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Influence of Chlorhexidine 2% and Sodium hypochlorite 5.25% on Micro-tensile Bond Strength of Universal adhesive system (G-Premio Bond)

Nafiseh Fazelian[1,*], Abbas Rahimi Dashtaki[2], MohammadAmin Eftekharian[2], Batool Amiri[3]

[1] Department of Restorative Dentistry, Dental School, Bushehr University of Medical Sciences, Bushehr, Iran

[2] Dental School, Bushehr University of Medical Sciences, Bushehr, Iran

[3] Clinical Research Development Center, Bushehr University of Medical Sciences, Bushehr, Iran

Corresponding author:
Nafiseh Fazelian
Assistant Professor, Department of Restorative Dentistry, Dental School, Bushehr University of Medical Sciences, Bushehr, Iran
n.fazelian@bpums.ac.ir
Tel: 09133804812

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Aim: One of the reasons for the failure of adhesion in composite restorations and secondary caries may be the chemical irrigants used during the endodontic treatment. NaOCl is widely used for the biomechanical preparation of root canals due to its antimicrobial properties and capacity to dissolve organic material. In addition, another very effective decontamination solution is chlorhexidine 2%. There are few studies about the effect of root canal irrigation solutions on bond strength of universal adhesives therefore, in this study we have investigated the influence of CHX 2% and NaOCl 5.25% on micro-tensile bond strength of G-Premio Bond. **Methods:** Twenty-four human teeth were randomly allocated to the following groups: G1, immersion in saline solution 0.9% for 30 minutes (control); G2, immersion in CHX 2% for 30 minutes; G3, immersion in NaOCl 5.25% for 30 minutes. After restoration, the dentin/resin interface was tested by micro-tensile bond strength. The surfaces morphology was analyzed by Scanning Electron Microscopy. Data were analyzed by ANOVA followed by Tukey test in SPSS software Version 24. **Results:** There was a statistically significant difference between G3 and G1 ($P < 0.05$). There were no statistically significant differences among G1 and G2, G2 and G3. **Conclusion:** Root canal irrigation with NaOCl 5.25% significantly reduced the micro-tensile bond strength in the G-Premio Bond at self-etch mode, but the use of CHX did not make a significant difference.

Keywords: Chlorhexidine. Dentin-bonding agents. Endodontics. Sodium hypochlorite.

Introduction

Today, tooth-colored restorations have become popular and the clinical success of resin composite restorations depends on effective bonding to enamel and dentin[1]. Because the oral cavity is a dynamic environment, the clinical durability of the bonding restoration is important[2].

The state in which two surfaces are held together through interfacial forces such as valence forces, interlocking action, or both is called adhesion. Dental-bonding agents are used to bonding the composites to tooth structures. A smear layer is created during tooth preparation and root canal treatment, which is the residual of organic and inorganic components. The orifices of dentinal tubules fill with the smear plug to reduce the dentin permeability by about 90%. The smear layer was removed with acidic solutions to increase the exposed dentin surface and bond strength[3].

Recently, universal adhesives were developed to simplify the adhesive bonding procedure[4]. These adhesive systems are classified as "universal" since they may be used as several bonding modification techniques: self-etch, etch-and-rinse, or selective enamel etch[5]. G-Premio is a universal adhesive that contains three functional monomers: MDTP, 10-MDP, and 4-MET, without HEMA (hydrophilic monomer), claimed that increases bonding stability. 10-MDP is a hydrophilic functional monomer that is capable of establishing a very intensive and stable chemical interaction with hydroxyapatite[6].

In successful endodontic treatments, effective cleaning and shaping are essential. Because of the complicated anatomy of root canal areas, applying various instrumentation techniques alone is not effective in removing bacteria from root canal areas; this emphasizes the necessity of chemical solutions for cleansing and disinfecting the root canal system. Sodium hypochlorite (NaOCl) is widely used as an irrigated solution in root canal treatments. It has an antibacterial effect; it can dissolve dentin's organic components with oxidizing and denatures the collagen content of the smear layer from root canals[7,8]. It has been stated that the elimination of the smear layer's collagen content resulted in a reduction in the smear layer compactness and enhanced the bond strength of the self-etching adhesive because acidic monomers can easily penetrate the demineralized dentin and interact with the underlying dentin surface[9].

Chlorhexidine 2% is another cleaning solution during root canal treatment[10]. Similar to NaOCl, it has an antimicrobial effect with a large spectrum of anti-bacterial activity. Currently, it is accepted that applying CHX after acid-etching of dentin may limit the collagen fibrils' degradation and act as a MMP inhibitor. Furthermore, in etch-and-rinse adhesives, the usage of CHX considerably improves the integrity of the hybrid layers and bond strength[11].

Numerous studies have stated that applying CHX to acid-etched dentin improved the integrity of the hybrid layers and bond strength[11].The application of CHX in combination with older dentin adhesive systems has been successful, and hydro-

philic primers containing chlorhexidine are effective in maintaining the hybrid layer over time. To date, there are not many studies to evaluate this effect in universal adhesives[4].

This in vitro study aimed to evaluate the influence of Chlorhexidine 2% and Sodium hypochlorite 5.25% on the micro-tensile bond strength of universal adhesive (G-Premio Bond).

The null hypothesis is that there is no significant difference on the micro-tensile bond strength of G-Premio Bond between chlorhexidine 2% and sodium hypochlorite 5.25% in the performance.

Materials and methods

Sample preparation

Twenty-four intact erupted, non-carious first maxillary premolars that had been extracted within the three last months for various reasons were collected, cleaned, and stored in thymol solution 0.5% at 37 ° C for 1 week. The auto-polymerizing acrylic resin (ProBase Hot; Ivoclar Vivadent, Amherst, NY) was used to mount teeth; then teeth were stored in distilled water at 24°C for 24 hours. The third occlusal surface of all teeth were horizontally sectioned by a disc (Leitz1600 saw microtome, Germany) with 0.3mm diameter and high-speed hand-piece under water coolant to obtain a smooth dentin surface. The exposed dentin surface was ground flat with #600 grit silicon carbide paper to create a uniform and standard smear layer. Then, the prepared teeth were divided into three groups randomly (n=8). G1, immersion in saline solution 0.9% for 30 minutes (control); G2, immersion in chlorhexidine 2% (Medicine Company, Wuhan, China) for 30 minutes; G3, immersion in NaOCl 5.25% (Xilong Chemical Factory, Shantou, China) for 30 minute[8]. After immersion, teeth blotted dry with tissue paper and adhesive (G-Premio Bond (GPB) GC Corp. Tokyo, Japan P) was applied using the disposable applicator and the surface remained wet for 10 seconds. Then adhesive was dried thoroughly for 5 seconds with oil-free air under maximum air pressure and was cured with an LED light-curing unit (Kerr, Orange, CA, USA) /1200 mW/cm^{2}for 10 seconds. Then composite (Filtek Z250; 3M ESPE, St. Paul, MN, USA) was applied and light-cured for 20 seconds with an LED light-curing unit, in layering method (three layers, dentin each layer had 2mm thickness).

Micro-tensile bond strength test

The specimens were stored in distilled water at 37°C for 24 hours, and each tooth was cut on the x and y-axis (mesiodistal and buccolingual) with a low-speed diamond saw (Leitz1600 saw microtome, Germany) at 300 rpm under water cooling to obtain dentin -composite sticks with an average cross-sectional area of 1 mm^2. Three central, non-trimmed beams from each tooth were selected for the μTBS test. (Figure 1).

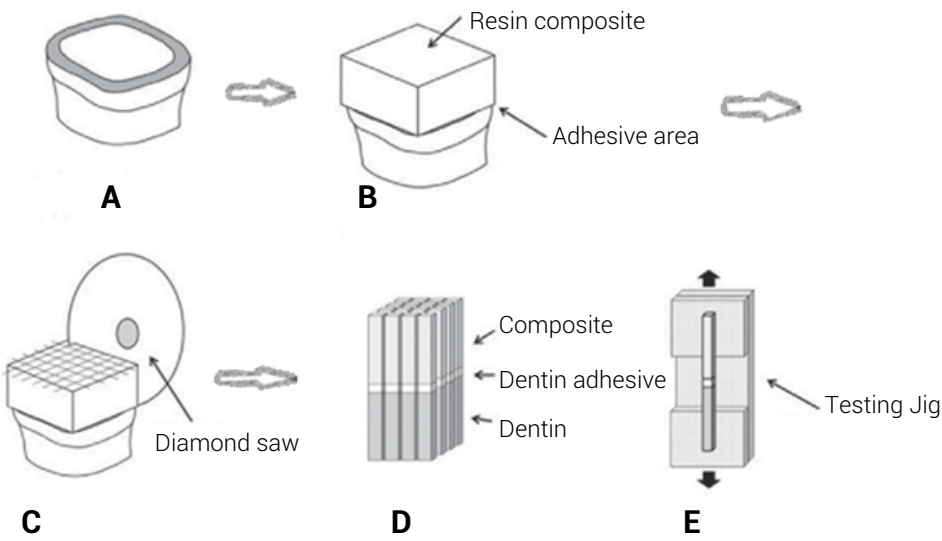

Figure 1. Sample preparation to micro- tensile bond strength test.

A universal testing machine (Zwick / Roell z020) was used for micro-tensile bond strength testing. The samples were fixed to the device using cyanoacrylate adhesive (Loctite Super Bonder Gel Control, Henkel Ltda, São Paulo, Brazil). The tensile load was applied to the resin-dentin interface at a 0.5 mm/min (ISO TR 1145) until failure. The data for each group were reported in megapascals (MPa)[12].

Scanning electron microscope analysis

SEM analysis was used to investigate the surfaces morphology of the sample. For this purpose, the selected sample surfaces were sputter-coated with the gold-palladium alloy for 10 minutes. The SEM images were captured by the SEM device (TESCAN VEGA3, Czech Republic) at 2000× and 10000× magnification.

Statistical analysis

Data were analyzed using SPSS software version 24. Comparison between experimental groups was performed using ANOVA followed by HSD and Tukey's post hoc tests. The accepted level of significance for all tests was $p < 0.05$.

Results

Micro-tensile bond strength

In this study, the Shapiro-Wilk test was used to evaluate the normality of the data. The highest and lowest mean values of tensile bond strength were obtained from G1 (11.704 Map) and G3 (7.791Mpa), respectively (Table 1).

Table 1. Mean tensile bond strength

Groups	mean	Standard deviation	max	min	pre-test failure
G1	11.704	4.395	20.60	0.07	1
G2	9.615	4.778	22.30	0	2
G3	7.791	4.625	15.30	0	4

Although the results obtained from HSD Tukey-test (Groups) showed a significant statistical difference between G1 and G3, there was no difference between G1 and G2. Also, there was no difference between G2 and G3 (Table 2).

Table 2. HSD Tukey test

Bonding System	Bonding System	Mean Difference	p-value
G1	G2	2.089	0.271
	G3	3.912	0.013*
G2	G3	1.823	0.367

*The mean difference is significant at ($p<0.05$).

Surface morphology by SEM

In teeth not treated with chlorhexidine (G1), G-Premio Bond agents produced a hybrid layer that was difficult to identify along most of the length of the interface and showed no presence of resin tags in the dentin tubules or were very scarce. Dentinal tubule orifices were visible with rounded tubule orifices' margins, which indicates the superficial demineralization of peri-tubular dentin. Areas of dentin inter-tubular dentin were smoothed, which may prove superficial demineralization of dentin in these areas. In teeth treated with chlorhexidine 2%(G2), the dentin surface was covered with a thick and homogenous smear layer. The dentinal tubule orifices were not visible (Figure 2-D). In the sodium hypochlorite 5.25% group (G3), some tubular apertures were visible (Figure 2-F). All the specimens of G2 and G3 groups showed the presence of a smear layer in all extensions of the specimen (Figure 2).

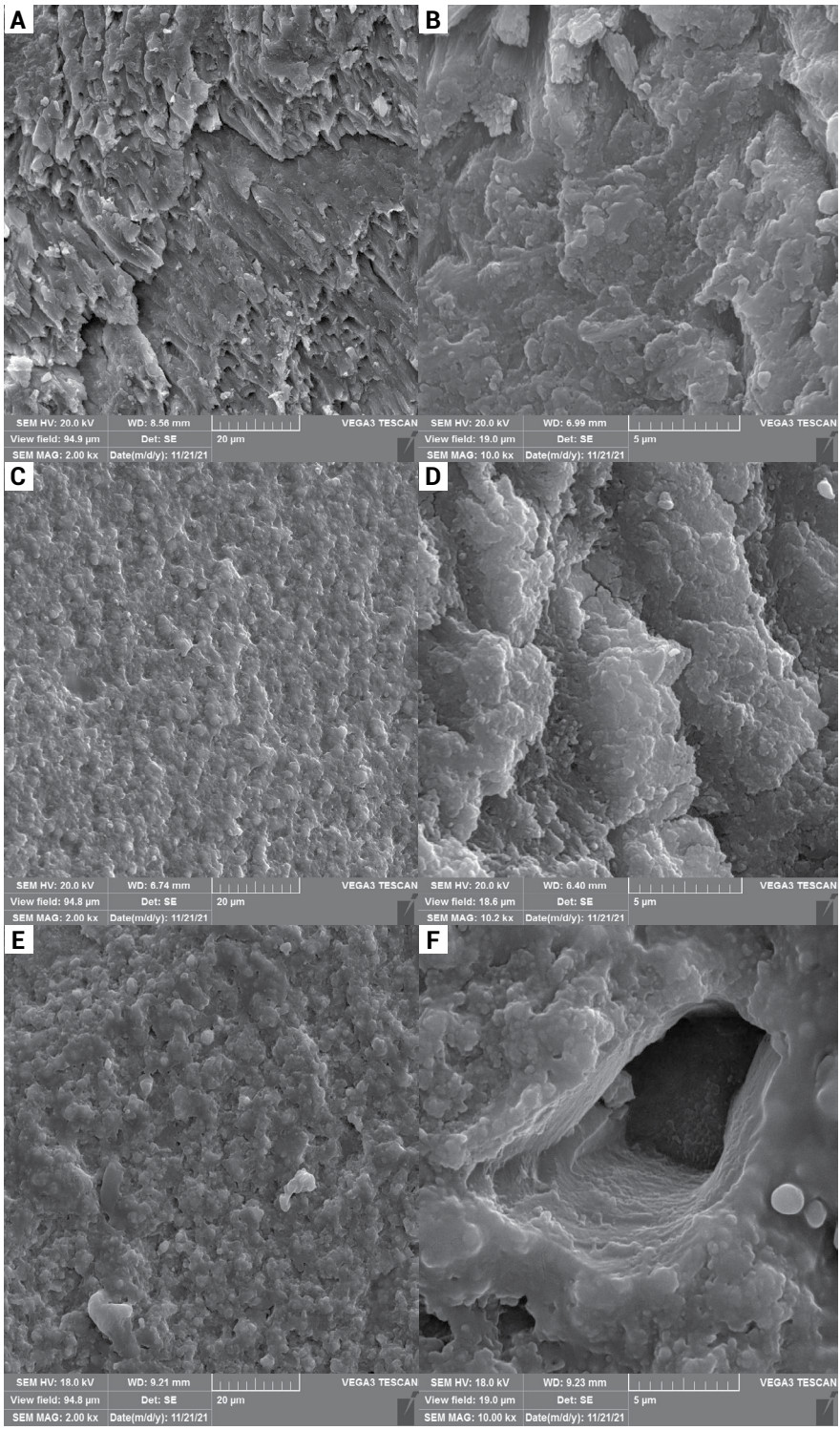

Figure 2. Representative SEM micrograms of the resin–dentin interfaces. A: G1, immersion in saline solution 0.9% for 30 minutes (control); B: Magnified image (10,000×) of (A); C: G2, immersion in CHX 2% for 30 minutes; D: Magnified image (10,000×) of (C); E: G3, immersion in NaOCl 5.25% for 30 minutes; F: Magnified image (10,000×) of (E).

Discussion

Adhesive bonding to dentin was differently affected by the endodontic chemical irritants. Two common irrigate used during endodontic treatments are chlorhexidine 2% and sodium hypochlorite 5.25%. Some studies have evaluated the bond strength after using canal irrigation solutions, which are different among bonding materials and methods (Etch and rinse / Self etch) and the different studies methods. The purpose of this study was to evaluate the influence of chlorhexidine 2% and sodium hypochlorite 5.25% on the micro-tensile bond strength of the G-Premio Bond in self-etch mode. Functional monomers such as 10-MDP, 4-MET, and Phenyl-P in "mild" self-etch adhesives, cause a chemical bond to calcium ions of the hydroxyapatite crystals. "Mild" self-etch adhesives interact only superficially with dentin, forming a thin hybrid layer. It is suggested that "mild" self-etch adhesives minimize nano-leakage, leave a substantial amount of hydroxyapatite around the collagen fibrils to mask the collagen cleavage site, and keep the enzymes "fossilized". Thus, the collagen could not be degraded[13].

According to our findings, there was a statistically significant difference in micro-tensile bond strength between the sodium hypochlorite 5.25% group and the saline solution 0.9% group. The micro-tensile bond strength in the sodium hypochlorite group was significantly lower than the control group (normal saline). These findings indicate that sodium hypochlorite can reduce micro-tensile bond strength. Thus, the null hypothesis that Sodium hypochlorite 5.25% has no significant difference on micro-tensile bond strength of G-Premio Bond, is refused.

This result is consistent with Erdemir et al.[14], and Dikmen et al.[15],' studies. Erdemir et al.[14] (2004) studied the effect of medications for root canal treatment on bonding to root canal dentin. They treated root canal dentin walls with 5% sodium hypochloride (NaOCl), 3% hydrogen peroxide (H_2O_2), the combination of H_2O_2 and NaOCl, or 0.2% chlorhexidine gluconate for 60 s; or calcium hydroxide or formocresol for 24 h. The teeth in the control group were irrigated with water. Micro-tensile bond strengths were measured after 24 h of storage in distilled water. The results indicated that NaOCl, H_2O_2, or a combination of NaOCl and H_2O_2 treatment decreased bond strength to root canal dentin significantly. Dikmen et al.[15] (2015) evaluated the effects of different antioxidant treatments (Accel, noni fruit juice and proanthocyanidin) on micro-tensile bond strength of a self-etching adhesive system (Single Bond Universal Adhesive) to sodium hypochlorite-treated dentin. They maintained that micro-tensile bond strength in NaOCl group was significantly lower than in all other groups.

On the one hand, Dontula et al.[1], (2012) reported the effects of different concentrations (2.5%, 5%, 10%) of sodium hypochlorite applied for 30 seconds on acid-etched dentin on shear bond strengths of an acetone-based adhesive (Prime and Bond NT / etch and rinse) and indicated that the highest shear bond strength values were demonstrated by the 5% sodium hypochlorite treatment group than other groups. They related this finding to partial de-collagenation and an optimum hybrid layer. The findings of Dontula et al.[1] are not in line with the present study, which may be related to the type of bond strength measurement and type of bonding. G-Premio Bond

contains a 10-MDP monomer, claiming that this monomer interacts with hydroxyapatite and increases bonding stability. The bonding mechanism in self-etch adhesives is micromechanical and chemical[16]. The removal of organic components, such as collagen fibers, which play an important role in forming the hybrid layer, form the weaker hybrid layer[7]. Also, the high pH of sodium hypochlorite neutralized the acidic monomers of the self-etch bonding, which prepares the substrate for better bonding by removing minerals components of dentin and the smear layer for better bond strength[13]. In addition, NaOCl interferes with the polymerization of the adhesive resin; because it interrupts and competes with the propagating vinyl-free radicals generated during polymerization, resulting in premature chain termination and incomplete polymerization adhesive[17]. However, NaOCl (a de-proteinizing agent) removed the organic content of the inter-tubular and peritubular dentin, which increases the opening of the dentin tubules (Figure 2), it removed the collagen network in the demineralized zone and reduced infiltration of dentin adhesive systems in the collagen network. Consequently, NaOCl reduces adhesive bond strength.

Based on the results of this study, there was no statistically significant difference between the chlorhexidine 2% group and the control group, and this finding accepts the null hypothesis. Kazemi-Yazdi et al.[18] (2020) reported that pretreatment with 2% CHX had no negative effect on the micro-tensile bond strength in Clearfill SE Bond. Clearfil SE Bond is mild self-etch two-step adhesive systems and has a mild acidic functional monomer, 10-MDP which agrees with the current study results may be since G-Premio Bond contains 10-MDP monomer.

According to Mapar et al.[19] study (2020) on comparison of the effect of Chlorhexidine and collagen cross-linking agent (Quercus Extract) on the tensile bond strength of composite to dentin, chlorhexidine 2% did not influence the bond strength of resin composites to dentin which is consistent with the present study. Shadman et al.[20] (2018) also compered the shear bond strength of Scotchbond Universal or Scotchbond Multi-purpose with/without chlorhexidine usage. They proved that CHX usage did not have any effect on immediate shear bond strength of Scotchbond Universal [self-etch (SE)] and Scotchbond Multi-purpose [Etch and rinse (ER)].

In this study, CHX decreases micro-tensile bond strength that is not statistically significant; this is maybe because CHX inhibits the infiltration ability of the hydrophilic monomers through the dentinal tubules. (Figure 2-B) confirms this finding, the specimen showed a very clear presence of smear plug in the dentin tubules. On the contrary, Fernandes et al.[21] (2020) stated that pre-application of 2% chlorhexidine gluconate did not reduce the immediate micro-tensile bond strength of a universal adhesive system (Single Bond Universal). Some studies reported a negative effect of CHX on the bond strength of adhesive systems.

Silva et al.[22] evaluated the effect of 2% chlorhexidine (CHX) application during the bonding protocol on micro-shear bond strength of two adhesive systems (Ambar and Single Bond 2/ two-step etch-and-rinse adhesives) after storage in different media (distilled water, mineral oil and 1% sodium hypochlorite-NaOCl). They concluded that the use of CHX in the bonding protocol did not cause a decrease of bond strength in any of the situations evaluated after 15 days when the bond protocol

without CHX application was used. However, the use of CHX in the protocol negatively influenced the bond strength for Single Bond 2 after 15 days of storage in distilled water and 1% NaOCl. This may be related to the MDP monomer in Ambar Bond (FGM, Joinville, SC, Brazil). Campos et al.[23] study (2009) also demonstrated that CHX-based cavity disinfectants in concentrations higher than 0.12% should be avoided before the self-etch adhesive systems. They used the Clearfil Tri S Bond – CTSB (Kuraray Co., Osaka, Japan) as a single-step self-etch adhesive system. They maintained interactions among CHX and the adhesive components, maybe decreasing their wettability and the level of dentin conditioning. Kaynar et al.[24] (2021) studied the effect of chlorhexidine and ethanol application on the bond strength of universal adhesive systems. They concluded that extra chlorhexidine or ethanol treatment may not directly influence the bond strength of universal adhesives in self-etch mode. They suggested that the lowest bond strength with the application of CHX can be due to the usage of 2% CHX before adhesive systems, which affect the monomer conversion degree of the material or lead to breakage before the micro-tensile bond strength test.

It should be stated that canal irrigation with sodium hypochlorite increases the success rate of root canal treatment. Although chlorhexidine has been recommended as a root canal irrigant because of its extensive spectrum antimicrobial action, its inability of tissue dissolution has been mentioned as its major disadvantage[25]. Furthermore, EDTA only modifies the inorganic part of the dentin and smear layer (hydroxyapatite). To completely remove the smear layer, NaOCl should be used before the final rinse with EDTA. EDTA has little or no antimicrobial activity[26].

Some studies have been done to achieve successful root canal treatment with adequate bond strength during the application of sodium hypochlorite. Abuhaimed and Abou Neel[7] (2017) suggested the usage of antioxidants such as ascorbic acid and sodium ascorbate after applying sodium hypochlorite. Also, Osorio et al.[27] (2002) recommended the usage of EDTA as a final root canal irrigant. Spicciarelli et al.[28] (2021) showed that continuous chelation (NaOCl+ etidronic acid in a single mixture) does not affect the bond strength of universal adhesives. When using NaOCl, it would be proper to do a final rinse with ethanol or delay post-endodontic restoration.

In conclusion, the findings of the present study showed that chlorhexidine 2% had no negative effect on the G-Premio Bond μTBS. However, Sodium hypochlorite 5.25% significantly decreased the bond strength of G-Premio Bond.

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Conflict of Interest

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Author contribution statement

Nafiseh fazelian: Conceived and designed the experiments and wrote the paper; Abbas Rahimi Dashtaki and Mohammad Amin Eftekharian Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools, or data; Wrote the paper. Batool Amiri: Analyzed and interpreted the data. All authors actively participated in the discussion of the manuscript's findings, and have revised and approved the final version of the manuscript.

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