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Efeitos da terapia fotodinâmica antimicrobiana com diferentes fotossensibilizadores e da medicação de hidróxido de cálcio na resistência de união, caracterização e vedação da interface adesiva da dentina intraradicular

Araçatuba
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Orientador: Prof. Assoc. Gustavo Sivieri de Araújo

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Dedicatória

A Deus,

"Senhor, tu me sondas, e me conheces.

Tu conheces o meu sentar e o meu levantar; de longe entendes o meu pensamento.

Esquadrinhas o meu andar, e o meu deitar, e conheces todos os meus caminhos.

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Tu me cercaste em volta, e puseste sobre mim a tua mão...

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(Salmo 139)

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não temais, nem vos espanteis diante deles;
porque o Senhor, teu Deus,
é quem vai contigo;
não te deixará, nem te desamparará."

- Deuteronômio 31:6

Resumo

Maltarollo TFHM. **Efeitos da terapia fotodinâmica antimicrobiana com diferentes fotossensibilizadores e da medicação de hidróxido de cálcio na resistência de união, caracterização e vedação da interface adesiva da dentina intraradicular.** 2024. 64 f. Dissertação (Mestrado)-Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2024.

RESUMO

OBJETIVO: O presente estudo *in vitro* teve como objetivo analisar a influência da terapia fotodinâmica antimicrobiana (TFDa) com os fotossensibilizadores (FSs) indocianina verde (IV), curcumina (CC) ou azul de metileno (AM), associados ou não a medicação intracanal de hidróxido de cálcio (HC), na resistência de união (RU) de pinos de fibra de vidro (PFV) à dentina intrarradicular, composição química do substrato dentinário e a vedação da interface adesiva nos diferentes terços da dentina intrarradicular. MATERIAIS E MÉTODOS: 112 dentes bovinos receberam o preparo biomecânico e foram alocados em oito grupos experimentais (n = 14): Controle negativo (CN) = Água Deionizada; Controle positivo (CP) = Água Deionizada + HC; IV = FS-IV 50mg/L (Laser Infravermelho λ808nm); IV + HC = FS-IV 50mg/L (Laser Infravermelho λ808nm) + HC; CC = FS-CC 500mg/L (Led Azul λ480nm); CC + HC = FS-CC 500mg/L (Led Azul λ480nm) + HC; AM = FS-AM 50 mg/L (Laser Vermelho λ660nm); e AM + HC = FS-AM 50 mg/L (Laser Vermelho λ660nm) + HC. A RU foi mensurada pelo teste de *push-out* por meio da máquina de ensaio universal (n=8), o padrão de fratura dessas amostras foi qualificado por uma lupa de alta magnitude e amostras representativas foram caracterizadas pela microscopia eletrônica de varredura. A identificação da composição química do substrato dentinário foi analisada pela espectroscopia de energia dispersiva (n=3), e a morfologia da interface entre o cimento obturador e a dentina caracterizada pela microscopia confocal de fluorescência (n=3). Os dados obtidos foram submetidos ao teste de normalidade e as médias dos dados da RU e composição química foram comparadas pela análise de variância a dois critérios, seguidos pelo teste de Tukey. Enquanto os dados da interface adesiva foi avaliado pelo teste Kappa interexaminadores e submetidos aos testes de Krskal-Wallis e Dunn`s ($\alpha = 0.05$). RESULTADOS: Os grupos tratados com diferentes FSs, bem como seus diferentes terços, e a presença do HC promoveram diferenças na RU de PFVs à dentina intrarradicular ($P<0.05$) e na concentração de elementos químicos como oxigênio, fósforo, cálcio, magnésio e sódio ($P<0.05$). O grupo AM + HC apresentou maior incidência de falha do tipo adesiva. Não houve diferenças estatisticamente significativas no selamento da interface adesiva para nenhum dos parâmetros avaliados ($P>0.05$). CONCLUSÕES: A TFDa com o FS CC 500mg/L ativado com LED azul (λ 480nm) apresentou resultados de RU constantes, favoráveis características químicas da dentina intrarradicular e adequada selamento da interface adesiva, tanto na presença quanto na ausência da medicação intracanal de HC, em qualquer profundidade do canal radicular.

Palavras-chave: Fotoquimioterapia, dentina, fármacos fotossensibilizantes, hidróxido de cálcio, resistência ao cisalhamento.

Abstract

Maltarollo TFHM. **Effects of antimicrobial photodynamic therapy with different photosensitizers and calcium hydroxide on bond strength, characterization, and sealing of the adhesive interface.** 2024. 64 f. Dissertation (master's degree) - School of Dentistry, São Paulo State University, Araçatuba, 2024.

ABSTRACT

OBJECTIVE: The present *in vitro* study aimed to analyze the influence of antimicrobial photodynamic therapy (aPDT) with photosensitizers (PSs) indocyanine green (IG), curcumin (CC), or methylene blue (MB), with or without intracanal medication of calcium hydroxide (CH), on the bond strength (BS) of glass-fiber posts (GFPs) to intraradicular dentin, chemical composition of dentin substrate, and sealing of the adhesive interface in different thirds of intraradicular dentin. MATERIALS AND METHODS: A total of 112 bovine teeth underwent biomechanical preparation and were allocated into eight experimental groups (n = 14): Negative control (NC) = Deionized water; Positive control (PC) = Deionized water + CH; IG = PS-IG 50mg/L (infrared Laser λ808nm); IG + CH = PS-IG 50mg/L (infrared Laser λ808nm) + CH; CC = PS-CC 500mg/L (blue LED λ480nm); CC + CH = PS-CC 500mg/L (blue LED λ480nm) + CH; MB = PS-MB 50 mg/L (red Laser λ660nm); and MB + CH = PS-MB 50 mg/L (red Laser λ660nm) + CH. Bond strength was measured using the push-out test with a universal testing machine (n=8), the fracture pattern of these samples was qualified by a high-magnitude magnifying glass and representative samples were characterized by scanning electron microscopy. The identification of the chemical composition of the dentin substrate was analyzed by energy dispersive spectroscopy (n=3), and the sealing of the adhesive interface between the obturation cement and dentin was characterized by fluorescence confocal microscopy (n=3). The data obtained were subjected to the normality test and the means of the RU data and chemical composition were compared by two-criteria analysis of variance, followed by the Tukey test. While the adhesive interface data was evaluated by the inter-examiner Kappa test and subjected to the Krskal-Wallis and Dunn`s tests ($\alpha = 0.05$). RESULTS: Groups treated with different PSs, as well as their different thirds, and the presence of CH, showed differences in the BS of GFPs to intraradicular dentin ($P<0.05$) and in the concentration of chemical elements such as oxygen, phosphorus, calcium, magnesium and sodium ($P<0.05$).The MB + CH group exhibited a higher incidence of adhesive failure. There were no statistically significant differences in the sealing of the adhesive interface for any of the evaluated parameters ($P>0.05$). CONCLUSIONS: PDT with the PS-CC 500mg/L activated with blue LED (λ 480nm) showed consistent BS results, favorable chemical characteristics of intraradicular dentin, and appropriate sealing of the adhesive interface, both in the presence and absence of intracanal CH medication, at any depth of the root canal.

Keywords: Photochemotherapy, dentin, photosensitizing agents, calcium hydroxide, bond strength.

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LISTA DE ABREVIATURAS, SÍMBOLOS E SIGLAS

% - Percent

°C – Degrees Celsius

± - More or less

< - Less than

= - Equal

> - More than

≤ - Less or equal

≥ - Bigger or equal

A - Adhesive interface area

ANOVA - Variance analysisa

PDT - Antimicrobial photodynamic therapy

BS - Bond strength

C – Carbon

Ca – Calcium

CC – Curcumin

CH - calcium hydroxide

EDS - Energy dispersive X-ray spectroscopy

F – Maximum force

GFPs – Glass-fiber posts

h - Height of the third slice

IG - Indocyanine green

Laser - Light Amplification by Stimulated Emission of Radiation

LED - Light Emitting Diode

MB – Methylene blue

Mg – Magnesium

mg/L – milligrams per liter

mL – Millilitre

mm – millimeter

mm^2 - Square millimeter

MPa – Mega Pascal

N – Newton

Na – Sodium

nm – nanometer

O – Oxygen

P – Phosphorus

pH - Potential of hydrogen

PS – Photosensitizer

R_a – Smallest interface

R_c – Largest interface

RCS – Root canal system

s – Seconds

SEM - Scanning Electron Microscopy

α – Alpha

λ – Lambda

µm – micrometer

µg - Microgram

× - Aumento

π – Pi

Sumário

SUMÁRIO

Article

This manuscript is according to the guidelines of the **Journal of Photochemistry and Photobiology B: Biology*

Effects of antimicrobial photodynamic therapy with different photosensitizers and calcium hydroxide on bond strength, characterization, and sealing of the adhesive interface

1. Introduction

Despite significant technological advancements in the field of Endodontics, the persistence of microorganisms within the root canal system (RCS) continues to pose a problem. This challenge is compounded by the intricate internal anatomy of the root canal and the resilience inherent to its structure. [1,2]. The contamination of RCS can extend beyond the pulp tissue space into dentinal tubules, making mechanical decontamination and chemical irrigation methods insufficient for completely eradicating bacterial contamination, especially in cases involving anatomical complexities such as accessory canals, isthmuses, and anastomoses [2]. Furthermore, other factors related to endodontic treatment itself, such as the use of irrigation solutions and intracanal medications, and whether the instrumentation is manual or mechanized, can compromise the success of this treatment [3], and as a consequence, the effectiveness of intraradicular support for prosthetic restorations [4,5].

Root canal treatment is a complex procedure that involves several stages. A commonly used strategy in this process is calcium hydroxide (CH), which acts as a routine intracanal antibacterial dressing [6]. This compound is recognized for its potentially beneficial anti-endotoxin properties due to its high pH, resulting in antibacterial and biological effects [6]. However, there is controversy regarding its impact on microbial biofilms, especially concerning *Enterococcus faecalis*, one of the main causes of persistent infections [7].

Antimicrobial photodynamic therapy (aPDT) represents a minimally invasive approach widely used in the healthcare field to treat various diseases [8]. In the field of endodontics, aPDT is recommended as an adjunctive treatment following biomechanical preparation and before intracanal medication, complementing the conventional therapy, offering a synergistic effects [5,9]. Studies indicate that aPDT achieves a substantial reduction in microorganism populations such as *Enterococcus faecalis* e *Porphyromonas gingivalis* [2,9], in addition to promoting a reduction in post-operative pain in teeth with necrotic pulp and asymptomatic periapical lesions, and improved healing of periapical tissue [10,11].

The mechanism of aPDT involves the chemical interaction of three components: a photosensitizer (PS), light source with specific wavelength, and oxygen presence in the environment. This interaction leads to a photochemical reaction that generates reactive oxygen

such singlet oxygen, and other toxic chemicals, resulting in the destruction of target cells or microorganisms [12].

PSs are chemical compounds that play a fundamental role in a variety of biological and technological processes related to light absorption and conversion [13]. To ensure the success of aPDT, careful consideration is required when selecting the photosensitizing agent, light source, and treatment approach. The light source should have emission at a wavelength suitable for the maximum absorbance of the PS and sufficient energy to its activation [14]. Additionally, it is important to use PSs approved in vivo and have sufficient *in vitro* and preclinical studies that analyze their toxicity, adequate tissue clearance rate, and safety for clinical applications in dentistry [15].

Some of the PSs considered non-toxic, approved for intraoral use, and with broad clinical dental application and proven antimicrobial action in aPDT are methylene blue (MB) and toluidine blue compounds [15]. Their structural cationic charge facilitates easy penetration of the outer membrane of gram-negative bacteria with a high affinity for bacterial cells over body cells [16]. Other PS used and regarded as safe is the curcumin (CC), a natural compound. Its chemical and pharmacological effects are widely known in the literature [12,17,18], and being also a photochemical compound, it has a broad absorption spectrum (300-500 nm) [15,19]. Indocyanine green (IG) is a synthetic fluorescent dye that can be used as a nearly non-toxic PS, approved by the US Food and Drug Administration. Indocyanine green is a PS for wavelengths in the infrared spectrum with high absorption between 800 nm to 810 nm with minimal scattering interference [20,21].

Recent research has shown that some PSs, as well as their concentration, lasers, and the light source, can affect the mechanical properties of root dentin, as well as the bond strength of resin cements to the dentin substrate during the luting procedure of glass-fiber posts (GFPs) [5,15,17,19]. However, there is a lack of studies in the literature that compare the effects of PS-IG with other PS, associated or not with intracanal medication for CH, on the bond strength (BS) of GFPs, on the chemical composition of intraroot surface or on the morphology of the adhesive interface. This fact is especially relevant for teeth that have undergone endodontic treatment and have considerable coronal destruction, as they require intraradicular retainers to restore function and aesthetics.

This in vitro study aimed to evaluate the effect of aPDT with distinct PSs (IG, CC, and MB), associated or not with intracanal medication CH, on BS and adhesive interface sealing of GFPs to intraradicular dentin, and the chemical composition of the root substrate. The null hypotheses tested were as follows: 1) The use of distinct photosensitizers in aPDT, whether

combined with intracanal medication or not, would not result in significant differences on bond strenght and adhesive interface sealing of GFPs to intraradicular dentin, as well as on chemical composition of root dentin; 2) The different root thirds would not cause a significant differences on bond strength, adhesive interface sealing, and chemical composition of intraradicular dentin.

2. Material and Methods

2.1. Experimental design

The information regarding the materials used in the present study is listed in Table 1. This study underwent review and approval by the local Ethics Committee on Animal Use (#0418/2022).

A total of one-hundred and twelve bovine incisor teeth, devoid of cracks, fractures, or curved roots, were extracted from approximately 3-year-old cattle [5,22] (Figure 1-A). The anatomical crown was removed using a low-speed diamond saw to standardize root length at 20 mm and maintain an average root canal diameter of around 4 mm (Figure 1-B). All specimens underwent biomechanical root canal instrumentation using manual K-Files #45 to #80 (Dentsply-Maillefer, Ballaigues, Switzerland) (Figure1-C). Root canals were irrigated with 10 ml of 2.5% sodium hypochlorite at each file change, followed by a 17% ethylenediaminetetraacetic acid (EDTA) (Biodinâmica Química e Farmacêutica LTDA, Ibiporã, PR, Brazil) treatment for 3 minutes which was subsequently rinsed away with 2.5% sodium hypochlorite irrigation to eliminate the smear layer. Apices were sealed with dental adhesive (Adper Single Bond 2; 3M ESPE, St Paul MN, USA) and resin composite (Filtek Z250 XT, 3M ESPE, St Paul MN, USA) to prevent PS extravasation and potential interference during root canal obturation, in accordance with established protocols (Figure 1-C) [23,24].

2.2. Antimicrobial photodynamic therapy and experimental groups

Table 2 provides details of the eight experimental groups (n = 14).

Both control groups groups did not receive PS and aPDT. The negative group (NC) received irrigation with deionized water exclusively, whereas the positive group (PC) received deionized water and intracanal medication for a duration of 14 days.

Groups subjected to aPDT with PS-IG 50 mg/L (Ophthalmos S/A, São Paulo, SP, Brazil) were filled with the PS, which remained within the root canal for 60 seconds (pre-irradiation period) [20]. Subsequently, activation was executed using an infrared Laser (λ 808 nm) (Laser Duo; MMO, São Carlos, SP, Brazil) for 60 seconds (20). Those receiving aPDT with PS-CC 500 mg/L (Sigma Aldrich, Merck KGaA, St. Louis, MO, US) were exposed to the

PS for 300 seconds prior to activation through a 480 nm blue LED (Radii Cal SDI, Victoria, Australia) for 240 seconds [5,23]. And the groups submitted to aPDT with the PS-MB 50 mg/L (Chimiolux, DMC, São Carlos, SP, Brazil) were filled with the PS for 180 seconds before being activated by the red Laser (λ 660 nm) (Laser Duo; MMO, São Carlos, SP, Brazil) for 60 seconds [22,34] (Figure 1-D).

During the pre-irradiation period of each PS, they were shaken for one minute, without refrigeration. An Irrisonic E1 ultrasonic insert (Helse Dental Technology, Santa Rosa de Viterbo, SP, Brazil) was used, positioned 2 mm below the working length, coupled to an ultrasound device (NEWTRON P5 XS, Satelec Acteon, Indaiatuba, SP, Brazil) adjusted to power scale 2. Care was taken to avoid contact with the root canal walls [23,22].

The activation of PSs was carried out via an optical fiber measuring 300 μm in diameter (DMC Equipamentos, São Carlos, SP, Brazil), employing helical movements in the apical-cervical direction (10 times/minute) to a depth 2 mm less than the root canal working length [24,25]. Subsequent to aPDT, a final irrigation step involved the use of 10 mL of deionized water to remove the PS, followed by root canal drying using air jets and sterile paper points (Dentsply Sirona, York, PA, USA).

2.3. Calcium hydroxide medication

The PC, IG + CH, CC + CH and MB + CH groups received CH intracanal medication (Biodinâmica Química e Farmacêutica, Ibiporã, PR, Brazil) and iodoform (Biodinâmica Química e Farmacêutica, Ibiporã, PR, Brazil) with propylene glycol (Farma Formula, Contagem, MG, Brazil). This mixture was introduced into the root canals using a #4 Lentulo instrument (Dentsply Sirona, York, PA, USA) (Figure 1-E), and the samples were sealed with interim cement (Septodont Ltda, Pomerode, SC, Brazil), and stored in an environment with 100% humidity at 37°C for 14 days. To certify the complete filling of the root canal extension with intracanal medication, radiographic analysis as performed on the tooth specimens (Digital X-Ray – 1.0.9.1; Micro Image, Indaiatuba, SP, Brazil). After this period, the coronal seal and intracanal medication were removed using 2.5% sodium hypochlorite irrigation and a K#80 file (Maillefer Instruments, Tulsa, OK, USA) [5]. To confirm the proper CH filling of the canal extension, a radiographic analysis was conducted.

2.4. Bonding strength analysis (Push-out)

Following the preceding procedures, the samples (n =8) were filled with MTA Fillapex endodontic sealer (Angelus, Londrina, PR, Brazil), #80 gutta-percha cones, and fine

(F) and medium-fine (MF) auxiliary cones (Dentsply Sirona, York, PA, USA) (Figure 1-F). The obturation was performed using Tagger's technique with a McSpadden Compactor #90 (Dentsply Sirona, York, PA, USA). The access to the coronal portion was sealed with provisional cement (Septodont Ltda, Pomerode, SC, Brazil), and the teeth were stored in 100% humidity at 37°C [16,24]. After 7 days, 9 mm of gutta-percha were removed using a #2 White Post DCE system drill (White Post DCE #2; FGM, Joinville, SC, Brazil) in accordance with the measurement of the working length of the tooth as a reference. Root canals were irrigated with 10 ml of physiological saline solution 0.9% to remove any debris or residual gutta-percha and dried with sterile paper points. The GFPs were prepared with 37% phosphoric acid (FGM, Joinville, SC, Brazil) for 60 seconds, washed with cooling water, dried with air jets, and treated with silane RelyX Ceramic Primer (3M ESPE, St Paul MN, USA) for 60 s, and intraradicular posts that would not be further manipulated were gently dried with an air jet, preventing surface contamination. Self-adhesive resin cement (RelyX U200; 3M ESPE, St Paul MN, USA) was spatulated and inserted into the root canals with the GFPs themselves (Figure 1-G). The assembly was polymerized for 40 s from the top root canal surface using a single wave LED light curing unit (Radii- Cal; SDI, Bayswater, Australia) at 1200 mW/cm2. Then they were left to rest for 7 days at 37°C and 100% humidity [19,26].

These samples were cut perpendicular to the long axis, resulting in approximately 1.3 mm slices (cervical, middle, and apical) (Figure 1-H). The thickness and internal base diameters (major and minor) of the sample sections were measured using a digital caliper (Mitutoyo, Aurora, IL, USA) [25,30]. In the push-out test, conducted on a universal testing (DL3000, EMIC, São José dos Pinhais, PR, Brazil), a metal tip with a specific diameter for each root third (cervical third - 1.90 mm; middle third - 1.40 mm; and apical third - 1.20 mm) was fixed at the upper portion, while the sample was secured on a stainless steel support at the lower portion. A compressive load was applied at a speed of 0.5mm/min (Figure 1-I), and the push-out bond strength values were calculated as follows:

$$POBS = F/A ,$$

where POBD represents the push-out bond strength, expressed in MPa; F is the maximum force, expressed in N; and A is the adhesive interface area, expressed in mm^2, calculated using the following equation:

$$A = \pi (RC + RA) \sqrt{(RC - RA)2 + h2} ,$$

where π is 3.14; RC and RA are the largest (coronal) and smallest (apical) post radii, respectively; and h is the height of the third slice.

Subsequently the push-out bond strength test, the slices were halved to analyze the adhesive interface under a stereomicroscope (Stemi SV11, Zeiss, NY, USA) at ×6 and ×66 magnifications to assess root canals failure modes, including (1) mixed failure; (2) adhesive failure; (3) cohesive failure in dentin; and (4) cohesive failure in resin cement. Representative samples from each group were coated with gold (Balzers SCD-050 Sputter Coater, Germany) and analyzed using SEM equipment (JEOL, JSM 5600LV, Tokyo, Japan) to illustrate fracture patterns [6,19].

2.5. Substrate Morphology and Chemical Composition Analysis

Twenty-four samples (n=3), after removal of intracanal medication, were sectioned perpendicularly to the long axis (Isomet 1000, Buheler, Ltd, Lake Bluff, III, USA), yielding approximately 1.3 mm slices (cervical, middle, and apical thirds). These slices were analyzed using the Stemi SV11 Microscope equipped with an energy dispersive spectroscopy (EDS) system, coupled with scanning electron microscopy. EDS analysis software automatically evaluated the relative amount of each element, including carbon (C), oxygen (O), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), silicon (Si) and zinc (Zn) detected in the region of interest. Five different positions of each specimen submitted to scanning electron microscopy (SEM) were pre-selected and evaluated, identifying the chemical composition for each root third, and an arithmetic mean was calculated for each root third (Figure 1J) [27,28].

2.6. Adhesive interface sealing analysis

A total of 24 specimens (n=3), prepared and subjected to GFPs post luting (as described in section 2.4), were examined using confocal fluorescence microscope. To facilitate visualization of the structures, two dyes were employed on the samples [5]. Fluorescein (0.1%) (FDA, Sigma Chemical Co, St. Louis, MO, USA), a fluorescent substance (Figure 1K), was diluted in distilled water and introduced into the root canals after removing 9mm of the filling material. This dye remained in the canals for four hours before being removed through irrigation with 10mL of deionized water. Additionally, Rhodamine B (FDA, Sigma Chemical Co, St. Louis, MO, USA), a fuchsia-colored dye, was added to the resin cement at a proportion of 0.16 µg/g prior to the post-luting procedure (Figure 1K). Then the samples were kept in an environment with 100% humidity at 37°C for 7 days. Subsequently, was obtained 1.3 mm slices (cervical, middle, and apical) and subjected to examination using a confocal laser scanning microscope (Figure 1L) (Leica TCS SP2; Leica Microsystems, Heidelberg, Germany). Excitation was achieved using an argon laser at 488 nm and a He-Ne laser at 453 nm [29]. The

confocal micrographs were registered in the fluorescent mode using an oil immersion objective lens (40 × , numerical aperture 1.25). Photos were taken of four regions of each sample that were assembled for analysis of the entire sample. The analysis was carried out independently by two pre-calibrated evaluators using a double-blind approach. In cases of disagreement between the two examiners, both reevaluated the image until a final consensus was reached.

The criteria used for image evaluation were based on previous studies [5,30]:

a) Quality of the dentin/cement adhesive interface

Score 0 - no cracks, clear, continuous adhesive interface;

Score 1 - partial presence of cracks, partially continuous adhesive interface, present in less than 50% of the interface;

Score 2 - presence of cracks with discontinuous interface, present in more than approximately 50% of the interface.

b) Formation of tags in intraradicular dentin:

Score 0 - not detectable;

Score 1- few tags visible;

Score 2- uniform formation of tags with few lateral branches;

Score 3- formation of long tags with many side branches.

c) Tag penetration depth:

Score 0 - no tags;

Score 1 - tags ≤ 3 μm on average;

Score 2 - tags = 3–8.9 μm on average;

Score 3 - tags ≥ 9-15 μm on average.

2.7. Statistical analysis

Bond strength, chemical composition and integrity of the adhesive interface data were submitted to normality (Shapiro-Wilk; Jamovi Software 2.3) and homogeneity (Bartlett; Jamovi Software 2.3) tests. Push-out bond strength data were analyzed using two-way repeated measures Analysis of Variance (ANOVA) and Tukey's post hoc test ($\alpha = 0.05$) (Jamovi Software 2.3). EDS analysis data were analyzed using two-way repeated measures Analysis of Variance (ANOVA) and Tukey's post hoc test ($\alpha = 0.05$) (Minitab Statistical Software, version

19.2020.1.0). The sealing of the adhesive interface was evaluated by the inter-examiner Kappa test and subjected to the Kruskal-Wallis and Dunn´s tests (α = 0.05) (SigmaPlot; Version 12.0).

3. Results

3.1. Push-out bond strength

The push-out bond strength values are presented in Table 3. When assessing differences among the distinct thirds, a statistically significant difference was observed in the NC group, wherein lower bond strength values were observed in the apical third relative to the cervical and middle thirds (P<0.05). In contrast, the IG group exhibited higher values in the apical third in comparison to its cervical region (P=0.007). The IG + CH group demonstrated elevated push-out values in the cervical third compared to its middle third (P=0.015).

Upon intergroup comparison for the cervical third, the IG + CH group displayed significantly higher bond strength values in relation to the PC, IG, CC, and MB + CH experimental groups (P<0.05). The cervical third of IG showed the lowest values of bond strength, which was significant different compared to the same third of the NC, IG + CH and CC + CH groups (P<0.05). Furthermore, in the cervical third, the MB + CH group presented lower bond strength values compared to the CC + CH group (P=0.021). Regarding the middle third, the sole statistically significant difference observed pertained to the NC group, which exhibited higher bond strength values in comparison to the PC group (P=0.027). In the apical third, the NC and PC groups demonstrated lower bond strength values in relation to the IG, CC, and MB groups (P<0.05).

the evaluation of the type of failure showed that in all experimental groups there was a higher incidence of mixed failure, except in the MB + CH group, where adhesive failure was more prominent, as can be seen in Figure 2. Figure 3 depicts the representative samples failure pattern from each experimental group, analyzed using scanning electron microscopy.

3.2. Substrate Morphology and Chemical Composition

Figure 4 illustrates the energy-dispersive X-ray spectra of the intraradicular dentin surface and scanning electron micrographs in accordance with each experimental group. Table 4 provides the percentage values of the mass of chemical elements identified on the dentin surface for each group and root third.

Regarding oxygen, the intergroup analysis in the apical third revealed significantly lower values in the NC group compared to PC, IG+ HC, CC + HC, and MB + CH (P<0.05). The PC, IG + CH, and MB + CH groups exhibited higher values in the apical third compared

to NC and MB (P<0.05). In the intragroup analysis of oxygen, the cervical third of the MB group showed lower values compared to the middle third (P=0.013), while the MB + CH group displayed higher levels in the apical third compared to the cervical third (P=0.022).

For phosphorus, in the comparison between groups, in the cervical third, the NC and CC groups showed higher values compared to PC, IG + CH (P<0.05), while PC exhibited lower values compared to NC, IG, CC, MB, and MB + CH (P<0.05). In the middle third, PC presented lower values compared to NC, IG, CC, and MB (P<0.05). In the apical third, NC and CC showed higher values compared to PC and CC + CH (P<0.05), while PC exhibited lower values compared to NC, IG, CC, and MB (P<0.05). In the intragroup analysis, only PC showed a difference in phosphorus values, where the middle third showed lower values compared to the cervical and apical thirds (P<0.05).

In relation to calcium, in the comparison between groups, for the cervical and apical thirds, PC presented higher values compared to IG (P<0.05). In the apical third, PC showed higher values when compared to the IC and CC groups (P<0.05). There was no statistical difference between the thirds for calcium (P>0.05).

For magnesium, in the intergroup comparison, in the cervical third, PC exhibited lower values compared to NC and CC (P<0.05). Additionally, in the cervical third, CC showed higher values compared to PC and CC + CH (P<0.05). There was no statistical difference between the thirds for magnesium (P>0.05).

In the analysis of sodium, in the comparison between groups, in the cervical third, NC presented higher values compared to PC, IG + HC, CC + HC, and MB + CH (P<0.05). Meanwhile, in the apical third, the NC and CC groups exhibited higher values compared to the PC, CC + HC, and MB + CH groups (P<0.05). There was no statistical difference between the thirds for sodium (P>0.05).

No statistically significant differences were found between groups and root thirds for the elements Carbon, Silicon, and Zinc (p>0.05). Despite of the non-statistical significant differences, silicon was detected only in the NC and CC groups, while Zinc was identified in some thirds of the groups that received intracanal medication with CH.

3.3. Adhesive interface sealing

The scores and micrographs of the adhesive interface sealing obtained through confocal laser scanning microscopy are presented in Table 5 and Figure 5, respectively. An inter-examiner agreement test was conducted, resulting in values of 0.78, 0.84, and 0.80 for the parameters of interface quality, formation of tags, and depth of tags penetration, respectively.

No significant differences were identified when comparing experimental groups or intraradicular thirds for any of the analyzed criteria (P>0.05) (Table 4). Observing the quality of the adhesive interface, a prevalence of score 0 was observed in all groups, except for the CC + CH, MB, and MB + CH groups, which exhibited a higher prevalence of score 1. Concerning the formation of tags in dentin, scores 1 and 2 prevailed. As for the depth of tags penetration, there was a predominance of score 2 in all experimental groups, except for the CC + CH group, where score 1 prevailed.

4) Discussion

The use of aPDT with different PSs (IG, CC, and MB), as well as the application of intracanal medication with calcium hydroxide, led to statistically significant differences in the BS of GFPs and in the chemical composition of intraradicular dentin. However, they did not demonstrate an influence on the morphology of the adhesive interface. Thus, the first hypotheses of this study were refuted. Additionally, the different depths of root dentin (cervical, middle, and apical thirds), treated with aPDT and intracanal medication with calcium hydroxide, influenced both the BS and the chemical composition of intraradicular dentin, leading to the rejection of the second null hypothesis of this study.

The group that received the PS-IG demonstrated the lowest BS values for the cervical third (Table 3). As indicated by recent studies, the aPDT with PS-IG exhibited a distinct mechanism of action compared to other PSs, resulting in significantly reduced BS values [31]. According to Mirhashemi et al., the likely explanation lies in the fact that PS-IG operates through a photothermal effect, unlike the common photochemical mechanism of other PSs [32]. Furthermore, the decrease in BS values may also be attributed to the anionic charge of this PS. When irradiated by infrared wavelength range (780-810nm), PS-IG associates with plasma protein, thereby hindering effective adhesion of resin cement to root dentin [21]. Alrefeai et al. further suggest that temperature changes resulting from the photoactivation of this PS can affect the physical and chemical properties of dentin, consequently leading to a reduction in BS values [31]. According to Ballal et al., the partial lack of surface calcium may reduce the BS of certain adhesive materials [33]. IG is an anionic compound which has a surface layer with cationic compounds, such as calcium atoms present in hydroxyapatite crystals, which can cause demineralization of the dentin surface and [32], together with the increase in temperature, can cause exacerbation of this interaction. Due to the greater concentration of this element in the cervical third, and the greater amplitude of this region in relation to the middle and apical third, there was greater action of FS-IV in this third, leading to lower values. Chemical changes

arising from PS-IG were confirmed in the EDS analysis, with the cervical third of the IG groupexhibiting lower concentrations of calcium (Table 4).

According to the studies by Al Ahdal et al. and Al-Kheraif et al., there was a decreasing trend in BS from the cervical to the apical third for the presented groups [15,34], contrary to the findings of this study, where higher BS values were observed for the apical third in the IG group compared to its cervical third (Table 3). In the cervical third, due to the higher concentration of PS, there is a greater interaction and affinity with calcium atoms, and due to the force of gravity, precipitation occurs, depositing in the middle third and apical third. This deposition would form a physical barrier, which would make it difficult for the PS-IG to come into close contact with the dentin wall, thus reducing the deleterious effect of the association of PS and infrared laser irradiation, justifying the higher BS values for this third.

The PS-CC, belonging to the natural class of photosensitizers, exhibited a significant influence on the BS of GFPs to root dentin in this study. The BS values associated with this photosensitizer were notably consistent with or without CH and remained high in all regions (cervical, middle, and apical thirds) (Table 3). *In vitro* research has supported that aPDT performed with PS CC resulted in higher BS values when luting GFPs with self-adhesive resin cements [17,23,34]. Like PS IG, PS CC is an anionic compound, which has an affinity for cationic molecules, facilitating binding to calcium ions (Ca^{++}) in intraradicular dentin. This binding between the PS and calcium increases porosity and promotes effective micromechanical retention of resin cement along the root canal [28]. It would be of great relevance to study and evaluate the degree of interaction between PSs CC and IG with dentin, which interaction is stronger, which could damage the substrate more. Martens hardness and modulus of elasticity tests could be valuable to determine the hardness and consequently the weakening of the dentin substrate.

Furthermore, its hydrophobic polyphenolic nature imparts adhesive properties to CC by consistently repelling moisture, a crucial aspect for achieving lasting adhesion [23,34]. Studies such as that by Strazzi-Sahyon et al., evaluated the use of PS CC, in the same concentration, however, in two sessions, one after biomechanical preparation and another before cementing the GFPs. The result was that two sessions with this PS led to a reduction in BS values compared to PS MB [19].

In this study, it was observed that the MB group did not show a significant difference in BS compared to the IG and CC groups in the cervical, middle, and apical thirds (Table 3). However, when this PS was associated with CH intracanal medication (MB + CH group), it exhibited lower values in its cervical third compared to groups that received other

PSs and CH (IG + CH and CC + CH groups) (Table 3). According to the literature, MB has a cationic charge that binds to anionic molecules such as phosphate (P) present in hydroxyapatite and can alter the calcium (Ca) and phosphorus (P) ratio. This variation in the Ca/P ratio may lead to the formation of phosphate precipitates on the surface of root dentin, acting as a physical barrier between the resin and dentin, compromising BS values, as demonstrated in other studies [17,31]. Another factor to consider is the hydrophilic nature of this PS, which contributes to the compromise of BS due to water absorption in both intertubular and intratubular dentin [32,35]. This could justify the higher incidence of adhesive failures in the MB + CH group (Figure 2).

This study assessed the action of PSs in the presence or absence of intracanal CH medication. In contrast to recent studies that employed a similar methodology and evaluated the action of PSs MB and CC at various concentrations with CH, all groups, except the negative control group irrigated solely with deionized water, received this intracanal medication [5]. Calcium hydroxide plays an essential role in endodontic treatment, and its notable alkalinity enables dentin repair through calcification, inhibiting the dissolution of the mineral component of root dentin [36] and aiding in the formation of mineralized tissue by releasing Ca ions [37]. These findings are consistent with the results of the intraradicular dentin chemical composition analysis, showing elevated Ca values in groups receiving intracanal calcium hydroxide medication (Table 4). According to Ballal et al., adhesion to dentin depends on the presence of residual Ca^2 on the bonding surface [33], which could justify the beneficial effect of CH used in conjunction with PS-IG on BS values in the cervical third of the IG + CH group compared to the group receiving only PS-IG (Table 3).

Among the promising methods for root canal cleaning are ultrasonic irrigation (PUI), easy clean tools (EC), and continuous ultrasonic irrigation (CUI) [38,35]. However, Oliveira et al. demonstrated that these tools were not effective in the satisfactory removal of intracanal medication [39]. The presence of remaining intracanal HC medication will not expose the dentinal tubules, and adhesion is made intimately between the dentin surface and the resin cement. Reducing the effectiveness of gaps and infiltration (Table 5). It can explain the lower BS values found in the middle third of PC group (deionized water + HC) and cervical third of the MB + CH group (Table 3). Furthermore, incomplete removal of CH may also justify why, when analyzing the different depths (cervical, middle, and apical thirds) of the IG + CH group, the middle third obtained lower BS values compared to the cervical and apical thirds of this group.

Additionally, the groups treated with intracanal CH medication also showed a decrease in phosphorus levels (Table 4). This is significant because P and Ca are essential

components of hydroxyapatite, one of the important chemical substances found in dentin within the root canal, with a molar Ca/P ratio of 1.67 [33]. Considering that the analysis conducted with EDS evaluates the superficial layer of dentin within the root canal [30,33], it is suggested that the persistence of intracanal CH medication on the surface of intraradicular dentin may interfere with the adhesion process, due to the fact that the dentinal tubules are blocked and adhesion occurs intimately with the dentin surface. In addition to being able to mask the amount of P, justifying the reduction in this chemical element. This fact is in line with the findings of Someya et al. [6] and can be observed through scanning electron micrographs (Figure 4), which show remnants of intracanal CH medication in the CP, IG + HC, CC + HC, and MB + CH groups (Figure 15B, D, F, and H). Antimicrobial activity occurs through the generation of reactive oxygen species, and as emphasized by Singh et al., photoreaction and, consequently, singlet oxygen production are intensified in an alkaline environment [14,40]. This is evidenced by the elevated values of the O observed in the groups that received HC (Table 4). According to the findings of Abu Zeid et al., root canal sealers, such as MTA Fillapex, exhibit Ca and Si in their composition, and are capable of releasing these elements [41]. This fact could justify the presence of Si in the PC group and in the middle third of the CC group (Table 1 and Table 4).

The adhesive interface was assessed through confocal microscopy to analyze the micromechanical interaction between resin cement and intraradicular dentin substrate. As observed in Table 5, there were no differences in the evaluated parameters of the adhesive interface. According to Strazzi-Sahyon et al., tubular penetration depth data should be interpreted carefully, as measurement techniques for tubular penetration depth can exhibit varied performance for intraradicular thirds [5].

This study employed specific tests for the analysis of the BS of GFPs, the characterization and morphology of the adhesive interface. The push-out test is a widely utilized procedure in Dentistry, as it aids in assessing the vitality of the interfacial bond between dentin structure, resin matrix, and GFPs. Its reliability is highlighted by its ability to replicate oral conditions and evenly distribute stress along the long axis of the root dentin, thus minimizing failure rates and allowing measurements on different portions of the root structure [19,42,43]. Energy dispersive spectroscopy, a microanalytical technique, was used for quantitative estimation of the chemical composition of the specimen under analysis, allowing for a rapid and non-destructive analysis [33]. Changes in this composition can impact crucial properties such as permeability, microhardness, and dentin solubility, as well as interfere with sealing capacity and adhesion to dentin [33,44]. The morphology of the adhesive interface was

evaluated through micromechanical interactions between the resin cement and intraradicular dentin substrate using laser scanning confocal microscopy. This methodology involves assessing micropermeability and the sealing capability of resin monomer infiltration into the dentin substrate, qualitatively representing the adhesive interface [5].

Dentin, a vital component of teeth, is a complex blend of organic and inorganic substances. The primary inorganic elements in this tissue are Ca and P, with approximately 22% of dental composition consisting of organic materials, primarily type I collagen [27]. In addition to Ca and P, Mg is also present in root dentin, playing an influential role in mineralization and hydroxyapatite crystal growth. Elements such as O, N, and Na are also identified in hydroxyapatite crystals, although the precise role of these minerals is not yet fully understood [27,33]. According to Moda et al., when dental tissues are deficient in calcium, ions such as Na, K, Mg, Cl, Zn, Pb, Cu, and Al may be present [30].

It has been observed that certain chemicals can induce changes in the chemical structure of human dentin [44]. Photosensitizers are fundamental chemical compounds for aPDT. Methylene blue, curcumin, and green indocyanine are among the most commonly used groups of PSs in aPDT. They serve as chemical agents, disinfectants, and antiseptics, applied as dyes or indicators of redox reactions. These PSs were activated using different light sources: PS-MB with red Laser (λ 660nm), PS-CC with blue LED (λ 480nm), and PS-IG with infrared Laser (λ 808nm) [13,31]. There is a lack of studies that compare these PSs with intracanal medication of CH in terms of their influence on the properties of intraradicular dentin.

The results of this study provide valuable insights for the practical application of aPDT in conjunction with different PSs and intracanal medication of CH for dental procedures. Notably, this study underscores that the choice of PS significantly influences the BS between resin cement and intraradicular dentin for GFP luting procedures. This revelation has direct implications for dental professionals, especially dentists and endodontists, when formulating treatment strategies for patients with substantial loss of coronal structure in endodontically treated teeth. It emphasizes the need for a personalized approach in choosing the PS, considering its effects on dentin properties in different clinical scenarios.

This study has notable limitations, including the complexity of instrumentation in deeper regions, uncertainty regarding the effective removal of intracanal medication containing calcium hydroxide, and the use of bovine teeth with a heterogeneous substrate. As an *in vitro* study unable to precisely replicate oral conditions, it is crucial to carefully analyze the correlation between its laboratory results and the clinical context. Further studies are necessary to complement discussions around aPDT and ensure it does not interfere with the retention

capacity of GFPs in root canals, regardless of the PS and light source used. Thus, aPDT can contribute to the long-term success of endodontic treatment, as well as the preservation of remaining dental structures and oral rehabilitation.

5. Conclusion

Based on the methodology employed and the results obtained in this *in vitro* study, the following conclusions can be drawn:

1. Antimicrobial photodynamic therapy using distinct photosensitizers, associated or not with intracanal medication of calcium hydroxide, influenced the bond strength of glass-fiber posts to intraradicular dentin and the chemical composition of the root substrate.
2. Intraradicular depth also influenced the bond strength of glass-fiber posts and the chemical composition of root dentin.
3. At a concentration of 500 mg/L, curcumin proves to be an effective photosensitizer for antimicrobial photodynamic therapy when exposed to blue LED irradiation (λ 480 nm). This treatment, whether used alone or in conjunction with calcium hydroxide intracanal medication, demonstrates favorable outcomes, including satisfactory bond strength and sealing of glass-fiber posts to intraradicular dentin, as well as maintaining consistent chemical composition throughout the depth of the root dentin.

References

[1] Gholami L, Shahabi S, Jazaeri M, Hadilou M, Fekrazad R. Clinical applications of antimicrobial photodynamic therapy in dentistry. Front Microbiol. 2023 Jan 5;13:1020995. doi: 10.3389/fmicb.2022.1020995.

[2] Asnaashari M, Veshveshadi O, Aslani F, Hakimiha N. Evaluation the antibacterial efficacy of sodium hypochlorite in combination with two different photodynamic therapy protocols against Enterococcus Faecalis in Infected root canals: An in-vitro experiment. Photodiagnosis Photodyn Ther. 2023 Sep;43:103722. doi: 10.1016/j.pdpdt.2023.103722.

[3] Barbosa-Ribeiro M, Arruda-Vasconcelos R, Louzada LM, Dos Santos DG, Andreote FD, Gomes BPFA. Microbiological analysis of endodontically treated teeth with apical periodontitis before and after endodontic retreatment. Clin Oral Investig. 2021 Apr;25(4):2017-2027. doi: 10.1007/s00784-020-03510-2.

[4] Papalexopoulos D, Samartzi TK, Sarafianou A. A Thorough Analysis of the Endocrown Restoration: A Literature Review. J Contemp Dent Pract. 2021 Apr 1;22(4):422-426.

[5] Strazzi-Sahyon HB, Banci HA, Melo FS, Soares LS, Spin-Neto R, Duarte MAH, Cintra LTA, Dos Santos PH, Sivieri-Araujo G. In vitro study on how photodynamic therapy and calcium hydroxide medication influence adhesive interface properties of glass-fiber posts to

intraradicular dentin. J Mech Behav Biomed Mater. 2023 May;141:105757. doi: 10.1016/j.jmbbm.2023.105757

[6] Someya T, Kinoshita H, Harada R, Kawada E, Takemoto S. Effects of calcium hydroxide reagent on the bond strength of resin cements to root dentin and the retention force of FRC posts. Dent Mater J. 2017 Sep 26;36(5):630-637. doi: 10.4012/dmj.2016-355.

[7] Asnaashari M, Ashraf H, Rahmati A, Amini N. A comparison between effect of photodynamic therapy by LED and calcium hydroxide therapy for root canal disinfection against Enterococcus faecalis: A randomized controlled trial. Photodiagnosis Photodyn Ther. 2017 Mar;17:226-232. doi: 10.1016/j.pdpdt.2016.12.009.

[8] Mahmoudi H, Bahador A, Pourhajibagher M, Alikhani MY. Antimicrobial Photodynamic Therapy: An Effective Alternative Approach to Control Bacterial Infections. J Lasers Med Sci. 2018 Summer;9(3):154-160. doi: 10.15171/jlms.2018.29.

[9] Alkahtany MF. Efficacy of curcumin-mediated photodynamic therapy for root canal therapy procedures: A systematic review. Photodiagnosis Photodyn Ther. 2023 Mar;41:103252. doi: 10.1016/j.pdpdt.2022.103252.

[10] Alves-Silva EG, Arruda-Vasconcelos R, Louzada LM, de-Jesus-Soares A, Ferraz CCR, Almeida JFA, Marciano MA, Steiner-Oliveira C, Bello-Silva MS, Shemesh H, Gomes BPFA. The effect of photodynamic therapy on postoperative pain in teeth with primary endodontic infection. Photodiagnosis Photodyn Ther. 2022 Mar;37:102700. doi: 10.1016/j.pdpdt.2021.102700.

[11] Alves-Silva EG, Arruda-Vasconcelos R, Louzada LM, de-Jesus-Soares A, Ferraz CCR, Almeida JFA, Marciano MA, Steiner-Oliveira C, Santos JMM, Gomes BP. Effect of antimicrobial photodynamic therapy on the reduction of bacteria and virulence factors in teeth with primary endodontic infection. Photodiagnosis Photodyn Ther. 2023 Mar;41:103292. doi: 10.1016/j.pdpdt.2023.103292.

[12] Sivieri-Araujo G, Queiroz ÍOA, Fabbro RD, Esteves F, Cintra LTA, Duarte PCT, Bagnato VS, Oliveira SHP, Gomes-Filho JE. Rat tissue reaction and cytokine production induced by antimicrobial photodynamic therapy. Photodiagnosis Photodyn Ther. 2017 Jun;18:315-318. doi: 10.1016/j.pdpdt.2017.04.002.

[13] Nagata JY, Hioka N, Kimura E, Batistela VR, Terada RS, Graciano AX, Baesso ML, Hayacibara MF. Antibacterial photodynamic therapy for dental caries: evaluation of the photosensitizers used and light source properties. Photodiagnosis Photodyn Ther. 2012 Jun;9(2):122-31. doi: 10.1016/j.pdpdt.2011.11.006.

[14] Strazzi-Sahyon HB, Cintra LTA, Nakao JM, Takamiya AS, Queiroz IOA, Dos Santos PH, Oliveira SHP, Sivieri-Araujo G. Cytotoxicity of root canal irrigating solutions and photodynamic therapy using curcumin photosensitizer. Photodiagnosis Photodyn Ther. 2022 Jun;38:102795. doi: 10.1016/j.pdpdt.2022.102795.

[15] Al Ahdal K, Al Deeb L, Al-Hamdan RS, Bin-Shuwaish MS, Al Deeb M, Maawadh AM, AlHelal A, Vohra F, Abduljabbar T. Influence of different photosensitizers on push-out bond

strength of fiber post to radicular dentin. Photodiagnosis Photodyn Ther. 2020 Sep;31:101805. doi: 10.1016/j.pdpdt.2020.101805.

[16] Strazzi Sahyon HB, Silva PPD, Oliveira MS, Cintra LTA, Dezan-Júnior E, Gomes-Filho JE, Jacinto RC, Dos Santos PH, Sivieri-Araujo G. Influence of curcumin photosensitizer in photodynamic therapy on the mechanical properties and push-out bond strength of glass-fiber posts to intraradicular dentin. Photodiagnosis Photodyn Ther. 2019 Mar;25:376-381. doi: 10.1016/j.pdpdt.2019.01.025.

[17] Almadi KH, Alkahtany MF, Almutairi B. Influence of synthetic and natural photosensitizers activated by photodynamic therapy on extrusion bond strength of fiber post to radicular dentin. Pak J Med Sci. 2021 Nov-Dec;37(7):1912-1917. doi: 10.12669/pjms.37.7.4331.

[18] Moradi M, Fazlyab M, Pourhajibagher M, Chiniforush N. Ação antimicrobiana da terapia fotodinâmica no biofilme Enterococcus faecalis usando luz de cura, curcumina e riboflavina. Aust Endod J. 2022 Ago;48(2):274-282. doi: 10.1111/aej.12565.

[19] Strazzi-Sahyon HB, da Silva PP, Nakao JM, da Silva PZ, Nunes LP, Seron MA, Cintra LTA, Dos Santos PH, Sivieri-Araujo G. Influence of two photodynamic therapy sessions and different photosensitizers on the bond strength of glass-fiber posts in different regions of intraradicular dentin. Photodiagnosis Photodyn Ther. 2021 Mar;33:102193. doi: 10.1016/j.pdpdt.2021.102193.

[20] Yamamoto LY, Loureiro C, Cintra LTA, Leonardo RT, Banci HA, Ribeiro APF, Sivieri-Araujo G, Jacinto RC. Antibiofilm activity of laser ablation with indocyanine green activated by different power laser parameters compared with photodynamic therapy on root canals infected with Enterococcus faecalis. Photodiagnosis Photodyn Ther. 2021 Sep;35:102377. doi: 10.1016/j.pdpdt.2021.102377.

[21] Maawadh AM, Al Ahdal K, Al Deeb L, Alshamrani AS, Almohareb T, Alrahlah A. Effectiveness of final cavity disinfectant terminalia chebula, malachite, and indocyanine green, against E. Faecalis and on the bond interface of fiber post to radicular dentin. Photodiagnosis Photodyn Ther. 2023 Jun;42:103538. doi: 10.1016/j.pdpdt.2023.103538.

[22] Sivieri-Araujo G, Strazzi-Sahyon HB, Jacomassi DP, Dos Santos PH, Cintra LTA, Kurachi C, Bagnato VS. Effects of methylene blue and curcumin photosensitizers on the color stability of endodontically treated intraradicular dentin. Photodiagnosis Photodyn Ther. 2022 Mar;37:102650. doi: 10.1016/j.pdpdt.2021.102650.

[23] Strazzi Sahyon HB, Pereira da Silva P, Silva de Oliveira M, Angelo Cintra LT, Gomes-Filho JE, Henrique Dos Santos P, Sivieri-Araujo G. Effect of photodynamic therapy on the mechanical properties and bond strength of glass-fiber posts to endodontically treated intraradicular dentin. J Prosthet Dent. 2018 Aug;120(2):317.e1-317.e7. doi: 10.1016/j.prosdent.2018.05.009.

[24] Banci HA, Strazzi-Sahyon HB, Duarte M, Cintra L, Gomes-Filho JE, Chalub LO, Berton SA, de Oliveira V, Dos Santos PH, Sivieri-Araujo G. Influence of photodynamic therapy on bond strength and adhesive interface morphology of MTA based root canal sealer to different

thirds of intraradicular dentin. Photodiagnosis Photodyn Ther. 2020 Dec;32:102031. doi: 10.1016/j.pdpdt.2020.102031.

[25] Allison RR, Moghissi K. Photodynamic Therapy (PDT): PDT Mechanisms. Clin Endosc. 2013 Jan;46(1):24-9. doi: 10.5946/ce.2013.46.1.24.

[26] Strazzi-Sahyon HB, de Oliveira MS, da Silva PP, Banci HA, de Melo FS, Martinez CMT, Cintra LTA, Gomes-Filho JE, Dezan-Júnior E, Dos Santos PH, Sivieri-Araujo G. Does photodynamic therapy with methylene blue affect the mechanical properties and bond strength of glass-fiber posts in different thirds of intraradicular dentin? Photodiagnosis Photodyn Ther. 2020 Jun;30:101673. doi: 10.1016/j.pdpdt.2020.101673.

[27] Küçükkaya Eren S, Uzunoğlu E, Sezer B, Yılmaz Z, Boyacı İH. Mineral content analysis of root canal dentin using laser-induced breakdown spectroscopy. Restor Dent Endod. 2018 Feb 4;43(1):e11. doi: 10.5395/rde.2018.43.e11.

[28] Wang Z, Maezono H, Shen Y, Haapasalo M. Evaluation of Root Canal Dentin Erosion after Different Irrigation Methods Using Energy-dispersive X-ray Spectroscopy. J Endod. 2016 Dec;42(12):1834-1839. doi: 10.1016/j.joen.2016.07.024.

[29] Piai GG, Duarte MAH, Nascimento ALD, Rosa RAD, Só MVR, Vivan RR. Penetrability of a new endodontic sealer: A confocal laser scanning microscopy evaluation. Microsc Res Tech. 2018 Nov;81(11):1246-1249. doi: 10.1002/jemt.23129.

[30] Moda MD, Dos Santos PH, Pini NIP, Furini LN, Briso ALF, Assmann A, Fagundes TC. Effects of Different Toothpastes on the Nanomechanical Properties and Chemical Composition of Resin-Modified Glass Ionomer Cement and Composite Resin Restorations. Dent J (Basel). 2023 Jul 17;11(7):173. doi: 10.3390/dj11070173

[31] Alrefeai MH, Aljamhan AS, Alhabdan A, Alzehiri MH, Naseem M, Alkhudhairy F. Influence of methylene blue, Riboflavin, and indocyanine green on the bond strength of caries affected dentin when bonded to resin-modified glass ionomer cement. Photodiagnosis Photodyn Ther. 2022 Jun;38:102792. doi: 10.1016/j.pdpdt.2022.102792.

[32] Mirhashemi A, Janani R, Bahrami R, Chiniforush N. Evaluation of the photodynamic therapy with riboflavin and curcumin on shear bond strength of orthodontic bracket: An in vitro study. Photodiagnosis Photodyn Ther. 2022 Jun;38:102787. doi: 10.1016/j.pdpdt.2022.102787.

[33] Ballal NV, Mala K, Bhat KS. Evaluation of decalcifying effect of maleic acid and EDTA on root canal dentin using energy dispersive spectrometer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011 Aug;112(2):e78-84. doi: 10.1016/j.tripleo.2011.01.034.

[34] Al-Kheraif AA, Mohamed BA, Khan AA, Al-Shehri AM. Role of Riboflavin; Curcumin photosensitizers and Ozone when used as canal disinfectant on push-out bond strength of glass fiber post to radicular dentin. Photodiagnosis Photodyn Ther. 2022 Mar;37:102592. doi: 10.1016/j.pdpdt.2021.102592.

[35] Fernandes KGC, Silva BBD, Boer NC, Mandarini DR, Moreti LCT, Kato AS, Bueno CEDS, Limoeiro AGDS, Pinheiro SL, Martin AS, Fontana CE. The Effectiveness of Three

Irrigation Systems in the Enterococcus faecalis Reduction after Instrumentation with a Reciprocating Instrument. Eur J Dent. 2020 Oct;14(4):539-543. doi: 10.1055/s-0040-1714760.

[36] Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J. 2011 Aug;44(8):697-730. doi: 10.1111/j.1365-2591.2011.01886.x.

[37] Zamparini F, Prati C, Taddei P, Spinelli A, Di Foggia M, Gandolfi MG. Chemical-Physical Properties and Bioactivity of New Premixed Calcium Silicate-Bioceramic Root Canal Sealers. Int J Mol Sci. 2022 Nov 11;23(22):13914. doi: 10.3390/ijms232213914.

[38] Souza CC, Bueno CE, Kato AS, Limoeiro AG, Fontana CE, Pelegrine RA. Efficacy of passive ultrasonic irrigation, continuous ultrasonic irrigation versus irrigation with reciprocating activation device in penetration into main and simulated lateral canals. J Conserv Dent. 2019 Mar-Apr;22(2):155-159. doi: 10.4103/JCD.JCD_387_18.

[39] de Oliveira RL, Guerisoli DMZ, Duque JA, Alcalde MP, Onoda HK, Domingues FHF, Vivan RR, Duarte MAH. Computed microtomography evaluation of calcium hydroxide-based root canal dressing removal from oval root canals by different methods of irrigation. Microsc Res Tech. 2019 Mar;82(3):232-237. doi: 10.1002/jemt.23164.

[40] Singh S, Nagpal R, Manuja N, Tyagi SP. Photodynamic therapy: An adjunct to conventional root canal disinfection strategies. Aust Endod J. 2015 Aug;41(2):54-71. doi: 10.1111/aej.12088.

[41] Abu Zeid S, Edrees HY, Mokeem Saleh AA, Alothmani OS. Physicochemical Properties of Two Generations of MTA-Based Root Canal Sealers. Materials (Basel). 2021 Oct 9;14(20):5911. doi: 10.3390/ma14205911.

[42] Strazzi-Sahyon HB, Oliveira AKL, Carvalho AP, Figueiredo RB, Cintra LTA, Gomes-Filho JE, Dos Santos PH, Sivieri-Araujo G. Influence of photodynamic therapy and intracanal medication on Martens hardness, elastic modulus and bond strength of glass-fiber posts to endodontically treated root dentin. Photodiagnosis Photodyn Ther. 2021 Dec;36:102571. doi: 10.1016/j.pdpdt.2021.102571.

[43] Almadi KH. Impact of antimicrobial photodynamic therapy on the bond-strength and penetration of endodontic sealers: A systematic review. Photodiagnosis Photodyn Ther. 2023 Mar;41:103249. doi: 10.1016/j.pdpdt.2022.103249.

[44] Capar ID, Aydinbelge HA. Surface change of root canal dentin after the use of irrigation activation protocols: electron microscopy and an energy-dispersive X-ray microanalysis. Microsc Res Tech. 2013 Sep;76(9):893-6. doi: 10.1002/jemt.22244.

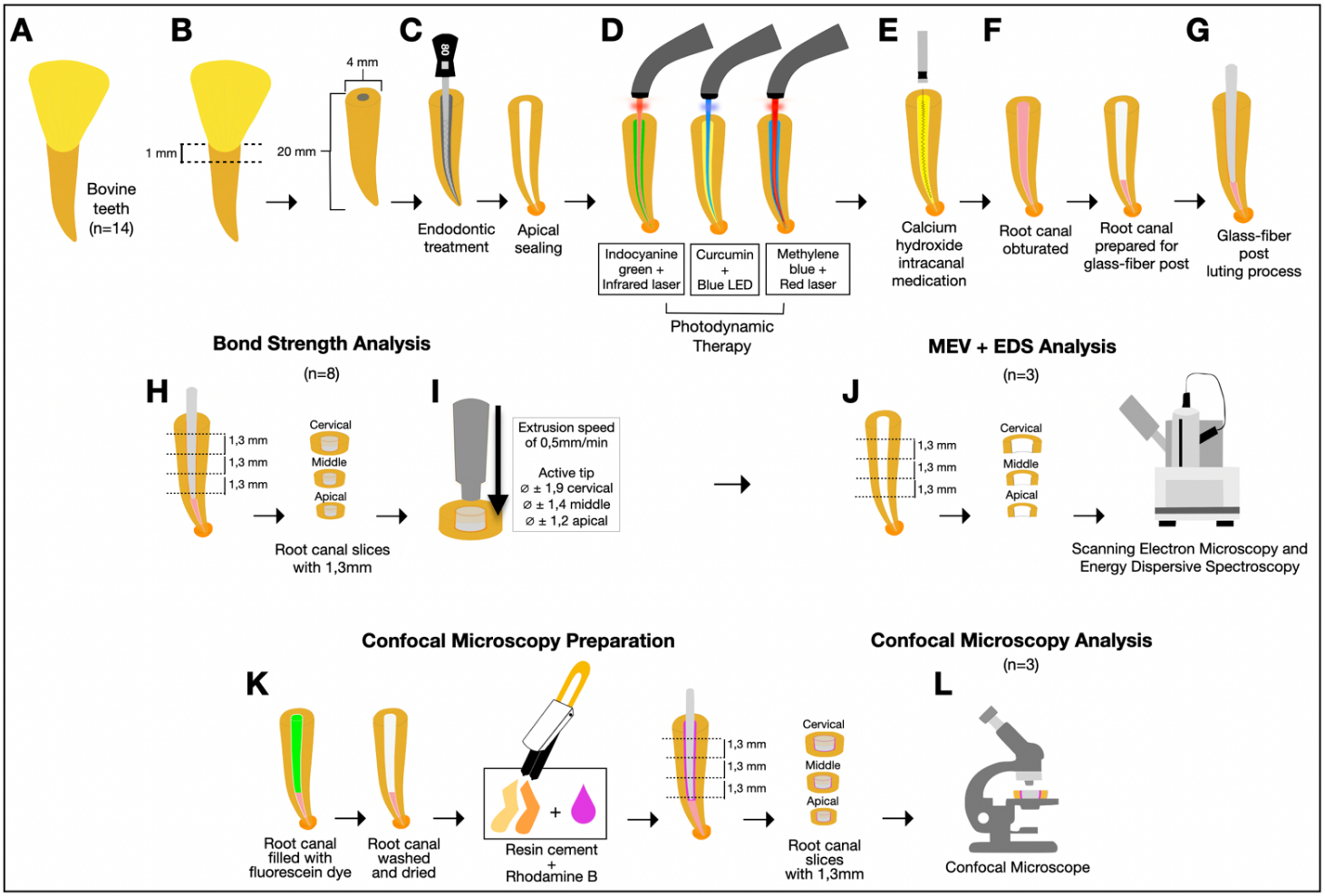

Figure 1. Sample preparation. A, Bovine teeth. B, Removal of anatomical crowns of the bovine teeth. C, Length and diameter standardization of root canals, and sealing of the apical region. D, Photodynamic therapy session with indocyanine green photosensitizer with infrared Laser (λ 808 nm); curcumin photosensitizer with blue LED light (λ 480 nm); methylene blue photosensitizer with red Laser (λ = 660 nm). E, Calcium hydroxide intracanal medication. F, Samples obturation. G, Post-luting process. H, Obtainment of 1.3-mm thick intraradicular slices for push-out bond strength test. I, Push-out bond strength test. J, Obtainment of 1.3-mm thick intraradicular slices for the scanning electron microscopy (MEV) and energy dispersive spectroscopy (EDS) analysis. K, Specimen preparation for the confocal microscopy analysis with fluorescein and rhodamine B dyes. L, Obtainment of 1.3-mm thick intraradicular slices for the confocal microscopy analysis.

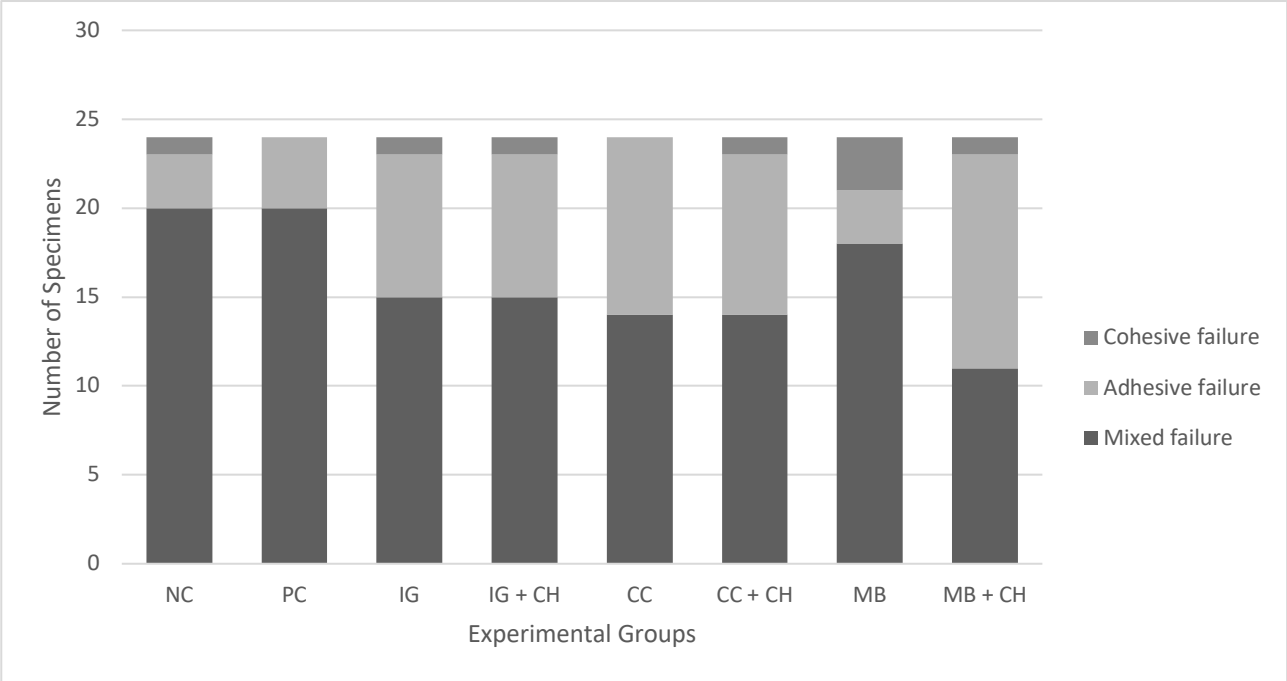

Figure 2. Occurrence of failure patterns of the specimens (number of sample thirds).

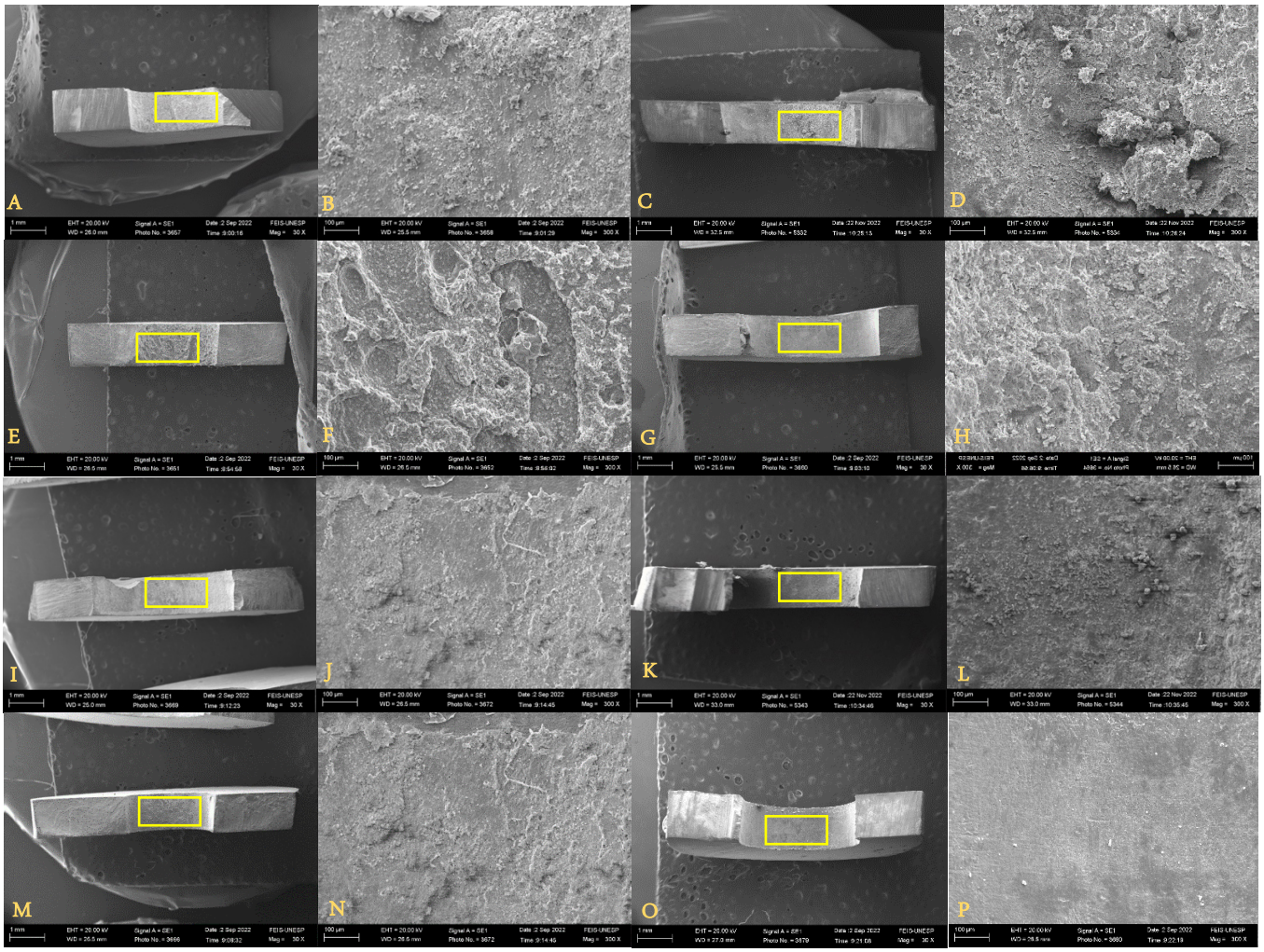

Figure 3. Scanning electron microscopy images with original magnification of 30× and 300×, respectively. A,B – Mixed-type failure of canals that received only deionized water (NC); C,D - Mixed-type failure of root canals that received deionized water and intracanal medication of calcium hydroxide (PC); E,F - Mixed-type failure of root canals treated with antimicrobial photodynamic therapy using the photosensitizer indocyanine green (IG); G,H - Mixed-type failure of root canals treated with antimicrobial photodynamic therapy using indocyanine green photosensitizer and intracanal medication of calcium hydroxide (IG + CH); I,J - Mixed-type failure of root canals treated with antimicrobial photodynamic therapy using the photosensitizer curcumin (CC); K,L – Mixed-type failure of root canals treated with antimicrobial photodynamic therapy using curcumin photosensitizer and intracanal medication of calcium hydroxide (CC + CH); M,N - Mixed-type failure of root canals treated with antimicrobial photodynamic therapy using methylene blue photosensitizer (MB); O,P - Adhesive-type failure of root canals treated with antimicrobial photodynamic therapy using methylene blue photosensitizer and intracanal medication of calcium hydroxide (MB + CH).

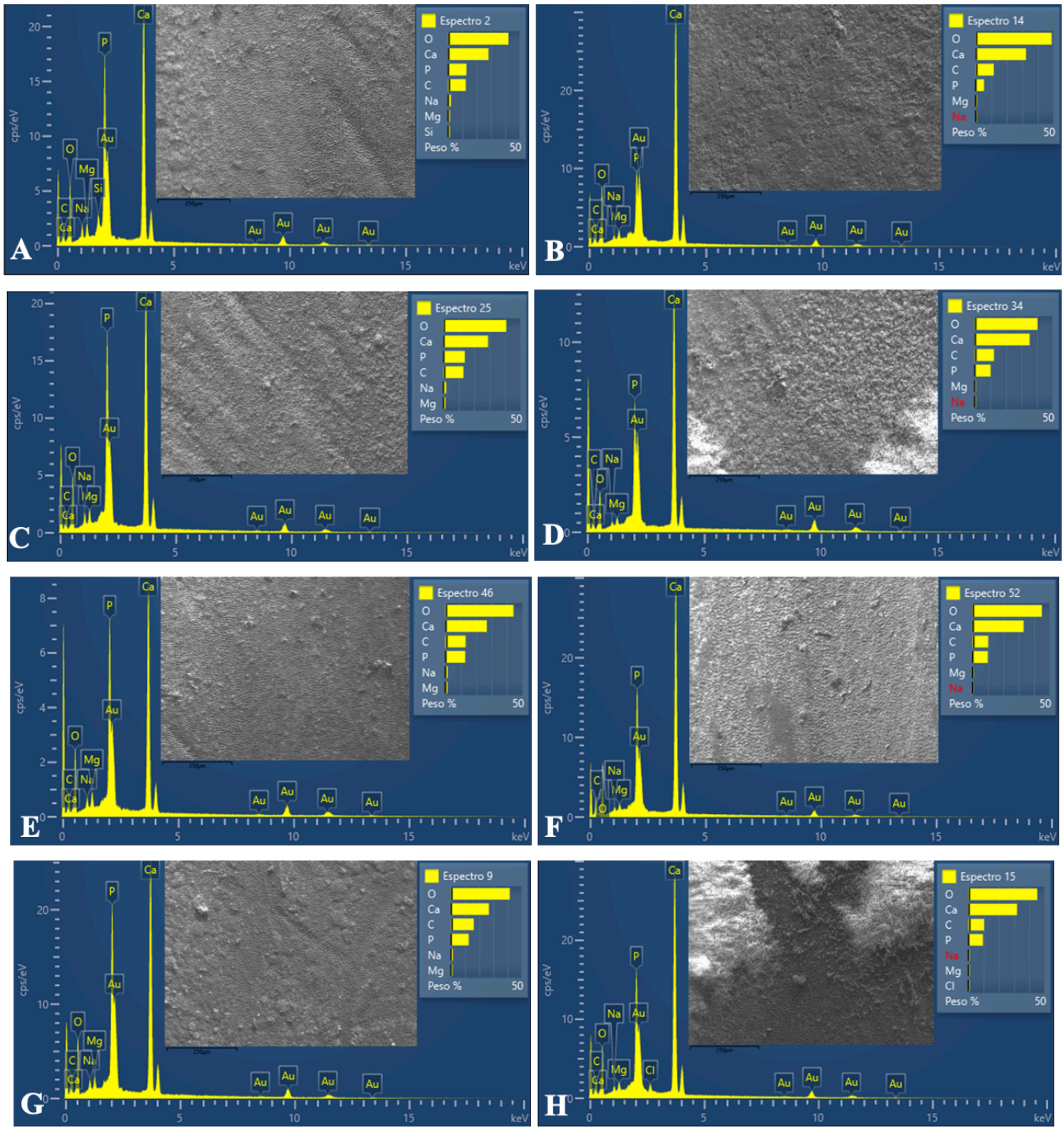

Figure 4. Energy-dispersive X-ray spectra of intraradicular dentin surface and scanning electron micrographs under original magnification (300×), according to each experimental group. A - Intraradicular dentin irrigated with deionized water solution (NC). B - Intraradicular dentin irrigated with deionized water and filled with intracanal medication of calcium hydroxide (PC). C - Intraradicular dentin after treatment with FS IV activated with infrared Laser (λ 808 nm) (IG). D - Intraradicular dentin after treatment with FS IV activated with infrared Laser (λ 808 nm) + intracanal medication of calcium hydroxide (IG + CH). E - Intraradicular dentin after

treatment with FS CC activated with blue LED (λ 480 nm) (CC). F - Intraradicular dentin after treatment with FS CC activated with blue LED (λ 480 nm) + intracanal medication of calcium hydroxide (CC + CH). G - Intraradicular dentin after treatment with FS AM activated with red Laser (λ 660 nm) (MB). H - Intraradicular dentin after treatment with FS MB activated with red Laser (λ 660 nm) + intracanal medication of calcium hydroxide (MB + CH).

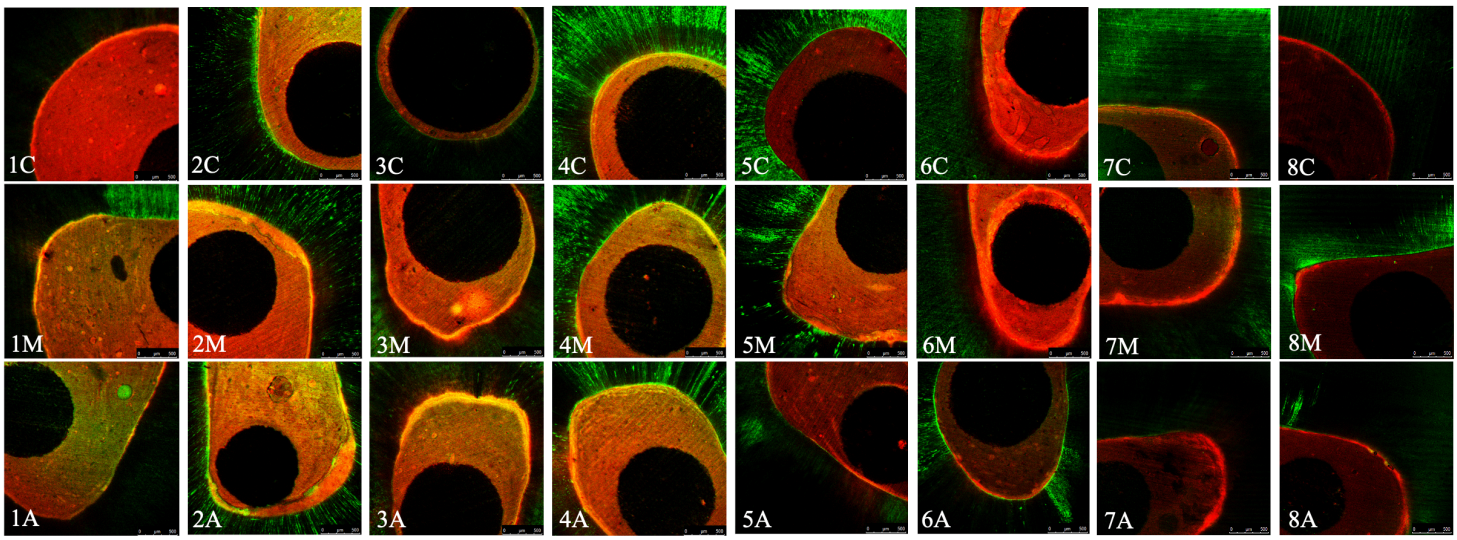

Figure 5. Confocal laser scanning micrographs of the adhesive interface sealing according to the experimental groups and intraradicular thirds under original magnification × 5. Figures 1C, 1M, 1A - Cervical, middle, and apical slices of intraradicular dentin irrigated with deionized water solution (NC). Figures 2C, 2M, 2A - Cervical, middle, and apical slices of intraradicular dentin irrigated with deionized water and filled with intracanal medication of calcium hydroxide (PC). Figures 3C, 3M, 3A - Cervical, middle, and apical sections of intraradicular dentin after treatment with FS IG activated with infrared Laser (λ 808 nm) (IG). Figures 4C, 4M, 4A - Cervical, middle, and apical sections of intraradicular dentin after treatment with FS IG activated with infrared Laser (λ 808 nm) + intracanal medication of calcium hydroxide (IG + CH). Figures 5C, 5M, 5A - Cervical, middle, and apical sections of intraradicular dentin after treatment with FS CC activated with blue LED (λ 480 nm) (CC). Figures 6C, 6M, 6A - Cervical, middle, and apical sections of intraradicular dentin after treatment with FS CC activated with blue LED (λ 480 nm) + intracanal medication of calcium hydroxide (CC + CH). Figures 7C, 7M, 7A - Cervical, middle, and apical sections of intraradicular dentin after treatment with FS MB activated with red Laser (λ 660 nm) (MB group). Figures 8C, 8M, 8A - Cervical, middle, and apical sections of intraradicular dentin after treatment with FS MB activated with red Laser (λ 660 nm) + intracanal medication of calcium hydroxide (MB + CH).

Table 1. Materials, classification, composition, and batch numbers of the materials used.

Material	Classification	Composition	Batch
Adper Single Bond 2 (3M ESPE)	Adhesive System	BisGMA, HEMA, glycerol 1,3-dimethacrylate, diurethane dimethacrylate, water, ethanol, photoinitiators, 5 nm silanized silica, polyacrylic and itaconic acid copolymer.	N820206
Filtek Z350XT (3M ESPE)	Resin Composite	Bis-EMA, Bis-GMA, TEGDMA, UDMA, silica and zirconia nanofillers, and, agglomerated zirconia silica nanoclusters.	HB004209993
Calcium Hydroxide (Biodinamica)	Intracanal Medication	38% of calcium hydroxide and, 62% of barium oxide.	36870
Iodoform (Biodinamica)	Intracanal Medication	Iodoform (99–100%)	005-22
Propylene Glycol (Farma Formula)	Intracanal Medication	100% propylene glycol	530080
MTA Fillapex (Angelus)	Endodontic Sealer	Salicylate resin, natural resin, diluting resin, bismuth oxide, nanoparticulated silica, MTA (mineral trioxide aggregate) and pigments.	102564
RelyX Ceramic Primer (3M ESPE)	Ceramic Primer	Ethyl alcohol, water, and 3-MPS.	2106800707
RelyX U200 (3M ESPE)	Resin Cement	Base: methacrylate phosphoric acid esters, glass-fiber, triethylene glycol dimethacrylate, sodium persulfate, silane-treated silica. Catalyst: substitute dimethacrylate, glass fiber, sodiump-toluenesulfonate, silane-treated silica, calcium.	2115100124

Table 2. Distribution of the experimental groups.

Groups Abbreviation	Treatment Details
NC	Root canals were irrigated only with deionized water (Negative control).
PC	Root canals were irrigated with deionized water and filled with intracanal calcium hydroxide medication (Positive control).
IG	Root canals were filled with indocyanine green photosensitizer at 50 mg/L activated by infrared Laser (λ 808 nm).
IG + CH	Root canals were filled with indocyanine green photosensitizer at 50 mg/L activated by infrared Laser (λ 808 nm) and filled with intracanal calcium hydroxide medication.
CC	Root canals were filled with curcumin photosensitizer at 500 mg/L activated by blue LED light (λ 480 nm).
CC + CH	Root canals were filled with curcumin photosensitizer at 500 mg/L activated by blue LED light (λ 480 nm) and filled with intracanal calcium hydroxide medication.
MB	Root canals were filled with methylene blue photosensitizer at 50 mg/L activated by red Laser (λ 660nm).
MB + CH	Root canals were filled with methylene blue photosensitizer at 50 mg/L activated by red Laser (λ 660nm) and filled with intracanal calcium hydroxide medication.

Table 3. Mean ± standard deviation values of push-out bond strength (MPa) of intraradicular dentin as function of the experimental groups and intraradicular thirds.

Groups / Thirds	NC	PC	IG	IG + CH	CC	CC + CH	MB	MB + CH
Cervical	5.37 ± 2.36 Aabc	3.93 ± 1.17 Abcd	2.64 ± 1.53 Bd	6.49 ± 2.92 Aa	4.18 ± 1.40 Abcd	6.03 ± 2.12 Aab	4.54 ± 2.02 Aabcd	3.44 ± 1.65 Acd
Middle	5.59 ± 4.21 Aa	3.10 ± 2.11 Ab	3.45 ± 2.11 ABab	3.50 ± 2.48 Bab	4.94 ± 1.80 Aab	4.95 ± 2.59 Aab	4.72 ± 2.84 Aab	4.14 ± 1.37 Aab
Apical	3.04 ± 1.26 Bb	3.36 ± 1.69 Ab	5.67 ± 2.42 Aa	4.51 ± 2.73 ABab	6.14 ± 1.66 Aa	4.56 ± 2.59 Aab	5.57 ± 2.53 Aa	4.02 ± 2.15 Aab

NC: Negative control; PC: Positive control; IG: Indocyanine green; IG + CH: Indocyanine green with calcium hydroxide medication; CC: Curcumin; CC + CH: Curcumin with calcium hydroxide medication; MB: Methylene blue; MB + CH: Methylene blue with calcium hydroxide medication.
*Different letters, uppercase in column and lowercase in row, indicate statistically significant differences ($P<0.05$).

Table 4. Mean values (± standard deviation) (%) of the intraradicular dentin chemical element content based on experimental groups and root canal depth.

Groups / Thirds	NC	PC	IG	IG + CH	CC	CC + CH	MB	MB + CH
Carbon (C)								
Cervical	10.10 ± 2.61 Aa	9.58 ± 0.59 Aa	12.98 ± 0.13 Aa	11.96 ± 0.81 Aa	10.51 ± 2.12 Aa	10.45 ± 0.66 Aa	12.38 ± 0.87 Aa	11.27 ± 0.69 Aa
Middle	11.34 ± 2.41 Aa	10.87 ± 0.97 Aa	13.45 ± 1.23 Aa	11.70 ± 0.73 Aa	17.57 ± 6.05 Aa	10.82 ± 1.49 Aa	13.44 ± 1.18 Aa	13.18 ± 1.61 Aa
Apical	12.61 ± 2.62 Aa	10.44 ± 0.80 Aa	13.58 ± 0.038 Aa	11.16 ± 2.93 Aa	12.78 ± 0.65 Aa	10.88 ± 2.23 Aa	14.36 ± 1.58 Aa	12.30 ± 1.76 Aa
Oxygen (O)								
Cervical	40. 82 ± 2.71 Aa	44.78 ± 3.02 Aa	42.47 ± 1.44 Aa	43.14 ± 3.11 Aa	41.64 ± 2.18 Aa	45.45 ± 1.32 Aa	39.47 ± 0.23 Ba	41.84 ± 1.28 Ba
Middle	42.34 ± 2.34 Aa	43.94 ± 6.83 Aa	43.00 ± 0.53 Aa	43.78 ± 1.07 Aa	41.57 ± 1.79 Aa	43.77 ± 0.92 Aa	41.63 ± 0.52 Aa	43.65 ± 0.72 ABa
Apical	40.51 ± 3.30 Ac	46.31 ± 1.46 Aa	43.02 ± 0.99 Aabc	45.30 ± 0.88 Aa	43.08 ± 1.04 Aabc	44.84 ± 0.24 Aab	40. 72 ± 0.87 ABbc	45.00 ± 0.93 Aa
Phosphorus (P)								
Cervical	14.13 ± 2.21 Aa	6.68 ± 0.60 Ac	13.47 ± 0.49 Aab	9.59 ± 2.24 Abc	14.08 ± 1.56 Aa	10.30 ± 1,96Aabc	13.58 ± 1.37 Aab	10.91 ± 1.67 Aab
Middle	13.56 ± 2.03 Aa	5.17 ± 0.14 Bb	13.36 ± 0.37 Aa	9.70 ± 1.81 Aab	12.37 ± 2.21 Aa	9.70 ± 1.96 Aab	13.33 ± 0.50 Aa	9.89 ± 2.53 Aab
Apical	13.67 ± 1.73 Aa	6.80 ± 0.57 Ac	13.17 ± 0.31 Aab	9.98 ± 1.90 Aabc	13.31 ± 0.64 Aa	9.09 ± 1.93 Abc	12.75 ± 0.94 Aab	9.89 ± 2.38 Aabc
Calcium (Ca)								
Cervical	31.56 ± 3.41 Aab	36.62 ± 1.55 Aa	28.21 ± 0.92 Ab	33.05 ± 1.99 Aab	31.00 ± 2.19 Aab	31.97 ± 2.08 Aab	32.28 ± 0.95 Aab	33.63 ± 1.76 Aab
Middle	29.38 ± 2.67 Aa	30.56 ± 5.68 Aa	27.71 ± 0.06 Aa	32.69 ± 1.42 Aa	25.92 ± 5.52 Aa	34.01 ± 2.44 Aa	29.35 ± 1.59 Aa	31.56 ± 1.16 Aa
Apical	30.02 ± 2.53 Aab	33.89 ± 1.62 Aa	27.80 ± 1.27 Ab	31.32 ± 1.55 Aab	27.91 ± 0.89 Ab	32.48 ± 2.26 Aab	29.83 ± 2.68 Aab	30.86 ± 2.48 Aab
Magnesium (Mg)								
Cervical	1.28 ± 0.23 Aab	0.79 ± 0.04 Ac	1.22 ± 0.27 Aabc	1.05 ± 0.14 Aabc	1.38 ± 0.21 Aa	0.82 ± 0.06 Abc	1.05 ± 0.16 Aabc	1.04 ± 0.16 Aabc
Middle	1.29 ± 0.22 Aa	0.91 ± 0.18 Aa	1.18 ± 0.30 Aa	1.23 ± 0.10 Aa	1.26 ± 0.12 Aa	0.88 ± 0.09 Aa	1.04 ± 0.09 Aa	0.97 ± 0.16 Aa
Apical	1.19 ± 0.22 Aa	0.95 ± 0.13 Aa	1.17 ± 0.28 Aa	1.09 ± 0.09 Aa	1.45 ± 0.12 Aa	0.93 ± 0.32 Aa	1.02 ± 0.16 Aa	0.90 ± 0.25 Aa
Sodium (Na)								
Cervical	1.73 ± 0.13 Aa	0.87 ± 0.06 Ab	1.26 ± 0.49 Aab	0.88 ± 0.24 Ab	1.38 ± 0.12 Aab	1.01 ± 0.17 Ab	1.23 ± 0.26 Aab	0.85 ± 0.03 Ab
Middle	1.72 ± 0.16 Aa	1.43 ± 1.19 Aa	1.30 ± 0.32 Aa	0.90 ± 0.06 Aa	1.15 ± 0.21 Aa	0.84 ± 0.14 Aa	1.19 ± 0.26 Aa	0.74 ± 0.17 Aa
Apical	1.63 ± 0.24 Aa	0.85 ± 0.11 Acd	1.26 ± 0.29 Aabcd	0.99 ± 0.18 Abcd	1.47 ± 0.16 Aab	0.78 ± 0.05 Ad	1.32 ± 0.10 Aabc	0.76 ± 0.21 Ad
Silicon (Si)								
Cervical	0.37 ± 0.65 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
Middle	0.38 ± 0.65 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.16 ± 0.28 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
Apical	0.36 ± 0.63 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
Zinc (Zn)								
Cervical	0.00 ± 0.00 Aa	0.50 ± 0.87 Aa	0.00 ± 0.00 Aa	0.33 ± 0.57 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.07 ± 0.12 Aa
Middle	0.00 ± 0.00 Aa	6.98 ± 12.08 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
Apical	0.00 ± 0.00 Aa	0.76 ± 1.32 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa	1.01 ± 1.74 Aa	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa

*Statistically significant differences for each chemical element are indicated by distinct letters, with uppercase letters in the column and lowercase letters in the row ($P<0.05$).

Table 5. Scores of adhesive interface sealing based on experimental groups and intraradicular thirds.

Parameters	Quality of the dentin/cement adhesive interface				Formation of tags in intraradicular dentin					Tag penetration depth				
Scores / Grups (Thirds)	0	1	2		0	1	2	3		0	1	2	3	
NC														
Cervical	3	-	-	Aa	-	3	-	-	Aa	-	1	2	-	Aa
Middle	2	1	-	Aa	-	3	-	-	Aa	-	-	3	-	Aa
Apical	-	3	-	Aa	-	3	-	-	Aa	-	-	3	-	Aa
PC														
Cervical	2	1	-	Aa	-	2	1	-	Aa	-	-	3	-	Aa
Middle	1	2	-	Aa	-	2	1	-	Aa	-	-	2	1	Aa
Apical	-	3	-	Aa	-	1	2	-	Aa	-	-	3	-	Aa
IG														
Cervical	3	-	-	Aa	-	2	1	-	Aa	-	1	2	-	Aa
Middle	2	1	-	Aa	-	2	1	-	Aa	-	-	2	1	Aa
Apical	3	-	-	Aa	-	2	-	1	Aa	-	1	1	1	Aa
IG + CH														
Cervical	3	-	-	Aa	-	2	1	-	Aa	-	1	2	-	Aa
Middle	2	1	-	Aa	-	1	2	-	Aa	-	2	1	-	Aa
Apical	3	-	-	Aa	-	1	2	-	Aa	-	1	2	-	Aa
CC														
Cervical	2	1	-	Aa	-	2	1	-	Aa	-	1	1	1	Aa
Middle	2	1	-	Aa	-	1	2	-	Aa	-	-	3	-	Aa
Apical	2	1	-	Aa	-	2	1	-	Aa	5	-	3	-	Aa
CC + CH														
Cervical	2	1	-	Aa	-	2	1	-	Aa	-	2	1	-	Aa
Middle	1	2	-	Aa	-	2	1	-	Aa	-	2	1	-	Aa
Apical	1	2	-	Aa	-	1	2	-	Aa	-	3	-	-	Aa
MB														
Cervical	1	2	-	Aa	-	1	2	-	Aa	-	-	3	-	Aa
Middle	1	2	-	Aa	-	-	3	-	Aa	-	-	3	-	Aa
Apical	1	2	-	Aa	-	-	3	-	Aa	-	-	3	-	Aa
MB + CH														
Cervical	1	2	-	Aa	-	2	1	-	Aa	-	2	1	-	Aa
Middle	-	3	-	Aa	-	1	2	-	Aa	-	1	2	-	Aa
Apical	-	3	-	Aa	-	2	1	-	Aa	-	1	2	-	Aa

NC: Negative control; PC: Positive control; IG: Indocyanine green; IG + CH: Indocyanine green with calcium hydroxide medication; CC: Curcumin; CC + CH: Curcumin with calcium hydroxide medication; MB: Methylene blue; MB + CH: Methylene blue with calcium hydroxide medication.

*Means followed by different letters, uppercase in the column and lowercase in the row, indicate statistically significant differences ($P<0.05$).

ATTACHMENT A

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado **"Influência da terapia fotodinâmica com fotossensibilizadores indocianina verde ou curcumina, e da medicação intracanal de hidróxido de cálcio, na adesão de pinos de fibra de vidro à dentina intrarradicular"**, Processo FOA nº 0418-2022, sob responsabilidade de Gustavo Sivieri de Araújo apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 30 de Maio de 2022.

VALIDADE DESTE CERTIFICADO: 01 de Agosto de 2025.
DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 01 de Setembro de 2025.

CERTIFICATE

We certify that the study entitled **"Influence of photodynamic therapy with indocyanine green or curcumin photosensitilizers, and intracanal medication of calcium hydroxide, on the adhesion of glass-fiber posts to intraradicular dentin"**, Protocol FOA nº 0418-2022, under the supervision of Gustavo Sivieri de Araújo presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on May 30, 2022.

VALIDITY OF THIS CERTIFICATE: August 01, 2025.
DATE OF SUBMISSION OF THE FINAL REPORT: September 01, 2025.

Prof. Dr. João Carlos Callera
Coordenador da CEUA
CEUA Coordinator

CEUA - Comissão de Ética no Uso de Animais
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