

RESEARCH AND EDUCATION

Effect of photodynamic therapy on the mechanical properties and bond strength of glass-fiber posts to endodontically treated intraradicular dentin



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Endodontically treated teeth with excessive loss of coronal structure are commonly restored with glass-fiber posts.¹⁻³ Disinfection of the root canal systems and a long-lasting restoration are essential goals for successful treatment. Microbial reduction is typically achieved with biomechanical instrumentation of the root canal,⁴ sodium hypochlorite irrigating solution,⁵⁻⁷ and calcium hydroxide intracanal medication.^{8,9} However, these procedures cannot ensure the total eradication of bacteria and their toxic products from the root canal.¹⁰⁻¹² Consequently, therapeutic approaches, including photodynamic therapy (PDT), have been introduced to reduce endodontic infections.

PDT involves the absorption of photons by photosensitizers

ABSTRACT

Statement of problem. The use of photosensitizers in photodynamic therapy promotes intraradicular microbial reduction during nonsurgical endodontic therapy. However, studies are lacking on the consequences of the application of these agents on the mechanical properties of intraradicular dentin and on the bond strength of glass-fiber posts.

Purpose. The purpose of this in vitro study was to evaluate the influence of photodynamic therapy on the bond strength of glass-fiber posts using a push-out test and, additionally, to measure the Martens hardness (MH) and elastic indentation modulus (Eit) of intraradicular dentin when different photosensitizers are used.

Material and methods. Eighty 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 + red laser; methylene blue 100 mg/L + red laser; curcumin 500 mg/L + blue LED; and curcumin 1000 mg/L + blue LED. The MH and Eit of intraradicular dentin were measured using an ultramicrohardness tester under a load of 3 mN (n=8). The push-out bond strength of glass-fiber posts to dentin was measured using a universal testing machine (n=8). Mechanical properties and bond strength data were subjected to the Kruskal–Wallis test, ANOVA, and Fisher least significant difference test ($\alpha=.05$). Images of representative specimens were obtained using a scanning electron microscope.

Results. The MH, Eit, and bond strength of intraradicular dentin were influenced by the photosensitizer used. In general, curcumin promoted lower mechanical properties values but higher bond strength values.

Conclusions. Photosensitizers influenced the mechanical properties of intraradicular dentin and the bond strength of glass-fiber posts, and methylene blue at 50 mg/L had no marked effect on the mechanical properties of the dentin or the bond strength values. (J Prosthet Dent 2018;120:317.e1-e7)

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Clinical Implications

The photodynamic therapy associated with methylene blue is a viable clinical procedure for the disinfection of root canals before the luting of glass-fiber posts to intraradicular dentin.

(PSs) from a light source,¹³ allowing the stimulation of electrons from the normal state to an excited phase. In the presence of oxygen, the PSs transfer this energy to the substrate. The return to its ground state promotes formation of highly reactive and short-lived species, related to singlet oxygen, affecting only the target cells and microorganisms through irreversible oxidation.¹⁴⁻¹⁶

However, the effects of PDT on the mechanical properties of dentin and its possible effect on adhesive restorative procedures are unclear. Therefore, the purpose of this *in vitro* study was to evaluate the mechanical properties (Martens hardness [MH] and elastic indentation modulus [Eit]) of intraradicular dentin as well as to determine the push-out bond strength of glass-fiber posts to dentin after PDT with curcumin and methylene blue PSs. The null hypothesis was that different PSs would not cause changes in the mechanical properties of dentin and the bond strength of glass-fiber posts to endodontically treated intraradicular dentin.

MATERIAL AND METHODS

The materials used in this project are listed in [Table 1](#). The study was approved by the ethics committee of the Araçatuba School of Dentistry, São Paulo State University (15-00688). Eighty bovine teeth were used in this study. Teeth that exhibited cracks or fractures were excluded. A low-speed diamond saw (IsoMet 5000; Buehler) was used to remove the anatomic crowns 1.0 mm coronal to the cementum-enamel junction. The working length was established to be 1.0 mm less than this length. The specimens were then endodontically treated. The root canals were instrumented using K-Files #80 (Dentsply Sirona), irrigated with 1% sodium hypochlorite, and subsequently dried with a jet of air and sterile paper points. The apical foramen was sealed to prevent escape of the PS. The apices were conditioned with 37% phosphoric acid (FGM) for 15 seconds, washed, and dried with a jet of air and sterile paper points. Two layers of adhesive (Adper Single Bond 2; 3M ESPE) were applied for 15 seconds to the etched surface, and the material was then polymerized for 20 seconds with a light-polymerization unit (Ultraled; Dabi Atlante). Finally, the apical foramen was sealed with composite resin (Filtek Z250 XT; 3M ESPE) using a Thompson spatula, followed by photoactivation for 40 seconds. The specimens were distributed into 5 experimental groups (n=16).

In the control group, the root canal was filled with deionized water and did not receive any type of PS or PDT. In the MB₅₀ and MB₁₀₀ groups, the root canals were filled with methylene blue (50 mg/L and 100 mg/L) for 3 minutes (period of preirradiation).¹⁷ In the C₅₀₀ and C₁₀₀₀ groups, the root canals were filled with curcumin (500 mg/L and 1000 mg/L) for 5 minutes.¹⁷ In all 4 groups (MB₅₀, MB₁₀₀, C₅₀₀, and C₁₀₀₀), the respective PS was carefully shaken for 1 minute in an ultrasonic unit (Nac Plus; Adiel), avoiding contact of the tip with the wall of the root canal.

In the MB₅₀ and MB₁₀₀ groups, the PS was activated by red laser (Laser Duo; MMO) of λ 660 nm for 1 minute with a flexible fiber optic of 300- μ m diameter, 2 mm apical to the working length.¹⁷ In the C₅₀₀ and C₁₀₀₀ groups, the PS was activated by blue LED of λ 480 nm for 4 minutes. Helicoidal movements were performed in the apicocervical direction with the optical fiber to ensure uniform diffusion of light throughout the root canal.^{18,19} The movements were repeated approximately 10 times/min.²⁰

The choice of light wavelength is directly related to the absorption characteristics of the PS used. The times of application of each light source (1 minute for laser and 4 minutes for blue LED) were chosen as the amount of time that demonstrated satisfactory antimicrobial activity according to the literature.^{13,19} In addition, MB and curcumin PSs present absorption peaks at 630 nm and 450 to 495 nm, with a final energy of 72 J/cm².^{13,14,19}

Immediately after the PDT had been performed, the root canals were irrigated with 10-mL deionized water to remove the PS and were subsequently dried with a jet of air and sterile paper points. All the teeth were stored at 100% relative humidity and 37°C for 7 days.^{21,22}

Cuts were made perpendicular to the long axis of the specimens (n=8 per group) using a low-speed diamond saw (IsoMet 5000; Buehler) under water cooling. Approximately 1.3-mm-thick slices from each of the three regions (cervical, middle, and apical) were fixed in acrylic resin (Classico). Slices were manually finalized using #320, #600, #800, and #1200 grit silicon carbide paper (Extex Corp) and polished using diamond pastes (6, 3, and 1 μ m; Buehler) for 4 minutes per phase. All the specimens were cleaned for 2 minutes in an ultrasonic unit (model 2210; Branson Ultrasonic Corp) containing deionized water between each silicon carbide paper and diamond paste polishing step and at the end of the process.^{23,24}

An ultramicrohardness tester (DUH-211; Shimadzu) was used to verify the MH and Eit under a load of 3 mN at a speed of 0.2926 mN/s with a holding time of 5 seconds. A Vickers diamond tip was used, and 5 indentations were made on the dentin.²³⁻²⁵ The MH and Eit values were calculated automatically by the software in the hardness tester, and the arithmetic mean between the different thirds was calculated for each specimen. The

Table 1. Materials, classification, composition, batch number of materials, and manufacturer

Material	Classification	Composition	Batch	Manufacturer
Filtek Z350 XT	Composite resin	Bis-GMA, Bis-EMA, UDMA, TEGDMA, silica and zirconia nanofillers, and agglomerated zirconia-silica nanoclusters.	HB004209993	3M ESPE
Sealer 26	Endodontic cement	Powder: bismuth trioxide, calcium hydroxide, hexamethylenetetramine, titanium dioxide. Liquid: bisphenol epoxy	260561	Dentsply Sirona
RelyX Ceramic Primer	Silane	3-MPS, ethyl alcohol, water.	H0001504424	3M ESPE
RelyX U200	Resin cement	Base: glass fiber, methacrylate phosphoric acid esters, triethylene glycol dimethacrylate, silane-treated silica, sodium persulfate. Catalyst: glass fiber, substitute dimethacrylate, silane-treated silica, sodium p-toluenesulfonate, calcium.	1518200189	3M ESPE

3-MPS, 3-methacryloxypropyl-trimethoxy silane; Bis-EMA, ethoxylated bisphenol-A glycol dimethacrylate; Bis-GMA, bisphenol-A diglycidyl ether dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

MH was established as the maximum load (P max) divided by the contact surface area (A):

$$MH = \frac{P \text{ max}}{A}$$

The Eit was measured according to the following equation:

$$\frac{1}{Er} = \frac{(1-v^2)}{Eit^*} + \frac{(1-vi^2)}{Ei}$$

where the Poisson coefficient of the specimen and the indenter are represented by v and vi, respectively, and the elastic indentation modulus of the indenter by Ei. The following equation was used to calculate the reduced modulus (Er):

$$Er = \frac{\sqrt{\pi}}{2\sqrt{A}} S,$$

where the designed area for contact printing is represented by A, and the stiffness of the material obtained from the slope of the unloading curve is represented by S; π is 3.14.

Eight specimens per group were used for push-out bond strength analysis. After PDT, the root canals were obturated by lateral condensation of gutta percha cones (Dentsply Sirona) and calcium hydroxide cement (Sealer 26; Dentsply Sirona). Coronal access was sealed with interim cement (White Cimpat; Septodont). The obturated teeth were stored at 100% relative humidity and 37°C for 7 days.

The glass-fiber post system used was WhitePost DCE #2 (FGM). For luting the glass-fiber posts, the post spaces in all the specimens were prepared with a #1 low-speed drill (Dentsply Sirona). A depth of ±9 mm with reference to the working length of the teeth was standardized to remove the gutta percha cones. For root canal standardization, the drill corresponding to glass-fiber post #2 was used. After removal of the gutta percha, the glass-fiber posts were placed in the post space to assess the adaptation of the posts. Before the adhesion process, the surfaces of the glass-fiber posts were etched with 35% phosphoric acid (3M

ESPE) for 60 seconds. The acid was removed from the glass-fiber posts by washing, and the posts were dried. A silane primer (RelyX Ceramic Primer; 3M ESPE) was applied to the surfaces of the posts for 60 seconds. Thereafter, a jet of air was gently applied to dry the glass-fiber posts, and the posts were not further manipulated to prevent contamination.²⁴

The canals were irrigated with physiological saline (0.9%) to remove any residual gutta percha. Then, a jet of air and sterile paper points were used to dry the post spaces before luting with a resin cement (RelyX U200; 3M ESPE) and polymerizing for 40 seconds from the occlusal surface using a light-polymerization unit (Ultraled; Dabi Atlante) with a light intensity of 1125 mW/cm².²⁶ After luting the fiber posts, the coronal access was sealed with composite resin (Filtek Z350 XT; 3M ESPE), and the specimens were stored at 37°C and 100% relative humidity for 7 days. To obtain slices of approximately 1.3-mm thickness, the experimental specimens were cut perpendicular to the long axis of the root with a low-speed diamond saw (Isomet 5000; Buehler) under water cooling.^{13,24} The slice thickness, which was used in the bond strength value calculations, was measured using digital calipers (Mitutoyo).

For the push-out test, a universal testing machine was used (DL 3000; EMIC). The active tip coincided with the center of the fiber post, applying a compressive load in a vertical direction with a speed of 0.5 mm/min.^{22,27-29} The bond strength values were calculated using an arithmetic mean between the different thirds of each dental specimen. The bond strength values were calculated according to the following equation³⁰:

$$Ru = F/A,$$

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 equation³⁰:

$$A = \pi(R_1 + R_2) \sqrt{(R_1 - R_2)^2 + h^2},$$

where π is 3.14, R₁ and R₂ are the coronal and apical arc radii of the post segment, respectively, and h is the height of the post measured using digital calipers.

Table 2. Mean \pm standard deviation (GPa) values of Martens hardness (MH) and elastic indentation modulus (Eit) of intraradicular dentin as a function of photosensitizer agents used in photodynamic therapy

Group	MH	Eit
Control	0.19 \pm 0.17 ^A	3.46 \pm 3.70 ^A
MB ₅₀	0.11 \pm 0.03 ^{AB}	2.89 \pm 2.23 ^A
MB ₁₀₀	0.10 \pm 0.02 ^{AB}	2.28 \pm 0.48 ^A
C ₅₀₀	0.08 \pm 0.01 ^B	1.67 \pm 0.29 ^{AB}
C ₁₀₀₀	0.05 \pm 0.01 ^C	1.27 \pm 0.25 ^B

C₅₀₀, curcumin 500 mg/L; C₁₀₀₀, curcumin 1000 mg/L; MB₅₀, methylene blue 50 mg/L; MB₁₀₀, methylene blue 100 mg/L. Different superscript letters indicate statistically significant differences for each mechanical property analyzed ($P < .05$).

A stereomicroscope was used at magnifications $\times 6$ and $\times 66$ to analyze the failure mode of the specimens. Failure modes were classified into 3 groups: (1) adhesive failure, (2) cohesive failure in dentin, and (3) mixed failure. Representative specimens were subjected to sputter coating with gold (BAL-TEC SCD 050; Balzers) and analyzed using scanning electron microscopy (SEM-JSM5600LV; JEOL) to demonstrate the patterns of fracture. ^{31,32} Data were submitted to a normality test (Shapiro-Wilk test). Mechanical properties data were submitted to the nonparametric Kruskal-Wallis test for comparison between the groups ($\alpha = .05$). Bond strength data were submitted to 1-way ANOVA and the Fisher least significant difference test ($\alpha = .05$).

RESULTS

The Kruskal-Wallis test of the mechanical properties indicated higher hardness values (MH) for the control group than the curcumin groups at both concentrations (C₅₀₀ and C₁₀₀₀) ($P < .05$). No statistically significant difference was found among the control group and the methylene blue groups at either concentration (MB₅₀ and MB₁₀₀) ($P > .05$). The effect of the application of the different PSs in the PDT was also supported by the Eit values, in which the C₁₀₀₀ group showed fewer Eit values than the MB₅₀, MB₁₀₀, and control groups ($P = .001$) (Table 2).

The results of ANOVA for push-out bond strength values indicated statistically significant differences among the PSs used ($P < .001$). Table 3 shows that the push-out bond strength values for the C₅₀₀ and C₁₀₀₀ groups were statistically significantly higher than those of the control and MB₁₀₀ groups ($P < .05$). A predominance of mixed-type failure was found in the C₁₀₀₀ and control groups, whereas in the C₅₀₀ and MB₁₀₀ groups, cohesive-type failure predominated (Fig. 1). Representative SEM images are presented in Figure 2.

DISCUSSION

The use of different PSs for PDT influenced the mechanical properties of intraradicular dentin, as well as the bond strength of glass-fiber posts to dentin (Tables 2, 3);

Table 3. Mean \pm standard deviation (MPa) values of extrusion bond strength (push-out strength test) of intraradicular dentin as function of photosensitizer agents used in photodynamic therapy

Control Group	MB ₅₀ Group	MB ₁₀₀ Group	C ₅₀₀ Group	C ₁₀₀₀ Group
1.64 \pm 0.96 ^B	1.71 \pm 0.85 ^{AB}	0.67 \pm 0.51 ^B	3.28 \pm 1.29 ^A	3.20 \pm 1.14 ^A

C₅₀₀, curcumin 500 mg/L; C₁₀₀₀, curcumin 1000 mg/L; MB₅₀, methylene blue 50 mg/L; MB₁₀₀, methylene blue 100 mg/L. Different letters indicate statistically significant differences ($P < .05$).

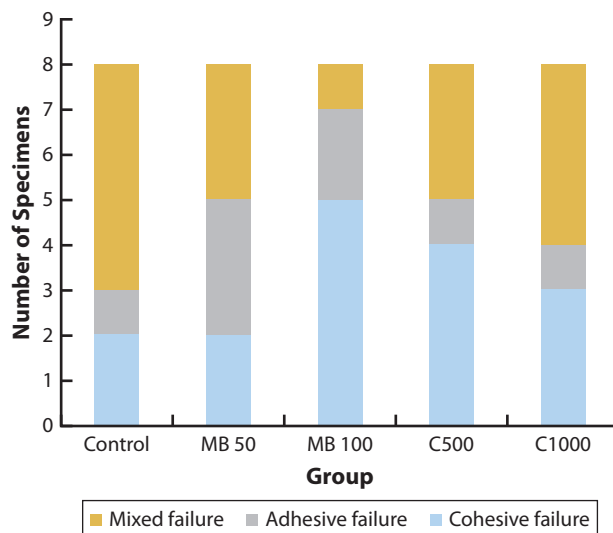


Figure 1. Incidence of fracture patterns (specimen numbers) according to type of failure. C₅₀₀, curcumin 500 mg/L; C₁₀₀₀, curcumin 1000 mg/L; MB₅₀, methylene blue 50 mg/L; MB₁₀₀, methylene blue 100 mg/L.

thus, the null hypothesis of the study was rejected. Although these PSs are commonly used at these concentrations in clinical practice, the authors are unaware of previous studies that have analyzed the effects of these PSs on intraradicular dentin after PDT. The PS used must not alter the mechanical properties of the dentin substrate during PDT because such alterations can influence the behavior of the dentin-restoration interface and reduce the fracture resistance of the root. ^{3,7}

The curcumin PS promoted significant surface changes in the dentin substrate; therefore, its photoactivation did not lead to formation of singlet oxygen (Table 2). However, the photoactivation of curcumin promotes hydrogen peroxide formation. ^{33,34} Some studies have reported that hydrogen peroxide formation is less cytotoxic than the formation of singlet oxygen, but its period of action is relatively longer, ^{33,34} supporting the results of this study.

The lowest MH and Eit values for intraradicular dentin were noticed for the groups in which curcumin was used as PS, at either concentration and at the higher concentration (Table 2). The products synthesized in the photodynamic action of this PS are anions of superoxide and hydrogen peroxide, as previously reported. ^{33,34} These anionic components are able to bind to cationic

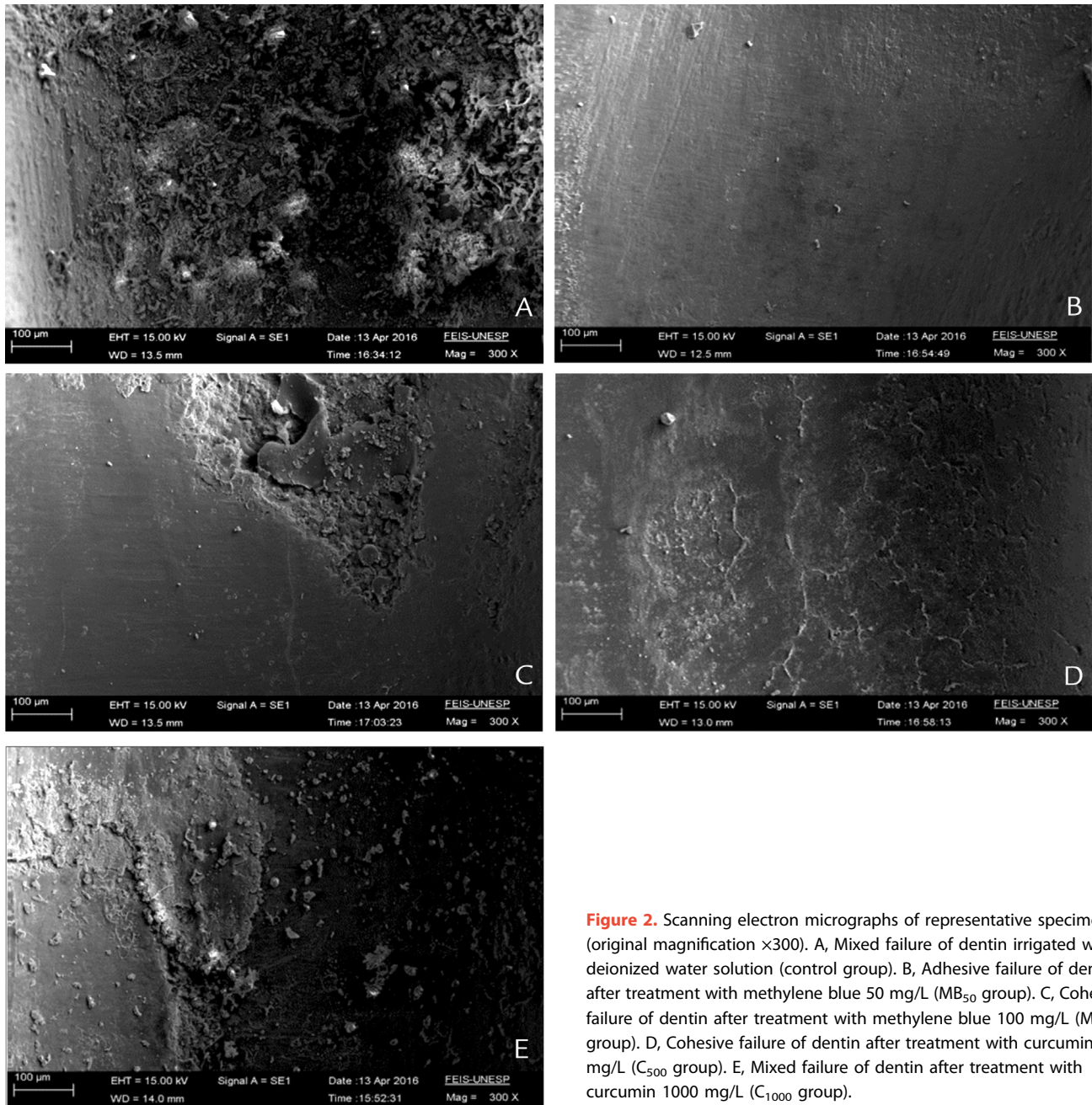


Figure 2. Scanning electron micrographs of representative specimens (original magnification $\times 300$). A, Mixed failure of dentin irrigated with deionized water solution (control group). B, Adhesive failure of dentin after treatment with methylene blue 50 mg/L (MB₅₀ group). C, Cohesive failure of dentin after treatment with methylene blue 100 mg/L (MB₁₀₀ group). D, Cohesive failure of dentin after treatment with curcumin 500 mg/L (C₅₀₀ group). E, Mixed failure of dentin after treatment with curcumin 1000 mg/L (C₁₀₀₀ group).

molecules, such as the calcium present in the hydroxyapatite crystals.¹² Considering that calcium is present in the calcium carbonate complex of intraradicular dentin, curcumin could induce alterations in the relation, of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, explaining the lower Eit values of dentin submitted to PDT with this PS.

Methylene blue is a cationic substance that binds to anionic molecules, such as the phosphate present in hydroxyapatite, thereby influencing the calcium/phosphate ratio. The reaction between this PS and phosphate would result in precipitates acting as a physical barrier

and reducing the interaction between the resin cement and the surface of the dentin.³² This phosphate-PS relationship could explain the lower bond strength values of the MB₁₀₀ group than those of the C₅₀₀ and C₁₀₀₀ groups. When the scanning electron microscopy images of the MB₅₀ and MB₁₀₀ groups were compared (Fig. 2B, 2C), a different fracture pattern could be seen, showing that the group exposed to the lowest concentration had a higher incidence of adhesive failures, whereas the group exposed to the highest concentration had predominantly cohesive-type failures (Fig. 1).

Methylene blue is a hydrophilic compound that allows water sorption to deteriorate the bond strength.³³ According to a study by Wainright et al,¹³ the intensity of water sorption is directly related to the concentration of the PS used, corroborating the results found in this study (Table 3). However, curcumin is a hydrophobic polyphenol, which is poorly soluble in water,³⁵ explaining the highest values of bond strength for this PS (Table 3). In addition, the resin cement used in this study has a hydrophobic character,²⁶ enhancing the integrity of the bond strength of the glass-fiber posts to the intraradicular dentin. The integrity of the adhesive interface can be seen in the image of the C₁₀₀₀ group (Fig. 2E), which showed a higher incidence of mixed failures (Fig. 1).

The higher values of bond strength obtained with curcumin as PS can also be explained by the anionicity of the component, which promotes changes in the calcium complex of the dentin substrate.^{33,34} Thus, the flow of the resin cement may have interacted with the changed surface of the intraradicular dentin, promoting micro-mechanical retention and consequently resulting in higher bond strength values in the push-out test.

However, different PSs, at various concentrations, will not always obtain satisfactory results in both the analyses. The use of methylene blue at 50 mg/L is an appropriate choice as a PS, as it promoted satisfactory mechanical properties of the dentin substrate and bond strength of the adhesive interface.

The study limitations include the use of bovine teeth, difficulty of standardizing the preparation in areas with difficult access, and nonhomogeneity of the substrate. Additional studies should investigate PDT further and should seek to identify different types and concentrations of PSs, different types of lasers and LED for use in PDT, and the time and technique used for the application of the PSs.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1. MH, Eit, and bond strength of intraradicular dentin were influenced by the PS used for PDT.
2. Methylene blue at a concentration of 50 mg/L is a suitable alternative for endodontic treatment involving PDT, as it does not alter the mechanical properties or bond strength of the intraradicular dentin and could therefore be implemented in PDT before luting the glass-fiber posts.

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