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LUIZ FELICIO FERNANDES LOUZADA FIOROTTI

EFEITO DO RECOBRIMENTO DE BRÁQUETES ORTODÔNTICOS COM FILME DE FOSFATO DE CÁLCIO NA DUREZA DO ESMALTE BOVINO SUBMETIDO À DESMINERALIZAÇÃO *IN VITRO*

VITÓRIA-ES
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Dissertação apresentada ao Curso de Mestrado Profissional em Clínica Odontológica do Programa de Pós-Graduação em Clínica Odontológica da Universidade Federal do Espírito Santo, como parte dos requisitos exigidos para a obtenção do título de mestre em Clínica Odontológica.

Orientadora: Profª Drª Juliana Malacarne Zanon
Coorientador: Prof. Dr. Anuar Antônio Xible

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Aprovada em 26 de março de 2019.

COMISSÃO EXAMINADORA

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Prof.ª Dr.ª Juliana Malacarne Zanon
Universidade Federal do Espírito Santo
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Prof.ª Dr.ª Liliana Aparecida Pimenta de Barros
Universidade Federal do Espírito Santo

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Prof. Dr. Américo Bortolazzo Correr
Universidade Estadual de Campinas

É preciso força pra sonhar e perceber que
a estrada vai além do que se vê.

Camelo, Marcelo

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RESUMO

Apesar do amplo emprego de aparelhos ortodônticos fixos para correção das maloclusões, a lesão de mancha branca (LMB) na superfície do esmalte adjacente aos bráquetes é um efeito adverso comumente associado ao tratamento. Ações de prevenção que diminuam essa ocorrência são importantes a fim de controlar o risco de cárie em pacientes ortodônticos. Este estudo *in vitro* teve por objetivo avaliar o efeito do recobrimento de bráquetes metálicos com filme de fosfato de cálcio (CaP) quanto a concentração de íons cálcio (Ca) no meio e na nanodureza Vickers (VHN) da superfície do esmalte bovino ao redor dos bráquetes após desafio desmineralizante. Inicialmente, vinte e oito bráquetes revestidos por eletrodeposição com o filme experimental de CaP foram armazenados em água destilada e a concentração de íons Ca no meio foi analisada após 7, 15 e 30 dias. Em seguida, bráquetes ortodônticos originais do fabricante e os revestidos (n=15) foram colados em blocos de esmalte bovino (8 mm x 10 mm) após polimento metalográfico. A VHN do esmalte ao redor de cada bráquete foi aferiada antes e após o desafio desmineralizante, empregando 50 mN de força por 20 segundos, em cinco pontos 100 micrometros distantes da área de colagem. Para a desmineralização *in vitro* os corpos de prova foram imersos em solução com pH 5,0 a 37 °C por 32 horas. O filme experimental foi caracterizado morfologicamente por microscopia eletrônica de varredura (MEV) e a espectroscopia de energia dispersiva (EDS) foi empregada para análise elementar (n=3). Os dados obtidos foram submetidos aos testes ANOVA e Bonferroni (α=5%). A superfície dos bráquetes revestidos apresentou-se amorfa, com formações sugestivas de cristais dispersos, e presença de íons cálcio, fosfato e oxigênio. Em 15 e 30 dias não foram detectados Ca na solução. O esmalte ao redor dos bráquetes revestidos manteve a dureza após o desafio desmineralizante, o que não foi observado no grupo unido com bráquete original do fabricante ($p<0.05$). O revestimento de bráquetes metálicos com filme de CaP, empregando deposição eletroquímica, promoveu aumento da disponibilidade de Ca no meio e, assim, evitou a desmineralização do esmalte adjacente durante o desafio desmineralizante.

Palavras chave: Dureza, Bráquetes Ortodônticos, Desmineralização.

ABSTRACT

Despite the widespread use of fixed orthodontic appliances to correct malocclusions, White spot lesion (WSL) is routinely adverse effects associated with this treatment modality. Thus, the aim of this in vitro study was to evaluate the calcium leaching ability of calcium phosphate (CaP) thin film coated orthodontic brackets in distilled water, as well to evaluate the surface Vickers nanohardness (VHN) of experimental bracket coated with calcium phosphate (CaP) after demineralizing challenge. For this, original manufacturer's orthodontic brackets were used as control (n=15) and fifteen were coated with CaP film by electrodeposition. The film was characterized morphologically by scanning electron microscopy (SEM) and dispersive energy spectroscopy (EDS) for elemental analysis. Twenty-eight coated brackets were stored in distilled water for 7, 15 and 30 days and analyzed at 7, 15 and 30 d for calcium leaching. Thirty blocks (8 x 10 mm) of enamel, obtained from the vestibular face of healthy bovine incisors and submitted to metallographic polishing, were used. The VHN of bovine enamels was evaluated before and after demineralizing challenge using 50 mN of force, for 15 seconds, in five previously determined peripheral areas. Then, the enamel blocks were divided into 2 groups (n = 15) according to the protocol that was attached to the dental enamel: test group, CaP coated bracket; and control group, manufacturer's original brackets. For the union of the rinses to the bovine bone, the system Transbond XT was used in all the collages. After storage in distilled water at 37 °C for 24 h, the specimens underwent a demineralization process in pH 5.0 solution for 32 hours at 37 °C. Soon after the VHN was remeasured. In the SEM the surface of the coating was amorphous and crystalline, in both structures there is presence of calcium and phosphate, proving the coating. Calcium leaching occurred with greater intensity in 7 d ($p < 0.05$). The group with orthodontic brackets covered with CaP showed lower hardness loss after the demineralization process than the control group ($p < 0.05$). Thus, the coating has been shown to be a means of maintaining bovine enamel hardness after in vitro demineralization.

Keywords: Orthodontic Brackets, Tooth Demineralization, Hardness test.

LISTA DE ABREVIATURAS E SIGLAS

CaP - Fosfato de cálcio

CPP-ACP - Fosfopeptídeos de caseína/fosfato de cálcio amorfo

d - Dias

DES-RE - Desmineralização-remineralização

EDS – Espetroscopy dispersive energy ou espectroscopia de energia dispersiva

Fig. – Figura

h - Horas

ICP-OES - Induductively coupled plasma – atomic emission spectrometry ou Espectrometria de emissão atômica com plasma acoplado indutivamente

LMB - Lesão de mancha branca

MEV - Microscopia eletrônica de varredura

min - Minuto

n° - Número

PVC - Policloreto de vinila

rpm – Rotações por minuto

s - Segundos

SEP - *Self-etching primer*

VHN – Vickers hardness number ou número de dureza Vickers

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1. INTRODUÇÃO GERAL

A maloclusão é definida como uma alteração do crescimento e do desenvolvimento que afeta a oclusão dos dentes. Ela está presente em aproximadamente 39% da população mundial (PINTO, 2015). Para sua correção, os aparelhos ortodônticos fixos são rotineiramente empregados (PAN et al., 2017). No entanto, apesar de sua extensa utilização, efeitos colaterais negativos como gengivite, periodontite e lesões de mancha branca em esmalte e cárie dentária são prevalentes nessa população (MEI et al., 2017; RICHTER et al., 2011). Essas alterações inicialmente reversíveis podem gerar sequelas que se mantem mesmo após a finalização do tratamento ortodôntico (PAN et al., 2017). Assim, é de fundamental importância a instituição de medidas preventivas e identificação precoce dessas alterações.

O tratamento ortodôntico fixo está frequentemente associado à desmineralização na superfície do esmalte ao redor do bráquete, a qual frequentemente se apresenta como lesão de mancha branca (LMB) (MUNJAL et al., 2016). A prevalência da LMB associada ao tratamento ortodôntico varia de 2 a 96% (MIZRAHI, 1982), e 24% podem se transformar em cavidades se permanecerem sem tratamento (SRIVASTAVA et al., 2013). Isso representa custos financeiros, emocionais e biológicos adicionais para os pacientes (YAP et al., 2014).

A LMB é caracterizada pela cor branca opaca do esmalte dental e representa o primeiro sinal visível da cárie. Ela pode se estabelecer em apenas 1 mês de tratamento com aparelho fixo (MILLER et al., 2016) e, eventualmente, pode levar à cavitação e a cárie em dentina (FEATHERSTONE et al., 2007). Comumente essas lesões ocorrem na superfície do esmalte vestibular dos dentes, fato menos comum em pacientes que não estão em tratamento com aparelhos fixos (WILLMOT, 2008). Além disso, há um aumento acentuado no número de LMB nos primeiros 6 meses de tratamento que continua progredindo lentamentamente até 12 meses, evidenciando que os meses iniciais do tratamento são críticos para o aparecimento das lesões (TUFEKCI et al., 2011).

Apesar do esforço dos ortodontistas, a incidência de LMB nos indivíduos com aparelhos ortodônticos fixos ainda permanece alto (BEERENS; TEN CATE; VAN DER VEEN, 2017; MUNJAL et al., 2016; SUNDARARAJ et al., 2015). Este fato tem sido atribuído aos bráquetes ortodônticos que apresentarem uma superfície complexa, o que dificulta o mecanismo de autolimpeza pela musculatura orofacial e saliva, e facilita o acúmulo de biofilme bacteriano (MEI et al., 2017), promovendo, por fim, o aumento do número de bactérias acidogênicas na cavidade bucal (MARET et al., 2014). Além disso, a presença desses dispositivos fixados aos dentes implica em maior dificuldade de higienização (PLISKA et al., 2012), exigindo maior tempo e dedicação por parte do paciente. Após tratamento ortodôntico, essas LMBs comprometem a estética e podem requerer intervenções invasivas (MAXFIELD et al., 2012).

Terapia com fluoretos por meio de pastas, enxaguantes e vernizes (KIRSCHNECK et al., 2016) são extensamente empregadas na prevenção e tratamento das LMBs. Os íons fluoretos, na presença de hidroxiapatita, podem promover a formação de fluorapatita, que possui menor solubilidade e pH crítico para dissolução inferior ao da hidroxiapatita (CURY et al., 2016; WIEGAND; BUCHALLA; ATTIN, 2006). Uma vez que os íons fluoreto estão no meio bucal, o fator limitante da remineralização pode ser a concentração de íons cálcio e fosfato (COCHRANE et al., 2010). Assim, dentifrícios a base de caseína fosfato de cálcio amorfo (CPP-ACP) (ELKASSAS; ARAFA, 2014) e outras fontes de liberação de íons cálcio e fosfato para o meio tem sido avaliados. Com a supersaturação desses íons em relação ao meio, há maior possibilidade de remineralização da estrutura dental.

Apesar da diversidade de tratamentos, o controle da LMB ainda permanece como um desafio para o ortodontista (KHOROUSHI; KACHUIE, 2017; MILLER et al., 2016). Este fato pode ser, em parte, atribuído ao fato de os métodos tradicionais para prevenção da cárie dentária dependerem da cooperação do indivíduo (SODAGAR et al., 2016). Dessa forma, novos meios de controle das LMBs, principalmente que atuem independentemente do paciente, são buscados.

2. OBJETIVOS

2.1 OBJETIVOS GERAIS

Os objetivos do presente estudo *in vitro* foram avaliar o efeito do recobrimento de bráquetes metálicos com filme de fosfato de cálcio (CaP) quanto a concentração de íons Ca no meio e a dureza Vickers (VHN) da superfície do esmalte bovino ao redor dos bráquetes após desafio desmineralizante.

2.2 OBJETIVOS ESPECÍFICOS

a) Avaliar a concentração de íons cálcio em água destilada após 7, 15 e 30 dias de armazenamento de bráquetes metálicos revestidos com filme de CaP;
b) Avaliar a nanodureza de superfície do esmalte bovino ao redor de bráquetes metálicos revestidos ou não com filme de CaP, antes e após desafio desmineralizante.

3. ARTIGO

Título: Efeito do revestimento de bráquetes ortodônticos com filme fino experimental de Fosfato de Cálcio (CaP) na dureza do esmalte bovino após desafio desmineralizante *in vitro*

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‘Conflitos de interesse: não há’.

RESUMO

O objetivo do estudo foi avaliar o efeito do revestimento de bráquetes metálicos com filme de fosfato de cálcio (CaP) na concentração de Cálcio (Ca) no meio e na nanodureza Vickers (VHN) da superfície do esmalte bovino ao redor dos bráquetes após desmineralização *in vitro*. Inicialmente, vinte e oito bráquetes revestidos por eletrodeposição foram armazenados em água destilada e a concentração de Ca no meio foi analisada após 7, 15 e 30 dias. Em seguida, bráquetes originais do fabricante e revestidos (n=15) foram colados em blocos de esmalte bovino (8 x 10 mm) empregando o sistema Transbond XT. A VHN do esmalte ao redor de cada bráquete foi aferiada antes e após o desafio desmineralizante, em cinco pontos, empregando 50 mN, por 15 s. O desafio desmineralizante consistiu da imersão dos espécimes em solução pH 5,0, a 37 °C, por 32 horas. O filme experimental foi caracterizado por microscopia eletrônica de varredura (MEV) e espectroscopia de energia dispersiva (EDS) (n=3). Dados foram submetidos à ANOVA e Bonferroni (α=5%). A concentração de Ca no meio foi maior em 7 dias. O esmalte ao redor dos bráquetes revestidos mostrou menor perda de dureza após o desafio desmineralizante ($p<0.05$). A superfície dos bráquetes revestidos apresentou-se amorfa, com cristais dispersos, e presença de Ca e fósforo (P). O revestimento de bráquetes com filme de CaP promoveu o aumento da disponibilidade de Ca no meio e evitou a desmineralização do esmalte durante o desafio ácido.

Palavras chave: Dureza, Bráquetes Ortodônticos, Desmineralização.

Destaques:

- Foi desenvolvido um revestimento de CaP para braquetes ortodônticos metálicos capaz de lixiviar íons Ca em água destilada
- A dureza de superfície do esmalte ao redor dos bráquetes revestidos com CaP não mostrou alteração significativa após um desafio severo de desmineralização

3.1 INTRODUÇÃO

A má oclusão é uma condição heterogênea que afeta populações em todo o mundo e resulta em comprometimento estético e funcional com redução da qualidade de vida (CLAUDINO; TRAEBERT, 2013). O tratamento com aparelho ortodôntico fixo é um método eficaz e comum no tratamento das más oclusões na ortodontia contemporânea (PAN et al., 2017). No entanto, apesar da ampla utilização, o controle dos efeitos colaterais negativos ainda permanece um desafio para o ortodontista (KHOROUSHI; KACHUIE, 2017; MILLER et al., 2016). Um dos efeitos adversos mais comuns associados a essa modalidade de tratamento é a desmineralização da superfície do esmalte em torno dos bráquetes, que rotineiramente se apresenta como uma lesão de mancha branca (LMB) (GORELICK; GEIGER; GWINNETT, 1982). A prevalência de LMBs em pacientes ortodônticos varia de 2% a 96% (MIZRAHI, 1982) e 24% podem se tornar cáries cavitadas se não forem tratadas (SRIVASTAVA et al., 2013).

O aspecto branco opaco da LMB é devido a um fenômeno ótico causado pela perda mineral no esmalte subsuperficial e representa o primeiro sinal de lesão de cárie que pode se desenvolver dentro de um mês após a colocação de bráquetes (MILLER et al., 2016) e eventualmente levar a interrupção do tratamento ortodôntico em casos mais generalizados. Além disso, um aumento acentuado no número de LMBs ocorre durante os primeiros 6 meses de tratamento (TUFEKCI et al., 2011). Dessa forma, o surgimento de LMBs nos meses iniciais de tratamento ortodôntico é um fator determinante para avaliar o grau de higiene bucal dos pacientes.

Embora existam vários métodos preventivos propostos na literatura científica, os estudos mostram que a incidência de LMB em indivíduos com aparelhos fixos ainda permanece elevada (BEERENS; TEN CATE; VAN DER VEEN, 2017; MUNJAL et al., 2016; SUNDARARAJ et al., 2015). Isso ocorre porque os bráquetes ortodônticos possuem várias reentrâncias em sua superfície, o que facilita o acúmulo de biofilme bacteriano (MEI et al., 2017) e aumenta o número de bactérias acidogênicas na cavidade bucal (MARET et al., 2014). Além disso, a presença desses dispositivos implica em maior dificuldade de higiene bucal (PLISKA et al., 2012), exigindo mais tempo e dedicação por parte do paciente.

Existem substâncias que conhecidamente interferem no processo de desmineralização-remineralização como o fosfato de cálcio (CaP), que é um dos principais constituintes dos ossos e dentes. Para que a remineralização ocorra, as substâncias remineralizantes devem ser capazes de liberar íons cálcio (Ca) e fosfato (PO^4) no meio ambiente (ELKASSAS; ARAFA, 2014). Para que isso ocorra, é necessário que o CaP seja supersaturado em relação ao meio para ter potencial remineralizador (COCHRANE et al., 2010). Assim, qualquer meio externo que aumente a concentração de íons Ca e PO^4, atua aumentando a capacidade natural de remineralização da saliva (COCHRANE; REYNOLDS, 2012). Isso seria particularmente importante em pacientes com alto risco de cárie, como aqueles em tratamento ortodôntico ou com deficiência salivar.

Vários procedimentos para a interrupção das LMBs são propostos, tais como: uso de fluoretos (KIRSCHNECK et al., 2016), dentifrícios com caseína de fosfato de cálcio amorfo (CPP-ACP) (ELKASSAS; ARAFA, 2014) e substâncias antimicrobianas (DEHGHANI et al., 2015). Dentre eles, o enxaguante bucal com flúor com ou sem substância antimicrobiana tem sido relatado como eficaz (DEHGHANI et al., 2015; KHOROUSHI; KACHUIE, 2017). No entanto, sua aplicação depende da colaboração do indivíduo. Dessa forma, a comunidade científica tem demonstrado interesse real na busca de novos meios para reduzir a ocorrência de LMB em torno de bráquetes que não dependam da colaboração do indivíduo.

Em laboratório, várias substâncias bioativas foram incorporadas aos materiais para colagem de bráquetes ortodônticos para reduzir a ocorrência de LMB em pacientes em tratamento com aparelhos ortodônticos fixos. Dentre eles podemos destacar as nanopartículas de prata (MOREIRA et al., 2015; SODAGAR et al., 2016; ZHANG et al., 2015), metacrilato à base de amônio (MELO et al., 2014; NASCIMENTO et al., 2017; ZHANG et al., 2016) e fosfato de cálcio amorfo (BURBANK et al., 2016; XIE et al., 2017; ZHANG et al., 2016). Estudos mostraram uma melhora nas propriedades mecânicas do esmalte ao redor dos bráquetes quando essas substâncias são incorporadas nas resinas para colagem dos dispositivos (NASCIMENTO et al., 2017; UYSAL et al., 2010). No entanto, há uma preocupação com o efeito limitado dessa ação, uma vez que a área de exposição do material de colagem na cavidade bucal é restrita (YAP et al., 2014).

Portanto, este estudo objetivou avaliar se a deposição de um filme experimental de CaP em bráquetes ortodônticos metálicos melhoraria a disponibilidade de íons Ca no meio, bem como a resistência do esmalte ao redor dos bráquetes revestidos à desmineralização *in vitro*. A hipótese nula é que o revestimento dos bráquetes ortodônticos com CaP não interfere na dureza do esmalte submetido a um desafio ácido desmineralizante.

3.2 MATERIAIS E MÉTODOS

Este estudo experimental *in vitro* foi realizado utilizando bráquetes metálicos revestidos com filme de CaP (grupo teste) e bráquetes metálicos originais do fabricante (grupo controle). A lixiviação de íons Ca em água destilada foi verificada pela avaliação da concentração desse íon no meio após armazenamento. A influência do revestimento na resistência do esmalte bovino à desmineralização foi verificada por meio de ensaio da dureza na superfície ao redor dos bráquetes, antes e após desafio desmineralizante.

3.2.1 Deposição de filme de CaP em bráquetes metálicos

Para o revestimento, foram utilizados bráquetes metálicos de aço inoxidável austenítico 304 da marca Morelli (Sorocaba, SP, Brasil), modelo caninos e pré-molares superiores, com slot de 0.022”, torque 0°, angulação 0°, comprimento de 2,5 mm.

Uma solução eletrolítica rica em íons cálcio (Ca^{2+}) e fosfato (PO_4^{3-}) foi preparada a partir de 50 mmol/L de KH_2PO_4, 100 mmol/L de $Ca(NO_3)_2.4H_2O$ e dissolvidos em água ultrapura. A síntese eletroquímica do filme de fosfato de cálcio sobre a superfície dos bráquetes metálicos foi realizada em uma célula eletroquímica contendo três eletrodos conectados a um potenciostato (Autolab; Metrohm, Utrecht, Holanda): (1) eletrodo de trabalho - bráquetes metálicos; (2) contra eletrodo - placa de platina e (3) eletrodo de referência - eletrodo de Ag/AgCl. A densidade de carga aplicada foi de -1,5 C/cm² em potencial de -1,5 mV em temperatura ambiente de ±21 °C. Após a deposição, os bráquetes foram armazenados em dessecador contendo sílica gel no interior de uma estufa a 37 °C por 24 h.

Para caracterização do revestimento, três bráquetes revestidos foram avaliados por microscopia eletrônica de varredura (MEV; JEOL JSM-6460LV, Akishima, Tokyo, Japão). Assim, foram fixados em suporte metálico (stub) com fita de carbono de revestimento duplo e metalizadas com ouro (Desc V; Denton vacuum, Moorestown, NJ, EUA). As análises foram realizadas com o microscópio operando a 30 kV e 20 mA. Adicionalmente, foi realizada a espectroscopia de energia dispersiva (EDS;

JEOL JSM-6460LV, Akishima, Tokyo, Japão) das regiões analisadas para caracterização química elementar do revestimento.

3.2.2 Análise quantitativa de íons Ca no meio

Vinte e oito bráquetes foram revestidos como descrito no item 3.2.1 e utilizados nesta análise. Após o dessecamento, foram imediatamente armazenados em recipiente contendo 250 mL de água destilada deionizada onde permaneceram por 7, 15 e 30 dias (d) imersos. Em 7 d, a água de armazenamento foi totalmente retirada e enviada para análise. Uma nova água destilada foi colocada para dar continuidade ao armazenamento. Nos dias subsequentes (15 e 30 d) o mesmo procedimento foi realizado. Juntamente com as águas de armazenamento, uma amostra da água destilada utilizada também era enviada para análise (branco). Assim, as águas de armazenamento foram enviadas e analisadas de acordo com o Standart Methods for the Examination of Water and Wastedwater, utilizando equipamento de espectrometria de emissão atômica com plasma acoplado indutivamente (ICP-OES) para investigação de cálcio.

3.2.3 Teste de nanodureza e desmineralização *in vitro*

Cinquenta incisivos permanentes bovinos foram selecionados para o estudo. Depois de limpos, eles foram armazenados em 0,5% de timol a 4°C até o tempo máximo de 30 d. Os dente selecionados estavam sem trincas, fraturas, defeitos de formação ou quaisquer desgaste na face vestibular. Os dentes foram obtidos de um frigorífico após aprovação do Comitê de Ética em Uso de Animais n° 74/2017.

A porção radicular foi separada da porção coronária ao nível da junção amelocementária com disco diamantado dupla face, em baixa rotação sob refrigeração. Blocos de esmalte com dimensão de 10 mm x 8 mm foram preparados com auxílio da politriz, utilizando discos de carbeto de silício com granulação #220 sob refrigeração constante operando a uma velocidade de 600 rpm.

Cilindros de PVC de ~8 mm de altura e diâmetro externo de 20 mm, com face superior e inferior paralelas entre si, foram utilizados para o embutimento dos blocos de esmalte em resina acrílica. Os blocos de esmalte foram posicionados na região central do cilindro de PVC sobre uma lâmina de cera utilidade sob placa de vidro para então ser incluídos em resina acrílica quimicamente ativada. Após a presa do material, os espécimes foram removidos dos cilindros de PVC e então planificados com discos de carbeto de silício #400, #600, #2000 e #4000 sob refrigeração abundante em politriz metalográfica com velocidade de 600 rpm durante 30 segundos (s) cada lixa. Em seguida, polidos com discos de feltro e suspensão de alumina com granulação extrafina de 3 µm durante 2 minutos (min) para obtenção de uma superfície plana, polida e padronizada. A cada troca de lixa, as amostras eram limpas em cuba ultrassônica por 5 min. Ao final, os espécimes foram armazenados em água destilada a 4 °C por 7 d.

Os blocos de esmalte foram alocados aleatoriamente entre dois grupos (n=15) de acordo com o bráquete metálico recebido: grupo teste, bráquetes com revestimento experimental; e grupo controle, bráquetes originais do fabricante.

Previamente à colagem de bráquetes, a dureza superficial foi avaliada utilizando um ultramicrodurômetro DUH-211S (Shimadzu Corporation, Kyoto, Japão) acoplado a um computador e um software específico. Foi utilizado um indentador de diamante piramidal invertido de base quadrada, tipo Vickers, empregando 50 mN de força, por 20 s, em cinco áreas 100 micrometros distantes da área de colagem. O valor de nanodureza Vickers (Kgf/mm^2) é o quociente da carga aplicada pelo quadrado das médias das diagonais (d_1 e d_2), calculado através da equação $VHN = 1,8544 \times P/d^2$, onde: P = carga aplicada (Kgf); d = média das medidas das diagonais d_1 e d_2 (mm). O valor de nanodureza de cada espécime foi dado pela média destas cinco impressões. O valor de nanodureza de cada grupo experimental foi dado pela média das médias dos valores de cada espécime.

Cada bráquete foi unido a um bloco de esmalte utilizando o adesivo Transbond Plus *Self Etching Primer* (SEP) (3M Unitek, Monrovia, Califórnia, USA) conforme orientação do fabricante. Em seguida a resina composta fotopolimerizável Transbond XT (3M Unitek, Monrovia, Califórnia, USA) foi dispensada diretamente sobre a base do bráquete e pressionada contra a superfície de esmalte em área previamente preparada com adesivo. O excesso de resina foi removido com o auxílio de sonda exploradora n° 5 precedendo a fotoativação com uma unidade fotativadora (Emitter C, Schuster, Guilin, Guangxi, China) por 20 s com irradiância de 1250 mW/cm^2. Logo após os esmaltes foram checados quanto à remoção dos excessos.

Após a união do bráquete aos blocos de esmalte bovino, os espécimes foram submetidos a desafio desmineralizante. Para isso, os corpos de prova foram imersos individualmente em 10 ml de solução desmineralizante contendo 100 mmol de tampão acetato, 1,28 mmol de Ca, 0,74 mmol de P_i, 0,03 µg F/mL, pH 5.0 a 37°C por 32 h. A solução foi preparada utilizando 1,3 mmol $Ca(NO_3)_2.4H_2O$; 0,78 mmol $Na_2HPO_4.2H_2O$; ácido acético glacial 50 mol; 0,03 ppm F^- (NaF) como preconizado por QUEIROZ et al., 2008.

Os bráquetes foram removidos dos blocos de esmalte, pois a presença do mesmo inviabiliza a indentação. Em seguida, os blocos de esmalte foram submetidos a novo teste de dureza do mesmo modo descrito anteriormente para o teste de VHN em cinco áreas periféricas não coincidentes com as indentações anteriores a 100 µm de distância da área de colagem do bráquete.

3.2.4 Análise estatística

Os dados foram tratados com o programa IBM SPSS Statistics 21.0. A normalidade dos dados foi verificada pelo teste Shapiro-Wilk. Foi utilizado o teste ANOVA dois critérios para medidas repetidas, considerando as condições Revestimento e Tempo de avaliação (antes e após o desafio desmineralizante) como fatores em estudo, e o teste Bonferroni para comparações múltiplas dos dados. Todos os testes foram realizados com nível de significância (α) em 5%.

3.3 RESULTADOS

3.3.1 Caracterização composicional e morfológica do filme

A Fig. 1 mostra a superfície do bráquete revestido. O revestimento apresentou uma superfície amorfa, com presença de algumas formas definidas na base do bráquete e próximo ao *slot*. O padrão EDS (Fig. 2) mostrou a presença de cálcio, fósforo e oxigênio, mas não de nitrogênio e potássio, que eram os contra íons de sais de cálcio e fosfato usados para preparar a solução de imersão (Tabela 1).

Tabela 1 – Composição e concentração de cada elemento presente no bráquete ortodôntico revestido com filme de CaP de acordo com a análise de EDS.

Elemento	**N° atômico**	**Massa (%)**	**%atômico**
Ferro	26	44.83	30.91
Cromo	24	13.01	9.64
Oxigênio	8	12.37	29.79
Cálcio	20	8.24	7.92
Níquel	28	7.64	5.01
Fósforo	15	7.93	9.86
Silício	14	1.17	1.60
Alumínio	13	2.70	3.85
Cobalto	27	1.10	0.72
Manganês	25	1.01	0.71

3.3.2 Concentração de Ca no meio

A concentração de íons Ca lixiviados do bráquete revestido por CaP pode ser visualizada na figura 3. Grande quantidade de Ca foi observado na análise de 7 d.

Em 15 e 30 d os valores desse íon na água de armazenamento dos bráquetes foi similar à do branco.

3.3.3 Nanodureza do esmalte

A nanodureza do esmalte após 32 h de imersão em solução desmineralizante está na Tabela 2 (média±desvio padrão; n=15). Foram encontradas diferenças significativas na nanodureza do esmalte antes e depois do ciclo de desmineralização para o grupo controle. No grupo teste, a dureza do esmalte foi mantida (p=0,0000). Após o desafio desmineralizante, a dureza do esmalte do grupo controle foi estatisticamente menor do que à do grupo teste (Fig. 4).

Tabela 2 – Nanodureza do esmalte ao redor dos bráquetes sem revestimento (controle) e revestidos (teste), antes (inicial) e após (final) o desafio desmineralizante.

Grupos	**Inicial**	**Final**
Controle	334,32 ± 25,93 ^{Aa}	204,73 ± 24,41 ^{Bb}
Teste	319,15 ± 17,65 ^{Aa}	308,11 ± 12,03 ^{Aa}
	p < 0,0000*	

*Post-hoc Teste de Bonferroni. Dados representam média ± desvio padrão. Letras maiúsculas diferentes significam diferenças entre as colunas. Letras minúsculas diferentes significam diferenças entre as linhas.

3.4 DISCUSSÃO

O esmalte do grupo teste que foi unido a bráquetes ortodônticos recobertos com filme de CaP manteve a dureza de superfície mesmo após desmineralização *in vitro*, enquanto o do grupo controle (não recoberto) apresentou-se significativamente inferior após o processo de desmineralização. Dessa forma, a hipótese nula foi rejeitada.

Bráquetes ortodônticos com diferentes tipos de recobrimentos (nanopartículas de prata, óxido de titânio, nitreto de titânio) já foram avaliados experimentalmente e mostraram efeito significante no controle do biofilme devido ao seu efeito antimicrobiano (ARASH et al., 2015, 2016; FATANI et al., 2017; GHASEMI et al., 2017). Revestimentos de fosfato de cálcio em implantes metálicos, ortopédicos e dentários, tem recebido atenção por sua capacidade de acelerar a osseointegração em estágios precoces após implantação (ARANYA et al., 2017; PIERRE et al., 2018). O revestimento de superfícies metálicas pode ser realizado por pulverização em plasma, pulverização catódica, métodos eletroquímicos e outros (GUASTALDI; APARECIDA, 2010). O método eletroquímico é particularmente atrativo para recobrimentos de amostras com forma complexa (BAN; MARUNO, 1995). Assim, bráquetes ortodônticos metálicos podem ser recobertos por esse método com baixa temperatura e custo. Além disso, íons cálcio e fosfato tem sido adicionados a dentifrícios e outros produtos preventivos sem efeitos prejudiciais ao corpo (HERAVI; AHRARI; TANBAKUCHI, 2018), indicando segurança no seu uso também em bráquetes.

O método de eletrodeposição tem sido utilizado para conferir efeito antimicrobiano a bráquetes metálicos recobertos com partículas de prata (Ag) com intuito de prevenir a formação de LMB. Estes bráquetes exibem adequado efeito antibacteriano (ARASH et al., 2016), sem prejuízo à resistência de união do bráquete ao esmalte ou à sua resistência friccional (ARASH et al., 2015). Nesse estudo, um novo revestimento de CaP foi desenvolvido para bráquetes ortodônticos metálicos utilizanto o método de eletrodeposição. Altos níveis de íons Ca lixiviados a partir do bráquete foram encontrados, indicando que esse pode ser um método promissor para combate a desmineralização e inibição de LMBs durante o tratamento ortodôntico. A figura 3 ratifica que bráquetes metálicos recobertos com CaP podem liberar íons cálcio para água destilada.

A análise por EDS confirma a deposição de filme de CaP na superfície dos bráquetes, pela presença do correspondente espectro dos elementos cálcio, fósforo e oxigênio. Os espectros dos elementos ferro, cromo, carbono, manganês, níquel e silício estão presentes em todas as avaliações por identificarem os elementos químicos constituintes do bráquete. CaP é uma denominação comum de uma família de minerais essenciais da vida humana. Dentes e ossos são compostos por cristais de cálcio e fosfato inorgânico. Os membros da família de fosfatos de cálcio são de interesse para aplicação biomédica, e podem ser classificados de acordo com a razão atômica de Ca/P. De maneira geral, baixas razões de Ca/P e uma organização amorfa dos íons resultam em maior solubilidade (GUASTALDI; APARECIDA, 2010). Por este motivo, a maior parte de íons cálcio dissolveu-se nos primeiros 7 d, demonstrando a alta solubilidade da camada de revestimento.

Desmineralização refere-se à dissolução de íons cálcio e fosfato da estrutura dental para a saliva, enquanto remineralização refere-se à precipitação dos minerais perdidos à estrutura dental. Apesar da saliva conter íons cálcio e fosfato, essa remineralização pode ser estimulada pela concentração local de íons cálcio e fosfato excedendo os existentes no fluido salivar oral (XU et al., 2011). Assim, o desenvolvimento de materiais à base de CaP para aumento local de íons cálcio e fosfato é uma maneira promissora de inibição de cáries recorrente e de preservação da progressão da lesão (NEEL et al., 2016). Revestimentos capazes de liberar íons cálcio e fosfato resultam em um estado de supersaturação desses íons no meio em relação à sua concentração no esmalte, prevenindo a desmineralização e podendo regenerar a perda mineral (JAHANBIN et al., 2017; UYSAL et al., 2010).

Para avaliar se a quantidade de íons lixiviados dos bráquetes revestidos com CaP interferiria na mineralização do esmalte, um modelo de desmineralização constante em pH 5,0 foi realizado ao invés de ciclagem de pH. A principal razão para isso foi para induzir lesões de cárie *in vitro* no esmalte bovino e simular uma condição clínica extrema. O período de 32 h cria lesões subsuperficiais de cárie sem erosão da superfície, permitindo a avaliação da perda ou ganho mineral por dureza de superfície (QUEIROZ et al., 2008). Assim, o efeito de bráquetes ortodônticos revestidos com CaP na perda mineral ao redor de bráquetes foi avaliado por meio do teste de nanodureza, tendo em vista a existência de uma relação linear entre conteúdo mineral e perfil de dureza da superfície do esmalte (LANGHORST; O'DONNELL; SKRTIC, 2009).

Os valores de dureza mostraram que o esmalte unido ao bráquete revestido com CaP tem significativamente menor perda mineral quando comparado com aqueles unidos ao bráquete metálicos convencionais. Isso sugere que, no mínimo em curto prazo, dentes com bráquetes revestidos com CaP são significativamente mais resistentes à desmineralização cariosa que aqueles com bráquetes tradicionais, mesmo em pacientes com alto risco de cárie. Com frequência, pacientes tem bráquetes em todos os dentes, com fio e elásticos que colaboram para o crescimento da placa, assim, a diferença de efeitos dos dois bráquetes (originais e revestidos) pode ser mais aparente.

Durante décadas, o flúor tem sido usado pelos dentistas para prevenção de cárie. Entretanto, materiais liberadores de flúor depositam significativamente mais mineral que materiais a base de fosfato de cálcio nos primeiros 30 % da lesão. Isso leva à oclusão dos poros da superfície da lesão, por onde ocorrem as trocas iônicas, e limita a extensão do reparo. Apesar de não fornecer vantagem significativa sobre o flúor na porção superficial da lesão, os materiais de CaP são capazes de fornecer minerais mais profundos no esmalte (LANGHORST; O'DONNELL; SKRTIC, 2009). Nesse caso, a incapacidade de penetração do fluoreto profundamente na estrutura dental o coloca em desvantagem quando comparado ao CaP, especialmente devido à evidência de que a saliva natural tem capacidade efetiva somente numa profundidade de até 100 μm (SILVERSTONE, 1977).

O foco desse estudo foi o desenvolvimento de um método capaz de revestir bráquetes ortodônticos metálicos com Ca/P e a investigação *in vitro* da capacidade desse revestimento em reduzir a perda mineral do esmalte ao seu redor. O revestimento de CaP, combinado com o uso de bioagentes sinérgicos para minimizar LMBs e preservar a dureza dentária, tem potencial para ser empregado para a prevenção das lesões de cárie comumente associadas ao uso de aparelhos ortodônticos. Todavia, considerando o fato de que os bráquetes na cavidade oral estão em contato com saliva, sofrendo abrasão causada pela escova e dentifrício, em contato com diferentes tipos de comida e bebidas com várias temperaturas e pH, novos estudos são necessários para melhorar a estabilidade da camada de revestimento.

3.5 CONCLUSÃO

O revestimento de CaP depositado sobre os bráquetes ortodônticos metálicos aumentou a disponibilidade de íons Ca no meio de armazenamento e, também, a resistência do esmalte contra a desmineralização *in vitro*. Assim, a dureza do esmalte ao redor dos bráquetes revestidos não mudou mesmo após um desafio severo de desmineralização.

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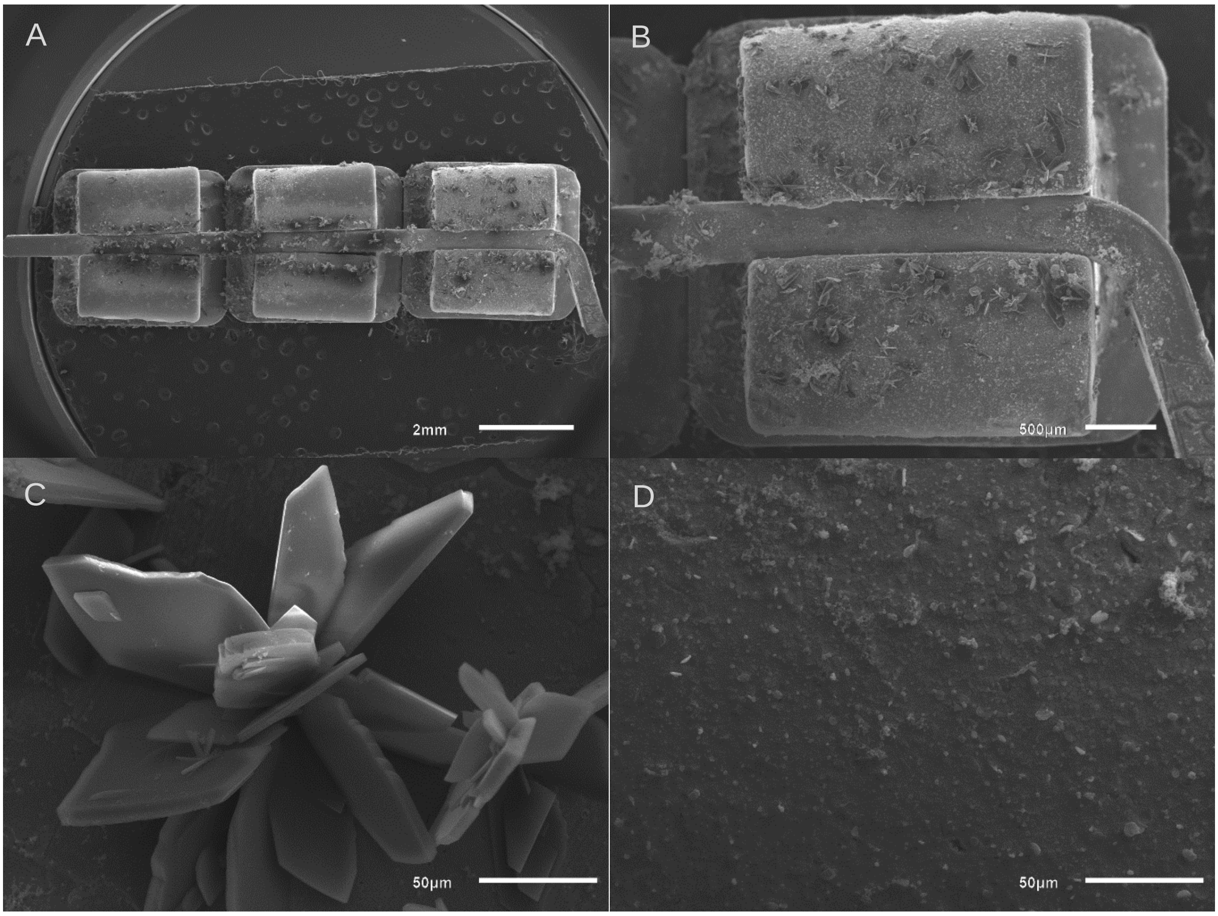


Figura 1 – Imagens de MEV da superfície dos bráquetes revestidos. (A) Aumento de 10x mostrando a superfície dos bráquetes avaliados. (B) Aumento de 30x evidenciando áreas com crescimentos semelhantes a um cristal e áreas sem morfologia definida. (C) Aumento de 500x mostrando o crescimento de formas sugestivas de cristal. (D) Aumento de 500x em região uniforme, sem morfologia definida.

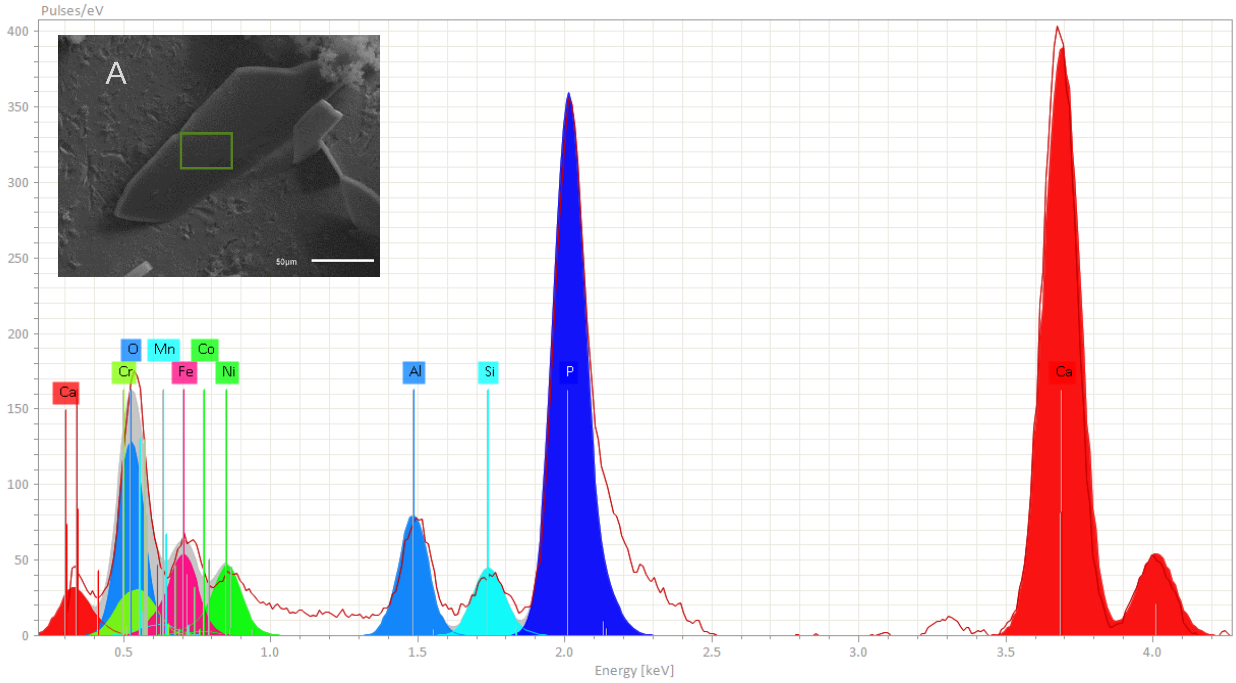


Figura 2 – EDS realizado sobre uma estrutura de forma definida presente sobre o bráquete revestido com CaP. Foram detectados sinais de cromo (Cr), manganês (Mn), cobalto (Co), ferro (Fe) e níquel (Ni), bem como de cálcio (Ca), fósforo (P) e oxigênio (O).

Figura 3 – Concentração (mg/L) de íons cálcio no meio em diferentes tempos de armazenamento.

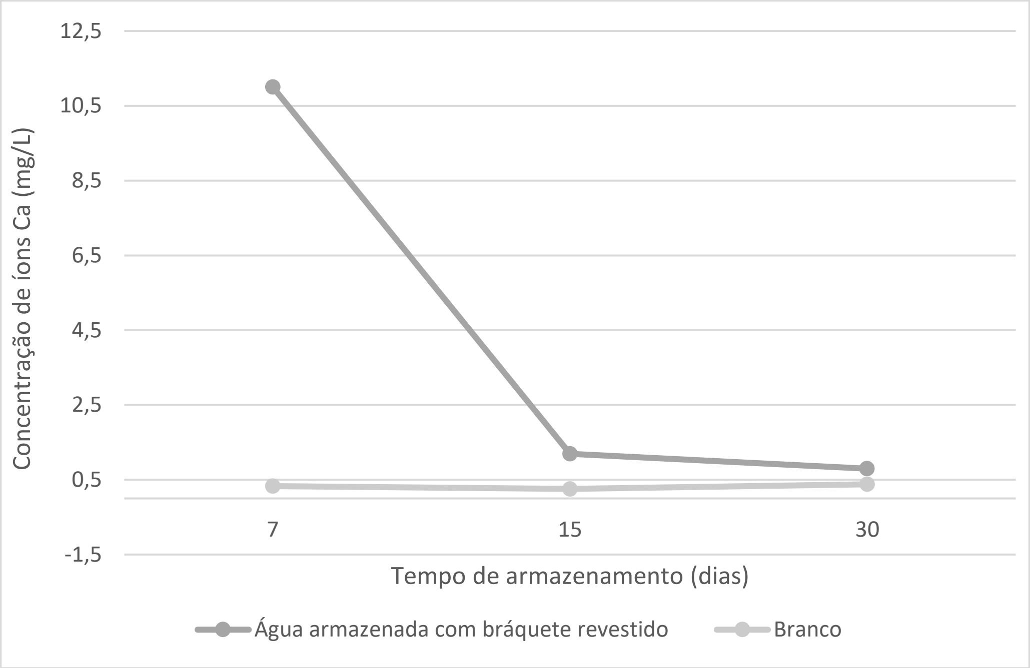
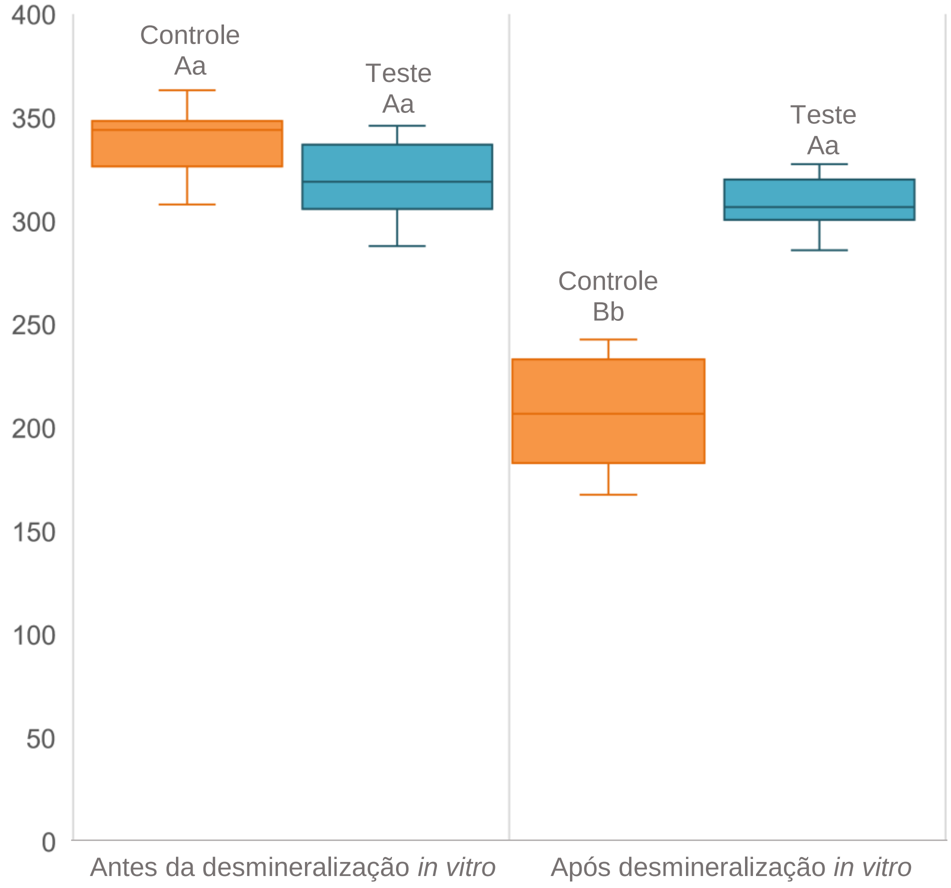


Figura 4 - Dureza do esmalte ao redor dos bráquetes com (teste) e sem revestimento (controle), antes e após o desafio desmineralizante.



*Post-hoc Teste de Bonferroni. Letras maiúsculas diferentes significam diferenças entre as colunas. Letras minúsculas diferentes significam diferenças entre as linhas.

4. CONCLUSÃO GERAL

De acordo com os objetivos propostos e a metodologia utilizada, conclui-se que o bráquetes revestidos com CaP possuem capacidade de lixiviar Ca em água destilada. Uma redução substancial na perda mineral quando estes bráquetes revestidos foram utilizados e maior dureza do esmalte foi obtida em comparação ao grupo controle após desmineralização *in vitro*.

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APÊNDICE 1 - DESCRIÇÃO METODOLÓGICA

Figura 1 - Bráquetes utilizados nos grupos controle e teste.

Figura 2 - Célula eletroquímica mostrando o eletrodo de trabalho (placa de platina) à esquerda, eletrodo de referência Ag/AgCl no centro e o contra eletrodo (bráquetes) à direita ligados a um potenciostato e imersos em solução eletrolítica rica em cálcio e fosfato.

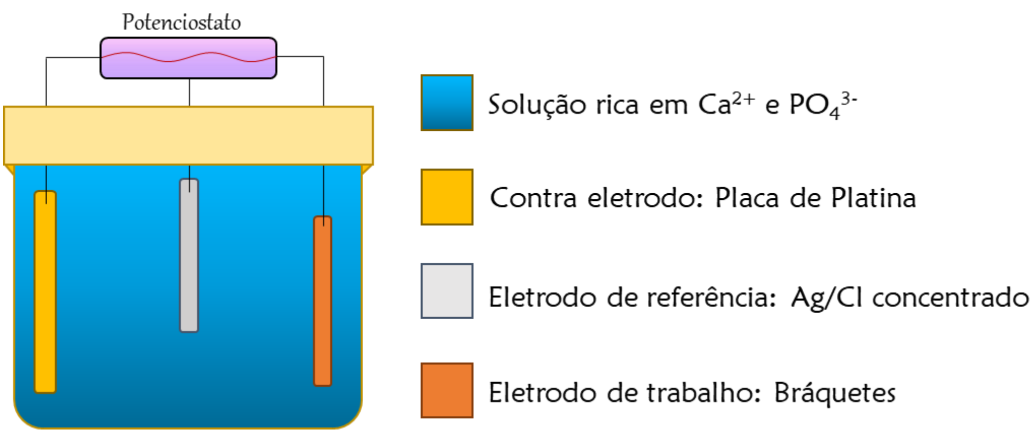

Figura 3 – Representação esquemática da eletrodeposição. Os eletrodos estão conectados a um potenciostato que é responsável pela aplicação da carga, permitindo que os fenômenos eletroquímicos ocorram.

Figura 4 – Aparência do bráquete antes (à esquerda) e após (à direita) o revestimento.

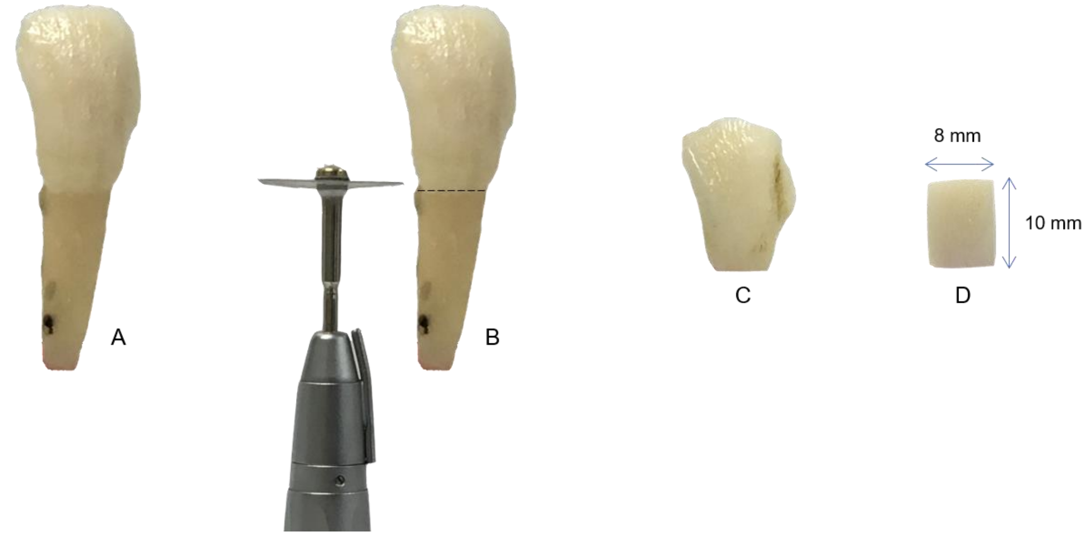

Figura 5 – Dente bovino utilizado no estudo. (A) Dente após extração e limpeza; (B) Corte na unção amelocementária com disco diamantado dupla face em baixa rotação; (C) Coroa de dente bovino pronto para corte; (D) Dente nas dimensões utilizadas.

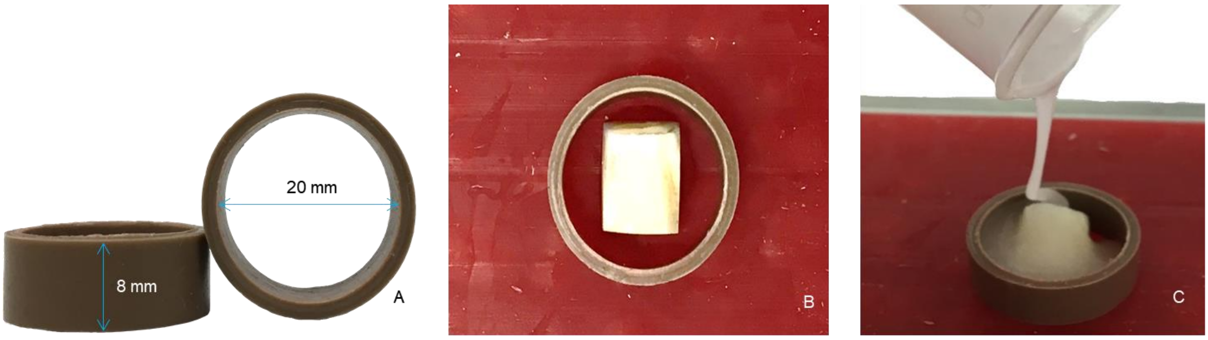

Figura 6 – (A) Cilindro de PVC utilizado na inclusão dos bloco de esmalte em resina acrílica; (B) Bloco de esmalte sobre cera utilidade com a superfície vestibular voltada para baixo no centro do cilindro de PVC; (C) Resina acrílica incolor quimicamente ativada é vertida no sobre o espécime de dente bovino.

Figura 7 – Politriz e lixas de carbeto de silício (#400, #600, #2000 e #4000) na planificação e polimento da superfície do esmalte após embutimento.

Figura 8 – Aspecto final Corpo de prova após polimento final com suspensão de alumina 3 µm.

Figura 9 – Sistema Transbond® para colagem ortodôntica utilizados para união do bráquete ao esmalte bovino.

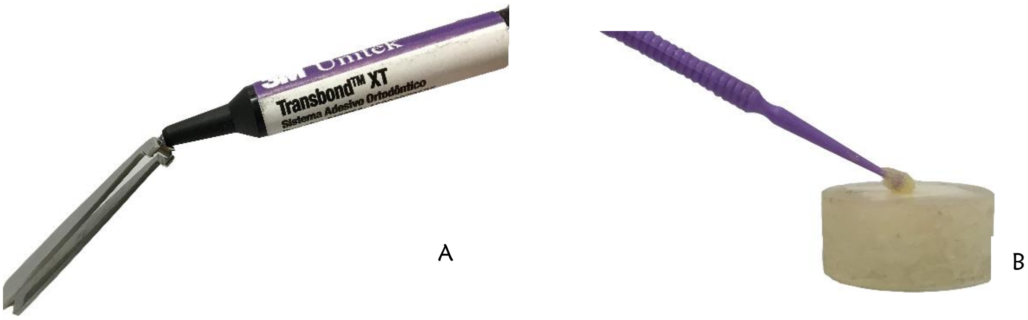

Figura 10 – A resina para colagem ortodôntica foi dispensada sobre a base do bráquete (A) e sobre o esmalte foi aplicado o adesivo *SEP* em área determinada previamente (B).

Figura 11 – Logo após a colagem os corpos de prova foram imersos em solução desmineralizante.

Figura 12 – Dispositivo utilizado para os ensaios de nanodureza (DUH-211S; Shimadzu Corporation, Kyoto, Japão).

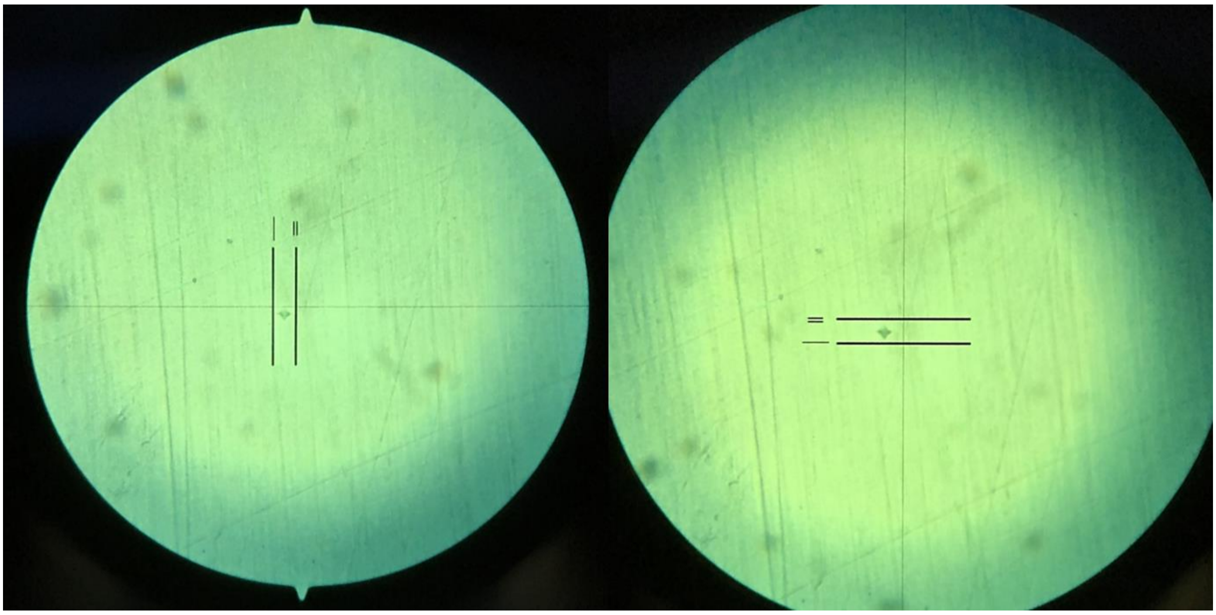

Figura 13 – Medida das diagonais da indentação no durômetro.

APÊNDICE 2 – ARTIGO PARA SUBMISSÃO

Title: Effect of CaP-coating on the hardness of bovine enamel around metallic brackets after demineralization *in vitro*

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‘Conflicts of interest: none’.

Abstract

Objectives: The aim of this *in vitro* study was to evaluate the calcium leaching ability of calcium phosphate (CaP) thin film coated orthodontic brackets in distilled water, as well to evaluate the surface Vickers nanohardness (VHN) of experimental bracket coated with CaP after demineralizing challenge. Methods: Original manufacturer's orthodontic brackets were used as control (n=15) and fifteen were coated with CaP film by electrodeposition. The film was characterized morphologically by scanning electron microscopy (SEM) equipped with dispersive energy spectroscopy (EDS). Twenty-eight coated brackets were stored in distilled water for 7, 15 and 30 days and analyzed at 7, 15 and 30 d for calcium leaching. Thirty blocks (8 x 10 mm) of bovine enamel were submitted to metallographic polishing used for hardness test. The VHN of bovine enamels was evaluated before and after demineralizing challenge using 50 mN of force, for 15 seconds, in five areas. Then, the enamel blocks were divided into 2 groups (n=15): test group, CaP coated bracket; and control group, manufacturer's original brackets. After storage in distilled water at 37 °C for 24 h, the specimens underwent a demineralization process in pH 5.0 solution for 32 hours at 37 °C. Soon after the VHN was remeasured. Results: The surface of the coating was amorphous and crystalline, in both structures there is presence of calcium and phosphate, proving the coating. Calcium leaching occurred with greater intensity in 7 d ($p < 0.05$).

The group with orthodontic brackets covered with CaP showed lower hardness loss after the demineralization process than the control group ($p < 0.05$). Significance: The coating has been shown to be a means of maintaining bovine enamel hardness after *in vitro* demineralization.

Keywords: Orthodontic Brackets, Tooth Demineralization, Hardness test.

Highlights

- A novel CaP-coating for metallic orthodontic brackets was developed
- The hardness of the enamel around the CaP-coated brackets didn't change after a severe demineralization challenge
- Substantial reduction in mineral loss when these coating brackets are used and greater enamel hardness were obtained compared to control

1. INTRODUCTION

Malocclusion is a heterogeneous condition that affects populations worldwide and results in impaired aesthetics, function and reduced quality of life [1]. Fixed orthodontic therapy is an effective and common method for treating malocclusions in contemporary orthodontics [2]. However, despite its widespread use, the control of negative side effects still remains a challenge for the orthodontist [3,4]. One of the most common adverse effects associated with this treatment modality is demineralization around brackets, which routinely presents as a white spot lesion (WSL) [5]. The prevalence of WSL among orthodontic patients ranges from 2% to 96% [6] and 24% can become cavities if left untreated [7].

The white opaque appearance of WSL is due to an optical phenomenon caused by mineral loss in the subsurface enamel and represent the first sign of a caries lesion which can develop within 1 month of bracket placement [3] and eventually lead to disruption of it in more generalized cases. Furthermore, a sharp increase in the number of WSLs during the first 6 months of treatment that continued to rise at a slower rate up to 12 months are of great importance to evaluate the oral hygiene status of patients during the initial months of orthodontic treatment [8].

Although there are several preventive methods proposed in the scientific literature, studies show that the incidence of WSL in individuals with fixed appliances still remains high [5,9,10]. This fact occurs because the orthodontic brackets have several recesses in their surface, which facilitates the accumulation of bacterial biofilm [11] and increases the number of acidogenic bacteria in the oral cavity [12]. In addition, the presence of these devices implies greater difficulty with oral hygiene [13], requiring more time and dedication on the part of the patient.

There are substances that are known to interfere in the demineralization-remineralization process. Among them, calcium phosphate is one of the main constituents of bones and teeth, and so it is of great interest in dentistry. For remineralization to occur, remineralizing substances must be able to release calcium and phosphate ions into the environment [14]. For this to occur, it is necessary that calcium phosphate is supersaturated relative to the medium in order to have remineralizing potential [15]. Thus, any external medium that increases the concentration of calcium and phosphate ions act by increasing the natural capacity of saliva remineralization [16]. This would be particularly important in patients with high risk of caries such as patients undergoing orthodontic or salivary deficiency treatment.

Several treatments for WSL are proposed in the literature as: use of fluorides [17], amorphous calcium phosphate casein paste (CPP-ACP) [14], and antimicrobial substances [18]. Among them, fluoride mouthwash with or without antimicrobial substance has been reported as effective [4,18]. However, its application is dependent on the individual, who may not collaborate. In this way, the scientific community has shown real interest in the search for new means to reduce the occurrence of WSL around brackets.

In laboratory, various bioactive substances have been incorporated in the materials for orthodontic brackets bonding to reduce the occurrence of WLS in patients under treatment with fixed orthodontic appliances. Among them: nanoparticles of silver [19–21], ammonium-based methacrylate [22–24] and amorphous calcium phosphate [24–26]. Studies have shown an improvement in the mechanical properties of the enamel around the brackets when these substances are incorporated in the resins for bonding the devices [23,27]. However, there is concern about the limited effect of this action since the area of exposure of the bond material in the oral cavity is restricted [28].

Therefore, this *in vitro* study aimed to evaluate if such deposition in metallic orthodontic brackets would improve Ca disponibility in the storage medium (or saliva) and enamel resistance against demineralization. The null hypothesis is that the CaP-coated orthodontic bracket would not interfere with enamel hardness, after a severe demineralization challenge.

2. MATERIALS AND METHODS

Standard maxillary canines and premolars metal brackets (Morelli, Sorocaba, SP, Brazil) were divided into two groups, namely test and control. In the test group, the brackets were coated with CaP, via electroplating. The control group did not receive any coating. The amount of calcium leached by the CaP-coating in distilled water and the influence of this experimental coating on bovine enamel nanohardness were verified.

2.1 Electroplating process

Fifty-one brackets were coated for the test group. Prior to electroplating, the brackets were cleaned with isopropyl alcohol, rinsed with ultrapure water and dried with hot air. After cleaning the brackets, they were then electroplated with CaP. The control group (n=15) was cleaned by the same process but not subjected to electroplating.

The electrolyte (supplier of Ca^{2+} and PO_4 ions) used in this process was composed of 50 mmol/L KH_2PO_4 and 100 mmol/L $Ca(NO_3)_2.4H_2O$, which had a pH of ~5.5. Deposition was performed using a potentiostat (Autolab; Metrohm, Utrecht, Netherlands) with a current density of 1.5 C/cm^2 at room temperature. The positively charged Ca^{2+} and PO_4 ions (anode) moved into the electrolyte solution and, over time, precipitated on the negatively charged bracket surface (cathode). The post-treated specimens were dried at 37 °C in desiccator with silica by 24 hours.

Surface morphology and composition of the CaP-coating were analyzed in a scanning electron microscope (SEM; JEOL JSM-6460LV, Tokyo, Japan) operating at 30 kV equipped with energy-dispersive spectroscopy (EDS). Eight coated brackets from test group were used for this purpose.

2.2 Measurement of ca ion release from CaP-coated brackets

Twenty-eight coated brackets were immersed in deionized water to simulate a mouth full of metal brackets. The concentrations of Ca ions released from the brackets were measured at 7, 15 and 30 days. At each time period, an aliquot of 250 mL was removed and replaced with fresh distilled water. The aliquots were analyzed for their Ca ion concentrations via a spectrophotometric method (Inductively Coupled Plasma Optical Emission Spectrometry - ICP-OES). Pure distilled water was used as blank.

2.3 Nanoindentation test and demineralization challenge

Thirty freshly extracted bovine incisor teeth were used in the experiment. The bovine incisors were obtained from a slaughterhouse after the Ethics Committee approval.

The specimens were cut, perpendicular to the buccal surface, with dimensions of 10x8 mm (height x width) using a water-cooled diamond precision saw (Labcut 1010; Extec Technologies, Connecticut, USA). The specimens were embedded in acrylic resin (Jet; Clássico, São Paulo, Brazil), leaving the enamel surface uncovered, which were further polished flat (silicon carbide paper: grit 400, 600, 1200, 2000 and 4000 for 30 seconds; alumina suspension 3 µm for 120 seconds), using a circular polishing machine, with speed of 600 rpm. During the exchange of grinding paper, the specimens were ultrasound cleaned for 5 minutes.

"Inicial" enamel nanohardness were measured with a Vickers nanoindenter (DUH-211S; Shimadzu Corporation, Kyoto, Japan) before the demineralization challenge. The measured parameter was the indentation length, often expressed in Vickers hardness numbers (VHN). The indenter was pressed perpendicular to the enamel surface with a 50 mN load for 15 sec at 0.01 mN/µs. Each indentation was repeated five times with a distance of 100 µm from each other, approximately 100 µm from the delimited area for the bracket. Hardness was calculated by the software of the nanoindentation apparatus.

Then, the teeth were randomly assigned to the test and the control groups (n=15). In both groups, brackets were bonded with Transbond Plus SEP (3M Unitek, California, USA) and Transbond XT composite to enamel block. All bonded teeth were immersed individually in a plastic vial with 10 mL of demineralizing solution (1.3 mmol/L $Ca(NO_3)_2.4H_2O$, 0.78 mmol/L KH_2PO_4 and 0.03 µg F/mL, with 50 mmol/L acetic acid to give a pH of 5.0) for 32 hours, at 37 °C [29].

After demineralization challenge, the brackets were debonded and the specimens were subjected to another nanoindentation test as previously described. Each indentation was repeated five times with a distance of 100 μm from each other, approximately 100 μm from the bracket edge in an unmatched previously location.

2.4 Statistical analysis

Statistical analysis was performed using the application IBM SPSS Statistics 21.0 for Windows.The data were subjected to a normality test (Shapiro-Wilk's). The mean values were analysed by repeated measured analyses of varience (ANOVA) test for both isolated factors, type of material and hardness before and after the cariogenic challenge. The Bonferroni *post-hoc* test was used. Statistical significance was established when $p<0.05$.

3. RESULTS

3.1 Morphological and compositional characterization of the film

Fig. 1 shows an amorphous coated surface with dispersed crystalline salts, specially on the base of the bracket and next to the slot. The EDS pattern showed the presence of calcium and phosphorus, but no nitrogen nor potassium which were the counter ions of calcium and phosphate salts used to prepare the soaking solution (Fig. 2).

3.2 Calcium leaching

The leached-ion concentrations of calcium from CaP-coated brackets are provided in Fig. 3. Greater amounts of ions were leached after 7 days of storage, but a sharp decline was observed in subsequent analyzes.

3.3 Enamel nanohardness

Enamel nanohardness after 32 hours of immersion in demineralizing solution is plotted in Table 1 (mean±standard deviation; n=15). There were significant differences in enamel nanohardness before and after the demineralization challenge (p=0.0000). After demineralization, measurement values showed significant decrease in enamel hardness in the uncoated group. However, the test group preserved surface hardness (Fig. 4).

Table 1. Means and standard deviation (SD) of enamel nanohardness for different solutions.

Groups	**Initial**	**Final**
Control	334.32 ± 25,93 ^{Aa}	204.73 ± 24,41 ^{Bb}
Testt	319.15 ± 17,65 ^{Aa}	308.11 ± 12,03 ^{Aa}
	p < 0,0000*	

*Bonferroni post-hoc test. Uppercase letters differ between columns. Lowercase letters differ between lines.

4. DISCUSSION

The novel CaP-coated orthodontic bracket yielded significantly greater enamel hardness after demineralization challenge than that of the original bracket, which exhibited effect of demineralization-prevention. Thus, the null hypothesis must be rejected.

Some different metal-coated orthodontic brackets have already been evaluated, showing significant effect on oral health by controlling the biofilm formation or by some antibacterial effect [30–33]. In medicine, calcium phosphate coatings have gained attention because of their apparent effect on accelerating bone fixation of metallic prostheses in the early stages after implantation [34,35]. There has been considerable work on calcium phosphate coatings and many coating methods have been reported, such as plasma spraying, sputtering, electrochemical methods and others [36]. Electrochemical methods are particularly attractive for coating irregularly shaped substrate [37]. Thus, for this method, metallic orthodontic brackets can be coated with low temperature and coast. In addition, Ca and P ions have been so long added to toothpastes and other caries prevention products without any indication of damage to the body [38].

The electroplating method has already been used to assess the antibacterial effect of stainless-steel orthodontic brackets coated with silver (Ag) particles to prevent the occurrence of WSL. Ag-coated stainless steel bracket exhibited adequate antibacterial effect [31], with no detrimental effect on the bracket's bond strength to enamel or any decreased friction in sliding mechanics [32]. In our study, a novel CaP-coating for metallic orthodontic brackets was developed using the electroplating method. High levels of Ca leaching from brackets were found for the first time (Fig.

1), indicating that this could be a promising method to prevent enamel demineralization and inhibit WSLs during orthodontic treatments.

CaP is the common name of a family of minerals essential to human life, since bones and teeth are composed of crystals of calcium and inorganic phosphate ions. The members of the CaP family that are of interest to biomedical applications can be classified according to their Ca/P atomic ratio. In general, the higher the Ca/P ratio results, the lower the solubility [36]. On the other hand, a higher amount of calcium was released of the CaP-coated bracket in 7 d, which demonstrates higher solubility of the coating layer.

Demineralization refers to the dissolution of calcium and phosphate ions from the tooth structure into the saliva, while remineralization refers to mineral precipitation into the tooth structure. Since saliva contains calcium and phosphate ions, remineralization can be stimulated by local concentrations of solution calcium and phosphate ions that exceed those existing in ambient oral fluid [39]. Therefore, the development of CaP materials to increase the local calcium and phosphate ion concentrations is a promising approach to the inhibition of recurrent caries and the prevention of lesion progression [40]. These materials can release Ca and P ions, resulting in a state of supersaturation with respect to enamel, thus preventing demineralization and even being able to regenerate lost tooth mineral [27,41].

To evaluate if the amount of ions leached from the CaP-coated brackets would interfere with enamel mineralization, a constant demineralizing model at pH 5.0 was carried out instead of pH-cycling. The reason for this was to induce *in vitro* caries-like lesions on bovine enamel blocks, simulating an extreme clinical condition. The 32 h cycle enabled measurable caries-like subsurface lesions without surface erosion, allowing for the evaluation of mineral loss or gain by determining surface hardness [29]. Afterwards, the effect of orthodontic bracket CaP-coating on mineral loss in enamel around brackets was assessed by a nanoindentation test, since a linear relationship had been shown between hardness profiles of enamel and mineral loss [42].

The hardness values of enamel showed that teeth bonded with CaP-coated brackets have significantly less enamel mineral loss when compared to teeth bonded with conventional metallic bracket. This suggests that, at least in the short term, teeth bonded with CaP-coated bracket are significantly more resistant to caries demineralization than those with traditional bracket, even in patients known for their high caries risk. Often, patients have brackets on all teeth, with wires and elastics compounding the plaque build-up, so that the difference in the effect of the two brackets would probably be even more apparent.

For decades, fluoride has been used in dentistry for caries prevention. However, fluoride-releasing materials deposit significantly more mineral than CaP materials in the initial 30 % of the lesion, although this may occlude surface pores and likely limits the extent to which the rest of the lesion may be repaired. While not providing significant advantage over fluoride in the superficial portion of the lesion, CaP materials are capable of delivering minerals deeper into the enamel [42]. In this case, the inability of the fluoride to penetrate deeply into tooth structures puts it at a distinct disadvantage compared to CaP, especially given the evidence that saliva's natural remineralizing ability is only effective to a depth of 100 μm [43].

It should be noted that the main focus of this study was the development of CaP-coated orthodontic bracket and the investigation of an *in vitro* ability to decrease mineral loss upon demineralization. Nonetheless, considering the fact that brackets in the mouth are in contact with saliva, abrasions caused by a toothbrush, toothpaste and different types of foods and drinks with various temperatures and pHs, the coated layers need to have their stability evaluated in future studies.

This study developed a novel CaP-coating for metallic orthodontic brackets for the first time, with the aim of avoiding WSLs in orthodontic treatments brackets. Substantial reduction in mineral loss when these coating brackets are used and greater enamel hardness were obtained compared to control. This method, combined with the use of synergistic multiple bio-agents to minimize lesions and preserve tooth hardness, has the potential to be used for applications in a wide range of orthodontic materials to combat caries.

5. CONCLUSION

CaP-coating deposited over the metallic orthodontic brackets improved Ca disponibility in the storage medium and, also, the enamel resistance against demineralization *in vitro*. Thus, the hardness of the enamel around the coated brackets didn't change even after a severe demineralization challenge.

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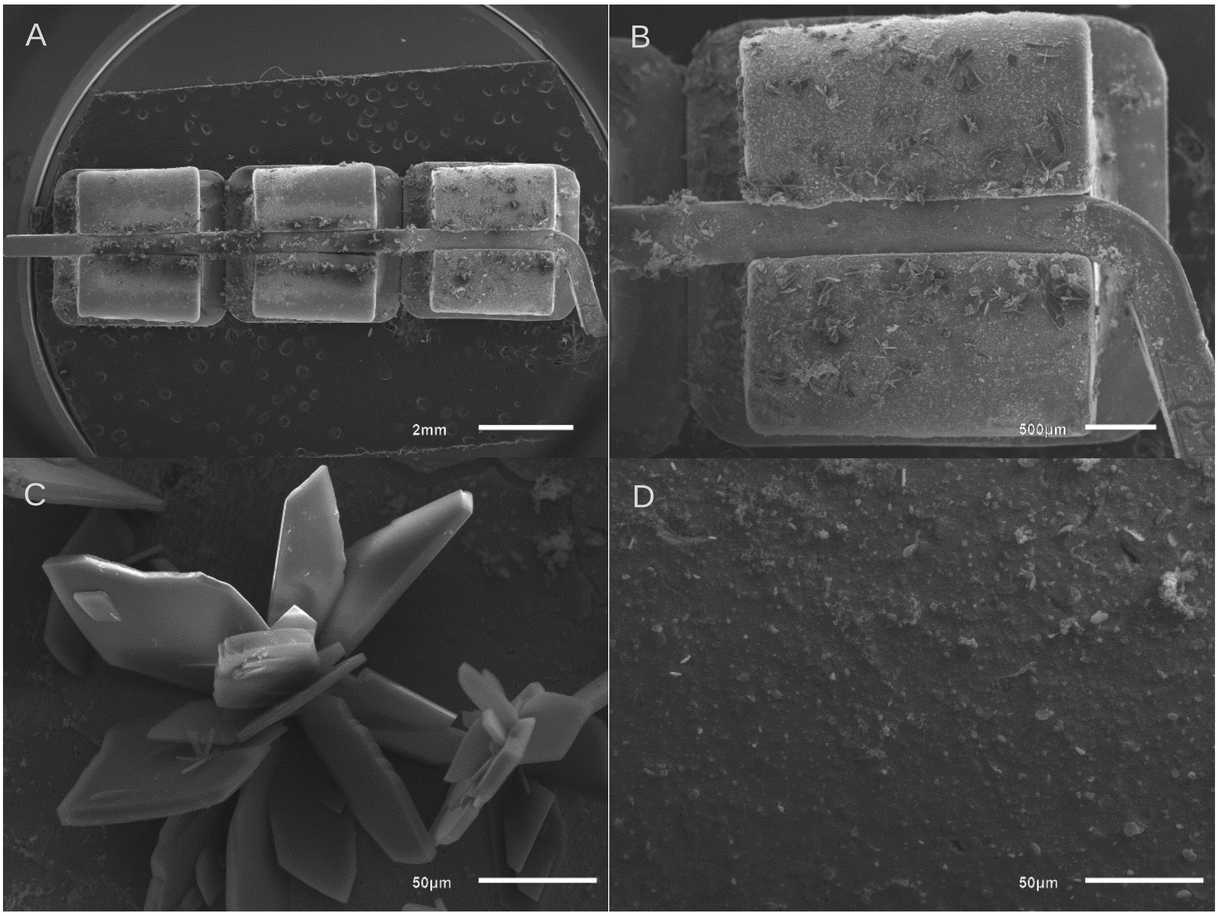

Figure 1 - SEM analysis of the surface of the coated brackets. (A) Lower magnification (10x) showing a surface of evaluated brackets. (B) Note of existence of areas with crystals and amorphous, including a base of the breta (30x). (C) Highest increase of a formed crystal (500x). (D) Higher incidence in the amorphous region (500x).

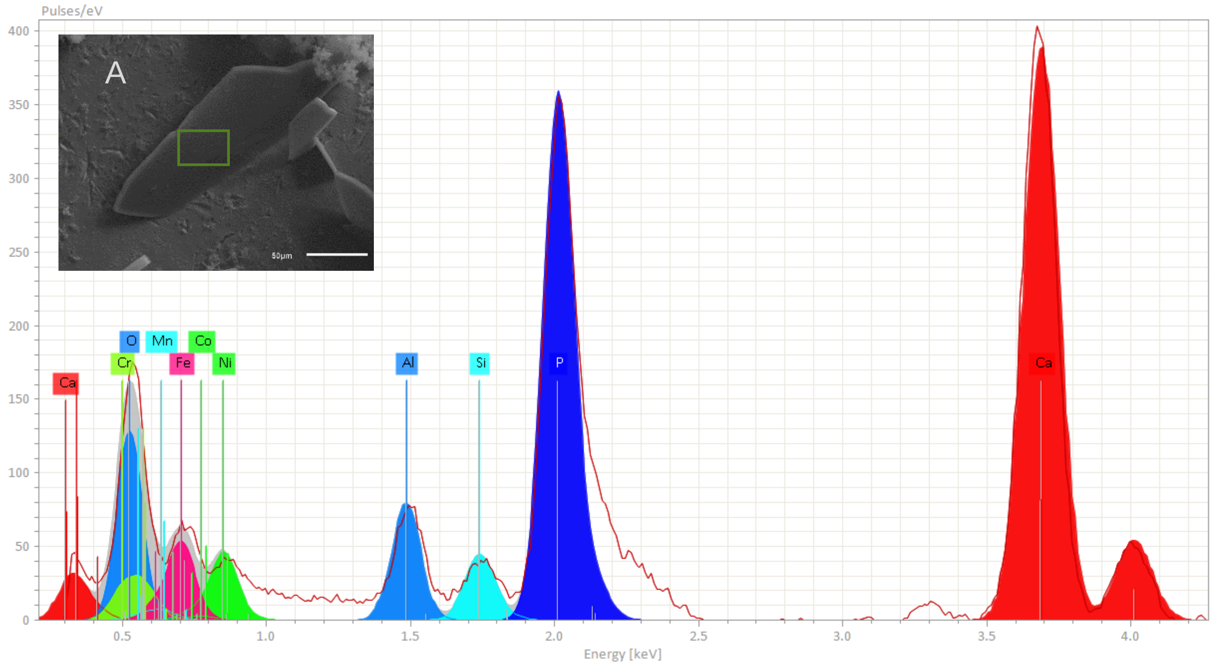

Figure 2 - EDS of a crystal. It is important to emphasize that there is X-ray penetration in the material and, therefore, part of the elements found are part of the metal alloy of the brackets.

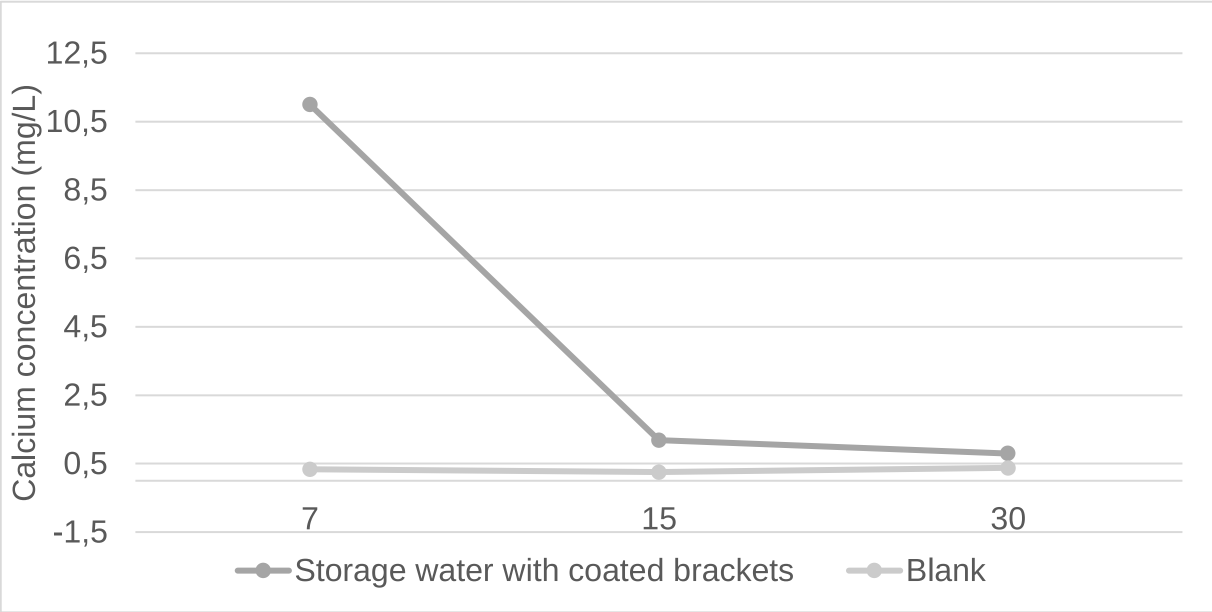

Figure 3 – Calcium concentration in the medium before and after the storage of coated brackets.

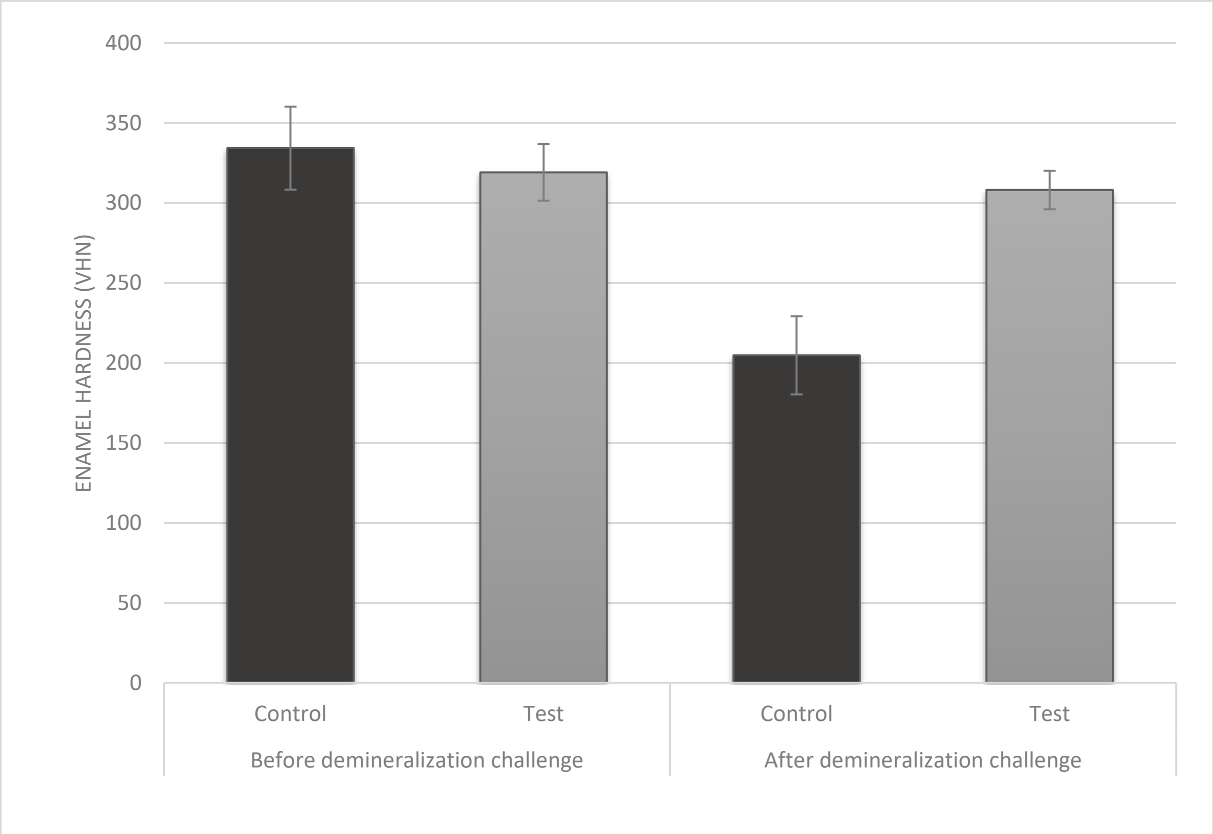

Figure 4 - Means and standard deviation (SD) for final nanohardness values in different groups (p=0.0000).

ANEXO 1 – CERTIFICADO DE TRADUÇÃO PARA LÍNGUA NATIVA

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São Paulo, January 10, 2019.

CERTI FI CATE

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Language: English

Document: EFEITO DO RECOBRIMENTO DE BRAQUETES ORTODÔNTICOS COM FILME EXPERIMENTAL NA DUREZA DE ESMALTE BOVINO SUBMETIDO A DESAFIO DESMINERALIZANTE" do Programa de Pós-Graduação em Clínica Odontológica.

....

Carlos Eduardo Chiba
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CNPJ: 15.267.097/0001-70

ANEXO 2 – CERTIFICADO DE APROVAÇÃO EM COMITÊ DE ÉTICA PARA USO ANIMAL

Universidade Federal do Espírito Santo

CEUA UFES

CERTIFICADO

Certificamos que a proposta intitulada Efeito do recobrimento de braquetes ortodônticos com filme fino experimental na dureza de esmalte bovino submetido a desafio cariogênico

Registrada sob o n.º 74/2017 sob a responsabilidade de JULIANA MALACARNE ZANON

que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei 11.794, de 8 de outubro de 2008, do Decreto n.167 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, em reunião no dia 08 de junho de 2018.

Vigência da autorização: junho/2018 a março/2019 Finalidade: Pesquisa

Espécie(s): Bovino

Linhagem(ns)/raça(s): Nelore

Peso: 18 a 20 arrobas Idade: 48 a 60 meses Sexo: Ambos

Número de animais: 56 (28 fêmeas, 28 machos)

Origem: Frigorífico Mafrical, Cariacica/ES

Vitória-ES, 08 de junho de 2018

Breno S. Salgado

Breno Souza Salgado

Coordenador da CEUA-Ufes

Comissão de Ética no Uso de Animais – CEUA
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Reference to a book: [3] Strunk Jr W, White EB. The elements of style. 4th ed. New York: Longman; 2000.
Reference to a chapter in an edited book:[4] Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.
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Note shortened form for last page number. e.g., 51–9, and that for more than 6 authors the first 6 should be listed followed by 'et al.' For further details you are referred to 'Uniform Requirements for Manuscripts submitted to Biomedical Journals' (J Am Med Assoc 1997;277:927–34) (see also Samples of Formatted References).

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