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Larissa dos Santos de Moraes

Influência da terapia fotodinâmica com diferentes fotossensibilizadores e do medicamento de hidróxido de cálcio no grau de conversão e na integridade da interface adesiva na cimentação de pinos de fibra de vidro

Araçatuba
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Dissertação apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do Título de Mestre em Ciências, Área de Concentração em Endodontia.

Orientador: Prof. Assoc. Gustavo Sivieri de Araújo
Coorientador: Prof. Tit. João Eduardo Gomes Filho

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“O sucesso é a soma

de pequenos esforços

repetidos dia após dia.”

(Robert Collier)

Resumo

Moraes, LS. **Influência da terapia fotodinâmica com diferentes fotossensibilizadores e do medicamento de hidróxido de cálcio no grau de conversão e na integridade da interface adesiva na cimentação de pinos de fibra de vidro.** 2024. 65 f. Dissertação (Mestrado) - Universidade Estadual Paulista (UNESP), Faculdade de Odontologia, Araçatuba, 2024.

RESUMO

Introdução: Este estudo *in vitro* avaliou a influência da terapia fotodinâmica antimicrobiana (TFD), usando curcumina (CC), indocianina verde (IV) ou azul de metileno (AM) como fotossensibilizadores (FSs) e hidróxido de cálcio (HC) como medicamento intracanal no grau de conversão do cimento resinoso e integridade da interface adesiva. *Material e métodos*: 112 dentes bovinos foram submetidos a tratamento endodôntico e foram distribuídos em 8 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. Após a cimentação dos pinos de fibra de vidro, as amostras foram submetidas à análise da integridade da interface adesiva (n=6) pela microtomografia de alta resolução (micro-CT) para a quantificação do número e volume de fendas e bolhas e ao grau de conversão pelo espectrômetro Raman (n=8), para identificação de alterações na polimerização do cimento. Os dados sobre a integridade da interface adesiva e o grau de conversão foram submetidos a testes de normalidade e homogeneidade. Posteriormente, eles foram analisados usando Anova *Two-Way* para medidas repetidas. A análise post-hoc foi realizada usando o teste de *Tukey* com um nível de significância (α) de 0,05. *Resultados*: A análise da integridade da interface adesiva evidenciou maior quantificação de gaps nos grupos: IV no terço cervical, médio e apical, seguido pelos grupos CC e AM; maiores volumes de bolhas: IV e CC no terço cervical, IV no terço médio, seguidos pelos grupos CC e AM e no terço apical CC+HC. Os menores valores de volume de bolhas e número de fendas foram encontrados nos grupos com FS+HC. Ao realizar a avaliação do grau de conversão nos terços cervical, médio e apical, constatou-se que não houve diferenças estatisticamente significativas entre os grupos experimentais (p>0,05). *Conclusões*: A indocianina verde com hidróxido de cálcio foram os melhores resultados em relação ao uso de medicamentos intracanais e fotossensibilizadores. A combinação de FS-IV e HC influenciou moderadamente as propriedades da dentina e do cimento.

Palavras-chaves: Tratamento do Canal Radicular, Fotoquimioterapia, Indocianina Verde, Azul de Metileno, Hidróxido de Cálcio.

Abstract

Moraes, LS. **Influence of photodynamic therapy with different photosensitizers and calcium hydroxide medication on the degree of conversion and integrity of the adhesive interface in the luting of fiberglass posts.** 2024. 65 f. Dissertation (master's degree) - São Paulo State University (UNESP), School of Dentistry, Araçatuba, 2024.

ABSTRACT

Introduction: This *in vitro* study evaluated the influence of antimicrobial photodynamic therapy (aPDT), using curcumin (CC), indocyanine green (IG), or methylene blue (MB) as photosensitizers (PSs), and calcium hydroxide (CH) as intracanal medications on the degree of resin cement conversion and adhesive interface integrity. *Materials and Methods*: 112 bovine teeth underwent endodontic treatment and were distributed into 8 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. After luting of fiberglass posts, the samples were subjected to the analysis of adhesive interface integrity (n=6) by high-resolution microtomography (micro-CT) to quantify the number of gaps and voids and to assess the degree of conversion by the Raman spectrometer (n=8), to identify changes in cement polymerization. Data on adhesive interface integrity and degree of conversion were subjected to normality tests and homogeneity. Subsequently, they were analyzed using Two-Way ANOVA for repeated measures. Post-hoc analysis was performed using the Tukey test with a significance level (α) of 0.05. *Results:* The analysis of the integrity of the adhesive interface revealed an increase in the quantification of gaps in the following groups: IG in the cervical, middle, and apical thirds, followed by the CC and MB groups. The highest void volumes were observed in the IG and CC groups in the cervical third, IG in the middle third, followed by the CC and MB groups, and in the apical third, CC+CH. The lowest values of void volume and number of gaps were found in the groups with PS+CH. When evaluating the degree of conversion in the cervical, middle, and apical thirds, it was found that there were no statistically significant differences between the experimental groups ($p>0.05$). *Conclusions:* Indocyanine Green with calcium hydroxide yielded the best results regarding the use of intracanal medication and photosensitizers. The combination of PS-IG and CH moderately influenced dentin and cement properties.

Key-words: Root Canal Therapy, Photochemotherapy, Indocyanine Green, Curcumin, Methylene Blue, Calcium Hydroxide.

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LISTA DE ABREVIATURAS, SÍMBOLOS E SIGLAS

% - Percent

°C - Degrees celsius

± - More or less

< - Less than

= - Equal

ANOVA - Variance analysis

aPDT - Antimicrobial photodynamic therapy

BS - Bond strength

CC - Curcumin

CH - Calcium hydroxide

F - Maximum force

GFPs - Glass-fiber posts

IG - Indocyanine green

Laser - Light amplification by stimulated emission of radiation

LED - Light emitting diode

LPS - Liposaccharides

MB – Methylene blue

mg/L -Milligrams per liter

mL -Millilitre

mm -Millimeter

mm^2 - Square millimeter

mm^3 - Cubed milliliter

MO - Microorganisms

nm –Nanometer

NC – Negative control

pH - Potential of hydrogen

PS – Photosensitizer

PC – Positive control

PDT - Photodynamic therapy

RCS - Root canal system

s - Seconds

α- Alpha

λ- Lambda

µm -micrometer

Sumário

SUMÁRIO

Article

*Article formatted according to the authors guidelines of the **Journal *of Photochemistry and Photobiology***

Influence of photodynamic therapy with different photosensitizers and calcium hydroxide medication on the degree of conversion and integrity of the adhesive interface in the luting of fiberglass posts

1.Introduction

Endodontic treatment in cases of pulp necrosis primarily aims to disinfect the root canal system (RCS) through mechanical instrumentation and irrigation to facilitate post-treatment healing [1-6]. Although root canal therapy significantly reduces the microbial load, the RCS's anatomical complexity often prevents the complete elimination of bacteria, a leading cause of endodontic treatment failures [7-12]. Consequently, adjunctive therapeutic strategies, such as intracanal medication and photodynamic therapy (PDT), have been developed [13-15].

Calcium hydroxide (CH) is the most commonly used intracanal medication in teeth that undergo more than one session of endodontic treatment. It possesses antibacterial properties and the ability to neutralize lipopolysaccharide (LPS) bacteria through hydrolysis of the lipid portion of bacterial LPS [16-22]. Additionally, due to its alkaline pH, it raises the medium's pH, rendering it inhospitable for the survival and proliferation of remaining microorganisms. Therefore, CH exhibits antiseptic properties, facilitates dissolution of organic matter, demonstrates osteogenic potential, shows absence of cytotoxicity, and can be used to alleviate postoperative pain, as recommended for endodontic treatment [16-22].

Antimicrobial photodynamic therapy (aPDT) involves a photosensitizer (PS), a specific wavelength light source, and oxygen to initiate a photobiochemical reaction [23,24]. The PS absorbs photons, generating singlet oxygen, a highly reactive and short-lived toxic agent that damage death target cells and irreversibly oxidizes bacteria and fungi [23-28].

The antibacterial efficacy of aPDT depends on the PS type and its singlet oxygen generation capacity [29,30]. Effective PSs, such as methylene blue (MB), curcumin (CC), and indocyanine green (IG), possess disinfectant and antiseptic properties [31]. MB, an amphiphilic compound effective against both gram-positive and gram-negative bacteria, has low toxicity and an analgesic effect, with an absorption wavelength of 600-660 nm [32-34]. CC, a natural polyphenol, exhibits anti-inflammatory, antioxidant, and antimicrobial properties, activated by blue light with an absorption peak between 300 and 500 nm [35-37]. IG, a water-soluble anionic cyanine dye, is activated by infrared light and has an absorption peak at 810 nm, with low toxicity [14, 38-40].

Despite the aPDT effectively reduces microbial loads without inducing resistance, it can adversely affect the mechanical properties of intraradicular dentin and the bond strength of fiberglass posts to dentin, potentially compromising root fracture resistance [41-45]. The interaction of PSs from aPDT and CH residues in dentinal tubules may contribute to failures in the bonding of fiberglass posts, a common issue in endodontic restorations [38,43,46]. However, literature lacks reports on the combined effects of aPDT with different PSs and CH as an intracanal medicament on the conversion degree and integrity of the fiberglass post and root dentin luting interface.

This study assessed the impact of aPDT using MB, CC, and IG, with or without CH as calcium hydroxide medicament, on the degree of conversion and adhesive interface integrity of fiberglass posts across different root thirds (cervical, middle and apical). The null hypotheses were: 1) aPDT with PS-MB, PS-IG, or PS-CC would not significantly affect the conversion degree and luting interface integrity of fiberglass posts in various intraradicular dentin thirds; 2) Different root thirds would not significantly influence the adhesive interface such as properties, degree of conversion, or luting interface integrity of fiberglass posts.

2.Material and Methods

2.1. Experimental design

The research received approval from the Animal Use Ethics Committee (0418/2022). Table 1 details the materials used in this study.

Previous studies were used to determine the sample size that should be used [25,47]. A total of 112 bovine incisors, aged between 28 and 36 months, were used. The specimens underwent evaluation under adequate lighting to identify any potential microcracks and/or fractures resulting from extraction. Teeth exhibiting cracks and/or fractures or apical curvature were discarded and replaced, selecting only those without fractures, cracks, or curved roots for inclusion (Fig. 1-A).

The roots were standardized to a length of 20 mm following the removal of the anatomical crowns, 1.0 mm above the dentin-enamel junction, through a transverse section using diamond discs in a precision cutter (IsoMet 1000; Buehler, Lake Bluff, USA) (Fig. 1-B). For biomechanical root canal preparation, the actual working length was set 1.0 mm shorter than the root length. The endodontic treatment of the samples involved mechanical instrumentation, starting with the K#50 file as the initial instrument and progressing to the K#80 to create the apical stop (Fig. 1-C).

Irrigation occurred with each file change, up to the K#80, using 10 mL of 2.5% sodium hypochlorite (Rioquímica, São José do Rio Preto, SP, Brazil) for 15 seconds. To eliminate the smear layer, using 10 mL of 17% EDTA (Biodinâmica Química e Farmacêutica LTDA, Ibiporã, PR, Brazil) was introduced into the root canals for 3 minutes [48]. Subsequently, the EDTA was removed through irrigation with 2.5% sodium hypochlorite, followed by aspiration and drying with absorbent paper points (Dentsply Sirona, Pirassununga, SP, Brazil). To prevent the PS from escaping, the apical foramina were sealed in the last 2 mm with 37% phosphoric acid (FGM, Joinville, SC, Brazil), adhesive (Adper Single Bond 2; 3M ESPE, St Paul, MN, USA) and composite resin (Filtek Z250 XT, 3M ESPE, St Paul, MN, USA) (Fig. 1-C).

2.2. Antimicrobial photodynamic therapy and experimental groups

The specimens were randomly distributed into 8 experimental groups (n=14), as shown in Table 2.

During the pre-irradiation period of the PSs, the lighting in the laboratory environment was dimmed to prevent potential interference with the results due to PS degradation [26].

After biomechanical preparation, the root canals in the negative control group (NC) were irrigated with deionized water, while those in the positive control group (PC) were irrigated with deionized water and then filled with intracanal CH medication for 14 days. The NC and PC groups did not receive PSs and were not subjected to aPDT.

The IG and IG+CH groups underwent aPDT using the PS-IG 50 mg/L (Ophthalmos S/A, São Paulo, SP, Brazil), which was retained in the root canal for 60 seconds (pre-irradiation period) with agitation using an Irrisonic E1 ultrasonic tip (Helse Dental Technology, Santa Rosa de Viterbo, SP, Brazil) connected to an ultrasound device (NEVTRON P5 XS, Satelec Acteon, Mérignac, France) set to power scale 2 [49]. The PSs were activated for 60 seconds with an infrared laser (Laser Duo; MMO, São Carlos, SP, Brazil; λ = 808 nm) using a flexible optical fiber (300 µm) (Guia de Luz PDT, DMC Equipamentos, São Carlos, SP, Brazil) resulting in a final energy of 200 J/cm^2 (Fig. 1-D).

The CC and CC+CH groups were exposed to aPDT using the PS-CC 500 mg/L (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) with a pre-irradiation period of 300 seconds and agitation for 60 seconds. The PS-CC was activated for 240 seconds by a blue LED light (Radii Cal SDI, Victoria Australia; λ = 480 nm), using a 300 µm flexible optical fiber, resulting in a final energy of 1200 mW/cm^2 (Fig. 1-D).

Finally, the MB and MB+CH groups underwent aPDT using the PS-MB 50 mg/L (Chimiolux, DMC, São Carlos, SP, Brazil) with a pre-irradiation period of 180 seconds and agitation for 60 seconds. The PS-MB was activated for 60 seconds by a red laser (Laser Duo, MMO, São Carlos, SP, Brazil; λ = 660 nm) using a 300 μm flexible optical fiber, resulting in a final energy of 72 J/cm^2 (Fig. 1-D).

The radiation was conducted by moving the optical fiber in a helicoidal motion along the cervical-apical direction at a rate of 10 times per minute, with a depth 2 mm less than the working length of the root canal. [9; 12]. Subsequently, a final irrigation with 10 mL of deionized water to remove any residual PSs, and drying of the canal with suction cannulas and #80 absorbent paper points [50]. All teeth were then stored at 100% relative humidity at 37°C for 7 days [51].

2.3. Calcium hydroxide medication

The IG+CH, CC+CH and MB+CH groups were given intracanal CH medication, which was left in the root canal for 14 days.

The NC, IG, CC, and MB groups did not have their root canals filled with intracanal CH medication; therefore, they remained empty until the root canal filling procedure [5]. In the groups medicated with CH (PC, IG+CH, CC+CH, and MB+CH), CH (Biodinâmica Química e Farmacêutica, Ibiporã, PR, Brazil) and iodoform (Biodinâmica Química e Farmacêutica, Ibiporã-PR, Brazil) were combined and introduced into the root canal using a Lentulo spiral #4 (Dentsply Sirona, Pirassununga, SP, Brazil) (Fig. 1-E) [52].

Subsequently, the teeth were sealed with a double sealant layer consisting of Cimpat Branco cement (Septodont do Brasil ImportadoraLtda, Barueri, SP, Brazil) and Maxxion R Glass Ionomer Cement (FGM, Joinville, SC, Brazil), and then stored at 100% humidity at 37°C.

2.4. Root channel filling and Fiberglass posts Luting

After 14 days, the coronary seal and the CH with iodoform were removed using 10 mL of 2.5% sodium hypochlorite irrigation with the aid of an K#80 2 mm less of the working length into the root canal. All root canals were then filled with #80 main and auxiliary gutta-percha cones (F and MF) (Tanariman Indústria Ltda., Manacapuru, AM, Brazil) and sealed with MTA Fillapex cement (Angelus, Londrina, PR, Brazil) (Fig. 1-F) [53]. The coronal seal was reapplied using a double sealing technique, and the teeth were stored at 100% humidity at 37°C for 7 days.

The intraradicular preparation of the samples was performed in all groups using a #1 wide drill (Dentsply Sirona, Pirassununga, SP, Brazil), equivalent to a #2 pin, to remove 9 mm of gutta-percha and create space for luting the posts (Fig. 1-F).

A White Post DCE No. 2 fiberglass posts (FGM, Joinville, SC, Brazil) was used, which was cleaned and conditioned with 37% phosphoric acid for 60 seconds, rinsed with water, and dried with an air jet. Subsequently, silane (RelyX Ceramic Primer, 3M ESPE, St. Paul, MN, USA) was applied with a microbrush (KG SORENSEN, Cotia, SP, Brazil) and air-dried. The root canals were irrigated with 2mL of 0.9% saline solution (FARMACE, Barbalha, CE, Brazil), followed by aspiration with endodontic cannulas and drying with absorbent paper points.

The posts were cemented using RelyX TM U200 cement (3M ESPE, St. Paul, MN, USA), and the luting process was carried out by dual activation using an automix tip for application in the root canal (Fig. 1-G) [26].

2.5 Degree of Conversion

The specimens were sectioned perpendicularly along their axis using a precision cutting machine (Isomet 1000, Buehler, Ltd., Lake Bluff, IL, USA), obtaining slices of 1.3 mm from each third [54] (Fig. 1-I). These slices were then embedded in acrylic resin (Clássico, São Paulo, SP, Brazil). Subsequently, they were manually abraded with abrasive sandpapers of 320, 600, 800, and 1200 grit (Extec Corp., Enfield, CT, USA), polished with diamond pastes of 6, 3, and 1 µm, and cleaned in an ultrasonic tank (Model 2210, Branson Ultrasonic Corp., Danbury, CT, USA) with deionized water for 2 minutes [26].

Measurements were conducted using a Micro-Raman spectrometer (Renishaw InVia Raman, Gloucestershire, United Kingdom) with a diffraction grating of 1800 grooves/mm, coupled to an optical microscope (Leica TCS SPE, Leica, Mannheim, Germany) with a spatial resolution of 1 µm. This setup recorded the Raman spectrum in the region of interest of the sample using a He-Ne laser (λ = 633 nm) (Fig. 1-I). Fourier transform spectra were analyzed by selecting a spectral region from 1550 to 1700 cm^{-1} (Fig. 1-I) [55, 56].

To ensure accuracy during the Raman readings, ambient light in the room was turned off to prevent interference, and the temperature was maintained at 23 ± 1 ºC, with the relative humidity controlled at ± 34% [25]. Raman readings were performed at 50x magnification, and each spectrum was recorded with an integration time of 10 seconds. Three spectra were obtained for each specimen, and an arithmetic average was subsequently calculated [55].

The absorbance peak of C=C (aliphatic) at 1638 cm^{-1} was identified (Fig. 2). The ratio used the absorption intensities of C=C and CC (% of double bonds that did not react) from pre- and post-polymerization, and the degree of conversion values were obtained using the following formula [25]:

DC = [1 – (aliphatic C/ aromatic C) / aliphatic U/ aromatic U)] x 100%,

Which can also be expressed by:

DC (%) = [1 – (R polymerized / R unpolymerized)] x 100,

"DC" is the acronym representing the degree of conversion. The "aliphatic C" had an absorption peak of 1638 cm^{-1} after curing, and the "aromatic C" had an absorption peak of 1607 cm^{-1} after curing. Similarly, "aliphatic U" had an absorption peak of 1638 cm^{-1} before curing, and "aromatic U" had an absorption peak of 1607 cm^{-1} before curing. The letter "R" denotes the ratio between the peaks of the polymerized and non-polymerized resin cement. Uncured resin cement was placed on a glass microscope slide for measurement using the Micro-Raman equipment [57].

2.6. Analysis of the Adhesive Interface Integrity

To analyze the integrity of the adhesive interface, the specimens (n=6) were evaluated for the volume of voids and quantification of gaps in the adhesive interface of the resin cement using high-resolution microtomography (Micro-CT) (SkyScan 1272; Bruker, Kontich, Belgium) (Fig. 1-H) [25, 58]. The microtomography was calibrated using the standard "ghost" function and operated at an energy of 80 kilovolts and 125 milliamps. Images were generated at a medium resolution (13 µm/slice) and at 180º, with specimens rotated in angular steps of 0.4º, and an exposure time of 3000 ms. Additionally, a 16.5 mm tube and a 1 mm thick aluminum filter were utilized [58].

The samples were scanned, and the data obtained from the scans were converted into DICOM files and rendered into 3D models (SolidWorks Corp., MA, USA). Subsequently, 3D image reconstruction was performed using NRecon software (SkyScan, Kontich, Belgium), extending from the cervical portion to the apex of the roots. The visualization and analysis of the images were conducted using Data Viewer software (SkyScan, Kontich, Belgium), in the coronal, sagittal, and transaxial axes, establishing a standard position for measuring the volume and quantifying voids and gaps [54].

To quantify the voids and gaps in the cement layer, CTAnalyser software (SkyScan, Kontich, Belgium) was employed, with the images positioned transaxially [59]. The area analyzed was determined by selecting an average of 1013 to 1200 slices, performing image segmentation to isolate the target object from other objects present in the image[59].A template of the area of interest was then drawn freehand, demarcating only the cement layer. Subsequently, the images were binarized, and the quantification and volume present in the root canals were calculated [59]. The CTVOX software (SkyScan, Kontich, Belgium) reconstructed the images into three-dimensional models, enabling the evaluation of the interface's integrity and the identification of discontinuities between the cement and the intraradicular dentin [59].

2.7 Statistical analysis

Data concerning the integrity of the adhesive interface and the degree of conversion were subjected to tests for normality (Shapiro-Wilk; Bioestat 2.0 Program) and homogeneity (Bartlett; Bioestat 2.0 Program). These data were then analyzed using repeated measures analysis of variance (ANOVA 2-way; SigmaPlot 12.0). Post-hoc analysis was conducted using the Tukey test, which was protected against least significant differences, with a significance level (α) set at 0.05.

3.Results

3.1 Adhesive Interface Integrity

Table 3 presents the quantification of gaps and the volume of voids at the adhesive interface, while Figure 3 provides illustrative microtomography images.

When comparing the groups regarding gap quantification, it was observed that in the cervical third, the IG group exhibited the highest number of gaps ($p<0.05$), whereas the other groups showed intermediate values ($p>0.05$). The MB+CH, IG+CH, CC+CH, NC, and PC groups had the lowest gap values ($p>0.05$). In the middle third, the IG group again presented the highest number of gaps, followed by the CC+CH, CC, MB+CH, MB, and PC groups ($p>0.05$), with the IG+CH and NC groups showing the lowest gap values ($p>0.05$). In the apical third, the IG group once more had the highest gap value, followed by the MB and IG+CH groups ($p>0.05$), and then by the CC+CH and CC groups ($p>0.05$). When evaluating the intraradicular thirds, only the NC group showed no statistically significant difference ($p>0.05$). In the PC, IG, CC+CH, and MB+CH groups, the cervical third was statistically different from the middle and apical thirds, having the fewest gaps ($p<0.05$). In the IG+CH and MB groups, the cervical and middle thirds were statistically different from the apical third, which exhibited the highest gap values ($p<0.05$). The CC group showed statistical differences across the cervical, middle, and apical thirds ($p<0.05$).

Regarding the volume of voids, in the cervical third, the IG and CC groups had the highest volumes ($p<0.05$), followed by the PC, NC, and IG+CH groups ($p<0.05$). The MB+CH and MB groups showed intermediate volumes ($p>0.05$), and the CC+CH group had the lowest volume ($p<0.05$). In the middle third, the highest void volumes were observed in the CC+CH group, followed by the IG, PC and CC groups ($p<0.05$). The NC and MB groups had intermediate volumes ($p>0.05$), and the IG+CH and MB+CH groups had the lowest volumes ($p<0.05$). In the apical third, all groups were statistically different, with the CC+CH group showing the highest void volumes ($p<0.05$), and the IG+CH group having the lowest volume of voids ($p<0.05$). The NC group's cervical third had the lowest void volume, differing statistically from the middle and apical thirds ($p<0.05$). In the PC, IG, IG+CH, CC, CC+CH, MB, and MB+CH groups, all thirds were statistically different, with a trend towards higher void volumes in the apical third, followed by the middle third, and the cervical third having the lowest volumes ($p<0.05$).

3.2 Degree of Conversion (DC)

The degree of resin cement conversion values is presented in Table 4. Upon conducting the conversion degree (CD) assessment across the cervical, middle, and apical thirds, it was determined that there were no statistically significant differences among the experimental groups (NC, PC, IG, IG+CH, CC, CC+CH, MB, and MB+CH) ($p>0.05$). However, regarding the intraradicular thirds, it was noted that only the NC group exhibited a significant difference between the cervical and middle thirds ($p<0.05$).

4.Discussion

Currently, strategies are used to achieve maximum bacterial eradication, such as the use of calcium hydroxide-based medications and aPTD, which minimize the occurrence of secondary and/or persistent infections responsible for failures in endodontic therapies [60, 61].

Authors such as Davies (2003) and Meire et al. (2012) suggest the use of phototherapy simultaneously with the use of CH, complementing conventional endodontic therapies and leading to the extermination of microorganisms [62,63]. However, the use of CH medication and aPDT PSs may have effects on the mechanical characteristics of root dentin and on the integrity of the adhesive interface of fiberglass post luting, a fact evidenced by the work of Strazzi-Sahyon et al. (2018) and Ramos et al. (2018) [43,64].

The use of aPDT with different types of PSs (PS-BM, PS-IG or PS-CC) and subsequent luting of a fiberglass post influences the integrity of the adhesive interface (Table 3), however, it was found that it did not there were statistically significant differences in relation to the degree of conversion (table 4), therefore, the first null hypothesis was partially accepted. The evaluation of the different root thirds demonstrated that there were significant differences in the degree of conversion and in the integrity of the post luting interface, refuting the second null hypothesis (table 3 and figure 3).

The PS-IG is a tri-carbocyanine dye, being an amphiphilic molecule with low toxicity and an absorption peak in the wavelength of the infrared spectrum, having the mechanism of action through the photothermal effect, which induces cell death by increasing of intracellular temperature [65-67]. When analyzing the adhesive interface in the experimental groups, it was found that the IG group presented the highest number of gaps in the cervical, middle and apical thirds when compared to the other groups and also showed a tendency towards greater volumes of voids in the cervical and apical thirds (table 3).

According to Schoop et al., the use of IG induces liquefaction and calcification of the dentin surface, partially destroying the structure of the dentinal tubules, resulting in an ineffective diffusion of the cement and a deficient hybridization of the dentin and dentinal tubules of the intraradicular dentin, causing a reduced binding integrity [15,68,69]. Furthermore, as IG has a photothermal and not a photochemical effect (like most PSs), it produces physical and chemical changes in dentin, reducing bond strength and leading to failure of the intracanal restoration [70]. Failure of the intracanal restoration leads to the appearance of gaps between the restoration interface and voids, as evidenced by our study.

As observed, the samples that used curcumin and methylene blue as PSs also showed relevant values of gaps and voids (table 3). Corroborating our study, Strazzi-Sahyon et al., (2018) showed that curcumin alters the behavior of the dentin-restoration interface, reducing fracture resistance [43, 71]. This occurs due to the formation of hydrogen peroxide by curcumin when activated by blue LED light, which binds to the calcium present in hydroxyapatite, producing a precipitate that acts as a physical barrier and reduces the interaction between the resin cement and the dentin surface [49].

Methylene blue, in turn, is a cationic substance that binds to the phosphate molecules present in hydroxyapatite crystals and changes the ratio of calcium and phosphate, also creating a precipitate that acts as a physical barrier [72].

When analyzing the experimental groups that had the use of CH medication, it was found that this medication promoted differences in the properties of the adhesive integrity of the luting interface of fiberglass posts, however, there were no statistically significant differences in relation to the degree of conversion.

When evaluating the root thirds of the groups with HC, it was observed that there was a tendency towards higher values of both the number of gaps and the volume of voids in the apical group, this fact, as observed by Savaris et Al. (2024), is explained by the greater difficulty in removing the HC, as the material in this third is more compressed against the walls of the root canal [73]. Furthermore, when comparing the different intra-radicular thirds of the different experimental groups, a predisposition of the dentin in the apical third to present the highest Gaps and void volume values was verified and, according to Strazzi-Sahyon et Al.(2019), it may be due to the configuration of the cavity, the presence of apical sclerosis and restricted penetration of the cement into the deeper portions of the root canal [49, 74-76].

It is worth noting that the results of void volume and number of gaps may have been influenced by the use of bovine teeth, as they have a larger root canal than human teeth and when using conventional fiberglass posts, there is a need to of a large amount of cement for luting, causing a greater propensity for the formation of voids and also for polymerization contraction [59].

Raman spectroscopy is a molecular spectroscopy that uses the interaction of light with matter to determine the constitution or composition of a material and the information provided is the result of a light diffusion process [77]. When analyzing the levels of light and vibrations of chemical bonds through the interaction of electromagnetic radiation, spectra are formed [77]. In this study, Raman spectroscopy was performed on samples with polymerized/cured cement (peak of 1638 cm^{-1}) from the cervical, middle and apical thirds of all groups and also on non-polymerized/uncured cement (peak of 1607 cm^{-1}) (Image 2).

When obtaining the degree of conversion (DC) from the data obtained (table 4), it was found that there were no statistically significant differences between the different experimental groups and also between the different intraradicular thirds, except for the NC group.

The study by Strazzi-Sahyon et al. (2024) has a very similar methodology in relation to the preparation of samples in this work and the use of aPDT with PSs [47]. In its results, statistically significant differences were found between the experimental groups and between the different thirds, with a higher DC value in the cervical third of the PC group. In relation to the apical third, Strazzi-Sahyon and collaborators (2024) observed that the experimental groups with CH presented the lowest DC values when compared to the negative and positive control groups, however, in this study there was a tendency towards higher DC values for the experimental groups with HC [47].

Despite the methodological similarities, there was a large difference in the results, which can be justified by the different use of PSs, in which Strazzi-Sahyon et al. (2024) used only MB and different concentrations of this PS [47]. Furthermore, in that study the same samples prepared for the analysis of mechanical characteristics were used, meaning that the samples were cemented for longer [47]. In this study, in addition to MB with a concentration of 50 mg/L, PS-CC and PS-IG were used.

The use of aPDT with IG, CC and MB as PSs associated with HC medication are important in clinical practice as complementary methods to conventional endodontic therapy, as they are effective against microorganisms present in root canals even after biomechanical preparation. The present study had limitations, such as the use of bovine teeth, which have differences in dentin root structure, root anatomy, size of dentin tubules, with difficulty in standardization and non-homogeneity of the substrate.

Furthermore, it is worth noting that glass posts cemented *in vivo* are subject to interference from occlusion, with the longevity of luting is influenced by the tooth's position in the arch and the amount of remaining tooth structure. Therefore, as this study was not able to simulate the oral environment (presence of saliva, temperature, microorganisms, pH, enzymes, biofilm and occlusion), caution must be taken when extrapolating data from the laboratory environment to the clinical environment.

More studies on the influence of different aPDT PSs on the properties of the adhesive interface of fiberglass posts should be carried out and evaluated for long-term durability, the use of different types of silanes in the preparation of fiberglass posts and different resin cements and, if possible, mimicking oral conditions.

5. Conclusion

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

1. The use of IG, CC, and MB as PSs, along with the HC intracanal medication, influences the properties of the integrity of the adhesive interface of fiberglass posts. However, it does not have significant effects on the degree of conversion of adhesive polymerization.
2. The parameters examined (voids and gaps) were also affected by the intra-root depth in

the analysis of the integrity of the adhesive interface. However, in relation to the degree of conversion, there were no influences in relation to the root depth.

3. IG with CH are the best choices in relation to the use of intracanal medication and PSs as complementary methods of disinfecting root canals. In general, the combination of PS-IG and HC moderately influenced the properties of dentin, cement and characteristics of the integrity of the adhesive interface and the degree of conversion.

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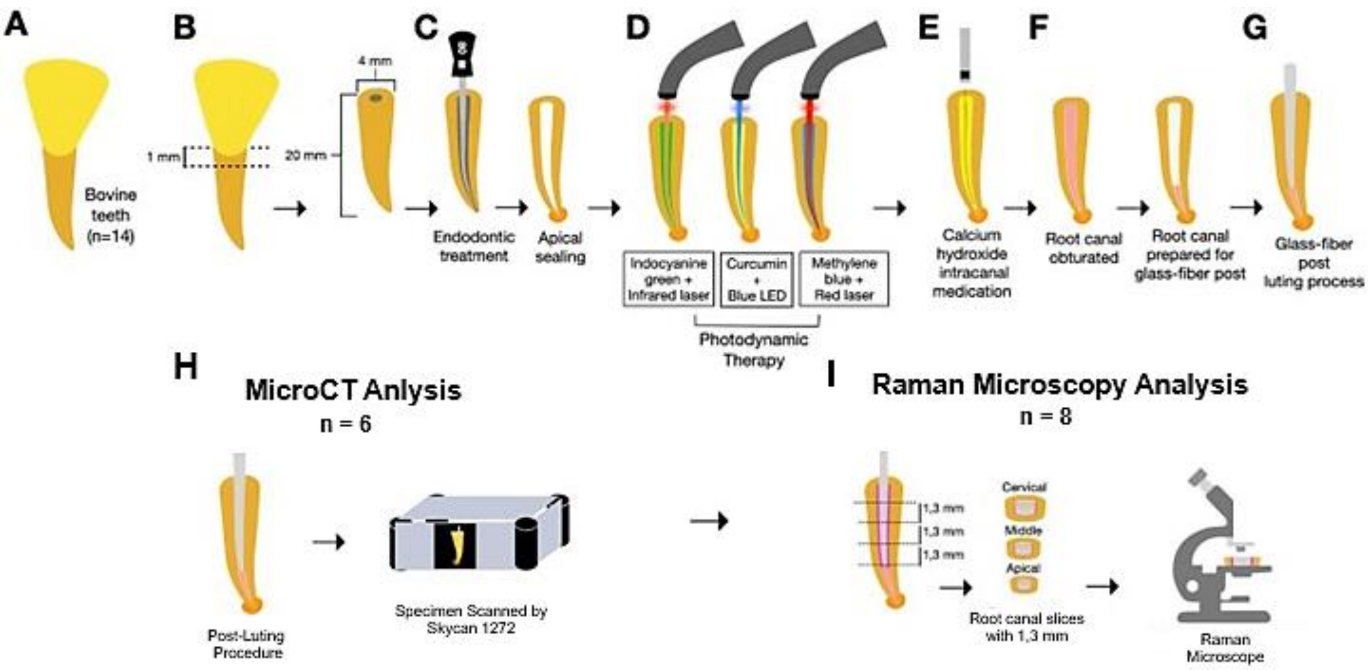


Figure 1. Sample preparation steps: A, Extraction of bovine incisors. B, Removal of the anatomical crowns. C, Standardization of root canal length and apical sealing. D, Carrying out

antimicrobial photodynamic therapy (aPDT) using the indocyanine green photosensitizer with an infrared laser (λ 808 nm); curcumin photosensitizer with blue LED light (λ 480 nm); methylene blue photosensitizer with red laser (λ 660 nm). E, introduction of intracanal calcium hydroxide medication. F, root canal filling. G, Unfilling and luting of fiberglass posts with dual resin cement. H, analysis of samples by high-resolution microtomography (Micro-CT). I, preparation of slices of the intraradicular thirds through 1.3 mm thick cuts and analysis using the *Micro-Raman* spectrometer.

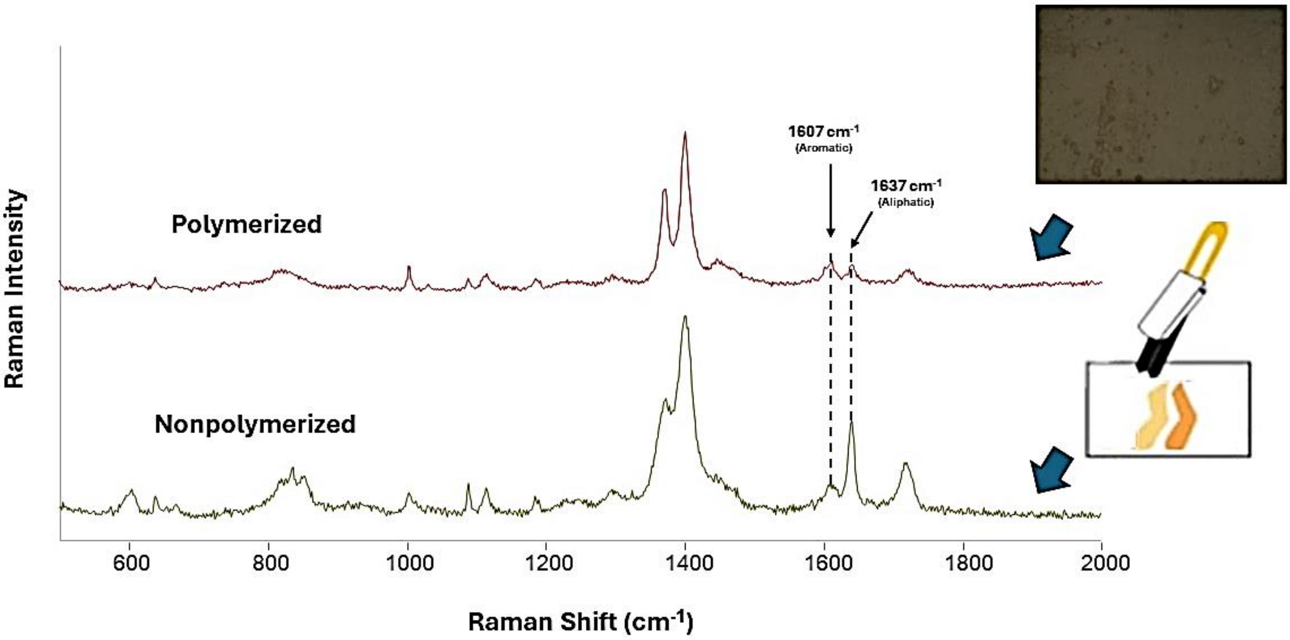
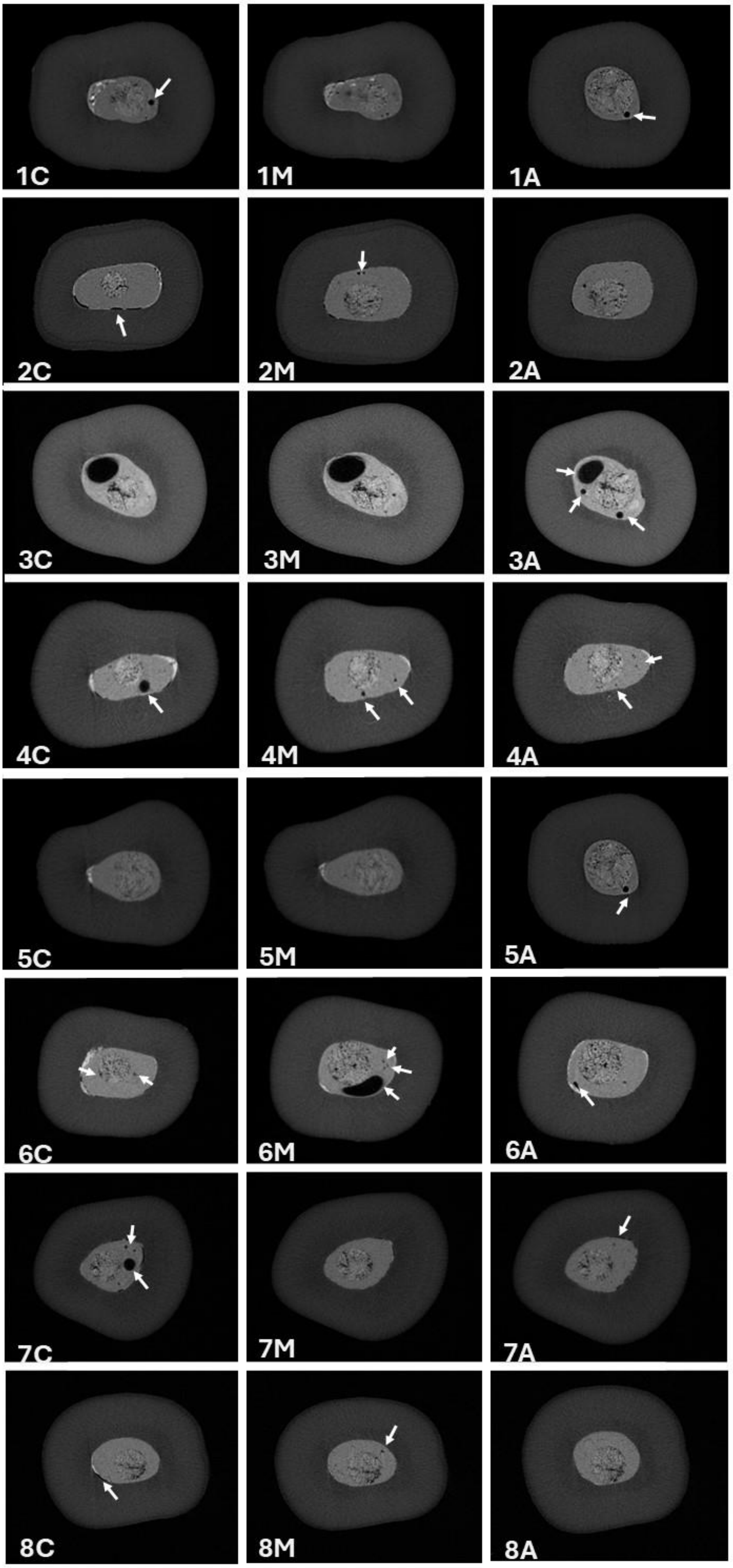


Figure 2. Height ratio between the peaks at) 1607 cm $^{-1}$ (aromatic) and 1637 cm $^{-1}$ (aliphatic) in both polymerized and nonpolymerized resin cement.


1C
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6C
6M
6A
7C
7M
7A
8C
8M
8A

Figure 3. Computed microtomography images were used to evaluate the integrity of the adhesive interface of fiberglass posts luting among different experimental groups and across different root thirds. 1C, 1M, 1A represent the cervical, middle, and apical thirds of intraradicular dentin irrigated with deionized water (negative control). 2C, 2M, 2A denote the cervical, middle, and apical thirds of intraradicular dentin irrigated with deionized water and filled with intracanal HC medication (positive control). 3C, 3M, 3A refer to the cervical, middle, and apical thirds of intraradicular dentin irrigated with deionized water, followed by aPDT with IG (50 mg/L) and activation by an infrared laser (λ 808 nm). 4C, 4M, 4A indicate the cervical, middle, and apical thirds of intraradicular dentin irrigated with deionized water, treated with aPDT using IG (50 mg/L), activated by an infrared laser (λ 808 nm), and filled with intracanal HC medication. 5C, 5M, 5A describe the cervical, middle, and apical thirds of intraradicular dentin irrigated with deionized water, treated with aPDT using CC (500 mg/L), and activated by a blue LED (λ 480 nm). 6C, 7M, 8A (note: it seems there might be a typo here, and you might mean 6C, 6M, 6A) are the cervical, middle, and apical thirds of intraradicular dentin irrigated with deionized water, treated with aPDT using CC (500 mg/L), activated by a blue LED (λ 480 nm), and filled with intracanal HC medication. 7C, 7M, 7A represent the cervical, middle, and apical thirds of intraradicular dentin irrigated with deionized water, treated with aPDT using MB (50 mg/L), and activated by a red laser (λ 660 nm). 8C, 8M, 8A denote the cervical, middle, and apical thirds of intraradicular dentin irrigated with deionized water, treated with aPDT using MB (50 mg/L), activated by a red laser (λ 660 nm), and filled with intracanal HC medication.

White arrows indicate the presence of discontinuities in adhesive luting.

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, diurethanedimethacrylate, water, ethanol, photoinitiators, 5 nm silanized silica, polyacrylic and itaconic acid copolymer.	N820206
Calcium Hydroxide (Biodinâmica)	Intracanal Medication	38% of calcium hydroxide and, 62% of barium oxide.	36870
Curcumin (SIGMA ALDRICH)	Photosensitizer	Curcuma Longa (Turmeric).	149460
EDTA (Biodinâmica)	Irrigating Solution	Disodium Ethylenediaminetetraacetic Acid, Sodium Hydroxide, Deionized Water and Thickener.	1108/22
Filtek Z250XT (3M ESPE)	Resin Composite	Bis-EMA, Bis-GMA, TEGDMA, UDMA, silica and zirconiananofillers, and, agglomeratedzirconia silica nanoclusters.	HB004209993
Indocyanine Green (OPHTHALMOS S/A)	Photosensitizer	5mg of indocyanine green sterilized by sterilizing filter.	112118
Iodoform (Biodinâmica)	Intracanal Medication	Iodoform (99–100%)	005-22
Methylene Blue (CHIMIOLUX DMC)	Photosensitizer	Purified water and methylene blue.	25015
MTA Fillapex (Angelus)	Endodontic Sealer	Salicylate resin, natural resin, diluting resin, bismuth oxide, nanoparticulated silica, MTA (mineral trioxide aggregate) and pigments.	102564
Phosphoric Acid 37% (FGM)	Conditioning Acid	37% phosphoric acid, thickener, coloring and deionized water.	130422
Propylene Glycol (Farma Fórmula)	Intracanal Medication	100% propylene glycol	530080
RelyX U200 (3M ESPE)	Resin Cement	Base: methacrylate phosphoric acid esters, glass-fiber, triethylene glycol dimethacrylate, sodiumpersulfate, silane-treatedsilica. Catalyst: substitutedimethacrylate, glass fiber, sodiump-toluenesulfonate, silane-treated silica, calcium.	2115100124
RelyX Ceramic Primer (3M ESPE)	Ceramic Primer	Ethyl alcohol, water, and 3-MPS	2106800707
Sodium Hypochlorite (RIOQUÍMICA)	Irrigating Solution	2.5% sodium hypochlorite, stabilizer and distilled water.	23034235

Table 2 - Distribution of the experimental groups.

Group abbreviation	Treatment Details
NC	Root canals that did not receive PS and aPDT received irrigation with deionized water.
PC	Root canals that did not receive PS and aPDT received irrigation with deionized water and subsequently filled with CH medication.
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 activated by infrared laser (λ 808 nm) and filled with intracanal calcium mg/L 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 660 nm).
MB+CH	Root canals were filled with methylene blue photosensitizer at 50 mg/L activated by red laser (λ 660 nm) and filled with intracanal calcium hydroxide medication.

NC: negative control; PC: positive control; PS: photosensitizer; aPDT: antimicrobial photodynamic therapy; CH: calcium hydroxide; IG: indocyanine green; CC: curcumin; MB: methylene blue.

Table 3. Mean ± standard deviation values of quantification of gap and volume (MM^3) of voids formation on resin cement as a function of the experimental groups and intraradicular thirds.

Groups/Thirds	NC	PC	IG	IG+CH	CC	CC+CH	MB	MB+CH
Quantification (Gaps)								
Cervical	19.6 ± 5.5 Aa	19.3 ± 7 Aa	224.5 ± 18.7 Ab	29.6 ± 6.6 Aac	38.6 ± 5.9 Ac	28.8 ± 8.9 Aac	53.5 ± 9.1 Ac	35 ± 9.4 Aac
Middle	13.6 ± 9.4 Aa	43.3 ± 7.6 Bab	250 ± 19.2 Bb	22.6 ± 7.2 Aad	95 ± 12.5 Bb	102.8 ± 7.2 Bb	44.5 ± 4.8 Aab	83.5 ± 9.14 Bbd
Apical	20 ± 6.6 Af	40.6 ± 9.1 Ba	238 ± 5.9 Ab	152 ± 11.7 Bc	109 ± 8.15 Cd	113.3 ± 9.5 Bd	161 ± 9.7 Bc	94.5 ± 8.55 Be
Volume (Voids)								
Cervical	276.6 ± 8 Aa	258.1 ± 7.8 Ab	352.4 ± 9.3 Ac	185.4 ± 9.6 Ad	347.5 ± 9 Ac	135 ± 8.6 Ae	137 ± 7.9 Ae	165.2 ± 7.3 Af
Middle	347.3 ± 9.7 Ba	422.2 ± 4.55 Bb	465.4 ± 7.6 Bc	248 ± 7.7 Bd	379.5 ± 5.2 Be	544.3 ± 8.1 Bf	347.3 ± 7.9 Ba	268.5 ± 4.6 Bg
Apical	353.5 ± 8.6 Ba	399.1 ± 3.8 Cb	492.6 ± 4.1 Cc	296.7 ± 3.8 Cd	390.3 ± 5 Cb	797.9 ± 7.9 Ce	715.6 ± 9.7 Cf	323.5 ± 8.1 Cg

Statistically significant differences are indicated by different letters, with uppercase letters in the column and lowercase letters in the row ($P<0.05$).

Table 4. Degree of conversion (in %) of resin cement are expressed as the mean ± standard deviation according to the experimental groups and intraradicular thirds.

Groups/Thirds	**NC**	**PC**	**IG**	**IG+CH**	**CC**	**CC+CH**	**MB**	**MB+CH**
Cervical	78.4 ± 7.28 Aa	70.3 ± 12.73 Aa	76.1 ± 5.39 Aa	77.7 ± 4.79 Aa	77.2 ± 6.87 Aa	75.6 ± 3.74 Aa	78.8 ± 75.6 Aa	78.5 ± 3.36 Aa
Middle	69.9 ± 6.14 Ba	72.4 ± 5.72 Aa	74.2 ±2.63 Aa	74 ± 9.97 Aa	72.0 ± 10.67 Aa	74.1 ± 4.85 Aa	75.6 ± 73.8 Aa	74.0 ± 8.52 Aa
Apical	73.9 ± 6.72 ABa	75.0 ± 8.13 Aa	75.1 ± 5.26 Aa	75.4 ± 7.64 Aa	73.8 ± 3.65 Aa	73.3 ± 5.27 Aa	73.8 ± 4.95 Aa	75.5 ± 4.09 Aa

Statistically significant differences are indicated by different letters, with uppercase letters in the column and lowercase letters in the row ($P < 0.05$).

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

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