Influence of Ozonated Water as an Irrigant and Dentin-cleaning Solution on the Bond Strength of Fiberglass Pins

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ABSTRACT

Aim and objective: This study aimed to evaluate the effect of ozonated water used as an irrigant and dentin-cleaning solution on the bond strength of fiberglass pins *in vitro*, comparing it with the commonly used solutions.

Material and methods: Seventy-seven bovine roots were randomly divided into seven groups according to the irrigant and dentin-cleaning solution to be used: HP/HP, 2.5% sodium hypochlorite; DA/DA, distilled water; CHX/CHX, 2% chlorhexidine; OA/OA, 4 ppm ozonated water; HP/DA; HP/CHX; and HP/OA. In each group, the root canals were endodontically prepared by using the corresponding irrigant and stored in DA; after 7 days, they were cleared and cleaned with the corresponding cleaning solution. The fiberglass pins were cemented by using self-adhesive cement. After 7 days, the roots were sectioned (six discs each) and submitted to the push-out test. The type of fracture was analyzed with a 4.5× stereoscopic magnifying glass. The data were analyzed by the analysis of variance and the Tukey test at a 5% significance level.

Results: The OA/OA (11.67), HP/HP (11.21), and HP/OA (9.71) groups showed the highest mean push-out bond strength (MPa) in the cervical third. The same trend was maintained in the middle and apical thirds.

Conclusion: Ozonated water and 2.5% sodium hypochlorite are the most relevant solutions for root dentin treatment.

Clinical relevance: Teeth treated with ozonated water, sodium hypochlorite, and a combination of the two showed greater bond strength than those treated with other solutions.

Keywords: Chlorhexidine, Ozone, Resin cements.

INTRODUCTION

One of the greater challenges in restorative dentistry is the esthetic and functional restoration of endodontically treated teeth and teeth with major structural loss.[1] The fiberglass pins are very effective in such situations because of their advantages such as corrosion resistance, esthetics, elastic modulus close to that of dentin, and the need for fewer clinical sessions for preparation.[1] Nevertheless, the dislodgment of fiberglass pins into the root canal remains a major drawback. To avoid this, it is essential to obtain satisfactory bond strength between the pin, bonding agent, and dental structure.[2]

The performance of fiberglass pins can be improved by using a joining technique that uses self-adhesive resin cements and, consequently, has fewer steps.[3] According to manufacturers, self-adhesive cements are used in a single clinical stage and do not require any dentin treatment. Bonding is achieved by a reaction between the phosphate groups of methacrylate in the material with the hydroxyapatite of the tooth.[4] However, previous studies have reported weak bonding between self-adhesive cements and dentin, probably because of their poor conditioning in this substrate and the presence of a smear layer.[5] Reports are indicating that dentin pretreatment can remove the smear layer and allow better interaction between dentin and self-adhesive cements.[2,3]

Several solutions are used for disinfecting root canal systems and cleaning dentin, including sodium hypochlorite, chlorhexidine, and, more recently, ozonated water.[6–9] In restorative dentistry, ozonated water is used for the treatment of carious lesions owing to its antimicrobial properties and ability to oxidize proteins within lesions, which leads to the diffusion of calcium and phosphate ions and remineralization of dental tissues.[10] In cavity preparation, it can be used as an irrigant and

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dentin-cleaning solution before application of an adhesive[11]; it does not interfere either with the bond strength between enamel/dentin and conventional or self-etch adhesive systems, resin cements, or composite resins or with the mechanical properties of these materials.[7] In endodontics, ozonated water has been proposed as a

intracanal medication or root canal irrigant, wherein the principle of using ozone consists on the release of oxygen promoting the destruction of bacteria without increasing their resistance, promoting the cleaning of dentin.[12]

What is not yet clear in the literature is the effect of the previous use of ozonated water alongside self-adhesive resin cements. Thus, this study aimed to evaluate the effect of ozonated water as an irrigant and dentin-cleaning solution on the push-out bond strength of fiber posts cemented with dual resin cement. The hypotheses of this study were dentin-cleaning or with irrigants solutions do not significantly influence the bond strength.

MATERIALS AND METHODS

This study was conducted on the premises of the Western State University of Paraná (UNIOESTE), Cascavel, Paraná, Brazil. For this study 77 bovine teeth ($n = 11$) were used for *in vitro* analysis. The experimental groups were divided in seven groups, a combination of the irrigating solution was also made, as well as the cleaning solution: HP/HP: 2.5% sodium hypochlorite; DA/DA, distilled water; CHX/CHX, 2% chlorhexidine; OA/OA, 4 ppm ozonated water; and also standardized the sodium hypochlorite irrigation solution as an irrigation solution associated with the other test solutions: HP/DA: 2.5% sodium hypochlorite irrigation solution and distilled water cleaning solution; HP/CHX: 2.5% sodium hypochlorite irrigation solution and 2% chlorhexidine cleaning solution; and HP/OA: 2.5% sodium hypochlorite irrigation solution and 4 ppm ozonated water cleaning solution. The treatment materials used in each group are described in Table 1.

Production of Ozonated Water

Ozonated water (4 ppm)[7] was prepared at room temperature ($25 \pm 1.0°C$) with an ozone generator (O&L3.0 RM; Ozone & Life, São José dos Campos, Brazil) by using pure oxygen, which was dispensed from a cylinder coupled to the generator and regulated at a flow rate of 1 L/minute. The ozonated water was produced by using 50 mL sterilized water in a sterile glass tube each time; it was prepared until 5 minute before use. The amount of ozone in the water was measured by direct iodometric titration, as recommended by the International Ozone Association. In this procedure, 50 mL potassium iodide (KI) solution (1 N) was added to the ozonated water. The ozone oxidized KI and promoted the release of I_2 according to the equation $O_3 + 2KI + H_2O \leftrightarrow I_2 + 2KOH + O_2$. To ensure I_2 production, it was necessary to acidify the medium by adding 2.5 mL H_2SO_4 (1 N) to the KI solution. Subsequently, the liberated I_2 was titrated with $Na_2S_2O_3$ (0.01 N) until its yellowish color became barely perceptible. Thereafter, 1 mL of a 1% starch indicator solution was added to the mixture, and the titration was resumed until the blue color of the solution disappeared. Because of the instability of ozone, its concentration was quantified during and immediately after the bubbling process.

Tooth Selection and Root Preparation

The teeth selected for *in vitro* analysis had a mean root length of 15.0 ± 1.0 mm and a canal diameter similar or close to 2.0 ± 0.5 mm in the cervical third. Teeth with curvature >5°, caries, cracks, or an open apex >2.5 ± 0.5 mm were excluded. The teeth were stored in 1% thymol solution in a refrigerator (approximately 4°C).[13] A previously calibrated operator removed the crowns 1 mm below the cementoenamel junction by using a diamond disc (KG Sorensen, Cotia, Brazil) at a low speed (Kavo, Joinvile, Brazil) under constant cooling with distilled water.

Biomechanical Preparation

For biomechanical preparation, the roots were surrounded with a rubber-like material and trapped in a vise to reduce mechanical stress. The root canals were prepared by using a high-precision electric drill (Bosch, Joinville, Brazil) with torque control and a 2-mm steel bit (Irwin, Carlos Barbosa, Brazil), which had previously been tested in a pilot experiment. During the preparation, the irrigant employed in each group was kept in contact with the dentinal surface for 5 minutes by using 5-mL syringes (Injex, Ourinhos, Brazil) and 27-gauge needles (Injex).

The canals were then washed with 5 mL distilled water and dried by using a medium-gauge vacuum cannula and absorbent paper cones (Maillefer/Dentsply). The roots were then sealed by

Table 1: Description of materials used

Material	Commercial brand	Manufacturer	Manufacturer's instructions	Composition
Ozonated water (OA)	Ozone & Life®/ O&L3.0RM	Ozone & Life, São José dos Campos, São Paulo, Brazil	Use up to 10 minutes after handling.	Sterilized water, 4 ppm ozone
Chlorhexidine 2% (CHX)	Clorhexidina®	Maquira, Maringá, Paraná, Brazil	Apply on the areas to be treated from 15 seconds.	Chlorhexidine Digluconate 2%, Distilled water
Distilled water (DA)	Eurofarma®	Eurofarma, São Paulo, São Paulo, Brazil		Distilled water
2.5% Sodium hypochlorite (HP)	Asfer®	Asfer, São Caetano do Sul, São Paulo, Brazil	Apply on the areas to be treated for 5 minutes.	2.5% sodium hypochlorite and deionized water
Self-adhesive resin cement (SRC)	Relyx U 200®	3M-ESPE, St. Paul, Minnesota, United States	Spread the dose for 20 seconds.	Methacrylate monomers containing groups of phosphoric acid, methacrylate monomers, stabilizers, additives, pigments, initiator component.
Resin sealant (RS)	Yseal/Yller®, Pelotas, Rio Grande do Sul, Brazil	Yseal, Pelotas, Rio Grande do Sul, Brazil	Apply the product over the area of interest and photopolymerize for 20 seconds.	Monomers methacrylates, ionometric charge, fluorides, silica nanoparticles, initiators, stabilizers, and pigments.

thermoplastic condensation by injecting plasticized alpha gutta-percha (Sistema TC; Tanaka de Castro & Minatel Ltda., Cascavel, Brazil) and endodontic cement (Sealer 26; Maillefer/Dentsply) following the manufacturers' instructions (Table 1). Then, the teeth were sealed both at the mouth of the canals and the apex by using a photopolymerizable resin sealant (Yseal; Yller, Pelotas, Brazil) following the manufacturer's instructions. The roots were stored in distilled water for 7 days at 37°C.

Cementation of Pins

After removing the seals, the teeth were clamped onto a vise and drilled with Gates Glidden drills to unclog the initial 10-mm depth of the roots (cervical to apical). A Kerr (K) 15-mm file was used to confirm that the 10-mm conduit was unclogged. The roots were calibrated to a depth of 10 mm by using fiberglass pin kits (number 3 [Ø = 2 mm]; Whitepost DC; FGM, Joinville, Brazil) mounted on a low-speed motor (Kavo), under irrigation with distilled water. After this process, the dentin-cleaning solution to be employed for each group was determined. The cleaning solutions were applied on the dentin surface for 20 seconds with a microbrush and then dried with light air jets for 10 seconds and with absorbent paper cones (Maillefer/Dentsply).

All roots were cemented with double-tapered fiberglass pins (number 3; Whitepost DC) by using self-adhesive cement (U200 Relyx; 3M ESPE, St. Paul, Minnesota, United States) following the manufacturer's instructions. The cement was inserted into the canal by using a syringe (Centrix; Nova DFL, Rio de Janeiro, Brazil). Subsequently, the pins were carefully inserted and held in position by digital pressure for 60 seconds. Any excess cement was removed with an insertion spatula (Quinelato, Rio Claro, Brazil), and the remaining cement was photoactivated by using an LED apparatus (Bluphase; Ivoclar Vivadent, Barueri, Brazil) at 1040 mW/cm^2 power for 60 seconds. Then, the exposed surface of the pins was covered by applying a photopolymerizable resin sealant (Yseal; Yller, Pelotas, Brazil) following the manufacturer's instructions. The teeth were then stored under relative humidity at 37°C for 7 days.

Specimen Preparation

The roots were fixed on a metal base in the ISOMET 1000 cutting machine (Buehler, Lake Bluff, Illinois, United States) and sectioned perpendicularly along the root axis (perpendicular to the Y-axis) by using a diamond disc (Extec-Erios, São Paulo, Brazil) under constant water cooling. Before cutting the discs, the cervical surface of each root was marked with an overhead marker to highlight it for subsequent positioning of the root for the mechanical test. A total of 10 discs, each with a thickness of 1.0 ± 0.2 mm, were sectioned from each root. The first cervical disc, which was approximately 1 mm in thickness, was discarded. Three discs were sectioned for each third (cervical, middle, and apical), and the third disc of each third was discarded, which meant that only six discs per root were used for further analysis. The thickness of the sections was determined by using digital calipers (Starret 727; Starret, Itu, Brazil). The specimens were stored under relative humidity at 37°C for 7 days.

Mechanical Testing

The coronary surface (largest diameter) of each disc was identified and positioned facing downward. For the push-out test, an adapted metal cylinder (Ø = 1 mm) was used to induce a load on the central portion of the pin–cement assembly. The test was performed in a universal test machine (EMIC DL-200 MF; São José dos Pinhais, Brazil), with a velocity of 1 mm/minute and a load cell of 50 kgf,

according to the protocol reported by Martinho et al.[13] The load was applied until displacement or fracture of the pin–cement assembly.

Bond strength was calculated by using the formula $R = L/A$, where L is the load required for fracture of the specimen (kgf) and A is the interfacial area (mm^2). The adhesive area (A) of the discs was calculated by using the formula for the lateral area of a straight circular cone with parallel bases.[14] Specimen diameter and height were determined with an accuracy of ±0.02 mm by using digital calipers (Starret 727; Starret) following the manufacturer's instructions. Adhesive strength was initially calculated in kgf/mm^2 and later converted to MPa by multiplying the R-value by 10 according to the following equation: 1 kgf/mm^2 = 10 N/mm^2 = 10 MPa.

Evaluation of Failure Type

After the mechanical test, all discs were observed in a stereoscopic loupe (Olympus SZ, São Paulo, Brazil) at a magnification of 4.5×. The type of failure was classified as type 1 (dentin cohesive), 2 (adhesive—pin–cement interface: pin without cement remnant), 3 (adhesive—dentin–cement interface: pin with remnant cement), or 4 (mixed: cohesive and adhesive).

Statistical Analysis

After the push-out test, statistical analyses were performed by using the BioEstat 5.3 program (Instituto Mamirauá, 2007). Initially, quantitative data (bond strength) were submitted to the Shapiro–Wilk test for evaluating the distribution type. Considering that the data did not adhere to the normality curve, intergroup interactions were analyzed by the Kruskal–Wallis test of variance, followed by the Dunn test (significance, p <0.05). Qualitative variables of the fractures were evaluated by descriptive statistical analysis.

RESULTS

Table 2 and 3 present the results of statistical analysis of bond strength according to the experimental group. Upon comparing bond strength among the different test solutions, with dental root depth being a fixed factor, the HP/HP and OA/OA combinations were found to show statistically similar results irrespective of root depth (Table 2). Upon comparing bond strength based on root depth, with the type of solution being a fixed factor, the cervical third were found to show higher bond strength values than the other two levels (in the order: cervical > medium > apical) in all groups except the OA/OA group; in the OA/OA group, the middle third presented bond strength values similar to the cervical and apical thirds.

Table 3 shows the combinations between the different solutions tested, with HP/HP being the control group. The results presented in Table 3 show a similar trend as those in Table 2. The HP/HP solution showed a statistically similar behavior as the HP/OA solution. Regardless of root depth, the HP/CHX group showed the worst bond strength. Among the different root depths, the bond strength values of the cervical portion were higher than those of the apical portion and similar to those of the middle portion in most groups, irrespective of the solutions used.

Figure 1 shows the distribution of failure type. Adhesive failure was the most predominant failure type in all groups, followed by the cohesive (in cement) and mixed types. Based on the graph, the groups HP/HP, AD/AD, and HP/CHX showed a predominance in adhesive failures followed by mixed and cohesive failures in cement. The CHX/CHX and AD/AD groups showed a predominance of adhesive failures followed by cohesive failures in cement. The most

Table 2: Mean value and standard deviation of the bond strength variable, according to the dental root depth (RD) and the combination of equal irrigation and cleaning solutions (MPa)

RP/solutions	HP/HP	DA/DA	CHX/CHX	OA/OA
Cervical	**10.71** + 1.74 Aa	**8.05** + 2.27 Ab	**8.09** + 1.21 Ab	**10.91** + 2.03 Aa
Medium	**9.47** + 2.29 Aa	**5.21** + 1.20 Bb	**4.88** + 1.12 Bb	**8.66** + 1.52 ABa
Apical	**6.3** + 1.98 Ba	**3.18** + 1.36 Bb	**3.82** + 1.29 Bb	**6.41** + 2.11 Ba

Different letters indicate statistically significant differences, lowercase letters for row and upper case for column, $p < 0.05$

Table 3: Mean value and standard deviation of the bond strength variable, according to the dental root depth (RD) and the combination of different irrigation and cleaning solutions (MPa)

RP/solutions	HP/HP	HP/DA	HP/CHX	HP/OA
Cervical	**10.71** + 1.74 Aa	**8.44** + 2.17 Aab	**6.93** + 1.13 Aa	**9.27** + 1.24 Ab
Medium	**9.47** + 2.29 Aa	**6.57** + 1.48 ABbc	**5.58** + 1.16 Ac	**7.38** + 1.12 Bab
Apical	**6.3** + 1.98 Ba	**5.69** + 1.36 Bb	**3.31** + 0.96 Ba	**5.6** + 1.24 Cb

Different letters indicate statistically significant differences, lowercase letters for row and uppercase for column, $p < 0.05$
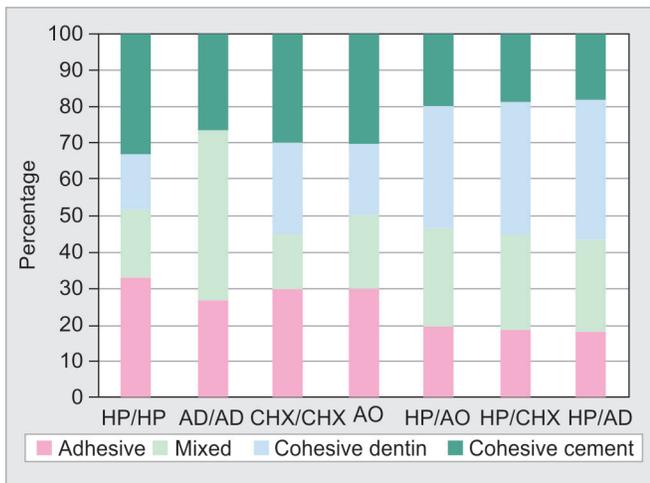

Fig. 1: Distribution of failure frequencies in the experimental groups according to the combination of evaluated solutions. HP, 2.5% sodium hypochlorite; DA, distilled water; CHX, 2% chlorhexidine; OA, ozonated water

predominant HP/AD group failure mode was adhesive followed by cohesive failure in cement and mix failure.

DISCUSSION

According to the results of the present study, irrigants and dentin-cleaning solutions can influence the bond strength of fiberglass posts cemented with the U200 self-adhesive resin cement, corroborating previous findings in the literature and rejecting the first hypothesis of this study.[15,16] The highest statistically significant bond strength values were observed in groups treated with ozonated water, sodium hypochlorite, and sodium hypochlorite in association with ozonated water. This indicates that the irrigant does not necessarily have to be the same as the dentin-cleaning solution, which is in agreement with the hypothesis of this study.

Sodium hypochlorite has a nonspecific proteolytic action, i.e., the ability to dissolve pulp tissue and organic components of the dentin.[17] It also has the ability to partially neutralize necrotic tissues

or any antigen or microbial component remaining in the root canal and remove all pulp remnants on the noninstrumented dentin surface.[18] The chemicals used during root-canal preparation might alter the composition and interaction of the dentin surface with restorative materials. In the literature, there are contradictory results on the effect of dentin treatment with sodium hypochlorite on bond strength. Sodium hypochlorite has the ability to degrade collagen and is, therefore, capable of affecting the bonding capability of root dentin.[8] The duration of dentin exposure to sodium hypochlorite has been reported to be negatively correlated with bond strength. Exposure of dentin to a high concentration of sodium hypochlorite for a long duration can reduce the adhesive strength and cause root fracture.[19] Nevertheless, the high bond strength values observed in the HP group in the present study can be explained by the moderate sodium hypochlorite concentration that was used (2.5%) as well as the low exposure time (20 seconds), which contributed to root-canal cleaning without collagen degradation. At a concentration of 2.5%, sodium hypochlorite acts on the organic components of dentin and improves the penetration ability of monomers into the dentin structure.[20] High tissue toxicity, unpleasant smell and taste, allergic potential, and inability to remove the smear layer are the disadvantages of NaOCl.[21] Thus, the important thing is to find an irrigating agent with an antibacterial effect without causing tissue damage.

The combination of sodium hypochlorite and ozonated water also provided significantly higher bond strength outcomes relative to the other groups in the present study. Considering the effect of dentin-cleaning solutions (in particular sodium hypochlorite) on dentin and the limited effect of acid conditioning of self-adhesive cements on the dentin surface ozonated water might have complemented the action of sodium hypochlorite, exporting more calcium to the surface, which might have improved the bond strength of the cementing agent.[22] This result can be explained by the antioxidant property of ozone. Ozone is produced by the dissociation of molecular oxygen (O_2) into free oxygen, which is capable of reacting with other oxygen molecules.[11] This anionic radical is protonated and generates hydrogen trioxide (HO_3), which, in turn, decomposes into a stronger antioxidant agent than the hydroxyl group (OH).[23] Previous studies have reported

similar results that ozonated water promotes dentin cleaning without affecting the bond strength of self-etching adhesive systems.[7,24] Thus, its high mechanical cleaning power is achieved by the release of oxygen, which exposes a greater number of dentin tubules to the possibility of mechanical bonding with the cementing agent.[25] The disadvantage of ozonized water is its chemical instability and the need for the ozonator for its production. Skowron et al.[26] reported the ozonated water, used immediately after preparation, was effective for all tested isolates antimicrobial tests. However after 1 and 2 hours of storage of ozonated water its activity decreased.[26]

However, in an evaluation of different forms of cavity cleaning before restoration with composite resin, Rodrigues et al.[27] reported contradictory results, where the groups that were treated with ozonated water after acid conditioning showed a statistically significant decrease in bond strength values relative to the other groups. Bitter et al.[28] evaluated the effects of root cleaning with ozone by the push-out test and found that the bond strength decreased significantly with the association of ozone and Rely X Unicem self-adhesive resin cement. This might have been due to the use of chlorhexidine as an irrigant for all groups and the use of ozone in gaseous form, which might have prevented the decrease in bond strength. In the present study, the use of chlorhexidine also resulted in significantly lower bond strength relative to the other combinations. In contrast, when Schmidlin et al.[25] performed a microcut test to evaluate the effects of ozone application to the dentin and enamel of bovine teeth on bond strength, and they did not observe a decrease in adhesion between the dental substrate and composite resin.

Previous reports on the effects of chlorhexidine on bond strength are controversial. Lenzi et al.[29] reported that chlorhexidine does not impair the adhesion capacity of dentin, whereas Hiraishi et al.[19] suggested that chlorhexidine has adverse effects on adhesion systems. Applying an MMP inhibitor on the dentin surface before adhesion or incorporating an MMP inhibitor into the adhesive system might help improve the stability and integrity of the adhesive interface over time.[25,30–32] Nevertheless, in the present study, the combination of chlorhexidine and distilled water produced the lowest bond strength values. It can be explained that the main limitation of CHX as an endodontic irrigant is its inability to dissolve pulp tissue and smear layer.[33] However, Guardins et al.[20] reported contradictory results, which revealed that aged samples pretreated with 2% chlorhexidine showed higher bond strength values than those that did not receive the treatment; this indicated that the use of chlorhexidine resulted in better adhesive interface stability. However, these tooth samples had been aged for 30 days before the bond strength test, unlike the samples used in the present research. To promote the stability of the hybrid layer, chlorhexidine requires some time to interact with the MMPs in dentin.[31] In the present study, the adhesive resistance test was performed within 7 days after treatment, which might have been insufficient for the action of chlorhexidine on MMPs. Further *in vitro* and *in vivo* studies are needed to clarify the effect of chlorhexidine on dentin adhesiveness.

In the present study, bond strength was found to be affected by the root level, with the lowest values being recorded in the apical region and the highest values in the cervical region, which is contradictory to our third hypothesis but in agreement with the results of previous studies.[11,17] The narrow and deep characteristics of the apical region are associated with the brittleness of the bond, restricted flow of the cementing agent, and difficulty of access to the apical region, which could explain this result.[32] However, some studies have not found any significant difference in bond strength according to the root region.[32,34] These conflicting findings indicate that adhesive procedures performed in the apical region are challenging and sensitive to the technique used.

The hybrid layer is more prone to adhesive failure due to wear, as seen in the present study.[35] The samples did show mixed and cohesive failures, albeit less predominantly. Despite the limitations of the present *in vitro* study, the clinical indication for the use of ozonized water is restricted due to ozone instability, requiring clinical studies to reinforce the results found in the present *in vitro* study.

CONCLUSION

Within the limitations of this study, we have demonstrated that among the different irrigants and dentin-cleaning solutions evaluated, ozonated water and sodium hypochlorite present the best results in terms of their effect on the bond strength of a self-degrading cement. In this sense, ozonated water—despite its difficult preparation process and limited usage time—might be a promising solution for both cleaning and irrigation, especially for patients allergic to sodium hypochlorite. More studies are needed on the use of ozonated water in dentistry.

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