LEONARDO FRANCHINI PAN MARTINEZ

ANÁLISE DA INTERFACE ADESIVA ENTRE PINOS DE FIBRA DE VIDRO E DENTINA INTRARRADICULAR SUBMETIDA À TÉCNICA ALCOÓLICA SIMPLIFICADA

Faculdade de Odontologia
Universidade Federal de Minas Gerais
Belo Horizonte
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Tese apresentada junto ao Colegiado de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do grau de Doutor em Odontologia – área de concentração em Clínica Odontológica

Orientador: Prof. Dr. Allyson Nogueira Moreira
Coorientadora: Prof. (a): Dra. Thaís Yumi Umeda Suzuki

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FOLHA DE APROVAÇÃO

Análise da interface adesiva entre pinos de fibra de vidro e dentina intrarradicular submetida à técnica alcoólica simplificada

LEONARDO FRANCHINI PAN MARTINEZ

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ODONTOLOGIA, como requisito para obtenção do grau de Doutor em ODONTOLOGIA, área de concentração CLÍNICA ODONTOLÓGICA.

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Dedico esse trabalho a **ELE**!

DEUS! O Criador onipotente, onisciente e onipresente.

Aquele que tudo sabe, tudo controla, tudo pode e ama incondicionalmente a todos nós.

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"Eu sou o Alfa e o Ômega, o Primeiro e o Último, o Princípio e o Fim." ***Ap 22:13***

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O que precisamos nessa vida é de coragem e fé!

Obrigado a todos vocês!

“O sucesso dos que triunfam é saber começar tudo de novo.”

Manuel Angel Pan Martinez

RESUMO

A cimentação de pinos de fibra de vidro à dentina intrarradicular, principalmente no terço apical, continua sendo um desafio na odontologia. O objetivo desse estudo foi avaliar a influência do controle de umidade com etanol na resistência de união de pinos de fibra de vidro em diferentes terços de dentina intrarradicular, 24 horas e 6 meses após o processo de cimentação adesiva. Sessenta e quatro incisivos bovinos extraídos foram submetidos ao tratamento endodôntico e divididos em dois grupos, de acordo com o tipo de controle de umidade (técnica convencional e alcoólica). Cada grupo foi dividido em 4 subgrupos (n=8) de acordo com a cimentação: RXU: Single bond Universal + RelyX Ultimate; PNV: primer de dentes Panavia V5 + Panavia V5; U200: RelyX U200; SET: Set PP. A resistência de união do pushout foi medida em diferentes áreas da interface entre o pino e a superfície radicular, 24 horas e 6 meses após o procedimento de união. Os dados foram submetidos ao teste de Bonferroni ($\alpha = 0,05$). Os menores valores de resistência de união foram encontrados para o grupo SET, com diferença estatisticamente significante entre os demais grupos para os terços cervical e médio. Os maiores valores de resistência de união foram encontrados para o grupo PNV com diferença estatisticamente significante para os demais grupos nos terços médio e apical em 24 horas. Avaliando os diferentes terços, em geral, os maiores valores de resistência de união foram encontrados para o terço cervical. O grupo PNV apresentou maiores valores de resistência de união para os terços cervical e médio, sem diferença estatisticamente significante entre eles. Quanto ao tipo de controle de umidade, pode-se observar que não há diferença estatisticamente significativa para o grupo PNV às 24 horas, U200 e SET aos 6 meses. Na comparação entre os tempos (24 horas e 6 meses), em geral, os valores de resistência de união diminuíram após o armazenamento. Diferenças significativas com menores valores foram observadas após o tempo de seis meses nos valores de envelhecimento para a resistência de união. Por meio de um microscópio óptico e um aparelho de microscopia eletrônica de varredura o padrão de fratura na interface adesiva foi caracterizado.

Palavras chave: Pinos dentários - Cimento resinoso – Dentina - Adesivos dentinários.

ABSTRACT

ANALYSIS OF THE ADHESIVE INTERFACE GLASS FIBER POSTS AND INTRARADICULAR DENTIN SUBMITTED TO THE SIMPLIFIED ALCOHOLIC TECHNIQUE

Cementation of fiberglass posts to intraradical dentin, especially in the apical third, remains a challenge in dentistry. The study was to evaluate the influence of moisture control with ethanol on the bond strength of fiberglass posts in different thirds of intraradicular dentin, 24 hours and 6 months after the adhesive cementation process. Sixty-four extracted bovine incisors were endodontically protected and divided into two groups, according to the type of moisture control (conventional and alcoholic technique). Each group was divided into 4 subgroups (n=8) according to cementation: RXU: Single bond Universal + RelyX Ultimate; PNV: Panavia V5 tooth primer + Panavia V5; U200: RelyX U200; SET: Set PP. The pushout bond strength was measured in different areas of the interface between the post and the root surface, 24 hours and 6 months after the bonding procedure. The data were confirmed by the Bonferroni test ($\alpha = 0.05$). The lowest bond strength values were found for the SET group, with a statistically significant difference between the other groups for the cervical and middle thirds. The highest bond strength values were found for the PNV group, with a statistically significant difference for the other groups in the middle and apical thirds at 24 hours. Evaluating the different thirds, in general, the highest bond strength values were found for the cervical third. The PNV group showed higher bond strength values for the cervical and middle thirds, with no statistically significant difference between them. As for the type of humidity control, it can be observed that there is no statistically significant difference for the PNV group at 24 hours, U200 and SET at 6 months. In the comparison between times (24 hours and 6 months), in general, the bond strength values decreased after storage. Minor elevation differences with values were observed after the six-month time in the ageing values for bond strength. Using an optical microscope and a scanning electron microscopy device, the fracture pattern at the adhesive interface was characterized.

Keywords: Dental pins - Resin cements – Dentin - Dentin-bonding agent.

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SUMÁRIO

1 CONSIDERAÇÕES INICIAIS

Dentes tratados endodonticamente requerem um tratamento restaurador diferenciado. A perda de dentina e de estruturas anatômicas, como cúspides e teto da câmara pulpar, podem resultar em fratura do tecido dentário após a restauração final. O uso de pinos intrarradiculares é necessário para fornecer retenção, caso a estrutura dentária coronal remanescente não seja suficiente (Bohrer *et al*., 2011; Cecchin *et al*., 2010; Cecchin *et al*., 2011;).

Diferentes materiais restauradores têm sido propostos para a correta união entre os pinos intrarradiculares e a dentina intrarradicular: cimento resinoso convencional em combinação com sistemas adesivos convencionais (três passos ou dois passos) ou autocondicionantes, e cimentos resinosos autoadesivos (Bitter et al., 2011; Bitter *et al*., 2017; Marchesi *et al*., 2013; Sarkis-Onofre *et al*., 2014).

Dentre os pinos pré-fabricados disponíveis, os pinos de fibras de vidro ganharam popularidade devido à resistência à flexão e ao módulo de elasticidade semelhante ao da dentina. Isto possibilita uma distribuição uniforme da tensão e reduz os riscos de fratura das raízes (Cecchin *et al*., 2011; Gomes *et al*., 2013). No entanto, podem ocorrer falhas na interface adesiva entre o pino, cimento e a dentina intrarradicular. A principal causa das falhas dos pinos de fibra é o descolamento, na interface dentina-cimento decorrente da degradação do cimento e a retentividade insuficiente do pino no conduto intrarradicular (de Carvalho *et al*., 2020; Gruber *et al*., 2020).

O uso de adesivos hidrofílicos, e a degradação das fibrilas de colágeno não encapsuladas presentes na camada híbrida, são alguns mecanismos responsáveis pela degradação do cimento (Hosaka *et al*., 2009; Malacarne *et al*., 2006). Com o intuito de se obter uma interface de qualidade na cimentação de pinos intrarradiculares, técnicas distintas de aplicação desses materiais têm sido buscadas afim de encontrar uma alternativa que possa influenciar na longevidade das restaurações pós-endodônticas com pinos de fibra de vidro (Bitter *et al*., 2017; Machry *et al*., 2020).

A técnica alcoólica foi desenvolvida afim de reduzir a degradação dos fatores envolvidos ao processo adesivo. O etanol teria como objetivo deslocar a água presente na dentina, fazendo com que as fibrilas de colágeno estivessem suportadas por esta substância, tornando-as menos hidrofílicas e mais resistentes, por meio da criação de possíveis camadas híbridas hidrofóbicas que absorvem menos água e geram ligações duráveis entre resina e a dentina (Sadek *et al*., 2007; Sadek *et al*., 2009; Pei *et al*., 2012).

Essa técnica é recomendada em associação aos sistemas adesivos de três passos quando usados previamente à aplicação do cimento resinoso convencional. Contudo, essa mesma técnica pode ser indicada para os cimentos autoadesivos, já que a adesão no canal radicular é um frequente desafio pela geometria dos canais que aprisionam água por tensão superficial, o que torna difícil deslocá-la sem tratamento prévio (Cecchin *et al*., 2011; de Carvalho *et al*., 2020; Hosaka *et al*., 2009; Pashley *et al*., 2007; Pei *et al*., 2012).

Quando analisamos os processos envolvidos nos pinos de cimentação, não devemos nos atentar apenas ao tipo de cimento resinoso usado, mas também as diferentes técnicas de abordagens que tentam melhorar a resistência de união no conjunto pino, cimento e dentina intrarradicular (Sarkis-Onofre *et al*., 2014; Skupien *et al*., 2015). A obtenção de uma interface cimento-dentina durável é crucial para a reabilitação de dentes tratados endodonticamente com pinos de fibra. A técnica simplificada de ligação úmida com etanol demonstra na literatura resultados promissores com relação à estabilidade da resistência de união do pino da fibra no interior do canal radicular (Pashley *et al*., 2007; Pei *et al*., 2012).

Levando em consideração a quantidade de estudos com cimentos resinosos autoadesivos, relacionados à cimentação dos pinos de fibra de vidro utilizando a técnica alcoólica e os diversos tipos de cimentos resinosos disponíveis atualmente, pesquisas para esclarecer qual seria a técnica mais adequada para o procedimento são necessárias. O objetivo deste estudo in vitro foi avaliar a influência da resistência de união de pinos de fibra de vidro cimentados em canais radiculares de dentes bovinos por dois diferentes pré-tratamentos (convencional e alcoólico) por meio dos cimentos resinosos testados.

2 OBJETIVOS

2.1. Objetivo Geral

Avaliar as propriedades físico-mecânicas, após 24 horas e 6 meses de quatro cimentos resinosos e respectiva interface de união entre pino e dentina radicular tratada de acordo com dois protocolos de pré-tratamento da dentina.

2.2 Objetivos Específicos

- Avaliar as resistências de união, 24 horas e 6 meses após o processo de união, de pinos de fibra de vidro cimentados à dentina intrarradicular nos terços cervical, médio e apical, por meio de diferentes agentes cimentantes e distintos protocolos de controle de umidade, utilizando o teste de *push-out*.

- Avaliar a área de união, imediata e tardia, de pinos de fibra de vidro cimentados à dentina radicular nos terços cervical, médio e apical, de diferentes agentes cimentantes e distintos protocolos de controle de umidade, por meio da microscopia eletrônica de varredura.

- Avaliar o padrão de fratura, imediata e tardia, de pinos de fibra de vidro cimentados à dentina radicular nos terços cervical, médio e apical, de diferentes agentes cimentantes e distintos protocolos de controle de umidadade por meio de um estereomicroscópio óptico com aumento de 40X.

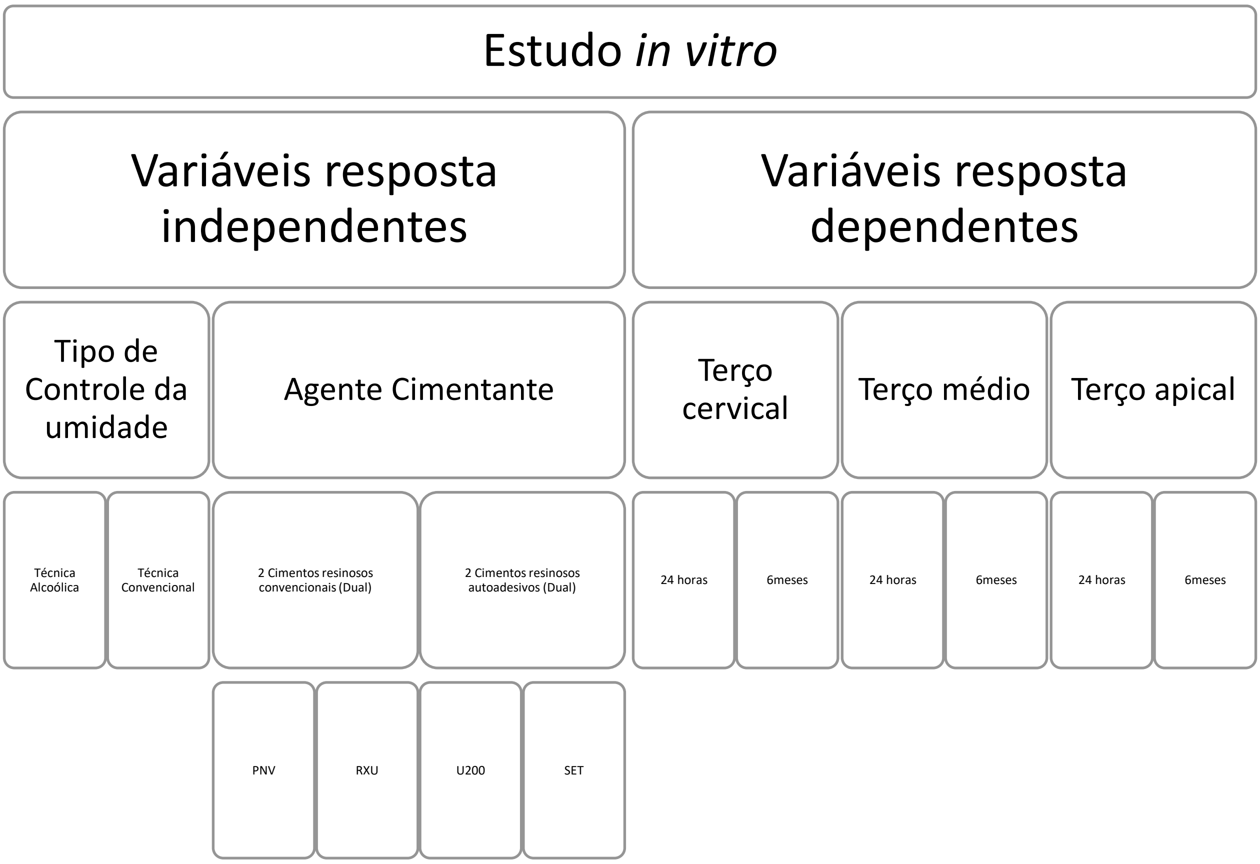
3 METODOLOGIA EXPANDIDA

3.1 Delineamento do estudo

Foi realizado um estudo *in vitro* qualitativo e quantitativo, de acordo com um desenho em blocos completos aleatorizados por meio de quatro agentes cimentantes. Os fatores de estudo independentes são: o tipo de controle da umidade em 2 níveis (técnica convencional X técnica alcóolica) e o agente cimentante em 4 níveis (2 cimentos resinosos convencionais e 2 cimentos resinosos autoadesivos). Os fatores de estudo dependentes são: tempo de armazenamento em 2 níveis (24 horas ou 6 meses) e a variabilidade regional da dentina intrarradicular em 3 níveis (terços apical, médio e cervical). As variáveis resposta analisadas no estudo contemplam resistência de união e qualidade da interface de união. Para o teste de resistência de união, foram utilizadas 64 raízes unirradiculares, divididas em oito grupos representativos (n=8).

Para o teste de microscopia eletrônica de varredura, amostras representativas foram utilizadas.

Figura 1: Esquema representativo do estudo in vitro realizado com suas respectivas variáveis resposta dependentes e independentes, técnicas utilizadas e agentes cimentantes (Metodologia e dados aplicados à pesquisa, 2022).



3.2 Seleção das amostras e preparação do canal de raiz

Um total de 64 dentes incisivos bovinos recém-extraídos com formas e tamanhos homogêneos foram coletados de animais abatidos em frigorífico na cidade de Bauru. Não houve a necessidade de submissão ao comitê de ética animal visto que os dentes são provenientes de animais abatidos.

Na análise morfológica foram estudados os seguintes fatores: faixa etária da dentina (jovem e madura/adulta), tipo de dente bovino (primeiro incisivo, segundo incisivo, terceiro incisivo e canino) e terço radicular (cervical, médio e apical).

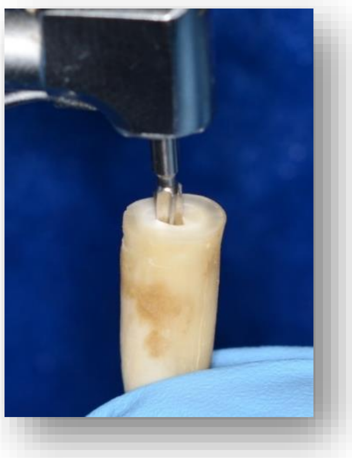
Foram selecionados os dentes incisivos bovinos com semelhança anatômica externa e interna (comprimento e diâmetro), com ápice dentário completamente formado e sem curvatura radicular. Foram excluídos do estudo dentes bovinos com curvatura radicular significativa e/ou com fratura radicular e/ou dentes com ápice aberto. As coroas foram removidas usando um disco de diamante em cortadeira de precisão (Isomet 1000, Buehler, Lake Bluff, EUA). Os dentes foram então fixados em placas de acrílico com cola quente e seccionados apicalmente à junção cemento–esmalte para a remoção da coroa na cortadeira de precisão (Isomet 1000). Todas as raízes tiveram um comprimento uniforme de 18 milímetros (mm), verificado por paquímetro digital (Mitutoyo, Miyazaki, Japão).

Figura 2: Espécimes de dentes bovinos selecionados para o estudo com mesma faixa etária, tipo de dente e formação apical radicular completa (Dados da pesquisa, 2022).

Para padronizar os espaços dos canais, foram selecionadas raízes com diâmetros de canal semelhantes. Previamente ao corte, os dentes foram submetidos a exame radiográfico (E–speed Kodak) em duas angulações a fim de verificar a presença de apenas um canal radicular e a conformação do conduto radicular (Neelakantan P et al., 2015).

Para garantir que diâmetros semelhantes de canais fossem selecionados, foram utilizadas apenas raízes que apresentaram alguma resistência à penetração da broca Largo #3 (Dentsply, Petrópolis, RJ, Brasil), afim de se padronizar o diâmetro do conduto, eram selecionadas as raízes nas quais o diâmetro no terço cervical eram de 1,5 mm e 0,9 mm nos terços médio e apical, além da ponta ativa broca de Largo nº 3 (Dentsply) não penetre completamente nos condutos (Delaprane B et al., 2014).

Figura 3: (a) Broca Largo nº3, broca DC 2 e Pino DC 2 (FGM), **(b)** Verificação da resistência do canal radicular bovino secionado (Dados da pesquisa, 2022).



(a) **(b)**

Um cirurgião dentista especialista em endodontia preparou os canais radiculares. Todas as raízes receberam tratamento endodôntico, utilizando equipamento rotatório (Xmart, Dentsply), com limas Easy Prodesign (Easy, Belo Horizonte, MG, Brasil) e irrigação com solução de hipoclorito de sódio a 2,5%. Após a instrumentação dos canais, os mesmos foram irrigados com solução EDTA trissódico (Biodinâmica, Ibiporã, PR, Brasil) e obturados com cone de guta-percha e cimento à base de resina epóxi (AH Plus, Dentsply, Petropólis, RJ, Brasil), por meio da técnica de condensação lateral (para garantir que o diâmetro do canal possa ser avaliado após o tratamento endodôntico). Uma radiografia final foi tirada afim de certificar a qualidade final da obturação. Em seguida, as raízes foram armazenadas em água destilada, a 37°C, por sete dias (Delaprane et al., 2014).

3.3 Preparo do espaço para cimentação do pino

Os canais radiculares foram preparados com brocas Largo # 3 (Maillefer-Dentsply) e broca DC 2 com baixa velocidade de rotação e comprimento de 14 mm, deixando 4 mm do canal apical preenchido. (Whitepost, FGM, Joinville, SC, Brasil). O pino utilizado na cimentação foi Whitepost DC 2 FGM (Whitepost, FGM, Joinville, SC, Brasil). Finalizado o preparo, os pinos foram provados para verificação da adaptação.

Figura 4: Preparo e desobstrução canal radicular do dente bovino seccionado (Dados da pesquisa, 2022).

3.4 Procedimento de preparo e tratamento da superfície do pino

Previamente ao procedimento adesivo, foi realizado o tratamento na superfície do pino de fibra de vidro, através do condicionamento com ácido fosfórico 37% por 60 segundos, seguido de lavagem abundante e secagem com jato de ar. Em seguida, a superfície do pino foi silanizada por 60 segundo (FGM), e seca com jato de ar. A partir desse momento, o pino não foi mais manipulado, para evitar contaminação.

3.5 Procedimento de preparo e tratamento da dentina intrarradicular e cimentação dos pinos

Previamente à cimentação dos pinos, os ápices das raízes foram posicionados perpendicularmente ao solo e fixados com cera pegajosa em uma base com resina composta para promover um apoio durante a cimentação. Em seguida, as raízes foram envolvidas, externamente, por uma fita adesiva preta para limitar o acesso de luz à entrada do conduto.

Os preparos foram irrigados com 2 ml de solução de água destilada, para remoção dos restos de guta-percha e manutenção da umidade do meio. Os espécimes foram então divididos, através de um sorteio aleatório, em 2 grupos, de acordo com tipo de pré-tratamento da dentina no controle da umidade (técnica convencional ou técnica alcóolica).

Na técnica convencional, o conduto foi seco com cones de papel absorvente. Já na técnica alcóolica, o excesso de água foi removido com cone de papel absorvente. Em seguida, o álcool absoluto à 99,5% foi aplicado no conduto radicular por 60 segundos e os excessos removidos com auxílio de cone de papel absorvente.

O Grupo RXU, seguindo as recomendações do fabricante foi submetido a irrigação com hipoclorito de sódio a 2,5% antes da irrigação com água destilada na técnica convencional e antes da aplicação do álcool na técnica alcoólica, pois havia a recomendação do uso do hipoclorito pelo fabricante.

Após o controle de umidade, os espécimes foram divididos aleatoriamente em 4 subgrupos (n=8), de acordo com a técnica adesiva utilizada:

- Grupo RXU: O adesivo autocondicionante Single Bond Universal (3M Espe) foi aplicado ativamente no conduto com um pincel por 20 segundos, seguido por leve jato de ar por 5 segundos para evaporação do solvente e fotoativado por 10 segundos, utilizando fotopolimerizador VALO® Cordless (Ultradent, UT, EUA). O cimento resinoso convencional RelyX Ultimate foi manipulado por 10 segundos e inserido no conduto radicular por meio de uma ponta aplicadora do tipo centrix número 2. Em seguida, foi aplicado o cimento sobre a superfície do pino e o mesmo foi levado em posição no interior do conduto, sendo o excesso de cimento removido. Por fim, o cimento resinoso foi fotoativado por 40 segundos utilizando o fotopolimerizador VALO® Cordless (Ultradent).

Figura 5: Cimento resinoso Dual Relyx Ultimate e adesivo autocondicionante universal 3m Espe (Fonte imagem: 3M Espe).

- Grupo PNV: O *primer* autocondicionante Panavia V5 *tooth primer* (Kuraray) foi aplicado no conduto com um pincel e deixado por 20 segundos antes de aplicar um leve jato de ar por 5 segundos. O cimento resinoso Panavia V5 (Kuraray) foi manipulado por 10 segundos e inserido no conduto radicular com auxílio uma ponta aplicadora do tipo centrix número 2. Em seguida, foi aplicado o cimento sobre a superfície do pino e o mesmo foi levado em posição no interior do conduto, sendo o excesso de cimento removido. Por fim, o cimento resinoso foi fotoativado por 40 segundos utilizando o fotopolimerizador VALO® Cordless (Ultradent).

Figura 6: Cimento resinoso Panávia V5 e adesivo autocondicionante universal e primer universal Kurarai Noritake (Fonte imagem: Kurarai Noritake).

- Grupo U200: O cimento resinoso autoadesivo RelyX U200 (3M Espe) foi manipulado, e inserido no conduto radicular por meio de uma ponta aplicadora do tipo centrix número 2. Em seguida, o cimento foi aplicado sobre a superfície do pino e levado em posição no interior do conduto, sendo removido os excessos de cimento. Por fim, o cimento resinoso foi fotoativado por 40 segundos pela superfície oclusal.

Figura 7: Cimento resinoso autoadesivo U200 3m Espe (Fonte imagem: 3M Espe).

- Grupo SET: O cimento resinoso autoadesivo Set PP (SDI) foi manipulado, e inserido no conduto radicular por meio de uma ponta aplicadora do tipo centrix número 2. Em seguida, o cimento foi aplicado sobre a superfície tratada do pino e levado em posição no interior do conduto, sendo removido os excessos de cimento. Por fim, o cimento resinoso foi fotoativado por 40 segundos.

Figura 8: Cimento resinoso autoadesivo SDI – Set-PP (Fonte imagem: SDI).

Após os procedimentos de cimentação, as raízes foram armazenadas em água em temperatura ambiente por 24 horas e 6 meses. Nesse período, as raízes foram submetidas ao teste de *push-out*, análise do padrão de fratura e análise da superfície por meio de microscopia eletrônica de varredura.

3.6 Testes de Push-Out

Raízes bovinas (n = 8) foram fixadas em placas de acrílico de 20 mm X 20 mm com cola quente e foram seccionadas perpendicularmente ao longo eixo da fibra do pino em seis (espessura aproximada de 1,0mm) usando uma cortadeira de precisão sob refrigeração a água (Isomet 1.000, Buehler), obtendo-se duas fatias para cada terço (terços cervical, médio e apical).

Figura 9: Espécimes fixados em placa de acrílico posicionadas para corte (Dados da pesquisa, 2022).

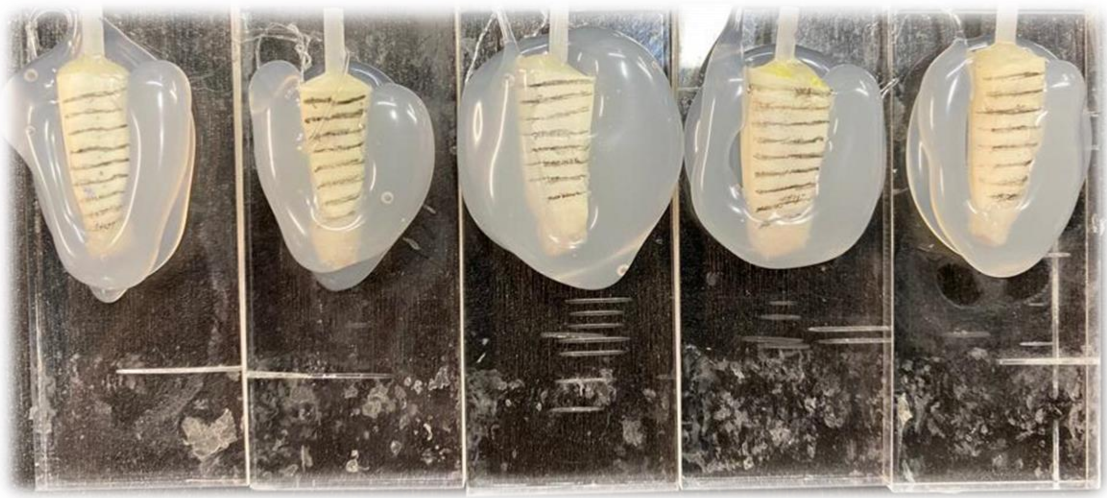
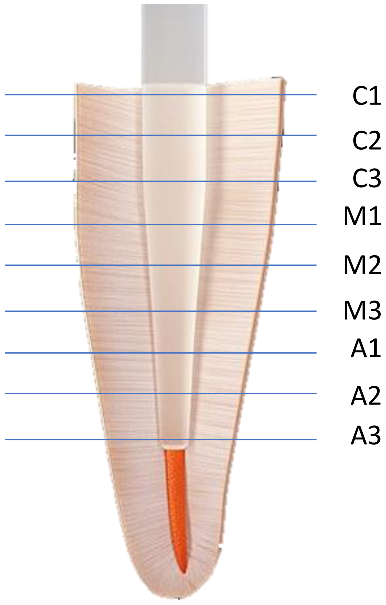
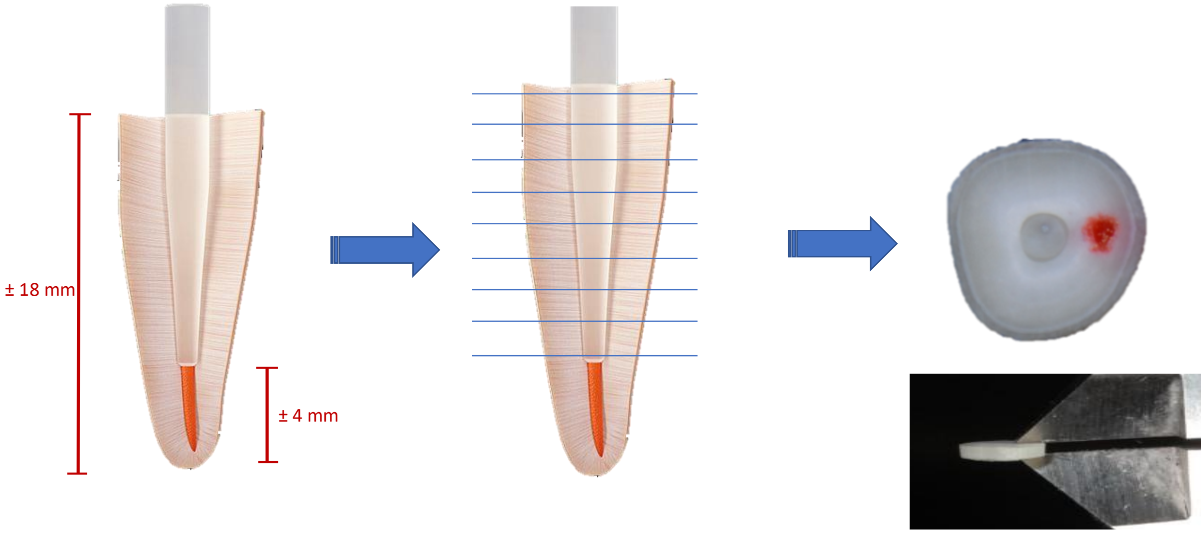


Figura 10: Desenho esquemático demonstrando o número de cortes realizado por unidade amostral (Elaborado pelo autor, 2022).



Cada corte foi marcado em seu lado apical com um marcador indelével, e a espessura foi medida com paquímetro digital (Mitutoyo Digimatic Caliper Serie 500, Mitutoyo).

Figura 11: Desenho esquemático demonstrando o número de cortes realizado por unidade amostral, marcação das amostras com marcador indelével e espessura conferida por meio de paquímetro digital (Elaborado pelo autor, 2022).



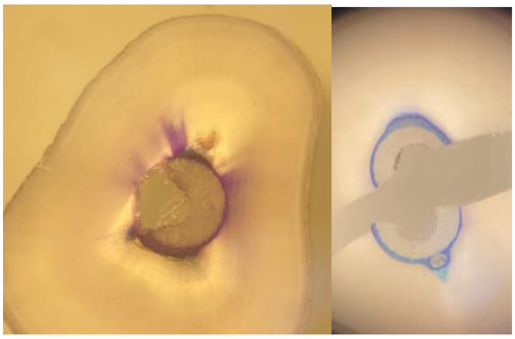
Os corpos-de-prova foram submetidos ao ensaio de união *push-out*, utilizando um êmbolo de 1 mm de diâmetro, e a carga foi aplicada a uma velocidade de 0,5 mm/min no sentido apical-coronal usando uma máquina de teste universal (EZ-LX Long-Stroke Model, Kyoto, Japão). O êmbolo foi centralizado para evitar o contato com a dentina. A resistência de união foi calculada em megapascais (MPa) dividindo a carga registrada em newtons (N) pela área (mm²) da interface pino / dentina (Delaprane B et al., 2014; Ekambaram et al., 2014).

Figura 12: Dispositivo usado na máquina de ensaio mecânico para o teste de *push-out* (Dados da pesquisa, 2022).

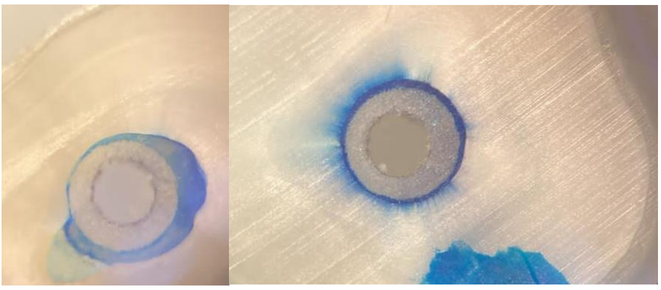
3.7 Análise do padrão de fratura

O padrão de fratura foi observado usando um estereomicroscópio (Carl Zeiss AG) com aumento de 40X. Esse padrão foi avaliado por dois operadores calibrados, e cada amostra foi categorizada da seguinte forma: falha adesiva, mista e coesiva em dentina (Delaprane B et al., 2014; Ekambaram et al., 2014).

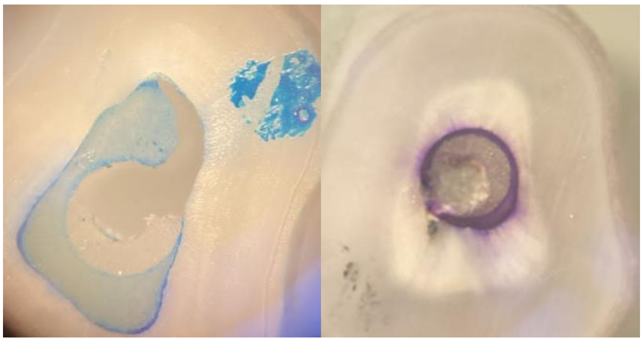
Figura 13: Tipos de fratura observados, **(a)** adesiva, **(b)** coesiva, **(c)** mista (Dados da pesquisa, 2022).



(a)



(b)



(c)

3.8 Avaliação de Microscopia Eletrônica de Varredura

As amostras foram levadas para análise por meio de um microscópio eletrônico de varredura (MEV). O padrão de fratura foi observado, na superfície dentinária em sua interface entre cimento.

3.9 Análise estatística

Os dados foram analisados para distribuições normais (Shapiro-Wilk, $p < 0,05$) e igualdades de variância (teste de Levene, $p < 0,05$) sendo observado a ausência de normalidade e homocedasticidade. Assim, a porcentagem do total sobre os valores do teste de push-out foram analisados utilizando o Teste de Bonferroni com o tipo de pré-tratamento (adesão úmida com tratamento convencional e adesão úmida alcoólica) e terços da raiz (coronal, médio, apical). As análises estatísticas foram realizadas no software SPSS, versão 21.0 (SPSS, Chicago, IL, EUA), com nível de significância de $\alpha = 0,05$ utilizado em todos os testes.

4 ARTIGOS CIENTÍFICOS

4.1 Artigo 1 – Artigo submetido em 26/11/2021 e aceito para publicação em 10/04/2022 - Journal of Adhesion Science and Technology

Title: Can bovine tooth replace human tooth in laboratory studies? A systematic review

Running title: Use of bovine tooth in replacement of human tooth

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Can bovine tooth replace human tooth in laboratory studies?
A systematic review

Introduction

There has been a continuous search for a suitable substitute for human teeth in laboratory dental research. The use of human teeth has been reduced mainly due to the ethical issues involved, aside from the increasing difficulty of obtaining freshly extracted healthy tooth elements and and insufficient quantity to allow standardization of samples [(1–5)].

Previous studies have presented descriptive and comparative data from human and non-human teeth, including goat, swine, sheep, and bovine teeth [(3,5)]. Besides being the most similar to human teeth [(2,4–7)], bovine teeth are convenient to use because of possible standardization of age, diet, size, color, and other environmental factors that may influence dental characteristics, minimizing the occurrence of bias [(8,9)].

Comparisons of physical-mechanical properties between human and non-human teeth showed great variability. The results of radiodensity [(2)], microhardness [(2,4,10)], bond strength tests [(11–13)], and marginal microleakage [(14)] tests are divergent. Bovine teeth, such as enamel, coronary, and root dentine, are suitable substitutes for human teeth [(10,12,13)].

Summarized scientific evidence to support the choice of bovine substrates as substitutes for human teeth in laboratory studies is lacking. The objective of this study was to perform a systematic review of articles comparing bovine teeth as substitutes for human teeth in laboratory dental research. The outcomes analyzed were morphology, organic and inorganic analysis, solubility, coefficient of thermal expansion, radiodensity, microleakage, surface roughness, hardness, bond strength, shear strength, compression, tensile strength, erosion, abrasion, optical aspects, microbiological analysis, and impedance of the substrates.

Material and Method

A systematic review of laboratory studies was conducted to provide the best evidence on this topic. This review can also be called “systematic search and review,” an adaptation of the *classic* systematic review of non-epidemiological studies [15]. When topics are out of the scope of epidemiology, the results of available carefully designed qualitative or methodological studies can be summarized to provide evidence on specific methods or social conditions.

This study followed the PRISMA statement criteria for systematic reviews and meta-analysis [16]. The research question was formulated to add the literature and outline the search strategy: For which properties testing can bovine teeth replace human teeth in laboratory studies?

Search strategy and selection criteria

PubMed, Web of Science, Lilacs (Bireme), BBO, and Scopus databases were searched to identify studies comparing the use of human and bovine teeth. Original articles published until April 18, 2021, in Portuguese, English, and Spanish without date restrictions were included. Search strategy included keywords from the *Medical Subject Headings* (*MESH*) and were appropriately modified for each database: Pubmed ("dentin"[mesh] and dentin or "dental enamel"[mesh] and enamel) and ("tooth root"[mesh] or “human tooth” or human tooth or tooth or teeth) and (“bovine tooth” or bovine tooth or “bovine teeth” or bovine teeth); Web of Science (bovine tooth and human tooth and radicular dentin) and (bovine tooth and human tooth and enamel) and (bovine tooth and human tooth and coronary dentin); Scopus (title-abs-key(*dentin*) and title-abs-key(*dental* and *enamel*) and title-abs-key(*tooth* and *root*)and title-abs-key(*human* and *tooth*) and title-abs-key(*tooth*) and title-abs-key(*teeth*) and title-abs-key(*bovine* and *tooth*) and title-abs-key(*bovine* and *teeth*)). In Lilacs (Bireme) and BBO, short combinations were made using the Boolean operators and/or, with the following terms: dentin, dental enamel, tooth root, human tooth, tooth, teeth, bovine tooth, and bovine teeth.

The inclusion criteria used for the studies selection were: (1) laboratory studies; and (2) studies that presented a comparison between human and bovine teeth. There were no restrictions regarding other animal species, tooth age, time, or storage media.

The exclusion criteria were as follows: descriptive articles that did not compare human and bovine teeth, review articles, academic theses, and studies published in languages other than English, Spanish or Portuguese.

Titles and abstracts were used to determine the eligible studies identified in each database. Articles presenting a comparison between bovine and human teeth in the title and abstract were selected for full reading. The retrieved data were imported by EndNote software (X5, Clarivate Analytics, Philadelphia, PA, USA), and a final list was analyzed by five calibrated researchers (ACNLL, FIRL, MCNSR, MFFC, NKLF). For the calibration exercise, the researchers discussed the criteria and applied them to a sample of 10% of the recovered studies, obtaining excellent inter-examiner agreement (kappa = 0.89). The calibration exercise was based on the classification of a 10% sample of the studies, in which all examiners classified the same studies and the results were compared. The examiners evaluated the substrate used, the variable response comparing different substrates, the laboratory test, and the tested property, the conclusion of the comparison, and the replaceability according to the author. After completing this step, the examiners scored the studies according to the methodological quality of the articles according to each property analyzed [(17)], ranking from scores comparing the results and reaching the value of kappa.

Data collection

All the tooth properties showed acceptable outcomes. Due to the large volume of data recovered from the selected studies, we decided to split the results into two different reports: this systematic review of 60 methodological/laboratory studies presenting a descriptive and qualitative analysis, and a meta-analysis of 18 bond strength studies with available quantitative data that was statistically summarized (recently published). A standard spreadsheet in Microsoft Office Excel 2013 (Microsoft Corporation, Redmond, WA, USA) was used for data extraction. Information concerning the authors, year of publication, title, journal, volume, page, language, country, study objective, outcome, laboratory test, dental substrate (enamel, dentin, cement), sample size, storage and disinfection solution, preparation of specimens, thermocycling, storage time, and conditions until evaluation, results, statistical analysis, and conclusions were independently extracted from the studies by two reviewers. All questions were resolved by consensus. When necessary, the first author was contacted to elicit further information during the data extraction process.

Two independent reviewers performed the quality assessment using a modification of the checklist/scale adapted from Faggion Jr. (2012) (Table 1) [(17)]. Disagreements were resolved through discussion and consensus between reviewers.

Data pooling was based on the characteristics of studies that used bovine and human teeth as substrates. Narrative synthesis of the data was conducted.

Results

Search and selection

Figure 1 shows the process of selecting articles according to PRISMA guidelines [16]. The online search retrieved 1438 references from PubMed, 22 from the Web of Science, 76 from Lilacs, and 8 from Scopus. After removing duplicate references [17], 1485 studies had their titles and/or abstracts read. A total of 1328 studies were excluded, and 157 were selected for full-text reading. Among these selected studies, 79 were excluded because they were concerned only with bovine or human substrates, no comparison between bovine and human substrates, review articles, articles in other languages (different from English, Spanish, or Portuguese), other methods/histological analysis, articles not available, and theses. Seventy-eight studies comparing bovine and human teeth were included. Another study under submission was a meta-analysis of 18 articles on bond strength. Ultimately, 60 methodological/laboratory studies were included in this review.

Descriptive analysis

Table 2 shows the characteristics of studies that compared human and bovine teeth in laboratory research. The studies were grouped according to the properties evaluated and described in ascending order of the year of publication. In the column "Replaceable according to the author" the articles were classified according to the results and conclusions presented in the complete text. The classification criteria are as follows:

- Indicated replacement: when the author clearly affirmed that the substrate could be replaced.
- Indicated replacement with caution: when the author indicated replacement with caution.
- Capable of replacing: when the author does not report clearly, but the text suggests that replacement may be possible.
- Did not specify replacement: when the author does not report replacement.
- Did not indicate replacement: when the impossibility of replacement was clearly reported.

Of the 60 articles evaluated, 15.5% indicated replacement, 15.5% indicated replacement with caution, 31% were capable of replacing, 29.3% did not specify replacement, and 8.7% did not indicate replacement.

Methodological quality analysis

In Table 3, the articles are classified according to their methodological quality. Considering the sum of the total scores obtained, the studies received the following attributes: high (score ≥ 5), moderate (3 ≥ score < 5), or low methodological quality (score < 3). Most of the studies described the intervention, results, and statistical analyses in detail. Increasing concern to describe the sample randomization was observed, although the description is not completely clear. However, these articles did not describe the sample size calculation, allocation, and blinding processes.

Discussion

This systematic review verified the evidence for substituting human teeth with bovine teeth as substrates in laboratory tests for several properties. The search showed the possibility of human tooth replacement by bovine in microleakage studies, organic and inorganic analyses, coefficient of thermal expansion, spectrofluorometry, hardness and microhardness, morphology, and radiodensity.

Elemental analysis showed that human substrates have greater similarity to bovine than to porcine and ovine substrates. Based on their chemical composition, bovine teeth should be the first choice as substitutes for human teeth (5). Studies have indicated the possibility of human tooth replacement in enamel evaluations based on morphology (18), organic (19), inorganic aspects (20,21), and optical reflection (22). By electrophoresis, the patterns for the human and bovine enamel matrix showed great similarities, although the human material presented a more complex molecular pattern with lower-weight components than the bovine material (19). Inorganic pyrophosphate has been detected in the enamel and dentin of human and bovine teeth (20).

MECHANICAL PROPERTIES

Studies assessing abrasion and erosion have shown that substitution should be performed with caution. Studies that evaluated the optical, solubility, and impedance aspects did not specify substitution. The possibility of replacing human teeth with bovine teeth was not specified in the analysis of hardness and microhardness (21), demineralization and remineralization (22, 23), erosion and abrasion (24), and histological aspects (25).

Comparing the hardness of human and bovine dentin (26-28), enamel and dentin superficial microhardness of permanent, deciduous human molar, and bovine teeth (28,29), the possibility of replacement of the human tooth by bovine teeth due to morphological similarity and superficial microhardness (26,27). Likewise, for the radiodensity and hardness assessment of enamel and dentin, the possibility of substitution when the age of bovine teeth is considered. Older bovine teeth are more similar to the human teeth (2). Using a microbiological model simulating occlusal caries lesions, the authors advised that in addition to human enamel, bovine teeth are another source to be used (27). When comparing the morphology and physical properties of the enamel structure of bovine, buffalo, and human teeth, human-like morphology, similar ultrastructural architecture, microhardness, and mineral composition equivalent to human dental tissue were observed, making them reference models for research [29]. Bovine enamel is a suitable substitute for a human substrate to assess the effect of irradiation with a CO2 laser and fluoride on caries inhibition, using hardness testing and SEM analysis [30]. On the other hand, the possibility of human tooth replacement for bovine teeth for the evaluation of the coefficient of thermal expansion using a thermomechanical analyzer [31].

Considering the morphological aspects of abrasion and erosion, the replacement was indicated [32,33] in the SEM evaluation and by the reflectometer system. This replacement was indicated with caution in the profilometric tests [4,34]. In the analysis of the photomicrographs, the human teeth are different from the bovine teeth in terms of resistance to diamond bur grinding, but the replacement is indicated with caution [35]. This replacement was indicated with caution for nanoindentation and optical profilometry tests [36], particularly at short acid exposure times in a laboratory study of erosion. Studies that evaluated the mineral loss and lesion depth [37], mass-loss method in cutting efficiency tests of diamond burs [38], effect of fluoride treatment on pellicle-covered enamel exposed to an acidic challenge simulating gastric reflux [39], effect of air polishing with combinations of abrasive powders [40], and enamel loss by Vickers hardness [41] did not specify the possibility of replacing human teeth with bovine teeth. In the test of enamel wear, in which human enamel demonstrated less wear than bovine enamel [42], the authors concluded that replacing human teeth with bovine teeth is not possible. The measurement of roughness parameters, surface microhardness, and SEM proved that there are highly significant differences in surface characteristics and tissue loss, and did not approve the use of bovine and ovine enamel as a substitute for human teeth in erosive and abrasive tests [4,34].

CHEMICAL PROPERTIES

Replacement of the human tooth with bovine was indicated for microleakage studies in enamel [43,44], dentin [43–45], and cement [46], as substrates behave similarly in laboratory tests. However, a replacement was indicated with caution in enamel [47,48] because the infiltration pattern was affected by the type of substrate evaluated. Bovine and porcine substrates allow marginal infiltration superior to human substrates [48].

Substitution was possible for the enamel [49,50] and cement-enamel junction [51], although the substitution was not clearly indicated.

The possibility of replacing human teeth with bovine teeth has not been specified in the analysis of enamel surface acid conditioning [22], demineralization and remineralization [23], erosion and abrasion [24], and histological aspects [25]. Data from studies that evaluated the dose response of fluoride for remineralization of human and bovine enamel [52] and salivary lubrication [53] demonstrated that bovine teeth are not adequate substitutes for human teeth. Tissue responses to anti-caries agents and remineralization challenges have not been elucidated [52], and further studies are needed to replace human teeth with bovine teeth [47, 53].

A substitution was also indicated in the comparison of the *in-situ* effects of salivary stimulation on erosion and abrasion in human and bovine enamel. Both human and bovine substrates are suitable for erosion/abrasion investigations [24]. A study has demonstrated that it is possible to quantify and distinguish the impedance characteristics between permanent, deciduous, bovine, and human enamel; however, the possibility of replacing human teeth with bovine teeth was not specified [54].

The authors did not specify the possibility of replacing human teeth with bovine teeth in solubility studies evaluating the kinetics of dissolution of human and bovine enamel [55], the release of fluoride from resin adhesive cement, and the resistance to acids in the surrounding dental structures [56].

For the study of morphological aspects of dental erosion, the use of bovine teeth was considered “capable of replacing”. The surface ultrastructures of erosive lesions in prismatic human enamel and bovine specimens did not differ. However, structural factors in enamel greatly modify the surface ultrastructure and the progression of erosion. These biological variations should be considered in enamel dissolution studies [57].

OPTICAL PROPERTIES

The possibility of substituting human teeth with bovine teeth in the assessment of absorption, dispersion, and emission of light through dental structures has not yet been specified [58]. Similarly, the possibility of substitution in both enamel and dentin opalescence [59] and color [60,22] evaluations were not specified. For thermoluminescence [61] and color change [62] evaluated in the enamel, the possibility of substitution was noted, although it was not clearly indicated. Bovine teeth are slightly more sensitive and convenient to thermoluminescence than humans (61). Bovine and human enamel substrates have similar behaviour in terms of staining and bleaching effects, although they present color differences between them (62).

CARIOGENICITY PROPERTIES

The replacement can be indicated with caution in intra-oral cariogenicity tests, considering the selected method of measuring enamel caries (63). There is no consensus on the substitution of humans by bovine enamel for microradiography mineral analysis of artificial carious lesion progression. Enamel lesions in bovine teeth are similar to those in humans, but those in equine and ovine teeth are markedly different (64). Moreover, lesions in bovines form faster than in human enamel, although the resulting lesions are almost indistinguishable in their mineral distribution (65). Therefore, replacement should be indicated with caution in caries experiments, and these differences should be considered (66). However, other authors do not specify (67) or indicate substitution by bovine substrates and affirm that human enamel acid gel lesions were found to be less demineralized and showed less variability than those formed in bovine enamel under virtually identical conditions. Moreover, biological variations within bovine enamel may overshadow any structural and chemical differences between tissues (52).

Also on the formation of artificial carious lesions, the spectrofluorometry showed that the lesions progressed more rapidly in deciduous than in permanent enamel, both in bovine and human enamel. Furthermore, lesions in bovine permanent, bovine permanently abraded, and ovine permanent enamel progressed three times faster than those in human permanent enamel. These results provide a comparative base for the use of bovine, ovine, or human enamel data in the quantitative discussion of artificial carious lesion formation (67).

MORPHOLOGICAL PROPERTIES

Considering the effect of irradiation on remineralization and demineralization of human and bovine enamel, neither irradiation nor abrasion influenced in vitro demineralization or in situ remineralization of human specimens, whereas abrasion hampers demineralization in irradiated bovine specimens. For studies focusing on the effects of irradiation, human enamel is preferred [68]. The investigation of the crystallographic nanoscale structures of human, bovine, porcine, and ovine enamels and dentin showed that there are differences in the organic and inorganic contents among the species. Crystallographic characteristics determine the behavior of enamel and dentin; moreover, the macroscopic physical and mechanical properties of teeth depend not only on their composition but also on the structure of the dental substrates. Therefore, when bovine, porcine, or ovine substrates are used as substitutes for human material in experimental studies, morphological differences, chemical composition, and structural differences should be taken into account to interpret the results correctly [69].

The radiodensities of the enamel, coronary dentin [3,70], and root dentin [71] from different species were evaluated. In the comparison among human, bovine, and swine substrates, human and bovine enamel presented similar radiodensity, higher than that of swine enamel, and only bovine dentin presented greater similarity to human dentin [3]. However, the higher radiographic density of bovine enamel than human enamel and lower radiodensity of bovine coronal and radicular dentin compared to humans determined that bovine teeth should be used with care in radiographic in vitro studies [70].

Replacement of human coronary dentin with the corresponding bovine substrate should be performed with caution. SEM analysis showed morphological differences between human and bovine dentin tubule structures. The number of tubules per area (tubules/mm2), regardless of depth, was significantly greater in human dentin than in bovine dentin. Therefore, caution must be exercised when using bovine dentin, which may influence the adhesive test results [71].

Replacement of human teeth with a bovine substrate has been indicated in laboratory studies involving enamel and dentin hardness and microhardness, as well as microleakage in enamel, dentin, and cementum[72]. The possibility of replacing human enamel with bovine enamel in studies involving abrasion and erosion was indicated with caution or not specified[73]. In general, there is the possibility of human tooth replacement by bovine, both for enamel and dentin substrates, in organic and inorganic analyses, coefficient of thermal expansion, spectrofluorometry, morphology, and radiodensity.

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Conflict of Interests Statement

The authors of the article named “Can bovine teeth replace human teeth in laboratory studies? A systematic review” declares no conflict of interest that may interfere with the impartiality of the scientific work.

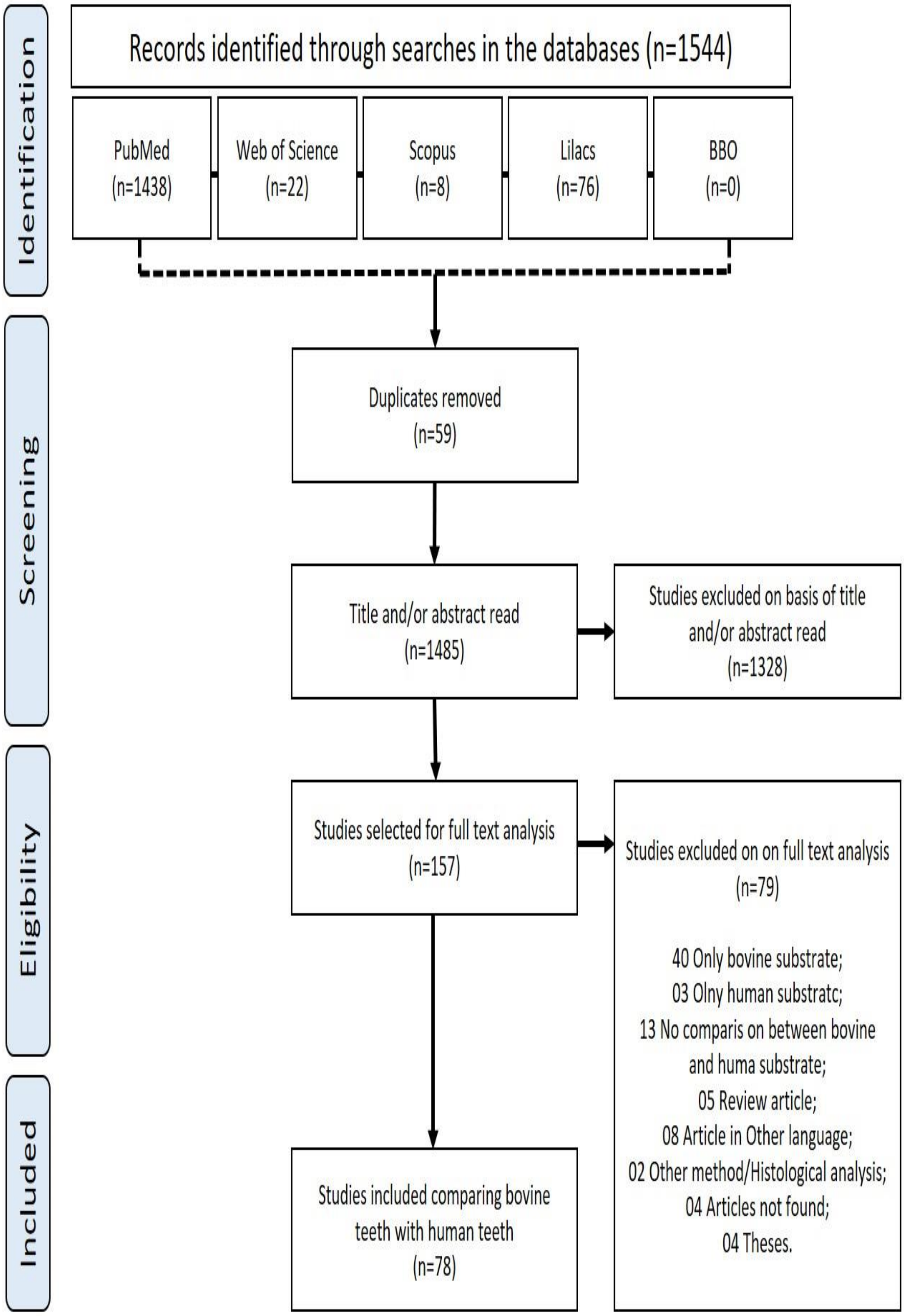
Identification
Screening
Eligibility
Included
Records identified through searches in the databases (n=1544)
PubMed (n=1438)
Web of Science (n=22)
Scopus (n=8)
Lilacs (n=76)
BBO (n=0)
Duplicates removed (n=59)
Title and/or abstract read (n=1485)
Studies excluded on basis of title and/or abstract read (n=1328)
Studies selected for full text analysis (n=157)
Studies excluded on on full text analysis (n=79)
40 Only bovine substrate;
03 Olny human substratc;
13 No comparis on between bovine and huma substrate;
05 Review article;
08 Article in Other language;
02 Other method/Histological analysis;
04 Articles not found;
04 Theses.
Studies included comparing bovine teeth with human teeth (n=78)

Table 1. Description of criteria for evaluating the quality of the selected articles.

Criteria	***Classification***	***Score***	***Definition***
1. Intervention	Adequate	1.0	The intervention for each group, including how and when it was administered, with sufficient detail to enable replication
	Inadequate	0.5	Data not clearly presented in the article
	Not described	0.0	Insufficient data
2. Outcomes	Adequate	1.0	Completely defined, pre-specified primary and secondary measures of outcome, including how and when they were assessed
	Inadequate	0.5	Insufficient data
	Not described	0.0	Data not clearly presented in the article
3. Sample size	Adequate	1.0	How sample size was determined
	Not described	0.0	Does not describe/present/communicate conducting sample calculation
4. Randomization	Adequate	1.0	Method used to generate and to implement the random allocation sequence, describing any steps taken to conceal the sequence until intervention was assigned
	Inadequate	0.5	Insufficient data
	Not described	0.0	Data not clearly presented in the article
5. Implementation	Adequate	1.0	Who generated the random allocation sequence, who enrolled teeth, and who assigned teeth to intervention
	Inadequate	0.5	Insufficient data
	Not described	0.0	Data not clearly presented in the article
6. Blinding	Adequate	1.0	If done, who was blinded after assignment to intervention and how
	Inadequate	0.5	Insufficient data
	Not described	0.0	Data not clearly presented in the article
7. Statistical methods	Adequate	1.0	Statistical treatment fully described and adequate
	Inadequate	0.5	Statistical treatment not fully described and inadequate
	Not described	0.0	No statistical treatment applied

Checklist/Scale adapted for this study from Faggion Jr (2012) (17).

Table 2. Characteristics of studies that used bovine and human teeth as substrate

Author,Year	Substrate	Number of teeth per group	Outcome	Laboratory test	Replaceable according to the author?*
		Microleakage			
Araújo et al. (50)	Enamel	30 third human molars and 20 bovine teeth	Marginal infiltration	Stereoscopic magnifying glass	Capable of replacing
Resende & Gonçalves (51)	Cement-enamel junction	20 third human molars and 20 bovine incisors	Marginal sealing	Stereoscopic magnifying glass	Capable of replacing
Abuabara et al. (48)	Enamel	60 bovine incisors, 60 molar swine and 60 human molars	Microleakage	Spectrophotometry	Replacement indicated with caution
Yavuz et al. (44)	Enamel and dentin	20 human premolars, 20 bovine incisors and 20 dog canines.	Dye penetration and microleakage	stereoscopic microscope	Replacement indicated
Guará K et al. (72)	Enamel	8 human premolars and 16 bovine incisors	Microleakage and marginal sealing	stereoscopic microscope	Capable of replacing
Simas et al. (46)	Dentin and cement	16 third molar human and 16 bovine incisors	Microleakage	Stereoscopic magnifying glass	Replacement indicated
Yavuz et al. (45)	Enamel and dentin	10 human deciduous canines 10 dog canines and 10 bovine incisors	Microleakage and marginal adaptation	Binocular microscope	Replacement indicated
		Optical aspects			
Spitze & Bosch (58)	Enamel	8 bovine and 4 human teeth	Absorption, dispersion, and emission of lightning	Computer Spectral Accuracy	Not Specified
Duran & Panzeri (61)	Enamel	6 human teeth and 3 bovine teeth	Termoluminescence TL	Thermoluminescence detector device	Capable of replacing
Lee & Yu (59)	Enamel and dentin	36 bovine teeth and 21 human teeth	Color and opalescence	Spectrophotometers	Not Specified
Yu, Ahn & Lee (60)	Enamel and dentin	20 bovine teeth and 20 human teeth	Color	Spectrophotometers	Not Specified
Attia et al. (62)	Enamel	7 third human molars and 7 bovine incisors	Change of color	Photoreflectance analysis	Capable of replacing
		Organic and inorganic analysis			
Bisaz, Russel & Fleisch (20)	Enamel and dentin	Does not mention	Inorganic analysis	Chromatography, infrared spectroscopy.	Capable of replacing
Buonocore & Sheykholeslam (18)	Enamel	24 bovine incisors and 30 human incisors.	Morphology of enamel	Scanning electron microscope.	Capable of replacing
Fincham (19)	Enamel	Primary teeth	Inorganic and organic analysis	Electrophoresis;	Capable of replacing
Shu et al. (73)	Enamel and dentin root	Human premolar bovine permanent incisors	Microradiography and microhardness	Microreadiography and microhardness	Not Specified
Hsu et al. (21)	Enamel	12 incisors bovine 10 third molars human	Optical reflectivity of penetration	OCT[1]; PS-OCT[2]; Polarized light microscopy; transverse microradiography	Capable of replacing
Teruel et al. (5)	Enamel and dentin	100 human incisors and molars, 100 of cattle, 100 pigs and 100 sheep.	Composition	TG-MS[3], EDXRF[4]	Replacement indicated
		Abrasion and erosion			
Amaechi et al. (37)	Enamel	10 bovine permanent, 10 permanent and 10 human deciduous	Mineral loss and lesion depth	Transverse microradiography	Not Specified

Antunes et al. (32)	Dentin	5 deciduous teeth, 5 permanent teeth and 5 bovine teeth	Effectiveness of high speed and air abrasion instruments	SEM (scanning electron microscopy)	Capable of replacing
Attin et al. (29)	Enamel	36 bovine (cattle and calves) 36 wisdom teeth and 36 deciduous teeth	Toothbrushing abrasion, erosion and the combination of erosion and toothbrushing abrasion.	Profilometrically	Replacement indicated with caution
Fais et al. (38)	Enamel	36 human and 26 bovine	Mass-loss method in cutting efficiency tests of diamond burs	Mass-loss method	Not specified
Hove et al. (39)	Enamel	16 bovine and 16 human	Effect of fluoride treatment on pellicle-covered enamel exposed to an acidic challenge simulating gastric reflux	Atomic absorption spectroscopy (the acid analysed for calcium)	Not specified
Khalefa et al. (40)	Enamel	60 from cows, 80 from calves and 80 human deciduous	Effect of air polishing	Profilometer	Not specified
Koulourides and Chien (63)	Enamel	Not related	Demineralization or remineralization of lesions. Surface microhardness	Intra-oral cariogenicity test (ICT)	Replacement indicated with caution
Mehl et al. (42)	Enamel	8 bovine and 8 human	Wear of enamel	Computed-aided laser profilometry.	Replacement not indicated
Pinelli et al. (35)	Enamel	36 bovine and 36 human	Photomicrographs the grinding of diamond burs	Stereomicroscope	Replacement indicated with caution
Putt et al. (33)	Enamel	Not related	Degree of polish	Reflectometer system	Capable of replacing
Sobral et al. (41)	Enamel	100 bovine and 20 human	Enamel loss	Vickers hardness	Not Specified
Turssi et al. (10)	Enamel and root dentin	45 bovine and 45 human *In situ:* 2 crown and 2 root slabs in 14 volunteers	Dental erosion models	Microhardness expressed in KHN	Bovine enamel Replacement indicated Bovine root dentin: no indicated
White et al. (36)	Enamel	120 specimens of bovine enamel and 130 specimens of human enamel	Acid type and concentration, pH, agitation rate	Nanoindentation and optical profilometry	Replacement indicated with caution
Field et al. (34)	Enamel	20 enamel slabs from bovine, human and ovine incisor crowns	Surface characteristics and tissue los subjected to an erosive and a abrasive challenge	Roughness parameters, height change and scanning electron microscopy	Bovine enamel: not capable Ovine enamel: not capable
Coefficient of thermal expansion					
Lopes et al. (31)	Dentin	15 bovine dentin slices and 15 human dentin slices	% dimensional and weight change and coefficient of thermal expansion	Thermomechanical analyzer	Capable of replacing
Microhardness and Hardness					
Castanho et al. (26)	Dentin	8 human incisors 8 bovine incisors	Microhardness	Vickers microhardness	Capable of replacing
Collys et al. (22)	Enamel	10 bovine and 10 human enamel blocks	Surface microhardness	Knoop hardness	Not Specified

Donassollo et al. (27)	Enamel and dentin	4 human deciduous molar, 4 human permanent molar and 4 bovine incisor	Surface microhardness	Knoop hardness	Capable of replacing
Feagin et al. (23)	Enamel	Does not mention	Demineralization and remineralization	Knoop hardness	Not Specified
Field et al. (4)	Enamel	20 bovine, 20 human and 20 ovine incisor crowns	Erosion and abrasion	Vickers microhardness	Not Specified
Fonseca et al. (2)	Enamel and dentin	10 human third molars and 40 bovine central incisors	Radiodensity and hardness	Knoop hardness and MEV	Capable of replacing
Lippert and Hara (52)	Enamel	Human permanent molars and bovine incisors	Dose-response of fluorine to remineralization	Vickers microhardness	Replacement not indicated
Masuda et al. (25)	Enamel	15 central bovine incisors and 15 human third molars	Histological evaluation and microhardness	Histological evaluation and transverse microhardness	Not Specified
Nogueira et al. (43)	Enamel	41 buffalo incisors, 41 bovine incisors and 30 permanent incisors of humans	Morphology and physical properties of enamel structure	Microhardness and surface roughness	Replacemen indicated
Reeh et al. (53)	Enamel	380 enamel human and bovine	Salivary lubrication	Knoop hardness	Replacement not indicated
Rios et al. (24)	Enamel	9 volunteers vigore intraoral palatal devices, with 12 enamel specimens (6 human and 6 bovine)	Erosion and abrasion	Knoop microhardness	Replacement indicated
Souza-Gabriel et al. (30)	Enamel	96 enamel slabs (48 from bovine and 48 from human teeth)	Microhardness e MEV	Knoop hardness	Replacement indicated
Strassler et al. (28)	Enamel	2 central incisors bovine 1 third molar human	Microbiological analysis and microhardness of cracks	Knoop hardness and microbiological test	Replacement indicated
Solubility					
Chen and Nancollas (55)	Enamel	Does not mention	Solubility	Spectrophotometry	Not Specified
Han et al. (56)	Enamel and dentin	20 human teeth and 20 bovine teeth	Fluoride release	Solubility test	Not Specified
Impedance					
Levinkind et al. (54)	Enamel	Does not mention	Electrochemical impedance	Potential difference	Not Specified
Spectrofluorometry/Hardness					
Featherstone and Mellberg (67)	Enamel	20 blocks of sound human and bovine deciduous/permanent teeth	Dye uptake	Spectrofluorometry	Capable of replacing
		10 bovine incisors, 5 ovine incisors and 10 human premolars permanent	Depth measurement	Microscope system of a Leitz minihardness tester	
Mineral Analyses/Morphology/Radiodensity					
Edmunds et al. (64)	Enamel	12 bovine, 11 equine, 15 human and 10 ovine incisors teeth	Appearance of the lesions Mineral level Depth of lesions	Polarized light Microradiography Scanning electron microscope (SEM)	Replacement indicated with caution
Meurman and Frank (57)	Enamel	Bovine permanent incisors and 2 human premolars teeth (12 specimens from each crown)	Progression and surface ultrastructure	Scanning electron microscope (SEM)	Capable of replacing

Fonseca et al. (3)	Enamel and dentine	10 bovine central incisors, 10 human third molars and 10 swine central incisors	Radiodensity	Digital radiographic system	Replacement indicated
Kielbassa et al. (68)	Enamel	24 bovine incisors and 24 human third molars (48 enamel slabs each group)	Mineral analyses	Microradiography	Replacement not indicated
Lynch and Cate (66)	Enamel	Bovine incisors and human premolars	Mineral analyses	Microradiography	Not Specified
Tanaka et al. (70)	Enamel Coronal and radicular dentine	30 sound bovine incisors and 20 sound human third molars	Radiodensity	Digital radiographic system	Replacement indicated with caution
Lopes et al. (71)	Dentin	10 human molars and 10 bovine incisors	Dentinal tubule diameter Dentinal tubule density	Scanning electron microscope (SEM)	Replacement indicated with caution
Lippert et al. (52)	Enamel	1709 specimens of bovine incisors teeth 1394 specimens of human permanent molars and premolars	Mineral analyses	Transverse microradiography	Replacement not indicated
Lippert and Lynch (65)	Enamel	90 specimens - bovine incisors teeth 90 specimens - human permanent molars and premolars	Mineral analyses Surface microhardness	Transverse microradiography Knoop and Vickers surface microhardness	Capable of replacing
Ortiz-Ruiz et al. (69)	Enamel and dentine	The study used 400 recently-extracted incisors and molars: 100 human, 100 bovine, 100 ovine,and 100 porcine	Crystallographic structure Relative content of organic and inorganic matter Combustion profile	X-ray diffraction (XRD) Fourier transform infrared spectroscopy (FTIR) Differential scanning calorimetry (DSC)	Replacement indicated with caution

[*]Indication of substitution according to the author of the article [1]Optical coherence tomography; [2] Optical coherence tomography with sensitive polarization; [3] Mass spectrometry coupled to mass spectrometry; [4] dispersive X-ray fluorescence wavelength

Table 3. Methodological quality of articles according to each property analyzed adapted of Faggion *et al*., (2012).

Author,Year	**Intervention**	**Outcomes**	**Sample size**	**Randomization**	**Implementation**	**Blinding**	**Statistical methods**	**Methodological quality***	**Score**
Microleakage									
Araújo et al. (50)	1**	1	0**	0	0	0	1	moderate	3
Resende & Gonçalves (51)	1	1	0	0,5**	0	0	1	moderate	3,5
Abuabara et al. (48)	1	1	0	0,5	0	0	1	moderate	3,5
Yavuz et al. (44)	1	1	0	0,5	0	0	0,5	moderate	3
Almeida (49)	1	1	0	0,5	0	0	1	moderate	3,5
Simas et al. (46)	1	0,5	0	0,5	0	0	0,5	low	2,5
Yavuz et al. (45)	0,5	1	0	0	0	0	1	low	2,5
Optical aspects									
Spitze & Bosch (58)	0,5	0,5	0	0	0	0	0	low	1
Duran & Panzeri (61)	0,5	0,5	0	0	0	0	0,5	low	1,5
Lee & Yu (59)	1	1	0	0	0	0	1	moderate	3
Yu, Ahn & Lee (60)	1	1	0	0,5	0	0	1	moderate	3,5
Attia et al. (62)	1	1	0	0,5	0	0	1	moderate	3,5
Organic and inorganic analysis									
Bisaz, Russel & Fleisch (20)	1	1	0	0	0	0	0	low	2
Buonocore & Sheykholesla (18)	1	1	0	0	0	0	0	low	2
Fincham (19)	1	1	0	0	0	0	0	low	2
Shu et al. (73)	0,5	1	0	0	0	0	1	low	2,5
Hsu et al. (21)	1	1	0	0	0	0	1	moderate	3
Teruel et al. (5)	1	1	0	0	0	0	1	moderate	3
Abrasion and Erosion									
Amaechi et al. (37)	1	1	0	0.5	0	0	1	Moderate	3.5
Antunes et al. (32)	1	1	0	0	0	0.5	1	Moderate	3.5
Attin et al. (29)	1	1	0	0	0	0	1	Moderate	3.0
Fais et al. (38)	1	1	0	0	0	0	0.5	Low	2.5
Hove et al. (39)	1	1	0	0.5	0	0	1	Moderate	3.5
Khalefa et al. (40)	0.5	0.5	0	0.5	0.5	0	1	Moderate	3.0
Koulourides and Chien (63)	0.5	0.5	0	0	0	0	1	Low	2.0
Mehl et al. (42)	1	1	0	0	0	0	1	Moderate	3.0
Pinelli et al. (35)	1	0.5	0	0	0.5	0	0.5	Low	2.5
Putt et al. (33)	0.5	0.5	0	0	0	0	0	Low	1.0
Sobral et al (41)	1	1	0	0	0	0	0.5	Low	2.5
Turssi et al. (10)	1	1	1	0.5	0	0	1	Moderate	4.5
White et al. (36)	1	1	0	0.5	0	0	1	Moderate	3.5
Field et al. (34)	1	0.5	0	0	0	0	1	Low	2.5
Coefficient of thermal expansion									
Lopes et al. (31)	1	1	0	0	0	0	0.5	Low	2.5
Microhardness and hardness									

Castanho et al. (26)	1	1	1	0	0	0	1	moderate	4
Collys et al. (22)	1	1	0,5	0	0	0	1	moderate	3,5
Donassollo et al. (27)	1	1	1	0	0	0	1	moderate	4
Feagin et al. (23)	1	1	0	0	0	0	1	moderate	3
Field et al. (4)	1	1	0	0	0	0	1	moderate	3
Fonseca et al. (2)	1	1	1	0,5	0,5	0	1	moderate	5
Lippert and Hara (52)	1	1	0	0	0	0	1	moderate	3
Masuda et al. (25)	1	1	0	0,5	0	0	1	moderate	3,5
Nogueira et al. (43)	1	1	1	0	0	0	1	moderate	4
Reeh et al. (53)	1	1	0	0,5	0	0	0,5	moderate	3
Rios et al. (24)	1	1	0	0	0	0	1	moderate	3
Souza-Gabriel et al. (30)	1	1	0	0,5	0	0	1	moderate	3,5
Strassler et al. (28)	1	1	0	0	0	0	1	moderate	3
Solubility									
Chen and Nancollas (55)	0,5	1	0	0	0	0	0	low	1,5
Han et al. (56)	1	1	0	0	0	0	1	moderate	3
Impedance									
Levinkind et al. (54)	1	1	0	0	0	0	1	moderate	3
Spectrofluorometry/Hardness									
Featherstone and Mellberg (67)	1	1	0	0	0	0	0	low	2
Mineral Analyses/Morphology/Radiodensity									
Edmunds et al. (64)	1	1	0	0	0	0	0	low	2
Meurman and Frank. (57)	1	1	0	0	0	0	NA***	low	2
Fonseca et al. (3)	1	1	0	0	0	0	1	moderate	3
Kielbassa et al. (68)	1	1	0	0	0	0	1	moderate	3
Lynch and Cate (66)	1	1	0	0	0	0	1	moderate	3
Tanaka et al. (70)	1	1	0	0,5	0	0	1	moderate	3,5
Lippert et al. (52)	0,5	1	0	0	0	0	1	low	2,5
Lippert and Lynch (65)	1	1	0	0	0	0	1	moderate	3
Lopes et al. (71)	1	1	0	0,5	0	0	0,5	moderate	3
Ortiz-Ruiz et al. (69)	1	1	0	0	0	0	1	moderate	3

*(High (≥5), moderate (≥ 3 and <5) or low methodological quality (<3) ** 1: Complete information; 0.5: Information incomplete; 0: Information not described ***NA: not applicable

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4.2 Artigo 2 – Artigo Submetido 30/09/2022 – Journal of Prosthetic Dentistry

Analysis of the bonding interface between glass fiber posts and intra-root dentin subjected to simplified alcohol technique

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ANALYSIS OF THE BONDING INTERFACE BETWEEN GLASS FIBER POSTS AND INTRA-ROOT DENTIN SUBJECTED TO SIMPLIFIED ALCOHOL TECHNIQUE

ABSTRACT

Statement of the Problem. The bonding of glass fiber posts to intraradicular dentin, especially at apical third, remains a challenge in dentistry.

Purpose. To evaluate the influence of humidity control with ethanol on the pushout bond strength between glass fiber posts in different thirds of intraradicular dentin, 24 hours and 6 months after the bonding process.

Materials and Methods. Sixty-four bovine incisors were submitted to endodontic treatment and divided into two groups, according to the type of humidity control (conventional and alcoholic technique). Each group was divided in 4 subgroups (n=8) according to cementation: RXU: Single bond Universal + RelyX Ultimate; PNV: Panavia V5 tooth primer + Panavia V5; U200: RelyX U200; SET: Set PP. Pushout bond strength were measured in different areas of post space, 24 hours and 6 months after the bond procedure. Data were subjected to Bonferroni test ($\alpha = 0.05$).

Results. The lowest bond strength values were found for the SET group, with a statistically significant difference between other groups for the cervical and middle thirds. The highest bond strength values were found for PNV group with statistically significant difference for other groups in the middle and apical thirds at 24 hours. Evaluating the different thirds, in general, the highest bond strength values were found for cervical third. The PNV group presented highest bond strength values for the cervical and middle thirds, with no statistically significant difference between them. Referring to the type of humidity control, it can be observed there is no statistically significant difference for PNV group at 24 hours, U200 and SET groups at 6 months. And in the comparison between the times (24 hours and 6 months), in general, the values of bond strength decreased after aging.

Conclusions. The highest bond strength was obtained when ethanol-wet bonding was associated with PNV and the lowest values were associated with SET cement. Significant differences were observed after the de time of six months in the values of bond strength ageing.

Key words. resin cement; dentin; push-out strength; dental bonding

CLINICAL IMPLICATIONS

It is possible to restore endodontically treated teeth that remaining tooth structure cannot provide adequate support and retention for the restoration with fiber posts. Conventional or self-adhesive resin cement are commonly used to lute the fiber post. In this case, the alcohol control of humidity in the root space could interfere and increase bond strength.

INTRODUCTION

Fiber posts have been used to restored endodontically treated teeth with excessive loss of dental structure. Endodontically treated teeth require a differentiated restorative treatment.[1,2] The total or partial loss of coronary structures, such as cusps and pulp chamber roof, can result in dental tissue fracture after the final restoration.[3] The use of intraradicular posts is necessary to provide retention when the remaining coronal tooth structure is not sufficient.[4]

Resin cements are normally used to lute glass fiber posts to intraradicular dentin. Different restorative materials have been proposed for the correct cementation: conventional resin cements associated with adhesive systems (etch-and-rinse or self-etch) or self-adhesive resin cements.[5-13]

Among the prefabricated posts available, glass fiber posts have been used due to their flexural strength and modulus of elasticity similar to the dentin.[14-17] This allows an uniform distribution of tension and reduces the risk of root fracture.[18,19] However, failures may occur at the cementation interface between the post and the post space.[20,21] I these cases, the debond of fiber post to intraradicular dentin is the most common failure, resulting from the degradation of the cement.[22-27]

The use of hydrophilic adhesive and the collagen fibrils degradation that were not protected by the hybrid layer are some causes of the cement degradation.[28] In addition, there is a lack of information on the kinetics of shrinkage and conversion of self-adhesive and conventional dual-polymerization resin luting agents.[29,30] To obtain a quality of adhesive interface for the cementation of intra-radicular posts, different techniques of application of these materials have been seeking to find an alternative can influence the longevity of endodontically treated teeth with glass fiber posts.[31-33]

The alcoholic technique was developed to reduce the degradation of the factors involved in the adhesive process.[27,34,35] Ethanol could displacing the water present in the dentin, and the collagen fibrils could be supported by the alcohol.[3,4] Then, the substrate would be less hydrophilic and more resistant. When the interface adhesive is formed, the hybrid layer could be more hydrophobic that absorb less water and make durable bonds between resin and dentin.[36-38]

This technique is recommended in association with three-step adhesive systems when used before the application of conventional resin cement.[39] However, this same technique can be indicated for self-adhesive resin cement, since adhesion in the root canal is a challenge due to the geometry of the root canal that trap water by surface tension, which makes it difficult to move it without prior treatment.[40]

When analyzing the processes involved the fiber post cementation in restorative dentistry, in addition to the type of resin cement used, the different techniques of approaches that try to improve the bond strength between post-cement-dentin interface are also important. The durable adhesive interface is crucial for the rehabilitation of endodontically treated teeth with fiber posts.[10-25] Since the scarce amount of studies related to the cementation of glass fiber posts using the alcoholic technique, studies addressing the topic are necessary.[26]

On the basis of these considerations, the purpose of this study was to evaluate the push-out bond strengths of glass fiber posts in different regions of the post space (cervical, middle, and apical) through two different pretreatments of the dentin (conventional and alcoholic), with different adhesive system/resin cement interactions at 24 hours and 6 months after the bonding procedure. The null hypotheses tested were: (1) the pushout bond strength of glass fiber post to intraradicular dentin does not depend on the adhesive system/resin cement interaction; (2) there is no difference in the bond strength of glass-fiber post to different thirds of the intraradicular dentin (cervical, middle, and apical); (3) the type of pretreatment of dentin (water-wet bonding - WWB or etanol-wet bonding - EWB) do not affect the bonding effectiveness; and (4) there would be no difference between the values of push-out bond strength when comparing the periods of 24 hours and 6 months.

MATERIALS AND METHODS

The materials used in this study are listed in Table 1. Sixty-four freshly extracted bovine incisors of homogeneous shape and size were selected, cleaned, and stored in water for 3 months at most.[7] Bovine teeth with significant root curvature and/or with root fracture, and/or teeth with open apex were excluded from the study.[41-43] The crowns were removed at the cement-enamel junction using a diamond saw in a precision cutting machine (Isomet 1000, Buehler) under constant water irrigation. The root length was standardized at 18 mm, verified by a digital caliper (Mitutoyo).[26]

The sectioned teeth were radiographed (E - speed Kodak) at 2 angulations to confirm the presence of a single canal.[22] Then, to standardize the canal space, root with similar canal diameters were selected. The canal aperture was tested with a Largo #3 drill (Dentsply Sirona) to ensure selection of similar canal diameters. Only roots that showed some resistance to Largo #3 drill penetration were used.

The specimens were then subjected to endodontic treatment by a single operator. The root canals were prepared using rotary instruments (VDW Silver Reciproc), under irrigation with 2.5% sodium hypochlorite solution (Asfer Indústria Química). After instrumentation, the canals were irrigated with 17% trisodium ethylenediamine tetra-acetic acid solution (EDTA Trissódico; Biodinâmica). The root canals were obturated using a gutta-percha thermoplastic technique (Thermo Pack WL; Easy) with lateral and vertical condensation by hydraulic compression, and AH Plus sealer (Dentsply Sirona). They were then stored in 100% humidity at 37°C for 7 days.

The glass fiber post used was Whitepost DC #2 (FGM). Before the adhesive procedure, treatment of the glass fiber post surfaces was performed with 35% phosphoric acid (Ultradent) for 60 seconds, and the surfaces were washed and dried with air. The post surfaces were silanized for 60 seconds (Ultradent) and gently dried with an air syringe. Finally, according to the treatment that was planned to be performed in the intraradicular dentin, if necessary, the same adhesive system used on the dentin surface was applied to the post surface. And then, the post was not further manipulated in order to avoid contamination.

The post spaces were prepared using Largo burr #3 (Dentsply-Maillefer) and DC drill #2 (FGM) at a slow rotary speed, yielding a 14-mm-long apical canal, 4-mm of which was filled.[7,25] The post spaces were irrigated with 2 mL distilled water to remove any gutta percha debris and to maintain the humidity of the environment. The root canal was dried with air and sterile paper points. The specimens were randomly divided in two groups (n=32) according to the type of humidity control of the dentin, water-wet bonding (WWB) or etanol-wet bonding (EWB). For the WWB, the post space were washed with distilled water (PNV and SET) or 2.5% sodium hypochlorite (RXU and U200) for 60 seconds and the excess were removed using sterile paper points. For the EWB, initially the same procedure for conventional technique were performed and then, the post space were completely filled with 100% ethanol for 60 seconds, and the excess ethanol was gently removed.

After the pretreatment of dentin, the specimens were divided by drawing lots into the following 4 subgroups (n=8), according to the luting procedure used [10]:

RXU group

The Single Bond Universal self-etching adhesive system (3M ESPE) was actively applied for 20 seconds in an even layer over the intraradicular dentin, and air-dried for 10 seconds. A second layer of adhesive was applied spread with gentle air, and light polymerized with VALO® Cordless light curing unit (1000 mW/ cm2; Ultradent) for 10 seconds. The RelyX Ultimate conventional resin cement (3M ESPE) was mixed for 10 seconds and placed in the post space using with a syringe and a needle tip #2 (Precision; Maquira). The resin cement was also applied to the post surface before it was brought into position within the post space; any excess cement was removed. The resin cement was light polymerized for 40 seconds.

PNV group

The Panavia V5 tooth primer self-etching primer (Kuraray Noritake Dental Inc.) was applied to the post space and left for 20 seconds before air dried for 5 seconds. Panavia V5 resin cement (Kuraray Noritake Dental Inc.) was manipulated for 10 seconds and placed in the root canal with a syringe and a needle tip #2 (Precision; Maquira). Then, the resin cement was applied on the post surface, and the fiber post was positioned within the post space; the excess cement was removed. Finally, the resin cement was light polymerized for 40 seconds.

U200 group

The RelyX U200 self-adhesive resin cement (3M ESPE) was mixed for 10 seconds and placed in the post space using with a syringe and a needle tip #2 (Precision; Maquira). The resin cement was also applied to the post surface, the post was properly positioned, and any excess cement was removed. Finally, the resin cement was light polymerized for 40 seconds.

SET group

The Set PP self-adhesive resin cement (SDI) was mixed and inserted into the canal with a syringe and a needle tip #2 (Precision; Maquira). The resin cement was also applied to the post surface, the post was properly positioned, and any excess cement was removed. Then, the resin cement was light polymerized for 40 seconds.

After 24 hours, the teeth were sectioned perpendicular to the long axis using a low-speed diamond saw under water cooling in Isomet 100 (Buehler) to obtain 2 slices of approximately 1.0 mm in thickness to be analyzed from each third (cervical, middle, and apical). One half of the specimens were used to evaluate the pushout bond strength 24 hours after the bonding process, while the other half were stored for 6 months in distilled water at 37°C, changed weekly. The coronal sides of the slices were marked with an insoluble ink.

The pushout bonding strengths were measured in a universal testing machine (EZ-LX Long-Stroke Model, Shimadzu). The post segment was loaded with a cylindrical plunger (0.8 mm in diameter), which was centered on the post segment without any contact with the surrounding dentin surface. A load was applied with a universal testing machine in an apical-to-cervical direction with respect to the specimens, tested at a cross-head speed of 0.5 mm/minute until the post was dislodged. The pushout bond strength was calculated for each specimen using the following formula: $Bs = N/2\pi rh$. Bond strength was calculated by dividing Bs (bond strength) by the division of maximum force (N) by area ($2\pi rh$), where (r) is the radius, (h) is the post height, and π is 3.14.[25]

The failure mode was observed using a stereomicroscope (Carl Zeiss AG) at 40X magnification. Each sample was categorized as follows: adhesive failure (at the cement-dentin or cement-post interface), mixed, and cohesive failure (within the cement).[20]

After the bond strength test, the specimens were sectioned to expose the adhesive interface, coated with gold (Q150T; Quorum Technologies). The specimens were also observed using scanning electron microscopy (SEM) (EVOLS15; ZEISS) to characterize the dentinal structure after bond failure.

The bond strength data were subjected to normality and homoscedasticity tests, and the means were analyzed by Bonferroni test ($\alpha = 0.05$). All statistical analyzes were performed using SPSS software, version 21.0 (SPSS).

RESULTS

In Table 2, comparing the resin cements for the same third, treatment and time, in general, the lowest bond strength values were found for the SET group, with a statistically significant difference between other groups for the cervical and middle thirds, except for the middle third, at 6 months, that there are no statistically significant different to RXU group ($p<0.05$). The highest bond strength values were found for PNV group with statistically significant difference for other groups in the middle and apical thirds at 24 hours.

Evaluating the different thirds, no statistically significant difference was observed for SET group regardless of time and treatment. In general, the highest bond strength values were found for cervical third, except for the SET group, at 24 hours and alcoholic treatment. The PNV group presented highest bond strength values for the cervical and middle thirds, with no statistically significant difference between them (Table 2).

Referring to the type of humidity control, it can be observed there is no statistically significant difference for PNV group at 24 hours, U200 and SET groups at 6 months. In the long term (6 months), EWB improved bond strength values for the PNV group in middle and apical thirds ($p<0.05$), when compared to conventional treatment. The same behavior can be observed for the SET group at 24 hours ($p<0.05$). For the RXU group, the EWB improved the bond strength values in the middle and apical thirds at 24 hours, and cervical and middle thirds at 6 months, with a statistically significant difference for the referred thirds by the WWB ($p<0.01$) (Table 2).

It is also possible to observe in Table 2, in the comparison between the times (24 hours and 6 months), in general, the values of bond strength decreased after aging. However, with a statistically significant difference in a few thirds. For the RXU group, in the cervical ($p<0.05$) and middle thirds ($p<0.05$) in the conventional treatment, and in the apical third for the EWB ($p<0.001$). The PNV group showed a statistically significant difference for the cervical and apical thirds for both types of humidity control (conventional and alcoholic treatment) ($p<0.05$). For the U200 group, there was only a statistically significant difference in the cervical third ($p<0.05$), for the EWB, where the bond strength values decreased after 6 months. Finally, for the SET group, the bond strength values decreased with a statistically significant difference after 6 months for the apical third ($p<0.01$) in the conventional treatment, and for the middle and apical thirds ($p<0.01$) for the EWB.

With respect to the fracture analysis at 24 hours, the main failure type of the all groups was adhesive failures, except for PNV group that showed more mixed failures in some occasions (Figures 2 and 3). After 6 months, there was a predominance of adhesive failure for self-adhesive resin cements, independent of the type of pretreatment of dentin and thirds analyzed. Only PNV group showed some variation in the fracture mode, with a significant number of dentin failures and mixed failures, in addition to adhesive failures (Figures 4 and 5).

DISCUSSION

In the present study, different intraradicular luting procedure approaches to bonding glass fiber posts to dentin were studied in two times. The results showed that the SET group presented the lowest pushout bond strength compared to the other groups for the cervical and middle thirds, except for the middle third in WWB for 6 months, that there are no statistically significant difference to RXU group (Table 2). The highest bond strength values were found for the PNV group with a statistically significant difference for other groups in the middle and apical thirds at 24 hours, resulting in the rejection of the first null hypothesis of the study. The Panavia V5 resin cement (PNF group) contain MDP in the primer, which could have attributed to the strong chemical bond, in addition to the micromechanical retention, that might resulted in significantly higher bond strength values.[13] The MDP interacts chemically with the hydroxyapatite of dentin, and forms a stable nanolayer.[27] This cement was development to simplify a conventional adhesive cement, incorporating a self-etch component and a multi-step adhesive into a single cement system. Then reduces technique-sensitivity and facilitates daily clinic routines.[13] In the specific case of the Set PP self-adhesive resin cement used in SET group presented lower values of bond strength. According to the manufacturer, this cement doesn't have Bis-GMA and HEMA. Bis-GMA has a greater number of covalent bonds, and thus better monomer conversion and greater post-gel polymerization,[23] which could justify the lower values for this cement.

In the analysis of the different thirds of the post space, only the SET group showed no statistically significant difference in the bond strength among the three thirds analyzed regardless of time and treatment, except for 24 hours in alcoholic treatment. In general, the highest bond strength values were found for the cervical third. The PNV group presented the highest bond strength values for the cervical and middle thirds, with no statistically significant difference between them (Table 2), rejecting the second null hypothesis of the study. In general, during the process of photoactivation, light intensity is reduced along the post space, and the cervical third that receives more light radiation. The deeper portions may be inaccessible to light, and a lower degree of conversion in the apical portion may affect the mechanical properties of resin cement. In addition, in apical third normally have a smaller number of dentinal tubules and a greater likelihood of presenting endodontic treatment remnants.[23]

Although all resin cements used in the study are dual-cured, their values were decreased in the course of intraradicular thirds. In places could not have received an adequate light energy (middle and apical thirds), the cements depend almost exclusively on chemical activation of the material. However, the self-adhesive cement had the lowest bond strength values when compared only the chemical activation of the materials,[27] which would explain the results of our study, as the deeper thirds could not received adequate light energy compared to the cervical region.

The results showed that the EWB improved bond strength values of some groups, when compared to WWB, resulting in the rejection of the third null hypothesis of the study. As dentin collagen is highly crosslinked and contains no cell membranes, it is envisaged that the same efficacy of water removal may be achieved by using 100% etanol as the sole chemical dehydrant. In addition, there is no need to worry about the risk of possible pulp complications caused by ethanol, as this is a non-vital tooth.[37,38] In the long term (6 months), PNV and group with EWB presented higher bond strength in middle and apical thirds, when compared to WWB. The same behaviour can be observed for the SET group at 24 hours. When the EWB technique was used, the spacing of collagen fibers after ethanol pretreatment seems to be slightly larger than WWB.[27,35,44] Besides that, the EWB procedure could results in a decreased diameter of collagen fibrils, an increased interfibrous volume of the dentine collagen network, and improved permeability of the adhesive materials and of encapsulation of collagen fibers, thus creating a hydrophobic and more stable hybrid layer with better bonding durability.[29,36,39,40]

For the RXU group, the EWB improved the bond strength values in the middle and apical thirds at 24 hours, and cervical and middle thirds at 6 months, with a statistically significant difference for the referred thirds by the WWB (Table 2). The Single Bond Universal adhesive system and RelyX Ultimate conventional resin cement are considered hydrophobic materials, since they contain Bis-GMA and/or TEGDMA.[38] The EWB strategy has been proposed to the ethanol is used instead of water to support demineralized dentin collagen fibers and to facilitate penetration of hydrophobic materials into ethanol-saturated etched dentin.[39]

Finally, the fourth null hypothesis was rejected because there was a significant difference between values obtained at 24 hours and 6 months. In general, the values of bond strength decrease after aging. It is known that the bond strength and the expression of interfacial nanoleakage of fiber posts cemented to intraradicular dentin with resin cement types are directly affected by the effects of ageing over time.[28,32,37,44] The low amount of light energy in the post space result in a lower degree of conversion of the resin materials. Then, a low degree of conversion can reduce the bond strength and increase the adhesive layer permeability. The aging in water for 6 months could result to the passage of water through polymer network leads to a decrease in the mechanical properties of the formed polymer. Over time, the polymer produces a relaxation process due the water uptake of the polymer. Thus, the polymer chains separate by a reduction in the frictional forces that leads to degradation of the material, and consequently a reduction in the long term bond strength.[33]

In the present study, when evaluating the bonding behavior of endodontically treated teeth restored with fiber posts, it was possible to observe different failure patterns as adhesive, mixed and dentin failure.[18,31,41-43] The failure mode was observed under a stereomicroscope (Carl Zeiss AG) at 40X magnification.[18,23] Most of the failures observed in the self-adhesive resin cements (U200 and SET groups) were adhesive failures (Figures 2 to 5), corroborating with the lowest bond strength values, mainly for the SET group. The scanning electron microscopy (SEM) images of U200 (Figure 6) and SET (Figure 7) groups showed the dentinal tubules with resin cement on their surface. For the SET group it is possible to observe a cleaner dentin surface, with more dentinal tubules, suggesting a worse adhesion of the resin cement to the substrate (Figure 7). When compared to the immediate bond strength (24 hours) of conventional and self-adhesive resin cements, self-adhesive cements have lower values.[11,12,14] After 6 months, a greater variety in fracture patterns could be observed with an increase in adhesive fractures for the self-adhesive cements (Figures 4 and 5). The PNV group showed more dentin and mixed failures, in EWB and WWB (Figure 8). These fracture patterns suggest better adhesive performance for PNV regardless of the type of treatment. The better performance could be justified by the formation of a hybrid layer that would provide better adhesion of the cement in the medium and long-term at the intraradicular dentin.[32]

CONCLUSION

The EWB improved bond strength values for fiber post cementation to the intraradicular dentin. The highest bond strength values was observed when EWB was associated with Panavia V5 resin cement and the lowest values were associated with Set PP resin cement. The light-curing access level influenced the bond strength between glass fiber posts to dentin. Statistically significant differences were observed after six months when compared to 24 hours.

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TABLES

Table 1. Adhesives and resin cements used in this study.

Product Name	Product Type	Batch No.	Composition[a]	Manufacturer
Single Bond Universal	Photo activation adhesive system	80284930291	Bis-GMA, HEMA, DGDMA, Ethanol, Water, MDP, Silane-treated sílica, 2-propenoic acid, 2-methyl-, reaction products with 1,10-decanediol and phosphorous oxide, Copolymer of acrylic and itaconic acid, Dimethylaminobenzoate, (Dimethylamino) ethyl methacrylate, Methyl ethyl ketone	3M ESPE
Panavia V5 Tooth Primer	Tooth primer	8R0081	2-hydroxyethyl methacrylate 10-Methacryloyloxydecyl dihydrogen phosphate Hydrophilic aliphatic dimethacrylate Accelerators Water	Kuraray Noritake Dental Inc.
Clearfil Ceramic Primer Plus	Ceramic Primer	3P0053	3-Methacryloxypropyl trimethoxysilane 10- Methacryloyloxydecyl dihydrogen phosphate (MDP) Ethanol	Kuraray Noritake Dental Inc.
Panavia V5	Dual adhesive system	9A0068	2-hydroxyethyl methacrylate, 10-Methacryloyloxydecyl dihydrogen phosphate, Hydrophilic aliphatic dimethacrylate, Accelerators, Water	Kuraray Noritake Dental Inc.
RelyX Ultimate	Dual adhesive system	2116900688	Base paste: Bis-GMA, TEGDMA, benzoyl peroxide; catalyst paste: Bis-GMA, TEGDMA, photoinitiator system, amine, peroxide, zirconia-silica filler 67.5% by weight	3M ESPE
RelyX U200	Self-adhesive resin cement	2125000120	Base paste: methacrylate monomers that contain phosphoric acid groups, methacrylate monomers, silanated fillers, initiator components, stabilizers; catalyst paste: methacrylate monomers, alkaline (basic) fillers, silanated fillers, initiator components, stabilizers, pigments	3M ESPE
Set	Self-adhesive resin cement	S0807171	Methacrylate ester phosphoric acids, UDMA, photoinitiator, glass of fluoride aluminum silicate (67 wt%), and pyrogenic silica (45 v%)	SDI Limited

Table 2. Values of pushout bonding strength [BS] (MPa) in designated thirds (cervical, middle, and apical) of intraradicular dentin treated with different type of humidity control (WWB and EWB) 24 hours and 6 months after the bonding process

		WWB			EWB		
		Cervical	Middle	Apical	Cervical	Middle	Apical
24 hours	RXU	11.9 Aa (9.6 - 14.8)	6.2 Bb (4.8 - 8.0)	3.2 Bc (2.4 - 4.4)	15.2 Aa (12.4 - 18.7)	11.3 Aa* (9.1 - 14.1)	7.1 Bb* (5.5 - 9.1)
	PNV	15.0 Aa (12.2 - 18.4)	10.9 Aab (8.7 - 13.7)	9.7 Ab (7.7 - 12.1)	15.8 Aa (12.9 - 19.4)	11.1 Aab (8.9 - 13.8)	10.4 Ab (8.3 - 13.1)
	U200	11.6 Aa (9.3 - 14.5)	5.8 Bb (4.5 - 7.5)	5.1 Bb (3.9 - 6.7)	14.8 Aa (12.0 - 18.2)	11.2 Aa* (9.0 - 14.0)	6.7 Bb (5.2 - 8.6)
	SET	3.0 Ba (2.2 - 4.0)	2.7 Ca (1.9 - 3.7)	4.1 Ba (3.1 - 5.5)	3.0 Bb (2.2 - 4.0)	5.1 Ba* (3.9 - 6.7)	6.2 Ba* (4.8 - 8.0)
6 months	RXU	7.5 Aa (5.9 - 9.5)	3.8 Bb (2.9 - 5.1)	2.4 Bb (1.7 - 3.3)	13.6 Aa* (11.0 - 16.8)	8.8 Ab* (7.0 - 11.2)	2.8 Bc (2.1 - 3.9)
	PNV	10.9 Aa (8.7 - 13.6)	8.5 Aa (6.7 - 10.8)	4.5 Ab (3.4 - 6.0)	10.9 Aa (8.7 - 13.6)	12.4 Aa* (10.0 - 15.4)	6.9 Ab* (5.3 - 8.8)
	U200	8.8 Aa (7.0 - 11.1)	7.9 Aa (6.2 - 10.1)	5.0 Ab (3.8 - 6.5)	10.22 Aa (8.2 - 12.8)	9.71 Aa (7.7 - 12.2)	5.97 Ab (4.6 - 7.7)
	SET	2.4 Ba (1.7 - 3.3)	2.5 Ba (1.8 - 3.4)	2.1 Ba (1.5 - 2.9)	2.36 Ba (1.7 - 3.3)	2.87 Ba (2.1 - 3.9)	3.07 Ba (2.3 - 4.2)

**Means followed by distinct letters. Capital letters compare cement for the same third and treatment. Lower case letters compare different thirds for the same cement and treatment. Means of the EWB followed by an asterisk (*) differ from the WWB, for the same third, cement and time. Means of 6-months in gray cells differ from the 24-hours for the same third, cement and treatment.

FIGURES LEGEND

Fig. 1. Specimen preparation: (A) Removal of anatomical crown of tooth at the cementum-enamel junction, (B) Endodontic treatment performed using a gutta-percha thermoplastic technique, (C) Intraradicular post space preparation (D) Post-luting procedure. After 7 days, slices of ± 1.0 mm were obtained from each third of the post space to be analyzed (cervical, middle, and apical). (E) Extrusion shear test (push-out) performed with active tip 0.8 mm in diameter and crosshead speed of 0.5 mm/min. (F) Specimens were sectioned to obtain SEM images.

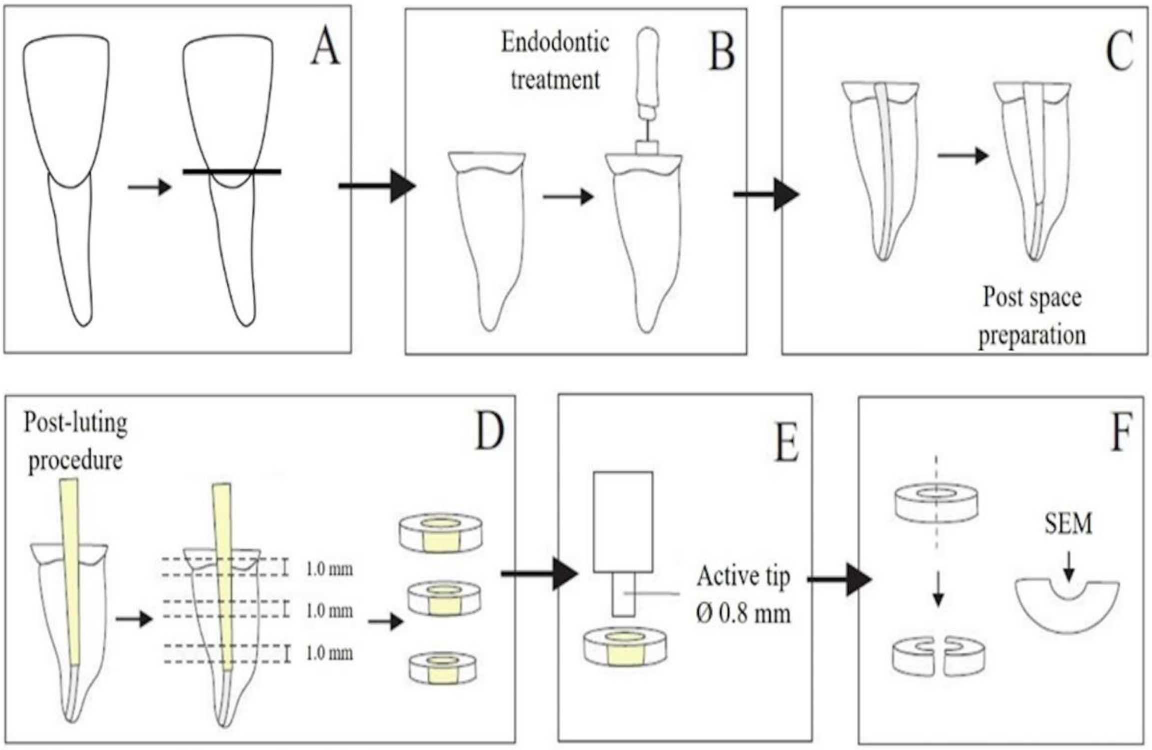


Fig. 2. Fracture pattern observed through the push-out test at 24 hours after the bond procedure in Water-wet bonding (WWB) in thickness to be analyzed from each third cervical (C), middle (M), and apical (A). Fracture adhesive failure, mixed failure and dentin failure. RXU: Single bond Universal + RelyX Ultimate; PNV: Panavia V5 tooth primer + Panavia V5; U200: RelyX U200; SET: Set PP.

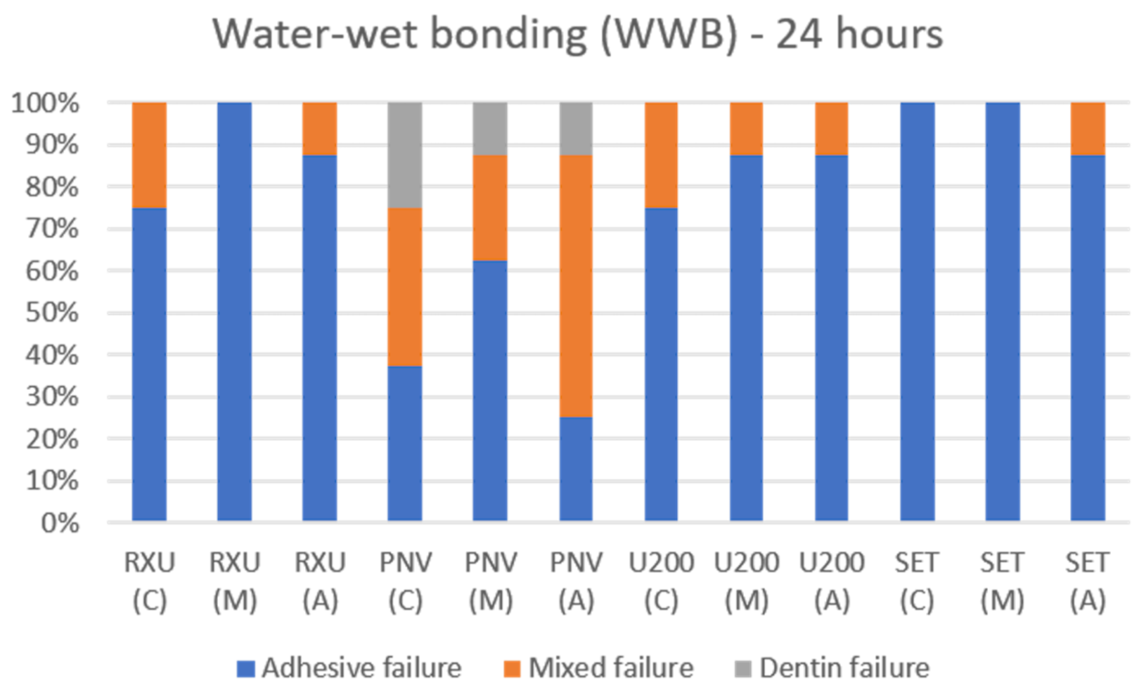


Fig 3. Fracture pattern observed through the push-out test at 24 hours after the bond procedure in Etanol-wet (EWB) in thickness to be analyzed from each third cervical (C), middle (M), and apical (A). Fracture adhesive failure, mixed failure and dentin failure. RXU: Single bond Universal + RelyX Ultimate; PNV: Panavia V5 tooth primer + Panavia V5; U200: RelyX U200; SET: Set PP.

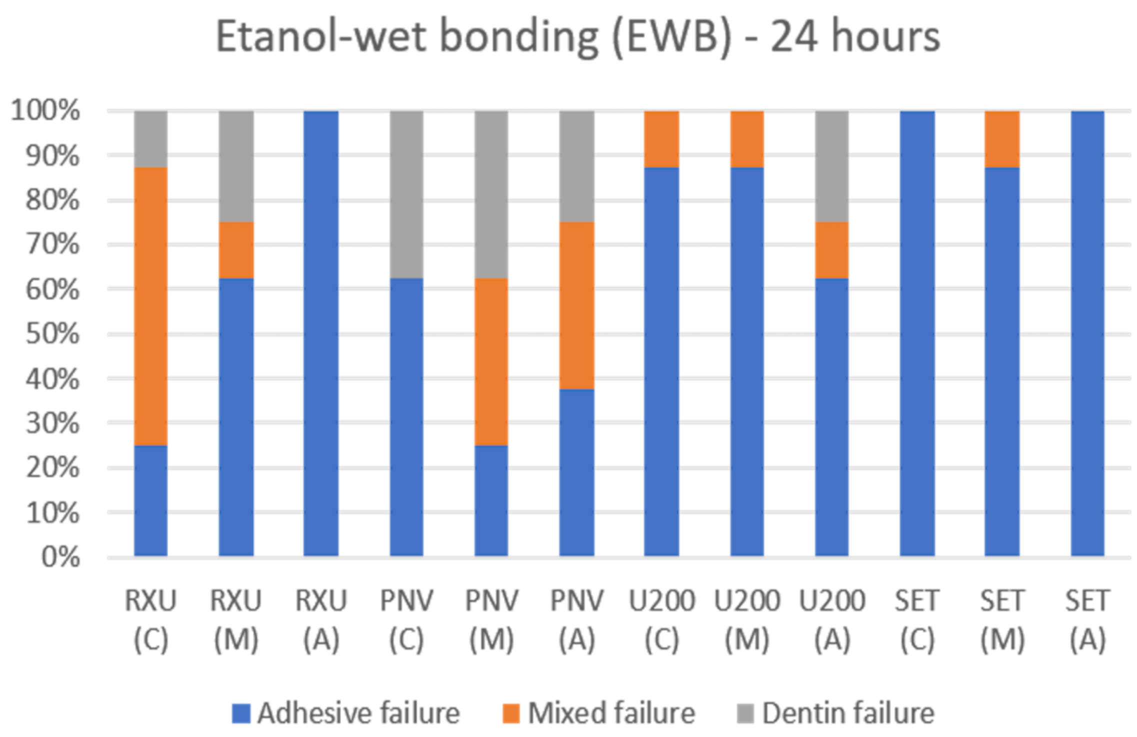


Fig. 4. Fracture pattern observed through the push-out test at 6 months after the bond procedure in Water-wet bonding (WWB) in thickness to be analyzed from each third cervical (C), middle (M), and apical (A). Fracture adhesive failure, mixed failure and dentin failure. RXU: Single bond Universal + RelyX Ultimate; PNV: Panavia V5 tooth primer + Panavia V5; U200: RelyX U200; SET: Set PP.

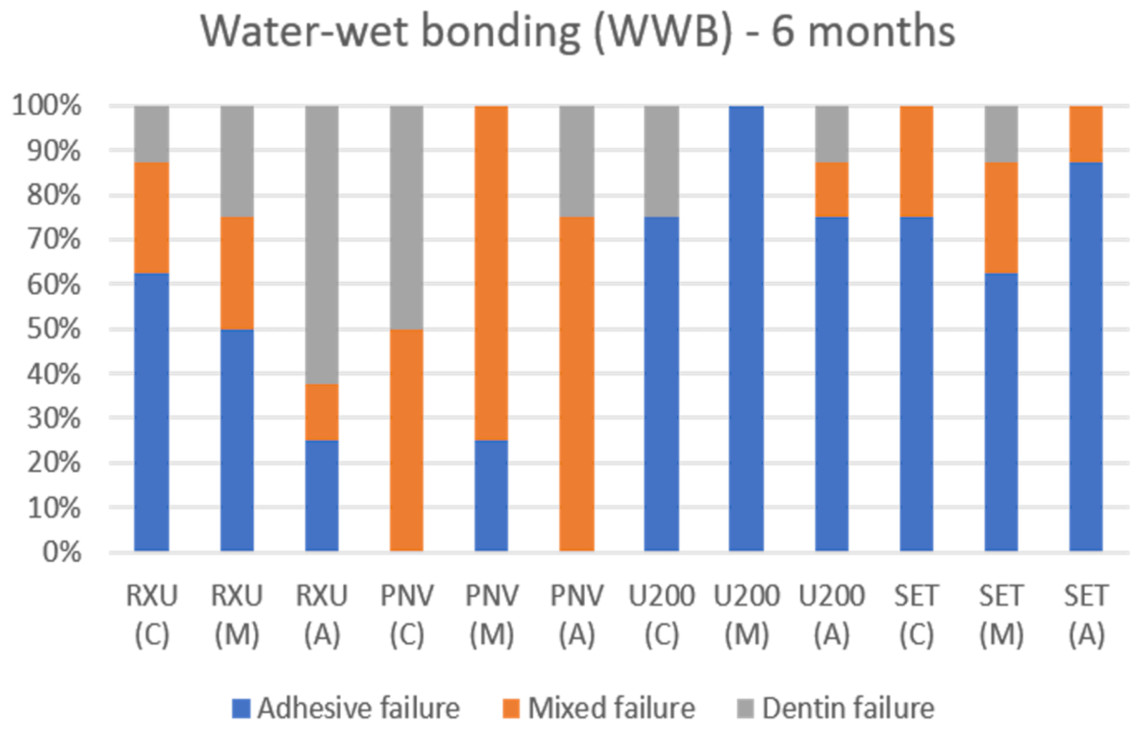


Fig 5. Fracture pattern observed through the push-out test at 6 months after the bond procedure in Etanol-wet (EWB) in thickness to be analyzed from each third cervical (C), middle (M), and apical (A). Fracture adhesive failure, mixed failure and dentin failure. RXU: Single bond Universal + RelyX Ultimate; PNV: Panavia V5 tooth primer + Panavia V5; U200: RelyX U200; SET: Set PP.

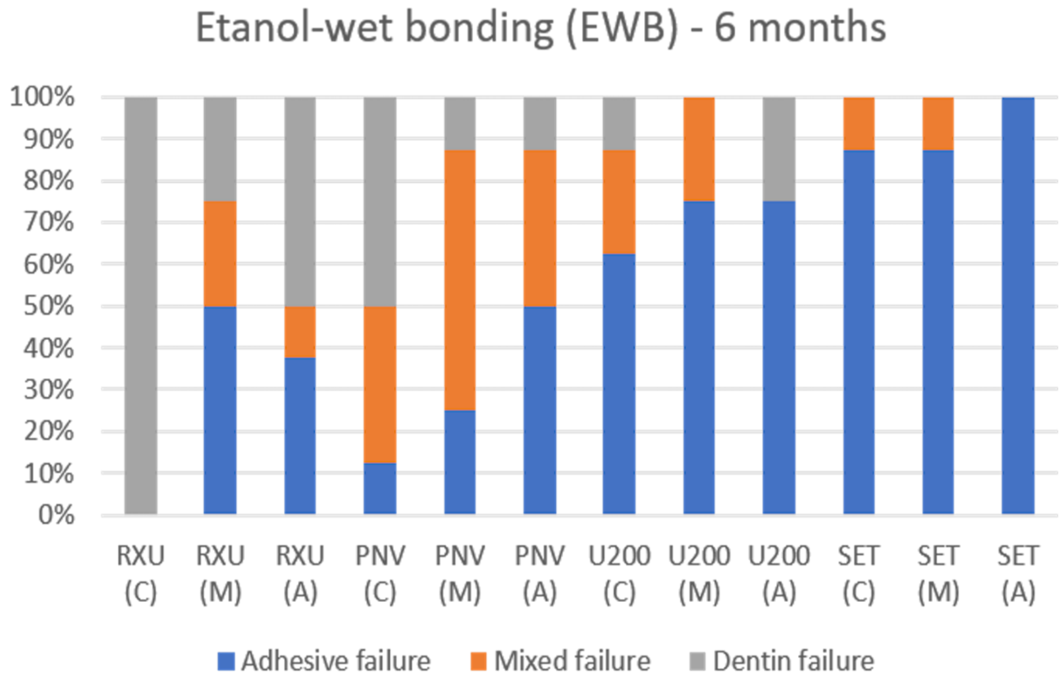


Fig. 6. Representative specimen of intraradicular dentin at 6 months in EWB. Obtained from the U200: RelyX U200 group in the middle third

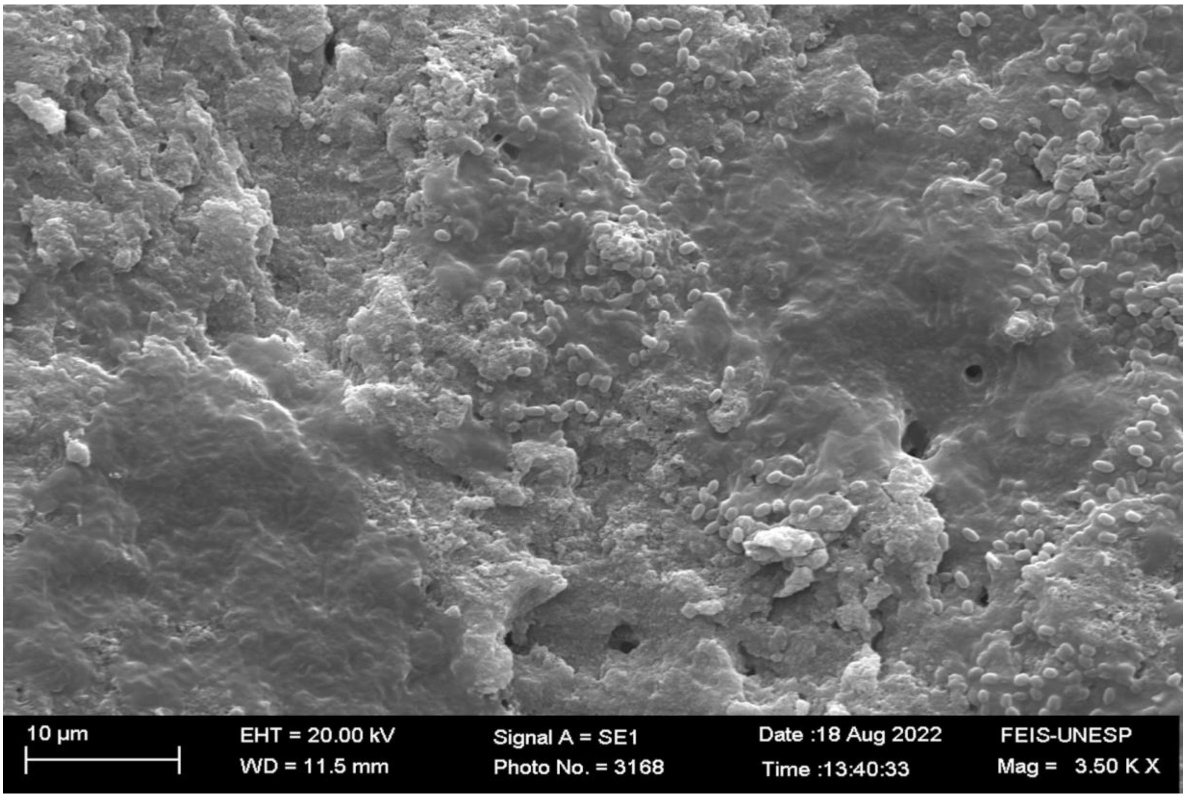


Fig. 7. Representative specimen of intraradicular dentin at 24 hours in EWB. Obtained from the SET: Set PP group in the middle third.

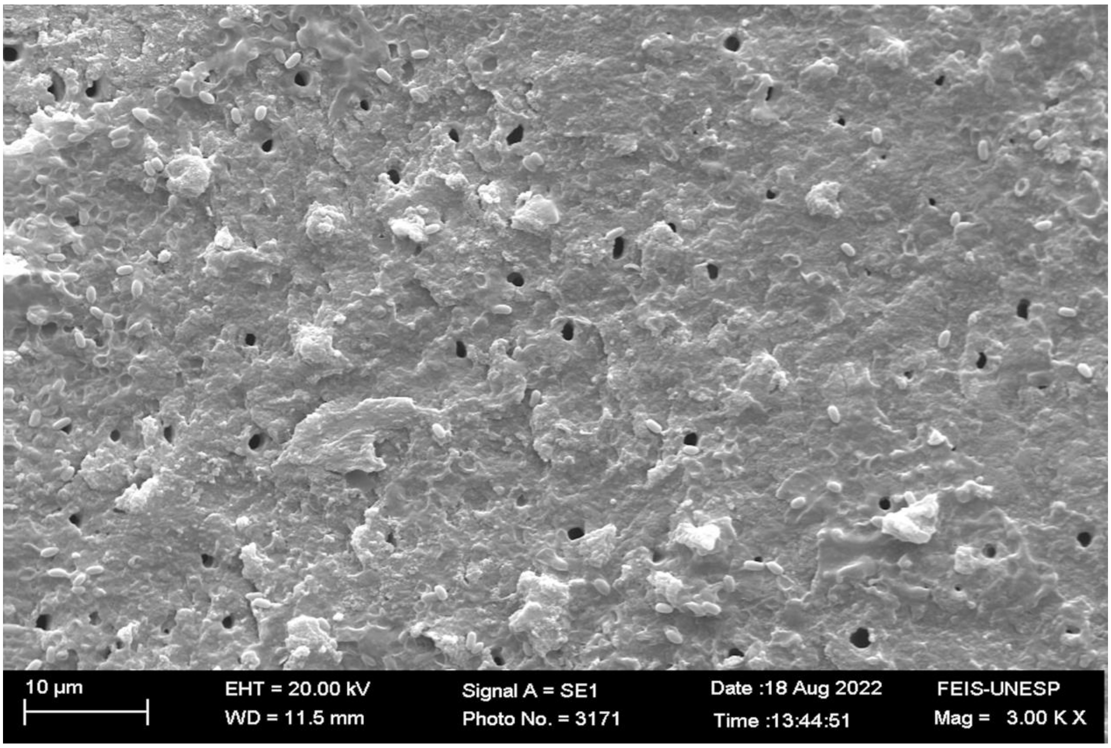
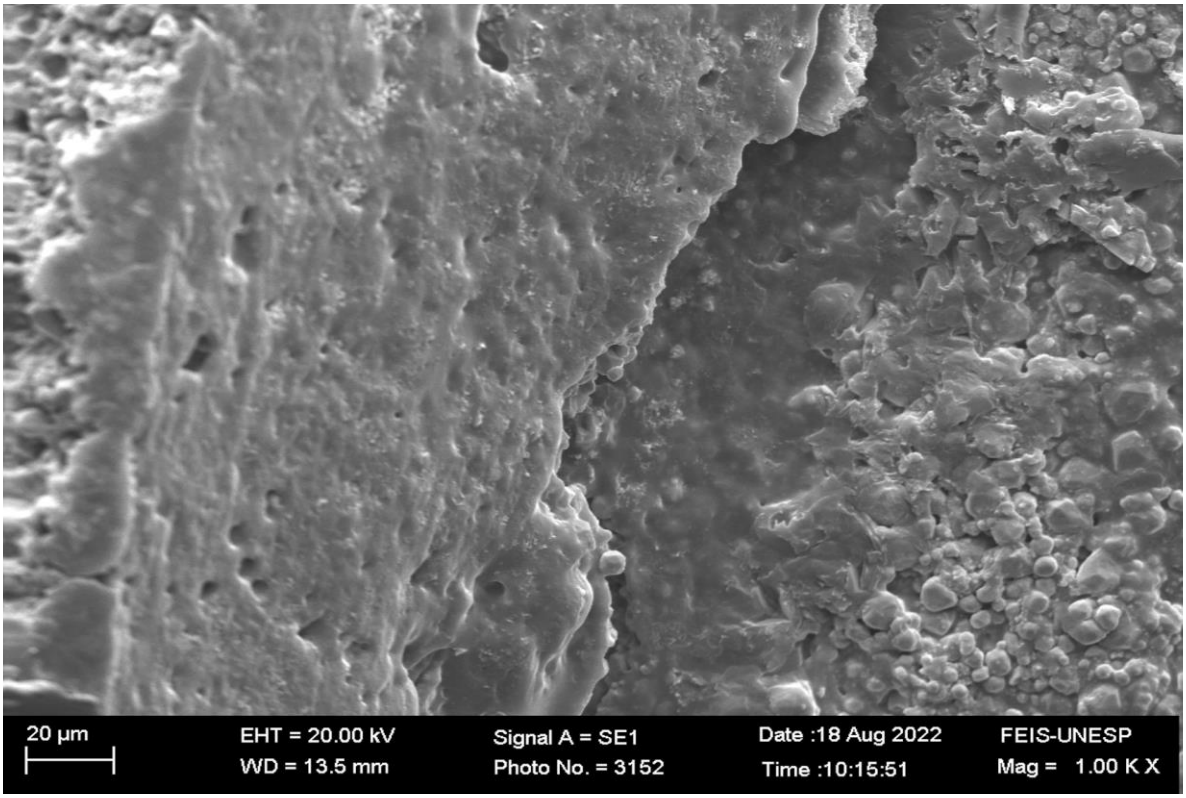


Fig. 8. Representative specimen of intraradicular dentin at 6 months in EWB. Obtained from the PNV: Panavia V5 tooth primer + Panavia V5 group in the apical third.



5 CONSIDERAÇÕES FINAIS

Dentro das limitações do estudo, as conclusões encontradas foram:

1. O tratamento alcoólico melhorou os valores de resistência de união para cimentação de pinos de fibra à dentina intrarradicular.
2. Os maiores valores de resistência de união foram observados quando o tratamento alcoólico foi associado ao cimento resinoso Panavia V5.
3. Os menores valores de resistência de união foram observados associados ao cimento resinoso Set PP.
4. O nível de acesso do aparelho fotopolimerizável influenciou a resistência de união entre pinos de fibra de vidro à dentina.
5. Diferenças significativas foram observadas após seis meses quando comparadas a 24 horas.

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