano} addition showed higher DC than 40% addition. However, all groups showed an increased in the degree of conversion after 7 and 14 days stored at 37ºC due the dual cure system added to the sealer Considering that the availability of light energy within the polymer depends on its refractive index (34), it's acceptable that the degree of conversion decreases with the increased of filler's concentration since the ZnO refractive index is approximately 2.00.

An ideal root canal sealer should present enough radiopacity to allow distinction from the adjacent anatomical structures for endodontic treatment evaluation. The ISO 6876:2001 (19) establishes 3 mmAl as the minimum radiopacity value for a material to be used as endodontic sealers. In this study, the addition of ZnO_{nano} in all concentrations did not promote a satisfactory radiopacity that enable its use as an endodontic sealer. Although the experimental sealers did not meet the ISO requirements, 40 wt% addition groups showed higher radiopacity values than control group ($p<0.05$). Therefore, others radiopaquing agents as barium sulphate or ytterbium trifluoride (8) should be added to the experimental endodontic root canal sealers to produce higher radiopacity values. The addition of ZnO_{nano} could add interesting properties to sealers developed with other radiopacifiers.

It is already well established that even after the root canal preparation for endodontic treatment with complete irrigation and shaping, there are still microorganisms impregnated at the root canal walls. This dentinal tubule infection can be even the cause of recurrent disease determining endodontic retreatment. In this study, all concentrations of ZnO_{nano} addition (20, 30 and 40%) influenced the *E. faecalis* growth at o direct contact inhibition. The use of a sealer exhibiting antibacterial properties may be useful to decrease or avoid growth of the remaining microorganisms in the root canal dentin. *Enterococcus faecalis* is a recognized pathogen in post-treatment endodontic infections and it is considered the most resistant bacteria species to chemo-mechanical preparation (35). Considering the antimicrobial effect

of ZnO_{nano} particles and the size dependent characteristic of this effect, we may predict that the smallest particles of this inorganic filler that penetrated into dentin tubules may contribute for its disinfection.

In the present study, the Raman spectra of sealer/dentin interface showed zinc oxide penetration in root canal dentin substrate, which could increase the antimicrobial potential of the sealer (11). Furthermore, ZnO_{nano} infiltration into dentin could decrease collagen degradation by reducing some matrix metalloproteinases (MMP) expression. When zinc molecules bind to collagen sites it produces conformational changes that makes the collagen fibers less prone to metalloproteinases effect (3, 14). Another important factor is that the presence of inorganic filler into this tissue could increase the stability of dentin-methacrylate sealer interface (3, 14). Thereby, penetration of zinc oxide nanoparticles in the dentin substrate could increase endodontic treatment longevity.

3.6 Conclusion

In the present study, the incorporation of 20%wt of ZnO_{nano} at an experimental methacrylate-based resin promoted satisfactory characteristics as radiopacity, flow, film thickness, degree of conversion, water sorption, solubility, presenting antimicrobial potential and the ability to infiltrate through the dentin tubules. Thus, the addition of ZnO_{nano} could add interesting properties to methacrylate-based endodontic sealers.

3.7 Acknowledgements

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3.8 Tables

Table 1: Mean and standard deviation of flow, film thickness, water sorption and solubility, specimens immersed water's pH and radiopacity of experimental sealers:

	Flow (mm)	Film thickness (µm)	Water sorption (µm/mm³)	Solubility (µm/mm³)	Water's pH	Radiopacity (pixels)
0%	*	26.7 (±5.0)^{B}	39.41 (±5.63)^{A}	- 4.16 (±0.09)^{A}	6.75 (±0.11)^{A}	66.8 (±5.3)^{C}
20%	19.77 (±0.99)^{A}	54.0 (±12.2)^{A}	32.30 (±1.71)^{A}	- 2.98 (±1.44)^{A}	6.35 (±0.13)^{B}	103.6 (±6.9)^{B}
30%	18.59 (±0.99)^{A}	48.0 (±7.2)^{A}	35.21 (±3.82)^{A}	- 1.73 (±1.52)^{A}	6.20 (±0.12)^{B,C}	110.6 (±7.9)^{A,B}
40%	17.20 (±2.08)^{A}	56.7 (±5.8)^{A}	36.50 (±6.51)^{A}	0.27 (±6.06)^{A}	6.14 (±0.03)^{C}	123.2 (±9.0)^{A**}

Different capital letters means significant statistical difference in columns ($p < 0.05$).

* Flow of experimental sealer without ZnO_{nano} addition was unable to measure.

** Radiopacity of 40wt% group had no statistical difference to 1mmAl (134.0 ± 4,35).

Table 2: Antimicrobial activity of experimental sealers against *E. faecalis* at Direct Contact Inhibition Test (DCT), expressed as $\log_{10}$ CFU/ml.

	0h	24h
0%	6.84 (±0.01) A, b	9.17 (±0.04) A, a
20%	6.85 (±0.09) A, b	8.33 (±0.17) B, a
30%	6.97 (±0.03) A, b	8.74 (±0.16) B, a
40%	6.94 (±0.06) A, b	8.37 (±0.32) B, a

Different capital letters indicate significant statistical difference in columns ($p<0.05$). Different small letters indicate significant statistical difference in lines ($p<0.05$).

3.9 Figures

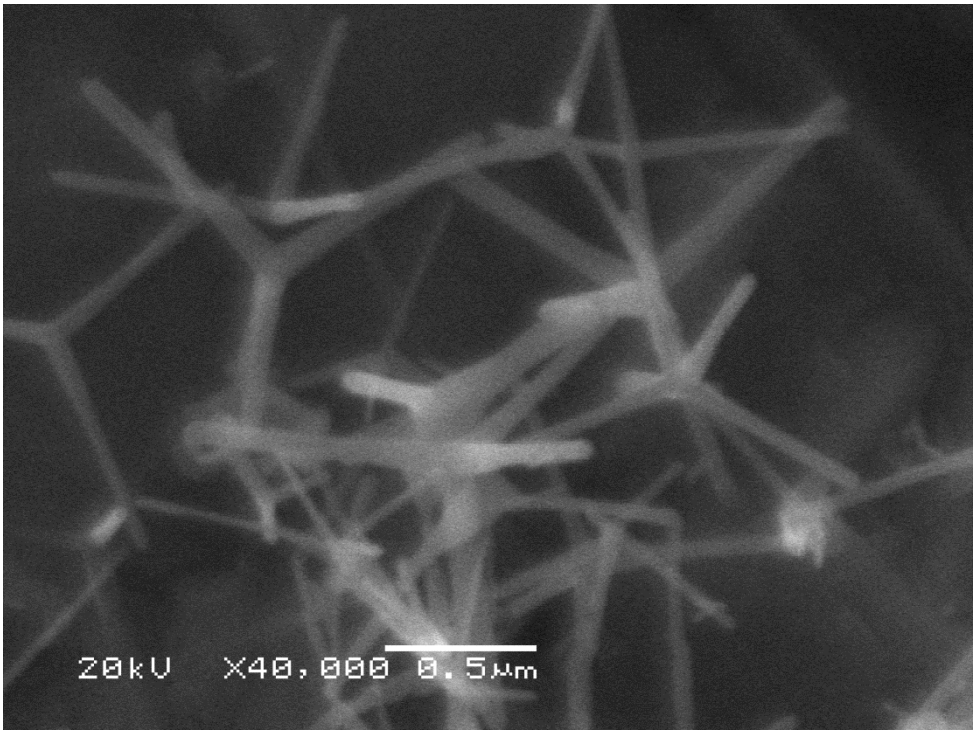

Figure 1. Scanning Electron Microscopy (SEM) of ZnO_{nano} showing the nanostructure after thermal evaporation process (40000x).

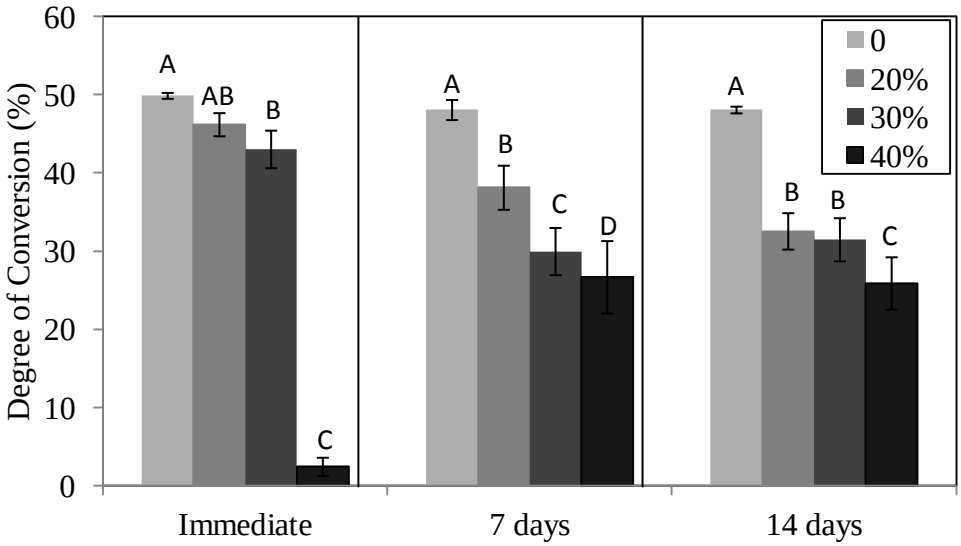

Figure 2. Degree of conversion of experimental sealers as a function of ZnO_{nano} concentration over time. Different letters indicate significant differences between different concentrations at the same time ($p<0.05$)

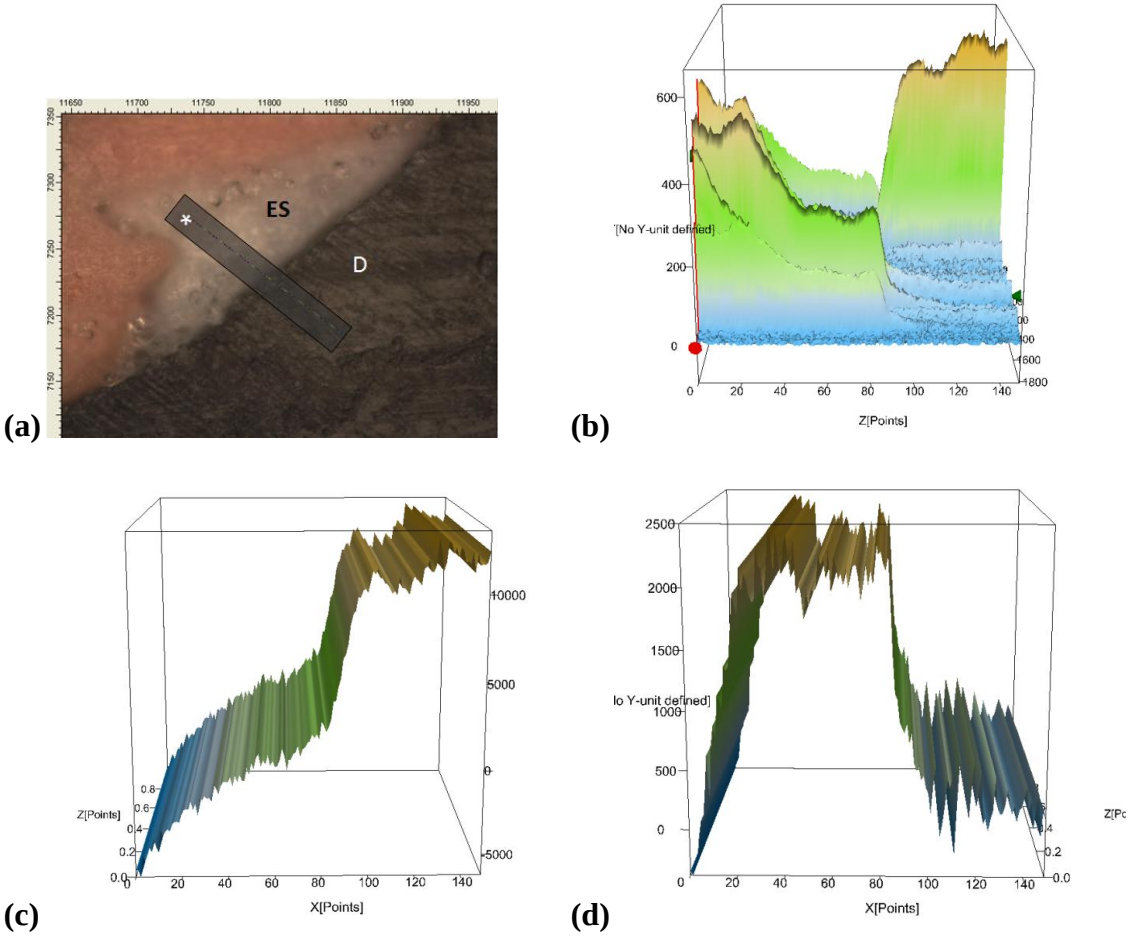

Figure 3. Interface analysis by micro-Raman. Representative Raman analysis of dentin-sealer interface. **(a)** Picture of the interface where ES is the experimental sealer, D is the dentine and the rectangle marked by * is the representation of the analyzed line. **(b)** Overall interface analysis. **(c)** Graph representing the integration of the hydroxyapatite corresponding peak (910 cm^{-1}). **(d)** Graph representing the integration of the corresponding zinc oxide peak (582 cm^{-1}).

4 CONSIDERAÇÕES FINAIS

Diversos estudos tem sido realizados com o objetivo de se desenvolver materiais odontológicos com melhores propriedades, através da incorporação de novas cargas a esses materiais[11, 14-16]. No presente estudo foi possível desenvolver um cimento endodôntico a base de metacrilato através da incorporação de partículas de óxido de zinco nanoestruturado. As partículas utilizadas no presente estudo apresentaram tamanho médio de 40 µm e área de superfície de 16 m^2/g.

Como resultado, foram obtidos cimentos endodônticos experimentais com melhor desempenho que grupo sem adição de carga em relação à radiopacidade, espessura de película, escoamento e atividade antimicrobiana. Além disso, a adição de ZnO_{nano} não influenciou na sorção de água e solubilidade e ainda resultou em materiais com grau de conversão e pH da água satisfatórios. Os resultados da análise de espectroscopia Raman indicaram a penetração da carga no interior do substrato dentário, permitindo interação do ZnO_{nano} com o tecido dentário. A estrutura nanométrica da partícula permite a penetração no interior dos túbulos dentinários. A presença de carga inorgânica no interior do substrato pode determinar o aumento da estabilidade dessa interface[32] contribuindo para o melhor selamento do canal radicular. Além disso, considerando a capacidade antimicrobiana do ZnO_{nano}, pode-se sugerir que a penetração da partícula no interior do tecido dentário pode contribuir para a desinfecção radicular, visto que, mesmo após o preparo químico-mecânico, bactérias residuais permanecem no interior do tecido[33].

Além disso, a penetração de óxido de zinco nanoestruturado no interior do substrato dentário pode representar aumento da longevidade do tratamento endodôntico, através do aumento da estabilidade da obturação e redução da degradação hidrolítica da superfície. Isso porque o zinco possui caráter inibidor da degradação de colágeno por metaloproteinases de

matriz (MMP)[24, 32, 34]. Essas enzimas, estruturalmente conhecidas como endopeptidases zinco-dependentes, estão presentes na dentina e contribuem para organização da matriz dentinária e sua mineralização. O zinco provoca modificações na estrutura do colágeno, diminuindo a degradação mediada pelas MMPs. Desta forma, a penetração de óxido de zinco no interior do substrato dentário torna o tratamento endodôntico menos suscetível a degradação hidrolítica e enzimática, aumentando sua longevidade[32, 34].

Dessa forma, podemos concluir que o óxido de zinco nanoestruturado, adicionado como carga a uma matriz resinosa a base de metacrilato, não modificou ou ainda melhorou suas propriedades. Além disso, foi capaz de penetrar no substrato dentário, podendo influenciar na estabilidade da interface entre a obturação e o substrato dentário[32,34], e obteve potencial antimicrobiano, características importantes para o seu uso como cimento endodôntico.

Com o estudo, buscou-se a geração de conhecimento científico na forma de artigo científico. Através da realização deste trabalho, com o desenvolvimento de novos cimentos com características promissoras, se prevê um impacto econômico positivo, através do desenvolvimento de materiais poliméricos inovadores nacionais com valor agregado e da substituição competitiva de importação através da produção desses materiais para uso biomédico. Pretende-se, ainda, estabelecer parceria com empresa de produtos odontológicos para a produção destes novos materiais, em prol do desenvolvimento do modelo no qual Universidade, Empresa e Governo promovam a transformação do conhecimento em inovação, de acordo com o Plano de Desenvolvimento Institucional da UFRGS[35].

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