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RESEARCH

Root canal irrigants and their role in the adhesion of pre-endodontic restorations

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Abstract

Objectives To evaluate the influence of root canal irrigating protocols on the micro-shear bond strength (µSBS) and endogenous enzymatic activity (MMPs) of pre-endodontic resin composite restorations at baseline and after artificial aging.

Materials and methods Deep dentin surfaces from human molars ($n = 16$) were exposed and embedded in acrylic resin. A universal adhesive (iBond Universal, Kulzer) and resin composite (Venus Pearl, Kulzer) simulated pre-endodontic restorations. The following groups were formed according to the endodontic irrigating protocol ($n = 20$/group): C (no treatment); SH (5.25% NaOCl + water rinse); CHX (SH + 2% chlorhexidine rinse); EDTA (SH + EDTA rinse). Additional teeth ($n = 3$) were used for in situ zymography. µSBS and MMPs activity were assessed after 24 h or 10,000 thermocycles (5–55 °C, 30s). Data were statistically analyzed ($p < 0.05$).

Results Irrigation, aging, and their interaction significantly influenced µSBS ($p < 0.001$). At baseline, EDTA showed the highest µSBS ($p < 0.05$), while C, SH, and CHX were statistically similar ($p > 0.05$). Thermocycling reduced µSBS in all groups, with no post-aging differences among irrigating protocols ($p > 0.05$). Enzymatic activity was affected by irrigation and aging. SH showed the highest MMPs activity while CHX the lowest ($p < 0.05$). No differences were observed between EDTA and C ($p > 0.05$).

Conclusions Irrigating solutions do not affect the adhesive bond strength of pre-endodontic resin composite restorations after aging. However, enzymatic activity increases after NaOCl, but is reduced when NaOCl is combined with EDTA or CHX.

Clinical relevance Clinically, this supports preserving interproximal pre-endodontic resin composite walls for final restorations.

Keywords Shear bond strength · Irrigating solutions · Endodontically treated teeth · Pre-endodontic restorations · Metalloproteinases · MMPs

Introduction

Among others, destructive caries, trauma and root resorption can undermine the structural integrity of the teeth [1]. In order to preserve as much of the tooth structure and avoid extraction, root canal treatments and resin composite restorations for subsequent prosthetic rehabilitation are routinely performed [2]. Endodontic treatment aims to eliminate or prevent infections within the root canal system by ensuring thorough cleaning, disinfection, and sealing of the canals, thereby maintaining tooth functionality over time. In a context where mechanically instrumentation alone cannot provide full decontamination, irrigation is considered a fundamental procedure for reliable root canal disinfection [3, 4]. Various irrigating solutions are used during both orthograde and retrograde procedures to enhance cleanliness, eliminate bacteria, and dissolve the smear layer. According to their mechanism of action, the root canal irrigants can be divided into antibacterial and decalcifying agents or both, aiming at overcoming anatomical complexities and

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structural variabilities. Among these, sodium hypochlorite (NaOCl) represents the gold standard solution either alone or in sinergy with agents like EDTA, citric acid, or chlorhexidine (CHX) to enhance antimicrobial efficacy and improve treatment outcomes [5] [6].

To ensure the proper use of irrigants in cases of significant coronal structure loss and protect the tooth's functional integrity, it is highly recommended to perform a pre-endodontic restoration before proceeding with root canal treatment [7]. Indeed, these restorations improve the predictability of root canal treatments by facilitating rubber dam placement [8], enabling safe irrigation [9], preventing fractures [10], maintaining reference points [7], improving esthetics between appointments, reducing the risk of temporary restoration loss [11] and, therefore, securing the marginal seal during or after the endodontic treatment [12].

Adhesive resin composite materials traditionally represent the first choice for pre-endodontic restorations [7]. However, numerous studies have shown a high propensity of morphological and chemical alterations of composite materials after contact with chemical substances, such as those routinely used for intra-canal irrigation during the endodontic treatment [13–18]. This issue can largely be attributed to the prolonged contact time between the irrigant solution and the composite resin restoration (Fig. 1), highlighting the potential role of chemical exposure duration in compromising the integrity of the composite material as well as its adhesion properties [17].

For instance, microscopic and spectroscopic investigations have demonstrated that NaOCl removes the superficial subsurface organic phase from mineralized dentin in a concentration-time dependent manner [19]. This results in a dentin substrate that is more brittle than untreated dentin [20]. Moreover, EDTA subsequently removes the mineral (apatite), leading to dentin

Fig. 1

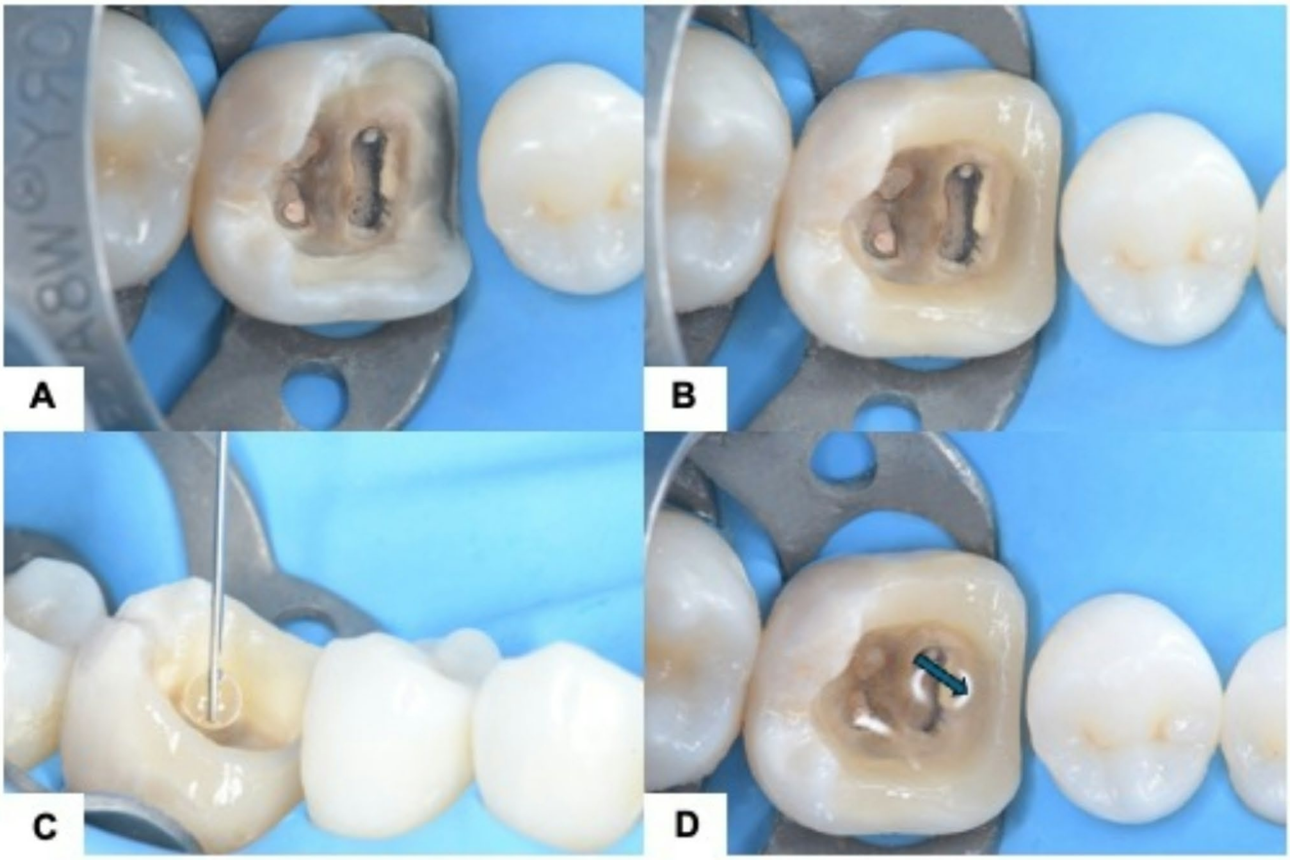

Fig. 1 Clinical case of maxillary first molar (#1.6) requiring endodontic re-treatment. Extensive carious involvement of the mesial wall necessitated its complete removal (**A**). Following adhesive procedures, the interproximal wall was reconstructed using a resin composite material (**B**). The re-establishment of the mesial wall allowed for improved rubber dam isolation and safer, more controlled endodontic procedures, particularly during the irrigation phase (**C**). Depending on the irrigation protocol adopted, irrigants may remain in contact with the adhesive interface for extended periods (blue arrow in **D**), raising concerns about their potential impact on the marginal integrity and bonding durability of the recent restoration. Due to this uncertainty, repeating the adhesive procedure and placing a new composite restoration might be required at the end of the endodontic treatment

erosion [19, 21]. The same irrigant solutions have been also proposed to influence the dentinal matrix metallo-proteinases (MMPs) activity within the hybrid layer (HL) [38]. The MMPs, and in particular MMP-2 and MMP-9, are enzymes that play a fundamental role in the degradation of the organic matrix of dentin, a process that can significantly compromise the stability of adhesive restorations [22]. This enzymatic activity represents a critical challenge in dentistry, as it contributes to the deterioration of HL, a zone where collagen fibrils, which are rich in water and poorly infiltrated by resin, provide the structural basis for adhesive bonding. To counteract this issue and enhance the durability of adhesive restorations, extensive research has focused on strategies to reinforce these collagen fibrils and make them more resilient [23–26]. In this context, various studies have explored potential substances that can inhibit the activity of MMPs, aiming to prevent the enzymatic breakdown that weakens the adhesive bond over time [27–38]. Among the inhibitors studied, CHX, as usually employed during the endodontic treatment, has emerged as particularly noteworthy due to its well-documented mechanism of action and its proven ability to preserve the integrity of the adhesive interface [39]. On the other hand, other agents such as EDTA and NaOCl have also been investigated [37, 38], but their impact on enzymatic activity has not been as thoroughly examined, leaving room for further exploration in this area.

All in all, the dentin substrate structural changes could possibly impact on the lifespan of the pre-endodontic resin composite restoration [2–13– [15–18]. According to this assumption, from a clinical point of view the decision on whether replace or maintain the composite resin restoration after the endodontic treatment can be operatively demanding. It seems that to date, the literature has not yet provided a definitive answer on this topic.

Therefore, this study aimed to evaluate the influence of different irrigating solutions used during endodontic treatment on the immediate (T0) and artificially aged (TC) micro-shear bond strength (μSBS) and endogenous dentinal enzymatic activity of pre-endodontic adhesive resin composite restorations. The null hypotheses tested were that: (1) intra-canal irrigants produced no effect on bond strength between the dentin and the pre-endodontic resin composite restoration immediately and after TC; (2) intracanal irrigants produced no effect on endogenous enzymatic activity immediately and after TC.

Materials and methods

Sample preparation

Sixteen freshly extracted non-carious human molars were obtained from anonymous individuals following their informed consent under a protocol approved by the Ethical Committee of the University of Bologna, Italy (protocol N°: 71/2019/OSS/AUSLBO). Teeth were collected and stored in a physiological solution at 4 °C until use, however no more than 1 month. Teeth were carefully observed under a stereomicroscope and those with signs of caries, posts, crowns, previous endodontic treatments and restorations, demineralization, abrasion, fractures and cracks were not included. Tooth crowns were removed using a low-speed diamond saw under water cooling (Microremet, Remet, Casalecchio di Reno, Italy) to expose deep coronal dentin, proximal to the pulp chamber. The cut was made 1 mm above the cemento-enamel junction (CEJ). Subsequently, the roots of the molars were removed with a second cut at a more apical level. Thus, the resulting tooth samples had an enamel portion very close to the CEJ and deep dentin, simulating clinical conditions (Fig. 2A). Afterwards, the specimens were examined under a stereomicroscope to ensure they were free from defects and embedded in acrylic resin (ProBase Cold resin, Ivoclar vivadent technical, Schaan Liechtenstein) using a cylindrical mold taking care to not touch the exposed dentin-enamel surface (Fig. 2B). Finally, the specimens were ground with a #180-grit wet silicon-carbide (SiC) abrasive paper until a flat enamel and dentin surfaces were obtained. Consequently, a standardized smear layer was created by #240-grit wet SiC paper. To closely replicate the clinical scenario of a pre-endodontic resin

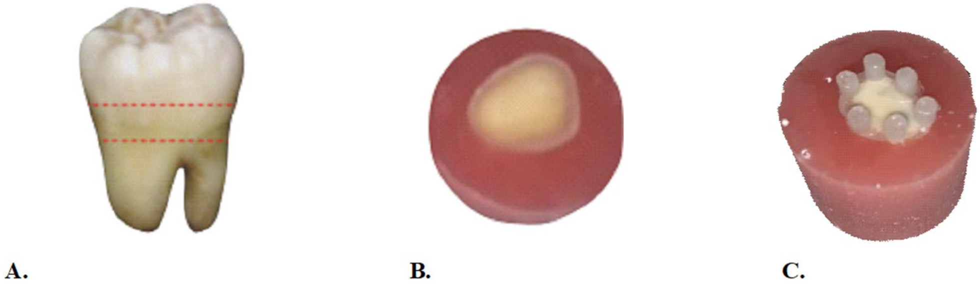

Fig. 2 Representation of specimens' preparation. **A**: discs of deep dentin were exposed **(A);** The dentin discs were embedded in self-curing acrylic resin **(B);** resin composite cylinders were bonded at the periphery of the deep dentin discs, to simulate clinical procedures of pre-endodontic restorations **(C).** In this conditions, the specimens came in contact with the irrigating solutions for the time estimated according to the testing group

composite restoration, the adhesive procedures were performed at the periphery of the teeth, both on the enamel and dentin (Fig. 2C).

The materials used for restorative procedures are represented in Table 1 and handled strictly following manufacturer's instructions.

A universal bonding system used in self-etch mode (IBOND Universal, Kulzer, Hanau, Germany) was applied with a micro brush, rubbed for 20 s, air-dried for 5 s and light-cured for 10 s with a light-emitting diode (LED) lamp (Curing Light Elipar™ DeepCure-L, 3 M™, St Paul, MN, USA; wavelength range 430–480 nm; light intensity 1.47 mW/cm²). A teflon ring mold (1.84 mm x 3 mm) [40] was placed on the periphery of the teeth and used to produce resin composite build-ups. The mold was filled with two 1.5-mm thick increments of a nanohybrid resin composite (shade A3; Venus Pearl, Kulzer, Hanau, Germany), and each layer was light-cured for 20 s. Excess of composite was meticulously removed with a dental explorer before final curing. Given that a sample size of $n = 10$ per group ensures at least 80% statistically significant differences with a standard error value of 0.05, a total of 20 resin composite build-ups per group were used. At the completion of the restorative procedures, the specimens were stored in distilled water in an incubator at 37 °C until use.

The following groups were formed based on the irrigation protocol performed ($n = 20$): **C**: No treatment; **SH**: 5.25% sodium hypochlorite for 10 min + final water rinse; **CHX**: SH + final 2% chlorhexidine rinse for 2 min; **EDTA**: SH + final EDTA rinse for 2 min. The irrigating solutions were then placed in specific containers, and the previously prepared samples were positioned upside down inside the containers, ensuring that they remained in constant and complete contact with the irrigants for the required duration. After each irrigating protocol, the specimens were washed with water. Then, half of the specimens ($n = 10$/group) were stored in artificial saliva and incubated in a humidified chamber at 37 °C overnight before being tested with the µSBS test (T0). The second half was subjected to thermal cycles (TC; THE100, Mechatronik, Feldkirchen-Westerham, Germany) with 10,000 cycles, dwell time of 30s at 5 to 55 °C and 5 s interval between each bath.

Micro-shear bond strength test (µSBS)

At the completion of each storage period, the specimens were mounted in a testing machine (Ultra Tester™, Ultradent, Cologne, Germany) and the µSBS test was executed. The machine's chisel-shaped blade was positioned at the adhesive interface to measure the bond strength at a crosshead speed of 0.5 mm/min. The µSBS was calculated as megapascals [MPa] using the following formula: stress [MPa] = force [N]/bonding area [mm^2] [40].

In situ zymography

Additional 3 teeth were used for the in situ zymography and MMPs activity quantified at baseline and after TC. The teeth used in this case were immediately frozen after extraction and thawed at the time of sample preparation. Two 1-millimeter-thick slabs of middle/deep coronal dentin were obtained from extracted human third molars using a low-speed saw (Micromet) under water-cooling. Two dentin slabs were obtained from each molar. Each slab was further divided into 4 pieces so that testing of the four experimental groups was performed using the same dentin substrate [41]. A standardized smear layer was created on each dentin surface using #240-grit silicon carbide paper under water cooling. Identical bonding procedures were performed as previously described for the µSBS test, forming the same 4 groups according to the different irrigating protocols. Resin-dentin interfaces were exposed by cutting the bonded specimens vertically into 1 mm-thick sticks using the slow-speed saw under water cooling. Half of the specimens were tested after 24 h (T0) and the other half after 10,000 TC. After aging, the sticks were fixed to glass slides with cyanoacrylate glue ground down and polished to obtain ~ 50 µm thick slabs using a series of wet silicon carbide papers. Self-quenched fluorescein-conjugated gelatin was used as the MMPs substrate (E-12,055, Molecular Probes, Eugene, OR, USA) for in situ zymography at T0 and TC [41]. The fluorescent gelatin mixture was placed on top of each slab and covered with a glass coverslip. The slides were incubated in a humidified chamber at 37 °C overnight or subjected

Table 1 Composition of iBond universal and Venus Pearl (Kulzer). DUDMA: polyurethane dimethacrylate; 10-MDP: 10-Methacryloyloxydecyl dihydrogen phosphate; 4-META: 4-methacryloxyethyl trimellitate anhydride; TCD-Urethane Acrylate: Toluene-2,4-Diamine-Urethane acrylate; UDMA: urethane dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; BHT: butylated hydroxytoluene

Product	Component	Description
iBond Universal	**Methacrylate Monomer**	DUDMA, 10-MDP, 4-META
	Solvents	Acetone, Water
	Additional Components	Stabilizers, Camphorquinone, Ethanol, Amine Accelerator
Venus Pearl	**Resin Matrix**	TCD-Urethane Acrylate, UDMA, TEGDMA
	Filler	Barium-Aluminum-Boro-Fluoro Silicate Glass, Silica, Titanium Dioxide
	Pigments	Fluorescent Pigments, Metal Oxide Pigments, Organic Pigments
	Initiators and Additives	Aminobenzoic Acidifying Agent, BHT, Camphorquinone

Table 2 μSBS test values in MPa (mean ± standard deviation). Different uppercase letters indicate statistically significant differences ($p < 0.05$) within the same row; different lowercase letters indicate statistically significant differences ($p < 0.05$) within the same column. C: control group; SH: sodium hypochlorite; CHX: chlorexidine; EDTA: Ethylenediaminetetraacetic acid

	T0	T1
C	18.1 ± 6.4 b, A	10.3 ± 5.3 a, B
SH	16.1 ± 5.2 b, A	7.1 ± 3.2 a, B
CHX	16.7 ± 6.4 b, A	8.5 ± 2.5 a, B
EDTA	22.7 ± 7.8 a, A	8.0 ± 3.3 a, B

to thermocycling. During incubation, the assemblies were prevented from direct contact with water and were protected from exposure to light. After incubation, the microscopic slides were examined using a confocal laser scanning microscope (laser excitation wavelength 488 nm; emission wavelength 530 nm; Leica SP8, Leica Microsystems GmbH, Wetzlar, Germany). To visualize the hydrolysis of the quenched fluorescein-conjugated gelatin substrate as an indicator of endogenous gelatinolytic activity, 3 z-stack images (~ 15 μm thick) were made per specimen. The steps between the optical sections were of 1 μm and the z-stacks were made from the top of the specimen and into the depth of ~ 15 μm. Enzymatic activity was quantified as the integrated density of the fluorescence signals by means of the ImageJ software (National Institutes of Health, Bethesda, MD, USA) using a rectangular selection (100 × 20 μm) placed over the HL and dentin (3 measurements per image). The quantification was performed after discarding several initial slices as to exclude parts of the specimen with the background noise in the signal attributed to the activated gelatin substrate on top of the specimen. After the removal of the initial slices also the differential interference contrast (DIC) image reached maximum focus and this point was considered repeatable and ideally suited for the execution of the measurements in all the specimens.

Statistical analysis

The data from the μSBS test (calculated in MPa) and in situ zymography were normally (Shapiro-Wilk test) and homogeneously (Brown-Forsythe test) distributed ($p > 0.05$). Consequently, two-way analysis of variance (2-way ANOVA) and pairwise multiple comparison procedures (Holm-Sidak method) were used to evaluate the effects of factors: "irrigating protocol" and "aging". Statistical significance was set at $\alpha = 0.05$ (SigmaPlot 14.0; Systat Software Inc., Berkshire, UK).

Results

The bond strength results are illustrated in Table 2.

The two-way ANOVA demonstrated that μSBS values were statistically influenced by both the irrigation protocol ($p = 0.011$) and aging ($p < 0.001$), with their interactions being also statistically significant ($p = 0.016$). The pairwise multiple comparison procedures (Holm-Sidak method) demonstrarted that at T0, irrigation with EDTA achieved the highest bond strength values compared to all the other groups (EDTA > SH, $p = 0.001$; EDTA > CHX, $p = 0.002$; EDTA > CTR, $p = 0.032$), while no differences were observed among C, SH, and CHX groups ($p > 0.05$). TC significantly reduced bond strength values in all groups ($p < 0.001$), with no differences observed between the various irrigating protocols ($p > 0.05$).

The results of gelatinolytic activity, expressed as green fluorescence intensity within the HL, are illustrated in Figs. 3 and 4.

The enzymatic activity was significantly influenced by the irrigation protocol ($p < 0.001$) and aging ($p < 0.001$), with their interactions being statistically significant ($p < 0.001$), as demonstrated by the two-way ANOVA test. At T0, the SH group exhibited the highest enzymatic activity (SH > C, $p = 0.009$; SH > CHX, $p < 0.001$; SH > EDTA, $p = 0{,}033$), while the EDTA

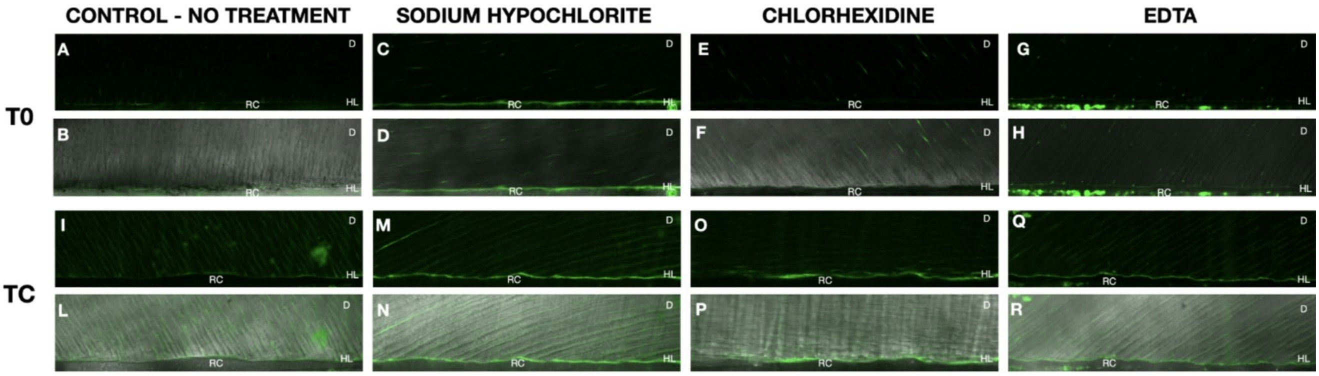

Fig. 3 In situ zymography analysis of the resin/dentin interfaces. RC: resin composite; HL: hybrid layer; **D**: dentin. **A, C, E, G, I, M, O, Q**: images acquired in green channel, showing fluorescence (the greater the green fluorescence, the higher the enzymatic activity) in dentinal tubules and within the HL. **B, D, F, H, L, N, P, R**: images obtained by merging differential interference contrast image (showing the optical density of the resin-dentin interface)

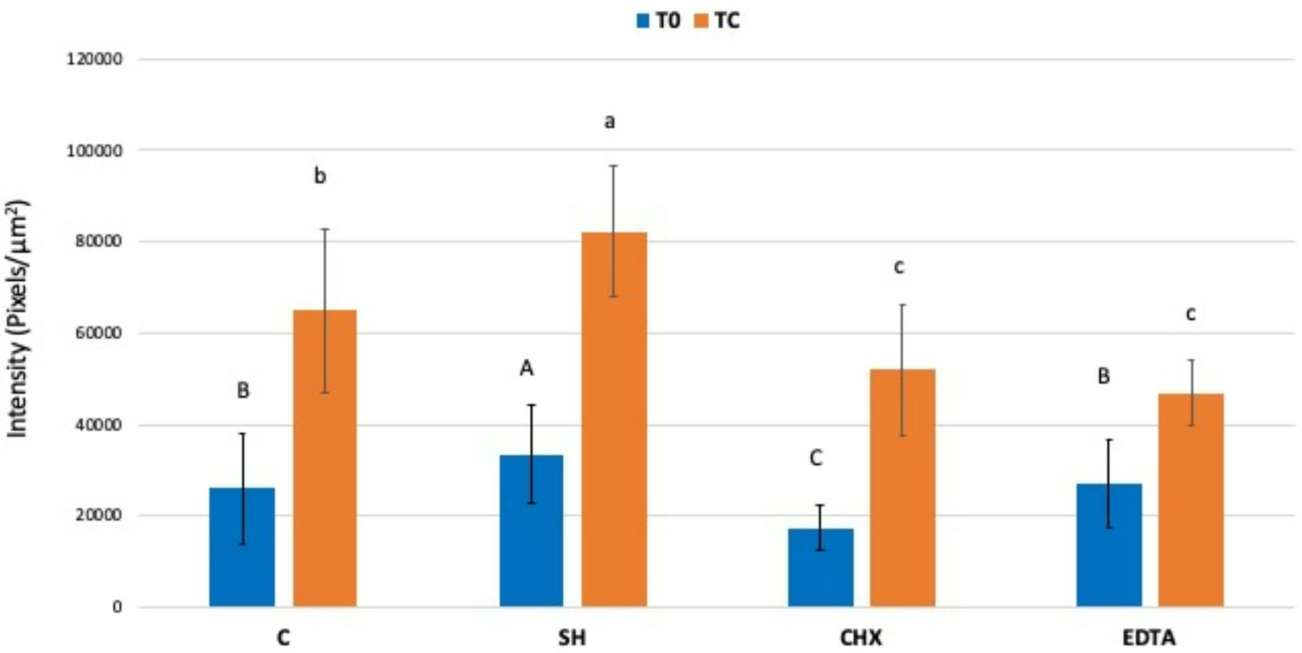

Fig. 4 Graphical representation of gelatinolytic activity values, expressed as the percentage of green fluorescence in the resin/dentin interfaces within the radicular dentin created in the experimental groups. The values are represented as means ± SD. Groups significantly different from each other ($p < 0.05$) were marked in different uppercase letters at T0 and different lowercase letters at TC. There was a significant increase in enzymatic activity in all the groups after aging ($p < 0.05$)

group showed similar values to the C group ($p = 0.525$). In contrast, the CHX group demonstrated the lowest fluorescence values (EDTA > CHX, $p < 0.001$; CTR > CHX, $p = 0.004$). After TC, the enzymatic activity increased across all groups ($p < 0.001$). Specifically, the post-hoc test demonstrated that the SH group recorded the highest overall values, followed by the C group (SH > C, $p < 0.001$; SH > CHX, $p < 0.001$; SH > EDTA, $p < 0.001$; C > CHX, $p < 0.001$; C > EDTA, $p < 0.001$). The CHX and EDTA groups exhibited lower and comparable enzymatic activity ($p = 0.079$).

Discussion

This study evaluated the influence of different intra-canal irrigation protocols on the adhesive performance and endogenous activity of dentin in pre-endodontic resin composite restorations. Our results showed that, initially, the irrigation protocol significantly influenced the adhesive properties only when EDTA was used, while no differences were observed between C, SH, and CHX. However, these differences disappeared after TC, with comparable bonding values between groups. Accordingly, over time, the irrigation protocol has no impact on the retention of resin composite pre-endodontic restoration. Therefore, the first null hypothesis must be partially accepted.

When a carious lesion extends subgingivally below the CEJ, reconstructing proximal walls is crucial for proper rubber dam isolation, facilitating safe and effective use of irrigants during the endodontic treatment (Fig. 1) [42, 43]. Considering the findings of this in vitro study, the irrigation solutions used after adhesive and restorative procedures do not significantly impact the bond strength performance of resin composite restorations. Clinically, this could suggest that the pre-endodontic interproximal wall reconstruction can be maintained and incorporated into the final restoration. This can be considered a beneficial aspect from a procedural standpoint, as the absence of significant effects on the restoration's behavior eliminates the need of a second intervention that would result in increased operative chair time and additional cost for the patient.

Regarding the use of NaOCl followed by a final water rinse, the data showed a slight decrease in bond strength values, but this was not statistically significant, both when comparing the groups within T0 and TC. NaOCl is believed to oxidize components in the dentin matrix [32, 33], producing protein-derived radicals that may compete with the vinyl-free radicals from resin adhesives during light activation [45]. This process could lead to premature chain termination and incomplete resin polymerization. Additionally, irrigation with 5% NaOCl has been shown to weaken the mechanical properties of dentin, such as elastic modulus, flexural strength, and microhardness [47]. According to some authors, these changes may further impair the micromechanical interaction between adhesive resins and NaOCl-treated dentin [46, 47]. Some studies have suggested that this irrigating solution may reduce the adhesive bond strength to composite materials [44–52]. On the other hand, other authors have claimed that, when used in a clinically-relevant scenario, NaOCl may improve or have no impact on adhesive bond strength [51]. Patil et al. [48] have tested resin composite bulk-fill samples, simulating pre-endodontic reconstruction and using different NaOCl concentrations (1%, 3%, and 5%, respectively) to analyze the influence of the irrigant compared to the control group treated with only distilled water. Although the mechanical characteristics of the restoration were not evaluated in this study, Patil et al. have observed changes in the composite structure and microhardness were observed [48]. However, it should be noted that the specimens were immersed in NaOCl for 40 continuous min, which represents a condition different from a clinical scenario and also from our in vitro study. Morris et al. [44] also found that the use of NaOCl alone decreases adhesive bond strength, and that this can improve when 10% ascorbic acid or 10% sodium ascorbate are applied. In their study, the irrigant was used for a longer duration than in our study (20 min) and rinsed with only 10 ml of water. These differences could have influenced the adhesion of the composite resin and explain the different results obtained. In another article [50], Previously, clinicians have been advised to carefully evaluate the pre-endodontic restoration after the use of NaOCl, as this irrigant has been shown to reduce adhesive bond strength [49, 50]. Similarly, our study also observed a decrease in bond strength when NaOCl was used as an irrigant, although not to a degree considered statistically significant. It should be, however, pointed out, that these studies presented methodologies completely different from each other, with particular, clinically challenging, increased time of immersion in irrigating solutions (from 30 to 40 min) [48–50]. The article by Cecchin et al. [51], on the other hand, achieved improvements in adhesive bond strength following the use of NaOCl by simulating clinical practice and rinsing the samples thoroughly with water for 60 s. This may emphasize the need of thoroughly water rinsing for an adequate amount of time in order to have the likelihood to maintain the pre-endodontic resin composite proximal wall as definitive reconstruction.

When using EDTA as irrigant solution, enhanced resin composite-tooth adhesion patterns have been observed [53]. EDTA is a molecule with 4 carboxylic acid groups capable of chelating calcium. It is widely used to dissolve the mineral component of dentin while preserving the protein content and maintaining the native fibrillar structure of collagen [54]. Studies [53– [55–57] have suggested that the collagen fibrils remain largely intact and retain most of their intrafibrillar minerals. As a result, these fibrils are less prone to dehydration, as the mineral structure provides essential support, which also facilitates resin infiltration [55]. Osorio et al. [57] have discovered in their in vitro study that specimens pre-treated with EDTA instead of phosphoric acid showed no significant decrease in bond strength after immersion in NaOCl. This may be due to an improved resin infiltration into the EDTA-demineralized collagen matrix, due to residual mineral in the collagen fibrils that increases the stability of the organic matrix. Other authors [50] also emphasize that the use of EDTA as a pretreatment does not appear to negatively affect the adhesive bond strength between deep dentin and composite. In the aforementioned study, different irrigating solutions (CHX, NaOCl, and combinations of these agents) were tested as dentin pretreatments. Their conclusion was that these solutions do not affect the adhesive bond of the final composite restoration, except for NaOCl, which, as previously explained, has been shown to reduce adhesive bond strength [44–50].

The activation of silenced endogenous dentinal enzyme has been considered one of the major factors affecting the durability of the bonds. Various MMPs (types 2-, 3-, 7-, 8-, 9-, and 20-) have been identified in dentin, pulp tissue, saliva, and carious lesions [58]. Gelatinases (MMP-2 and MMP-9) are particularly studied for their role in degrading the HL [32, 34, 59, 60], especially in low pH conditions like acid etching [28, 58, 61]. These enzymes target the type I collagen matrix, which is a major component of dentin extracellular matrix, thus playing a key role in adhesive dentistry [35, 36]. MMP-2 and MMP-9 were identified also in root dentin [62], with MMP-2 present across all dentin compartments, while MMP-9 required extensive demineralization for detection. Agents like EDTA and citric acid have been shown to inhibit MMPs by exposing dentin proteins and collagen fibers [34]. Similarly, CHX has been proven to inhibit MMP activity, reducing their impact on the adhesive interface and stabilizing the bond [39, 62]. Two immunohistochemistry studies [38, 39] confirmed that common irrigants (NaOCl, EDTA, citric acid and CHX) inhibit MMPs expression. When CHX is used as a final rinse, MMP activity is significantly reduced, preventing HL degradation and enhancing bond longevity [39]. Using 2% CHX after acid etching has been particularly effective in stabilizing the adhesive interface and preventing bond failure [32, 38, 39, 63–66]. This aligns with the findings of our in vitro study, where the specimens treated with CHX showed the lowest fluorescence levels (Fig. 2). However, after thermocycling, enzymatic activation values increased across all groups (Fig. 3), confirming that MMPs activity consistently rises with sample aging. Accordingly, the second null hypothesis, regarding the effect of the tested irrigating solutions on dentin enzymatic activity, had to be rejected, as they can influence the metalloproteinases activity both at T0 and TC.

Nonetheless, it was observed that in the samples treated with CHX, enzymatic activation was still lower compared to the other groups (Fig. 4). CHX is a cationic bis-biguanide effective against microbes within a pH range of 5.5 to 7.0, acting by disrupting cell walls and internal components [67]. CHX is widely employed as a disinfectant, preservative, and antiseptic across medical, pharmaceutical, and dental applications [40, 69]. As a bis-biguanide, it is a strongly basic salt, and its original forms (CHX acetate and hydrochloride) are the most stable. However, due to its poor water solubility [69, 70], CHX digluconate has become the preferred alternative [70]. Erdemir et al. [71] found that using CHX for endodontic irrigation improved bond strength to root dentin, possibly due to its adsorption enhancing resin penetration into dentinal tubules. In their study, CHX did not influence the SE adhesive system's performance in pulp chamber dentin, being considered a non-oxidizing agent [71]. Furthermore, a group of authors demonstrated that the adhesive bond strength between dentin and composite is not affected by the irrigating solution, irrespective of the delivery form (liquid or gel) [50]. A recent literature review by Josic et al. [40] has concluded that, despite several in vitro findings, currently there is still no evidence that supports or discourages the use of CHX to improve the prognosis of adhesive-bonded composite restorations. Discrepancies in methodology and conclusions between laboratory and clinical studies were observed. Results from our study indicate the effectiveness of CHX as an MMPs inhibiting agent. These results were not translated directly to the bond strength properties, demonstrating that CHX does not influence bonding efficacy at baseline or after 1-year of simulated aging. However, due to the MMPs inhibitory activity, its beneficial effects might become notable only after a prolonged aging time.

The effects of the irrigants observed on dental substrates in this study may be specific to the particular universal adhesive and composite materials used. Different adhesive systems or composites might interact differently with the same irrigating solutions, potentially leading to variations in outcomes. In particular, the present study focused on the universal adhesive used in the SE mode. It could be interesting to investigate whether, in EAR mode, enzymatic activity and adhesive bond strength might change. Additionally, the experimental conditions in this study may not fully replicate the complexities of the oral environment, such as variations in pH, temperature, or mechanical stresses, as well as insertion methods (i.e. agitation, ultrasound activation etc.) or the structural changes that occurred after endodontic treatments. Therefore, clinical trials and further in vitro studies are necessary to better understand the broader implications of irrigating solutions on the functional, biological, and esthetic properties of pre-endodontic restorations.

Conclusions

Irrigating solutions do not affect the adhesive performance of composite restorations after artificial aging. However, enzymatic activity increases when using NaOCl followed by rinsing with distilled water or decreases when NaOCl is combined with EDTA or CHX. Clinically, this supports the possibility of preserving the pre-endodontic resin composite reconstruction of the interproximal wall for the final restoration.

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Declarations

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