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Departamento de Odontologia Restauradora
Programa de Pós-Graduação em Ciências Odontológicas - Endodontia

**HENRIQUE AUGUSTO BANCI**

# Influência da terapia fotodinâmica na resistência de união do cimento obturador à dentina intrarradicular e na morfologia da interface adesiva

**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 a obtenção do título de Mestre em Endodontia.

Orientador: Prof. Ass. Dr. Gustavo Sivieri de Araújo

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"As pessoas felizes lembram o passado com gratidão, alegram-se com o presente e encaram o futuro sem medo."

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*"O discípulo não está acima do seu mestre; todo aquele, porém, que for bem instruído será como o seu mestre."* **(Lucas 6:40)**

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# *Epígrafe*

*"Only the gentle are ever really strong".*

*(James Byron Dean)*

# *Dados Curriculares*

## Dados curriculares

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## Defesa de Dissertação de Mestrado

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BANCI, H.A. **Influência da terapia fotodinâmica na resistência de união do cimento obturador à dentina intrarradicular e na morfologia da interface adesiva**. 2020. 41f. Dissertação (Mestrado) – Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba, 2020.

# RESUMO

**Introdução:** A terapia fotodinâmica (TFD) é utilizada como coadjuvante ao tratamento endodôntico para potencializar a redução microbiana no sistema de canais radiculares. No entanto, a literatura carece de estudos sobre as consequências da TFD na resistência de união do cimento obturador à dentina intrarradicular. O objetivo deste estudo foi avaliar a influência da TFD com azul de metileno na resistência de união do cimento obturador (MTA Fillapex) utilizando o teste de *push-out*, analisar o padrão de fratura das amostras representativas por microscopia eletrônica de varredura (MEV) e a morfologia da interface adesiva intrarradicular pela microscopia confocal de fluorescência (MCF). **Material e Métodos:** Cinquenta e cinco dentes bovinos foram utilizados para simular o tratamento endodôntico. O preparo biomecânico foi realizado em todos os canais radiculares e os espécimes foram distribuídos em 5 grupos: controle (água deionizada); azul de metileno 50 mg/L (AM50SL); azul de metileno 100 mg/L (AM100SL); azul de metileno 50 mg/L + laser vermelho 660nm (AM50L) (TFD); e de azul de metileno 100 mg/L + laser vermelho 660nm (AM100L) (TFD). A resistência de união do cimento obturador à dentina foi mensurada usando uma máquina de teste universal (n=8) e as amostra representativas analisadas em MEV. A morfologia da interface adesiva intrarradicular foi analisada pela MCF. Os dados de resistência de união foram submetidos ao teste de Kruskal-Wallis para comparação entre grupos e ao teste de Friedman para comparação entre terços ($\alpha$=.05). **Resultados:** Houve diferença estatística significante para o grupo azul de metileno 100 mg/L + laser vermelho 660nm (TFD) quando comparando os terços radiculares, onde o terço apical apresentou maiores valores de resistência de união em relação ao terço médio (P=.0302). Nos demais grupos e terços não houve diferença estatística significante (P>.05). Quanto a morfologia da interface adesiva foi possível observar uma menor penetração do cimento obturador no grupo AM100L. **Conclusão:** Pode-se concluir que o uso de TFD com o fotossensibilizador azul de metileno na concentração de 50 mg/L não alterou negativamente a resistência de união do cimento obturador MTA Fillapex na dentina intrarradicular.

**Palavras-chaves:** Fotoquimioterapia, Resistência ao cisalhamento, Cimentos dentários, Materiais restauradores do canal radicular.

BANCI, H.A. **Influence of photodynamic therapy on the bond strength of an MTA based root canal sealer to intraradicular dentin and morphology of the adhesive interface**. 2020. 41f. Dissertação (Mestrado) – Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba, 2020.

# ABSTRACT

**Introduction:** Photodynamic therapy (PDT) is used as an adjunct to endodontic treatment to enhance microbial reduction in the root canal system. However, studies are lacking on the consequences of the application of the application of PDT on the bond strength of the root canal sealer. Therefore, the objective of this study was to evaluate the influence of photodynamic therapy with methylene blue on the bond strength the root canal sealer using a *push-out* test, to analyze the fracture pattern of representative samples using scanning electron microscopy (SEM) and the morphology of the adhesive interface of the intraradicular dentin using Confocal Laser Scanning Microscopy (CLSM). **Material and Methods**: Fifty-five bovine teeth were used to simulate experimental endodontic treatments. Biomechanical instrumentation was performed for all root canals, and the teeth were distributed into 5 groups: control deionized water; methylene blue 50 mg/L (MB50WL); methylene blue 100 mg/L (MB100WL); methylene blue 50 mg/L + red laser 660nm (MB50L) (PDT); and methylene blue 100 mg/L + red laser 660nm (MB100L) (PDT). The push-out bond strength of the root sealer to dentin was measured using a universal testing machine (n=8), representative scanning electron microscopy images were obtained. Images of the morphology of the adhesive interface were obtained using CLSM (n=3). Bond strength data were submitted to the non-parametric Kruskal-Wallis test for comparison between groups and the Friedman test for comparison between thirds ($\alpha$=.05). **Results:** Comparing the root thirds, for the methylene blue group with the higher concentration activated by Red Laser, there was significant difference, the apical third presented higher values of bond strength in relation to the middle third (P=.0302). In the other groups and thirds there was no statistically significant difference (P>.05). As for the morphology of the adhesive interface, it was possible to observe a lower penetration of the sealer in the MB100L group. **Conclusion:** The application of PDT with the photosensitizer methylene blue at a concentration of 50 mg/L, regardless of the activation by red laser, did not alter negatively the bond strength of the MTA Fillapex sealer to intraradicular dentin.

**Keywords:** Photodynamic therapy, Bond Strength, Root Canal Filling, Endodontic Sealer.

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**CLSM** – Confocal Laser Scanning Microscopy

**Laser** – Light Amplification by Stimulated Emission of Radiation

**LED** – Light Emitting Diode

**MO** – Microorganisms

**MB** – Methylene Blue

**MTA** – Mineral Trioxide Aggregate

**PDT** – Photodynamic Therapy

**PS** – Photosensitizer

**RCS** – Root Canal System

**SEM** – Scanning Electron Microscopy

# SUMÁRIO

# *Article*

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**Influence of photodynamic therapy on the bond strength of an MTA based root canal sealer to intraradicular dentin and morphology of the adhesive interface**

## 1) INTRODUCTION

The main purpose of the endodontic treatment is cleaning, shaping and sealing hermetically the root canal system (RCS) [1,2]. In addition, in teeth with pulp necrosis the disinfection of the RCS must be effective [3]. Different therapeutic strategies such as: root canal biomechanical preparation [2], the use of irrigating solutions like sodium hypochlorite [2,3] and intracanal medication of calcium hydroxide are known methods to reduce quantity of microorganisms (MO) and their toxic products [5-6]. However, studies indicate the ineffectiveness of these methods, particularly in cases of persistent infections [7]. Therefore, new therapeutic strategies are constantly being investigated to eradicate the endodontic infections.

With the development of Laser (Light Amplification by Stimulated Emission of Radiation) and LED (Light Emitting Diode) devices, new therapeutic approaches, including photodynamic therapy (PDT) [8,9], have been introduced to enhance disinfection in the endodontic treatment. PDT involves the absorption of photons by a photosensitizer (PS) activated by a light source in a specific wavelength (Laser or LED) promoting a selective destruction at a specific site (target-cell) [10-12]. The mechanism of action of PDT resides on the process of absorption of light by the PS stimulating the electrons from the normal state to an excited state [13-15]. The PS in presence of oxygen, transfers the energy generated to the substrate [16]. On the process of returning to its normal state the electron promotes formation of highly reactive and short-lived oxygen species, called singlet oxygen, affecting the target cells and MO through irreversible oxidation [13-17].

Studies demonstrates that PDT increases root canal disinfection because unlike antibiotics, which have a specific mechanism of action, singlet oxygen generated from a photodynamic reaction has a nonspecific physical-chemical mechanism of action, preventing the development of microbial resistance [18-23]. Methylene blue (MB) has photosensitizing characteristics, being used in PDT as a PS [24]. It has an absorption band range of 500nm to 700nm, therefore it is activated by red light [25,26]. When activated MB reacts resulting in singlet oxygen, which could influence on the polymerization process and mechanical properties of the root canal sealer and intrarradicular dentin, compromising directly the longevity and success of the endodontic treatment [27].

A study by Strazzi-Sayhon et al. 2018 [27] demonstrated that PDT affected negatively the bond strength of the resin cement (used in glass fiber-posts) to intraradicular dentin effecting directly the longevity of the restorative procedure. Few studies investigated the influence of PDT with MB as a PS on the bond strength of the root canal sealer to intraradicular dentin [28,29]. It is understood that the adhesion of the filling material to intraradicular dentin is one of the most important properties for maintaining the integrity of the obturation [30].

Knowing that the effects of PDT on the bond strength of the root canal sealer to intraradicular dentin are still unclear, the purpose of this in vitro study was to determine the *push-out* bond strength of the root canal sealer (MTA Fillapex) to intraradicular dentin after PDT with MB as a PS. Using confocal laser scanning microscopy (CLSM) qualitative images of the morphology of the adhesive interface were obtained in order to compare visually the penetration of the sealer. Two null hypotheses were tested: 1) That the different concentrations of MB, activated by laser or not, would not cause a significant difference in the intraradicular dentin, and 2) That different thirds of the intraradicular dentin would not promote a significant difference for the treatment with PDT.

## 2) MATERIAL AND METHODS

### *2.1) Experimental design*

Table 1 describes the materials used in this study. The study was approved by the institutional Ethics Committee (#19-00611) (Attachment 1). Fifty-five bovine incisors that had been extracted from cattle approximately 3 years old (28–36 months) were used [27,31,32]. All teeth that presented fractures, cracks, and curved roots were excluded. The anatomic crowns of all teeth were removed at 1.0 mm above the cementum–enamel junction using a machine cutter Isomet 1000 (Buehler, Lake Bluff, IL, USA) [27,31,32]. To standardize teeth used in this study, only teeth with a mean root canal length of approximately 20 mm and mean root canal diameter of approximately 4 mm were included. The radicular canals were instrumented with K-files #80 (Maillefer Instruments, Tulsa, OK, USA) after the working length had been determined to be 1.0 mm less than this length; the canals were then irrigated with 10 mL of 1% sodium hypochlorite once per 15s, aspirated and dried with sterile paper points [27,31,32].

In order to prevent escape of the PS, the apical portion of all the roots were etched with 37% phosphoric acid (FGM) for 15s, washed and dried. Dental adhesive (Adper Single Bond 2, 3M ESPE, St Paul, MN, USA) was applied to the conditioned surface for 15s and was then activated for 20s using a light-polymerization unit (Poly Wireless, Kavo, Joinville, SC, Brazil). The apical foramens were sealed with a composite resin (Filtek Z350 XT, 3M ESPE, St Paul MN, USA) [27,31,32].

*2.2) Experimental groups*

The endodontically treated roots were randomly distributed into 5 groups (n=8). Deionized water was inserted into the root canal and no PS or PDT was administered in the control group. In the remaining 4 groups, the root canals were filled with MB (50 mg/L [$MB_{50}$] or 100 mg/L [$MB_{100}$]) for 3min (period of pre-irradiation) and the PS was slowly agitated for 1 min using an ultrasonic Irrisonic E1 tip (Helse Dental Technology, Santa Rosa de Viterbo, SP, Brazil) coupled to an ultrasonic unit (Nac Plus, Adiel LTDA, São Paulo, SP, Brazil) [31,32]. Care was taken to avoid contact of the tip with the dentin substrate [27,31,32]. In the $MB_{50WL}$

(without Laser light activation) and MB100WL groups, the PS was not activated. In the MB50L (PS activated by Laser light) and MB100L, the PS was activated by 1 min of red Laser light (λ 660 nm) irradiation (Laser Duo, MMO, São Carlos, SP, Brazil), using a fiber optic of 300µm diameter (DMC, São Carlos, SP, Brazil), inserted into the canal to a level 2 mm apical to the teeth working length [27,32]. To ensure homogeneous diffusion of light throughout the canal, the flexible optical fiber was moved in the apico-cervical direction, using helicoidal movements, that were performed 10 times/min [27,31,32].

The wavelength of the light source used was thus determined by the absorption property of the PS, and the duration of light action was determined according to the satisfactory anti-microbial activity reported in previous studies [27,31,32]. Subsequently, 10 mL of deionized water was inserted into the intraradicular canals to remove the MB PS and the root canals were aspirated and then dried with sterile paper points.

Immediately after treatment with MB PS, with or without PDT, the canals were filled with only the MTA based sealer (MTA Fillapex, Angelus, Londrina, PR, Brazil) using #70 McSpadden instruments (Maillefer Instruments, Tulsa, OK, USA) until the complete fill of the specimens confirmed by radiography. The coronal access was then sealed with composite resin (Filtek Z350 XT, 3M ESPE, St Paul MN, USA) and the obturated root canals were stored at 37 °C and 100% humidity for 7 days [31,32].

*2.3) Bonding strength analysis*

The specimens were sectioned with a low-speed diamond saw Isomet 1000 (Buehler, Lake Bluff, IL, USA) under water cooling, to obtain slices of the cervical, middle, and apical thirds. Slice thickness measured using a digital caliper (Mitutoyo, Suzano, SP, Brazil), was approximately 1.3mm [27,31,32]. A universal testing machine (DL 3000, EMIC, Sao José dos Pinhais, PR, Brazil) was used for the push-out test. A compressive load was applied in a vertical direction with an active tip with a cross-head speed of 0.5 mm/min, in the center of the slice. Each third had a different tip diameter due to the taper of the RCS: cervical tip 1,90 mm, middle tip 1,40 mm and apical tip 1,20 mm. The bond strength values were calculated using an arithmetic mean between the different thirds of each dental specimen [27,31,32]. The bond strength values were calculated according to the following equation:

$$Ru = F/A;$$

where the bond strength is $Ru$, maximum force is $F$, and the area of the bonding interface is $A$. $A$ was obtained according to the following equation:

$$A = \pi (R1 + R2) \sqrt{(R1 - R2)^2 + h^2}.$$

where $\pi$ is 3.14, $R1$ and $R2$ are the coronal and apical arc radii of the slice, respectively, and $h$ is the height of the slice measured using a digital caliper (Mitutoyo, Suzano, SP, Brazil) [27,31,32]. The failure mode of the root canals was analyzed using a stereomicroscope of all specimens. Classification of the failure modes was: (1) mixed failure; (2) adhesive failure; (3) cohesive failure in dentin. The most representative failure specimens were submitted to coating with gold (Baltec SCD 050, Balzers, Liechtenstein, Austria) and the fracture patterns were evaluated by scanning electron microscopy (SEM - JSM5600LV, LEOL, Tokyo, Japan) [27,31,32].

*2.4) Confocal Laser Scanning Microscopy*

Fifteen specimens (n=3) were filled with Rhodamine B (FDA, Sigma Chemical Co, San Luis, MI, USA) at a proportion of 0.01% incorporated to the sealer. Prior to obturation the RCS of the specimens were filled with Fluorescein (FDA, Sigma Chemical Co, San Luis, MI, USA) (0.1%) for four hours. Specimens were stored at 100% humidity and 36°C. After 7 days, the roots were transversally cleaved in three slices, as described, at a cut machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water cooling. Slices were assessed from coronal to the apex surfaces with a scanning confocal microscope (Leica Microsystens, Wetzlar, Germany). The emission wavelengths were 545/740 nm. Dentin samples were analyzed using the ×5 lens [33,34]. The imagens obtained were used for a qualitative comparison.

*2.5) Statistical analysis*

Bond strength data were submitted to the Shapiro-Wilk normality test (Sigma Plot 12.0, Systat Software Inc.) which indicated non-standard data. Proceeding the non-parametric Kruskal-Wallis (Sigma Plot 12.0, Systat Software Inc.) test was used for comparison between groups and the Friedman test (Sigma Plot 12.0, Systat Software Inc.) for comparison between thirds ($\alpha$=.05).

**3) RESULTS**

According to Table 2 it can be observed that there was no significant difference between the groups submitted to the action of the PS MB of higher and lower concentration, regardless of the activation or not by the red Laser in relation to the control group, regardless of the intraradicular depth evaluated (P>.05).

However, comparing the root thirds, for the methylene blue group with the higher concentration activated by Red Laser (MB100L), there was significant difference, the apical third presented higher values of bond strength in relation to the middle third (P=.0302). The other groups it can be observed that in general there was no statistically significant difference (P>.05).

When analyzing the thirds for each group, there was a predominance of mixed-type failure in all groups (Fig. 1 and 2). The images from the morphology of the adhesive interface suggested a qualitative lower penetration of the sealer on the MB100L Group compared to the other groups (Fig. 3).

**4) DISCUSSION**

The use of MB as a PS in both concentrations (50 mg/L; 100 mg/L) did not influence the bond strength of the root canal sealer to intraradicular dentin thus, the first null hypothesis of the study was accepted. However, on the MB100L group the apical third presented higher values than the middle third leading to the rejection of the second null hypothesis.

MB is a cationic compound that binds itself to anionic molecules, such as phosphate present in hydroxyapatite and intraradicular dentin, which could influence the calcium/phosphate ratio [25,27,29]. The reaction between this PS could result in precipitates that act as a physical barrier between the root canal sealer and the intraradicular dentin [25-27,35]. This phosphate-PS relationship could explain the lower bond strength values between thirds in the MB100L group (Table 2).

The push-out bond strength analysis showed no statically significant difference between the thirds in any of the evaluated groups, except for the MB100L group, which showed lower bond strength values for the middle than the apical third (Table 2). The fact that the other groups did not have any statistical difference may be because of the use of a lower concentration, due to the fact that the only group that had a degradation of the bond strength was the MB100L group (Table 2) which had the high concentration activated by red laser.

It could be speculated that the cervical and middle thirds would be more susceptible to PS action, and due to the cationicity of the substance, phosphate precipitates were accumulated in the middle third, reducing the interaction between the root canal sealer and the surface of the root dentin [31]. In the apical third the adhesion can be defined as a mechanical connection by mechanical interlocking which could explain the higher bond strength values of the MB100L group in the apical region compared to the middle due to its shape and taper [36].

Furthermore, MB is a hydrophilic compound that causes water sorption, which may potentially deteriorate bond strength according with the findings of Strazzi-Sayhon et al. 2020

[32]. Wainwright et al. 1997 described that water sorption is directly related to the concentration of the PS used [24]. This could explain, in general, the qualitative and visual difference of penetration of the sealer between the $MB_{100L}$ and the control group (Fig. 3).

Different concentrations of the MB PS, with or without PDT, will not always obtain satisfactory results in bond strength analyses [26,27,32]. A recent study has demonstrated that the use of PDT with methylene blue PS at 50 mg/L is a suitable alternative for endodontic treatment, as it does not interfere with the mechanical properties of the bovine tooth or with the bond strength between glass-fiber posts and the intraradicular dentin, at any root depth [32]. Although no improvement in the bond strength values could be evidenced in this study, the use of MB at 50 mg/L, with or without PDT, could be an appropriate alternative to promote antisepsis of the RCS, since it has satisfactory antimicrobial activity, antioxidant and anti-inflammatory effects, as shown in previous studies [16-18].

The MTA based sealer MTA Fillapex contains salicylate resin, solvent resin, nanoparticle silica, bismuth oxide, pigments and MTA (Table 1) [37]. It has high radiopacity, low solubility, a small expansion during setting and also promotes the deposition of mineralized tissue on its surface, according to the manufacturer [37]. The low bond strength values of the MTA Fillapex found in literature can be explained by a weak adhesion of the apatite layer formed between the sealer and intraradicular dentin [38]. Studies have evaluated other characteristics of the MTA Fillapex sealer like Borges et al. (2012) [39] which obtained lower values of solubility than those required by the American Dental Association (ADA), where MTA Fillapex presented the worse values in comparison to the AH Plus, Sealapex and White MTA. Further studies must be encouraged, using other sealers and different analyses studying Physicochemical properties of those to test the influence of PDT using different sources of light to further understand the action and response of this therapy on the obturation process.

The bovine teeth model, selected in this study, is a suitable and acceptable alternative. A study recently published by De Deus et al. 2019 [40] showed that when using an intra-tooth model, bovine teeth did not significantly influence the bond strength of the various sealers tested (AH Plus, MTA Fillapex and BC Sealer) [40].

In addition, some limiting factors of this study should be taken into consideration, such the difficulty of performing the procedures in regions of difficult access and inhomogeneity of the substrate. Thus, future studies are needed and complement each other as discussions around PDT.

## 5) CONCLUSION

Based on the methodology used and the results obtained by the present study, it can be concluded that the use of PDT with the photosensitizer MB at a concentration of 50 mg/L did not alter negatively the bond strength of the MTA Fillapex canal sealer to intraradicular dentin. Therefore, it can be implemented in PDT before obturation as a suitable alternative for the endodontic treatment.

## CONFLICT OF INTEREST

The authors do not have any conflicts of interest related to this study.


## ACKNOWLEDGMENTS

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## Tables

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

**Table 2.** Mean ±standard deviation (MPa) values of extrusion bond strength (push-out strength test) of intraradicular dentin as function of methylene blue in two concentrations used in photodynamic therapy.

## Figure Legends

**Figure 1.** Incidence of fracture patterns (number of thirds of samples) according to the failure type.

**Figure 2.** A,B—Mixed failure of intraradicular dentin irrigated with deionized water solution (control group) (original magnification ×30 and ×150). C,D—Mixed failure of intraradicular dentin after treatment with methylene blue at 50 mg/L (MB50WL ×30 and ×150). E,F—Mixed failure of intraradicular dentin after treatment with methylene blue at 100 mg/L (MB100WL ×30 and ×150). G,H—Mixed failure of intraradicular dentin after treatment with methylene blue at 50 mg/L activated with red Laser (MB50L ×30 and ×150). I,J— Mixed failure of intraradicular dentin after treatment with methylene blue at 100 mg/L activated with red Laser (MB100L ×30 and ×150).

**Figure 3.** 1C,1M,1A—Cervical, middle and apical slices of intraradicular dentin irrigated with deionized water solution after obturation respectively (control group) (original magnification ×5). 2C,2M,2A—Cervical, middle and apical slices of intraradicular dentin after treatment with methylene blue at 50 mg/L and obturation respectively (MB50WL ×5). 3C,3M,3A—Cervical, middle and apical slices of intraradicular dentin after treatment with methylene blue at 100 mg/L and obturation respectively (MB100WL ×5). 4C,4M,4A—Cervical, middle and apical slices of intraradicular dentin after treatment with methylene blue at 50 mg/L activated with red Laser and obturation respectively (MB50L ×5). 5C,5M,5A—Cervical, middle and apical slices of intraradicular dentin after treatment with methylene blue at 100 mg/L activated with red Laser and obturation respectively (MB100L ×5).

**TABLE 1**

Materials, classification, composition, and batch numbers of materials.

| Material | Classification | Composition | Batch |
|---|---|---|---|
| **Filtek Z350XT (3M ESPE)** | Resin Composite | Bis-EMA, Bis-GMA, TEGDMA, UDMA, silica and zirconia nanofillers, and agglomerated zirconia-silica nanoclusters | HB004209993 |
| **MTA Fillapex (Angelus)** | Endodontic Sealer | Salicylate resin, natural resin, diluting resin, bismuth oxide, nanoparticulated silica, MTA and pigments | 36870 |

Bis-EMA, ethoxylated bisphenol A glycol dimethacrylate; Bis-GMA, bisphenol-A diglycidyl ether dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; MTA, mineral trioxide aggregate.

**TABLE 2**

Mean ±standard deviation (MPa) values of bond strength of intraradicular dentin as function of treatments and photodynamic therapy in different root thirds.

| Bond Strengh and Region | Control Group | MB50WL | MB100WL | MB50L | MB100L |
|---|---|---|---|---|---|
| **Cervical** | 1,16 ± 1,25 A a | 1,10 ± 1,91 A a | 2,81 ± 2,95 A a | 1,81 ± 2,59 A a | 1,45 ± 1,77 AB a |
| **Middle** | 0,66 ± 1,01 A a | 0,23 ± 0,29 A a | 1,05 ± 1,11 A a | 1,26 ± 1,77 A a | 0,45 ± 0,62 B a |
| **Apical** | 0,70 ± 0,97 A a | 0,61 ± 0,46 A a | 1,46 ± 1,22 A a | 2,36 ± 2,09 A a | 0,69 ± 0,38 A a |

MB50WL, methylene blue 50 mg/L without red Laser; MB100WL, methylene blue 100 mg/L without red Laser; MB50L, methylene blue 50 mg/L activated with red Laser; MB100L, methylene blue 100 mg/L activated with red Laser.
Different superscript uppercase letters in columns and lowercase letters in rows indicated statistically significant differences (P<.05).

**FIGURE 1**

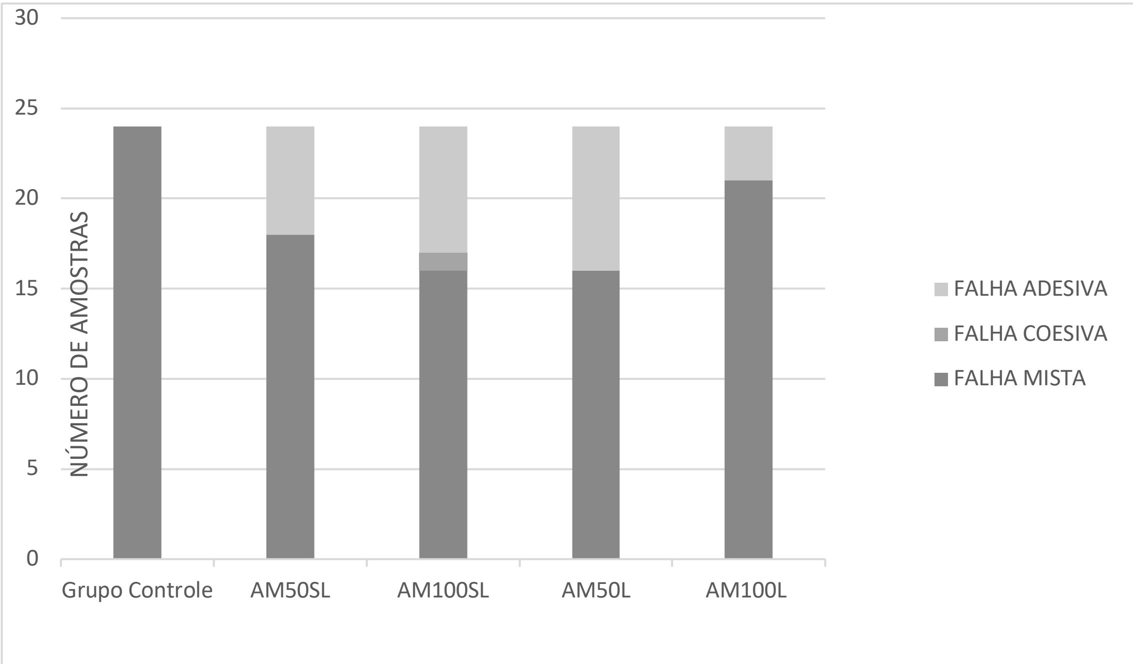

30
25
20
15
10
5
0
NÚMERO DE AMOSTRAS
Grupo Controle
AM50SL
AM100SL
AM50L
AM100L
FALHA ADESIVA
FALHA COESIVA
FALHA MISTA

**FIGURE 2**

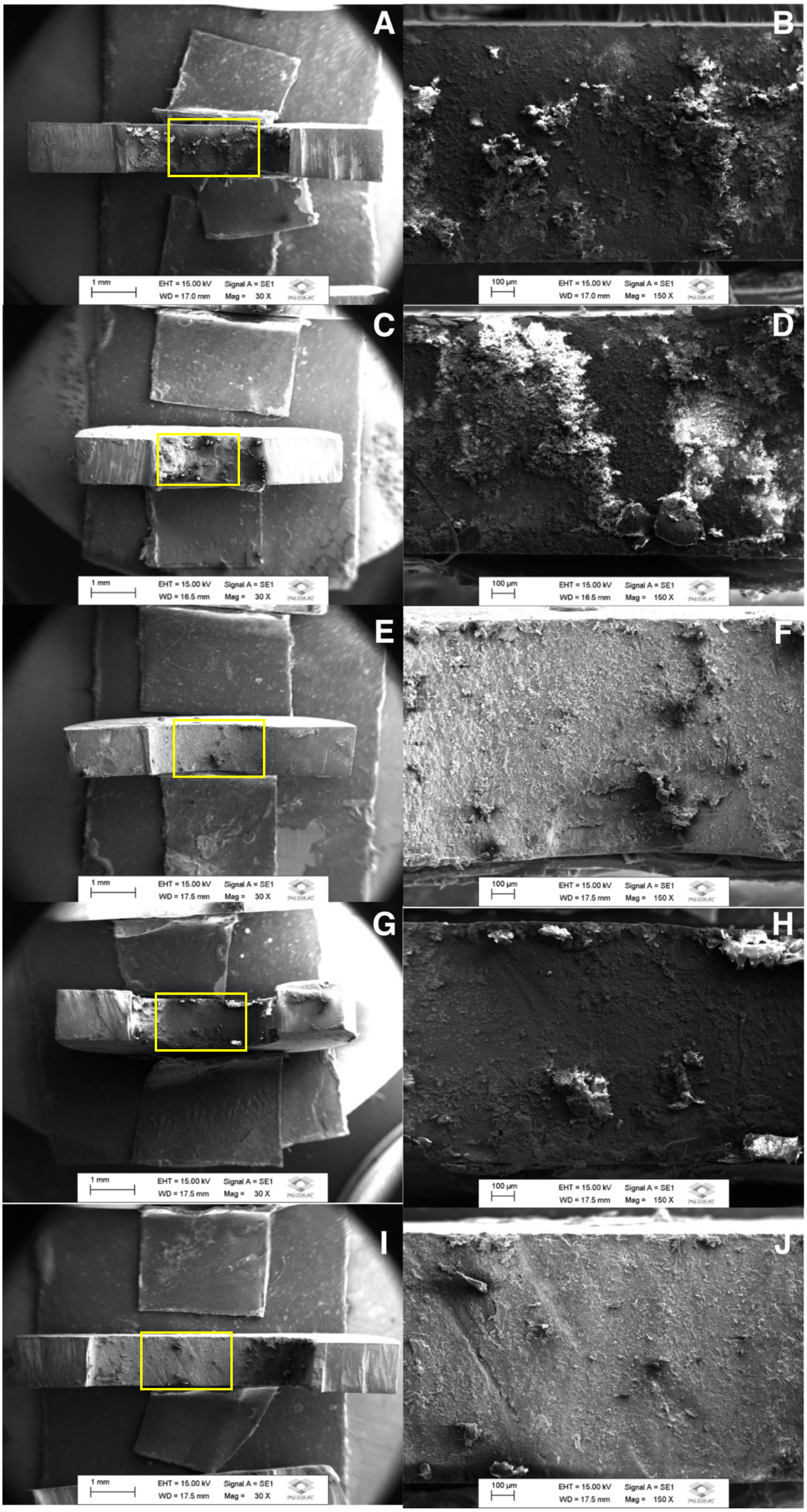
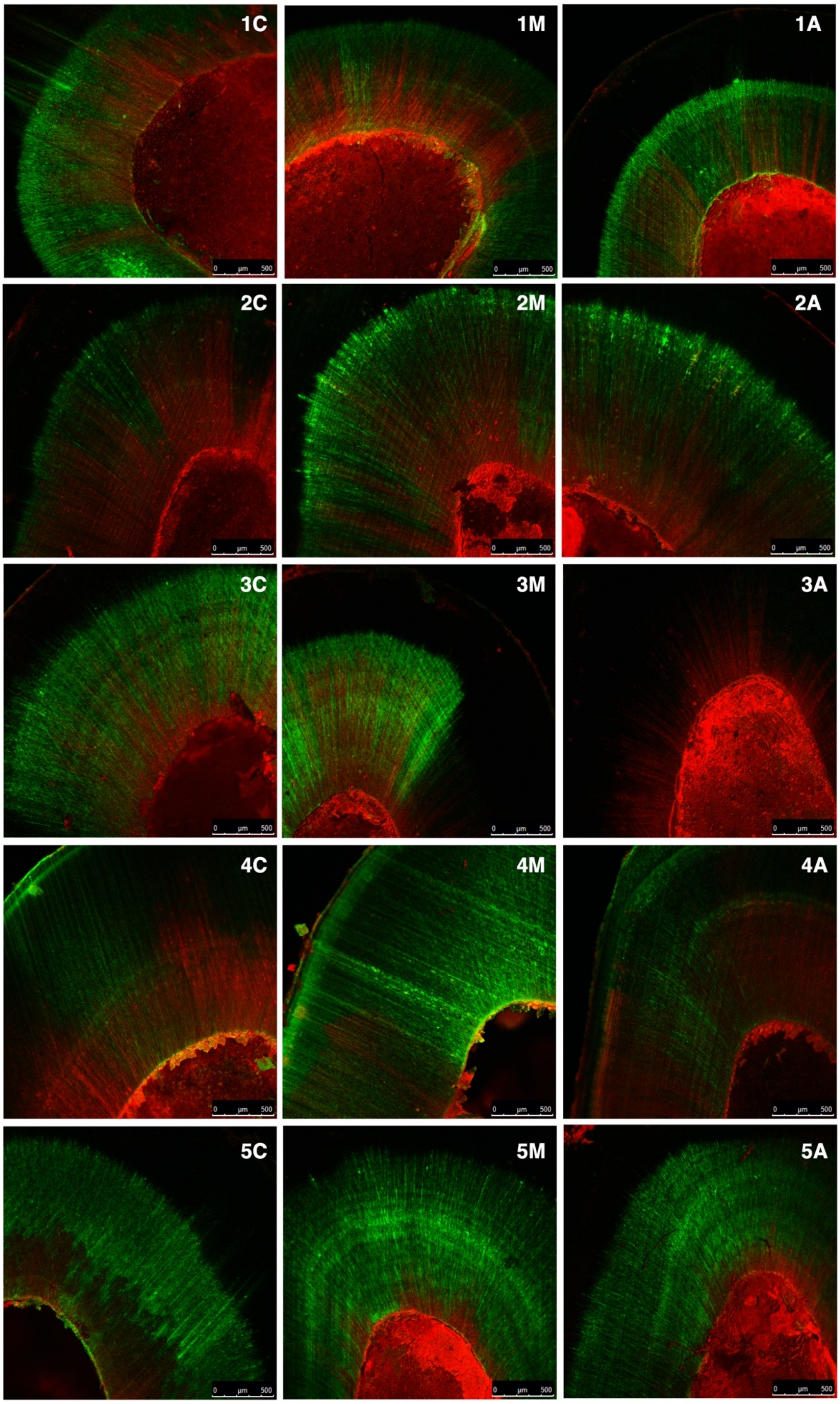

A
B
C
D
E
F
G
H
I
J
1 mm
EHT = 15.00 kV
Signal A = SE1
Mag = 30 X
100 µm
Mag = 150 X

**FIGURE 3**



**ATTACHMENT 1**

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 na resistência de união do cimento obturador a dentina intrarradicular"**, Processo FOA nº 00611-2019, 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 08 de Outubro de 2019.

**VALIDADE DESTE CERTIFICADO:** 08 de Dezembro de 2020.
**DATA DA SUBMISSÃO DO RELATÓRIO FINAL:** até 08 de Janeiro de 2021.

## CERTIFICATE

We certify that the study entitled **"Influence of photodynamic therapy on the bond strengh of the filling material to intraradicular dentin"**, Protocol FOA nº 00611-2019, 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 October 08, 2019.

**VALIDITY OF THIS CERTIFICATE:** December 08, 2020.
**DATE OF SUBMISSION OF THE FINAL REPORT:** January 08, 2021.

**Profa. Associada Maria Cristina Rosifini Alves Rezende**
Coordenador da CEUA
CEUA Coordinator

**CEUA - Comissão de Ética no Uso de Animais**
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