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NARA JULIANA CUSTÓDIO DE SENA

PROPRIEDADES FÍSICO-QUÍMICAS E ANTIMICROBIANAS DE UMA RESINA ORTODÔNTICA MODIFICADA PELA INCORPORAÇÃO DE NANOPARTÍCULAS DE PRATA COM FOSFATO DE CÁLCIO

FORTALEZA/CE
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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como requisito parcial para a obtenção do Título de Mestre em Odontologia.

Área de Concentração: Clínica Odontológica.

Orientador: Prof. Dr. Vicente de Paulo Aragão Saboia
Co-orientador: Prof Dr. Victor Pinheiro Feitosa

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Aprovada em ____/_____/_____

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FORTALEZA – CE

2017

A minha filha Júlia, que me fez ver que não existe nada maior, melhor e mais verdadeiro que o amor por ela.

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RESUMO

O presente estudo tem objetivo de avaliar propriedades físico-químicas, resistência ao cisalhamento em esmalte bovino e efeito antibacteriano de uma resina ortodôntica (RO) incorporada com nanopartículas de prata (AgNPs) com ou sem fosfato de cálcio (CaP). As RO foram preparadas com 1 ou 5% em peso de AgNP e 1 ou 5% em peso de AgNP e CaP. As propriedades físico-químicas avaliadas foram grau de conversão (GC), sorção (WS) e solubilidade (SO) em água, flexão de três pontos (resistência à flexão, FS e módulo, E), rugosidade superficial (Ra) e microdureza Knoop (KHN). A adesão ao esmalte bovino foi avaliada por ensaio de resistência ao cisalhamento (SBS). A atividade antimicrobiana da resina contra Streptococcus mutans foi medida por contagem de unidades formadoras de colónias. Os dados foram analisados estatisticamente por ANOVA unidirecional e teste de Tukey ($p<0,05$). Os resultados mostraram que os testes GC, FS, KHN, SBS e antimicrobiano não mostraram diferença estatística entre os grupos controle e experimentais. O valor de WS diminuiu em RO 1% em peso de AgNP e o valor de SO também diminuiu em RO 5% em peso de AgNP. FS obtiveram maior resultado em RO 5% em peso de AgNP. RO 1% e 5% em peso de AgNP e 5% em peso de AgNP com CaP atingiram uma rugosidade estatisticamente inferior à RO.

Palavras-chaves: Nanopartículas de prata, resina ortodôntica e atividade antimicrobiana.

ABSTRACT

Objectives: To assess physicochemical properties, enamel strength and antibacterial effect of an orthodontic resin (OR) incorporated with silver nanoparticles (AgNPs) with or without calcium phosphate (CaP). Methods: ORs were prepared with 0, 1 or 5wt% of AgNP and 0, 1 or 5wt% AgNP and CaP. Physicochemical properties were evaluated in terms of degree of conversion (DC), water sorption (WS) and solubility (SO), three point bending (flexural strength, FS and modulus, E) and surface roughness (Ra) and Knoop microhardness (KHN). Enamel adhesion was evaluated by shear bond strength test (SBS). Resin's antimicrobial activity against *Streptococcus mutans* was measured by counting colony-forming units. The data was statistically analyzed by one-way ANOVA and Tukey's test ($p<0.05$). Results showed that the DC, FS, KHN, SBS and antimicrobial tests showed no statistical difference between control and experimental groups. WS value decrease in OR 1wt% AgNP and SO value also decrease in OR 5wt% AgNP. FS achieve higher result in OR 5wt% AgNP. OR 1wt% and 5wt% AgNP and 5wt% AgNP with CaP attained statistically lower roughness than OR. Significance: The incorporation of AgNP in OR alters some physical-chemical properties, but does not interfere in its adhesion to the enamel. The use of CaP associated with AgNP might attain remineralization when undergoing acid challenges. Its antimicrobial potential has not been verified, but more studies are necessary to confirm this fact.

Key word: Silver nanoparticles, orthodontic resin, antimicrobial activity

SUMÁRIO

Introdução Geral

1. INTRODUÇÃO GERAL

A Ortodontia apresentou grandes mudanças com a substituição do procedimento de bandagem de peças ortodônticas pela colagem realizada através de condicionamento ácido e materiais adesivos (BUONOCORE, 1955; NEWMAN, 1965; RENSCH, 1973). Braquetes ortodônticos colados são mais vantajosos que os bandados por não possuírem contato interproximal, são mais fáceis de posicionar e remover, e são mais estéticos, higiênicos e causam menos irritação gengival (GORELICK,1977; THANOS, MUNHOLLAND & CAPUTO, 1979).

Os componentes do aparelho e o material de cimentação geralmente promovem acúmulo de micro-organismos formadores de placa que podem causar descalcificação com formação de manchas brancas na superfície do esmalte (DERKS et al., 2007; ECKSTEIN, HELMS, KNOSEL, 2015). Lesão de mancha branca pode ser definida como a redução do volume dos cristais e aumento dos poros que ocorre abaixo da camada superficial do esmalte. Pesquisas recentes encontraram que a incidência de pelo menos uma lesão de mancha branca em pacientes que foram tratados com aparelhos fixos foi entre 46% e 73% (RICHTER et al., 2011; TUFEKCI et al., 2011).

Com intuito de inibir a adesão de micro-organismos e o acúmulo de placa na superfície do esmalte dentário, alguns autores têm sugerido materiais para colagem de braquetes ortodônticos contendo agentes antimicrobianos ou associados a esses (KORBMACHER et al., 2006; MOREIRA et al., 2015; POOSTI et al., 2013). Os aditivos que são mais comumente utilizados são os materiais com flúor e clorexidina. Embora estes, inicialmente sejam muito eficazes, suas concentrações vão diminuindo ao longo do tempo (COHEN et al., 2003; EVRENOL et al., 1999; RIBEIRO & ERICSON, 1991).

As nanopartículas de prata têm mostrado que possuem baixa citotoxicidade às células humanas (PANACEK et al., 2009; YUDOVIN-FARBER et al., 2008) e exibem uma forte atividade antimicrobiana contra bactérias cariogênicas (BALAZS et al., 2004; BÜRGERS et al., 2009; MONTEIRO et al., 2009; YAMAMOTO et al., 1996). A ação da nanopartícula de prata na célula bacteriana não está totalmente elucidada, mas formação de espécies de

oxigênio reativas e a inibição da síntese do DNA bacteriano é o mais provável mecanismo antimicrobiano dessa substância (ALLAKER, 2010; MARAMBIO-JONES & HOEK, 2010).

Diversos estudos que utilizam nanopartícula de prata associada a material odontológico no intuito de obter ação antimicrobiana (AHN et al., 2009; DEGRAZIA et al., 2016; ZHANG et al., 2013) não apresentam nenhum material que remineralize a superfície dentária em caso de desmineralização, como o fosfato de cálcio por exemplo. A adição dessas substâncias a materiais dentários pode trazer ambos efeitos, antimicrobiano e remineralizante.

Os pacientes ortodônticos podem ser considerados de alto risco de lesão de mancha branca e cárie e, tendo em vista a importância de um material que possa prevenir e diminuir a desmineralização inicial do esmalte nesses casos, o objetivo do presente estudo foi avaliar os efeitos da incorporação de nanopartículas de prata em diferentes concentrações, com e sem cobertura de fosfato de cálcio, nas propriedades físico-químicas e antimicrobianas de um cimento ortodôntico para fixação de braquetes. As hipóteses deste estudo são que (1) tanto a resina ortodôntica com nanopartículas de prata e fosfato de cálcio, bem como a que apresenta somente associação com nanopartículas de prata não sofrem alterações de suas propriedades físico-químicas e (2) a adição de nanopartículas de prata a resina ortodôntica com e sem fosfato de cálcio reduzem a viabilidade bacteriana.

Proposição

2 PROPOSIÇÃO

O presente trabalho teve como objetivos:

2.1 Objetivo Geral

Avaliar os efeitos da incorporação de nanopartículas de prata em diferentes concentrações, com e sem fosfato de cálcio, nas propriedades físico-químicas e antimicrobianas de uma resina ortodôntica para fixação de braquetes.

2.2 Objetivos Específicos

2.2.1. Avaliar as propriedades físico-químicas da resina ortodôntica incorporada com diferentes concentrações de nanopartículas de prata e fosfato de cálcio através dos ensaios de grau de conversão, sorção e solubilidade, flexão de três pontos, rugosidade e microdureza.

2.2.2. Avaliar potencial antimicrobiano da resina ortodôntica incorporada com diferentes concentrações de nanopartículas de prata e fosfato de cálcio.

2.2.3. Avaliar a resistência de união da resina ortodôntica incorporada com diferentes concentrações de nanopartículas de prata e fosfato de cálcio, ao esmalte bovino, através de teste de cisalhamento de braquetes metálicos cimentados nesse substrato.

Capítulo

3.CAPÍTULO

Esta dissertação está baseada no Artigo 46 do Regimento Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado, e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Assim sendo, esta dissertação é composta de um artigo científico que será submetido ao periódico *DENTAL MATERIALS*, conforme descrito abaixo:

PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF AN ORTHODONTIC RESIN DOPED WITH SILVER/CALCIUM PHOSPHATE NANOPARTICLES

SENA NJC, FEITOSA VP, MORAIS W, FECHINE PBA, RUEGGEBERG F, SABOIA VPA

PHYSICOCHEMICAL AND MICROBIOLOGICAL ASSESSMENT OF AN ORTHODONTIC RESIN DOPED WITH SILVER/CALCIUM PHOSPHATE NANOPARTICLES

ABSTRACT

Objectives: To assess physicochemical properties, enamel strength and antibacterial effect of an orthodontic resin (OR) incorporated with silver nanoparticles (AgNPs) with or without calcium phosphate (CaP). Methods: ORs were prepared with 0, 1 or 5wt% of AgNP and 0, 1 or 5wt% AgNP and CaP. Physicochemical properties were evaluated in terms of degree of conversion (DC), water sorption (WS) and solubility (SO), three point bending (flexural strength, FS and modulus, E) and surface roughness (Ra) and Knoop microhardness (KHN). Enamel adhesion was evaluated by shear bond strength test (SBS). Resin's antimicrobial activity against *Streptococcus mutans* was measured by counting colony-forming units. The data was statistically analyzed by one-way ANOVA and Tukey's test ($p<0.05$). Results showed that the DC, FS, KHN, SBS and antimicrobial tests showed no statistical difference between control and experimental groups. WS value decrease in OR 1wt% AgNP and SO value also decrease in OR 5wt% AgNP. FS achieve higher result in OR 5wt% AgNP. OR 1wt% and 5wt% AgNP and 5wt% AgNP with CaP attained statistically lower roughness than OR. Significance: The incorporation of AgNP in OR alters some physical-chemical properties, but does not interfere in its adhesion to the enamel. The use of CaP associated with AgNP might attain remineralization when undergoing acid challenges. Its antimicrobial potential has not been verified, but more studies are necessary to confirm this fact.

Key word: Silver nanoparticles, orthodontic resin, antimicrobial activity

1. INTRODUCTION

The introduction of enamel acid-etching for orthodontic fixed appliances to bond brackets [1-3] may induce changes on number and composition of oral bacteria. High levels of *Streptococcus mutans* are capable of decreasing pH of plaque in orthodontic patients to a greater extent than in non-orthodontic patients [4-6]. White spot lesion is caused by mineral loss from enamel surface or subsurface, and can occur whenever bacterial plaque is retained for a prolonged period [7,8]. The development of these lesions is an undesirable side effect during orthodontic treatment with an incidence and prevalence rate of 45.8% and 68.4%, respectively [9-12].

To prevent caries initiation, some authors [13-18] recommend the use of orthodontic resins containing some antimicrobial material. Fluoride and chlorhexidine are the most common preventive additives. Although initially strong, the released amounts of fluoride and chlorhexidine do not last for long periods [19-21]. Composites containing silver nanoparticles (AgNP) also possess antibacterial effects on oral streptococci [17,22-27] without affecting the cytotoxicity and human gingival fibroblast viability [28,29]. In addition, nanofillers can decrease surface roughness (SR) of orthodontic resins, which is one of the most significant factors for bacterial adhesion [30].

Most studies investigating AgNP in dental materials [22, 23,28] do not present any material able to remineralize the enamel, such as calcium phosphate. The addition of these compounds during the synthesis of silver nanoparticles may achieve both antimicrobial and remineralizing effects. However, to our knowledge, such nanoparticles were never evaluated incorporated in orthodontic resins.

Therefore, the aim of this study was to evaluate effects of the incorporation of silver nanoparticles in different concentrations, with and without calcium phosphate coating, on the physicochemical, enamel bonding and antimicrobial properties of an orthodontic resin for fixation of brackets. The study hypotheses are that (1)both orthodontic resins with AgNP and AgNP with calcium phosphate (CaP) do not suffer changes on physicochemical properties in comparison with AgNP-free resin, (2) the addition of AgNP and AgNP-CaP to

orthodontic resin reduces bacterial viability, and (3) incorporation of nano-silver with and without CaP does not influence enamel bonding.

2. MATERIALS AND METHODS

2.1. Synthesis of Nanoparticles and Experimental Design

AgNPs synthesis was performed using glucose as a reducing agent and sodium dodecyl sulfate (SDS) as a stabilizing agent. Briefly, 1.0 g of glucose and 0.5 g of SDS were added to 500 mL of $AgNO_3$ solution (5 mM). The solution was continuously stirred and the temperature was maintained at 50°C to favor the reaction. Then, 1.0 mL of 0.2 M NaOH was added to the mixture. The reaction was maintained under these conditions for 30 min, then stirred, heated and filtered. The AgNPs were purified by ultracentrifugation at 8,500 x for 20 min, and characterization was carried out using spectrophotometric reading at 300 to 700 nm (UV-Vis) [31]. To add calcium phosphate to silver nanoparticles, during the stirring, calcium nitrate solution (1M) and sodium phosphate solution (1M) were added in order to obtain Ca/P ratio of 1.67 (hydroxyapatite ratio). Therefore, calcium phosphate nanocompound was attached to silver nanoparticles.

Commercial orthodontic resin (Orthocem, FGM, Joinvile, Brazil) was used and incorporated with 1wt% or 5wt% AgNP or AgNP-CaP. For the physicochemical and antimicrobial tests 10 disk-shaped specimens for each group (n = 50) were prepared with 6 millimeters diameter and 1 millimeter thickness, using polyvinylsiloxane molds (Aquasil LV, Dentsply DeTrey, Denver, USA). Each resin was covered with a polyester strip. The specimens were light-cured for 60 s with a LED curing unit (1100 mW/cm^2, DB85, Dabi Atlante, Ribeirao Preto, Brazil), and then carefully removed from the molds. They were checked by stereomicroscopy and those with defects were discarded. The experimental design and groups were:

- **Control** - Orthocem® orthodontic resin.
- **AgNP 1%** – Orthocem® orthodontic resin incorporated with 1wt% silver nanoparticles.
- **AgNP 5%** – Orthocem® orthodontic resin incorporated with 5wt% silver nanoparticles.

- **AgNP+CaP 1%** – Orthocem® orthodontic resin incorporated with 1% of whitish silver nanoparticles containing calcium phosphate.
- **AgNP+CaP 5%** – Orthocem® orthodontic resin incorporated with 5% of whitish silver nanoparticles containing calcium phosphate.

2.1.1. Degree of Conversion (DC)

The degree of conversion (DC) of orthodontic resins with AgNP was performed following the protocol described by Ogliari et al. [32]. Briefly, resins were analyzed using Micro-Ramam spectrophotometer Xplora (Horiba, Paris, France) before and subsequent light-activation (40 s; DB85). The acquisition was ten seconds with three accumulations. The laser had 3.2 mW of power and wavelength of 532nm.All spectra were obtained in the range of 1580–1670 cm^{-1}. The rate of unreacted carbon–carbon double bonds (% C=C) was determined from the ratio of absorbance height intensities of aliphatic C=C (peak at 1637 cm^{-1}) against an internal standard (aromatic carbon–carbon bond peak at 1608 cm^{-1}) before and 2 minutes after curing. 10 segundos de aquisição.3 Acumulações.DC was determined by subtracting the C=C% from 100%. The analyses were performed in triplicate.

2.1.2. Water sorption and solubility

For water sorption and solubility testing [33] the discs were placed in a desiccator and weighed until a constant mass (m_1) was obtained. The discs were immersed in distilled water at 37°C for 7 days, then using dried cloth to remove the water absorbed on the surfaces of samples and weighed (m_2). A constant mass (m_3) was further obtained by subsequently drying the specimens. The diameter and the thickness of the specimen were measured at five points and the volume (V) was calculated in cubic millimeters. The values of water sorption (WS) and solubility (SO) were calculated for each disc using the following formula:

$$WS = \frac{m2 - m3}{V} \qquad SO = \frac{m1 - m3}{V}$$

2.1.3. Flexural three-point bending

According to Hojati et al., the resins with 25×2×2mm (bars), dimensions distance between the two supports was 20 mm apart and the crosshead speed was set at 0.5 mm/min. A three-point bending test was performed using a universal testing machine Instron (Instron, model 4466, Canton, USA). The flexural strength (*FS*) in MPa was calculated as:

$$FS = \frac{3PL}{2bd^2}$$

Where P stands for load at fracture (N), L is the span length (20 mm), and b and d are, respectively, the width and thickness of the specimens in millimeter. The elastic modulus (*E*) was also determined from the slope of the initial linear region of stress–strain curve [34].

2.1.4. Micro-hardness

Knoop hardness (KHN) measurements were performed on the irradiated surface (outer) using an indenter (Duramin 2, Ballerup, Denmark), using a load of 10 kgf and a dwell time of 5 s using a digital micro-hardness tester (FM100 Future-Tech Corp, Kawasaki, Japan). Hardness was measured at five locations on each specimen, and the mean KHN was subsequently determined for each specimen [35].

2.1.5. Surface Roughness

The measurements were performed using Stylus perfilometer (HommelTesterT1000 Hommelwerke GmbH, Schwenningen, Germany). On each specimen, three readings (1.5 mm in length) were realized with intervals of 100 μm between each other and the roughness evaluating parameters (R_a) were obtained in appropriate software [36].

2.2 Shear Bond Strength

2.2.1 Preparation of specimens

Fifty bovine incisors with sound buccal enamel were used. After extraction, the teeth were cleaned, polished with brushes in low-speed rotation handpiece.

Teeth were included in PVC pipes (3 cm high x 4 cm in diameter) filled with acrylic resin (DimClay, Brazil). At the time of inclusion, a square of tooth enamel was positioned in the center of the pipe, so that these (buccal) faces were perpendicular to the pipe orientation. Then, prophylaxis was performed with rubber cup, pumice and water. After drying with air spray, the metallic brackets (Morelli, Sorocaba, Brasil) with 9.63 mm^2 base area were bonded with 37% phosphoric acid etching enamel for 15s, 30s water rinsing and active drying with air-blast. Orthodontic resins were then employed to cement brackets and photo-cured for 40s with DB85 unit. The whole protocol was undertaken following manufacturers' instructions regardless the presence of AgNPs in resin [38].

2.2.2 Shear Bond Test

The universal testing machine (Instron) was used for the shear bond strength test at a speed of 0.5 mm/min. Each tooth was positioned with buccal surface parallel to the direction of the force during the shear bond strength test. The force was applied directly on the tooth-bracket interface by a flat steel knife. The value of the load on which the bracket debonded was recorded by Instron software. The values of shear bond strength were calculated in MPa, dividing the force (N) by the base area of the bracket. The fractures were analyzed by stereomicroscopy (40X magnification) and classified into adhesive, mixed or cohesive [38].

2.3 Microbiological test

2.3.1. Inoculum and Biofilm Model

To analyze the antibacterial effect, four discs (6.0 mm diameter x 1 mm thick) of resins incorporated with 1wt% and 5wt% AgNP and AgNP-CaP were produced under aseptic conditions using a silicone molds. Hydrogen peroxide plasma sterilized samples before starting biofilm formation.

Streptococcus mutans UA159 (ATTCC) was obtained from single colonies isolated on blood agar plates, inoculated in Tryptone yeast-extract broth containing 1% glucose (w/v) and incubated for 18-24 h at 37 ºC under microaerophilic conditions in partial atmosphere of 5% CO_2. Mono-species *S. mutans* biofilms were formed on saliva-coated orthodontic resin discs placed in

same bath cultures at 37 ºC in 5% CO_2 up to 5 days in 24-well polystyrene plates and once daily the discs were dip-washed three times in a plate containing of NaCl 0.89% solution and they were transferred to new 24-well plates with sterile medium [36].

2.3.3 Biofilm Analysis

The biofilm formed on the discs were removed after 5 days of initial biofilm formation and were transferred to pre-weighed microtubes containing NaCl 0.89% solution. Biofilms were then dispersed using three 15-s pulses (Branson Sonifier 150; Branson Ultrassonics, Danburry, USA) and an aliquot (0.05 mL) of the homogenized biofilm was serially diluted (10^{-1}–10^{-7}) and plated in triplicate onto blood agar (Blood agar base with 5% sheep blood), plates were then incubated at 37 ºC, 5% CO_2 during 48 h before enumerating viable microorganisms. Results were expressed as colony forming units (CFU)/mL and transformed in $\log_{10}$ CFU in order to reduce variance heterogeneity [37].

2.4 Statistical analysis

All data were submitted to statistical analysis by normality test and after approval ($p>0.05$), they were analyzed using one-way ANOVA and Tukey's test ($\alpha=5\%$). In the case of rejecting normality, Kruskal Wallis test was undertaken with same significance level.

3. RESULTS

The outcomes (means and standard deviations) of physicochemical experiments are presented in Table 1 and 2. The degree of conversion (DC) showed no statistical difference between control and experimental groups ($p = 0.133$). The addition of 1wt% AgNP resulted in a significant decrease in water sorption, followed by AgNP 5wt% and AgNP-CaP 1wt% groups. The control and AgNP-CaP 5wt% groups achieved the same statistical results and obtained highest water sorption. The silver nanoparticle and calcium phosphate had influence on the solubility of the composites ($p < 0.05$). The solubility in water decreased more significantly in the AgNP 5% group. The groups AgNP 1% and AgNP-CaP 5% that obtained the same statistical results showed the second best values for this test. After these groups come the AgNP-CaP 5% and the control

groups with the worst result respectively. The flexural strength of AgNP 5wt% resin was higher than further groups (p=0.08); however, the flexural modulus (E) had no statistical difference among groups. Also, the micro-hardness results were not significantly different among all groups (p=0.161). And the roughness test showed that AgNP-CaP 5wt%, AgNP 1wt% and 5wt% resins attained statistically lower roughness than Control resin (p<0.05).

Results of microbiological test are depicted in Fig. 1. No statistically significant differences (p=0.798) were found between control and experimental groups. The outcomes of shear bond strength are presented in Table 2. Again, no significant differences were found among groups (p=0.252). In control group, the predominant type of fracture was cohesive, which also occurred in AgNP 5wt% and AgNP-CaP 1wt% groups. In the groups AgNP 1wt% and AgNP-CaP 5wt%, the predominance of fracture was adhesive.

4. DISCUSSION

The incorporation of silver nanoparticles and calcium phosphate into orthodontic resin induced several modifications on physicochemical properties. Thus, the first hypothesis of this study, that both orthodontic resins with AgNP and AgNP-CaP do not suffer changes on physicochemical properties in comparison with AgNP-free resin, should be rejected, because water sorption and solubility, flexural strength and roughness depicted statistical differences. The second hypothesis, that the addition of AgNP and AgNP-CaP to orthodontic resin reduces bacterial viability, is rejected because no experimental group has demonstrated antimicrobial action against the bacteria in this set-up. Furthermore, the third hypothesis that the incorporation of nano-silver with and without CaP does not influence enamel bonding should be accepted, as the shear bond strength test showed no statistical difference between control and experimental groups.

The addition of AgNP and AgNP-CaP to orthodontic resin induced no significant effect (p = 0.133) on the DC compared to the control filler-free resin. This occurred likely because the silver and calcium phosphate nanoparticles did not react with the camphoroquinone or the tertiary amine present in the orthodontic resin. In addition, the nanoparticles used might possess low ability to

transmit/scatter light and the amount of nanoparticles added to the material was not sufficient to change their degree of conversion. This result was different from that found in study [22] that showed *in situ* decreased DC with higher concentrations of silver nanoparticles. However, one studies [39] using enamel infiltrants and another one [40] in self-etching adhesives showed an increase of the degree of conversion when adding nanoparticles of hydroxyapatite.

For water sorption, AgNP 1wt% and AgNP 5wt% resins obtained lower sorption in relation to the control group. A similar result was found in other studies [24,41] using only silver nanoparticles. They found that the addition of AgNP resulted in a significant decrease of water sorption. Concerning the solubility, AgNP 5wt% group presented lower outcomes in comparison to the control group. The same occurred in one investigation [41] in which the incorporation of silver nanoparticles decreased solubility. Conversely, in one study [42] the addition of silver nanoparticles in an acrylic resin increased both water sorption and solubility of the material.

No statistical difference was found on flexural modulus between the experimental groups and control, thanks to the high standard deviations (Table 2). This could be solved by increasing the number of specimens. However, the flexural strength results showed higher outcomes for AgNP 5wt% resin in comparison to the control group. This may have been caused by the increase of nanoparticles concentration in the dental material, which might improve the mechanical properties, especially for high strength fillers like nano-silver. One study [43] showed the flexural strength and modulus of the nanocomposite increased by adding 21.7 and 30.8 % nano-filler.

The microhardness results depicted no statistical difference among the resins, in contrast to other study [44] which verified that the microhardness was improved in the resin-based composites modified with TiO2 nanoparticles. In the present study, the experimental groups showed no effect on the microhardness values, despite the addition of nanoparticles to the orthodontic resin. This may be explained by the mixture of nanoparticles with high viscosity orthodontic resin. Indeed, with manual mixture, most fillers could be incorporated only in the central portion of the resin; thus, the superficial zone should be devoid of nanoparticles,

thereby yielding less benefic effects on surface experiments such as microhardness.

Nevertheless, in higher concentration (5wt%), some nanoparticles should be present on the surface and the addition of AgNPs and AgNP-CaPs decreased roughness (Table 2). In fact, lower surface roughness clinically may be correlated with lower bacterial retention and reduced caries activity. This improvement on roughness was different from that found in another study [23], which showed experimental (nanoparticles-containing) composites with higher roughness than the control group. Although roughness is the physical property most related to bacterial adhesion, this study [23], bacterial adhesion was decreased even with higher surface roughness, likely due to the antimicrobial effect of their nanoparticles.

An alternative to reducing bacterial adhesion on dental materials is to focus on materials that release antimicrobial agents. The most important shortcoming caused by the chemical antimicrobial agents is multidrug resistance and various microorganisms have evolved drug resistance over time. Silver nanoparticles showed efficient antimicrobial property due to their extremely large surface area and production of oxidative species [22] and the microorganisms are unlikely to develop resistance against silver nanoparticles as compared to antibiotics as silver attacks a broad range of targets in the microbes [45]. Studies [22,28,17] showed an effective antimicrobial activity with the addition of silver nanoparticles in dental adhesives.

The present outcomes depicted experimental groups with no significant differences in relation to the control group in terms of antibacterial efficacy. This difference may be explained by the fact that the thickness of the dental adhesives, in the case of another studies [28,17] is thinner than that of the orthodontic resin used in this study. Indeed, this facilitates the exposure of silver nanoparticles on the surface of the material and their contact with the bacteria. Additionally, orthodontic resin is a more viscous material and this may cause greater retention of the nanoparticles inside the material, and perhaps no Ag leached out from the material. In fact, in the clinical scenario, the wear of orthodontic resin by tooth brushing may remove the superficial resin thereby exposing more silver nanoparticles which would advocate antimicrobial effects.

In-vitro high shear bond strength cannot be completely correlated with clinical efficiency, once several limitations of laboratory assessments and the different intraoral factors are present. The addition of AgNPs and AgNP-CaPs did not induce significant difference on enamel bonding, what might be represented as satisfactory outcomes because these nanoparticles are not impairing the orthodontic resin bond to enamel. One study [46] evaluated the shear bond strength of orthodontic resin with addition of several concentrations of silver nanoparticles, and they found that only the addition of 10wt% AgNPs dropped the enamel bond strength. Therefore, concentrations of 1wt% and 5wt% are adequate in terms of bracket bonding to enamel and their results corroborate the present outcomes. The study [47] also found no statistical difference on shear bond strength between the materials with the addition of quaternary ammonium methacrylate and AgNP in comparison with control group. In a clinical view, practioners introduce combinations of shear, tensile, and torsion forces when performing bracket debonding, but shear strength evaluation might provide a guidance towards the selection of the bracket/resin choice [14].

The presence of white spot lesions during and after removal of orthodontic appliances is a discouraging finding to a dental field whose goal is to improve facial and dental esthetics. Orthodontic resin with AgNP and AgNP-CaP can enhance remineralization of the enamel surface located around the bracket and consequently aid the prevention of dental caries. The study of Andrade Neto et al., evaluated the incorporation of hydroxyapatite nanoparticles in enamel infiltrants for the treatment of white spot lesion, but only these fillers cannot rule out the effects of oral bacteria and their organization in a mature biofilm. For this reason, this study presented a material containing both a compound able to assisting in dental remineralization (CaP) and further (AgNP) with antimicrobial potential. Besides, the addition of CaP make the orthodontic resin with nano-silver more esthetic, with light gray to white appearance, which could be more easily accepted by the patients than dark tone of AgNP-containing orthodontic resin. Further investigations are needed to better evaluate the microbiological effects and biocompatibility of the incorporation of silver and silver-calcium phosphate nanoparticles into orthodontic resin.

5. CONCLUSION

Within the limitations of this study, it may be concluded that orthodontic resins with the addition of 5wt% silver nanoparticles without calcium phosphate achieve lower water sorption, solubility and higher flexural strength. These benefits were achieved without compromising enamel shear bond strength. However, the orthodontic resin containing 5wt% nano-silver presented no antibacterial effects when compared with control resin, likely due to lack of intimate contact with the bacterial cells to cause damage. Adjuct use of calcium phosphate to silver nanoparticles induced intermediate outcomes, but might attain remineralization when undergoing acidic challenges.

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TABLES & FIGURES

Table 1. Results of chemical proprieties, Degree of Conversion (DC), Water Sorption (WS) and Solubility (Sol).

	DC (%)	WS (mg/mm^3)	Sol (mg/mm^3)
Control Resin	84.4 (9.6)	85.0 (31.5) A	54.0 (19.3) A
AgNP 1%	69.5 (6.2)	48.1 (8.9) B	7.3 (5.5) BC
AgNP 5%	83.6 (8.7)	63.4 (22.5) AB	- 22.0(32.3) C
AgNP+CaP 1%	72.1 (9.6)	78.8 (30.1) AB	19.9 (9.9) AB
AgNP+CaP 5%	77.5 (7.5)	94.4 (34.7) A	16.3 (12.4) BC

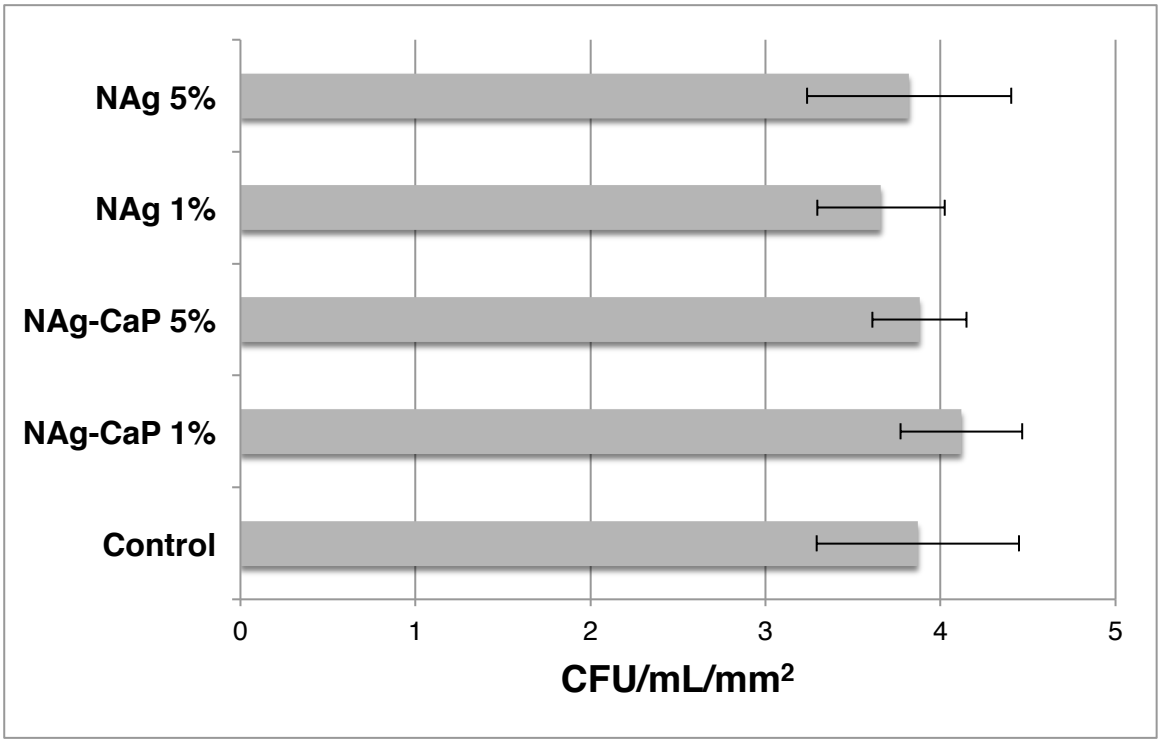
*Different capital letters in column indicate statistical difference ($p<0.05$). No letters in a column represent absence of significant difference ($p>0.05$).

Table 2. Results of physical proprieties, Flexural Modulus (E) and Flexural Strength (FS), Microhardness (KHN), Roughness (Ra), Shear Bond Strength (SBS).

	E (Mpa)	FS (Mpa)	KHN	Ra	SBS(Mpa)
Control Resin	349.0 (130.5)	74.3 (21.4) AB	30.2 (7.8)	3.59 (0.79) A	4.51 (1.0)
AgNP 1%	381.5 (164.9)	43.5 (7.9) BC	29.5 (7.7)	2.06 (0.37) BC	6.71 (1.7)
AgNP 5%	344.5 (100.4)	80.1 (17.8) A	30.9 (2.9)	2.05 (0.24) BC	4.56 (0.8)
AgNP+CaP 1%	186.9 (71.6)	30.0 (5.3) C	28.9 (6.5)	2.33 (0.55) AB	3.74 (1.2)
AgNP+CaP 5%	283.3 (210.2)	45.4 (13.1) BC	24.4 (4.6)	1.60 (0.41) C	5.11 (1.9)

*Different capital letters in column indicate statistical difference ($p<0.05$). No letters in a column represent absence of significant difference ($p>0.05$).

Figure 1. Results of microbiological tests, as colony forming units (CFU)/mL/mm^2



*Different capital letters in column indicate statistical difference ($p<0.05$). No letters in a column represent absence of significant difference ($p>0.05$).

Conclusão Geral

3. Conclusão geral

Uma resina ortodôntica comercial com adição de Nag e NAg-CaP foi avaliada neste estudo em suas propriedades físico-químicas e antimicrobianas. Este material não deve apenas prevenir a iniciação da cárie, mas ao mesmo tempo tem de proporcionar uma força de ligação suficiente entre o braquete e o dente. Dentro das limitações deste estudo, pode-se concluir que as resinas ortodônticas com a adição de 5% em peso de nanopartículas possuem melhor desempenho porque obtiveram os melhores resultados em solubilidade em água, nos testes de resistência à flexão e apresentaram a segunda melhor marca no teste de sorção de água. Estes benefícios foram alcançados sem comprometer a resistência ao cisalhamento do esmalte. No entanto, estes resultados mostraram que a resina ortodôntica contendo 5% em peso de NAg não tinha efeitos antibacterianos quando comparada com o controle de resina comercial. As nanopartículas de prata devem ser liberadas da matriz da resina e entrar em contato com as células bacterianas para causar danos a elas. A retenção das nanopartículas em sua estrutura pode ter sido a causa da ineficiência da atividade antimicrobiana dos grupos avaliados. Compreender os processos de libertação de nanopartículas a partir de matriz de resina pode melhorar as estratégias terapêuticas para evitar a lesão de manchas brancas e cáries durante e após o tratamento ortodôntico em superfícies de esmalte. Futuros estudos in vivo e in vitro devem considerar mais sistematicamente os vários efeitos da química das nanopartículas de prata, transporte e toxicidade.

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