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CECÍLIA VILELA VASCONCELOS BARROS DE ALMEIDA

AVALIAÇÃO DA REMINERALIZAÇÃO E ESTABILIDADE DE COR DO BIOSILICATO NO TRATAMENTO DE CÁRIE RADICULAR: UM ESTUDO COMPARATIVO

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de doutora em Odontologia. Área de concentração em Clínica Integrada.

Orientador: Anderson Stevens Leônidas Gomes

Coorientadora: Cláudia Cristina Brainer de Oliveira Mota

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A Deus que dá sentido a todas as coisas na minha vida;
Ao meu marido que sonha meus sonhos;
Aos meus pais que me apresentaram a fé em Cristo e sempre fizeram tudo pela minha educação;

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RESUMO

O objetivo do presente trabalho foi avaliar o efeito do Biosilicato (BIOS) no tratamento da cárie radicular e na estabilidade de cor em comparação com o diamino fluoreto de prata (SDF – *Silver Diamine Fluoride*). No primeiro estudo (E1) a remineralização foi avaliada através da tomografia de coerência óptica (OCT). As amostras de dentina radicular foram distribuídas em dois grupos: BIOS (n = 10), e SDF (n = 10), e avaliadas em três momentos: antes (T0) e após a desmineralização (T1), e após a remineralização (T2). Testes complementares de microdureza, e microscopia de luz polarizada (PLM – *Polarized Light Microscopy*) foram realizados. No segundo estudo (E2), a estabilidade de cor foi avaliada em três momentos: inicial (T0), após a remineralização: 24h (T1) e 5 semanas (T2). As amostras de dentina radicular foram distribuídas em dois grupos: BIOS (n = 15), e SDF (n = 15). No E1, foi observado na OCT que ambos os grupos recuperaram os níveis iniciais de remineralização, mas o SDF obteve maiores níveis de remineralização em comparação ao BIOS ($p < 0,05$). Quanto aos testes complementares, não houve diferença entre os grupos em T2 para microdureza, e na PLM observou-se aspectos de remineralização nos dois grupos. No E2, houve uma leve alteração de cor para o BIOS durante o período do estudo, entretanto, em comparação com o SDF, a estabilidade de cor se apresentou muito superior ($p < 0,05$). Portanto, pode-se concluir a partir destes estudos que o potencial remineralizador e discreta alteração de cor tornam o BIOS um forte candidato para substituir o SDF no tratamento da cárie radicular.

Palavras-chave: biomaterial; tomografia de coerência óptica; remineralização dentária; cárie radicular, colorimetria.

ABSTRACT

The aim of the present work was to evaluate the effect of Biosilicate (BIOS) in the treatment of root caries and in the color stability in comparison with silver diamine fluoride (SDF). In the first study (S1) remineralization was evaluated using optical coherence tomography (OCT). The root dentin samples were divided into two groups: BIOS (n = 10), and SDF (n = 10), and evaluated at three moments: before (T0) and after the demineralization (T1), and after the remineralization (T2). Complementary tests of microhardness and polarized light microscopy (PLM) were performed. In the second study (S2), the color stability was evaluated in three moments: initial (T0), after the remineralization: 24h (T1) and 5 weeks (T2). Root dentin samples were distributed into two groups: BIOS (n = 15), and SDF (n = 15). In S1, it was observed in OCT that both groups recovered the initial levels of remineralization, but SDF obtained higher levels of remineralization when compared to BIOS ($p < 0.05$). As for the complementary tests, there was no difference between the groups in T2 for microhardness, and remineralization was observed for both groups under PLM. In S2, there was a slight color change for BIOS during the study period, however, with higher color stability, when compared to SDF ($p<0.05$). Therefore, it is concluded from these studies that the remineralizing potential and slight discoloration make BIOS a strong candidate to replace SDF in the treatment of root caries.

Keywords: biomaterial; optical coherence tomography; tooth remineralization; root caries; colorimetry.

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1 INTRODUÇÃO

O envelhecimento populacional associado a uma menor taxa de perdas dentárias é um dos fatores responsáveis pelo aumento da prevalência da cárie radicular (ALQRANEI *et al*., 2021). Esta é uma doença multifatorial, podendo se apresentar como uma lesão amarronzada, amolecida, cavitada ou não (ALQRANEI *et al*., 2021). A superfície radicular é mais vulnerável à desmineralização do que o esmalte, pois a dentina e o cemento que a recobre contêm um menor percentual de minerais em sua composição (ZHANG *et al*., 2019), o que facilita a instalação e desenvolvimento da doença cárie em sua superfície quando exposta na cavidade bucal.

De forma geral, a abordagem para o tratamento da doença cárie tem mudado ao longo dos anos. No lugar da excisão cirúrgica completa de tecidos acometidos (dentina infectada e afetada), a filosofia contemporânea se baseia na Odontologia Minimamente Invasiva (OMI), isto é, na máxima preservação tecidual (GAO *et al*., 2022). Além da remoção seletiva da dentina infectada, e subsequente restauração, a doença cárie deve ser tratada pelo uso de agentes antimicrobianos e remineralizantes (HORST *et al*., 2016). Muitos agentes estão disponíveis para serem utilizados neste propósito, como o fluoreto de sódio, clorexidina, caseína fosfato de cálcio fosfopeptídeo amorfo e o diamino fluoreto de prata (SDF – *Silver Diamine Fluoride*) (LI *et al*., 2016).

Em especial, os produtos à base de SDF têm sido amplamente explorados no tratamento da cárie radicular por causa da sua eficácia em inibir o início e reduzir a progressão da doença cárie além da sua técnica simples e baixo-custo (GREENWALL-COHEN *et al*., 2020). Como o próprio nome diz, o SDF é composto principalmente de amônia, prata e flúor. O flúor é responsável pela remineralização e os íons de amônia se ligam aos íons de prata formando um complexo estável (GREENWALL-COHEN *et al*., 2020). O íons de prata, por sua vez, são responsáveis pelo potencial antimicrobiano, os quais interagem com os grupos sulfidrilas das proteínas e com o DNA das bactérias, ocasionando a inibição de processos vitais como a respiração, e por fim leva à morte e lise da parede celular (DÚRAN *et al*., 2016; ROZALYONOK; SIDORIN, 2016; KALWAR; SHAN, 2016). Entretanto, os íons prata remanescentes se depositam nos túbulos dentinários, e geram um manchamento do tecido dentário (PATEL *et al*., 2018), o que diminui a sua aceitação pelos pacientes

(CRYSTAL *et al*., 2016) e limita a sua indicação apenas para dentes posteriores (FUNG *et al*., 2016). Além disso, após a sua aplicação, ocorre uma redução na resistência de união da dentina aos materiais resinosos (FERREIRA *et al*., 2022). Dessa forma, faz-se necessária a busca por materiais que unam propriedades biológicas importantes presentes no SDF, como remineralização e propriedades antimicrobianas e estéticas.

Os biovidros e as vitrocerâmicas bioativas, pertencentes à terceira geração de biomateriais, podem ser considerados alternativas potenciais ao SDF. Hench (2002) descreve três mecanismos de ação desse grupo de materiais: proliferação celular, indução de resposta genética, e adesão a um tecido vivo. Tendo como base esses três mecanismos, os biovidros e as vitrocerâmicas bioativas têm um importante papel quando aplicados para inativar o processo carioso, que é o desenvolvimento de uma camada de hidroxiapatita bioativa semelhante em estrutura e composição química à fase mineral da apatita presente no osso (HENCH *et al*., 2015) e no dente (TALAL *et al*., 2020). Essa produção de hidroxiapatita é necessária para a adesão entre esses biomateriais aos tecidos vivos, e justifica os melhores resultados encontrados em comparação com outros grupos de biomateriais quando da sua aplicação para regeneração de tecidos mineralizados (CROVACE *et al*., 2016). Dessa forma, os biovidros e as vitrocerâmicas bioativas podem ser considerados como excelentes candidatos para a inativação do processo desmineralização e regeneração de esmalte e dentina.

O Biovidro 45S5 foi desenvolvido por Larry Hench em 1969, apresentando propriedades bioativas importantes, incluindo o seu alto índice de biotividade (HENCH, 2006). Entretanto, por possuir baixas propriedades mecânicas, a sua aplicação se tornou limitada, especialmente em casos onde há grande incidência de cargas, como no caso dos implantes (MONTAZERIAN; ZANOTTO, 2016). Em 1995, o Biosilicato, uma vitrocerâmica completamente cristalizada do sistema Na_2OCaO-SiO_2-P_2O_5 com adição de Li_2O e K_2O foi desenvolvida (RENNO *et al*., 2013). O Biosilicato também possui alto índice de bioatividade, semelhante ao biovidro 45S5, e excelentes propriedades mecânicas, em comparação com as vitrocerâmicas disponíveis comercialmente (PEITL *et al*., 1996; PEITL *et al*., 2001). Essa vitrocerâmica possui propriedades fundamentais para inativar o processo carioso: remineralização (FERREIRA *et al*., 2022), propriedades antimicrobianas (MARTINS *et*

al., 2011), além de atuar no aumento da resistência de união de adesivos ao remanescente dentário (PIRES-DE-SOUZA *et al*., 2022; VIVANCO *et al*., 2021).

Com o intuito de avaliar o potencial de remineralização, a tomografia de coerência óptica (OCT – *optical coherence tomography*) é uma ferramenta desejável, dado o seu caráter não-invasivo, não-destrutivo e não-ionizante (DARLING *et al*., 2012). Através da OCT é possível obter imagens com cortes transversais de alta resolução dos tecidos biológicos (HUANG *et al*., 1991). O seu funcionamento é semelhante ao ultrassom, mas ao invés de utilizar som em alta frequência, a luz é utilizada (NAKAJIMA *et al*., 2012). Dessa forma, a OCT tem sido selecionada para avaliação de uma variedade de propósitos médicos (LAVINSKY; LAVINSKY, 2016; SU *et al*., 2016; CAO; TEY, 2015) e odontológicos (SHIMADA *et al*., 2015; MOTA *et al*., 2015). Os estudos que utilizaram essa ferramenta para avaliar remineralização confirmaram a sua eficácia e validaram este método (KITASAKO *et al*., 2019; SUGIURA *et al*., 2016; COLSTON *et al*., 1998a; COLSTON *et al*., 1998b; YAVUZ; KARGUL, 2021; OKUWAKI *et al*., 2021; THANNAING *et al*., 2022; DARLING *et al*., 2012; CHEN *et al*., 2020; ABUNA *et al*., 2021).

A imagem obtida através do OCT pode ser analisada quanto aos níveis de remineralização de forma qualitativa e quantitativa. Esse último pode ser executado através da mensuração do coeficiente de atenuação óptica (OAC – *Optical Attenuation Coefficient*) (CARA *et al*., 2014; MAIA *et al*., 2016; MUJAT *et al*., 2003). O OAC caracteriza o decaimento do sinal de luz do OCT no tecido irradiado, e assim consegue discriminar diferentes tipos de tecidos e seu estado de saúde (CHANG; BOWDEN, 2019). Qualitativamente podemos avaliar uma imagem de OCT através da variação do reflexo e espalhamento de luz os quais são modificados de acordo com os níveis de desmineralização e remineralização (ABDELAZIS *et al.*, 2022).

Assim como a descoloração produzida pelo SDF é uma de suas desvantagens, na procura de um material que o substitua é necessário entender também o seu comportamento na estabilidade de cor do substrato dentinário. Os estudos que avaliaram a estabilidade de cor do Biosilicato o fizeram quando este biomaterial esteve sob materiais restauradores (FERREIRA *et al*., 2022; PINTADO-PALOMINO *et al*., 2019), permancecendo obscuro o seu real impacto no tecido dentário. Para medir precisamente a alteração de cor, o espectrofotômetro é um método confiável (SAKIROFF *et al*., 2022). O equipamento lê a cor e a quantifica em três parâmetros:

L* (luminosidade), a* (eixo verde-vermelho), e b* (eixo azul-amarelo). A partir dessa informação, conseguimos calcular a mudança de cor total através de diversas opções de fórmulas matemáticas, como CIEL*a*b*, CIEDE2000 e Hyab. Na presença de uma grande mudança de cor, como após a aplicação de SDF, a fórmula Hyab é a opção mais adequada (ABASI *et al*., 2020).

Portanto, o presente estudo teve como objetivos: avaliar os níveis de remineralização e a estabilidade de cor do Biosilicato quando usado no tratamento da cárie radicular e compará-lo com o SDF.

2 METODOLOGIA

2.1 ESTUDO 1. AVALIAÇÃO DA REMINERALIZAÇÃO DA CÁRIE RADICULAR APÓS TRATAMENTO COM UMA VITROCERÂMICA BIOATIVA: UM ESTUDO COMPARATIVO

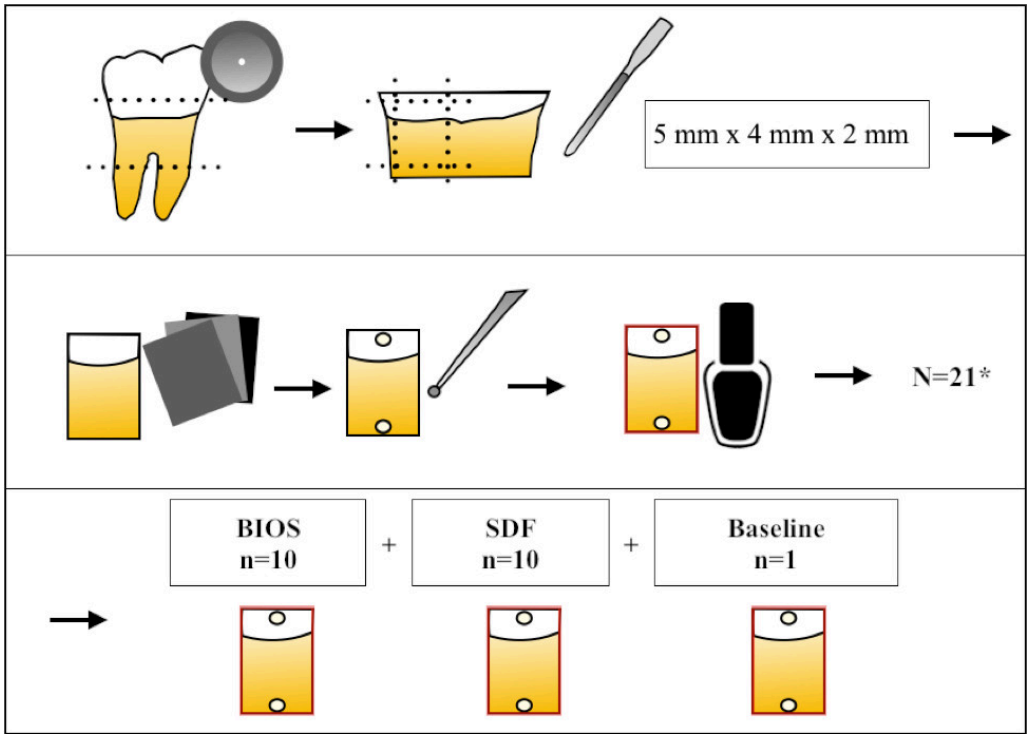
O presente estudo foi aprovado pelo Comitê de Ética em Pesquisa (CEP) da Universidade Federal de Pernambuco (UFPE), nº do processo 4.381.523. O mesmo foi desenvolvido no laboratório de Fotônica e Biofotônica do departamento de Física e no Instituto Nacional de Tecnologia em União e Revestimento de Materiais (INTM), ambos na UFPE. Para este estudo, a amostra consistiu em 7 molares superiores e inferiores obtidos no banco de dentes humanos (UFPE), com a superfície radicular íntegra, sem fraturas, fissuras ou lesões cariosas. Os dentes foram limpos e armazenados em uma solução de 0,1% de Timol a 4 ºC anteriormente ao preparo das amostras.

2.1.1 Preparo das amostras

Os dentes foram seccionados em duas partes, com disco diamantado dupla face 7020 (KG Sorensen, São Paulo, Brasil) sob irrigação abundante, removendo 2/3 da parte superior, e 1/3 da parte inferior, permanecendo aproximadamente 2 mm da coroa e 3 mm da raiz. Depois deste procedimento, os dentes foram seccionados novamente, agora com a ponta diamantada 3216 (KG Sorensen, Cotia, São Paulo) em alta rotação e sob refrigeração abundante. Assim, obteve-se o total 21 amostras (3 blocos a partir de cada dente), provenientes das faces vestibular e lingual, medindo 5 mm de altura x 4 mm de largura x 2 mm de espessura (ZHOU *et al*., 2017). A superfície externa (vestibular ou lingual) dos blocos de dentes foram polidas com lixas d'água de granulação crescente (320, 600, 1200, 2500) com o propósito de remover a camada de cemento e expor a superfície radicular. A superfície interna dos blocos (porção da dentina voltada para a câmara pulpar), também foi lixada para sua regularização. Com auxílio de uma ponta diamantada 1010 (KG Sorensen, São Paulo, Brasil), duas cavidades semicirculares de aproximadamente 0,5 mm de profundidade foram confeccionadas na porção mais superior e na porção mais inferior da superfície externa dos blocos de dente, para padronizar a área de escaneamento pelo OCT. Todas as superfícies de cada bloco, exceto a superfície externa, foram cobertas com uma fina camada de esmalte ácido-resistente (Risqué, Savoy, Goiás, Brasil). Após

completo preparo das amostras, elas foram distribuídas aleatoriamente em dois grupos: Biosilicato (BIOS) (n=10) e SDF (n=10); uma amostra extra foi utilizada como *baseline* no microscópio de luz polarizada antes de qualquer tratamento. Os detalhes do preparo das amostras podem ser visualizados na Figura 1.

Figura 1 - Diagrama esquemático do preparo das amostras e alocação nos grupos.

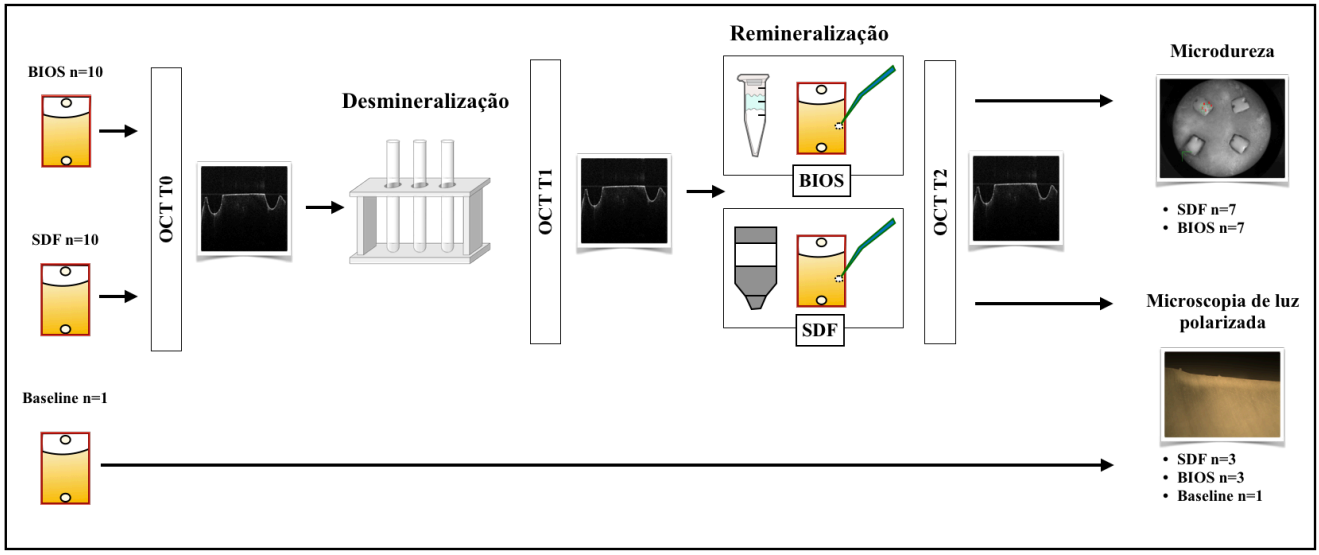


Fonte: a autora (2022)

2.1.2 Fase experimental

As amostras foram submetidas a um processo de desmineralização, e depois de remineralização de acordo com a sua alocação nos grupos: BIOS ou SDF. A cronologia deste processo pode ser visualizada na Figura 2.

Figura 2 – Cronologia da fase experimental.



Fonte: a autora (2022)

2.1.2.1 Desmineralização in vitro

Cada amostra foi imersa individualmente em 20 ml de solução ácida tamponada (50 mmol/L de ácido acético, 1,5 mmol/L de $CaCl_2$ e 0,9 mmol/L de KH_2PO_4 e pH ajustado para 5,0 com KOH) e armazenada a 37 °C em estufa (FANEM, São Paulo, Brazil) por 5 dias (CAI *et al*., 2019).

2.1.2.2 Remineralização in vitro

Após a desmineralização, foi realizada profilaxia das amostras com pedra pomes (Maquira, Paraná, Brasil) e água destilada, com auxílio da escova de Robinson em micromotor e contra ângulo. Para o grupo SDF, utilizou-se a concentração de 30% (Cariestop, Biodinâmica, Paraná, Brasil) seguindo as instruções do fabricante: o tempo de aplicação total foi de 3 minutos, esfregando o produto de maneira vigorosa com o microbrush sobre a superfície externa da amostra. Após este período, a superfície de cada amostra foi lavada com água destilada em seringa tríplice (D700, São Paulo, Brasil).

Para o grupo BIOS, uma suspensão de Biosilicato (Vitrovita, São Paulo, Brasil) a 10% foi preparada com água destilada e deionizada. Em um microtubo de 1,5 ml foi adicionado 0,1 mg de Biosilicato e 1,0 ml de água imediatamente antes da sua aplicação. A suspensão de Biosilicato a 10% foi aplicada ativamente com um

microbrush na superfície da amostra, permanecendo em contato com a mesma durante 1 minuto. Após este período, a suspensão foi gentilmente removida com papel absorvente.

Após a aplicação dos agentes remineralizantes (SDF e BIOS), as amostras foram armazenas individualmente em saliva artificial (1% carboximetilcelulose; 0,12% cloreto de sódio; 0,005% cloreto de cálcio; 1% metilparabeno; 97,8% água deionizada) por 24 horas.

2.1.3 Análise das amostras

As amostras foram analisadas através da OCT, microscopia de luz polarizada e teste de microdureza de Vickers (Figura 2). Todas as amostras foram escaneadas pela OCT em três momentos: inicial (T0), após desmineralização (T1), após remineralização (T2). A microscopia de luz polarizada e o teste de microdureza, são métodos destrutivos, por essa razão as amostras só foram submetidas a estas análises após o terceiro escaneamento por OCT, ou seja, após a remineralização, cada teste analisou 3 e 7 amostras de cada grupo, respectivamente. Uma amostra *baseline*, que não recebeu nenhum tipo de tratamento, foi também submetida à microscopia de luz polarizada.

2.1.3.1 Tomografia de coerência óptica (OCT)

O sistema de OCT selecionado para este estudo foi o Lumedica OQ Labscope (Lumedica, Carolina do Norte, EUA), operando no domínio espectral (SD-OCT). Este sistema utiliza um diodo superluminescente com 840 nm de comprimento de onda central e 0,75 mW de potência de saída. As imagens geradas apresentam resolução axial de 7/5 µm (ar/tecido), resolução lateral de 15 µm e 100 dB de sensibilidade. O sistema captura 12 imagens por segundo formando uma matriz numérica de 512 x 512 pixels e 5 mm de amplitude de varredura. Para análise de OCT, as amostras foram fixadas em cera pegajosa, com a face externa perpendicular ao scanner. O semicírculo na parte superior e inferior de cada amostra permitiu o escaneamento das amostras na mesma posição em todos os períodos de avaliação. As imagens geradas foram salvas no formato JPEG.

Após o escaneamento com OCT, as imagens foram processadas no MatLab para quantificar o OAC. Para tal, foi determinada a largura da região de interesse (ROI)

que deveria ser avaliada em toda imagem. A localização da ROI poderia variar entre as imagens, com o propósito de evitar os pontos brancos formados na imagem, os quais poderiam gerar vieses. Além da avaliação do OAC, foi realizada uma avaliação qualitativa dos padrões de refletividade em cada imagem.

2.1.3.2 Microscopia de luz polarizada

A amostra *baseline* e três amostras remineralizadas de cada grupo foram seccionadas em fatias de 1 mm com disco diamantado em cortadeira de precisão (IsoMet Low Speed, Buehler, Illinois, EUA). Cada fatia foi lixada com lixas d'água de granulação crescente (320, 600, 1200, 2500), para obter uma espessura média de 80 μm. Cada fatia foi analisada pelo microscópio de luz polarizada (Olympus BX51, Tóquio, Japão) com 20x de magnificação. As imagens obtidas foram salvas no formato TIFF, para posterior análise qualitativa.

2.1.3.3 Teste de Microdureza de Superfície

Sete amostras remineralizadas de cada grupo foram incluídas em resina acrílica autopolimerizável (VIPI Flash, São Paulo, Brasil) e submetidas ao teste de microdureza Vickers (DuraScan, EMCO-TEST, Salisburgo, Áustria) com uma carga de 100 g por 15 segundos a uma magnificação de 50 x. Cinco endentações aleatórias na superfície da dentina radicular de cada amostra foram realizados. A média da microdureza de superfície dos cinco pontos de endentação foram registrados como o valor da dureza Vickers (VHN).

2.1.4 Análise estatística

A análise estatística foi realizada usando o software Prism 7 (GraphPad Software, Inc). A significância estatística de todos os testes foi considerada $p < 0,05$.

Para as imagens de OCT, foram calculados os dados descritivos (média e desvio padrão) do OAC de cada grupo. O teste de normalidade Shapiro-Wilk indicou uma distribuição não-normal. O teste não-paramétrico de Friedman foi realizado, seguido pelo teste de múltiplas comparações de Dunn para determinar as diferenças intragrupo entre T0, T1 e T2. O teste de Mann-Whitney U foi realizado para determinar se havia diferença intergrupos.

O teste T não pareado avaliou a significância da microdureza entre os grupos.

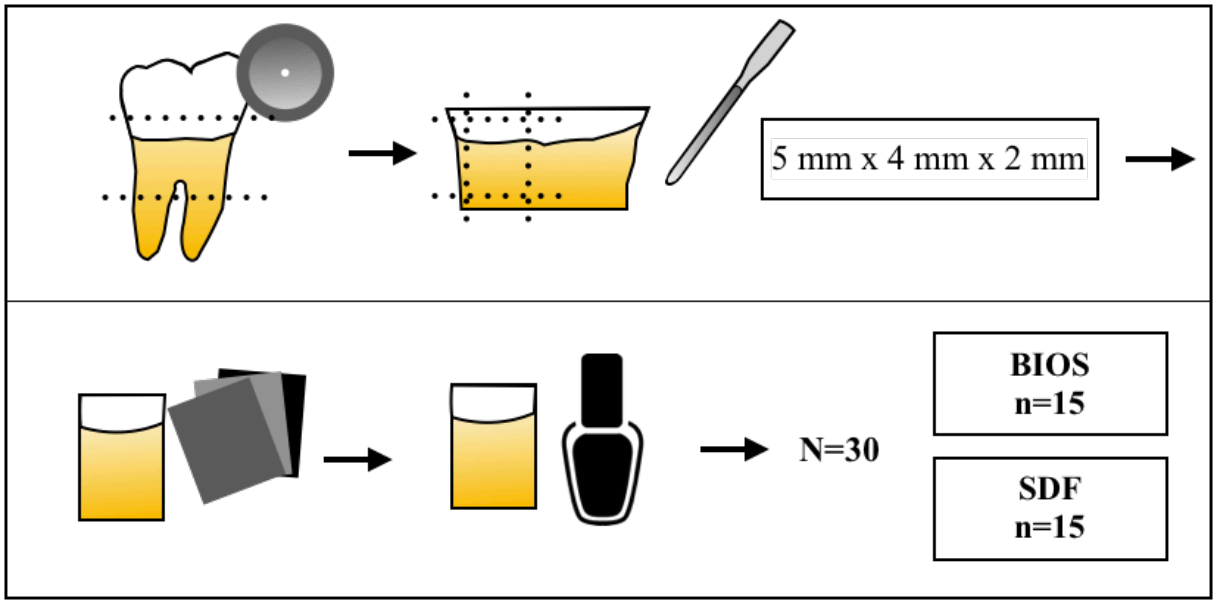
2.2 ESTUDO 2. ESTUDO DA ALTERAÇÃO DE COR APÓS APLICAÇÃO DO BIOSILICATO COMO TRATAMENTO REMINERALIZANTE DE CÁRIE RADICULAR: UM ESTUDO COMPARATIVO

Este estudo in vitro foi aprovado pelo comitê de ética em pesquisa da Universidade Federal de Pernambuco (UFPE), número do processo 4.381.523. A amostra consistiu em 10 molares permanentes obtidos no Banco de Dentes Humanos da UFPE, os quais deveriam ter a porção radicular íntegra, sem fraturas, trincas ou lesões cariosas. Os dentes foram limpos e armazenados em uma solução de Timol a 0,1% a uma temperatura de 4 ^{o}C previamente à preparação das amostras.

2.2.1 Preparo das amostras

Os dentes foram seccionados em duas partes com disco diamantado dupla face 7020 (KG Sorensen, São Paulo, Brasil) sob irrigação abundante, removendo 2/3 da parte superior, e 1/3 da parte inferior, permanecendo aproximadamente 2 mm da coroa e 3 mm da raiz. Depois deste procedimento, os dentes foram seccionados novamente, agora com a ponta diamantada 3216 (KG Sorensen, Cotia, São Paulo) em alta rotação e sob refrigeração abundante, resultando em 30 blocos (3 blocos a partir de cada dente) provenientes da face vestibular e lingual, medindo: 5 mm de altura x 4 mm de largura x 2 mm de espessura (ZHOU *et al*., 2017). As superfícies externas (vestibular ou lingual) dos blocos de dentes foram polidas com lixas d'água de granulação crescente (320, 600, 1200, 2500) com o propósito de remover a camada de cemento e expor a superfície radicular. A superfície interna dos blocos (porção da dentina voltada para a câmara pulpar) também foi lixada para sua regularização. Todas as superfícies de cada bloco, exceto a superfície externa, foram cobertas com uma fina camada de esmalte ácido-resistente incolor (Risqué, Savoy, Goiás, Brasil). Em seguida, as amostras foram distribuídas aleatoriamente em dois grupos: Biosilicato (BIOS) (n=15) e SDF (n=15). Os detalhes do preparo das amostras podem ser visualizados na Figura 3.

Figura 3 – Diagrama esquemático de preparação das amostras.



Fonte: a autora (2022)

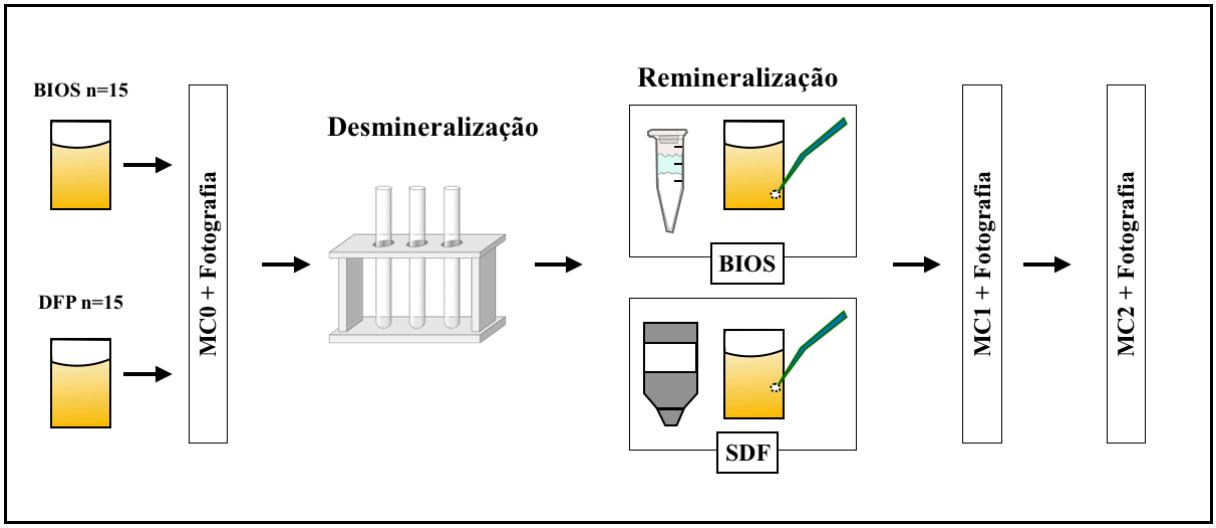
2.2.2 Fase experimental

As amostras foram submetidas ao processo de desmineralização, e subsequente remineralização, de acordo com a sua alocação nos grupos BIOS ou SDF. Foram três os períodos de mensuração de cor (MC): inicial (MC0), 24 horas após a remineralização (MC1) e 5 semanas após a remineralização (MC2) (Figura 4).

2.2.2.1 Desmineralização in vitro

Cada amostra foi individualmente imersa em 20 ml de solução ácida tamponada (50 mmol/L de ácido acético, 1,5 mmol/L de $CaCl_2$ e 0,9 mmol/L de KH_2PO_4 e pH ajustado para 5,0 com KOH) e armazenada a 37 ^oC em estufa (FANEM, São Paulo, Brazil) por 5 dias (CAI *et al.,* 2019) (Figura 4).

Figura 4 – Distribuição das amostras nos grupos e cronologia dos testes executados.



Fonte: a autora (2022)

2.2.2.2 Remineralização in vitro

Após a desmineralização, foi realizada profilaxia das amostras com pedra pomes (Maquira, Paraná, Brasil) e água destilada, com auxílio da escova de Robinson em micromotor e contra ângulo. Para o grupo SDF, foi aplicado o produto a 30% de concentração (Cariestop, Biodinâmica, Paraná, Brasil) seguindo as instruções do fabricante: O produto foi aplicado durante 3 minutos de forma vigorosa com um microbrush. Após esse período, o produto foi lavado da superfície com água destilada em seringa tríplice (D700, São Paulo, Brasil).

Para o grupo BIOS, uma suspensão de Biosilicato microparticulado (Vitrovita, São Paulo, Brasil) a 10% foi preparada imediatamente antes da sua aplicação: em um micro tubo de 1,5 ml foi adicionado 0,1 mg de Biosilicato e 1 ml de água destilada e deionizada. A suspensão foi aplicada ativamente na superfície externa da amostra com um microbrush, e permaneceu 1 minuto em contato com a superfície. Após este período, a suspensão foi gentilmente removida com papel absorvente (TIRAPELLI *et al.*, 2011).

Após a remineralização dos grupos BIOS e SDF, as amostras foram individualmente imersas em saliva artificial (1% carboximetilcelulose; 0,12% cloreto de sódio; 0,005% cloreto de cálcio; 1% metilparabeno; 97,8% água deionizada) por 24 horas até MC1 e por 5 semanas até MC2.

2.2.3 Análise das amostras

2.2.3.1 Colorimetria

A colorimetria foi realizada com um espectrofotômetro (Konica Minolta CR-400, illuminant D65, Tóquio, Japão), cujo mecanismo é baseado na emissão e reflexão de luz. A luz refletida é lida e processada de acordo com o sistema CIE L*a*b*. A definição de cor é baseada nas coordenadas cartesianas de cor: L* vai do 0 (preto) ao 100 (branco), representando a luminosidade; a* vai do -80 ao + 80, representando o matiz que varia do verde ao vermelho, respectivamente; e b*, varia do -80 ao +80, representando os matizes do azul ao amarelo, respectivamente.

As amostras foram posicionadas sobre uma placa cerâmica branca que faz parte do sistema comercial do espectrofotômetro utilizado (Konica Minolta CR-400, illuminant D65, Tóquio, Japão), e o feixe de luz do espectrofotômetro foi mantido perpendicular a esta placa sobre a superfície externa do bloco de dente. Em cada período de avaliação de cor (CM0, CM1, CM2), a medição de cor foi realizada três vezes em cada amostra e a sua média foi usada para posterior análise.

Para acomodar o aspecto psicofísico, relevante em uma diferença de cor em larga escala, a distância do espaço de cor (CD) foi realizada através da fórmula Hyab (ABASI *et al*., 2020):

$$\mathrm{CD} = \sqrt{(a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} + |L_1^* - L_2^*|$$

Foi obtida uma média do CD para cada grupo em dois períodos de tempo: de CM0 para CM1 e de CM0 para CM2. As médias do CD encontradas nesses dois períodos de tempo foram comparadas dentro do mesmo grupo (CM0-CM1 vs. CM0-CM2). Comparações intergrupo também foram realizadas, comparando os mesmos períodos de tempo entre os grupos (CM0-CM1 do BIOS vs. CM0-CM1 do SDF; CM0-CM2 do BIOS vs. CM0-CM2 do SDF).

2.2.3.2 Imagens fotográficas

O método fotográfico é uma ferramenta de diagnóstico válida (MONCADA *et al*., 2014), e foi usado neste estudo para ilustrar os achados encontrados na mensuração de cor. Cada amostra foi fotografada nos mesmos períodos em que a colorimetria (CM0, CM1 e CM2) foi realizada. Foi usada uma câmera digital T3i EOS

Rebel (Canon, Tóquio, Japão), com lente Macro 100 mm f/2.8 e Flash Twin YN24EX (Yongnuo, Shenzhen, China).

Para padronizar a aquisição de imagens, alguns parâmetros de configuração foram estabelecidos: ISO 100, abertura F22, e modo do flash em TTL com máxima potência. As amostras foram posicionadas em um fundo branco, sempre no horário de 14:00, a uma distância aproximada de 50 cm entre amostra e lente, próximo a uma janela para que as amostras fossem expostas à luz natural, e a lente estava posicionada a 90º em relação à amostra.

2.2.4 Análise estatística

A análise dos dados foi feita utilizando o programa GraphPad Prism 7.0 (GraphPad Software, Inc). para Windows (2017). As médias e desvios-padrões foram calculados para o espaço de cor (CD), e os parâmetros de cor L*, a*, b* em cada período tempo, assim como os seus deltas (ΔL, Δa, Δb), para cada grupo. O teste de Shapiro-Wilk foi usado para verificar a normalidade dos dados.

Para CD, ΔL, Δa, Δb, comparações intergrupos foram feitas utilizando o teste t e o teste de Mann-Witney. Na análise intragrupo, a significância estatística dos dados pareados foi analisada usando um teste t pareado e teste de Wilcoxon para amostras pareadas.

Para análise dos parâmetros de cor (L*, a*, b*) em cada período de tempo (MC0, MC1 e MC2), o teste t foi usado para análise inter e intragrupo. Significância estatística foi considerada quando $p < 0,05$.

3 RESULTADOS E DISCUSSÃO

Os resultados e discussão destes estudos serão apresentados a seguir por meio de 2 artigos científicos. O estudo 1 está descrito no artigo "*Optical coherence tomography in the assessment of conventional and experimental treatments for root caries*", que será submetido ao periódico *British Dental Journal* (Fator de impacto: 2.727, Qualis: B1). Nesta pesquisa, fui responsável pelo desenho de estudo, execução de todos os experimentos, análise de resultados e construção do artigo.

O estudo 2, intitulado *"Color stability after root caries treatment with a bioactive glass-ceramic: a comparative study"*, será submetido no periódico Journal of Esthetic and Restorative Dentistry (Fator de impacto: 3.63, Qualis A1). Neste artigo, eu desenvolvi o desenho do estudo, realizei o experimento desde o preparo das amostras até a avaliação de cor e fotografias, e redigi o artigo.

3.1 ARTIGO 1 - OPTICAL COHERENCE TOMOGRAPHY IN THE ASSESSMENT OF CONVENTIONAL AND EXPERIMENTAL TREATMENTS FOR ROOT CARIES

Abstract

Introduction: Root caries are extensively addressed through the application of silver diamine fluoride (SDF). However, it stains the tooth surface and reduces bond strength, limiting its application. Due to the remineralizing potential, antibacterial and mechanical properties, Biosilicate (BIOS) application could be considered as an alternative approach.

Aim: To assess the remineralizing potential of BIOS and SDF through optical coherence tomography (OCT).

Materials and Methods: 7 teeth were obtained for the production of 21 root dentin samples, randomly allocated in two groups (n=10): SDF and BIOS; one specimen was preserved as baseline. Both groups were submitted OCT at: initial stage (T0), after demineralization (T1), and after remineralization (T2). After T2, three specimens from each group and the baseline underwent to polarized light microscopy (PLM), and seven to Vickers Microhardness (VMh).

Results: OCT and VMh evidenced that BIOS and SDF samples were remineralized to their initial state. Also, a high-density layer suggestive of remineralization was observed on remineralized samples under PLM.

Conclusion: BIOS is able to fully remineralize in vitro root caries, and can be considered an alternative approach to treat root caries. Even though SDF showed greater capability to remineralize through OCT, PLM and VMh suggested similar potential for both products.

Key-points :

- Based on the results of our study, both Biosilicate and silver diamine fluoride were effective in remineralizing in vitro root caries.
- When both products were compared, silver diamine fluoride presented higher remineralization potential than Biosilicate.
- Biosilicate could be considered as an alternative for silver diamine fluoride in the treatment of root caries.

1. Introduction

Optical coherence tomography (OCT) has become an interesting approach for monitoring changes in the structure of caries lesions overtime since it is a non-invasive, non-destructive, and uses a non-ionizing short-wavelength infrared (SWIR) light.[1] This method provides high resolution cross-sectional images of biological tissues.[2] The functioning is similar to the ultrasound, but instead of high frequency sound it uses light.[3] In terms of remineralization studies, OCT has been used in enamel on carious and non-carious lesion testing a variety of experimental products including dentifrice containing sodium calcium silicate, and sugar-free gum containing bio-available calcium and fluoride.[4,5] As well, OCT studies assessed dentin remineralization after SDF application and experimental products, such as fluoride/S-PRG filler-containing gel (PRG).[6,7] It can differentiate healthy from demineralized tissue by assessing the changes in the tissue's optical properties, as for example through the measurement of the OAC.[8-10] Studies in literature proposed the OCT use for assessment of tooth remineralization,[4,6,7,11-17] confirming the validity of the method to study this subject.

Root caries is identified as a multifactorial, discolored lesion, softened, ill-defined, cavited or not, located on the root surface.[11] Root area is more susceptible to

demineralization than the enamel due to the lower mineral content in cementum.[18] Also, the increase in the elder population associated to natural tooth retention will turn root caries into a great challenge in the future decades.[11] And yet, there is no ideal product for its treatment. The regular application of silver diamine fluoride (SDF) became the preferable choice to arrest carious lesions, including on the root surface,[19-22] since it is an effective, simple, low cost, easy of application, non-invasive treatment.[23] However, it causes a dark staining on the tooth surface,[24] and diminishes dentin bond strength due to obliteration of dentin tubules.[24-27] Thus, experimental treatments have been proposed to overcome the SDF disavantages. Biosilicate, a fully crystallized glass-ceramics (Na2O-CaO-SiO2-P2O5, and addition of Li2O and K2O),[28] is a prospective candidate to fulfill this gap.

Several studies using this glass-ceramic showed good results on remineralization,[24,29,30] as it increases the bond strength in sound and demineralized dentin,[30,31] presents wide spectrum of antimicrobial activity, including anaerobic bacteria,[32] is efficient against dentin hypersensitivity,[33] presents biocompatibility,[34] and has no esthetics impairment.[24,35] However, its application on root caries has not been studied yet.

Root caries have gained evidence in the last decades and so the methodologies involved in testing the treatments for arresting such a type of lesion. OCT images allow qualitative and quantitative data to assess tooth remineralization. Quantitatively it is possible to measure, for instance, the attenuation coefficient (OAC).[8-10] The OAC is a quantitative measurement that characterizes the decay of the OCT light signal into the irradiated tissue. Thus, through OAC, it is possible to quantitatively discriminate between different tissue types and their status of health.[36] Qualitatively, the variation of the reflectivity and scattering of light allow to understand the remineralization and demineralization levels on different tooth surfaces.[37] When there is a caries arrest, the lesion is remineralized in the outer surface of the lesion and inhibits the fluids diffusion between the surface and most part of the lesion.[38] The lesion activity is perceived by the amount of water diffusion out of the lesion which changes the reflectivity and scattering.[38] Sound and demineralized dentin are expected to strong scatter SWIR light, the latter more than the first.[37] The application of SDF increases the reflectivity of dentin due to the deposition of silver metal,[37] showing that scattering patterns can vary between materials.

The aim of this study was to compare an experimental treatment for arresting root caries (Biosilicate) with SDF through OCT. The Null hypothesis is that there is no difference between BIOS and SDF regarding remineralization of in vitro root carious lesions by any methods applied.

2. Materials and methods

The present experimental in vitro study was approved by the Ethics Committee at Universidade Federal de Pernambuco (UFPE) under process number 4.381.523. The sample consisted of 7 upper and lower permanent molars obtained from the Human Teeth Bank at UFPE, whose root surface should be sound, without cracks, fracture or carious lesions. The teeth were cleaned and stored in a 0.1% Timol solution at 4 ^{o}C before sample preparation.

2.1 Sample preparation

Every tooth had 2/3 of the crown and 1/3 of the root removed with a low-speed flex 7020 diamond saw (KG Sorensen, Cotia, São Paulo) under refrigeration, remaining a portion about 2 mm of crown and 3 mm of root surface for each sample. Then the teeth were sectioned with a 3216 diamond burr (KG Sorensen, Cotia, São Paulo) in high speed under refrigeration, to obtain 21 blocks (3 blocks from each tooth) measuring 5.0 mm of length x 4.0 mm of width x 2.0 mm of thickness from the buccal and lingual surfaces of the teeth. The external surface of blocks were worn out with sandpaper with increasing grain (320, 600, 1200, 2500) aiming to remove the cement layer over the root surface, exposing the root dentin. The internal surface (the one turned to the pupal chamber) were also worn to obtain a plain surface. Two semi-round of approximately 0.5 mm depth were made on the buccal surface with a 1010 diamond bur (KG Sorensen, Cotia, São Paulo), on the top and bottom of the surface to standardize the area of OCT scanning. All surfaces, except the external one of each block, were covered with a thin layer of acid-resistant varnish (Risqué, Savoy, Goiânia, Goiás). The specimens were randomly allocated in two groups: Biosilicate (n = 10) and SDF (n = 10); one extra specimen was used as baseline for comparison with the samples submitted to polarized light microscopy (PLM). Detailed steps of the sample preparation are shown in Fig. 1.

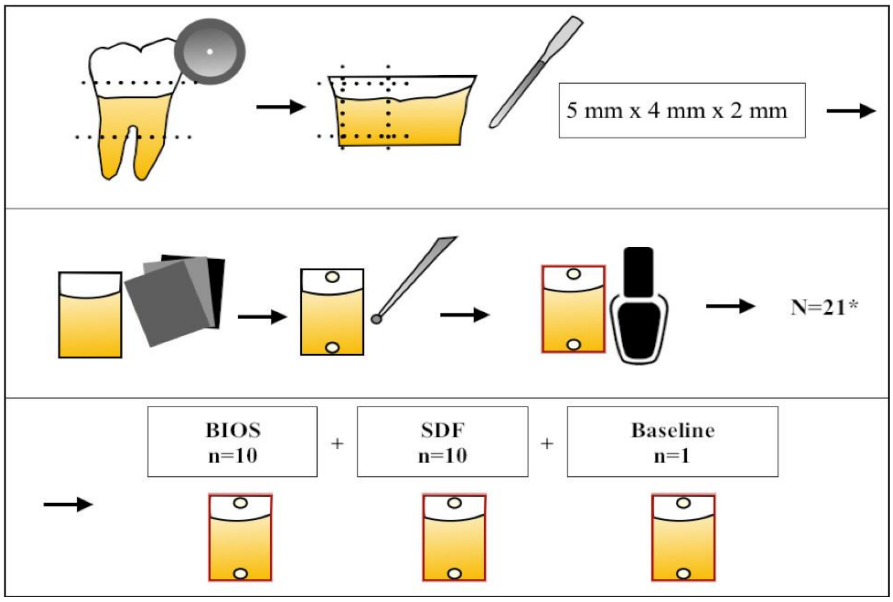


Fig. 1. Schematic diagram of sample preparation and allocation in groups.

2.2 Experimental phase

The specimens underwent a demineralization process, and then, they were remineralized according to their allocation in groups BIOS or SDF. Additional information is shown in Fig. 2.

2.2.1 In vitro demineralization

Each tooth was individually immersed in a 20 mL buffered acid solution (50 mmol/L acetic acid, 1.5 mmol/L $CaCl_2$ and 0.9 mmol/L KH_2PO_4, pH adjusted to 5.0 with KOH) and stored at 37 ^oC in a kiln (FANEM, São Paulo, Brazil) for five days.[39]

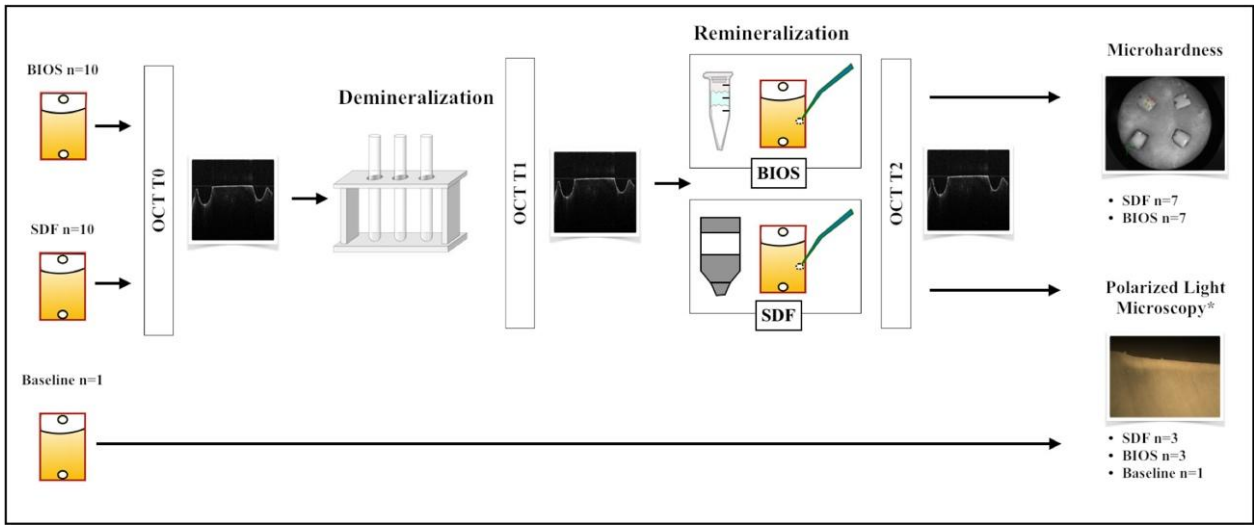


Fig. 2. Schematic diagram of sample allocation and chronological sequence of tests. *To submit the samples under Polarized Light Microscopy, they were prepared according to the description in section 2.2.3.2.

2.2.2 In vitro remineralization

The samples were cleaned with pumice stone and water previous to the remineralization, according to the experimental groups.

For SDF group, the silver diamine fluoride at 30% (Cariestop, Biodinâmica, Ibiporã, Paraná) was applied following the manufacturer's instructions: the demineralized surface, after cleaned, was dried with an absorbent paper keeping the dentin humidity. The application time should be at least one minute to be considered efficient,[40] however, as we followed fabricant's instructions, the product was applied for 3 minutes vigorously with a microbrush. After this period, the surface was washed with distilled water from the triple syringe (D700, Ribeirão Preto, São Paulo).

For BIOS group, a Biosilicate (Vitrovita, São Carlos, São Paulo) suspension at 10% was prepared with distilled and deionized water. A microtube of 1.5 mL containing 0.1 mg of Biosilicate and 1.0 mL of water were mixed immediately before its application. The 10% Biosilicate suspension was actively applied with a microbrush on the surface, remaining for 1 minute in contact with the surface. Then, it was gently removed with absorbent paper.[33]

After the application of silver diamine fluoride and Biosilicate, the samples were individually stored in artificial saliva (carboxymethylcellulose 1%; sodium chloride

0.12%; calcium chloride 0.005%; methylparaben 1%; deionized water 97,8%) for 24 hours.

2.2.3 Sample analysis

The samples were analyzed by OCT, polarized light microscopy (PLM) and Vickers microhardness (VMh) (Fig. 2). All samples were scanned by OCT in three moments: previous (T0) and after the demineralization (T1), and after the remineralization (T2). PLM and VMh were performed only after the remineralization, using 3 and 7 samples of each group, respectively. A sound tooth, with no treatment, was used as a baseline sample for PLM.

2.2.3.1 Optical Coherence Tomography

The selected OCT system for this study was the Lumedica OQ Labscope (Lumedica, Durham, North Carolina), operating in the spectral domain (SD-OCT). This SD-OCT uses a superluminescent diode operating at 840 nm central wavelength, with tissue depth resolution air/tissue of 7/5 μm, A-scan line rate of 13.000 lines/second, B-scan image rate of 20 lines/second, transverse resolution of 15 μm and 5 mm x 5 mm scanning range. The samples were fixed in wax with the superficial surface positioned perpendicular to the scanning piece. The upper and lower marks done on the specimens guided the OCT scanning. The generated images were saved in JPEG format.

After OCT scanning, the images were processed through MatLab to quantify the OAC. Before image assessments, we set the width of the region of interest (ROI) that should be observed in every image. The position of the ROI would vary between images in order to escape from the white spots formed in the image, which could lead to a biased result. Also OCT images were submitted to visual and qualitative analysis of their reflectivity patterns.

2.2.3.2 Polarized light microscopy

The extra sample for baseline and three remineralized ones from each experimental group were cut in slices of 1 mm with precision diamond saw (IsoMet, Buehler, Lake Bluff, Illinois). Each slice was worn out with sandpaper with increasing grain (320, 600, 1200, 2500), in order to obtain around 80 μm thickness. Then the

sample slices were analyzed by a polarized light microscope (Olympus BX51, Tokyo, Kanto) with 20x magnification, and the images were saved in TIFF format. All sections underwent qualitative analysis.

2.2.3.3 Surface Microhardness

The selected remineralized samples of each group were included in self-curing acrylic resin (VIPI Flash, Pirassununga, São Paulo) and submitted to Vickers microhardness tester (DuraScan, EMCO-TEST, Kuchl, Salzburg) with a load of 100 g for 15 s at 50x magnification. Five random points on each sample of root dentin were indented. The mean surface microhardness in five points of samples was recorded as Vickers hardness number (VHN).

2.3 Statistical analysis

Statistical analysis was performed using Prism 7 (GraphPad Software, Inc). The statistical significance of all tests was considered as $p<0.05$.

For OCT imagens, descriptive data (mean and standard deviation) of the OAC were calculated for each group. Shapiro-Wilk normality test indicated a non-normal distribution. A non-parametric Friedman test of differences among repeated measures followed by Dunn's multiple comparisons test determined the differences intra-group between T0, T1 and T2 according to the remineralizing materials studied (SDF 30% and Biosilicate). Mann-Whitney U test was performed to determine whether there is a difference in of OAC between materials.

For VMh data, the unpaired t test was used to evaluate the significance of the difference in microhardness between SDF 30% and Biosilicate.

3. Results

3.1. Optical Coherence tomography

Fig. 3 shows summary statistics for the OAC in each group tested. Closer inspection reveals higher mean values of OAC in T2 in both materials measured.

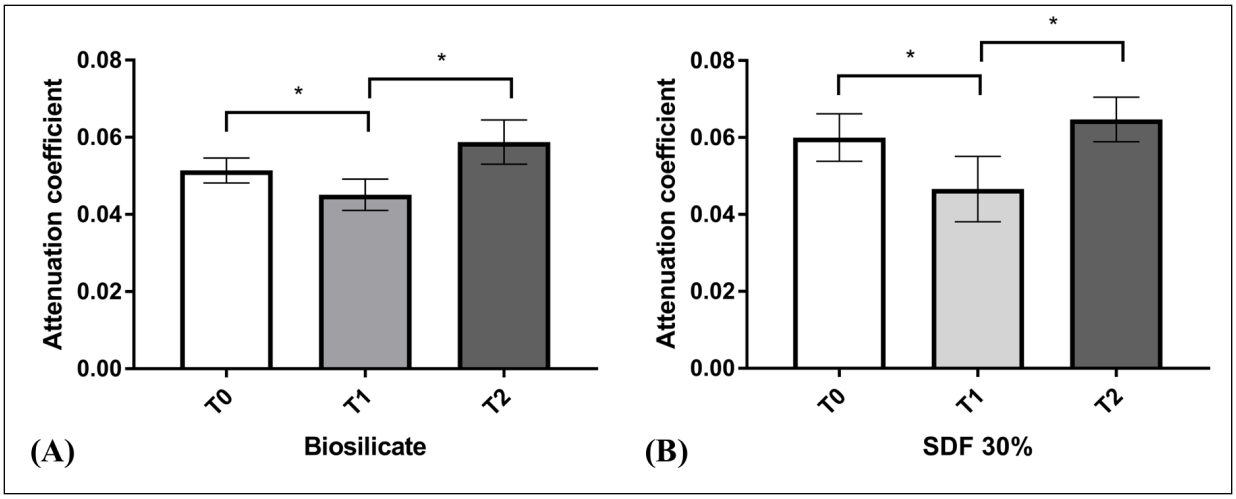


Fig. 3. (A) Analysis of OAC measurement obtained from OCT images of Biosilicate at T0, T1 and T2. Bars show mean and ± OAC. * indicates a difference for which p = 0.0417 between T0 and T1 and p < 0.0001 between T1 and T2. Between T0 and T2 there was no statistical difference (p= 0.2209). (B) Analysis of OAC measurement obtained from OCT images of SDF 30% at T0, T1 and T2. Bars show mean and ± OAC. * indicates a difference for which p = 0.0219 between T0 and T1, and p = 0.0002 between T1 and T2. Between T0 and T2 there was no statistical difference (p = 0.5391).

There was a significant difference in mean values between T0 vs. T1 and T1 vs. T2 for BIOS and SDF groups (Fig. 3). These results indicate the remineralizing potential of BIOS and SDF in caries treatment in the OCT analysis. Also, multiple comparisons test did not reveal significant differences between the T0 and T2, revealing the OAC similarity and the accomplishment of remineralization in T2 for both materials.

When comparing the mean values of OAC between groups, Mann Whitney analysis identifies significant differences between BIOS and SDF before (T0, p=0.0029) and after remineralization treatment (T2, p= 0.043). More details about the Mann-Whitney analysis can be seen in Fig. 4.

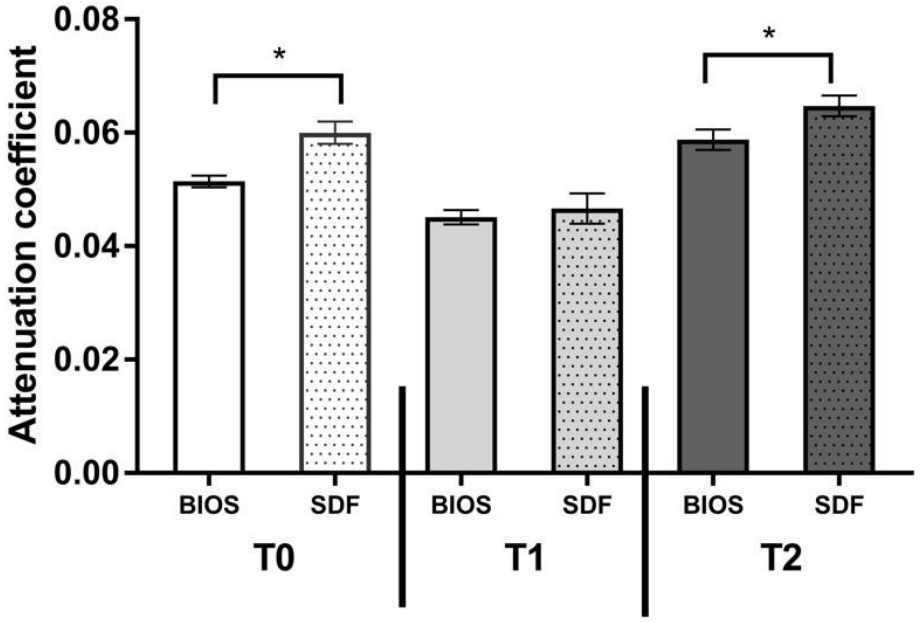


Fig. 4. Comparison of mean values and ± OAC between Biosilicate and SDF 30% in T0, T1 and T2. * indicates a difference for which p = 0.0029 between BIOS and SDF at T0, and p = 0.0433 between BIOS and SDF at T2. At T1 we found no significant difference between groups.

Fig. 5A and Fig. 5D show initial stage (T0) of selected samples of Biosilicate and SDF 30% respectively. The sound enamel in both images showed a high reflectivity of the outer surface that diminishes on the internal enamel layer. However sound dentin has a different optical behavior by increasing the reflectivity from the deeper layers of dentin. In Figures 5B and 5E it is possible to see a discontinued degree between root dentin and enamel characterizing the effect of the demineralization process (T1). The demineralized enamel showed higher reflectivity in the outer surface than the sound enamel. And for the demineralized dentin the reflectivity was found as slightly higher than the sound dentin. And finally, in T2 different behavior between materials are seen, in which SDF has a high reflectivity in almost complete surface involving root dentin and enamel. On the other hand, Biosilicate sample showed a higher reflectivity only at the root dentin on the semi-round mark, meaning residual Biosilicate particles. Besides this specific location, most of the surface presented only a gentle higher reflectivity than sound and demineralized

dentin, with lower intensity than SDF sample. When comparing the light depth penetration, it seems that SDF achieved deeper layers of dentin than Biosilicate.

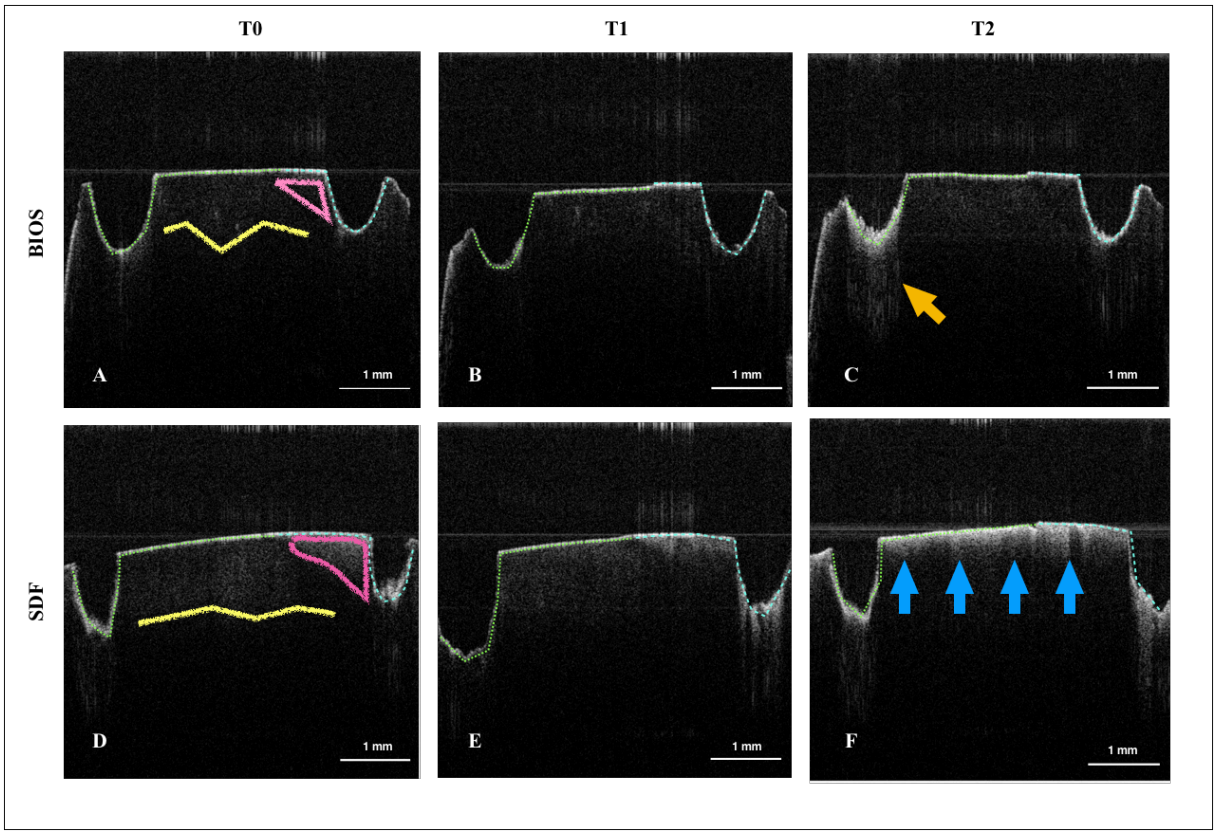


Fig. 5. Representative OCT images from BIOS and SDF groups, at T0, T1 and T2. The green dots are representing the root dentin area, and the blue dashes for the enamel. The (A) and (D) images are representing the moment of no treatment, in which the yellow line indicate the reflex present in dentin, and the pink triangle indicates the enamel with the reflex present more on the surface. The (B) and (E) images indicate the moment after demineralization. The (C) and (F) are representative of the remineralized samples; the orange arrow indicates the biosilicate particles that remained in the cavity, however the area of cavities was not used for assessment; the blue arrows indicate the greater reflex produced by the silver particles deposition.

3.2 Polarized light microscopy

Visual and qualitative analysis showed differences between the images obtained in the baseline, Biosilicate and SDF samples (Fig. 6). Biosilicate showed a cleared range close to the root surface (Fig. 6B). On the other hand, on SDF samples,

a brownish stain discolored the whole extension of the sample, and near to the surface a most strong stain suggestive of remineralization (Fig. 6C).

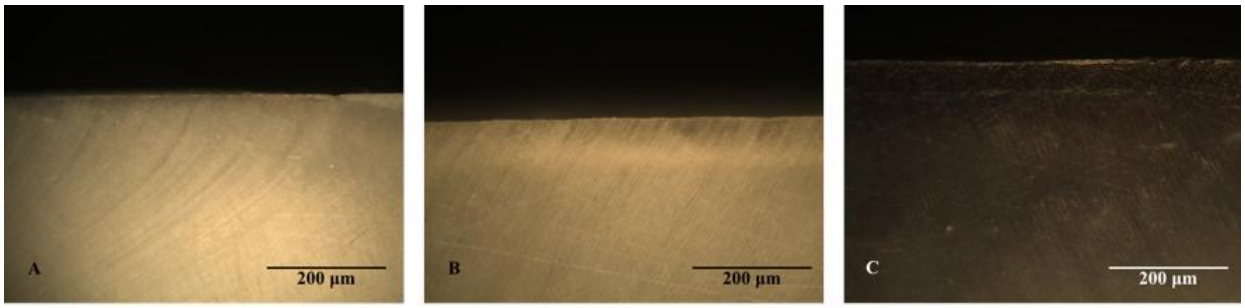


Fig. 6. Dark field reflected PLM images of root dentin. (A) baseline sample; (B) After Biosilicate application, an opaquer and clearer range near to the root surface is suggestive of remineralization; (C) After SDF application, the image shows the stain produced by the silver present in its composition. A darker range is seen closer to the surface, suggestive of remineralization.

3.3 Vickers Microhardness

There was no statistical difference (p = 0.9543) of VMh between the samples treated with BIOS and SDF (Fig. 7).

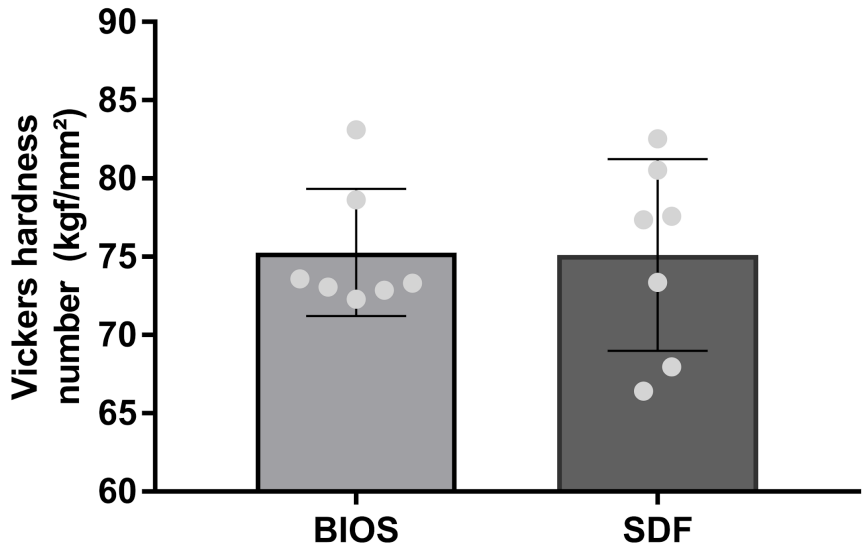


Fig. 7. Vickers hardness number of selected samples after remineralization with Biosilicate and SDF 30%. Means and standard deviation of each group is represented by the bars.

4. Discussion

In the attempt to find an alternative for SDF in the treatment for root caries lesion, a new strategy was proposed by the application of a remineralizing bioactive glass-ceramic, called Biosilicate. Based on the results found in our study, the null hypothesis was partially accepted. To the best of our knowledge, this is the first time that Biosilicate has been investigated for root caries treatment in comparison to SDF, currently used to treat and prevent root caries,[19] as well as it is the first time that Biosilicate has been assessed through PLM, enabling the better understanding of its optical behavior related to remineralization.

Active root caries is rapidly developed leaving unsupported enamel near to the cement-enamel junction.[21] Thus, the first clinical management to control the progression of root caries is to educate and assist the patient to achieve a better oral hygiene through the correct brushing associated with a fluoridated dentifrice. However, this management is not always enough, especially in patients that have limited motor skills.[21] Therefore, different approaches were suggested, such as the use of clorexidine-containing products, which was considered inconclusive.[41] In this way, SDF has shown beneficial effects in the treatment of root caries lesion.[21,22]

Different SDF concentrations are available (10%, 12%, 30%, 38%) to arrest caries non-invasively.[21,22] In Brazil, the maximum concentration of silver compound allowed by the regulatory agency is 30%. Nevertheless, lower concentrations of SDF (30% and 12%) have been considered as efficient as 38% in remineralization.[24,40] To reach this efficiency at the concentration of 30% the application time is at least one minute,[40] as occurred in the present study. The specific composition of 30% SDF (Cariestop, Biodinâmica, Ibiporã, Paraná) is fluoridic acid (35,400 ppm), silver nitrate, ammonia hydroxide and deionized water. It has been understood that the fluoride present in the SDF products is responsible for increasing the tooth resistance against the acid produced by bacterial carbohydrate fermentation.[42]

Biosilicate was produced in accordance with the process described in the patent deposit.[29] The concentration of 10% Biosilicate suspension was chosen based on

previously reported studies with successful outcomes in dentin, concerning adhesion,[30,31] dentin hypersensitivity,[33] and more recently microhardness.[24] It is expected that hydroxycarbonate apatite (HCA) formation occurs 24 hours after contact of the bioactive glass with a body fluid,[28] which determined the time of assessment after Biosilicate application on the root dentin surface.

OCT is a non-invasive, non-ionizing, non-destructive and real time imaging technique.[11] This high-resolution imaging method has been used to several applications in dentistry, such as the early caries detection as well as monitoring its progression.[25] The OCT technique has already been used in laboratory studies and in a clinical setting.[4,12] Therefore, it allows the comparison between data of clinical and in vitro studies, which may clear strengths, weakness, or gaps of the latter.

The remineralization potential is presented through OAC results (Fig. 3). For both materials, there was no significant difference when comparing T0 with T2, which allows us to suggest that those samples were fully remineralized to their initial stage. The significant difference between T0 vs. T1 and T1 vs. T2 for both materials, suggests that the demineralization process was efficient and emphasizes that the remineralization results of the present study are strong. Additionally, the decrease seen in OAC during demineralization is in agreement with other studies, showing that the demineralization promoted by acids create empty spaces in dentin, increasing the number of interfaces, and thus increases the scattering of light, decreasing the OAC.[8-10]

In this study, Biosilicate and SDF remineralization was also compared (Fig. 4). In T0 there was a significant difference between groups, due to the inherent higher minerals content in SDF samples, even though the samples were distributed randomly. The mineralization content of healthy teeth can vary,[41] and thus, produce this difference from sample to sample. In T1, we can see that samples were homogenized, presenting no significant difference between them. T2 shows a significant difference between groups, where SDF had a higher remineralization capacity, however descriptively they were very close to each other (0.05754 for BIOS group and 0.06391 for SDF).

OCT images of sound enamel and dentin shows different optical behaviors due to their composition. The aspects of sound and demineralized enamel and root dentin found in our study is in accordance with a previous study.[25] The marked differences between sound enamel and dentin are justified by the dentin tubules which strong scatter the SWIR light.[25] Also, this previous report was the first to submit SDF under OCT and assessed its optical properties. Our SDF sample (Fig. 5) shows the same pattern found in this study which is explained by the deposition of silver on dentin causing the increase of reflectivity.[25] Biosilicate showed lower reflectivity intensity when compared to SDF. This substantial difference must be attributed to the optical characteristics of the silver particles, as metal reflects more than crystalized glass-ceramic particles and it cannot be directly related to the remineralization potential. However, the reflectivity of metal particles helps to the visualize the depth penetration of SDF. The higher depth penetration of SDF demonstrates the ability of silver to penetrate the dentin, which is known to occlude the dentinal tubules and generates an insoluble layer,[26] interfering on the ability of the bonding agent to impregnate the peritubular and intratubular dentin and the infiltration within the collagen matrix,[27] thus the hybrid layer is not formed.[24]

When submitting the selected samples to PLM it was possible to visually compare the initial stage (T0) with after remineralization (T2). The sample treated with SDF (Fig. 6C), in which a high dense layer suggestive of remineralization appears near to the surface is in accordance with initial studies describing that the SDF application forms a highly mineralized layer on the lesion surface, resulting from the reaction of its components, silver and fluoride, with the hydroxyapatite.[43] The stain seen (Fig. 6C) is indicative of silver precipitation. As seen in the OCT image (Fig. 5F), through the intense reflectivity, the stain extended in depth can be seen in PLM image as well (Fig. 6C). The PLM image of BIOS sample showed a clear range near to the surface, suggestive of remineralization (Figure 6B). For both materials, this remineralization zone appears to be integrated to the root dentin, and not simply above the surface, confirming the results of the microhardness test, that pointed to the increase in this mechanical property.

According to a mapping study of the root dentin, Vickers Hardness of the cervical root dentin is about 63.5 ± 19.13,[44] which is close, but not as high as the results

found in our groups after remineralization (BIOS group = 75.27 ± 4.064, and SDF group = 75.11 ± 6.131). Also, VMh results showed no significant difference between BIOS and SDF and confirm that BIOS remineralization has a comparable remineralization potential. This is in agreement with a previous study which compared Biosilicate (10% suspension) with SDF (38%) and found no difference in microhardness,[24] even though the concentration of SDF was higher than the one used in this study.

This study has inherent limitations as it was conducted in vitro, therefore is necessary to test this hypothesis in vivo and clinically to extrapolate this findings to the clinical practice. There are still some questions that need to be answered before establishing Biosilicate as the gold standard for treating root caries: the protocol of application for prevention and treatment, including how many times in a year it should be applied, and test different concentrations and particles sizes. Beyond treatment application, Biosilicate should be investigated for non-carious lesion on root dentin as well. Therefore, other laboratory studies are urged to investigate these matters and then, clinical studies should be carried out to input a safe and efficient protocol of application in the treatment of root caries.

4. Conclusions

The application of 10% Biosilicate in root caries presented an efficient remineralizing potential comparable to SDF 30% and could be considered for root caries treatment instead of SDF 30%.

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Declaration of Interests

The authors declare no conflict of interest in this work.

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3.2 ARTIGO 2 - COLOR STABILITY AFTER ROOT CARIES TREATMENT WITH A BIOACTIVE GLASS-CERAMIC: A COMPARATIVE STUDY

Abstract

Objective:

Silver diamine fluoride (SDF) is considered the preferable approach for arresting dental caries, however, it stains the remineralized surface. In order to find an esthetical approach, Biosilicate was tested for root caries treatment regarding color stability in comparison to SDF. **Materials and methods**: Thirty blocks of root dentin were produced and randomly divided in two groups: SDF and Biosilicate. We registered the color with a spectrophotometer and performed digital high-quality photographic images in three moments: baseline (CM0), 24h (CM1) and 5 weeks (CM5) after

remineralization. Color difference (CD), and L*a*b* parameters were calculated and data were statistically analyzed ($p<0.05$). **Results:** For CD and ΔL we found significant difference in the inter and intragroup analysis for both groups. Regarding Δa, significant difference between groups was found as well in the intragroup analysis for Biosilicate. Δb presented significant difference only between groups. L*a*b* were significant for SDF at CM1 and CM2. For Biosilicate, L* and a* presented significant differences at CM2. **Conclusions:** Biosilicate produced lower color change compared to SDF.

Clinical relevance: Biosilicate, a remineralizing bioactive glass-ceramics, showed that it can be an esthetical option to replace silver diamine fluoride in the treatment of root dentin caries.

Keywords: Root caries; Biosilicate; Silver Diamine Fluoride; Tooth Remineralization; Color; Tooth Discoloration.

1. Introduction

In general, management of carious lesions has changed over the years. The contemporary philosophy has moved from the complete excision of the diseased tissues and posterior restoration, to minimally invasive dentistry (MID) that relies on maximum tissue preservation.[1] Besides removal of the infected dentin and subsequent restoration, the carious disease must be treated by the local use of products that have antimicrobial and remineralizing properties.[2]

Many agents have been used to prevent and arrest root carious lesions, such as sodium fluoride, chlorhexidine, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and silver diamine fluoride (SDF) products.[3] SDF have been exploited in the treatment of root caries,[4-7] due to its success in decreasing the progression and initiation of root caries,[8,9] simple technique and low-cost.[10] Nevertheless, the application on the tooth surface causes a discoloration of the tooth surface,[11,12] and impairment of bond strength.[13] SDF products are composed mainly by silver ions, ammonia, and fluoride.[5,14] Fluoride produces remineralization[15] and silver ions are responsible for the antimicrobial property of this product which is fundamental to caries arrest,[16] however its deposition on dentinal tubules is the reason of tooth staining.[17]

Biosilicate, a fully crystallized glass-ceramics of the Na2OCaO-SiO2-P2O5 system, with additions of Li2O and K2O was developed.[18] It has a high bioactivity index, similar to Bioglass 45S5, and the best mechanical performance between the commercially available glass-ceramics with a bending strength of 210 MPa, very close to 215 MPa of A/W glass-ceramic.[19,20] This material has fundamental properties to produce caries arrest, such as: remineralization capability,[21-23] antimicrobial properties,[24] and increases the bond strength.[25-28] Also, the protocol of application is simple, and can be used as a non-invasive treatment.[29] However, only few previous reports investigated color change after the use of Biosilicate particles and they did it under restorative materials, therefore the direct impact on dental esthetics remains obscure.[21,30]

In order to measure precise color change, the spectrophotometer is a reliable method of assessment.[31] The equipment reads color, and quantify three parameters: L* (luminosity), a* (green-red axis) and b* (blue-yellow axis), in which L* and b* are the parameters mostly associated to a whitened aspect.[32] From color measurement, we can calculate the overall color change by a diverse options of mathematical formulas, such as CIEL*a*b*, CIEDE2000 and Hyab.[33] Nevertheless, in the presence of a great color change, which occurs after application of SDF products, the Hyab formula is the suitable choice.[33] The photographic method is a valid diagnostic tool,[34] and associated to the spectrophotometry will allow us to compare the results found by both methods.[35,36]

Therefore, our study aims to apply Biosilicate directly on the root surface, and investigate if there is any color change over the time through the use of a spectrophotometer associated to the photographic method. The null hypotheses tested were that (1) there is no difference between groups on color change at the same time periods; (2) there is no difference on color change along the time periods in each group.

2. Materials and methods

This in vitro study was approved by the Ethics Committee at Universidade Federal de Pernambuco (UFPE) under process number 4.381.523. The sample consisted of 10 upper and lower permanent molars obtained from the Human Teeth Bank at UFPE, whose root surface should be sound, without cracks, fracture or carious

lesions. The teeth were cleaned and stored in a 0.1% Timol solution at 4 oC before sample preparation.

2.1 Sample preparation

Every tooth had the upper portion of the crown and the approximately 1/3 of the roots removed with a low-speed flex 7020 diamond saw (KG Sorensen, Cotia, São Paulo) under refrigeration, remaining a portion of about 2-mm of crown and 3-mm of root surface for each sample. Then the teeth were sectioned with a 3216 diamond burr (KG Sorensen, São Paulo, Brazil) in high speed under refrigeration, to obtain blocks measuring 5.0 mm of length x 4.0 mm of width x 2.0 mm of thickness from the buccal and lingual surfaces of the teeth. The external surfaces of blocks were worn out with sandpaper with increasing grain (320, 600, 1200, 2500) aiming to remove the cement layer over the root surface, exposing the root dentin. All surfaces, except the external one, were covered with a thin layer of acid-resistant varnish (Risqué, Savoy, Goiás, Brazil). The specimens were randomly allocated in two groups: BIOS (n = 15) and SDF (n = 15). Detailed steps of the sample preparation are shown in Figure 1.

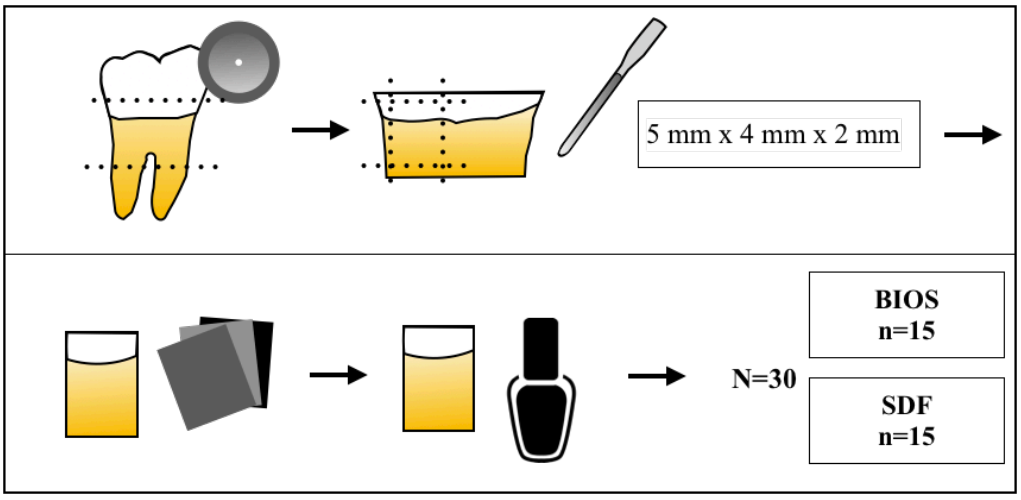

Figure 1. Schematic diagram of sample preparation and allocation in groups.

2.2 Experimental phase

The specimens underwent a demineralization process, and then, they were remineralized according to their allocation in groups BIOS or SDF. The color measurements (CM) and photographs were at initial state (CM0), after 24 h (CM1) and after 5 weeks (CM2) (Fig. 2).

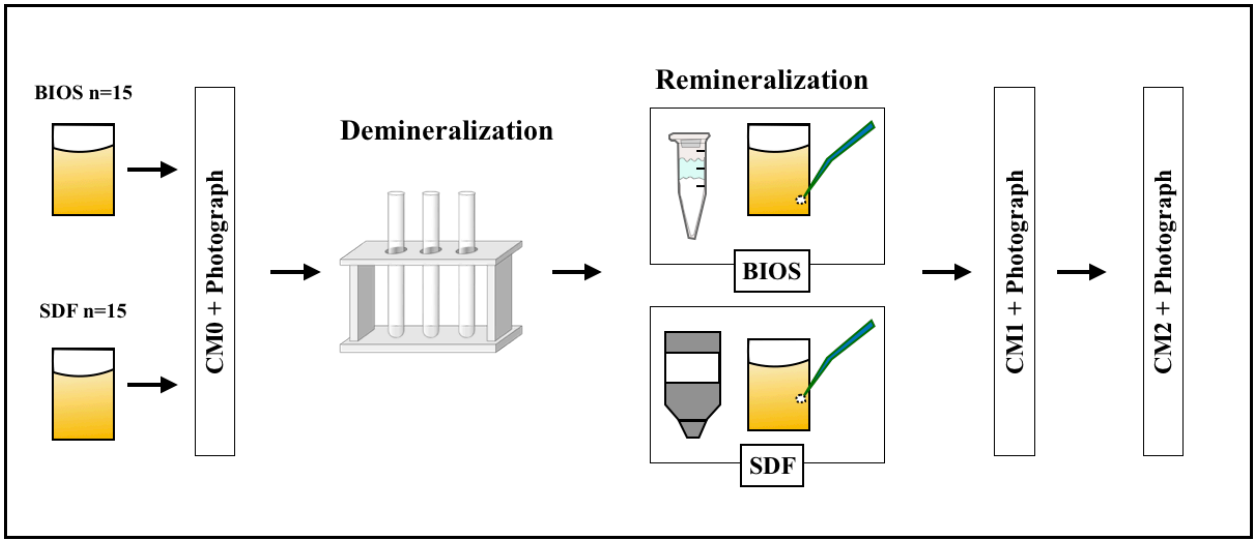

Figure 2. Schematic diagram of sample allocation and chronological sequence of tests.

2.2.1 In vitro demineralization

Each specimen was individually immersed in a 20 mL buffered acid solution (50 mmol/L acetic acid, 1.5 mmol/L CaCl2 and 0.9 mmol/L KH2PO4, pH adjusted to 5.0 with KOH) and stored at 37°C in a kiln (FANEM, São Paulo, Brazil) for five days.[37]

2.2.2 In vitro remineralization

Previous to remineralization, the samples were cleaned with pumice stone and water. For SDF group, the silver diamine fluoride at 30% (Cariestop, Biodinâmica, Ibiporã, Paraná) was applied following the manufacturer's instructions: the demineralized surface was dried with an absorbent paper keeping the dentin humidity, then the product was applied for 3 minutes vigorously with a microbrush. After this period, the surface was washed with distilled water from the triple syringe (D700, Ribeirão Preto, São Paulo).

For BIOS group, a Biosilicate (Vitrovita, São Carlos, São Paulo) suspension at 10% was prepared with distilled and deionized water. A microtube of 1.5 mL containing 0.1 mg of Biosilicate and 1.0 mL of water were mixed immediately before its application. The 10% Biosilicate suspension was actively applied with a microbrush on the dentin surface, remaining for 1 minute in contact with the dentin surface. Then, it was gently removed with absorbent paper.[38]

After the application of silver diamine fluoride and Biosilicate, the samples were individually stored in artificial saliva (carboxymethylcellulose 1%; sodium chloride 0.12%; calcium chloride 0.005%; methylparaben 1%; deionized water 97,8%) for 24 hours until the CM1, and stored for more 5 weeks until CM2.

2.3 Sample analysis

The samples were analyzed by color measurements (CM) with a commercial system (Konica Minolta CR-400, illuminant D65, Tokyo, Japan) and visually by photographic images of the samples at three different moments: initial state (CM0), 24h after remineralization (CM1), 5 weeks after remineralization (CM2).

2.3.1 Colorimetry

Colorimetry was performed with a spectrophotometer (Konica Minolta CR-400, illuminant D65, Tokyo, Japan) which the mechanism is based on light emission and reflection. The reflected light is read and processed in accordance to the CIE L*a*b* system. The definition of color is based on cartesian color coordinates, which are: L* axis from 0 (black) to 100 (white) representing the lightness, the hue axis that varies from -80 to +80 represented by a* (green-red axis) and b* (blue-yellow axis).

The samples were positioned over the white ceramic calibration plate to the color measurement. The spectrophotometer's tip was held perpendicular to the external tooth surface. The measurement in each sample was performed three times, for reliability, and the average of them was used for further analysis.

The color differences were then analyzed for both groups from CM0 to CM1 and from CM0 to CM2. With this averages we did an intragroup analysis between time periods. Also, we did an intergroup analysis at the same time periods (CM0-CM1 or CM0-CM2). To accommodate the psychophysical aspect, relevant in large color-difference, the distance in the color space (CD) were presented in HyAB,[33]

$$\mathrm{CD} = \sqrt{(a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} + |L_1^* - L_2^*|$$

2.3.2 Photographic images

To observe color change, each sample was photographed at the same time intervals of color measurements using an EOS Rebel T3i digital camera (Canon, Tokyo, Japan), EF 100-mm f/2.8 Macro USM Lens (Canon) and YN24EX Twin Macro

Flash (Yongnuo, Shenzhen, China). To standardize the image acquisition some parameters were defined: ISO 100, F22 aperture, and TTL flash mode with maximum power. The samples were positioned in a white background at 14:00, at a distance of 50 cm between the lens and samples, near to a window so the samples could be exposed to natural light, then photographs were taken.

2.4 Statistical analysis

Data management and analysis were performed using GraphPad Prism 7.04 for Windows (2017). Means and standard deviations for Color difference, L*a*b* parameters and their deltas were calculated for each group. Shapiro-Wilk test was used to verify the normality of the data. For the Color difference (CD) and ΔL, Δa, Δb, comparisons between the two groups were made using t test and Mann-Whitney test. For the intragroup analysis paired data statistical significance was analyzed using Paired t test and Wilcoxon matched-pairs signed rank test. For the color parameters (L*a*b*) in each time period (CM0, CM1 and CM2) t test was used in the inter and extra group analysis. The statistical significance was considered as $p < 0.05$.

3. Results

3.1 Color difference (CD):

The average color changes, CM0 - CM1 were $CD_{SDF} = 34 \pm 2$ and $CD_{BIOS} = 4.2 \pm 0.9$. Meanwhile, BIOS falls under the regimen of color change noticeable only under close inspection. At CM2 the color difference with respect to the initial color were respectively $CD_{SDF} = 40 \pm 3$ and $CD_{BIOS} = 6.2 \pm 0.8$. The broader color variation within samples in the SDF can also be noted by the higher standard error of the mean. Statistical analysis showed significant differences in the intergroup (comparison of CD ate the same time periods) and intragroup analysis (comparison of time periods of each group) (Figure 3).

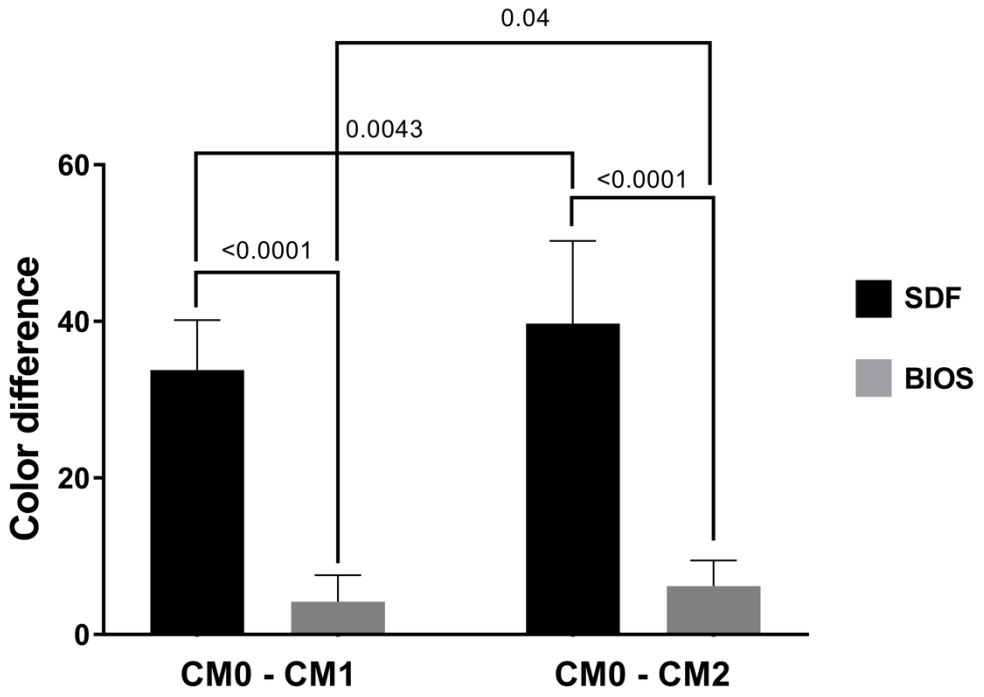

Figure 3. Intergroup and intragroup analysis of CD at CM0-CM1 and CM0-CM2.

3.2 Color parameters L*a*b*

With the purpose to observe the color shift, in this study we statistically analyzed ΔL, Δa and Δb in both time periods: CM0 to CM1 and CM0 to CM2 comparing intra and intergroup differences. For both groups, we observed between both time periods a ΔL direction to negative values, which leads to a darker tone. Differences in ΔL were found in the intragroup analysis between both time periods for SDF (p=0,0022) and BIOS (p<0,0001) group. Comparing SDF and BIOS at the same time period, we observed differences in both CM0-CM1 (p <0,0001) and CM0-CM2 (p <0,0001). Therefore, SDF group presented in a short period a considerably higher reduction in lightness when compared to BIOS. BIOS group also had a reduction in lightness, however, much more discrete (Figure 4).

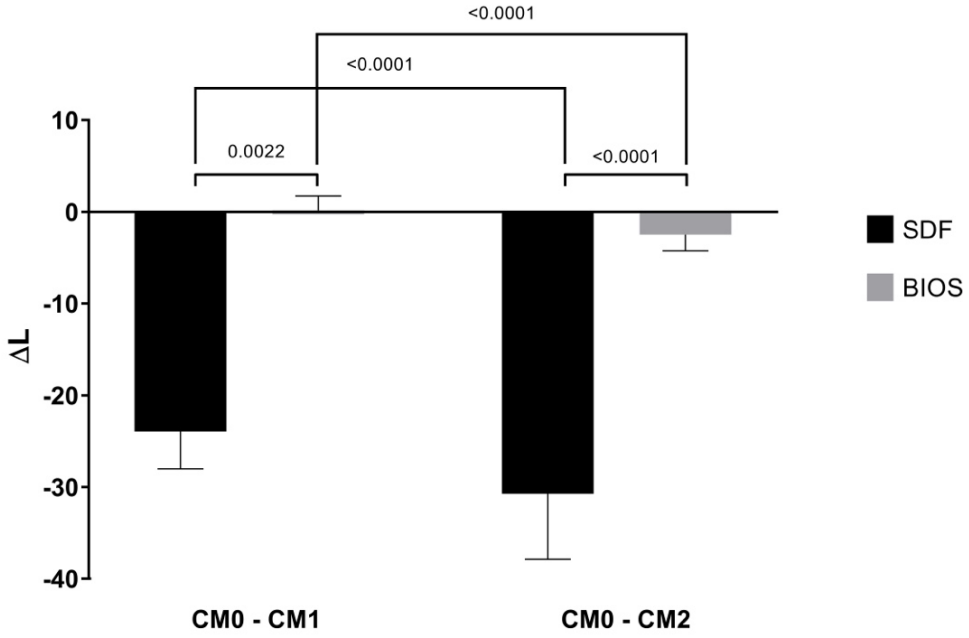

Figure 4. Intergroup and intragroup analysis of ΔL at CM0-CM1 and CM0-CM2.

Looking at the L parameter more closely we performed a T-test to analyze intergroup differences at the same time periods, and intragroup differences between time periods, comparing CM0 with CM1 and CM2 (Table 1). An abrupt decrease in the L* parameter is seen with significant differences between CM0 with CM1 ($p = 1.9 \times 10^{-17}$) and CM2 (p = 2.1×10^{-14}) for SDF. BIOS presented a minor decrease in lightness from CM0 to CM1 with no significant difference (p = 7.4×10^{-1}) but from CM0 to CM2 with significant difference (p = 2.5×10^{-4}). In the intergroup analysis no significant differences were found at CM0 (p= 9.0×10^{-1}), which means that the initial color of samples were homogenic between groups. After remineralization, differences at CM1 (p = 1.1×10^{-17}) and CM2 (p = 1.9×10^{-13}) confirms the much higher darker tone of SDF group.

Table 1: Mean, standard deviation (±) of L* a* and b* for SDF and BIOS groups.

	L*		a*		b*	
	SDF	BIOS	SDF	BIOS	SDF	BIOS
CM0	89.24 ± 0.41a	89.17 ± 0.36a	-0.54 ± 0.27a	-0.16 ± 0.36a	14.68 ± 0.74a	15.41 ± 0.79a
CM1	65.09 ± 0.21*a	89.33 ± 0.34b	2.64 ± 0.30*a	-0.58 ± 0.18b	6.17 ± 0.68*a	13.79 ± 0.79b
CM2	59.33 ± 2.05*a	86.78 ± 0.44*b	2.48 ±0.37*a	0.28 ± 0.13*b	5.94 ± 0.60*a	13.86 ± 0.44b

Asterisk indicate significant difference (p<0.05) between time periods (CM0 with CM1 or CM0 with CM2) in intragroup analysis (columns); different lower case letters indicate significant differences (p<0.05) between groups at the same time periods in extra group analysis (lines).

Regarding Δa analysis, in the intragroup comparison, no differences were found in the SDF group, however in Biosilicate there was a positive shift which lead to significant difference between time periods. In the intergroup analysis, we found significant differences between groups at both time periods. Figure 5 shows intra and intergroup analysis.

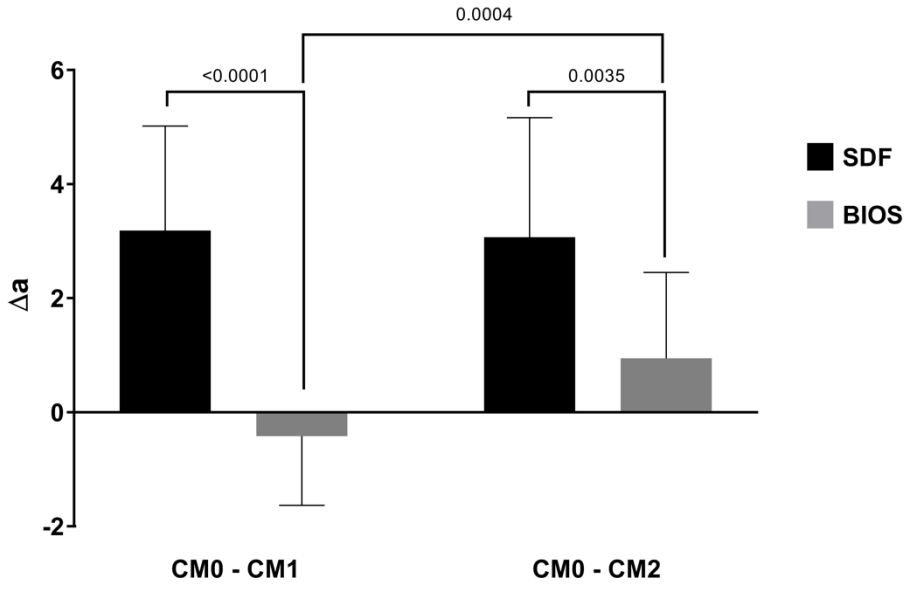

Figure 5. Intergroup and intragroup analysis of Δa at CM0-CM1 and CM0-CM2.

The a* parameter of SDF group had an increase in the value of the a* coordinate with significant differences when comparing CM0 with CM1 (p<0.0001) and CM2 (p<0.0001). BIOS group also presented a color variation in this aspect. From CM0 to CM1 there was a slight decrease with no significant difference (p = 0.96). From CM0 to CM2 the color shifted in the opposite direction, increasing the value of a* coordinate,

with significant difference (p = 0.0065). When both groups are compared, initially, at CM0, there is no significant difference between them (p=0,95), but after treatment, there are significant differences at CM1 (p<0.0001) and CM2 (p<0.0001), in which SDF has an increased a* coordinate (Table 1).

Δb results showed no significant difference in the intragroup analysis for both groups. In the intergroup analysis differences were found for both time periods (Figure 6).

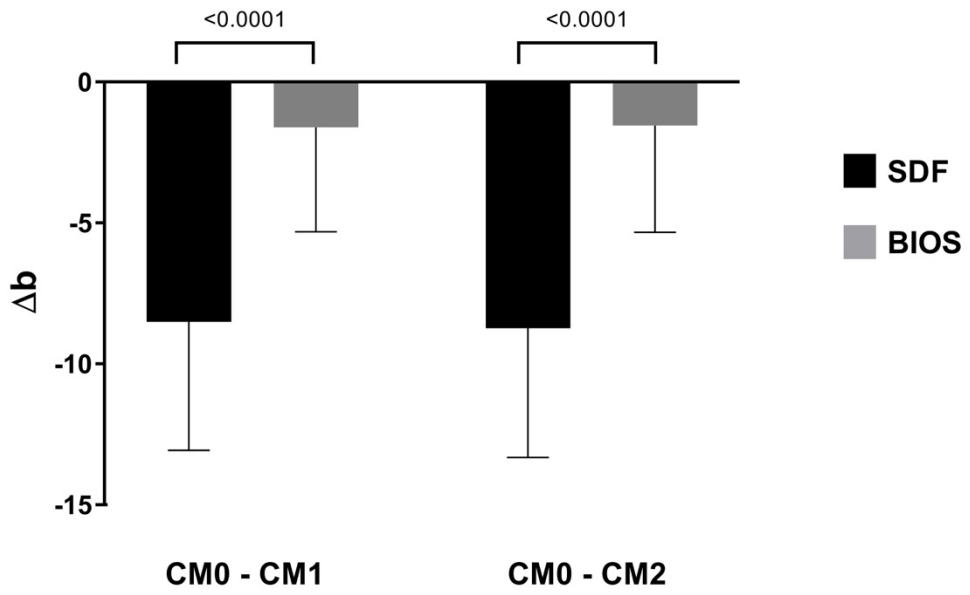

Figure 6. Intergroup and intragroup analysis of Δb at CM0-CM1 and CM0-CM2.

Regarding the b* coordinate, SDF had a decrease which lead to significant difference between CM0 with CM1 (p<0.0001) and CM0 with CM2 (p<0.0001). BIOS group had only a minor decrease, and no significant differences were found between CM0 with the posterior time periods (CM0 X CM1 p = 0,16; CM0 X CM2 p = 0,14). When BIOS and SDF groups are compared, we found no significant difference at CM0 (p = 0,51), but after treatment at CM1 and CM2 significant differences were found (p<0.0001), with a much lower b* value for SDF group (Table 1).

3.3. Photographic images:

The photographic assessment revealed that only slight color change was detected at CM2 for BIOS group, especially in the cervical area a more saturated

coloration. In the other hand, SDF showed great color change immediately after remineralization at CM1, and at CM2 the darkened stain visually looked the same.

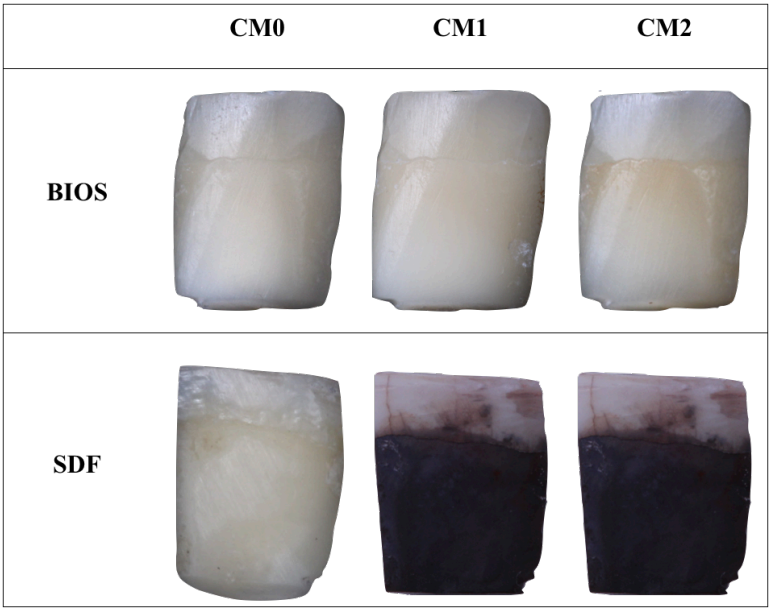

Figure 7: Photographic images of tooth color change at CM0, CM1 and CM2 for BIOS and SDF group.

4. Discussion

According to the results, both of the null hypothesis are rejected since it was found significant difference between groups at the same color measurement periods and also difference in color stability along of the time for both materials. The photographic images confirmed the results found in colorimetry analysis.

The action of fluoride in enamel and dentin it is well-known to reduce their solubility in acid, produce remineralization, and decrease carious lesions.[15] The use of ammoniacal silver fluoride was first introduced with the purpose to combine the action of F- and Ag+.[14] After this discovery, many similar products were developed around the world with different brands and concentrations. In Brazil the highest concentration found in the market is 30%, which we used in this study. A systematic review on the application of SDF on adults found three studies on prevention and arrest of root carious lesion.[9] From them, the authors came to the conclusion that SDF products are recommended to seniors with a high risk of caries.[9] However, the application of this product have been indicated only at posterior teeth due to the stain caused by the deep

penetration on dentin.[12] Therefore, there is an esthetic limitation that narrows the application of SDF, besides the decrease of bond strength.[13]

The research on Biosilicate is crescent, due to the excellent results found in many areas of dentistry.[21-28] The contact of Biosilicate microparticles with the tooth surface is capable to increase enamel and dentin microhardness,[21,22] and increase mineral matrix ratio.[23] With a higher amount of minerals, the levels of bond strength are higher as well, as the deposition of minerals on dentin tubules can act as an additional receptor to the monomer component of the adhesive system.[27,28] Biosilicate was tested as pre-treatment of dentin prior to restorative procedures and its influence on color change of theses restorative materials was studied.[21,30] However, Biosilicate microparticles can be used directly on tooth surface, without subsequent restoration, and the remineralization will be achieved as well.[22] But color change was never assessed in this context. Therefore this is the first study that assessed the influence of Biosilicate color change directly on root dentin surface.

To obtain an accurate data in color measurement the use of a digital spectrophotometer is fundamental to assess color change, since the visual analysis alone can be subjected to bias, and differ between observers.[31] A previous report analyzed color selection through visual analysis, photographic method and spectrophotometer. The high agreement between photographic method and spectrophotometer lead the authors to conclude the reliability of digital photography in color assessment.[36] Therefore, in our study, both methods were used.

The assessment of color change in a scenario of a large color difference with CIELAB and CIEDE2000 formula would not correlate well,[33] even though they are commonly used in dental studies. Thus, in our study, we used Hyab formula to calculate the color difference produced by the products because it precisely accommodates large color difference.[33] Our results showed that after treatment with SDF, at CM1 a significant color change was found, and this difference increased when CM0 was compared to CM2 (Figure 3). The responsible for this stain is the silver ions precipitation, that should be continuous which lead to a greater color difference at CM2. These findings corroborate with previous studies that assessed SDF application and staining as a function of time, and therefore considered a determinant factor for the extent of dentin color change.[35,36] The color change presented after Biosilicate

application was significant over the time (Figure 3), however the digital photographs confirmed that CD was much more evident after SDF application (Figure 7).

In the observation of the photographic images (Figure 7), the black stain is easily seen, with not much difference from CM1 to CM2, however in the Biosilicate treated sample, the cervical area presented a saturated reddish coloration that is likely to come from the storage and ageing of the samples.

The Hyab formula calculates overall color change, however it does not indicate which color parameter specifically changed. Therefore we decided to analyze the parameters individually, and their deltas, comparing them along the time and between groups. L* and b* coordinates appears to be the parameters of more significance in color studies, since luminosity and blue hue are more related to a whitened aspect.[32] The L* coordinate decrease was responsible for the higher color change in the SDF samples (Table 1). The negative values show the great darkening of the samples in this group. In Biosilicate group we also identified a significant ΔL change from the first to the second time period (Figure 4). When both groups were compared at the same time periods, the significant differences found confirm the major darkening in the SDF group.

SDF samples had a greater decrease in the b* axis, which means an increased saturation of blue chroma (Table 1). Biosilicate samples also had a decrease in the b* axis, but very small, and not statistically significant when compared with the time periods, which could mean that the samples remained unaltered in this aspect (Table 1). This drastic blue shift of SDF samples, that lead to a statistical difference between Biosilicate and SDF, could imply that in a large color difference, the shift to blue is not always an esthetical parameter, and the others coordinates must be considered in color assessment to obtain a correct interpretation of the results. In respect to the Δb, the intragroup analysis showed that after treatment there was no difference for both groups at both time periods, nevertheless the intergroup analysis showed significant difference for both time periods (Figure 6).

The a* axis increased for both groups (Table 1), which means a shift for the red hue. For SDF, this increase was much more perceivable than Biosilicate, with a significant difference between groups at the same time periods (Table 1). In the intragroup analysis, Δa showed difference only for Biosilicate, confirming the reddish saturation observed in the cervical portion of the root sample (Figure 5). The intergroup

analysis of Δa was significant in both time periods, due to the remarkable black stain produced by SDF (Figure 5).

The two studies that assessed Biosilicate did not find any effect on color stability, however, this remineralizing product was under restorative materials.[21,30] It seems that when applied directly on the tooth surface, a slight color change could be found, but more laboratory and clinical studies must be conducted to confirm these results and why this color change is being produced. Due the limitations of this in vitro study, research on this topic must be developed, specially clinical studies to observe the actual behavior of Biosilicate when in contact with root dentin. To find the best protocol of application, different concentrations and vehicles, Biosilicate should be clinically tested with the purpose to observe color change.

5. Conclusions

Within the limitations of the current study it was concluded that:

1. The application of 10% Biosilicate in root caries presented only a slight color change along of the time, while SDF product had a great overall color change.
2. When both products were compared at the same time periods, Biosilicate produced significantly lower color change than SDF.

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4 CONSIDERAÇÕES FINAIS

Dentro das limitações dos estudos, podemos concluir que:

- Estudo 1: O Biosilicato é um excelente candidato para substituir o SDF como agente remineralizante de cárie radicular.
- Estudo 2: O Biosilicato, produz mínima alteração de cor ao longo do tempo, e pode ser considerado para uma abordagem mais estética no tratamento da cárie radicular em substituição ao SDF.

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