UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO DE CIÊNCIAS DA SAÚDE
CURSO DE GRADUAÇÃO EM ODONTOLOGIA

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TRABALHO DE CONCLUSÃO DE CURSO

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CURSO DE GRADUAÇÃO EM ODONTOLOGIA

EFEITO DA ATIVAÇÃO ULTRASSÔNICA DO CIMENTO OBTURADOR NA RESISTÊNCIA DE UNIÃO À DENTINA IRRADIADA

Trabalho de conclusão apresentado ao curso de Odontologia da Universidade Federal de Santa Catarina para obtenção do título de cirurgiã-dentista.

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Luana Duart Jordani

Efeito da Ativação Ultrassônica do Cimento Obturador na Resistencia de União a Dentina Irradiada

Este Trabalho de Conclusão de Curso foi julgado adequado para obtenção do título de Cirurgião Dentista e aprovado em sua forma final pelo Curso de Graduação em Odontologia.

Florianópolis, 12 de maio de 2023.

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DAS UTOPIAS

“Se as coisas são inatingíveis... ora!
Não é motivo para não querê-las...
Que tristes os caminhos, se não fora
A presença distante das estrelas!”

- Mario Quintana

RESUMO

O objetivo do presente estudo *in vitro* foi avaliar o efeito da ativação ultrassônica do cimento endodôntico na resistência de união em dentina irradiada. Quarenta dentes humanos foram distribuídos aleatoriamente em 4 grupos (n=10), de acordo com a exposição à radiação ionizante (70 Gy) e a ativação ultrassônica do cimento obturador, a saber: RT/AU - dentes irradiados e ativação ultrassônica do cimento obturador; RT/sem AU - dentes irradiados e sem ativação ultrassônica do cimento; Sem RT/AU - dentes não irradiados e ativação ultrassônica do cimento e Sem RT/sem AU - dentes não irradiados e sem ativação ultrassônica do cimento. A resistência de união *push-out* do cimento obturador à dentina intrarradicular foi realizada em Máquina Universal de Ensaios (Instron) (0,5 mm/min). Os padrões de falha e a interface adesiva foram analisadas em MEV. Os dados obtidos foram comparados estatisticamente (*push-out* - *two-way*-ANOVA e teste *post-hoc* de Games-Howell; padrão de falha - teste exato de Fisher - α=0,05). As diferentes condições experimentais (irradiação e ativação ultrassônica) e o terço radicular tiveram efeito significativo na resistência de união *push-out*, e a interação desses fatores foi significativa ($p<0,05$). A ativação ultrassônica do cimento aumentou significativamente a resistência de união nas amostras irradiadas e não-irradiadas ($p<0,05$). Os grupos irradiados apresentaram em sua maioria falha do tipo adesiva do material obturador ($p<0,01$). Independentemente da condição experimental (radiação), os grupos ativados apresentaram prolongamento mais numerosos, em maior densidade e profundidade, e interface adesiva mais integra e homogênea. A ativação ultrassônica aumentou a penetração intratubular, e consequentemente, a resistência de união do cimento obturador à dentina intrarradicular irradiada e não-irradiada.

Palavras-chave: Radioterapia. Tratamento Endodôntico. Ultrassom. Cimento Obturador. Resistência de União.

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LISTA DE ABREVIATURAS E SIGLAS

CCP Câncer de cabeça e pescoço

PUI Irrigação ultrassônica passiva

Gy *Gray*

SEM *Scanning Electron Microscopy*

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1. INTRODUÇÃO

O câncer é um grande grupo de doenças que se iniciam quando células anormais crescem descontroladamente (OMS, 2018). O câncer de boca é um dos mais frequentes entre aqueles que acometem a região de cabeça e pescoço (OMS, 2018). Pode envolver lábios, gengiva, mucosa jugal, palato, língua e assoalho da cavidade oral, sendo mais frequente em homens acima dos 40 anos (OMS, 2018). O tabagismo e o consumo excessivo de álcool estão entre os principais fatores de risco, sendo uma doença que pode ser prevenida focando na promoção de saúde e acesso aos serviços de diagnóstico precoce (OMS, 2018).

Para o tratamento dessas neoplasias, o protocolo, na maioria dos casos, envolve quimioterapia e cirurgia, independentemente do tamanho da lesão (OMS, 2018). No entanto, em casos mais complexos, é necessária a complementação do tratamento com radioterapia para garantir a completa eliminação das células neoplásicas (OMS, 2018).

A radiação ionizante entregue durante o tratamento radioterápico não afeta somente as células neoplásicas, mas também células saudáveis adjacentes ao local irradiado (CANCELIER *et al.*, 2023). A radioterapia em pacientes portadores de câncer de cabeça e pescoço (CCP) leva à hipovascularidade local e hipóxia celular, prejudicando a reconstituição do tecido ósseo e favorecendo a ocorrência de osteorradionecrose (PARAHYBA *et al.*, 2016). Além disso, a radioterapia afeta as estruturas dentais como um todo, tanto esmalte quanto dentina (KIELBASSA *et al.*, 1999; SPRINGER *et al.*, 2005; DE SIQUEIRA MELLARA *et al.*, 2014; GONÇALVES *et al.*, 2014), comprometendo a efetividade de procedimentos restauradores.

A radiação está associada a uma diminuição significativa da resistência de união de cimentos obturadores à dentina intrarradicular (MARTINS *et al.*, 2015; PAIOLA *et al.*, 2018). O protocolo clínico de tratamento endodôntico em pacientes portadores de CCP submetidos a radioterapia continua sendo muito similar ao de pacientes saudáveis (CANCELIER *et al.*, 2023). Verifica-se, porém, uma pequena quantidade de estudos laboratoriais que avaliem materiais, instrumentos e técnicas operatórias que proporcionem melhores resultados do ponto de vista clínico, na dentina irradiada.

Em 1957, Richman publicou o primeiro estudo empregando o ultrassom na endodontia, utilizando-o como equipamento auxiliar na instrumentação e limpeza do canal radicular (MOZO; LLENA; FORNER, 2011). Uma das aplicações mais efetivas atualmente é a ativação ultrassônica da solução irrigadora (MONTASER *et al*., 2023; XU *et al*., 2023). Quando comparados a irrigação ultrassônica passiva (PUI) e o sistema convencional de irrigação com seringa e agulha, os resultados mostram que a PUI promove maior alcance das soluções irrigadoras na região apical e irregularidades do canal radicular, acarretando numa maior limpeza da região (MUNOZ, CAMACHO-CUADRA, 2012). A PUI produz um fenômeno acústico chamado cavitação, que desloca a solução irrigante em direção às paredes do canal radicular com alto impacto, removendo a *smear layer* e atingindo áreas em que os instrumentos não alcançam (ORLOWSKI *et al*., 2020).

Além da irrigação, outra aplicação estudada atualmente é a ativação ultrassônica do cimento obturador. Arslan, Abbas & Karatas *et al*. (2016) demonstraram que a ativação ultrassônica do cimento obturador aumenta significativamente sua penetração nos túbulos dentinários. Wiesse *et al*. (2018) avaliaram a eficácia da ativação sônica e ultrassônica do cimento obturador na sua adaptação e união à dentina do canal radicular. Tal estudo demonstrou que o cimento, quando ativado ultrassonicamente, apresenta maior resistência de união quando comparado àquele submetido a agitação sônica e quando não ativado.

Um dos efeitos produzidos sobre o cimento obturador quando este é ativado é a alteração em sua reologia devido ao calor produzido pela ativação ultrassônica (BITTMANN; HAUPERT; SCHLARB, 2009; LIONETTO & MAFFEZZOLI, 2013). Sendo assim, a viscosidade do cimento diminui significativamente, melhorando sua capacidade de escoamento (BITTMANN; HAUPERT; SCHLARB, 2009; LIONETTO & MAFFEZZOLI, 2013). Outro fenômeno decorrente da ativação é o aumento da pressão sobre o cimento obturador gerada pelo inserto ultrassônico, impulsionando-o contra as paredes do canal radicular, e fazendo com que este alcance regiões de maior complexidade anatômica, melhorando sua capacidade de selamento (WIESSE *et al.*, 2018). Esses fenômenos permitem um preenchimento mais eficaz de irregularidades nas paredes do canal radicular (GUIMARÃES *et al.*, 2014), de canais acessórios (ARSLAN, ABBAS & KARATAS, 2016) e maior penetração nos túbulos dentinários (GUIMARÃES *et al.*, 2014).

Embora esteja consolidado na literatura que a ativação ultrassônica do cimento obturador aumente sua capacidade de selamento e aumente sua resistência de união à dentina intrarradicular (GUIMARÃES *et al*., 2014; ARSLAN *et al*., 2016; WIESSE *et al*., 2018; DE BEM *et al*., 2020), essa relação não está estabelecida quando se trata de dentina irradiada. Portanto, é necessária uma avaliação quantitativa da interferência da ativação ultrassônica na resistência de união do cimento à dentina exposta à radiação ionizante, proveniente do tratamento radioterápico em pacientes portadores de CCP.

2. OBJETIVOS

2.1 Objetivo geral

Avaliar o efeito da ativação ultrassônica do cimento endodôntico na resistência de união à dentina intrarradicular irradiada.

2.2 Objetivo específico

Avaliar o efeito da ativação ultrassônica do cimento endodôntico à base de resina epóxica na resistência de união à dentina intrarradicular irradiada com 70 Gy através do teste *push-out.*

2.3 Hipótese

A ativação ultrassônica do cimento obturador aumentará a resistência de união do cimento obturador à dentina intrarradicular irradiada.

3. ARTIGO CIENTÍFICO

Este estudo foi preparado e escrito na forma de artigo científico de acordo com as normas para submissão no periódico *International Endodontic Journal* (Qualis A1, Fator de Impacto 5.165).

ORIGINAL ARTICLE

The ultrasonic activation of sealer increases its bond strength to intraradicular irradiated dentine - a laboratory study

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Abstract

Aim: The purpose of the present *in vitro* study was to evaluate the effect of ultrasonic activation of the root canal sealer on its bond strength to irradiated dentine.

Materials and methods: Forty human teeth were randomly distributed into 4 groups (n=10), according to exposure to ionizing radiation (70 Gy) and ultrasonic activation of the sealer, as follows: RT/UA - irradiated teeth and ultrasonic activation of the sealer; RT/no-UA - irradiated teeth and no ultrasonic activation of the sealer; no-RT/UA - non-irradiated teeth and ultrasonic activation of the sealer and no-RT/no-UA - non-irradiated teeth and no ultrasonic activation of the sealer. The push-out bond strength of the sealer to the intraradicular dentine was performed in a Universal Testing Machine (Instron) (0.5 mm/min). Failure modes and adhesive interface were analyzed under Scanning Electron Microscopy (SEM). The data were statistically compared (push-out - two-way-ANOVA and posthoc Games-Howell test; failure mode - Fisher's exact test - $\alpha = .05$).

Results: The different experimental conditions (radiation and ultrasonic activation) and the root third had a significant effect on the push-out bond strength, and the interaction of these factors was significant ($p < .05$). The ultrasonic activation of the sealer significantly increased its bond strength to both irradiated and non-irradiated dentine ($p < .05$). The irradiated groups mostly presented adhesive-type failure of the sealer ($p < .01$). Regardless of the experimental condition (radiation), the activated groups showed a more homogeneous adhesive interface to the root canal dentine, with the presence of sealer tags in greater density and depth.

Conclusions: Ultrasonic activation increased intratubular penetration and, consequently, the bond strength of the sealer to irradiated and non-irradiated intraradicular dentine.

KEYWORDS

radiotherapy, endodontic treatment, ultrasound, root canal sealer, bond strength.

INTRODUCTION

Cancer is a large group of diseases that may initiate when abnormal cells grow uncontrollably (World Health Organization, 2018). Oral cancer is one of the most frequent among those that affect the head and neck (World Health Organization, 2018). It may involve lips, buccal mucosa, palate, tongue and the oral cavity floor, being more frequent in men over 40 years of age (World Health Organization, 2018). Smoking and excessive alcohol consumption are the main risk factors for oral cancer, and they may be prevented by focusing on health promotion and access to early diagnosis services (World Health Organization, 2018).

The protocol for cancer treatment, in most cases, involves chemotherapy and surgery, regardless of the size of the lesion (World Health Organization, 2018). However, in more complex cases, the treatment must be complemented with radiotherapy to ensure the complete elimination of neoplastic cells (World Health Organization, 2018).

The ionizing radiation delivered during radiotherapy does not only affect the neoplastic cells but also healthy cells adjacent to the irradiated site (Parahyba et al., 2016). Radiotherapy in head and neck cancer patients leads to local hypovascularity and cell hypoxia, hindering the reconstitution of bone tissue and favouring osteoradionecrosis occurrence (Parahyba et al., 2016). In addition, radiotherapy affects the dental structures, both enamel and dentine, compromising the effectiveness of restorative procedures (Kielbassa et al., 1999; Springer et al., 2005; de Siqueira Mellara et al., 2014; Gonçalves et al., 2014). Radiotherapy is highly associated with a decrease in the bond strength of root canal sealers to intraradicular dentine (Martins et al., 2016; Paiola et al., 2018).

Several studies have reported that ultrasonic activation of the root canal sealer increases its penetration into the dentinal tubules, showing promising results (Arslan et al., 2016; Wiesse et al., 2018; De Bem et al., 2020). One of the main effects when the sealer is activated is the alteration of its rheological properties due to the heat produced (Bittmann et al., 2009; Lionetto & Maffezzoli, 2013; De Bem et al., 2020). The viscosity of the sealer decreases, improving its flow capacity (Bittmann et al., 2009; Lionetto & Maffezzoli, 2013; De Bem et al., 2020). Another phenomenon resulting from activation is the pressure generated by the ultrasonic insert on the sealer, pushing it towards the root canal walls and hard-to-reach areas, improving its sealing capacity (Guimarães et al., 2014; Arslan et al., 2016; Wiesse et al., 2018).

Although it is consolidated in the literature that ultrasonic activation of the sealer increases its sealing capacity and its bond strength to intraradicular dentine (Guimarães et al., 2014; Arslan et al., 2016; Wiesse et al., 2018; De Bem et al., 2020), this relationship has not been established when it comes to irradiated dentine. Studies confirm changes in dentine as a consequence of radiotherapy (Gonçalves et al., 2014; Martins et al., 2015; Paiola et al., 2018; Cancelier et al., 2023; Coelho et al., 2023). Therefore, the purpose of the present *in vitro* study was to answer the following research question: Does the ultrasonic activation of the sealer increases its bond strength to irradiated dentine?

MATERIALS AND METHODS

The manuscript of this laboratory study has been written according to Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu et al., 2021) (Figure 1).

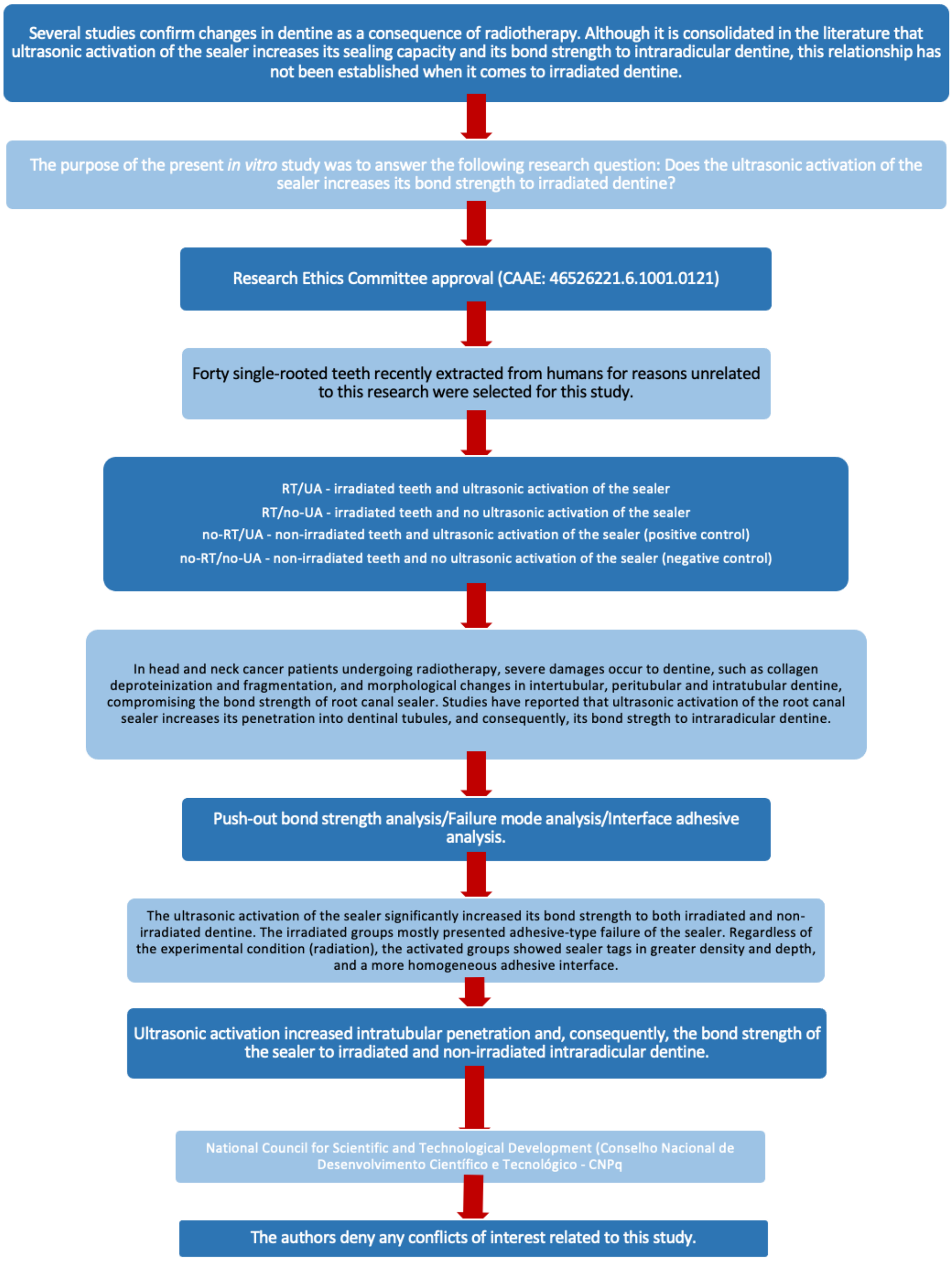

FIGURE 1 PRILE 2021 flowchart.

Ethical approval and sample size calculation

Following prior approval from the Research Ethics Committee (CAAE: 46526221.6.1001.0121), and under the ethical standards laid down in the 1964 Declaration of

Helsinki and its later amendments, forty teeth freshly extracted from human subjects for reasons unrelated to this study were selected.

The sample size was based on the study by Guimarães et al. (2014). The G*Power version 3.1.9.6 software (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/) was used for the sample size calculation, and the following parameters were considered: α error = 0.05 (5% significance level), test power (1-β) = 0.80 and an effect size = 0.5. Wilcoxon signed-rank statistical test (matched pairs) was performed. The type of power analysis was set *a priori* (compute required sample size - given α, power, and effect size). The calculation was based on two factors and normal parental distribution. The sample calculation demonstrated a total number of ten specimens per experimental group for the proper application of the statistical tests.

Sample selection and preparation

After rigorous visual and radiographic inspection, single-rooted human teeth, with complete rhizogenesis, a single and straight canal, a total root length of 15 mm and a ratio of buccolingual and mesiodistal dimensions ≤ 1.5 were included in this study (Pereira et al., 2017). In this inspection, the following exclusion criteria were adopted: the presence of carious lesions, root fractures, a root canal with previous endodontic treatment, internal and/or external calcifications and resorptions. The teeth were immersed for 48 hours in a 0.5% Chloramine T solution for disinfection, followed by washing in running water for 24 hours. After, the teeth were stored individually in containers with distilled water in an oven at 37ºC to avoid dehydration, until the beginning of the experiment.

Samples were randomly distributed (www.random.org) into 4 experimental groups (n=10), according to teeth exposure to ionizing radiation and ultrasonic activation of the sealer. The following experimental groups were formed: RT/UA - irradiated teeth and ultrasonic activation of the sealer; RT/no-UA - irradiated teeth and no ultrasonic activation of the sealer; no-RT/UA - non-irradiated teeth and ultrasonic activation of the sealer; and no-RT/no-UA - non-irradiated teeth and no ultrasonic activation of the sealer. The teeth were decoronated at the cementoenamel junction using a double-sided diamond disk Nº 7016 (American Burrs, Palhoça, SC, Brazil), under copious water cooling.

Teeth irradiation

The irradiation of teeth from the RT/UA and RT/no-UA groups was carried out at the Department of Radiotherapy of the Oncological Research Center (CEPON - Florianópolis, SC, Brazil); under the supervision of a physicist and a radio-oncologist. The entire protocol was performed in a linear accelerator (Clinac 2100C; Varian Medical Systems, Inc., Palo Alto, CA, USA) with dynamic collimators (Dynamic Multileaf Collimator - DMLC), by the Intensity Modulated Radiotherapy Technique (Cancelier et al., 2023; Coelho et al., 2023). A dose distribution map was prepared before radiotherapy to ensure that the total predicted radiation dose uniformly reach all teeth. The teeth were placed inside a plastic receptacle and immersed in distilled and deionized water, aligned and equidistant from the centre of the beam to allow a correct distribution of the dose rate (400 UM/min) (da Cunha et al., 2016).

A total dose of 70 Gy was delivered to each tooth, divided into 2 Gy daily, 5 days a week, for 7 weeks. (Yamin et al., 2018). At the end of each irradiation cycle, the distilled and deionized water was replaced by artificial saliva and the teeth were kept at 37ºC to simulate the oral conditions. At each new cycle of irradiation, the artificial saliva was replaced by distilled and deionized water (Cancelier et al., 2023; Coelho et al., 2023). Once the irradiation protocol is finished, the teeth were stored again in artificial saliva at 37ºC until use.

Endodontic treatment

After irradiation, the endodontic treatment of all teeth was performed. The root canal was initially negotiated with a size 10 K-type instrument (Dentsply-Maillefer, Ballaigues, Switzerland). The working length was established 1 mm short of the apical foramen. Root canal preparation was performed with the instrument R40 (40/.06) from the Reciproc system (VDW GmbH, Munich, Germany) coupled to a 6:1 reducing contra-angle (VDW Silver Reciproc, Sirona Dental Systems GmbH, Bensheim, Germany) and driven by a Silver Reciproc electric motor (VDW, Sirona Dental Systems) in the “RECIPROC ALL” mode, according to the manufacturer's recommendations. The instrument gradually advanced in the apical direction, in pecking movements. After every three pecks, the instrument was removed from the root canal and cleaned with sterile gauze soaked in alcohol. At this moment, the root canal was irrigated with 2 mL of 2.5% NaOCl solution (Asfer, São Caetano do Sul, SP, Brasil). The irrigation solution was accomplished using a 30-gauge tip (NaviTip, Ultradent Products Inc., South Jordan, UT, USA) coupled to a 5-mL syringe in back-and-forth movements of 2-3 mm amplitude. Apical patency was checked throughout the preparation with a size 10 K-type instrument (Dentsply-Maillefer).

For the final irrigation protocol, 3 mL of 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil) were applied for 3 minutes, followed by 3 mL of 2.5% NaOCl solution. Next, the root canals were dried with R40 sterile absorbent paper cones (Reciproc, VDW GmbH). Each reciprocating instrument was used to prepare a maximum of four root canals.

The root canals were obturated using the lateral compaction technique. An epoxy resin-based sealer (AH Plus; Dentsply Sirona, York, PA, USA) was manipulated according to the manufacturer's recommendations and placed into the canal using a size 30 lentulo spiral (Dentsply-Maillefer) positioned 2 mm short of the working length, rotating clockwise. After the complete root canal filling, ultrasonic activation of the sealer was performed in the RT/UA and no RT/UA groups. The sealer activation was performed with an ultrasonic insert (E1 Irrisonic; Helse, Santa Rosa do Viterbo, SP, Brazil) positioned 2 mm short of the working length, coupled to an ultrasound device (JetSonic Four Plus; Gnatus, Ribeirão Preto, SP, Brazil) in the "ENDO" mode (50% power), for 20 seconds, in both mesiodistal and buccolingual directions (Guimarães et al., 2014).

A master gutta-percha cone (Reciproc; VDW GmbH) was inserted into the root canal and its correct positioning on the working length was carefully verified. A size M finger spreader (Dentsply Sirona, São Paulo, SP, Brazil), positioned 1 mm short of the working length, was inserted inside the root canal to create space for the placement of B7 size gutta-percha accessory cones (Tanari; TANARIMAN INDUSTRIAL LTDA, Manacapuru, AM, Brazil). This process was repeated until it was no longer possible to place more gutta-percha accessory cones. Next, a pre-heated plugger (Odous de Deus, Belo Horizonte, MG, Brazil) was used to remove the excess filling material, followed by vertical compaction with a cold plugger. Radiographic examination in both directions (mesiodistal and buccolingual) was performed to ensure the quality of the root canal obturation. Root canals with voids and gaps were discarded from the final sample and replaced.

After cleaning with 70% alcohol and sealing the entrance of the root canals with a temporary restorative material (Coltosol; Coltene, Rio de Janeiro, RJ, Brazil), the samples were stored at 37°C, at 100% humidity, for a time corresponding to three times the setting time of the root canal sealer. All procedures described above were performed by a previously trained operator.

Push-out test

After the sealer set, the samples were individually placed in silicone moulds and embedded in colourless self-curing acrylic resin (JET, Clássico, São Paulo, SP, Brazil) forming blocks (25 mm x 10 mm). The samples were sectioned perpendicular to their long axis with a double-sided diamond disc (Buehler, Lake Bluff, IL, USA) mounted on a metallographic cutter (Isomet 1000; Buehler), under copious water cooling to prevent frictional heat. Two 1.0-mm-thick ($\pm$ 0.1 mm) dentine slices from each root third (cervical, middle and apical) were obtained (Martins et al., 2016).

One dentine slice per root third was placed in the Universal Testing Machine (Model 3345; Instron, Canton, MA, USA), on a stainless-steel metal base containing 2.5 mm (dentine slices from the cervical and middle thirds) or a 1.0 mm in diameter (apical third dentine slices) hole in the centre. Each dentine slice was placed with its cervical surface towards the hole in the metal base. A cylindrical metal plunger (0.6 to 1.2 mm) compatible with the diameter of the root canal was positioned near the area corresponding to the filling material. The plunger was activated with a crosshead speed of 0.5 mm/min in the apical-cervical direction until the filling material displacement.

The force applied to displace the filling material was recorded in Newton (N) and converted into MegaPascal (MPa), according to the formula $\sigma = F/A$, where "σ" is the stress in MPa, "F" the force in N and "A" is the bonded surface area in mm^2. To calculate the bonding interface area between the filling material and the root canal walls, the following formula was used:

$$A = \pi\,(R + r)\sqrt{h^2 + (R - r)^2}$$

where R = canal radius at the coronal portion; r = canal radius at the apical portion; and h = thickness of the dentine slice (Cancelier et al., 2023). The thickness of each dentine slice was measured with a digital caliper, and the canal radius at the coronal and apical portion were measured using a stereomicroscope (SteREO Discovery V12; Carl Zeiss, Jena, Germany) before the push-out test.

Failure mode analysis

After the push-out test, both sides of all samples were analyzed under a stereomicroscope (SteREO Discovery.V12; Carl Zeiss) at ×15 and ×40 magnifications to classify the failure modes into adhesive (failure at the sealer-dentine interface); cohesive (failure within sealer or dentine) or mixed (adhesive and cohesive failures) (Martins et al., 2016; De Bem et al., 2022). Representative samples of the failure modes were selected for image acquisition under Scanning Electron Microscope (SEM) (Jeol JSM 5410, Sony, Tokyo, Japan) at ×500, ×1000 and ×1500 magnifications. The analysis of the images obtained was performed by a single, blinded and previously calibrated examiner.

Adhesive interface analysis

The dentine slices that were not submitted to the push-out test were used for the adhesive interface analysis under SEM (Jeol JSM 5410, Sony). After fixation and dehydration (Dos Santos et al., 2021), each dentine slice was positioned and attached with a double-sided tape into Teflon rings (5.0-mm-thick x 2.0-cm in diameter) with the cervical surface facing downwards. Then, the dentine slices were embedded in epoxy resin. After resin polymerization, the samples were polished with abrasive sandpaper (Norton, São Paulo, SP, Brazil) in decreasing order of abrasiveness (#400, 600 and 1200), followed by polishing with abrasive disks and pastes (0.3 and 0.1 μm) (Arotec, São Paulo, SP, Brazil). After fixation and dehydration, the samples were subjected to demineralization with 6 mol/L hydrochloric acid (Dermus, Florianópolis, SC, Brazil) for 30 seconds, and deproteinization with 2% NaOCl solution (Asfer) for 10 minutes. The samples were washed in distilled water, dried, and placed in a vacuum chamber to be sputter-coated with a layer of gold of 300 Å (BalTec SCD 005, BalTec Co., Canonsburg, PA, USA) (Tedesco et al., 2018). Images of distinct areas of each dentine slice were obtained at ×100, ×300, ×1000 and ×1500 magnifications. The following structures were considered during the qualitative analysis (Tedesco et al., 2018): integrity of the adhesive interface between intraradicular dentine and sealer (presence of gaps or voids, or a continuous and homogeneous interface); and density and depth of penetration of the sealer tags into the dentinal tubules.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 8.0.1 software (GraphPad Software, La Jolla, CA, USA). Normal distribution and homogeneity of data variance were confirmed by the Shapiro-Wilk and Levene test ($p > .05$), respectively. A two-way analysis of variance (two-way-ANOVA) was performed to identify the effects of the experimental conditions (radiation and ultrasonic activation) and root thirds, as well as the interaction of these factors, on bond strength. The Games-Howell post hoc test was applied for pairwise comparisons. The failure mode was analyzed using Fisher's exact test. The significance level was established at 5%. ($\alpha = .05$).

RESULTS

Push-out bond strength

No specimens were lost during testing. The push-out bond strength test values may be seen in Figure 2.

The different experimental conditions (radiation and ultrasonic activation) and the root third had a significant effect on the push-out bond strength ($p < .05$). The interaction of these factors was significant ($p < .05$). When comparing irradiated and non-irradiated groups, there was a significant decrease in bond strength for the irradiated samples ($p < .05$). Conversely, the ultrasonic activation of the sealer significantly increased the bond strength for both conditions (irradiated and non-irradiated), compared to non-activated samples ($p < .05$). When the root thirds were compared, there was a significant decrease in bond strength in the apical third when the sealer was ultrasonically activated, regardless of whether the sample was irradiated or not ($p < .05$). There was no significant difference between the RT/UA and no-RT/AU groups in the middle third ($p > .05$).

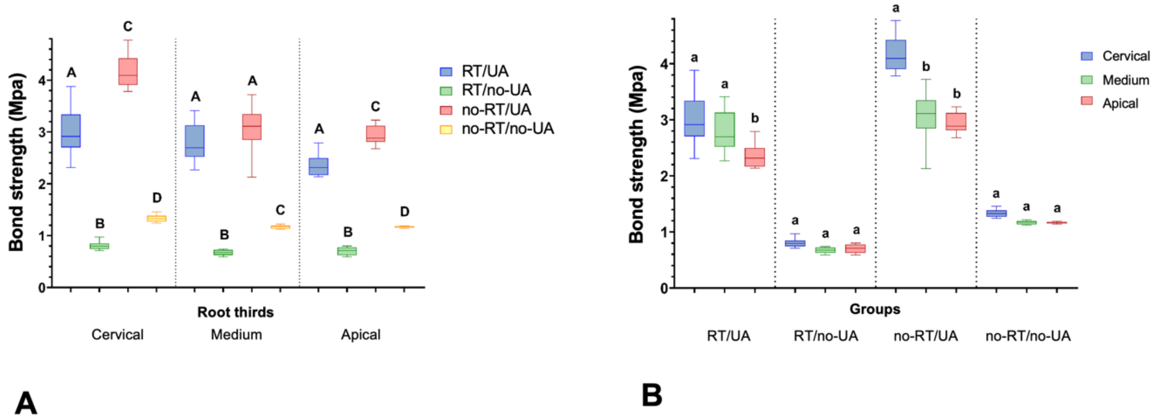

FIGURE 2 Box plot graphical representation of bond strength (MPa) for the different groups and root thirds. (A) Different capital letters represent a statistically significant difference among the experimental groups. (B) Different lowercase letters represent a statistically significant difference among the root thirds. Two-way ANOVA test, Games-Howell posthoc test, $p < .05$.

Failure mode

The incidence and percentage of the different failure modes observed in each experimental group may be seen in Table 1 and Figure 3, respectively. Representative SEM images of the failure modes may be seen in Figure 4. Overall, the failure modes were shown to be significantly different among groups ($p < .01$). RT/UA and RT/no-UA showed the predominance of adhesive failures, whereas no-RT/UA showed a higher prevalence of mixed failure. No-RT/no-UA showed a uniform distribution between the adhesive and mixed failures. It was observed a relatively low incidence of cohesive failures in all experimental groups (Figure 4). Regarding the root thirds, there were significant differences among groups in the cervical and middle root thirds ($p < .01$). There was no difference in the apical root third ($p > .05$).

TABLE 1 Frequency of failure mode according to the groups and root thirds.

Root thirds	Groups	Failure Mode		
		Adhesive	Cohesive	Mixed
Cervical*	RT/UA	7	1	2
	RT/no-UA	10	0	0
	no-RT/UA	2	2	6
	no-RT/no-UA	4	2	4
Middle*	RT/AU	8	0	2
	RT/no-UA	10	0	0
	no-RT/UA	2	2	6
	no-RT/no-UA	4	0	6
Apical	RT/UA	7	1	2
	RT/no-UA	8	1	1
	No-RT/UA	4	2	4
	no-RT/no-UA	6	1	3
Overall*	RT/UA	22	2	6
	RT/no-UA	28	1	1
	no-RT/UA	8	6	16
	no-RT/no-UA	14	3	13

* Indicates significant statistical difference among groups - $p < .01$ Fisher's exact test.

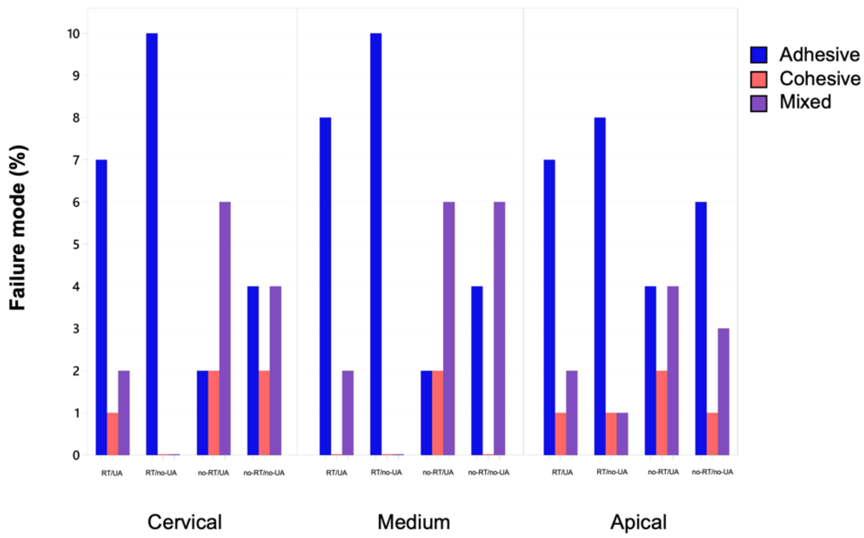

FIGURE 3 Graphical representation of the failure mode (%).

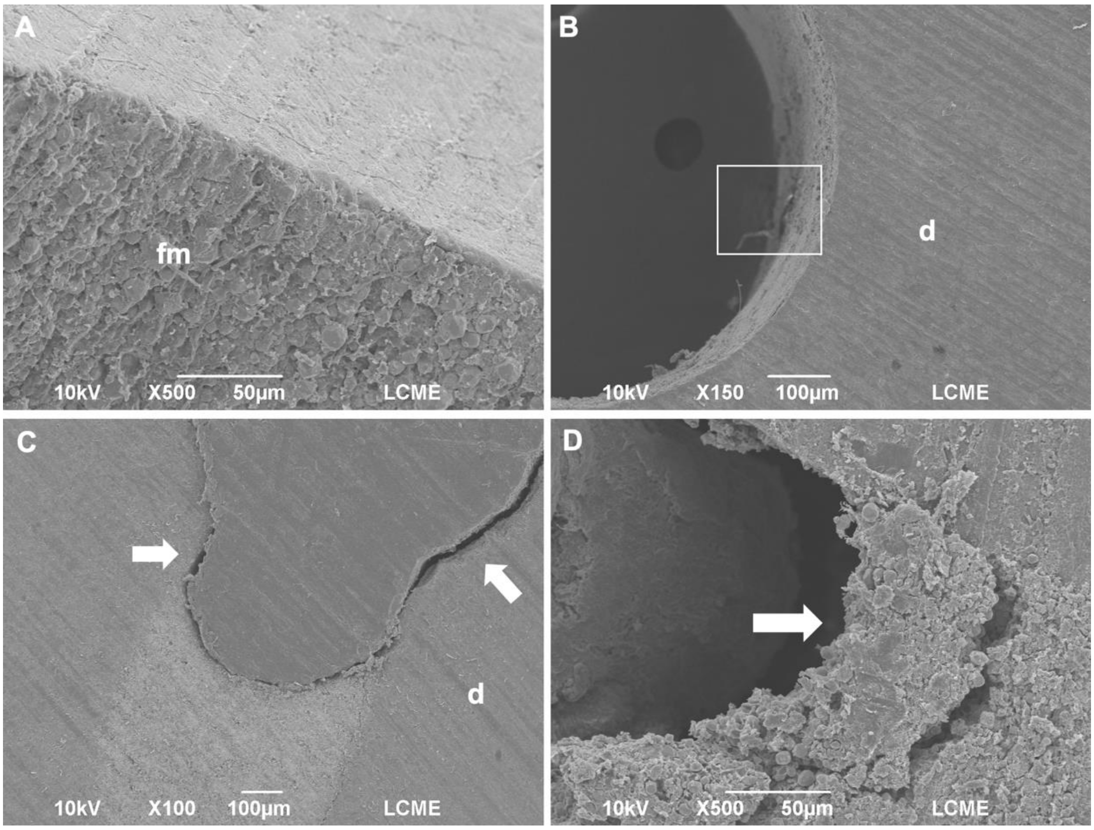

FIGURE 4 Representative SEM images of the failure mode after the push-out test. (A) RT/UA (mixed) - note the presence of remnants of filling material (fm) attached to the root canal wall; (B) RT/no-UA (adhesive failure of the sealer in relation to the gutta-percha cone) - remaining filling material attached to the root canal wall (square), dentine surface (d); (C) no-RT/UA (mixed) - areas corresponding to adhesive failure (arrows), dentine surface (d); and (D) no-RT/no-UA (mixed) - note the cohesive failure of the filling material (arrow).

Adhesive interface analysis

The qualitative analysis of the SEM images revealed differences and similarities in the adhesive interface of the different experimental groups (Figure 5). Gaps at the adhesive interface were observed in both irradiated and non-irradiated groups. However, in irradiated samples, the incidence of this feature was higher. Non-irradiated samples presented a more continuous and homogeneous adhesive interface, with a higher prevalence of areas of juxtaposition between sealer and intraradicular dentine. Irradiated teeth showed areas of dentine fracture in some samples. Regarding the sealer tags, noticeable differences were observed among the experimental groups, especially when the sealer was ultrasonically activated. Regardless of the experimental condition (radiation), the activated groups showed a greater density of sealer tags, penetrating more deeply into the dentinal tubules.

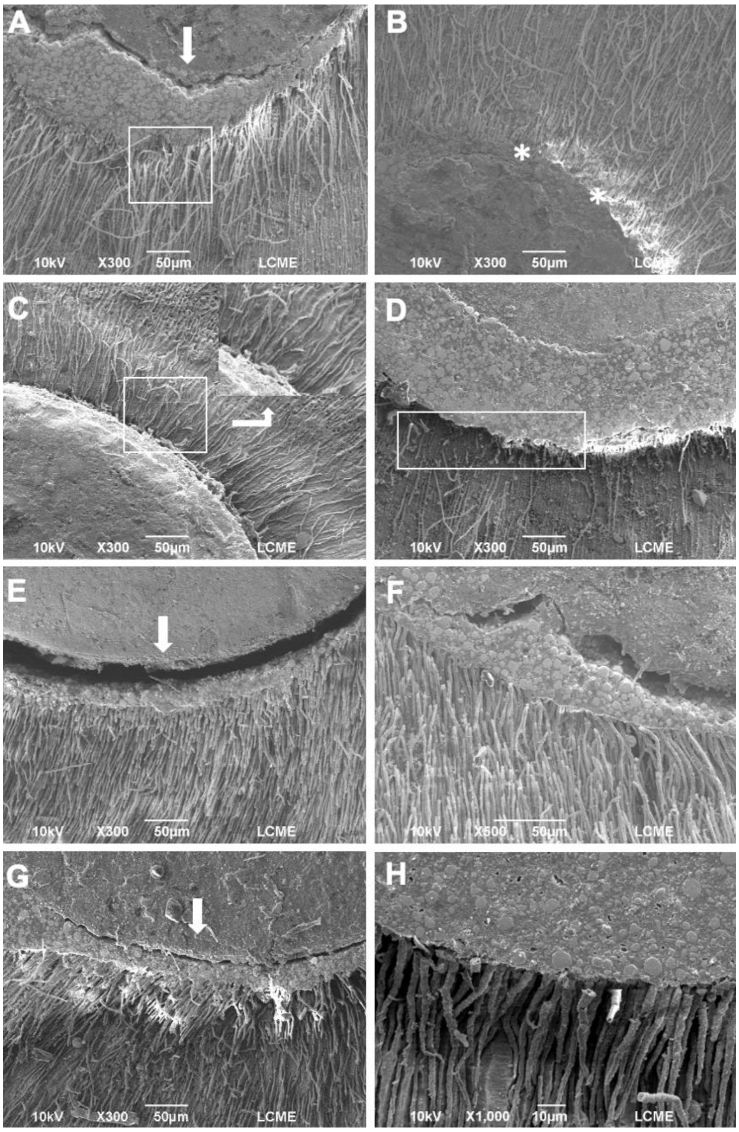

FIGURE 5 Representative SEM images of the adhesive interface between the sealer and the intraradicular dentine. (A) RT/UA (cervical third) - intratubular penetration of the sealer. Note the density of the sealer tags (square). Failure at the interface between sealer and gutta-percha core (arrow). (B) RT/UA (apical third) - area of juxtaposition between filling material and intraradicular dentine (*). Lower density of sealer tags at this portion of the root canal. (C) RT/no-UA (cervical third) - sealer tags in smaller quantity and depth. Note details at higher magnification (highlighted area); (D) RT/no-UA (apical third) - note the small number of sealer tags in this portion of the root canal (square), with many gaps and a non-continuous interface. (E) no-RT/UA (cervical third) - discontinuous interface between filling material and

intraradicular dentine (arrow). (F) no-RT/UA (middle third). Note the sealer tags in greater density and depth. (G) no-RT/no-UA (cervical third) - discontinuous interface between gutta-percha core and sealer (arrow). Sealer tags in lower density. (H) no-RT/no-UA (apical third) - sealer tags into the dentinal tubules at higher magnification.

DISCUSSION

One of the most deleterious effects of radiotherapy on dentine is the degeneration of the odontoblastic processes (Gonçalves et al., 2014), followed by obliteration of the dentinal tubules (Kielbassa et al., 1999). Therefore, the mechanical retention of the sealer to the root canal walls in irradiated dentine is highly compromised (Martins et al., 2016; Paiola et al., 2018). Several studies have already reported that ultrasonic activation of the sealer increases its ability to penetrate the dentinal tubules, creating denser and deeper tags, a more homogeneous interface between sealer and intraradicular dentine, and consequently, greater resistance to dislodgement (Guimarães et al., 2014; Arslan et al., 2016; Wiesse et al., 2018; De Bem et al., 2020). However, the effect of ultrasonic activation of the sealer on teeth undergoing radiotherapy is a gap in the scientific literature that needs to be bridged.

The purpose of the present *in vitro* study was to evaluate the effect of ultrasonic activation of the sealer on its bond strength to irradiated intraradicular dentine. Based on the results obtained, the tested hypothesis was confirmed, since the ultrasonic activation increased the bond strength of the sealer to both irradiated and non-irradiated dentine.

Clinically, the radiation therapy protocol adopted for the treatment of head and neck cancer uses doses ranging from 50 to 70 Gy. In this study, a maximum dose of 70 Gy was delivered to the samples, in a fractional mode, following the protocol carried out in other laboratory studies of similar purpose (Verdonck *et al*., 2009; Thariat *et al*., 2012; Velo *et al*., 2018; Cancelier et al., 2023, Coelho et al., 2023). Furthermore, the Intensity Modulated Radiotherapy Technique was used, as it is widely adopted in clinical conditions (Verdonck *et al*., 2009; Parahyba et al., 2016). This technique allows high doses of irradiation to be delivered to neoplastic cells without exposing healthy tissues to irradiation (Verdonck *et al*., 2009; Parahyba et al., 2016).

Root canals with similar buccolingual and mesiodistal dimensions (ratio $\leq$1.5) were selected for this research (Pereira et al., 2017). The push-out test was performed using a cylindrical metal plunger with a tip similar in diameter to the central area of the filling material in the different root thirds (Martins *et al*., 2016). Therefore, it was possible to estimate the adhesiveness of the filling material to the intraradicular dentine in the different portions of the root canal, simulating axial displacement forces to which these materials are subjected during mastication (Martins et al., 2016).

Root canal obturation was performed by the lateral compaction technique, as it is considered a reference for comparison with other techniques (Araujo et al., 2016). Even so, the lateral compaction technique may lead to the formation of voids and gaps in the filling material, an excessive amount of sealer in specific areas of the root canal and low apical density (Rossetto et al., 2014). The ultrasonic activation of the sealer tends to minimize such limitations of the technique (Guimarães et al., 2014, Arslan et al., 2016).

The root canals were obturated with an epoxy resin-based sealer (AH Plus; Dentsply Sirona). AH Plus is considered the “gold standard” sealer, as it has excellent physicochemical properties, such as dimensional stability, sealing capacity and flow ability (Zhou et al., 2013). Only one type of sealer was used in this study to avoid that no other variables, besides the factors, “radiation” and “ultrasonic activation”, were tested.

The results of the present study pointed to a significant decrease in the bond strength of the sealer to the irradiated teeth. This finding is in line with the scientific literature (Martins et al., 2016; Paiola et al., 2018) since radiotherapy causes remarkable structural changes in dentine (Kielbassa et al., 1999; Springer et al., 2005; Gonçalves et al., 2014; de Siqueira Mellara et al., 2014). Conversely, our results reaffirm the positive effects of ultrasonic activation of the sealer on its bond strength to intraradicular dentine (Wiesse et al., 2018), especially, for the experimental condition in which the teeth were irradiated. Our study is the first one to assess this topic, and its positive findings point to the adoption of an important clinical protocol for patients with head and neck cancer undergoing radiotherapy.

According to Oral et al. (2012), ultrasonic activation at high frequency and small amplitude generates sufficient acoustic energy to promote a more homogeneous adaptation of the sealer to the root canal walls. The heat generated by the process decreases the viscosity of the sealer, improving its flow ability, and consequently, its capacity to fill hard-to-reach areas of the root canal, such as isthmuses and dentinal tubules (Oral et al., 2012). In the present study, this phenomenon was confirmed in the adhesive interface analysis, for both irradiated and non-irradiated samples. A more homogeneous interface, with sealer tags in greater number and density, was observed in the ultrasonically activated samples.

It is also worth noticing that after ultrasonic activation, the filler particles of epoxy resin-based sealers, such as AH Plus, are more efficiently incorporated into its organic matrix, reinforcing its mechanical properties, such as cohesive strength (Bittmann et al., 2009; Lionetto & Maffezzoli, 2013). This phenomenon is highly relevant, since ionizing radiation promotes the breakdown of the collagen fibrils network of the dentine organic matrix (Cancelier et al., 2023), which harms the filling material's bond strength (de Siqueira Mellara et al., 2014; Yaduka et al., 2021).

Epoxy resin-based sealer chemically bonds to the collagen fibrils network of intraradicular dentine (Yaduka et al., 2021). AH Plus chemically interacts with this collagen network through covalent bonds between its epoxy rings and the amine groups of the dentine substrate (Sousa-Neto et al., 2005; Yaduka et al., 2021). It is already consolidated in the literature that radiation is more deleterious to the organic components of dentine than the inorganic ones, leading to a decrease in bond strength (Soares et al., 2010). The ultrasonic activation in this case proved to be an important strategy for maintaining a proper adhesion of the sealer to the irradiated dentine.

Despite the ultrasonic activation having significantly increased the bond strength of the sealer in the irradiated samples, in general, the bond strength was lower in comparison with non-irradiated/ultrasonically activated samples. Conversely, it is important to emphasize that this clinical protocol should be considered for root canal obturation in head and neck cancer patients undergoing radiotherapy.

Another trend observed in this study was the decrease in the bond strength of the sealer towards apical in the ultrasonically activated samples, regardless of their experimental condition (irradiated and non-irradiated). The cervical third has a greater number of dentinal tubules and a greater area of intertubular dentine, making this portion of the substrate more appropriate for epoxy resin-based sealers adhesion to the collagen network (Wiesse et al., 2018). On the other hand, the apical third is considered a critical zone due to the lower density of dentinal tubules and the greater presence of sclerotic dentine (Chadha et al., 2012; Ubaldini et al., 2018). Furthermore, the small amplitude of the apical third may limit the action of the ultrasonic insert, which leads to a decrease in the bond strength of the sealer in this portion of the root canal (Guimarães et al., 2014).

Failure mode analysis was performed to complement the push-out test findings. Most irradiated samples showed adhesive failures. Despite the proper results obtained with epoxy resin-based sealers in bond strength tests, its ability to adhere to the organic portion of the intraradicular dentine may be considered a weak point (Sousa-Neto et al., 2005; De Bem et al., 2020). This phenomenon corroborates the findings of the present study since the organic portion of dentine is significantly affected by ionizing radiation (Yaduka et al., 2021).

Laboratory studies have several limitations. Among the limitations of this research, the use of single-rooted teeth, with straight and circular canals must be considered before extrapolating our findings to clinical situations. Other studies using multirooted teeth, with flattened root canals and the presence of isthmuses, should be performed to assess the tested obturation protocol in a more challenging condition.

The validation of clinical protocols based on scientific evidence is essential to meet the endodontic needs of head and neck cancer patients. The obturation technique assessed in the present *in vitro* study showed promising results concerning the sealing capacity of root canal sealers. However, further studies must be carried out to scientifically validate this new clinical protocol for oncological patients.

CONCLUSIONS

This is the first study assessing the effect of ultrasonic activation of the root canal sealer on its bond strength to irradiated dentine. Within the limits of this *in vitro* study, the following conclusions may be drawn:

1. The ultrasonic activation of the sealer positively affected its intratubular penetration.
2. Therefore, ultrasonic activation significantly increased the bond strength of the sealer to irradiated and non-irradiated intraradicular dentine.

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ETHICAL APPROVAL

This research involved human samples. The study was approved by the Research Ethics Committee of the Federal University of Santa Catarina (CAAE: 46526221.6.1001.0121).

CONFLICT OF INTEREST

The authors deny any conflicts of interest with this article.

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4. CONSIDERAÇÕES FINAIS

Apesar das limitações deste estudo *in vitro*, podemos concluir que a ativação ultrassônica afetou positivamente a penetração do cimento obturador nos túbulos dentinários. Desta forma, a ativação ultrassônica aumentou significativamente a resistência de união do cimento obturador à dentina intrarradicular irradiada e não-irradiada.

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APENDICE A - PARECER DO COMITE DE ÉTICA EM PESQUISA COM SERES HUMANOS DA UFSC

UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DA AGITAÇÃO ULTRASSÔNICA DO CIMENTO OBTURADOR NA RESISTÊNCIA DE UNIÃO EM DENTINA IRRADIADA

Pesquisador: LUCAS DA FONSECA ROBERTI GARCIA

Área Temática:

Versão: 1

CAAE: 46526221.6.1001.0121

Instituição Proponente: CENTRO DE CIÊNCIAS DA SAÚDE

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.730.649

Apresentação do Projeto:

Segundo pesquisador: " Serão selecionados 40 dentes humanos unirradiculares extraídos e armazenados em solução de cloramina T a 5% por 7 dias. As amostras serão distribuídas aleatoriamente em 4 grupos (n=10), de acordo com a exposição à radiação ionizante dos elementos dentais e a ativação ultrassônica do cimento obturador, a saber: G1 - dentes irradiados com agitação ultrassônica do cimento obturador; G2 - dentes irradiados sem agitação ultrassônica do cimento; G3 - dentes não irradiados com agitação ultrassônica do cimento e G4 - dentes não irradiados sem agitação ultrassônica do cimento. Após, será feita a avaliação da resistência de união do cimento à dentina radicular utilizando o teste de push-out em Máquina Universal de Ensaios (Instron) (0,5 mm/min). Os padrões de fratura serão classificados em adesivo, coesivo ou misto após análise em estereomicroscópio e Microscópio Eletrônico de Varredura (MEV). "

Objetivo da Pesquisa:

Segundo pesquisador: "Avaliar o efeito da agitação ultrassônica do cimento endodôntico na resistência de união em dentina irradiada."

Avaliação dos Riscos e Benefícios:

Segundo pesquisador:

"Riscos: os dentes utilizados na pesquisados serão extraídos por motivos de interesse do paciente (tratamento ortodôntico ou por não haver mais meios de recuperar o dente devido a cárie ou

problemas periodontais). Tais dentes seriam descartados ou armazenados, por isso solicita-se a cessão dos dentes ao paciente após extração para realização desta pesquisa. Os riscos ao paciente são praticamente inexistentes. Poderá haver desconforto e sensibilidade no local devido à cirurgia, e necessidade de pontos para auxiliar na cicatrização, mas essa sensibilidade está presente no pós- operatório de qualquer cirurgia e é resolvida com o uso de analgésicos que serão receitados pelo profissional que realizará a cirurgia e que podem ser obtidos gratuitamente nos postos de saúde.
Benefícios: apesar do paciente não ter benefícios diretos com a pesquisa, ele poderá contribuir com seu dente para determinar a melhor técnica de obturação para dentes submetidos aos efeitos da radioterapia."

Comentários e Considerações sobre a Pesquisa:
Vide campo "Conclusões ou Pendências e Lista de Inadequações".

Considerações sobre os Termos de apresentação obrigatória:
Vide campo "Conclusões ou Pendências e Lista de Inadequações".

Recomendações:
Vide campo "Conclusões ou Pendências e Lista de Inadequações".

Conclusões ou Pendências e Lista de Inadequações:
Recomendamos as seguintes modificações:
1. Remover a seguinte frase no campo "Riscos" no formulário base da Plataforma Brasil: "Os riscos ao paciente são praticamente inexistentes."
2. Quanto ao TCLE:
2.1. Substitua a palavra cópia por via na seguinte frase: "Além disso, o paciente que concordar em participar ainda receberá uma cópia deste termo de consentimento (TCLE) assinada e rubricada pelos pesquisadores responsáveis."
2.2. Frases de teor declaratório devem ser evitadas, uma vez que podem reduzir a capacidade de consentimento do participante. Portanto, orientamos remover o seguinte trecho, não sendo necessário manter consentimento pós-informado: "Eu, ________________________________, declaro optar por livre e espontânea vontade participar desta pesquisa e que recebi todas as orientações sobre os riscos e objetivos da pesquisa, que todos os meus dados serão mantidos em sigilo e que serão obedecidas as normas para pesquisa envolvendo seres humanos, assim como, poderei remover o consentimento da pesquisa sem haver penalidade alguma."
3. O presente projeto não se configura um projeto multicêntrico, visto que o CEPON parece apenas

ser o local de recrutamento da amostra. Corrigir.
4. Visto não haver submissão de TALE, pressupõe-se que os dentes extraídos que constituirão a amostra serão apenas de participantes adultos. Solicitamos incluir nos critérios de inclusão a idade dos participantes ou, caso menores de idade sejam incluídos, anexar o TALE.

Considerações Finais a critério do CEP:

A análise foi realizada com base em todos os documentos apresentados, incluindo o projeto em sua íntegra. Os pesquisadores deverão enviar uma carta respondendo todos os questionamentos do parecer. Quando a resposta alterar o projeto e/ou TCLE, enviar uma nova versão do(s) documento(s) com as modificações em destaque e estas modificações deverão estar citadas na carta resposta identificando o local da alteração ao longo do texto (página e/ou título do item).

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1748283.pdf	04/05/2021 17:03:08		Aceito
Folha de Rosto	Folha_de_Rosto.pdf	04/05/2021 17:02:47	LUCAS DA FONSECA ROBERTI GARCIA	Aceito
Outros	Declaracao_de_Cessao_Dentes.pdf	04/05/2021 16:33:55	LUCAS DA FONSECA ROBERTI GARCIA	Aceito
Declaração de concordância	Declaracao_Cepon.pdf	04/05/2021 16:32:27	LUCAS DA FONSECA ROBERTI GARCIA	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_da_Instituicao_UFSC.pdf	04/05/2021 16:30:34	LUCAS DA FONSECA ROBERTI GARCIA	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	04/05/2021 16:27:49	LUCAS DA FONSECA ROBERTI GARCIA	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO.pdf	04/05/2021 16:26:20	LUCAS DA FONSECA ROBERTI GARCIA	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

FLORIANOPOLIS, 24 de Maio de 2021

Assinado por:
Nelson Canzian da Silva
(Coordenador(a))

Endereço: Universidade Federal de Santa Catarina, Prédio Reitoria II, R: Desembargador Vitor Lima, nº 222, sala 401
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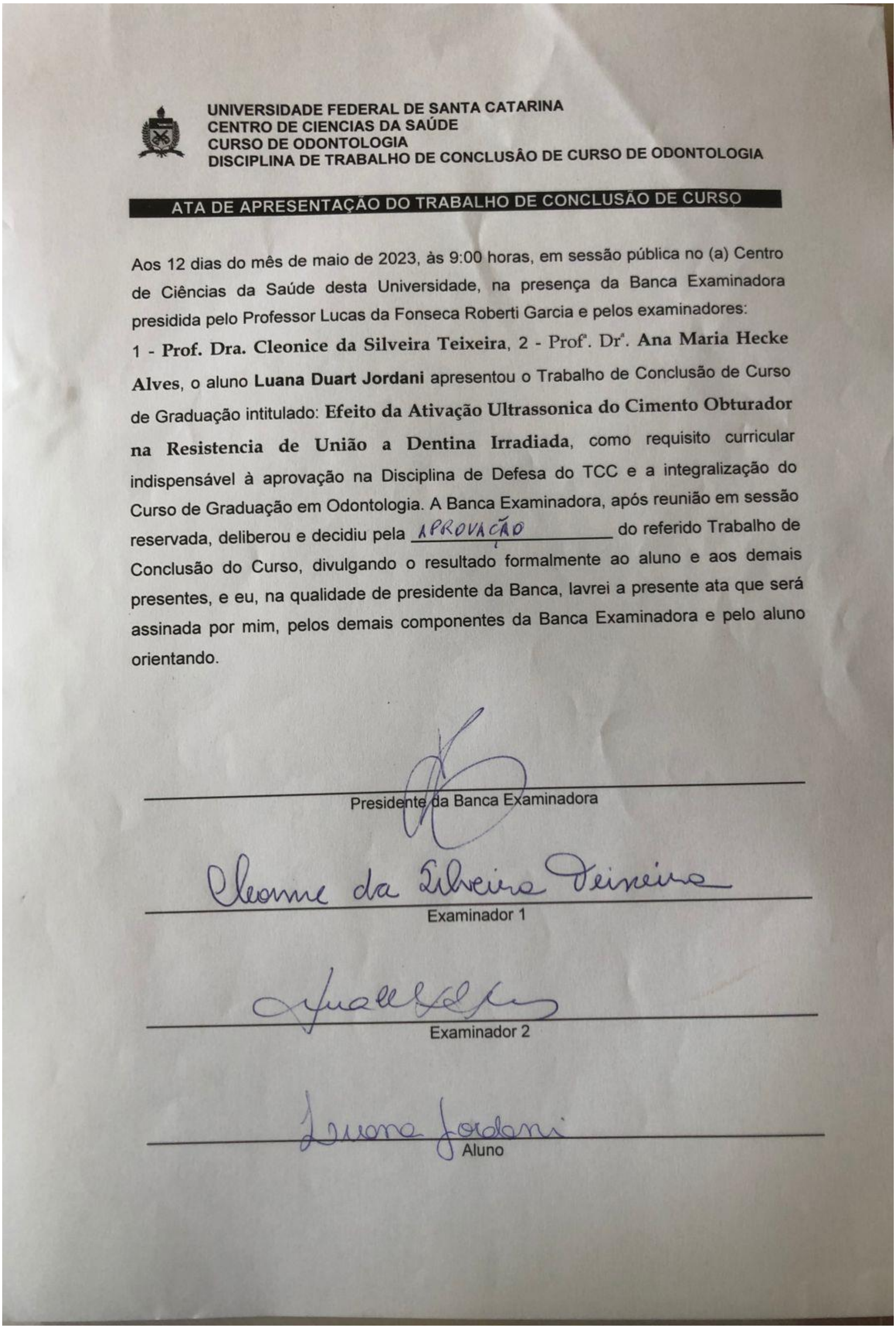
ANEXO 1 - ATA DA DEFESA



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ATA DE APRESENTAÇÃO DO TRABALHO DE CONCLUSÃO DE CURSO

Aos 12 dias do mês de maio de 2023, às 9:00 horas, em sessão pública no (a) Centro de Ciências da Saúde desta Universidade, na presença da Banca Examinadora presidida pelo Professor Lucas da Fonseca Roberti Garcia e pelos examinadores: 1 - **Prof. Dra. Cleonice da Silveira Teixeira**, 2 - Profª. Drª. **Ana Maria Hecke Alves**, o aluno **Luana Duart Jordani** apresentou o Trabalho de Conclusão de Curso de Graduação intitulado: **Efeito da Ativação Ultrassonica do Cimento Obturador na Resistencia de União a Dentina Irradiada**, como requisito curricular indispensável à aprovação na Disciplina de Defesa do TCC e a integralização do Curso de Graduação em Odontologia. A Banca Examinadora, após reunião em sessão reservada, deliberou e decidiu pela APROVAÇÃO do referido Trabalho de Conclusão do Curso, divulgando o resultado formalmente ao aluno e aos demais presentes, e eu, na qualidade de presidente da Banca, lavrei a presente ata que será assinada por mim, pelos demais componentes da Banca Examinadora e pelo aluno orientando.

Presidente da Banca Examinadora

Examinador 1

Examinador 2

Aluno

ANEXO 2 – NORMAS DA REVISTA

Author Guidelines

Sections

1. Submission and Peer Review Process
2. Article Types
3. After Acceptance

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Review Articles (systematic, scoping and narrative):

These are accepted for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should generally include a clearly defined search strategy and take a broad view of the field rather than merely summarizing the literature. Extensive or unbalanced citation of the authors´ own publications is not acceptable.

Clinical Commentaries:

These can describe significant improvements in clinical practice such as the report of a novel technique, a breakthrough in technology or practical approaches to recognised clinical challenges. They should conform to the highest scientific and clinical practice standards.

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Article Type	Structure Description	Abstract / Structure
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Systematic, Scoping and Narrative Review Articles	Main text includes: Introduction, method, results, discussion, conclusions, funding, Conflict of Interest	Structured: Background, Objectives, Method, Results, Conclusion, Funding, Conflict of interest, Registration No more than 350 words.
Case Reports and Clinical Commentaries	Main text includes: Introduction, report, discussion and conclusion	Structured: Aim, summary, key learning points
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2.1 Reporting Guidelines

2.1.1 Case reports/case series

Case reports should be written to comply with the Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines (Nagendrababu et al. 2020, doi:10.1111/iej.13285).

When submitting manuscripts that have been written using the PRICE 2020 guidelines, authors should include the following statement in the beginning of "Report" section: "This case report has been written according to Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines.

A PRICE checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The PRICE 2020 checklist and flowchart can be viewed by clicking the appropriate link. PRILE guidelines can be found here, and PRAISE checklist link here.

It is recommended that authors consult the following papers when writing case reports, which explains the rationale for the PRICE 2020 guidelines and their importance:

Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Setzer FC, Sunde PT, Dummer PMH (2020) PRICE 2020 guidelines for reporting case reports in Endodontics: a consensus-based development. International Endodontic Journal 53, 619-26. (**https://www.ncbi.nlm.nih.gov/pubmed/32090342**)

Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH (2020) PRICE 2020 guidelines for reporting case reports in Endodontics: Explanation and elaboration. International Endodontic Journal 53, 922-47.

A PRIASE 2021 checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The PRIASE 2021 checklist and flowchart can be downloaded from: http://pride-endodonticguidelines.org/priase/

It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the PRIASE 2021 guidelines and their importance:

Nagendrababu V, Kishen A, Murray PE, Nekoofar MH, de Figueiredo JA, Priya E, Jayaraman J, Pulikkotil SJ, Camilleri J, RM S, Dummer PMH (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: a consensus-based development. International Endodontic Journal 54, 848-57. (https://onlinelibrary.wiley.com/doi/10.1111/iej.13477)

Nagendrababu V, Kishen A, Murray PE, Nekoofar MH, de Figueiredo JA, Priya E, Jayaraman J, Pulikkotil SJ, Jakovljevic A, Dummer PMH (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: Explanation and Elaboration. International Endodontic Journal 54, 858-86. (https://onlinelibrary.wiley.com/doi/10.1111/iej.13481)

2.2.6. Laboratory studies

Laboratory studies should be reported using the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu et al. 2021, doi: 10.1111/iej.13542).

When submitting manuscripts that have been written using the PRILE 2021 guidelines, authors should include the following statement in the beginning of "Materials and Methods" section: "The manuscript of this laboratory study

2.2.4. Diagnostic accuracy studies

Diagnostic accuracy studies should be written using the Standards for Reporting of Diagnostic Accuracy Studies (STARD) 2015 guidelines. Compliance with this should be detailed in the "Materials and Methods" section. A STARD checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The STARD checklist and flowchart can be downloaded from: https://www.equator-network.org/reporting-guidelines/stard/

It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the STARD guidelines and their importance:

Cohen JF, Korevaar DA, Altman DG, Bruns DE, Gatsonis CA, Hooft L, Irwig L, Levine D, Reitsma JB, de Vet HCW, Bossuyt PMM. (2016) STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. BMJ Open 6, e012799

http://bmjopen.bmj.com/content/6/11/e012799.abstract

2.2.5. Animal studies

Animal studies should be written using the Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines (Nagendrababu et al. 2021, doi: 10.1111/iej.13477).

When submitting manuscripts that have been written using the PRIASE 2021 guidelines, authors should include the following statement in the beginning of "Materials and Methods" section: "The manuscript of this animal study has been written according to Preferred Reporting Items for Animal studies in Endodontology (PRIASE) 2021 guidelines.

Clinical Trials

The *International Endodontic Journal* asks that authors submitting manuscripts reporting a clinical trial register the trial a priori in any of the following public clinical trials registries: www.clinicaltrials.gov, https://www.clinicaltrialsregister.eu/, http://isrctn.org/. Other primary registries if named in the WHO network will also be considered acceptable. The clinical trial registration number and name of the trial register should be included in the Acknowledgements at the submission stage.

2.2.3. Epidemiological observational trials

Observational studies should be written using the STrengthening the Reporting of OBservational studies in Epidemiology' (STROBE) guidelines. Compliance with this should be detailed in the "Materials and Methods" section. (www.strobe-statement.org). A STROBE checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material.

It is recommended that authors consult the following papers when writing manuscripts, which explains the rationale for the STROBE guidelines and their importance:

Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, Strobe Initiative (2014) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. International Journal of Surgery 12, 1495-9.

Vandenbroucke JP, von Elm E, Altman DG, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, Poole C, Schlesselman JJ, Egger M, STROBE Initiative. (2014) Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. International

2.2.2. Randomised clinical trials

Randomised clinical trials should be reported to comply with the Preferred Reporting Items for Randomised Trials in Endodontics (PRIRATE) 2020 guidelines (Nagendrababu et al. 2020, doi: 10.1111/iej.13294).

When submitting manuscripts that have been written using the PRIRATE 2020 guidelines, authors should include the following statement in the beginning of "Materials and Methods" section: "This randomised clinical trial has been written according to Preferred Reporting Items for RAndomised Trials in Endodontics (PRIRATE) 2020 guidelines.

A PRIRATE 2020 checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The PRIRATE 2020 checklist and flowchart can be downloaded from: http://pride-endodonticguidelines.org/prirate/.

It is recommended that authors consult the following papers when writing manuscripts, which explains the rationale for the PRIRATE 2020 guidelines and their importance:

Nagendrababu V, Duncan HF, Bjørndal L, Kvist T, Priya E, Jayaraman J, Pulikkotil SJ, Pigg M, Rechenberg DK, Vaeth M, Dummer PMH (2020) PRIRATE 2020 guidelines for reporting randomised trials in Endodontics: a consensus-based development. International Endodontic Journal 53, 764-73. (https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13294)

Nagendrababu V, Duncan HF, Bjørndal L, Kvist T, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH (2020) PRIRATE 2020 guidelines for reporting strials in Endodontics: Explanation and elaboration. International Endodontic Journal 53, 774-03.

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The abstract and main body of the systematic review should be reported using the PRISMA for Abstract and PRISMA guidelines respectively (http://www.prisma-statement.org/). Authors submitting a systematic review must register the protocol in one of the readily-accessible sources/databases at the time of project inception and not retrospectively (e.g. PROSPERO database, OSF registries). The protocol registration number, name of the database or journal reference should be provided at the submission stage in the "Registration" section in the abstract and 'Methods' section in the main body of the text.

A PRISMA checklist and flow diagram as a Figure (to be included in the manuscript for readers) should also be included in the submission material. Source of funding (grant number, if available) should be added in the 'Acknowledgements' section.

It is recommended that authors consult the following papers, which help in the production of high quality reviews:

Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLOS Medicine 6, e1000097.

Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Journal of Clinical Epidemiology 62, e1-34.

Nagendrababu V, Duncan HF, Tsesis I, Sathorn C, Pulikkotil SJ, Dharmarajan L, Dummer PMH (2019) PRISMA for abstracts: best practice for reporting abstracts of systematic reviews in Endodontology. International

2.2.8 Scoping reviews

Reviews should be reported using the PRISMA guidelines. A checklist for scoping reviews should also be included in the submission material - see: http://www.prisma-statement.org/Extensions/ScopingReviews.

2.2.9 Guidelines for reporting of microarray and next-generation sequencing data

Submission will be assessed according to MIAME and MINSEQE standards. The complete current guidelines are available at

Also, manuscripts will be published only after the complete data has been submitted into the public repositories, such as GEO (http://www.ncbi.nlm.nih.gov/geo/) or ArrayExpress (http://www.ebi.ac.uk/microarray/submissions_overview.ht and the data accession number (the identification number of the data set in the database) quoted in the manuscript. Both databases are committed to keeping the data private until the associated manuscript is published, if requested.

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A PRILE checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material The PRILE 2021 checklist and flowchart can be downloaded from: http://pride-endodonticguidelines.org/prile/

It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the PRILE 2021 guidelines and their importance:

Nagendrababu V, Murray PE, Ordinola-Zapata R, OA Peters, IN Rôças, JF Siqueira Jr, E Priya, J Jayaraman, SJ Pulikkotil, J Camilleri, C Boutsioukis, G Rossi-Fedele, PMH Dummer (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontics: a consensus-based development. International Endodontic Journal May 3. doi: 10.1111/iej.13542. (https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13542)

Nagendrababu V, Murray PE, Ordinola-Zapata R, OA Peters, IN Rôças, JF Siqueira Jr, E Priya, J Jayaraman, SJ Pulikkotil, N Suresh, PMH Dummer (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontics: Explanation and elaboration. International Endodontic Journal (https://onlinelibrary.wiley.com/doi/abs/10.1111/iej.13565)

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Appendix

Abbreviations:

The International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

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Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8.

Wittke, M. (2006) Design, construction, supervision and long-term behaviour of tunnels in swelling rock. In: Van Cotthem, A., Charlier, R., Thimus, J.-F. and Tshibangu, J.-P. (Eds.) Eurock 2006: multiphysics coupling and long term behaviour in rock mechanics: proceedings of the international symposium of the international society for rock mechanics, EUROCK 2006, 9–12 May 2006, Liège, Belgium. London: Taylor & Francis, pp. 211–216.

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

The University Encyclopedia (1985) London: Roydon.

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.

Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html [accessed on 7 November 2003].

European Space Agency. (2015) Rosetta: rendezvous with a comet. Available at: http://rosetta.esa.int [Accessed 15th

Examples of correct forms of reference

Standard journal article

Jakovljevic, A., Duncan, H.F., Nagendrababu, V., Jacimovic, J., Milasin, J. & Dummer, P.M.H. (2020) Association between cardiovascular diseases and apical periodontitis: an umbrella review. *International Endodontic Journal,* 53, 1374–1386.

Selman, P. (2016) The global decline of intercountry adoption: what lies ahead? Social Policy and Society, 11(3), 381–397.

Corporate author

British Endodontic Society (1983) Guidelines for root canal treatment. *International Endodontic Journal* 16, 192-5.

Department of Health. (2009) Living well with dementia: a national dementia strategy.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). Blood 54 (Suppl. 1), 26a.

Holding, M.Y., Saulino, M.F., Overton, E.A., Kornbluth, I.D. & Freedman, M.K. (2008) Interventions in chronic pain management. 1. Update on important definitions in pain management. Archives of Physical Medicine and Rehabilitation, 89 (3, Supplement 1), S38–S40.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) Surgical Endodontics, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Barnes, R. (1995) Successful study for degrees, 2nd edition, London: Routledge.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. Endodontics in Clinical Practice, 3rd edn; pp. 186-223. London, UK: Butterworth.

Partridge, H. & Hallam, G. (2007) Evidence-based practice and information literacy. In: Lipu, S., Williamson, K. & Lloyd, A. (Eds.) Exploring methods in information literacy research. Wagga Wagga, Australia: Centre for Information Studies, pp. 149–170.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Wittke, M. (2006) Design, construction, supervision and long-term behaviour of tunnels in swelling rock. In: Van Cotthem, A., Charlier, R., Thimus, J.-F. and Tshibangu, J.-P. (Eds.) Eurock 2006: multiphysics coupling and long term behaviour in rock mechanics: proceedings of the international symposium of the international society for rock mechanics, EUROCK 2006, 9–12 May 2006, Liège, Belgium. London: Taylor & Francis, pp. 211–216.