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ORIGINAL ARTICLE

Effect of smear layer removal using different chelators on push-out bond strength of bioceramic sealer

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ABSTRACT

Aim: *The aim of this study was to evaluate the effects of phytic acid (IP6), ethylenediamine-tetraacetic acid (EDTA) and glycolic acid (GA) used as a final irrigation solution on the push-out bond strength (POBS) of a bioceramic-based root canal sealer.*
Methodology: *The study included 60 single-root human mandibular premolars. After the teeth were decoronated, they were cleaned and shaped using the #25.08 Reciproc R25 single file system. Throughout preparation, each canal was irrigated using 20 ml 5.25% sodium hypochlorite (NaOCl). The teeth were divided into 4 group, each consisting of 15 (n=15) according to the final irrigation agent [1% IP6, 10% CI, 17% EDTA, and distilled water (DW)]. The final irrigation protocol consisted of application of 5 ml chelating agent for 1 minute followed by 5 ml 5.25% NaOCl application. Root canals were filled using Well Root ST canal sealer and Reciproc R25 gutta-percha, based on the single-cone technique. A 2-mm section was extracted from the middle part of the roots to test for POBS. Values were recorded in MPa and fracture types were examined. Groups were compared using one-way ANOVA (Welch's) test.*
Results: *The GA, EDTA, and IP6 groups showed no significant intergroup differences (p>0.05). EDTA and GA groups had significantly higher POBS than the DW group (p<0.05). No significant difference was observed between IP6 and DW groups (p>0.05).*
Conclusion: *GA increased the bond strength of the bioceramic-based canal sealer to the root canal dentin at least as much as EDTA; IP6 exhibited similar strength to these chelators, it was not better than DW.*

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KEYWORDS *Phytic acid, glycolic acid, EDTA, push-out bond strength, Well Root ST*

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Introduction

Root canal sealers are used to provide a bond between the root canal wall and the core filling material. A strong and long-lasting bond between the root canal wall and the filling material is an important factor in preventing root canal infection caused by proliferation of microorganisms or reinfection owing to coronal/apical leakage (1, 2). Push-out bond strength (POBS) is considered a prognostic factor relevant to assess the bond provided by a root canal sealer between the canal wall and core filling material (3).

Root canal instrumentation causes the formation of a smear layer (SL), which contains organic and inorganic components that occlude the orifices of dentinal tubule and remarkably prevent irrigants and medications from reaching the dentin surface (4). Furthermore, SL creates an interface between the filling material and dentin, preventing the sealer from adhering to the dentin (5). Chelating agents are recommended for use during root canal treatment to improve chemo-mechanical preparation by removing SL and demineralized and softened dentin from the root canal. To some extent, this procedure exposes a large number of dentinal tubules, which increases the contact zone and provides stronger bonding between the sealer and root canal dentin (6). Removal of SL using this procedure may improve the adhesion of sealers to dentin. An effective chelating agent should remove SL by acting only on the superficial dentin without damaging the interior region of the root dentin. Ethylenediaminetetraacetic acid (EDTA) has good SL-removal capacity, but (7) has some undesirable effects when used for more than 3 minutes, including denaturation of collagen fibrils (8), and erosion of peritubular and intertubular dentin (7). This resulted in a need for searching alternative irrigants that are biocompatible and effective in removing SL without damaging the structure and properties of the root dentin to ensure a successful root canal treatment. Phytic acid (inositol hexaphosphate-IP6), recognized in the endodontic literature as an alternative to EDTA, is a chelating agent that is biocompatible with osteoblasts, able to remove SL and release TGF Beta in regeneration (9, 10). Glycolic acid ($C_2H_4O_3$ -GA) is an organic chelator that is highly soluble in water and that induces collagen and fibroblast proliferation. Furthermore, this acid is suggested as a substitute for phosphoric acid, which is abrasive on enamel and dentin (11). Additionally, GA is relatively less toxic than EDTA and readily biodegradable (12).

However, various chelating agents may cause different superficial dentin modifications (removing different parts of major inorganic elements such as calcium [Ca^{++}] (13, 14) and this may give rise to variable interference with the bonding of calcium silicate-based sealers to dentin. Against this background, the aim of this study was to evaluate the effect of IP6, EDTA and GA used as chelating agents on the bonding strength of calcium silicate-based root canal sealer (Well Root ST) to root canal dentin. The DW group was used as the reference agent for comparison purposes. The null hypothesis tested was that various chelating agents would not affect the bonding strength of the calcium silicate-based root canal sealer.

Materials and Methods

This study received approval from Ethical Committee of Dicle University, Faculty of Dentistry, Diyarbakır, Türkiye (no: 2021-39).

Calculating the sample size

The sample size was calculated based on a previous study (15). According to this study, the minimum sample size for each group was found to be 6 observations. In the present study, we used 15 teeth per group and 60 teeth in total.

A total of 60 single-root mandibular premolar teeth were used; these teeth had completed their apical development, had been extracted for periodontal or orthodontic reasons, and had no fractures or cracks on them. The teeth were decoronated to a root length of 15±1 mm using a diamond disk under water cooling. Then, working length was determined using a 15 K-type (VDW, Munich, Germany) hand file for standardization. The working length was determined such that it would be 1 mm short of the file going beyond the apical foramen. Root canal preparation was performed using a Reciproc R25 (VDW, Munich, Germany) single file system in accordance with the manufacturer's instructions. Irrigation was performed using 30 gauge irrigation needles (Dentsply Sirona, Maillefer, Ballaigues, Switzerland) with two lateral vents. Throughout the preparation, each canal was irrigated using 20 ml 5.25% NaOCl (Promida, Eskişehir, Turkey). Following enlargement, the teeth were randomly divided into 4 groups for final irrigation (n=60). The groups were as follows:

EDTA Group (n=15): The root canals were irrigated with 5 mL 17% EDTA (Promida, Eskişehir, Turkey) (1 minute) and 5 ml 5.25% NaOCl.

GA Group (n=15): The root canals were irrigated with 5 mL 10% GA (Sigma Aldrich Co. LLC, St Louis, MO, USA) (1 minute) and 5 ml 5.25% NaOCl.

IP6 Group (n=15): The root canals were irrigated with 5 mL IP6 (Sigma Aldrich Co. LLC, St Louis, MO, USA) (1 minute) and 5 ml 5.25% NaOCl.

DW Group (n=15): The root canals were irrigated with 5 mL DW (1 minute) and 5 ml 5.25% NaOCl.

After final irrigation procedures were performed according to the irrigation regimen for each group, all teeth were rinsed using 5 ml DW and dried with paper points. Root canals were filled with bioceramic-based Well Root ST (Vericom, Gangwon-Do, South Korea) sealer using Reciproc R25 gutta-percha cones based on the single-cone technique. The coronal chamber was obturated using a temporary restorative material. The teeth were embedded in square silicone molds filled with cold-curing acrylic (Imicyl, Konya, Türkiye). After acrylic polymerization, the samples were stored at 37 °C and 100% humidity for 7 days to ensure that the canal sealer was fully cured.

The acrylic was marked with an acetate pen 4 and 6 mm above the apical section. Then, samples were cut using the ISOMET device (Isomet, Buehler, Lake Bluff, IL, USA), with a blade rotating at 200 rpm (ATM GmbH, Mammelzen, Germany) under water cooling to obtain a sample of approximately 2 mm from the middle 1/3 of each sample. Section thickness was measured with a digital caliper (Mitutoyo Corp, Tokyo, Japan) with 0.01 mm precision. The push-out bonding strength of the samples was tested using the Instron device (Instron 3382, Instron Corp., Memmingen, Germany). A stainless-steel cylindrical tip with a diameter of 0.75 mm was mounted on the device and positioned to touch only the canal filling. The prepared device was used to apply a load at a constant speed of 1 mm/min until debonding of the root canal filling and dentin was achieved. Owing to the increasing taper of the sections in the apico-coronal direction, the load was applied to the canal filling material in the apico-coronal direction. Fracture strength values were recorded in Newton (N) and converted to Megapascals (MPa) to calculate the bond strength. After the bond strength test, samples were examined to identify their fracture types (Figure 1). Adhesive failure is the type of failure that occurs at the interface between the filling material and dentin, whereas a cohesive failure is the failure that occurs within the filling material itself. Mixed failure indicates frac-

Figure 1
Visualization of failure types after POBS testing under a stereo microscope
A) Adhesive failure, **B)** Ccohesive failure, **C)** mixed failure.

ture both at the filling material-dentin interface and within the filling material.

Statistical analysis

Data were analyzed using SPSS 21.0 Software (IBM Corp, Armonk, NY) and were normally distributed according to the Shapiro-Wilk test. The variances were not found to be homogeneous according to Levene's test, and the data were compared using the one-way ANOVA (Welch's) test. Multiple comparisons were performed using Tamhane's test. Inter-rater agreement was calculated using Cohen's Kappa statistic. The alpha type error was set at 0.05.

Results

Descriptive statistics of the push-out test are given in Table 1. No significant difference was observed between the IP6 and EDTA groups, and these two groups exhibited significantly higher bond strength than the DW group. Although the IP6 group showed lower bond strength than the GA and EDTA groups, this difference was not statistically significant. No significant difference was observed between the IP6 group and the DW group.

The graph of intragroup values by type of failure is given in Figure 2. Kappa test results showed statistically high inter-rater agreement in determining types of failure after bond strength test in each group (Kappa value=0.824). Mixed failure was more common in the IP6 group (53.3%), while cohesive failure was more common in the EDTA group (46.7%). The rate of adhesive failure was higher in the GA group (40%) and DW group (66.7%).

Discussion

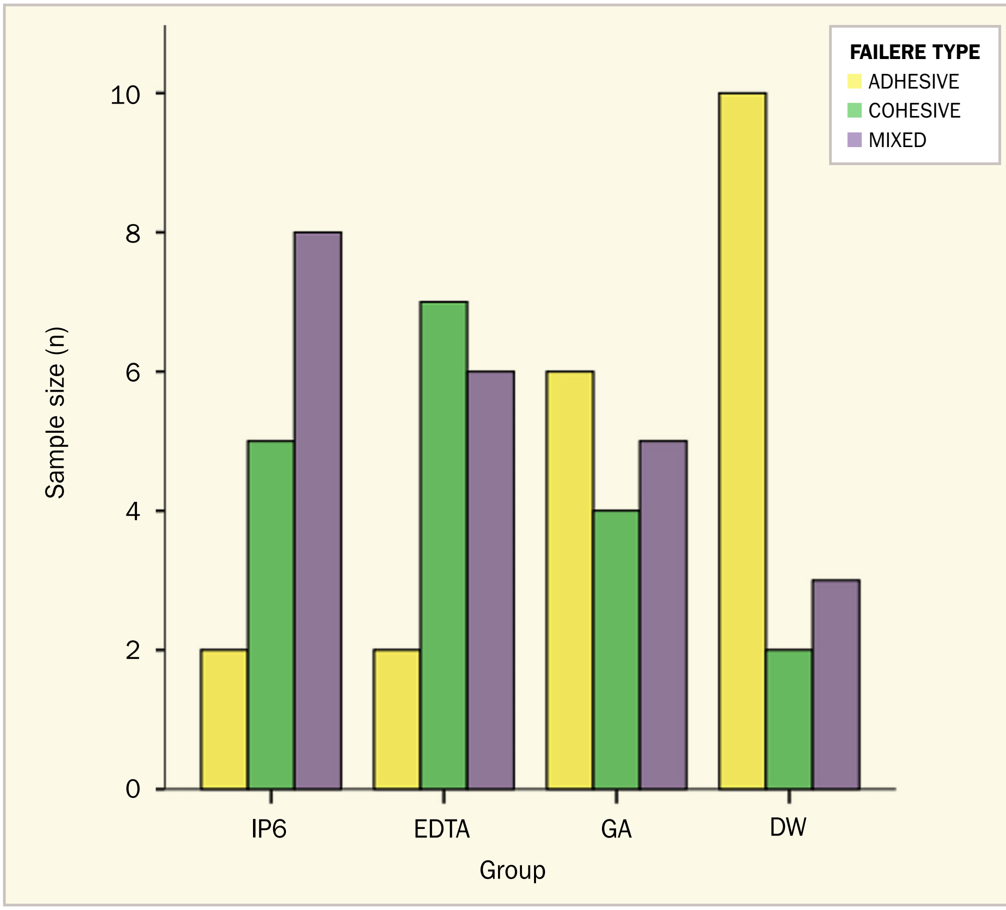
Removing the inorganic component of SL using EDTA and the organic component using NaOCl is generally considered a standard clinical practice. However, this combined irrigation protocol causes abrasion on the dentin surface (16). Furthermore, prolonged exposure of the root dentin to EDTA can reduce dentin's modulus of elasticity and flexural strength (17). Therefore, there is a search for alternative chelating agents with less or no harmful effect on root dentin. The effect of chelating agents is generally not limited to SL; these agents also change the ratio of Ca^{++} / phosphate present in the dentinal walls (18). Furthermore, they expose collagen fibers and reduce dentin hardness (19). These changes may affect the bonding between dentin to calcium silicate-based sealers, which use Ca^{++} ions in dentin for biomineralization. The results showed no significant difference in the bond strength of the Well Root ST bioceramic sealer to root dentin after removal of SL with different chelation solutions, therefore, the null hypothesis of the study was accepted. Compared to the samples treated with chelating agents, DW-treated (control) samples had significantly lower bond

Table 1
Descriptive statistical values for push-out bond strength in groups

Group	n	Mean ± SD	Median (Min-Max)	p
GA	15	3.9 ± 1.19^{A}	4.31 (1.71-5.56)	0,004*
EDTA	15	4.12 ± 1.45^{A}	4.13 (1.79-6.73)	
IP6	15	3.39 ± 1.19^{AB}	3.17 (1.95-5.71)	
DW	15	2.83 ± 0.59^{B}	3.02 (1.72-3.54)	

$^{A-B}$*Groups with the same letter have no significant difference between them.]*

Figure 2
Intergroup and intragroup comparison of failure types.



strength values. This finding strongly suggests that the presence of SL has a negative effect on adhesion between dentin and the Well Root ST. The increase in surface roughness caused by removal of SL may be clinically beneficial because retention is achieved by the micromechanical interaction of the sealer with the dentinal tubules (20). The fact that the bonding between bioceramic sealer and radicular dentin is reportedly brought about by mechanical interlocking of the sealer plug in the dentinal tubules rather than chemical interaction (21). All chelating agents used were kept in the canal for 1 minute. 1 minute of EDTA application has been reported to be effective in removing SL (22). A longer contact time may result in excessive peritubular and intertubular erosion and destruction of root dentin (22, 23).

In this study, the highest POBS value was observed in the GA and EDTA groups. This may be owing to the fact that GA at 10% concentration has an SL-removal ability similar to EDTA at 17% concentration. In addition, its acidic pH (2.36 and 2.18) reportedly causes demineralization of dentin, and this may result in increased surface roughness, which has a clinical benefit in micromechanical bonding of adhesive materials (24). The results of our study are in line with that of a POBS evaluation by Veeramachaneni et al. (25) conducted with a similar method. This study evaluated the POBS of bioceramic (Bio C sealer) and epoxy resin (Dia-Proseal) sealers after using a variety of final irrigants and found that both sealers exhibited higher push-out bond strength after treatment with GA with no significant difference between 5% and 17% GA has been demonstrated. Furthermore, bioceramic sealer with GA as final irrigant showed higher bond strength than epoxy resin. Our study only used bioceramic-based

Well Root ST sealer, and could not make any comparison with other sealers.

IP6 contributes to various cellular functions, has multiple negative charges, making it an effective chelator of polyvalent cations such as Ca++, magnesium and iron (26). Studies have found IP6 to be effective in removing SL (10, 27). A confocal laser scanning microscopy study by Eskander et al. (28) found deeper sealer penetration with 17% EDTA, compared to 1% IP6 and reported that the IP6 group had moderate tubular penetration. In contrast, Nassar et al.(10) reported that both IP6 and EDTA removed SL, but the 1% IP6 group exhibited cleaner surfaces. This study found no significant difference between the IP6 group and the other groups, including the control group. We think that the discrepancy in the findings is due to the different amounts of irrigants used and the size of contact with the dentin surface. There is no other study with a similar methodology that investigated the effect of IP6 on the POBS of bioceramic sealer.

Well Root ST is a tricalcium silicate-based, premixed and injectable bioceramic sealer. The zirconium oxide, calcium silicate, filler and hydrophilic thickeners it contains initiate the setting reaction by using the moisture in the canal (29). A study with a similar methodology to ours investigated the effect of 17% EDTA, 18% etidronic acid (HEPB) and 0.2% chitosan on the POBS of AH Plus and Well Root ST sealer and found that the bond strength of Well Root ST sealer was affected by chelating agents (15). Our study achieved a similar result in that the POBS of Well Root ST sealer did not differ significantly with three different chelators. A study by Kaki and Genç Şen (15) mostly observed adhesive failure in all experimental groups, this study achieved a different result. The control group using DW exhibited mostly adhesive failure (66.7%), the samples using chelator had 42.22% mixed failure. According to the authors of the study, the greater proportion of adhesive failure in the DW group may be the result of insufficient sealer-dentin bonding. The greater proportion of mixed failure in the samples using chelators compared to the control group can be attributed to the fact that sealer-dentin bonding has an effect comparable to sealer-gutta-percha bond (30). There was no significant relationship between the effect of different chelating agents and the types of fracture.

Using sealer without a core filling material is not an appropriate representation of actual clinical conditions and the absence of gutta-percha in the root canal filling may affect the results of the POBS test (31). The bond between gutta-percha and sealer is weak, and this low adhesion reduces the mechanical properties of root canal filling materials compared to the use of endodontic sealers alone and exhibits low resistance to elastic deformation (32). Concerning obturation techniques, lateral condensation and warm filling techniques can have an impact on POBS and are less reproducible than the single-cone technique (33, 34). Therefore, in this study, obturation was performed using gutta-percha cones suitable for the enlarged canals, based on the single-cone technique. Because standardization of the POBS test is important to investigate bonding issues in the sealer-dentin interface (35). This study investigated the effect of three different chelating agents on the bonding between bioceramic sealer and dentin, and therefore, the use of core material should be considered invariant.

POBS test determines dislocation resistance of materials and thus provides an effective and reliable measure of adhesion of sealers to root canal walls (36). The density of dentinal tubules and the strength of sealer bonding to dentin decrease along the root from the coronal to the apical third (37). Therefore, this study only used sections obtained from the middle third of each root for POBS testing. One of the limitations of this study is thus the use of sections from only the middle third of the roots, instead of the coronal or apical third.

Conclusion

GA exhibited a POBS that was significantly higher than DW and similar to EDTA. IP6, on the other hand, exhibited a POBS similar to GA, but had no significant difference with DW, which is unable to remove SL. Therefore, GA, as an alternative chelator, can increase the POBS of bioceramic-based Well Root ST root canal sealer. Further studies are needed to investigate the chelating effect of IP6.

Clinical Relevance

The knowledge about the influence of the chelator on POBS of bioceramic-based root canal sealers is essential. Also, GA, an organic chelator, increased POBS as much as EDTA.

Conflict of Interest

All authors declare no conflicts of interest.

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