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Influence of Ozonated Water and Photodynamic Therapy on the Bond Strength of Anatomical Fiberglass Posts to Root Dentin. An *In Vitro* Study

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ABSTRACT

This study evaluates, *in vitro*, the effects of ozonated water as auxiliary endodontic irrigating and photodynamic therapy on the bond strength of fiberglass posts to root dentin. Sixty bovine incisors were prepared and divided into six groups ($n=10$): Oxidation only G1 Distilled water G2 5.25% NaOCl G3 10–15mg/L O₃. Ox+PDT G4 DW+PDT G5 NaOCl+PDT G6 O₃+PDT. Anatomical fiberglass posts were cemented and the roots were sectioned and submitted to the push-out test. Bond strength values were analyzed with two-way ANOVA and Tukey 5%. Failure patterns were analyzed. Sodium hypochlorite and ozonated water without photodynamic therapy showed the highest bond strength, and sodium hypochlorite with photodynamic therapy had the lowest bond strength. The O₃+PDT association showed intermediate results. Considering that the main purpose of the O₃ is disinfection, it was noted that ozonated water did not interfere on the bond strength of fiberglass posts to root dentin. Photodynamic therapy affected bond strength, mainly when associated with sodium hypochlorite. The ozonated water increases the PDT bond strength results.

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Dentin Adhesives; root canal irrigants; ozone; photodynamic therapy

Introduction

Microorganisms are essential in inducing and perpetuating pathological changes affecting the pulp and periapical tissues (Bukhary and Balto 2017). Thus, root canal therapy success highly depends on eliminating these microorganisms through correct instrumentation and using irrigating solutions and appropriate intracanal medications (Arneiro et al. 2014). Sodium hypochlorite (NaOCl) is the “gold standard” irrigating solution in endodontics due to its broad-spectrum antimicrobial activity, ability to dissolve organic tissues, and oxidizing and hydrolyzing capacity (Bukhary and Balto 2017). However, high concentrations and long-time exposure to NaOCl are required to eliminate persistent bacteria, making it potentially cytotoxic (Blattes et al. 2017).

Alternative irrigating substances to NaOCl have been researched in endodontics to help decontaminate the root canal system, such as ozonated water (O₃), known as a potent antimicrobial agent (Oznurhan, Ozturk and Ekci 2015). OW has 99.9% antibacterial efficacy resulting from its oxidation potential capable of destroying the cell wall and cytoplasmic membrane of bacteria and fungi in the oral cavity (Nogales et al. 2016; Nogales, Ferreira and Lage-Marques 2014). Ozone in the aqueous phase also

has the advantage of biocompatibility with dental structures and no cellular mutagenicity (Oznurhan, Ozturk and Ekci 2015). However, there is still no consensus in the literature on the direct influence of O₃ on the bond strength of filling/restorative materials to root dentin (Akturk et al. 2019; de Macedo et al. 2021).

For Santana et al. (2015), the high instability of ozone, rapidly transforming into oxygen, may inhibit the polymerization of the resin cement, reducing bond strength to root dentin. However, in the study by de Macedo et al. (2021) the authors reported an increase in the bonding strength results of fiberglass posts with the use of ozonated water. According to them, despite its instability, ozonated water has a high cleaning power due to the release of oxygen, which leads to greater exposure of dentinal tubules, increasing the area of contact with adhesive agents.

Photodynamic therapy (PDT) has also represented a promising auxiliary resource in endodontics for intracanal decontamination (Souza et al. 2018), inducing insignificant cytotoxicity levels (Gomes-Filho et al. 2016). PDT associates a low-power laser light source and a nontoxic photosensitizer (Souza et al. 2018), exciting electrons that, in the presence of oxygen (O₂),

form short-lived reactive O₂ species, which enter the bacterial cell wall causing microorganism lysis through irreversible oxidation (Ramos et al. 2018). However, studies have shown the harms of PDT on bond strength and dentin penetrability in cementation protocols of fiberglass posts, which would impair the longevity of cemented intracanal dental restorations (Garcia et al. 2018; Ramos et al. 2018).

Considering that the durable bond between polymeric restorative materials and dental structures is significant for restoration success, their mechanical resistance is usually measured by determining bond strength (Escribano, Del-Nero and De La Macorra 2003). In this sense, bond strength tests are the most frequently used to assess the adhesive quality of the interface between the dentin structure and adhesive systems. Therefore, the present study aimed to evaluate, *in vitro*, the influence of O₃ and PDT on the bond strength of fiberglass posts to root dentin. The hypotheses of this study were that dentin irrigation with aqueous solution of O₃ or PDT does not significantly influence the bond strength of fiberglass posts relined to root dentin using a self-adhesive resin cement.

Materials and methods

The manuscript of this laboratory study has been written according to Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines Collection and preparation of samples (Nagendrababu et al. 2021). This project did not require submission to the Ethics Committee, as the used bovine teeth were extracted from carcasses of animals previously slaughtered for meat consumption and its derivatives.

This study selected 60 single-rooted bovine teeth extracted in licensed commercial slaughterhouses, with similar external dimensions and narrow root canals. Immediately after extraction, the teeth were stored at -17 °C to prevent the degradation of collagen and dentin structures. The teeth were stored in plastic bags every 10 units to be thawed gradually, according to use.

Initially, the crown portion of the teeth was sectioned with a double-sided diamond disc (KG Sorensen, Cotia, SP, Brazil) attached to a straight low-speed piece, under constant irrigation, below the cemento-enamel junction, so that the root remnant presented a 15-mm length. Teeth received reference markings on the root surfaces using a projector pen. The first marking was 2 mm below the cemento-enamel limit, the second 2 mm above the root apex, and the last was halfway between both previous marks. These measurements were

performed with a digital caliper (Vonder Electronic Digital Caliper, Curitiba, PR, Brazil).

Hence, teeth were selected with an initial anatomical file (#45), standardizing it for all studied dental elements. All roots were prepared using the same protocol. The pulp tissue was removed with K #45 endodontic files (Dentsply Maillefer, Ballaigues, Switzerland) under abundant irrigation with distilled water (DW). Then, the apical portion of all samples was sealed with light-activated composite resin (Vittra APS, FGM Dental Group, Joinville, SC, Brazil), and the root canals were enlarged in the cervical-apical direction using #6 Largo drills (Dentsply Maillefer, Ballaigues, Switzerland) at low speed, under abundant irrigation with DW, up to a 10-mm length. The internal root canal diameter of the samples was standardized at 2 mm and the external diameter at 5 mm (Carrera et al. 2016).

After standardizing the roots, the samples were randomly divided in six study groups according to according to the characteristics of Oxidation (Ox) and the treatment or not with Photodynamic Therapy (PDT) ($n = 10$):

Oxidation only G1 Distilled water G2 5.25% NaOCl G3 10–15 mg/L O₃

Ox + PDT G4 DW + PDT G5 NaOCl + PDT G6 O₃ + PDT

The 5.25% NaOCl and DW solutions were manipulated in a commercial pharmaceutical establishment (Natupharma™, Passo Fundo, RS, Brazil). OW was prepared by converting bidistilled water with ozone gas for 10–15 minutes using a Philozon Medplus™ ozone generator (Philozon, Balneário Camboriú, SC, Brazil). Distilled water was ozonated to prepare a stock of ozonated water. Since the solubility in water is governed by Henry's law, a gas-phase ozone concentration of 40–60 mg/L will result in a concentration in water of 10–15 mg/L depending on temperature (Van Leeuwen 2015). The collected water was stored in a dark temperature-proof container and used within 24–48 hours from production to preserve ozone properties.

Irrigation protocol and photodynamic therapy (PDT)

Irrigation cycles were performed in all groups to simulate the clinical use of auxiliary irrigating substances inside the canal, leaving the tested substance in each group for 30 minutes, which was agitated with a manual file in the first minute and renewed every five minutes, up to 30 minutes. In groups 4 to 6 - DW+PDT, 5.25% NaOCl+PDT, and O₃+PDT -, the PDT protocol was applied immediately after the chemomechanical preparation and before the final irrigation.

For the PDT protocol, the root canals were filled with 0.01% (0.1 mg/mL) methylene blue (Chimio Lux DMC, São Carlos, SP, Brazil) until overflowing at the root canal entrance. The dye remained in the root canal for five minutes (pre-irradiation time). After that, a low-intensity laser (Therapy XT™ DMC, São Carlos, SP, Brazil) with 100 mW of power, continuous emission, and red light spectrum (690-nm wavelength) was used, with a 600- μ m-diameter intracanal optical fiber inserted 3 mm short of the working length. Root canals were irradiated for 90 seconds, providing a total dose of 9 J and an energy density of 320 J/cm², while the intracanal fiber remained in a static position, as recommended by the manufacturer.

For all studied groups, a final irrigation with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) was performed for 30 seconds, in order to remove the smear layer. After the final irrigation, a new irrigation was performed with 5 mL of DW and the root canals of all groups were aspirated and dried with #45 absorbent paper cones (Tanari, Manaus, AM, Brazil).

Preparation of fiberglass posts

Prefabricated intraradicular retainers Exacto™ fiberglass posts #2 (Angelus Odonto, Londrina, PR, Brazil) were used after light-activated composite resin relining (Vittra APS™, FGM Dental Group, Joinville, SC, Brazil). Initially, the posts were prepared by applying 37% phosphoric acid (Angelus Odonto, Londrina, PR, Brazil) on their surface for one minute, washing for one minute, drying by air spray, and silanizing (Angelus Odonto, Londrina, PR, Brazil) with a microbrush (Angelus Odonto, Londrina, PR, Brazil), rubbing over the post structure and waiting one minute to volatilize the alcohol in its composition. A thin layer of the Ambar APS™ adhesive system (FGM Dental Group, Joinville, SC, Brazil) was applied on the post surface, and light activation was performed on two sides for 40 seconds each (Haragushiku et al. 2015).

The canal was first isolated with water-soluble gel (KY, Semina, São Paulo, SP, Brazil) to prepare the relining of posts. The fiberglass post was wrapped in composite resin, and the set was taken inside the root canal, removed and replaced twice, removing excess composite and light-activating it positioned inside the canal for three seconds. Next, a marking was made on the buccal region of the post and the tooth to identify the correct position for inserting the post during the cementation stage.

The modeled post was removed from the root canal, careful not to touch the canal walls, and immediately light-activated on the buccal and palatal surfaces for 40

seconds on each side. Finally, the canal and relined post were abundantly irrigated with DW for one minute to eliminate the water-soluble gel. The canal was aspirated with a narrow endodontic cannula until completely removing the water. Then, a #45 absorbent paper cone moistened with DW was gently passed on the root canal walls to leave the dentine moist.

After modeling, the post was cemented with dual self-adhesive resin cement RelyX U200™ (3 M-ESPE, St. Paul, MN, USA), which was poured onto an impermeable paper block and manipulated for 10 seconds. Cêntrix syringes and Acudosse #2 needles (DFL, Rio de Janeiro, RJ, Brazil) were used to insert the cement into the root canal. The post was positioned, manually stabilized for 20 seconds, and light-cured for five seconds. Excess cement was removed, and right after, a final light activation was performed for 40 seconds on four sides of the tooth (mesial, distal, buccal, and lingual) (Haragushiku et al. 2015).

Preparation of samples and push-out bond strength test

The roots were fixed on an acrylic resin plate and then adapted to a metallographic cutter (Miniton™, Struers, Copenhagen, Denmark) with a double-sided diamond disc, driven at a speed of 250 rpm under refrigeration. Ten teeth per group were used, and seven slices of each tooth were obtained, always discarding the first slice of each tooth. Six slices per tooth, with a thickness of 2 mm, were used for analysis: two from each cervical, middle, and apical third. Thus, 20 slices per third were obtained for the evaluated method, according to a preliminary study (Carrera et al. 2016). All slices were marked on their most apical surface to allow the correct positioning of the sample in the push-out test machine.

The discs were placed with the cervical part of the section facing down on the table (perforated in the center) of the device. A top-to-bottom vertical force was applied perpendicularly to the post/resin/cement set using a metallic piston with a circular section, which diameter covered the largest possible area of the restorative set without touching the dentin walls. The metallic piston was connected to a universal testing machine (Instron, Norwood, MA, USA) with a 500-Newtons (N) load cell. The test was performed at 0.5 mm/min with the load applied until post/resin/cement set extrusion. After that, the force measure required for displacing the material was obtained.

After the push-out test, the dentin discs were verified individually in an optical microscope (Carl Zeiss, São Paulo, SP, Brazil) at a 50 \times magnification. The adhesive

surface area was calculated using the cylinder area (A) formula $A = 2\pi R(R+H)$, considering the canals with an internal diameter of 2 mm, radius (R) of 1 mm, and height (H) of each specimen of 2-mm slices. Bond strength was calculated in Megapascal (MPa) by dividing the value obtained in the push-out test by the surface area of the canal.

The failure patterns of each sample were also observed and classified under optical microscopy (Cecchin, Farina and Bedran-Russo 2018) into five types: (1) adhesive between composite and resin cement (cement not visible around the composite); (2) mixed, with cement resinous covering 0 to 50% of the total diameter of the composite; (3) mixed with cement resinous covering 50 to 100% of the composite surface; (4) adhesive between cement resinous and root dentin (composite surrounded by resin cement) and (5) cohesive in dentin (Figure 1).

Statistical analysis

After a normality test, the values for cervical, middle, and apical thirds obtained in all groups showed normal distribution, but the variances were not homogeneous (verified by the Levene test). The three thirds were evaluated separately (after the logarithmic transformation of the data) by two-way ANOVA (irrigating substance and PDT), followed by Tukey's test, at a 5% significance level. Two sample power calculations were performed ($n = 10$, $\alpha = 0.05$, and mean standard deviation). Power was 1.00 for groups DW and O_3 and 0.99 for groups O_3 and DW+PDT. Comparison of root thirds was performed using one-way ANOVA and Tukey ($\alpha = 0.05$).

Results

The overall bond strength results evaluated in each root third (cervical, middle, and apical) of the studied sample showed a statistically significant difference, with the means decreasing from cervical to apical. Table 1 describes push-out bond strength values for all experimental groups.

The same result pattern occurred within each root third, with the highest bond strength values in the groups using 5.25% NaOCl and O_3 without PDT, followed by O_3 with PDT, and DW without and with PDT, respectively. NaOCl associated with PDT showed the lowest bond strength values in all root thirds (Table 1).

Table 2 describes the percentage of failure modes for each group. As for the groups of DW associated with PDT or not, the most frequent failure was type 3 (mixed, with resin cement covering 50 to 100% of the composite surface). Group NaOCl without PDT showed a higher frequency of type 1 failures (adhesive, between composite and resin cement, with cement not visible around the composite), and NaOCl associated with PDT showed type 3 failures as the most frequent. O_3 groups followed the same pattern, which without PDT, showed more type 1 failures, and when associated with PDT, showed more type 3 failures.

Discussion

The results of the present study confirm partially the hypothesis, considering that ozonated water (O_3) did not harm the adhesive strength of fiberglass posts to

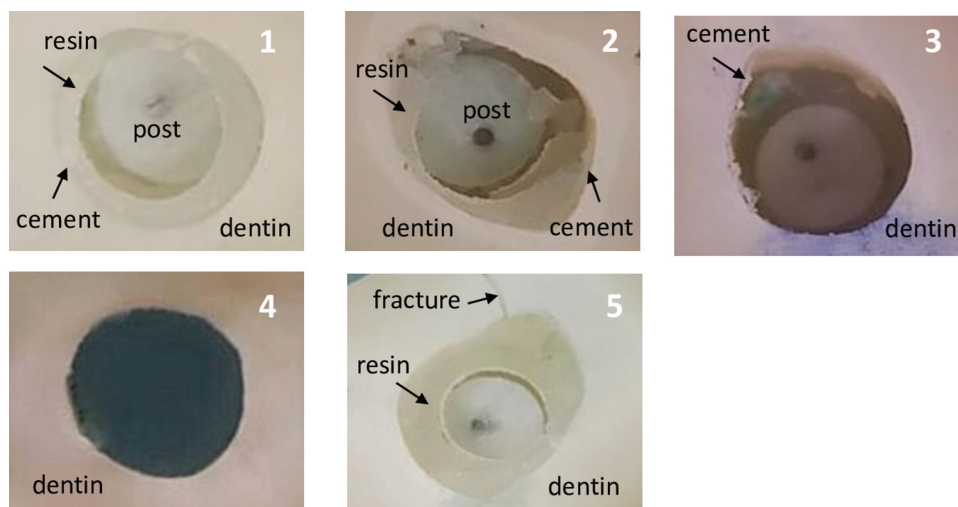


Figure 1. Failure modes (1) adhesive, between the composite and resin cement (cement not visible around the composite); (2) mixed, with resin cement covering from 0 to 50% of the total composite diameter; (3) mixed, with resin cement covering 50 to 100% of the composite surface; (4) adhesive, between the resin cement and root dentin (composite wrapped in resin cement); (5) cohesive in dentin.

Table 1. Mean and standard deviation (M±SD) of the bond strength of fiberglass posts to root dentin of the studied groups in the cervical, middle, and apical root thirds.

Group Classification	Ox	Ox	Ox	Ox + PDT	Ox + PDT	Ox + PDT	
Root third	DW (G1)	NaOCl (G2)	O ₃ (G3)	DW+PDT (G4)	NaOCl+PDT (G5)	O ₃ +PDT (G6)	p-values
Cervical	3.43 ± 0.7 cA	6.72 ± 0.9 aA	6.53 ± 1.2 aA	2.74 ± 0.4 dA	2.19 ± 0.2 eA	4.37 ± 0.3 bA	0.000*
Middle	2.59 ± 0.6 cB	6.15 ± 1.5 aAB	5.30 ± 0.7 aB	2.09 ± 0.5 dB	1.65 ± 0.2 eB	3.74 ± 0.2 bB	0.000*
Apical	1.79 ± 0.4 dC	5.50 ± 0.8 aB	4.44 ± 0.5 bC	1.32 ± 0.3 eC	1.15 ± 0.1 eC	3.22 ± 0.4 cC	0.000*
p-values	0.000**	0.005**	0.000**	0.000**	0.000**	0.000**	

Group Classification – Ox- oxidation only; Ox+PDT- oxidation+photodynamic therapy.

a, b, c, d, e - Equal letters in the same row indicate statistically similar results and different letters indicate statistically different results for distinct groups in the same root third.

A, B, C - Equal letters in the same column indicate statistically similar results and different letters indicate statistically different results for the same groups in distinct root thirds.

DW - distilled water; NaOCl - sodium hypochlorite; O₃ - ozonated water; PDT - photodynamic therapy.

*The p-value was 0.000 for the factors irrigating substance, PDT, and for the interaction (two-way ANOVA).

** One-way ANOVA.

Table 2. Failure mode distribution in each group.

Substance	PDT	Failure modes				
		1	2	3	4	5
DW	No	0%	33%	43%	20%	4%
	Yes	0%	30%	43%	20%	7%
NaOCl	No	57%	23%	0%	0%	20%
	Yes	0%	20%	57%	23%	0%
O ₃	No	45%	25%	17%	0%	13%
	Yes	0%	10%	70%	20%	0%

DW - distilled water; NaOCl - sodium hypochlorite; O₃ - ozonated water; PDT - photodynamic therapy.

Failure modes: 1-adhesive between composite and resin cement (cement not visible around the composite); 2-mixed, with cement resinous covering 0 to 50% of the total diameter of the composite; 3-mixed with cement resinous covering 50 to 100% of the composite surface; 4-adhesive between cement resinous and root dentin (composite surrounded by resin cement) and 5-cohesive in dentin.

root dentin. That agrees with the findings of other authors (Akturk et al. 2019; Oznurhan, Ozturk and Ekci 2015) and (Garcia et al. 2012), who reported that O₃ cleans dentin without affecting the bond strength of self-etching adhesive systems. Although there are reports of controversial results (Bitter et al. 2008; Cadenaro et al. 2006; Murugesan et al. 2022; Santana et al. 2015), a high cleaning power occurs by O₂ release, which probably opens the tubular structure, exposes more dentinal tubules, and removes organic debris, thus improving bond strength (Oznurhan, Ozturk and Ekci 2015).

For (Akturk et al. 2019), the lack of consistency in the results may be due to different methodologies, O₃ concentrations, and dentin substrates used in each investigation, justifying the continuity of studies using O₃ as an auxiliary irrigating substance and seeking to elucidate these issues.

In contrast, the hypothesis of the present study was partially rejected in the sense that PDT harmed the bond strength of fiberglass posts to root dentin, mainly when associated with NaOCl and, to a lesser extent, with O₃. That may have occurred due to a large amount of singlet

oxygen generated from the blue dyes stimulated by PDT, which may have reduced the adhesive system capacity, interfering with the bond strength of fiberglass posts to the root dentin. Menezes et al. (2017) evaluated the effect of PDT on cement adhesion and penetration in root canals, verified the lowest bond strength values of AH Plus™ and MTA Fillapex™ cements when used after PDT, but related them to a possible interference or the presence of traces of the photosensitizing agent on the dentin surface.

Hashem et al. (2021) raised this same hypothesis when noting that the bond strength values of fiberglass posts to dentin were higher in the cervical than in the apical third and related this result to a more effective removal of the PDT photosensitizer at the cervical end of the root segment. Ferreira et al. (2015) explain that adhesion is more problematic in apical than cervical dentin due to morphological differences, the density reduction of dentinal tubules, and the altered collagen expression in root dentin.

In the present study, the combination of ozonated water and PDT (G6) resulted in an increase in bond values compared to the use of PDT alone (G4) (Table 1). This important outcome of this study can potentially be attributed to the action of residual oxygen from ozonated water, which potentially decreased the presence of residues of the photosensitizing agent in the dentinal tubules. Subsequently, new studies must be carried out to molecularly confirm this reduction and verify whether it affects the antimicrobial efficacy of PDT.

Divergent results may also occur due to methodological differences, including different photosensitizers, pre-irradiation times, and light sources. Alonzaian et al. (2019) evaluated the bond strength and failure modes of fiber posts after PDT with a diode laser; erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser; neodymium-doped yttrium aluminum garnet (Nd:YAG) laser; and conventional cleaning

and modeling. The lasers varied regarding wavelength and output power. This study verified, with significant differences, a higher mean adhesion force in the PDT group with a diode laser, followed by Er,Cr:YSGG and Nd:YAG lasers. That suggests that PDT with different lasers and irradiations can produce distinct bond strengths of fiber posts to root dentin. Although our study did not aim to compare various photosensitizers and light sources, it is worth mentioning the use of 0.01% methylene blue with a pre-irradiation time of five minutes and a low-intensity laser source with 100 mW of power, continuous emission, and red light spectrum (690-nm wavelength). Root canals were irradiated for 90 seconds, providing a total dose of 9 J and an energy density of 320 J/cm².

The highest bond strength values in the present study occurred with 5.25% NaOCl, corroborating studies that relate the treatment with NaOCl to the increased bond strength of adhesive systems due to the effects of this irrigant on smear layer removal, which may benefit some resinous materials in providing adequate adhesion to dentin (Inoue et al. 2002). Cecchin et al. (2012), for instance, compared 5.25% NaOCl to 2% chlorhexidine and 100% ethanol and demonstrated that 5.25% NaOCl presents the best bond strength values of fiberglass posts relined with composite resin to root dentin, also maintaining these values for 12 months.

The favorable results of NaOCl used as an auxiliary endodontic irrigant probably occur because this substance removes the superficial fibrils of dentin collagen (Cecchin et al. 2012). Considering these fibrils are inherently wet due to their high water affinity, adhesive components are more prone to degradation over time due to water absorption, resin leaching, and other water-mediated aging phenomena that weaken the polymeric structure of the adhesive and cause adhesive interface failure (Breschi et al. 2008). Thus, partially removing the demineralized collagen network changes the dentin surface and its hydrophilic properties, decreasing the sensitivity of the hybrid layer technique without compromising adhesion effectiveness (Cecchin et al. 2012).

Macedo et al. (2021) evaluated the effect of ozonated water used as an irrigant and dentin-cleaning solution on the bond strength of fiberglass posts *in vitro*. These authors observed that the high bond strength values observed in the NaOCl group can be explained by the moderate sodium hypochlorite concentration used (2.5%), as well as the low exposure time, which contributed to root canal cleaning without collagen degradation. Moreover, the authors state that the combination of sodium hypochlorite and ozonated water also provided significantly higher bond strength results

compared to the other groups. These results are compatible with ours in the sense that O₃ and NaOCl do not harm the bond strength to dentin.

As for failure modes, the present study showed a prevalence of type 1 failures (adhesive, between composite and resin cement, with no visible cement around the composite) for groups NaOCl and O₃ without PDT, while groups irrigated with DW (with or without PDT) and NaOCl and O₃ associated with PDT had a prevalence of type 3 failures on a larger scale (mixed, with resin cement covering 50 to 100% of the composite surface). Thus, type 3 failures were more common in all groups using PDT. Such results demonstrate that the PDT protocol established in the present study precipitated the occurrence of more unfavorable failure modes, considering that Cecchin, Farina and Bedran-Russo (2018) classified these modes, stating that 3 and 4 are the most unfavorable and 1, 2, and 5 are the most favorable because the largest cement portion remained in contact with dentin.

Among the limitations of this study is that it is laboratory research using bovine teeth, which present similar composition and morphology to human teeth (de Carvalho et al. 2018). Although this experiment aimed to reproduce, as much as possible, the procedures performed in a clinical setting, the findings must be interpreted with caution, requiring further clinical tests to evaluate the studied variables and confirm our results. Additionally, more studies should compare different photosensitizers and light sources to prove or disprove their interference with the bond strength of fiberglass posts to root dentin. In future studies, special attention should be paid to the combination of dentin treatments with ozonated water and PDT, since interesting results were reported in this work.

Conclusion

Based in this *in vitro* study, the use of ozonated water as an auxiliary endodontic irrigating solution did not harm the adhesive strength of fiberglass posts to root dentin. The application of photodynamic therapy interfered negatively in bond strength, especially when associated to sodium hypochlorite. The ozonated water increase the PDT bond strength results.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Authorship declaration

All authors state that have contributed significantly and all authors are in agreement with the present manuscript.

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