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ATIVAÇÃO ULTRASSÔNICA DE DIFERENTES SISTEMAS ADESIVOS AUTOCONDICIONANTES –RESISTÊNCIA DE UNIÃO DE PINOS DE FIBRA DE VIDRO E PENETRAÇÃO INTRATUBULAR

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Orientador: Prof. Dr. Marcus Vinicius Reis Só

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Dedico essa dissertação aos meus pais, que dedicam muito de suas vidas a mim.

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RESUMO

ARRUÉ, THALITA AYRES. 2024. Ativação ultrassônica de diferentes sistemas adesivos autocondicionantes – resistência de união de pinos de fibra de vidro e penetração intratubular. Dissertação (Pós-Graduação em Odontologia - Clínica Odontológica - Endodontia) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2024.

Objetivos: Avaliar o efeito da ativação ultrassônica de sistemas adesivos autocondicionantes (Clearfil SE, Prime & Bond Universal e Single Bond Universal) na penetração intratubular destes adesivos e na resistência de união de pinos de fibra de vidro à dentina radicular através de Microscopia Confocal de Varredura a Laser (MCVL) e teste de *push-out*. **Materiais e métodos:** A amostra foi composta de 60 dentes bovinos extraídos, os quais tiveram suas raízes seccionadas e padronizadas. Os canais radiculares foram preparados e tiveram seus terços apicais obturados. Três adesivos autocondicionantes foram selecionados para o estudo (Clearfil SE, Prime & Bond Universal e Single Bond Universal). A amostra foi dividida em seis grupos com 10 raízes. Em três grupos, foi relizado o protocolo adesivo indicado pelo fabricante de cada sistema e, nos demais, foi realizada a ativação ultrassônica do sistema adesivo, sendo 20 segundos de ativação do primer e 10 segundos de ativação do adesivo (sistema Clearfil SE) e 20 segundos de ativação do adesivo nos dois outros grupos. Em todos os dentes, foi realizada a cimentação de pinos de fibra com o cimento RelyX ARC. As raízes tiveram sua porção do pino dividida em três fatias de 2mm de espessura. Para análise da penetração intratubular, as fatias referentes ao terço apical do pino foram submetidas à MCVL e tiveram suas imagens examinadas. Para análise da resistência de união dos pinos de fibra, todas as fatias foram submetidas ao teste de *push-out*. Para determinação do padrão de falha ocorrido no teste de *push-out*, todos os cortes foram analisados sob microscópio óptico em aumento de 10x. Dados de resistência de união foram analisados estatisticamente por ANOVA (1 via) e teste *post hoc* de *Tukey*. Para comparação dos protocolos padrão e com ativação ultrassônica, foi utilizado teste *t de Student*. Para a penetração intratubular dos adesivos foi realizada uma análise descritiva das imagens geradas por MCVL de cada grupo estudado. **Resultados:** A penetração intratubular foi visualmente superior nos grupos submetidos à ativação ultrassônica dos sistemas adesivos. Quanto à resistência de união, não houve diferença entre os terços do canal protético. No protocolo padrão, o adesivo Prime & Bond Universal apresentou menor resistência de união ($P<0.05$). No protocolo de ativação ultrassônica, o adesivo Clearfil SE apresentou superior resistência de união ($P<0.05$). Quando comparados os protocolos padrão e com ativação ultrassônica, os adesivos Clearfil SE e Prime & Bond Universal apresentaram maiores valores de resistência de união quando submetidos à ativação ultrassônica ($P<0.05$). **Conclusões:** A ativação ultrassônica aumentou a penetração intratubular dos adesivos autocondicionantes e a força de união do Clearfil SE e Prime & Bond Universal. Ativação ultrassônica influenciou na prevalência dos padrões de falha observados após teste de *push-out.*

Palavras-chave: Adesivos Dentinários, Análise do Estresse Dentário, Endodontia, Pinos Dentários, Ultrassom

ABSTRACT

ARRUÉ, THALITA AYRES. 2024. Ultrasonic activation of different self-etch adhesive systems – dentin tubule penetration and push-out bond strenght of fiber posts. Dissertation (Postgraduate in Dentistry, Dental Clinic - Endodontic) – School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, 2024.

Objectives: To evaluate the effect of ultrasonic activation on self-etch adhesive systems (Clearfil SE, Prime & Bond Universal, and Single Bond Universal) on the dentin tubule penetration of these adhesives and on the bond strength of fiberglass posts to root dentin using confocal laser scanning microscopy (CLSM) and push-out test. **Material and methods:** Sixty extracted bovine teeth had their roots sectioned and standardized. The root canals were prepared, and their apical thirds were filled. Three self-etching adhesives were selected for this study (Clearfil SE, Prime & Bond Universal, and Single Bond Universal). The sample was divided into six groups with ten roots each. The manufacturer's adhesive protocols were applied in three groups. In three groups, the adhesive systems were ultrasonic activated for 20 seconds on primer, 10 seconds on bond (Clearfil SE system), and 20 seconds on the other systems' adhesives. Fiber posts were cemented with RelyX ARC cement in all roots. The prosthetic portions of the roots were cut into three 2mm thick slices. The images of the apical third of the posts were examined with CLSM to observe dentin tubule penetration. All slices were submitted to a push-out test to obtain the bond strength of fiber posts to root dentin. The failure patterns were analyzed under an optical microscope at 10x magnification. Bond strength values were statistically analyzed using 1-way ANOVA and Tukey's *post hoc* test. The students' t-test was used to compare the standard and the ultrasonic activation protocols. The dentinal tubule penetration of the adhesives was described after CLSM's image observation and analysis. **Results:** Adhesive dentin tubule penetration was visually superior in ultrasonic-activated adhesive systems. There was no difference between the thirds of the prosthetic canal's bond strength. In the standard adhesive protocols, the Prime & Bond Universal system presented lower bond strength ($P<0.05$). In the adhesive ultrasonic activation protocols, the Clearfil SE system presented higher bond strength ($P<0.05$). Comparing standard and ultrasonic activation protocols, Clearfil SE and Prime & Bond Universal adhesive systems presented higher bond strength values when ultrasonic activated ($P<0.05$). **Conclusions:** Ultrasonic activation improved self-etch adhesives dentinal tubule penetration and bond strength of Clearfil SE and Prime & Bond Universal. Ultrasonic activation of self-etch adhesive systems influenced the prevalence of adhesive failure patterns.

Keywords: Dental Pins, Dental Stress Analysis, Dentin-Bonding Agents, Endodontics, Ultrasonics

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LISTA DE ABREVIATURAS E SIGLAS

% – porcentagem

ºC – graus celsius

ANOVA – análise de variância

CLSM – confocal laser scanning microscopy

EDTA – Ácido etilenodiaminotetracético

Fig – figure

Hz – hertz

MCVL – microscopia confocal de varredura a laser

MHz – megahertz

mL – mililitro

mm – milímetro

mPa – megapascal

NaOCl – hipoclorito de sódio

nm – nanômetro

UFRGS – Universidade Federal do Rio Grande do Sul

SUMÁRIO

1 ANTECEDENTES E JUSTIFICATIVA

No processo de reabilitação de dentes endodonticamente tratados com extensa perda de estrutura, a cimentação de pinos intrarradiculares é muitas vezes indicada (PEROZ et al., 2005; FERRARI et al., 2007; CARVALHO et al., 2018), uma vez que o reduzido volume de dentina perde elasticidade e resistência na ausência do tecido pulpar (SOARES et al., 2007). Pinos de fibra de vidro apresentam módulo de elasticidade similar ao da dentina, o que possibilita uma distribuição mais homogênea das forças que incidem sobre o dente reabilitado (MACCARI et al., 2007; CARVALHO et al., 2018). Tal característica do pino de fibra é a principal razão de sua ampla indicação nestes casos, uma vez que as taxas de falhas catastróficas, que levariam necessariamente à perda do dente, são reduzidas; enquanto falhas passíveis de reparo são mais prevalentes (COELHO et al., 2009; SCHMITTER, HAMADI, RAMMELSBERG, 2011; OZTURK et al., 2019).

É necessário o uso de um agente cimentante para adequada acomodação do pino de fibra à superfície dentária. Tendo em vista as características do pino de fibra e frente às propriedades dos sistemas adesivos atuais, os cimentos resinosos têm sido a escolha nesses casos (BERGOLI et al., 2012; KHEUR et al., 2020; LEUNG et al., 2022).

Os cimentos resinosos apresentam alta resistência a forças flexurais e de compressão, baixos coeficientes de expansão térmica, alta retenção e baixa permeabilidade marginal, além de um maior apelo estético quando comparados aos cimentos utilizados anteriormente, como o cimento de fosfato de zinco (WIEDENMANN et al., 2021; HEBOYAN et al., 2023). Sua classificação é feita de acordo com diferentes características. Podem ser autopolimerizáveis, ou seja, quimicamente ativados; fotopolimerizáveis, quando ativados pela luz; ou de dupla ativação, sendo necessárias tanto a fotoativação quanto a ativação química. Além disso, podem ser classificados como convencionais ou autoadesivos. Os cimentos resinosos convencionais requerem a aplicação prévia de um sistema adesivo ao tecido dentário, enquanto os autoadesivos o dispensam (HEBOYAN et al., 2023).

O RelyX ARC (3M ESPE, St. Paul, MN, USA) é conceituado como padrão-ouro entre os cimentos resinosos convencionais de dupla ativação (GIACHETTI et al., 2009; AMARAL et al., 2011; NESELLO et al., 2022).

Os sistemas adesivos apresentam, também, diferentes classificações de acordo com suas características. Classificam-se, basicamente, como convencionais, autocondicionantes e universais. Os sistemas adesivos convencionais apresentam-se em formulações de dois ou três passos clínicos. Caracterizam-se pela aplicação do ácido fosfórico à estrutura dentária previamente aos demais passos. O passo subsequente é a aplicação de um primer e de um adesivo (três passos), ou de uma solução em que ambos estão presentes (dois passos) (ARINELLI et al., 2016). A aplicação do ácido fosfórico tem como objetivo promover uma zona de desmineralização do tecido dentário e da *smear layer*, quando presente. Em esmalte, o ácido cria microporosidades onde haverá penetração de monômeros resinosos. Em dentina, causa a abertura dos túbulos dentinários e a formação da camada híbrida pela penetração dos monômeros e sua relação com as fibras colágenas expostas (WU et al., 2010; OZER; BLATZ, 2013; DRESSANO et al., 2020 SAIKAEW et al., 2022).

Os sistemas adesivos autocondicionantes dispensam a aplicação do ácido fosfórico. Surgiram com o objetivo de reduzir o risco de erros na aplicação da técnica adesiva e simplificá-la, além de diminuir a sensibilidade da técnica em relação à umidade dentinária. Estão disponíveis em um ou dois passos clínicos, sendo eles a aplicação de um *primer* acídico seguido do adesivo (dois passos) ou em apenas uma solução adesiva que inclui ambos (um passo) (ARINELLI et al., 2016; SOFAN et al., 2017). Sem a ação isolada do ácido fosfórico, um residual da *smear layer* que não foi totalmente desmineralizada pelo *primer* permanece sobre a estrutura e é incorporada à camada híbrida, podendo afetar a performance da adesão química do adesivo. Apesar disso, com o passar do tempo, a evolução dos sistemas e técnicas de aplicação possibilitaram que os adesivos autocondicionantes se tornassem eficazes e amplamente utilizados (SOFAN et al., 2017; SAIKAEW et al., 2020; SAIKAEW et al., 2022).

Os sistemas adesivos universais são aqueles em que é possível que se faça tanto a aplicação convencional quanto a autocondicionante. A criação dos adesivos universais teve o intuito de simplificar ainda mais a técnica adesiva e contemplar as particularidades da adesão ao esmalte e à dentina com um mesmo produto. Ainda assim, demanda atenção com relação à adesividade à dentina na técnica autocondicionante, pela reduzida desmineralização da *smear layer* (SOFAN et al., 2017; DRESSANO et al., 2020; SAIKAEW, 2022).

O padrão de falha mais prevalentemente observado na reabilitação com pinos de fibra e cimento resinoso é a falha adesiva. No intuito de reduzir a incidência de falhas adesivas, busca-se a constante evolução tecnológica dos materiais e também novas técnicas de aplicação dos mesmos (AMARAL et al., 2011; SCOTTI et al., 2014; LOPES et al., 2021; VERDUM et al., 2022).

O emprego do ultrassom tem crescido em diferentes áreas e procedimentos na odontologia. Com relação à Endodontia, técnicas envolvendo a ativação ultrassônica durante a irrigação do sistema de canais, na aplicação de medicações intracanais, em cimentos endodônticos, na resolução de intercorrências e em cirurgias têm sido amplamente estudadas e praticadas na clínica (ALCALDE et al., 2017; PAIXÃO et al., 2022; SETZER; KRATCHMAN, 2022; SILVA et al., 2022).

A ativação ultrassônica é realizada com insertos de formatos específicos para cada finalidade acoplados a um aparelho de ultrassom. A ativação com insertos ultrassônicos apresenta efeitos como agitação de soluções e cavitação de superfícies, além do aumento de pressão e temperatura do ambiente, o que pode alterar propriedades de materiais (BOTSIOUKIS; ARIAS-MOLIZ, 2022; LIU et al., 2022).

Estudos já foram feitos avaliando a aplicação sônica de sistemas adesivos na cimentação de pinos de fibra à dentina radicular. Entretanto, com frequências entre 1000 e 6000 Hz (CUADROS-SANCHEZ et al., 2014; ZARPELLON et al., 2016), inferiores a frequências acima de 20.000 Hz geradas pelos aparelhos ultrassônicos. Estudos que avaliaram a ativação ultrassônica de sistemas adesivos são também encontrados na literatura (BAGIS et al., 2008; BAGIS et al., 2009), embora sejam dedicados a realizar análises em dentina coronária.

Verdum et al., em 2022, testaram o efeito da ativação ultrassônica em diferentes sistemas adesivos e cimentos na cimentação de pinos de fibra. Como resultados, observaram que a ativação ultrassônica do sistema adesivo previamente à cimentação com cimento resinoso aumentou a penetração intratubular do adesivo autocondicionante (Clearfil SE), diferentemente do adesivo convencional de 2 passos (Scotch Bond Multiuso) e a força de união do adesivo autocondicionante à dentina radicular foi superior ao convencional e ao cimento de ionômero de vidro.

Até o presente momento, não há mais estudos em que se avalie a ativação ultrassônica de sistemas adesivos na cimentação de pinos de fibra. Tendo em vista o estudo de Verdum et al. (2022), as propriedades de cada material e técnica envolvidos na cimentação adesiva de pinos de fibra e sendo a falha adesiva identificada como uma das maiores fragilidades no processo, a ativação ultrassônica do sistema adesivo autocondionante pode ser uma técnica que venha a aprimorá-lo.

2 OBJETIVOS

2.1 Objetivo Geral

Avaliar o efeito da ativação ultrassônica de sistemas adesivos autocondicionantes (Clearfil SE, Prime & Bond Universal e Single Bond Universal) na penetração intratubular destes adesivos e na resistência de união de pinos de fibra de vidro à dentina radicular.

2.2 Objetivos Específicos

- Comparar a penetração intratubular dos sistemas adesivos de acordo com os protocolos de aplicação utilizados.
- Comparar os valores de resistência de união dos pinos de fibra à dentina radicular após utilização de diferentes sistemas adesivos autocondicionantes e diferentes protocolos de aplicação.
- Descrever a penetração intratubular dos sistemas adesivos na região apical do pino de fibra.
- Avaliar os valores de resistência de união nas diferentes regiões do pino de fibra (cervical, médio e apical).
- Descrever os padrões de falha após o teste de resistência de união com o auxílio de microscopia óptica.

3 ARTIGO CIENTÍFICO

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ULTRASONIC ACTIVATION OF DIFFERENT SELF-ETCH ADHESIVE SYSTEMS – DENTIN TUBULE PENETRATION AND PUSH-OUT BOND STRENGHT OF FIBER POSTS

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Abstract

This study evaluated the dentin tubule penetration of three self-etching adhesive systems and the bond strength of fiber posts cementation using standard protocols and ultrasonic activation of the adhesive system. The 60 extracted bovine teeth sample had its canals prepared and the apical thirds filled. The sample was divided into six groups. Clearfil SE adhesive system was applied in two groups, the Prime & Bond Universal system in two groups and the Single Bond Universal system in two groups. The standard adhesive application protocol was executed in one group, and ultrasonic activation of the adhesive system was executed in one group for each adhesive system. Afterward, fiber posts were cemented in all canals using resin cement (RelyX ARC). The samples were prepared and analyzed under confocal laser scanning microscopy (CLSM) to observe the adhesive's dentin tubule penetration. The samples were also submitted to a push-out test to evaluate the bond strength values of the posts. Failure patterns were determined by optical microscopy analysis. Post-bond strength values were analyzed using 1-way ANOVA and Tukey's *post hoc* test. The Student's T test was performed to compare the standard application protocols and those with ultrasonic activation of the adhesive systems. The dentin tubule penetration of the adhesives was more visible on the ultrasonic-activated adhesive groups. Using the standard adhesive protocol, Prime & Bond Universal presented the lowest bond strength values on the apical and cervical thirds and mean value ($P<0.05$). Clearfil SE presented the highest bond strength values among ultrasonic-activated adhesive systems groups ($P<0.05$). The most prevalent failure was the adhesive failure between cement and dentin in all groups. However, there was a prevalence reduction in groups with ultrasonic activation of the adhesive system. Ultrasonic activation improved self-etch adhesives dentinal tubule penetration and bond strength of Clearfil SE and Prime & Bond Universal. Ultrasonic activation of self-etch adhesive systems influenced the prevalence of adhesive failure patterns.

Keywords: Dental Pins, Dental Stress Analysis, Dentin-Bonding Agents, Endodontics, Ultrasonics

1. Introduction

Fiber posts have been used as an alternative in rehabilitating endodontically treated teeth with little structure remaining. The adaptation of the posts into the root canal is achieved using resin-based cement with an adhesive system [1-3].

Adhesive systems can be classified as 2- or 3-step or self-etch. Conventionally, the adhesive system's first step consists of the application of phosphoric acid in order to demineralize the smear layer and the dentin's top layer. This demineralization exposes collagen fibers that will bond to the adhesive's resin monomers and form the so-called hybrid layer [4-6]. Self-etch adhesive systems are those that do not require a phosphoric acid step. In this system, an acid primer performs a partial demineralization. The smear layer's residual portion on the dentinal surface is incorporated into the hybrid layer [5-7].

Adhesive failure between resin cement and the dentinal surface is the most prevalent failure observed in the fiber posts cementation process, mainly because of the hybrid layer's composition and weaknesses. In order to improve adhesive techniques, different technologies and protocols have been studied and developed [8-10].

Ultrasonic devices in dentistry are widely studied and applied in different dentistry areas. In endodontics, ultrasonic activation can be used on irrigation solutions, intracanal medications, endodontic sealers, mechanical canal preparation, and surgeries [11-15]. There are a few studies that evaluated the sonic application of adhesive systems.

Knowing the characteristics of adhesive systems and the ultrasonic activation effects on materials' physical and chemical properties, Verdum et al. (2022) tested the ultrasonic activation influence on different adhesive systems in resin cementation of fiber posts. They concluded that this technique improved bond strength and dentinal tubule penetration of a self-etch adhesive, unlike a 3-step adhesive system and glass ionomer cement.

Ultrasonic activation of different self-etch adhesive systems used on resin cementation of fiber posts has not been studied to date, so this study aims to observe and evaluate the influence of this protocol on dentinal tubule penetration and bond strength of different self-etch adhesive systems on fiber post cementation.

The null hypothesis was that ultrasonic activation of self-etch adhesives would not influence the dentinal tubule penetration and bond strength of fiber posts to root dentin.

2. Material and methods

This study was approved by the Research Committee of the Federal University of Rio Grande do Sul’s School of Dentistry.

The parameters indicated by Rosa et al. (2013) [16] were considered for the sample size calculation, referring to the bond strength test. ANOVA test and Tukey test were used. The number of treatments was six. The minimum difference between the treatment means was 1.65, and the standard deviation of the error was 0.92. The power of the test was 0.80, and the significance level was 0.05.

The study was conducted using 60 extracted bovine mandibular incisors (n=60). These were cleaned and sectioned at the cementoenamel junction to obtain a standardized root length of 15mm. The roots were examined and radiographed to confirm the complete root formation, the presence of a single canal, and the absence of fractures, cracks, lacerations, and internal or external resorption.

The root canals were instrumented using #15 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) until the file tip was visualized at the foramen. The file measure was noted at this point, and the working length was determined to be 1mm shorter than that was. The root canal was prepared with a R50 instrument (Reciproc Blue, VDW Dental, Munich, Germany) and irrigation with 10mL of 2.5% sodium hypochlorite (NaOCl). After the preparation, the canals were irrigated with 5mL of 17% EDTA solution for 3 minutes, followed by 2mL of saline solution. The irrigation was performed with a 5mL syringe (Ultradent Products Inc., UT, USA) and a 27-gauge tip (Endo-Eze, Ultradent Products Inc., UT, USA). The canals were dried with paper points the same size as the final apical diameter.

The prosthetic canal length was determined by dividing the working length measure by 3. The obturation of the apical third of the canal was performed using the single-cone technique with Sealer Plus (MK Life Products, Porto Alegre, Brazil) endodontic sealer and gutta-percha cones (MK Life Products, Porto Alegre, Brazil). The roots were stored at 37ºC and 100% humidity for 15 days to allow the sealer polymerization.

Before adhesive protocols and post-cementation, final irrigation of the prosthetic canal was performed by 3mL of 2.5% NaOCl, followed by paper points drying. The prosthetic canal preparation was made using the fiberglass post's matching drill and the dentin's surface was cleaned with 70% ethyl alcohol. The fiberglass posts (Exacto #2, Angelus, Londrina, Brazil) were cleaned with 70% ethyl alcohol and coated with silane (Angelus, Londrina, Brazil), and the solvent was allowed to evaporate for 5 minutes.

2.1 Experimental Groups

The roots were randomly divided into six groups according to the adhesion strategy (Table 1).

Table 1
Experimental groups according to adhesive system, brand, composition and adhesion strategy

Adhesive System	Brand	Composition	Adhesion Strategy	n
Clearfil SE	Kuraray Medical Inc.	Primer: MDP, HEMA, dimethacrylate monomer, water, catalyst Bond : MDP, HEMA, dimethacrylate monomer, microfiller, catalyst	Standard (CFS)	10
			Ultrasonic activation (CFU)	10
Prime & Bond Universal	Dentsply Sirona	Bi- and multifunctional acrylate, phosphoric acid modified acrylate resin, initiator, stabilizer, isopropanol, water	Standard (PBS)	10
			Ultrasonic activation (PBU)	10
Single Bond Universal	3M	MDP phophate monomer, dimethacrylate resins, HEMA, vitrebond copolymer, filler, ethanol, water, initiators, silane	Standard (SBS)	10
			Ultrasonic activation (SBU)	10

2.2 Adhesion protocols and post cementation

The adhesives were handled according to the manufacturer's guidelines;

however, 0,1% Rodhamine B dye was added to provide fluorescence for visualizing the adhesive penetration into dentinal tubules during confocal laser scanning microscopy.

In the CFS group, the primer was applied using a micro brush (KG Sorensen, SP, Brazil) for 20 seconds, followed by five seconds of the air jet. The bond was also applied by micro brush for 10 seconds, and light polymerized (Valo Cordless Grand, Ultradent Prod. Inc., UT, USA) for 10 seconds. In the CFU group, the same adhesive strategy was applied in CFS; however, after micro brush application, ultrasonic activation was performed for 20 seconds on the primer and 10 seconds on the bond.

In PBS and SBS groups, the adhesives were applied using a micro brush for 20 seconds and light polymerized for 20 seconds. In PBU and SBU groups, after 20 seconds of micro brush application, the adhesives were ultrasonically activated for 20 seconds before 20 seconds of light polymerization.

For ultrasonic activation, a 0.2mm diameter tip (E1 Irrisonic, Helse, São Paulo, Brazil) was placed in a device (UDS-E LED Woodpecker, WAK, São Paulo, Brazil) and used in the Endo setting, with a potency of 5. The ultrasonic tip was positioned 1mm shorter than the prosthetic canal length and moved toward the canal walls.

After adhesion protocols, the resin cement RelyX ARC (3M ESPE, Sumaré, Brazil) was handled according to the manufacturer's guidelines and inserted into the canal with a Lentulo drill. The fiber post was immediately placed, the cement excess was removed with a micro brush, and light polymerization was performed for 40 seconds.

All roots were stored at 37ºC and 100% humidity for 24 hours.

2.3 Specimens preparation

All roots were sectioned by using a section machine (LabCut 1010, Extec Corp., CT, USA) (Fig 1) to obtain three slices (2 ±0.3~mm thick), each one corresponding to the apical, middle, and cervical thirds of the fiberpost. The slices had their surfaces polished in a polishing machine using Arotec paste (Arotec, Cotia, SP, Brazil) under water cooling.

2.4 Dentin tubule penetration

The adhesive systems dentin tubule penetration was evaluated by CLSM (Olympus Corp., Tokyo, Japan) (Figure 1). The apical slices of all roots were placed, one by one, under the microscope, and the wavelength absorption and emission of the rhodamine B dye were 540 nm and 494nm, respectively. The images were obtained at x10 magnification using a software program (FluoView 10 ASW 4.2, Olympus Corp., Tokyo, Japan) and examined with the Adobe Photoshop 25.1 (Adobe Inc., San José, California, USA) software program for a descriptive analysis.

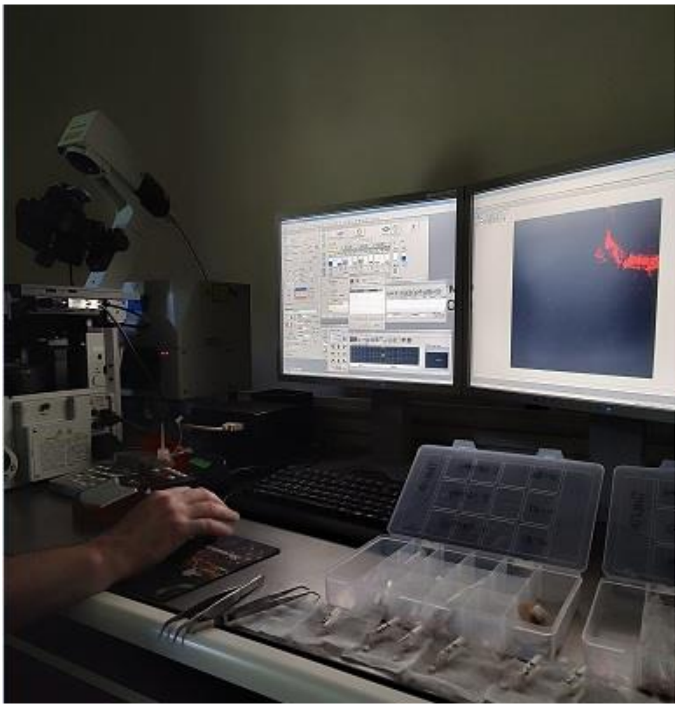

Fig 1. CLSM analysis

2.5 Push-out test

After dentin, tubule penetration analysis of the apical slices, all the cervical, middle, and apical slices of all roots went through a push-out test performed in a universal test machine (EMIC, Instron, São José dos Pinhais, Brazil) (Figure 2). Each slice coronal portion was placed in contact with a metallic device with a central opening larger than the root canal diameter. A metallic cylinder (Ø=0.8mm) induced a load on the fiberpost surface at a 0.5mm/min speed. The bond strength values (mPa) were obtained by the ratio between the required load for specimen rupture (in Newton) and the adhesive area (mm²).

The experimental groups were compared for push-out bond strength using 1-way ANOVA and Tukey post-hoc tests. The comparison between the thirds of the prosthetic canal within the same group was also made using a 1-way ANOVA test. The Student's T test compared the groups that used manual adhesive protocols and those that added ultrasonic activation of the adhesive systems.

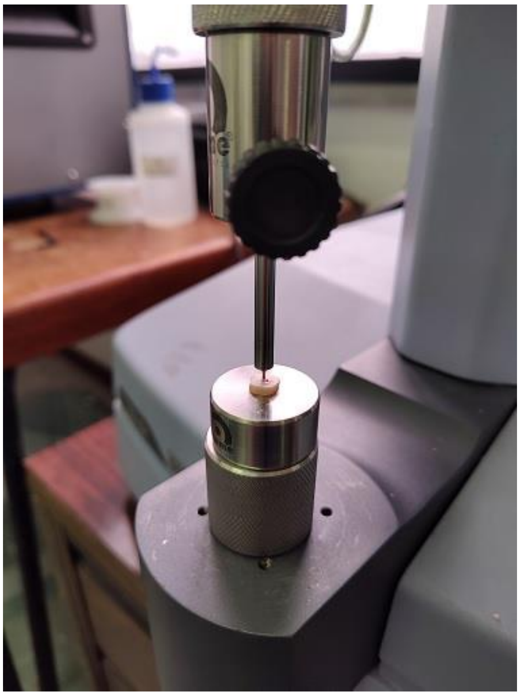

Fig 2. Push-out test.

2.6 Failure pattern

The failure patterns were classified as adhesive between cement and dentin (ACD), adhesive between cement and post (ACP), cohesive for the cement (CC), cohesive for the post (CP), and cohesive for the dentin (CD). After the push-out test, all specimens were analyzed under an optical microscope (BX60M, Olympus Corp., Tokyo, Japan) (Figure 3) with 10x magnification to determine their predominant failure pattern.

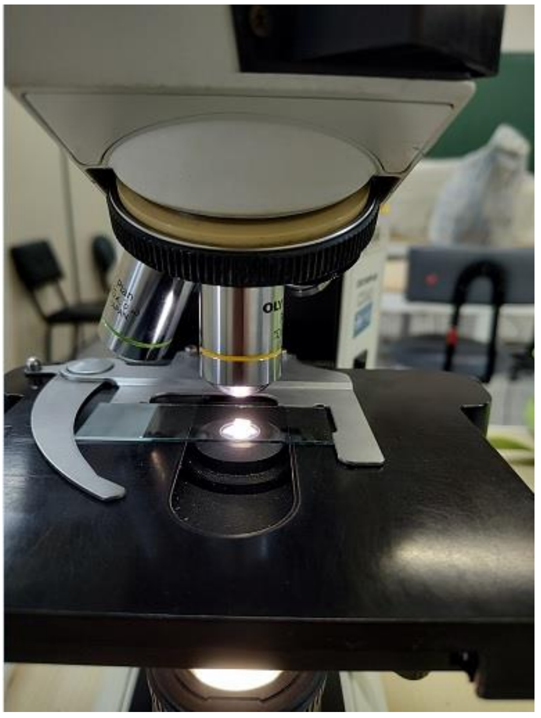

Fig 3. Failure pattern analysis under optical microscope.

3. Results

3.1 Dentin tubule penetration

The ultrasonic activation of the adhesive systems improved its dentin tubule penetration in apical slices of experimental groups. Dyed adhesives appear red in the CLSM images presented in Figure 4.

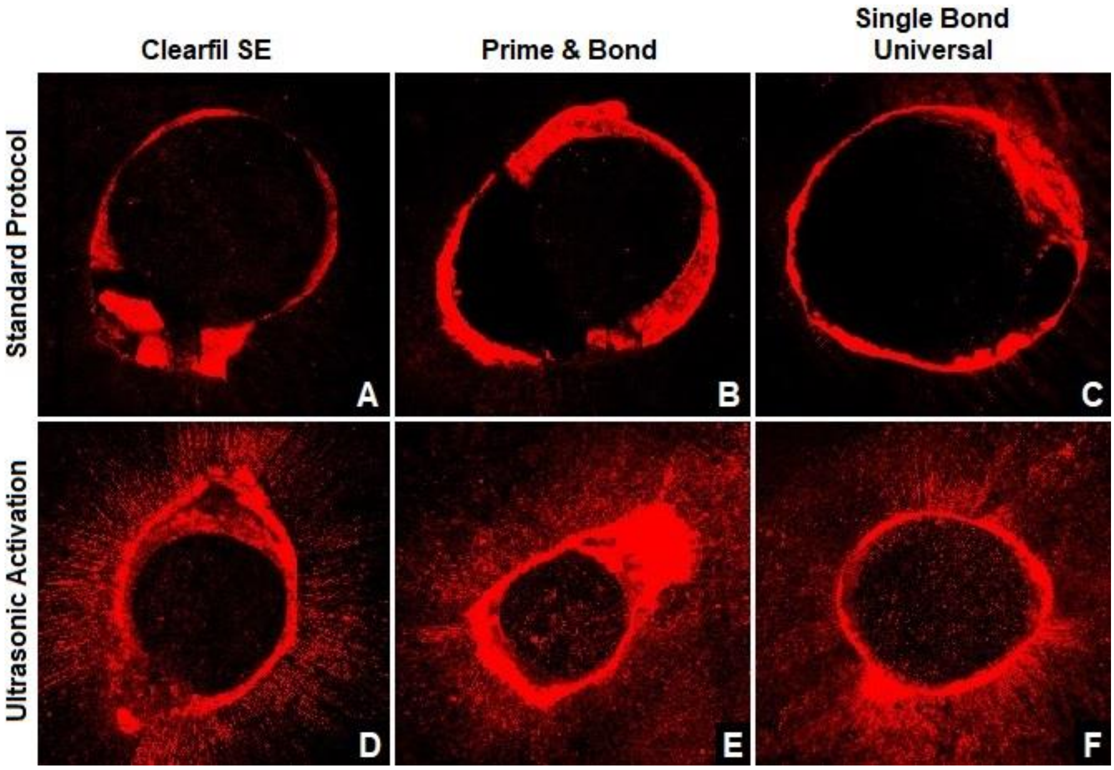

Fig 4. Representative image of dentinal tubule penetration of adhesives in experimental groups apical slices in CLSM

3.2 Push-out bond strength

The intragroup analysis showed no significant difference in bond strength values between the fiberpost's three levels in the experimental groups (P>0.05). About standard adhesive protocol groups, PBS presented lower bond strength values in the apical and cervical thirds of the post (P<0.05), and there was no difference in the middle third (P>0.05). PBS showed the lowest bond strength mean value (P<0.05). Between ultrasonic-activated adhesive protocol groups, there was no difference in the cervical third of the post (P>0.05). PBU presented no difference compared to CFU and SBU in the apical third and the mean value of the three-thirds (P>0.05). In the middle third, CFU presented higher bond strength values when compared to PBU and SBU (P<0.05). CFU's bond strength values were higher than SBU in the apical third and the mean value (P<0.05). Ultrasonic activation improved bond strength when applied to Clearfil SE and Prime & Bond in the apical and middle thirds of the post and the mean value of the three-thirds (P<0.05).

Table 2 presents the values of bond strength after the push-out test.

Table 2
Mean and standard deviation for bond strength values after push-out test (mPa)

	CFS	PBS	SBS	CFU	PBU	SBU
Apical	10.9250^{aAB} ±4.9164	7.4156^{aB} ±4.4896	13.6189^{aA} ±4.9485	19.7513^{aA*} ±4.5585	14.6930^{aAB*} ±8.7412	10.8220^{aB} ±4.7759
Middle	12.6610^{aA} ±4.4707	8.1189^{aA} ±4.9905	11.7833^{aA} ±5.0357	19.7075^{aA*} ±6.6145	13.2280^{aB*} ±5.7839	11.4630^{aB} ±3.9160
Cervical	14.9930^{aA} ±6.5405	9.1678^{aB} ±2.9212	14.8611^{aA} ±3.6461	17.5075^{aA} ±5.6981	12.6890^{aA} ±7.3889	12.7290^{aA} ±4.0827
Mean	12.8730 ^{A} ±4.4796	8.1311 ^{B} ±3.8461	13.2478 ^{A} ±2.9423	18.9888 ^{A*} ±4.5948	13.5550 ^{AB*} ±6.1366	11.8960 ^{B} ±3.7744

Lowercase letters compare the thirds of the prosthetic canal within the same group after 1-way ANOVA test ($p<0.05$)
Uppercase letters compare standard and ultrasonic activation protocols groups separately within each third of the prosthetic canal after 1-way ANOVA and Tukey's post-hoc test ($p<0.05$)
* indicate differences between the manual and ultrasonic activation protocols for each adhesive system after T test ($p<0.05$)

3.3 Failure Pattern

The adhesive failure between cement and dentin was the most commonly observed failure mode in all groups (59.44%), followed by an adhesive failure between cement and post (30%) (Table 3). The adhesive failure between cement and dentin had a 65.55% prevalence in manual adhesive protocol groups. This value decreased to 53.33% in the ultrasonic activation groups (Table 4).

Figure 5 presents pictures of each failure mode observed under the optic microscope.

Table 3
Failure patterns observed after push-out bond strength test.

Adhesion Protocol		ACD	ACP	CC	CP	CD
CFS	**Apical**	6	4	-	-	-
	Middle	5	4	-	1	-
	Cervical	4	6	-	-	-
CFU	**Apical**	5	3	-	-	2
	Middle	5	2	-	1	2
	Cervical	3	3	1	2	1
PBS	**Apical**	5	3	1	-	1
	Middle	10	-	-	-	-
	Cervical	9	1	-	-	-
PBU	**Apical**	6	3	1	-	-
	Middle	3	6	1	-	-
	Cervical	6	3	-	1	-
SBS	**Apical**	7	2	-	1	-
	Middle	6	2	1	1	-
	Cervical	7	2	-	-	1
SBU	**Apical**	8	2	-	-	-
	Middle	5	5	-	-	-
	Cervical	7	3	-	-	-
Total		**107 (59.44%)**	**54 (30%)**	**5 (2.78%)**	**7 (3.89%)**	**7 (3.89%)**

Table 4
Prevalence of failure patterns in standard adhesive protocol groups and ultrasonic activation adhesive protocol groups

	Standard Protocol	Ultrasonic Activation
ACD	59 (65.55%)	48 (53.33%)
ACP	24 (26.67%)	30 (33.33%)
CC	2 (2.22%)	3 (3.34%)
CP	3 (3.34%)	4 (4.44%)
CD	2 (2.22%)	5 (5.56%)

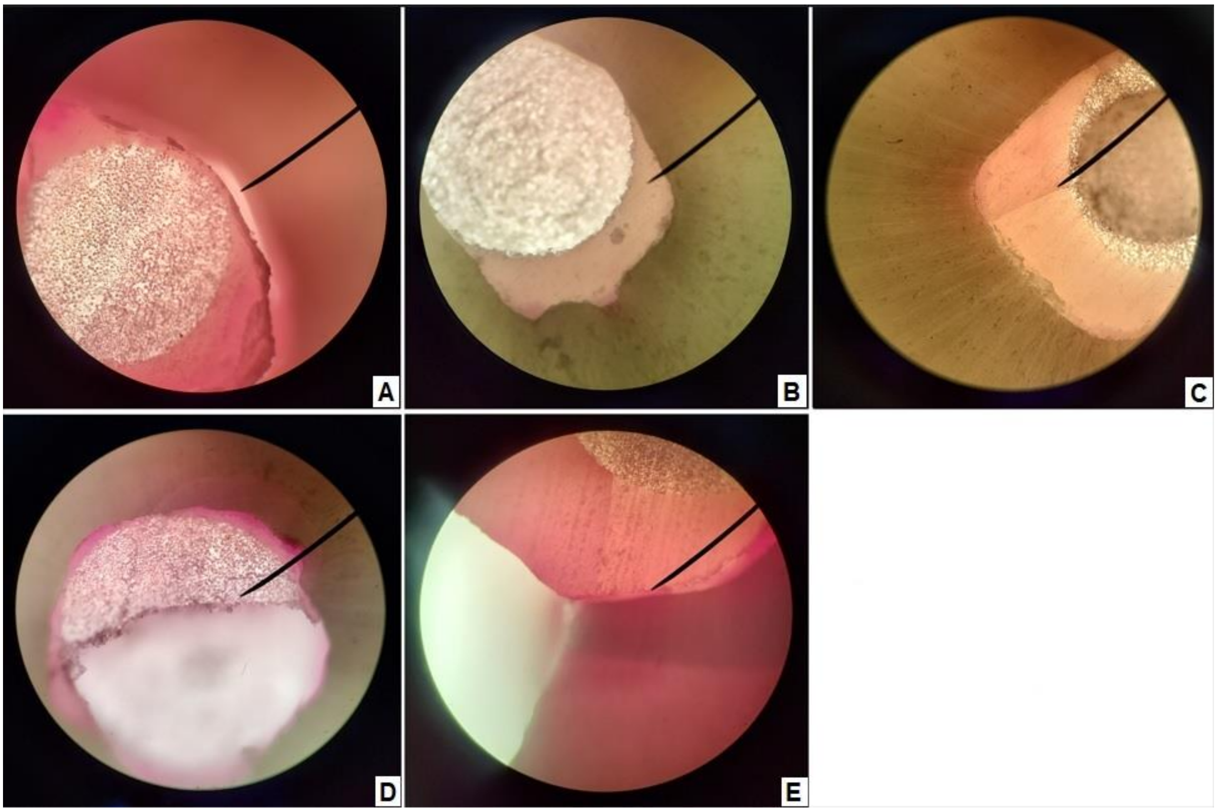

Fig 5. Representative pictures of failure patterns after *push-out* test.
A= ACD; B= ACP; C= CC; D= CP; E= CD

4. Discussion

This study evaluated the effect of ultrasonic activation on dentinal tubule penetration and bond strength on fiber post cementation. The null hypothesis was rejected.

Bovine teeth can be used as a substitute of human teeth in studies that aim to assess bond strength, once its dentin and enamel presents similar results [17].

The study of Verdum et al. (2022) is the only one that aimed to observe different adhesion techniques, including ultrasonic activation and different adhesive systems tubule penetration in fiber post cementation. In this study, the authors observed that ultrasonic activation increased the bond strength of a self-etch adhesive system (Clearfil SE) for resin cementation of fiber posts. The present study method was designed to observe if Cleafil SE's results found by Verdum et al. (2022) would also be seen in different self-etch adhesive systems.

Verdum et al. (2022) performed a statistical analysis of the adhesive's dentinal tubule penetration and concluded that ultrasonic activation improved Clearfil SE's dentinal tubule penetration in apical and cervical fiber post thirds. Our study's dentinal tubule penetration of adhesives was performed by observing CLSM images of standard adhesive and ultrasonic activation protocols and their visual comparison. Our choice to perform a descriptive analysis of the adhesive's dentinal tubule penetration was due to the different diameters of the roots. In some root slices, it was impossible to observe its hole surface on CLSM, which could lead to underestimated values of dentinal tubule penetration if a statistical analysis was done.

The bond strength of self-etch adhesive systems can be compromised by the lower dentinal and smear layer demineralization and the smear layer's incorporation in the adhesive hybrid layer [5-7]. These facts can explain the lower bond strength values found on the standard adhesive protocols groups in the present study ($P<0.05$). About bond strength values of Clearfil SE, Prime & Bond Universal, and Single Bond Universal in standard application protocols under push-out test, Single Bond Universal presented the higher values, and Prime & Bond Universal presented the lowest bond strength values in our study, in disagreement with other authors that observed the lowest values in Single Bond Universal and the highest values on Clearfil SE. However, these studies compared different adhesive systems in composites bond to coronal dentin [18]. They differ from our aim to observe the adhesives on resin cementation of fiber posts to root dentin.

Despite the results on bond strength of standard adhesive protocols, the ultrasonic activation groups reached different mean values. Clearfil SE and Prime & Bond Universal had their bond strength improved after ultrasonic activation ($P<0.05$), and Single Bond Universal did not ($P>0.05$). Verdum et al. (2022) also observed the improvement of Clearfil SE bond strength after ultrasonic activation. It might be related to the fact that this adhesive system is a 2-step self-etch, and the primer was separately ultrasonic activated before the bond's activation. Single Bond Universal is an adhesive system that has a large and varied formulation. The effects of ultrasonic activation on its composition may have influenced the results of this system [6,7,15,18].

Ultrasonic vibration causes a local pressure and temperature increase and also solution agitation. These properties may lead to a more efficient solution spread through dental tissues and consequently increase the primer's dentinal and smear layer demineralization and adhesion capacity of the system. The increase in local temperature during ultrasonic activation may have improved adhesive’s solvents volatilization, improving the polymerization of the adhesives [19-21].

Cementation of fiber posts is a technique with some technical fragile points. In the present study, only the apical thirds of the canals were filled after chemical-mechanical preparation, leaving less residual tissue and materials on the canal walls before adhesive system application, which is different from what usually occurs in clinical practice [22,23]. The difficult access to adhesive system application is another challenge on post cementation, especially in deeper portions of canal walls. An adhesive protocol that requires fewer steps, like the self-etch adhesives, is an attempt to minimize this adversity. Despite being one more step in the process, ultrasonic activation of the adhesive system could reduce the technical difficulties of fiber post resin cementation, possibly increasing its longevity and decreasing its clinical failures. More studies must be performed in order to confirm these hypotheses.

Other studies evaluated techniques applied to adhesive systems, including sonic application and ultrasonic agitation [24-27]. However, sonic application differs from ultrasonic according to each frequency range. The frequency of sonic devices ranges from 1000 to 6000 Hz, different from ultrasonic devices' frequencies, which are 20.000 Hz or higher.

Bagis et al. (2008) observed no difference in the bond strength of fiber post's cementation when self-etch adhesive systems were ultrasonically activated. However, Bagis et al. (2009) performed a high-frequency (1MHz) ultrasonic activation of self-etch adhesives and observed improved bond strength. It is essential to mention that the ultrasonic agitation performed by Bagis et al. in 2008 and 2009 [26,27] observed this event on the coronal dentin surface. To date, no studies other than ours have evaluated the effects of ultrasonic activation on self-etch adhesive systems on fiber post cementation.

The present study observed that the most prevalent failure after the push-out test of resin-cemented fiber posts was adhesive between cement and dentin, followed by adhesive between cement and post in all groups, in line with other author's similar studies [8-10]. Interestingly, after ultrasonic activation of adhesive systems, the incidence of adhesive failure between cement and dentin had a 12,22% decrease, and adhesive failure between cement and post and cohesive failures had increased. These findings may be explained by the higher adhesive penetration in the dentinal tubule and higher bond strength of fiber posts resin cementation on dentin after ultrasonic activation, leading to a more robust adhesive power between resin cement and dentinal surface.

Despite the limitations of this in vitro study, our findings are a starting point for new studies in this promising research topic. Further investigation should be developed to compare and confirm our results, and posterior clinical trials and long-term follow-up of clinical protocols must be done to evaluate and observe its results.

Conclusion

The findings of this in vitro study lead to the following conclusions about the resin cementation of fiber posts:

1. Ultrasonic activation improved self-etch adhesives dentinal tubule penetration.
2. Ultrasonic activation improved the bond strength of Clearfil SE and Prime & Bond Universal.
3. Ultrasonic activation of self-etch adhesive systems influenced the prevalence of adhesive failure patterns.

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4 CONSIDERAÇÕES FINAIS

A ativação ultrassônica dos sistemas adesivos autocondicionantes Clearfil SE, Prime & Bond e Single Bond Universal aumentou sua penetração intratubular na dentina radicular durante o processo de cimentação de pinos de fibra de vidro com o cimento resinoso RelyX ARC. Além disso, a ativação ultrassônica dos sistemas Clearfil SE e Prime & Bond aumentou a força de união da cimentação dos pinos de fibra à dentina radicular. O presente estudo demonstrou, ainda, que a prevalência de falhas adesivas entre cimento resinoso e dentina radicular foi reduzida após ativação ultrassônica dos sistemas adesivos autocondicionantes.

Tendo em vista os resultados observados nesse estudo, pode-se considerar que a ativação ultrassônica dos sistemas adesivos autocondicionantes pode ser um passo clínico que aumentará a qualidade da cimentação adesiva de pinos de fibra de vidro. Para embasar tal conclusão, são necessários mais estudos versando sobre a aplicação da técnica, visto que não há metodologias semelhantes ou estudos clínicos e longitudinais que a avaliem e observem na literatura até o momento.

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APÊNDICE 1 – NORMAS DA REVISTA INTERNATIONAL JOURNAL OF ADHESION AND ADHESIVES

Link de acesso para as normas completas:
https://www.sciencedirect.com/journal/international-journal-of-adhesion-and-adhesives/publish/guide-for-authors

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Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

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State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

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Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Theory/calculation
A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section,

which may stand alone or form a subsection of a Discussion or Results and Discussion section.

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If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

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Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

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Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

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Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, Crossref and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.
A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

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As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

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There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

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Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:
[1] Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. J Sci Commun 2010;163:51–9. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
[2] Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. Heliyon. 2018;19:e00205. https://doi.org/10.1016/j.heliyon.2018.e00205
Reference to a book:
[3] Strunk Jr W, White EB. The elements of style. 4th ed. New York: Longman; 2000.
Reference to a chapter in an edited book:
[4] Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.
Reference to a website:
[5] Cancer Research UK. Cancer statistics reports for the UK, http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/; 2003 [accessed 13 March 2003].
Reference to a dataset:
[dataset] [6] Oguro M, Imahiro S, Saito S, Nakashizuka T. Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1; 2015. https://doi.org/10.17632/xwj98nb39r.1.
Note shortened form for last page number. e.g., 51–9, and that for more than 6 authors the first 6 should be listed followed by 'et al.' For further details you are referred to

'Uniform Requirements for Manuscripts submitted to Biomedical Journals' (J Am Med Assoc 1997;277:927–34) (see also Samples of Formatted References).
Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

ANEXO 1 – PARECER COMISSÃO DE PESQUISA DE ODONTOLOGIA

De: compesq_odo@ufrgs.br
Data: 27 de julho de 2022 17:41:16 BRT
Para: marcus.so@ufrgs.br
Assunto: Projeto de Pesquisa na Comissão de Pesquisa de Odontologia
Responder A: compesq_odo@ufrgs.br

Prezado Pesquisador MARCUS VINICIUS REIS SO,

Informamos que o projeto de pesquisa Ativação ultrassônica de diferentes sistemas adesivos autocondicionantes ? penetração intratubular e resistência de união de pinos de fibra. encaminhado para análise em 07/06/2022 foi aprovado quanto ao mérito pela Comissão de Pesquisa de Odontologia com o seguinte parecer:

O projeto de pesquisa ATIVACAO ULTRASSONICA DE DIFERENTES SISTEMAS ADESIVOS AUTOCONDICIONANTES: PENETRACAO INTRATUBULAR E RESISTENCIA DE UNIAO DE PINOS DE FIBRA, número 42716 é coordenado por Marcus Vinicius Reis Só, e conta com a participação de Ricardo Abreu da Rosa, Lucas Silveira Machado e Thalita Ayres Arrué.
Foi apresentada revisão de literatura quanto ao tema, discorrendo sobre cimentos resinosos e ativação ultrassônica. O objetivo geral da pesquisa é avaliar, in vitro, o efeito da ativação ultrassônica dos sistemas adesivos autocondicionantes (Clearfil SE, Prime & Bond e Single Bond Universal) na penetração intratubular dos adesivos e da resistência de união de pinos de fibra vidro em diferentes terços do conduto radicular.
O estudo será realizado no Laboratório de Materiais Dentários (LAMAD) e Centro de Microscopia Eletrônica da UFRGS. Os pesquisadores anexaram carta de anuência do CME e do LAMAD.
Sessenta dentes bovinos monorradiculares serão selecionados e seccionados em nível da junção amelo- cementária, padronizando o comprimento de raiz em 14 mm. Os condutos serão instrumentados com o sistema rotatório ProTaper até o instrumento F3, obturados com cimento endodôntico AH Plus e cones de guta-percha F3 do mesmo sistema, pela técnica do cone único. Os condutos serão parcialmente desobturados, mantendo-se os 4 mm apicais de material obturador. As raízes serão distribuídas aleatoriamente em 6 grupos (n=10) de acordo com o uso do ultrassom e o adesivo autocondicionante: G1)Clearfil SE aplicação manual, G2)Clearfil SE com ativação ultrassônica, G3)Prime & Bond Universal aplicação manual, G4) Prime & Bond Universal com ativação ultrassônica, G5) Single Bond Universal aplicação manual e G6) Single Bond Universal com ativação ultrassônica. Após a manipulação, o cimento resinoso (RelyX ARC) será levado ao conduto com espiral lentulo. Imediatamente, os pinos de fibra serão posicionado s e fotopolimerizados por 40 segundos. Os corpos de prova serão seccionados na máquina de corte, obtendo-se 3 slices por espécime, sendo 1 slice para cada porção do pino (cervical, média e apical) e o teste de push- outserá realizado. Para análise da penetração intratubular, os espécimes serão analisados em microscopia confocal de varredura a laser (MCVL). A resistência adesiva e o percentual de dentina penetrada pelos sistemas adesivos serão calculados e analisados através do teste ANOVA e Tukey. O nível de significância será estabelecido em 5%.
O projeto será desenvolvido em 16 meses. O orçamento total é de R$ 2712,83, com financiamento pelo pesquisador principal.

Após a análise da proposta, a pesquisa encontra-se em condições de aprovação quanto à pertinência, ao valor científico, à adequação de metodologias e objetivos, e à exequibilidade.

Devido as suas características este projeto foi encaminhado nesta data para avaliação por .

Atenciosamente, Comissão de Pesquisa de Odontologia