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ORIGINAL ARTICLE

Influence of photosensitizing agent and number of photodynamic therapy sessions on resistance of fiberglass posts to displacement within the canal

ABSTRACT

Aim: *To evaluate the influence of the type of photosensitizing agent and the number of photodynamic therapy (PDT) sessions on the resistance of cemented fiberglass posts to displacement within the root canal.*
Methodology: *Fifty bovine primary incisors were randomly divided into five experimental groups according to the type of photosensitizing agent and to the number of PDT sessions: CG without PDT (control); GF1M one PDT session with methylene blue; GF2M two PDT sessions with methylene blue; GF1T one PDT session with toluidine blue; and GF2T two PDT sessions with toluidine blue. Exacto® fiberglass posts were cemented with RelyX U200® in the root canal and kept for 15 days in distilled water. The specimens were sectioned with an average thickness of 1.56 mm at the cervical, middle, and apical root thirds and subjected to the push-out test. After the test, the fractured specimens were analyzed under a stereomicroscope to determine the fracture pattern. The data obtained were treated by one-way ANOVA (α=0.05).*
Results: *There was no statistical difference in the comparison of the proposed treatments and the analyzed root thirds (P>0.05).*
Conclusions: *The type of photosensitizing agent used and the number of PDT sessions do not influence the resistance of cemented intraradicular fiberglass posts to displacement.*

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Introduction

Two objectives are essential for achieving clinical success in endodontic treatment: control over root canal disinfection and placement of long-lasting restorations. The correct planning of the restorative treatment has provided high survival and restoration success rates of approximately 85% (1, 2). According to the degree of impairment of the dental crown structure, fiberglass posts have shown to be commonly used in direct restorative treatment, providing adequate support and retention for the restorations (3). However, the success of this procedure depends on dentin morphology, on the materials used during endodontic treatment, and on the adhesive cementation of intraradicular posts. Most of the failures occur in the adhesion between the root canal walls and the resin cement (4).

An attempt is usually made at eradicating bacteria from the root canal using chemomechanical preparation (5) associated with intracanal medication (6). However, these procedures cannot guarantee complete disinfection since the complex anatomy of the root canal system and the organization of microorganisms in highly complex biofilms contribute a lot to the persistence of the infectious process, with regions not accessible to instrumentation and irrigation (7). Thus, changes in therapeutic approaches with the associated use of other auxiliary resources, e.g. photodynamic therapy (PDT), have been tested to improve the treatment of endodontic infections.

PDT is performed with the aid of a low power laser at a wavelength between 630 and 980 nm and of a non-toxic photosensitizing agent that can eliminate endodontic pathogens through the formation of reactive oxygen species (8). Photosensitizers are heterocyclic light-absorbing molecules. They must have a resonant absorption band with the wavelength of the light source to be used (9). Photosensitizers derived from phenothiazines are the most widely used in PDT (10). Phenothiazines are tricyclic heteroaromatic compounds, such as toluidine blue and methylene blue. Photosensitizing agents absorb photons from the radiation source and conduct their electrons to an excitatory state. In the presence of oxygen, the energy transfer from the photosensitizing agent generates reactive oxygen molecules, such as singlet oxygen and free radicals, which cab damage cellular components such as lipids and nucleic acids through irreversible oxidation, causing bacterial death (11). PDT has shown to be a promising auxiliary resource for eradication of oral pathogenic bacteria that cause endodontic diseases, and periodontitis (12). On the other hand, as photosensitizing agents are viscous substances used in aqueous solutions, they can adhere to the root canal walls and dentinal tubules, forming a chemical smear layer, as described by Souza et al (13), which could influence the bond strength of intraradicular posts. It creates a favorable environment for microbial microleakage and inadequate adhesion of the root filling material to root canal dentin (14). According to Lima et al (15) and Akman et al (16), the photosensitizing chemical agents present a negative effect on the hybrid layer formation and on the adhesive interface between the fiber post cementation system and root dentin.

Therefore, the present study aims to assess whether the type of photosensitizing agent and the number of PDT sessions influences the resistance of cemented intraradicular fiberglass posts to displacement. The null hypothesis is that photosensitizers and the number of PDT sessions do not cause changes in the bond strength of fiberglass posts within the intraradicular dentin.

Materials and Methods

Sample selection and preparation

Fifty primary bovine incisors were selected and standardized to the initial apical diameter of the root canal, equivalent to a K-type #20 endodontic instrument (Dentsply/Maillefer Instruments S.A., Ballaigues, Switzerland). The root canals had circular sections. After cleaning, the dental crowns were sectioned at the cementoenamel junction with the aid of a low-speed silicon

Table 1
Experimental groups

Group	n	Photodynamic therapy
GC	10	Without PDT
GF1M	10	One PDT session+methylene blue
GF2M	10	Two PDT sessions+methylene blue
GF1T	10	One PDT session+toluidine blue
GF2T	10	Two PDT sessions+toluidine blue

carbide disc. The length of the root remnant was standardized to 17 mm and the working length (WL) was 1 mm below this standardized measurement (WL=16 mm). The apical foramina were previously sealed with composite resin to prevent leakage of the irrigating and photosensitizing agents. The restorative procedure was performed with the use of 37% phosphoric acid (FGM, Joinville, SC, Brazil) and Single Bond Universal® adhesive system (3M ESPE, St Paul, MN, USA), with later placement of Filtek Z250® composite resin (3M ESPE, St Paul, MN, USA).

Experimental groups

The teeth were divided into five experimental groups (Table 1) by the simple random sampling using Excel (Microsoft Excel, Microsoft, USA).

Endodontic preparation of samples

All samples were prepared manually with first and second series K-type stainless steel endodontic instruments (Dentsply/Maillefer Instruments S.A., Ballaigues, Switzerland). Chemomechanical preparation was carried out in the following sequence of K-type instruments: #20, #25, #30, #35, #40, and #45 (Dentsply/Maillefer Instruments S.A., Ballaigues, Switzerland). All instruments were used along the WL. At each instrument change, the canals were irrigated with the aid of a plastic syringe (BD Solumed, São Paulo, SP, Brazil) and 25 mm 30-gauge NaviTip needles (Ultradent, Indaiatuba, SP, Brazil), containing 2.5% sodium hypochlorite (Iodontec Indústria e Comércio de Produtos Odontológicas Ltda., Porto Alegre, RS, Brazil) in a standard amount of 2 mL.

After the preparation, the final toilet was made with 17% trisodium EDTA (Biodinamica, Ibiporã, PR, Brazil) for three minutes and with agitation of #45 instrument. The canals were then washed with distilled water (Iodontosul, Industrial Odontológica do Sul LTDA, Porto Alegre, RS, Brazil) and dried with absorbent paper points (Tanari Indústria Ltda., Manaus, AM, Brazil).

For the endodontic filling, the canals were filled with gutta-percha cones and AH Plus® epoxy resin-based cement (Dentsply/Maillefer Instruments SA, Ballaigues, Switzerland), using Tagger's hybrid technique and #60 McSpadden® compactor (Dentsply/Maillefer Instruments SA, Ballaigues, Switzerland).

After filling, all samples were provisionally restored with Cimpat® restorative material (Septodont, Saint Maur des Fosses, France) and immersed for two days in a flask containing distilled water, at 37 °C and 100% relative humidity, for complete setting of the endodontic sealer.

After that, the canals were cleared to prepare the space needed for the post to be cemented. The root canal filling was removed along 13 mm with the bur provided with the post kit and which corresponds to the diameter of the used post, leaving 3 mm of apical sealing.

Photodynamic therapy (PDT)

A flexible optical fiber with a diameter of 500 μm (MMOptics Ltda., São Carlos, SP, Brazil) coupled to the Duo® laser device (MMOptics Ltda., São Carlos, SP, Brazil) was used for PDT.

Initially, the dry canals were filled with the photosensitizing agent. The photosensitizing agents used were aqueous solutions of 0.01% methylene blue (Sigma-Aldrich, Sigma-Aldrich Brazil, SP, Brazil), 0.1 mg/mL, (Groups GF1M and GF2M) and toluidine blue (Sigma-Aldrich, Sigma-Aldrich Brazil, SP, Brazil) at 0.01%, 0.1 mg/mL, (Groups GF1T and GF2T). The teeth were filled with the photosensitizing agent, which was kept in the canal for 5 minutes (pre-irradiation period).

After the pre-irradiation period, the photosensitizing agent was activated by red

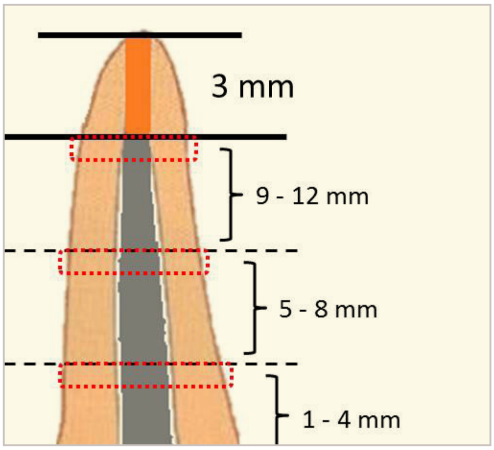

Figure 1
Schematic diagram of root slices.

visible light at 660 nm, 18 J of energy, for 3 minutes, with the aid of a flexible optical fiber, advanced 2 mm below the WL. The fiber was introduced in helical movements in the apical-cervical direction for uniform diffusion of light along the root canal length. The movements were repeated approximately 10 times/minute. Immediately after PDT, the root canals were finally irrigated with 10 mL of deionized water to remove the photosensitizing agent and subsequently dried with an aspiration cannula and absorbent paper points. In groups GF1M and GF1T, PDT was performed in a single session. This session took place after the final rinse and before the endodontic filling procedure. In specimens from groups GF2M and GF2T, PDT was performed in two sessions. The second session was carried out after unblocking and preparing the canal for post cementation.

Post cementation and specimen preparation

After the canals were cleared, the placement of Exacto® #1 or 2 fiberglass posts (Angelus, Londrina, PR, Brazil) followed the cementation protocol and the manufacturer's instructions. The posts were disinfected with 70% alcohol (Icarai, São Paulo, SP, Brazil) prior to use and subsequently dried. Single Bond Universal® adhesive was applied for 20 seconds and then dried with air jets for 5 seconds. The posts were luted with self-adhesive cement (RelyX U200®, 3M ESPE, St. Paul, MN, USA). The resin cement was applied to the root canal with the aid of a centrix syringe (DFL, Rio de Janeiro, RJ, Brazil) with a fine metal tip. The post was inserted into the root canal and filled with cement to the most coronal portion to hermetically seal the entrance and photoactivated with the aid of an EC450 device (ECEL, Ribeirão Preto, SP, Brazil), with light intensity greater than 400 mW/cm^2, for 20 seconds, and chemical polymerization for 6 minutes.

After 15 days of cementation and storage in distilled water, the roots were sectioned perpendicularly to the long axis, and three thick slices (1.56 mm±0.37 mm) were obtained with the aid of a cutting machine (Labcut 1010, Extec Corp., Enfield, CT, USA). The slices were obtained in a standardized manner at 4 mm (cervical third), 8 mm (middle third), and 12 mm (apical third) away from the cervical edge of the root (Figure 1), identified, and stored in an oven at 37 °C and 100% relative humidity for 7 days.

Push-out test

The specimens were placed on a stainless steel metal support with a 2 mm central hole. Given the conical shape of the posts, the load was applied in the apical-cervical direction from the apical surface, so that the post could be pushed towards the widest portion of the root canal.

The load was applied only on the post surface with a tip of approximately 1 mm in diameter coupled to the EZ-SX (Shimadzu Corp., Kyoto, Kyoto, Japan) universal testing machine. The selected load cell was 500 kg (50 N) and the loading speed was 0.5 mm/min. The values were recorded in N and displacement resistance in MPa.

To measure the area of the canal and calculate resistance, the diameter of the upper and lower circle of the canal and the thickness of the section (area of a cone trunk) were measured. After the push-out test, the fractured specimens were analyzed under an X20 stereomicroscope (Stemi 2000, Karl Zeiss, Germany) to determine the adhesive, cohesive, or mixed failure pattern.

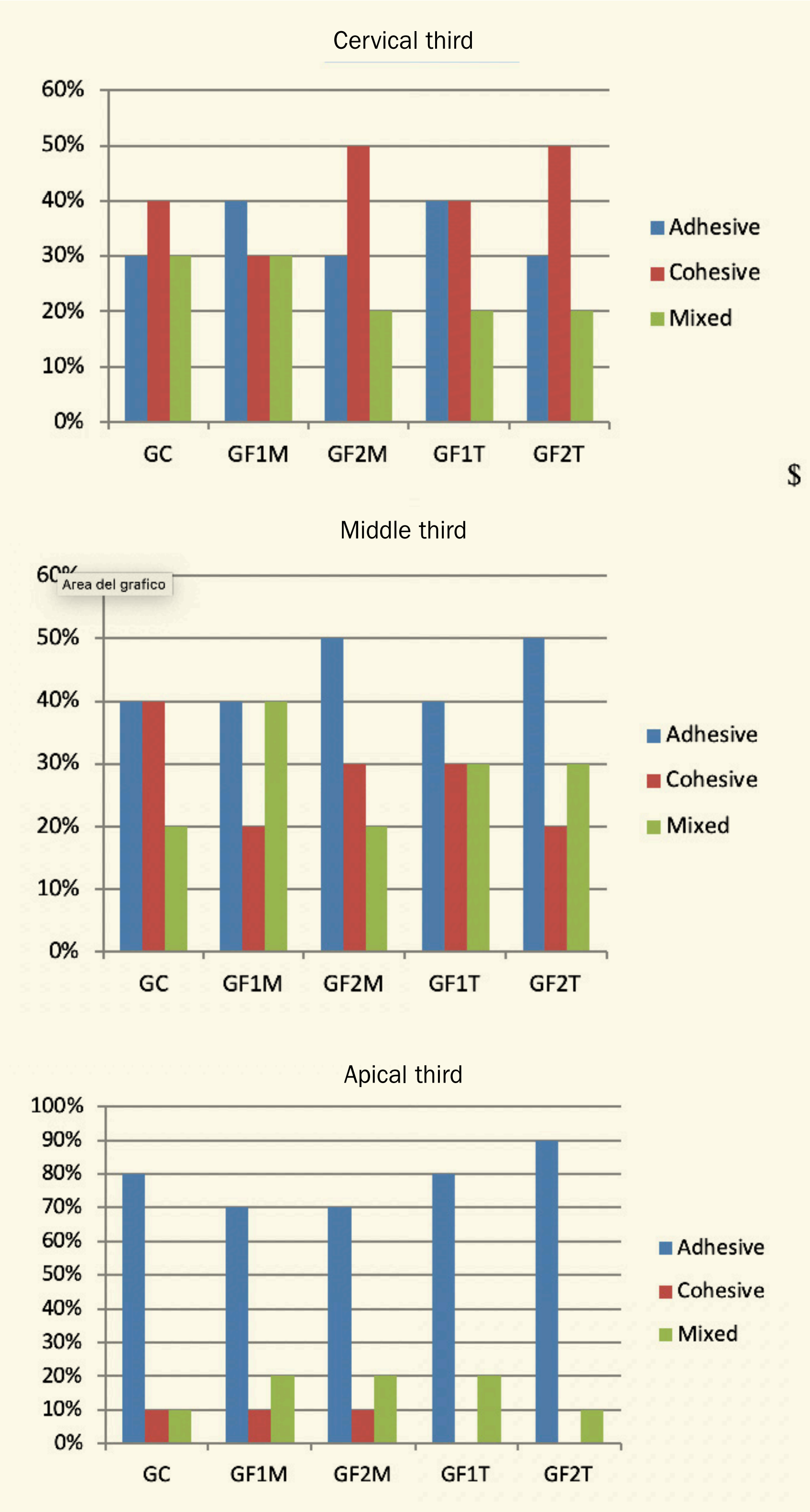

Figure 2 Failure patterns (%) after tested protocols.

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of the data. One-way ANOVA was used to assess bond strength. The level of significance was set at 5% ($P \leq 0.05$). Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA).

Results

The means of displacement resistance (MPa) for the different experimental groups in different regions of the canal are shown in Table 2. There was no statistical difference between the groups regarding the different root positions analyzed, that is, the number of PDT sessions and the type of photosensitizing agent used did not influence the bonding of the intraradicular post.

Graph in Figure 2 shows a homogeneous distribution of fracture patterns among the experimental groups in the different regions analyzed, with a higher rate for the adhesive pattern in the most apical region of the root canal.

Discussion

Intracanal preparation prior to cementation of an intraradicular retainer requires partial removal of the endodontic filling material. During this procedure, there could be breaches of the aseptic field, compromising endodontic success and/or rehabilitation treatment (17). Some substances, such as sodium hypochlorite and chlorhexidine digluconate, are used to clean and disinfect the dentin space prepared for the retainer. However, negative effects on the bond strength of resin cements to root dentin under these conditions have been described in the literature (15, 18, 19). Accordingly, the present study sought to analyze the behavior of PDT, used as an auxiliary resource for intraradicular disinfection, in the adhesive bond strength of fiberglass post on the dentinal wall. In the present study, only methylene blue and toluidine blue were tested, as they are commonly used in association with red low-intensity lasers, also used in our study,

Table 2
Bond strength in root segments in the push-out test

Experimental Group	Root thirds			P
	Cervical	Middle	Apical	
	MPa (±SD)	MPa (±SD)	MPa (±SD)	
GC	8.61^{Aa} ± (4.32)	7.53^{Aa} ± (5,23)	6.87^{Aa} ± (4.30)	P=0.707
GF1M	10.39^{Aa} ± (7.68)	8.96^{Aa} ± (7.17)	7.15^{Aa} ± (5.21)	P=0.574
GF2M	11.07^{Aa} ± (4.92)	9.39^{Aa} ± (3.27)	8.27^{Aa} ± (3,56)	P=0.302
GF1T	10.52^{Aa} ± (5.22)	9.83^{Aa} ± (5.78)	6.96^{Aa} ± (2.90)	P=0.230
GF2T	14.04^{Aa} ± (5.32)	9.67^{Aa} ± (5.65)	8.52^{Aa} ± (7.05)	P=0.117
P	P=0.311	P=0.890	P=0.901	

Means followed by different uppercase letters in the row and means followed by different lowercase letters in the row differ significantly in the analysis of variance at the 5% significance level.

and because these associations with PDT have antimicrobial effects that have already been confirmed in the literature (20). The push-out test, one of the main resources for quantification of the bond strength between different materials and structures (21), according to the literature, was applied. A disadvantage of the push-out test is that voltage is not distributed evenly. To overcome this problem, sections should be prepared with a thickness of approximately 1 mm (22). The push-out test is still the most reliable and reproducible method when compared to microtensile, shear, and traction tests (22).

According to the results obtained, the null hypothesis of the present study was accepted, as PDT and the number of sessions did not influence the bond strength of the fiberglass post to the dentinal wall. These findings are consistent with the study by Ramos et al (23) who, regardless of the root third assessed, observed that PDT did not affect the bond strength of fiberglass posts cemented with the RelyX U200® self-adhesive system. On the other hand, in the study by Ramos et al (24), PDT negatively affected the bond strength of the cemented post with the conventional Relyx ARC® system. According to Konopka and Goslinski (25), the use of PDT within the root canal promotes the release of reactive oxygen species, mainly singlet oxygen, which have negative effects on the formation of the hybrid layer and on the polymerization and bonding of the adhesive system on the dentin surface. It is believed that the result obtained with RelyX U200® cement was different because of its bonding to the dentin substrate. For Pisani-Proença et al (26), the acidic monomers of RelyX U200® demineralize and infiltrate the dentin substrate, providing micromechanical retention. Simultaneously, the reaction between the acidic monomers of the cement and the hydroxyapatite of the dental substrate also leads to chemical retention. This, to some extent, explains our results. Another factor that might have influenced the results obtained in the study by Ramos et al (24) was the use of the optical fiber in a static position for 30 seconds; consequently, the irradiation might have been concentrated in only one region (cervical root third). Garcez et al (27) reported that light distribution and oxygen formation are uniformly generated when the optical fiber is used in spiral and non-static movements.

The type of photosensitizing agent also did not interfere with the adhesive bond strength of the posts to the root canal. According to Di Hipólito et al (28), methylene blue is a cationic substance that binds to anionic molecules, such as the phosphate present in hydroxyapatite. This reaction results in the formation of a precipitate that acts as a physical barrier and can thus influence the interaction between the resin cement and the dentin surface. However, it is believed that because methylene blue and toluidine blue are hydrophilic compounds, the type of cement used (Relyx U220®) exhibits the same behavior which, in a way, may have led to the favorable results obtained in our study.

The failure pattern was also an interesting finding. Failures (adhesive, cohesive, or mixed) occurred homogeneously at the cervical and middle thirds. Only at the apical third did a higher percentage of adhesive-type failures occur, regardless of the group analyzed. The dentinal wall has a smaller amount of dentinal tubules in the most apical regions of the root canal when compared to the middle and cervical thirds (29), and cementation poses some challenges in regions closer to the apex of the tooth (30). Other studies, such as that by Rengo et al (31), found that this is due to the greater probability of cement accumulation in this area. Another factor that may be correlated with this type of failure is the difficulty in removing the photosensitizing agent from deeper regions of the root canal. Although deionized water was used with the aid of a plastic syringe coupled to a 30-gauge needle to remove methylene blue or toluidine blue from the root canal, the dentin surface still exhibited some pigmented areas at the apical thirds. According to Lima et al (15), the pigmentation of these chemical agents can have negative effects on the formation of a hybrid layer and on the adhesive interface between the fiberglass post and the root dentin surface. Ethylenediaminetetraacetic acid (EDTA) is considered the most effective chelating agent in endodontic therapy, showing the ability to very effectively remove the inorganic component, especially in the coronal and middle third of the canal (32). However, prolonged activity of chelating agents on the inorganic dentine structure may reduced tooth microhardness (33). The use of passive ultrasonic irrigation (PUI) instead of the traditional syringe irrigation method could be tested for more effective removal of photosensitizing agents from the root canal. PUI consists of the activation of the irrigating chemical solution within the root canal by means of a smooth ultrasonic tip that, when activated in a passive back-and-forth movement, respecting the WL (34), creates an acoustic flow of the irrigating solution with energy transmission through ultrasonic waves within the canal (35). This agitation of the irrigation solution by ultrasound waves improves its ability to dissolve tissues, also contributing to the removal of the smear layer (36) and promoting antimicrobial activity as a result of the physical disruption of bacterial aggregations, such as biofilm (37).

PDT can be a good alternative for promoting root canal disinfection prior to cementation of intraradicular posts and as a substitute for sodium hypochlorite and chlorhexidine which, according to the literature, have still questionable and deleterious effects on the root canal prior to the cementation of retainers (11). On the other hand, other clinical protocols should be investigated for a more effective removal of photosensitizing agents from the root canal.

Conclusions

The bond strength of cemented intraradicular fiberglass posts was not influenced by the type of photosensitizing agent used and by the number of PDT sessions.

Clinical Relevance

The methylene blue and toluidine blue do not influence intraradicular posts adhesion.

Conflict of Interest

The authors declares that there is no conflict of interest.

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