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Original research

The effects of final irrigants on the push-out bond strength of two calcium silicate-based root canal sealers: an *in vitro* study

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Purpose

The purpose of the present study was to investigate the effect of the different irrigant combinations used in final irrigation on the push-out bond strength of root canal sealers that have different compositions.

Materials and Methods

In total 60 dentinal slices in 1 mm thickness were collected from 15 extracted mandibular premolar teeth; 4 slices from each tooth. 3 canal-like artificial cavities were opened on each dentinal slice. Samples were divided into 4 experimental groups, each of which consisted of 15 samples. In group 1, samples were immersed in 5.25% NaOCl and 17% EDTA solutions respectively; in group 2, immersed in 5.25% NaOCl and 2% CHX solutions respectively; in group 3, immersed in 5.25% NaOCl, 17% EDTA and 2% CHX solutions respectively; and in group 4 immersed in distilled water. After drying with absorbent papers, each cavity in dentinal slice sample was filled with different sealer (Endoseal MTA, Tech Biosealer Endo or AH Plus). Two days later, the push-out bond examination was performed.

Results

AH Plus showed higher push-out bond strength value in two combinations (group 2 and 3) in which final irrigants contained CHX ($p<0.001$). Dentinal push-out bond strengths of root canal sealers from Endoseal MTA and Tech BioSealer Endo were not affected by final irrigant ($p=0.965$).

Conclusion

Using CHX after NaOCl in final irrigant increases push-out strength of epoxy resin-based sealer but, did not create any difference in dentinal push-out bond strength of calcium silicate-based sealers.

Keywords: *Calcium silicate-based sealer, chlorhexidine gluconate, precipitate, push-out bond strength, root canal irrigants*

Introduction

It is widely accepted thaat there is a positive correlation between the outcome of endodontic treatment and the technical quality of the root canal sealing (1, 2). One of the main aims of root canal filling is to seal the prepared canal to prevent the tissue fluids, bacteria and/or bacterial products to enter into it (3). In order to achieve this aim in root canal filling procedure, it is critical that the sealer used with gutta-percha provides an optimum adhesion to root canal walls (4, 5).

Final irrigation of the root canal is performed after root canal shaping in order to reduce the pre-obturation microbial load within the canal system and to minimize future failure (6). Successive administration of sodium hypochlorite (NaOCl) solution and ethylenediaminetetraacetic acid (EDTA) is the most common final irrigation method used all around the world in order to ensure the chemical debridement of root canals and to remove the smear layer take place on dentinal surfaces after the

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instrumentation (7, 8). On the other hand, it was claimed that it is not expected to see a positive therapeutic effect of NaOCl solution while there is EDTA in the canal (9). Besides the high concentrations of NaOCl is toxic and can irritate the periapical tissues (10). Therefore, chlorhexidine gluconate (CHX) was suggested as an alternative irrigant solution for NaOCl (11). Even though CHX has substantivity property along with antimicrobial activity, it does not have the capability of dissolving vital or necrotic tissues (12). In fact, an irrigant with all the desired physiochemical and ideal microbiological properties has yet to be introduced (13, 14). Therefore, different irrigation solutions that have positive effects on antimicrobial properties of NaOCl can be used after NaOCl for effective irrigation (13, 15). However, it is important to be aware of the possibility that irrigation solutions used successively can chemically react with each other. For example, a mixture of NaOCl and CHX produces an orange-brown, hard-to-remove precipitate that colors the walls of the pulpal cavity (16). Previous researches reported that this precipitate contains para-chloroaniline (PCA), a toxin produces methemoglobin and has a potential carcinogenic effect in time (17, 18). However, recent studies that used advanced analysis techniques showed that the precipitate produced by a mixture of these solutions do not contain PCA (6, 19, 20).

There is a limited data on the effect of precipitates that accumulated in canal walls on the push-out bond strength of sealers to the canal walls. Graziele Magro *et al.* (14) reported that the produced precipitates after final irrigation, which used 2% CHX solutions with different formulas, did not change the push-out bond strength of AH Plus, an epoxy-based sealer. Neelakantan *et al.* (21) reported that using EDTA in final irrigation increased the adhesion force of AH Plus sealer to the root dentin significantly when it is compared with a final irrigation that used NaOCl.

Recently, a number of calcium-silicate based root canal sealers that have the positive attributes of bioceramic cement were introduced. Endoseal MTA and Tech Biosealer Endo are the two examples of these type of sealers. In literature, there is limited knowledge about whether final irrigants affects the push-out bond strength of calcium silicate-based sealers to root canal dentin. The purpose of the present study was to evaluate influence of NaOCl+EDTA, NaOCl+CHX or NaOCl+EDTA+CHX final irrigation regimens on the bond-strength of two calcium silicate based (Endoseal MTA and Tech Biosealer Endo) and one epoxy resin based (AH Plus) sealer. The null hypothesis is that the order of the irrigant solutions used in final irrigation does not affect the dentinal push-out bond strength of these root canal sealers.

Materials and Methods

Ethical statement

This study was reviewed and approved by the Research Ethics Committee of Karadeniz Technical University (Protocol Number: 2021/75). The manufacturers and the material contents of root canal sealers tested in this study can be found in Table 1.

Table 1. *The ingredients and manufacturers of the tested materials.*

Sealer	Manufacturer	Composition
Endoseal MTA	Maruchi, Wonju, Korea	Calcium silicates, calcium aluminates, calcium aluminoferrite, calcium sulfates, radiopacifier, thickening agent
Tech BioSealer Endo	Isasan SRL, Revello Porro, Italy	*Powder*: White Portland cement, bismuth oxide, anhydride, sodium fluoride *Liquid*: Alfacaine SP solution (4% articaine + 1/100.000 epinephrine)
AH Plus	Dentsply DeTrey GmbH, Konstanz, Germany	**Epoxy paste:** diepoxy, calcium tungstate, zirconium oxide, aerosol, and dye **Amine paste:** 1-adamantane amine, N.N'dibenzyl-5 oxanonandiamine-1,9, TCD-diamine, calcium tungstate, zirconium oxide, aerosol, and silicone oil

Sample preparation

Samples were prepared by following the technique described by Scelza *et al.* (22). Newly extracted 15 mandibular premolars were chosen. Teeth were stored in 0.02% sodium azide solution at 4°C until the experiment. Soft tissue residuals on teeth were removed by using a scalpel and crowns were removed by using a low-speed diamond disc (Micracut 125; Metkon, Bursa, Turkey) under continuous water wash. The same low-speed diamond disc was used in order to create 4 horizontal cross-sections (1 ± 0.1 mm thick) in each root in coronal to apical direction. 60 root slices were obtained through this protocol. In each root slice, 1 mm thick cylindrical carbide bur was used in order to open three canal-like cavities parallel to the root canal (Figure 1).

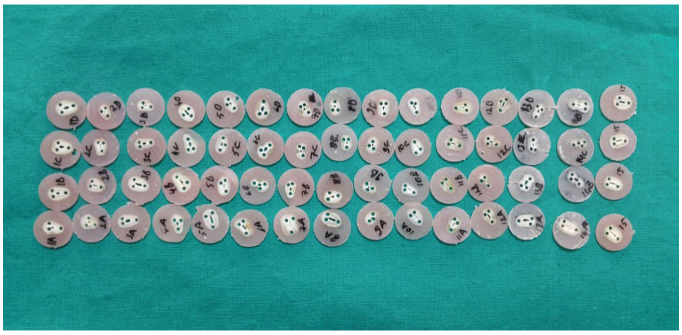

Figure 1. *1.0 mm thick slices were obtained from the roots and three canal-like cavities were opened on each slice.*

The cavities were opened perpendicular to the surface under continuous water wash. The cavities were preserved so as to the distance between the external cement and root canal wall to be 1 mm in minimum. All root slices were divided into 4 different experimental groups according to the final irrigant administration, so each group has 15 slices:

Group-1; the samples were immersed in 5 mL 5.25% NaOCl (Wizard; Rehber Kimya, Istanbul, Turkey) for 15 minutes and then in 5 mL 17% EDTA (Wizard; Rehber Kimya, Istanbul, Turkey) for 3 minutes.

Group-2; the samples were immersed in 5 mL 5.25% NaOCl for 15 minutes, then after drying with absorbent papers, they were immersed in 5 mL 2% CHX (Consepsis; Ultradent, South Jordan, UT, USA) for a minute.

Group-3; the samples were immersed in 5 mL 5.25% NaOCl for 15 minutes and then in 5 mL 17% EDTA for 3 minutes. After drying with the absorbent paper, they were immersed in 5 mL 2% CHX for a minute.

Group-4; the samples were immersed in 5 mL distilled water for 15 minutes.

Then the cavities were dried with absorbent papers and each of the cavities in each of the root slice was randomly filled with one of the chosen sealers: Endoseal MTA (Endoseal; Maruchi, Wonju, Korea), Tech Biosealer Endo (Isasan; Rovello Porro, Italy) or AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany). All of the sealers were mixed and put in cavities according to the protocols provided by the manufacturers. In order to prevent the bubble formation, a weak vibration was applied while placing the sealers into cavities. Lastly, root slices filled with 3 different sealers was incubated at 37° C and 95% relative humidity for 48 hours before push-out analysis in order to harden them completely (Figure 2).

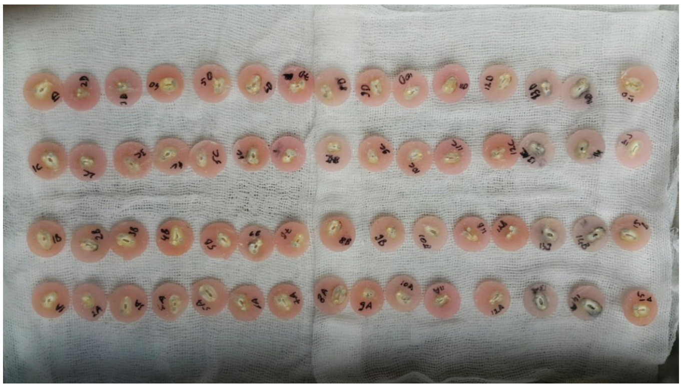

Figure 2. *Each cavity in the dentinal slices was filled with a different sealer.*

Push-out bond strength test

A plunger tip (0.8 mm in diameter) was placed in a way that it was on top of only the test material. The force was always applied in coronal-apical direction. Loading was performed on a universal testing machine (Lloyd Instruments, Foreham, UK) at 0.5 mm/min speed until material dislocation occurred (Figure 3). The load at failure was recorded (in Newtons) and the values were used to calculate the push-out strength in megapascals according to the formula used by Bitter *et al.* (23).

Statistical analysis

The collected data from all groups were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 17.0 (SPSS Inc., Chicago, IL, USA). The fitness of the data to a normal distribution was analyzed with the Kolmogorov-Smirnov test. The mean, and standard deviation values of the data belong to the independent variables that affect the push-out bond strength (irrigants and canal sealers) were calculated. The mean values of these groups were compared by using one-way ANOVA test ($p<0.05$) and

Figure 3. *Loading was performed on a universal testing machine at 0.5 mm/min speed until material dislocation occurred.*

the irrigation protocol values corrected by Bonferroni test with a new threshold level, $p=0.00833$.

Results

The dentinal push-out bond value for each group and the comparison of the groups are summarized in Table 2. AH Plus showed higher push-out bond strength value on CHX groups (Group 2 and 3) than the other two groups ($p<0.001$). The push-out bond strength of Endoseal MTA and Tech BioSealer Endo root canal sealers to dentine were not influenced by the final irrigation protocol ($p=0.965$).

Regardless of the root canal sealer, the use of CHX in final canal irrigation resulted in higher bond strength than

Table 2. *Push-out bond strength (MPa, means±standard deviations) of root canal sealers in canal-like cavities irrigated with different regimes.*

Sealer	NaOCl + EDTA	NaOCl + CHX	NaOCl + EDTA + CHX	Distilled water	Total
AH Plus	8.12±2.37^{a}	12.87±3.89^{b}	10.54±4.65^{b}	6.68±2.59^{a}	9.55±4.16^{A}
Endoseal MTA	5.98±2.2	6.07±2.25	6.87±3.63	5.22±1.6	6.04±2.53^{B}
Tech Biosealer Endo	4.02±0.96	5.17±2.1	5.19±2.11	4.41±1.39	4.7±1.74**B**

CHX: chlorhexidine; EDTA: ethylenediamine tetra acetic acid; NaOCl: sodium hypochlorite. The different small letters indicate significantly differences between final irrigation groups (p<0.05).The different capital letters indicate significantly differences between sealers (p<0.05) the other two groups (p=0.003). No difference occurred between the two CHX combinations, likewise, the push-out bond strengths of the other two groups were also similar to each other (p=0.26).

AH Plus showed stronger dentinal push-out bond strength than bioceramic based root canal sealers independently of the final irrigation solutions (p<0.001).

Discussion

The present findings indicate a significant difference in the performance of the tested materials. AH Plus produced stronger push-out bond-strength to the root dentin than Endoseal MTA and Tech Biosealer Endo. Moreover, the push-out bond strength of AH Plus was affected by final irrigant. AH Plus presented stronger push-out bond strength when NaOCl-CHX used as a final irrigant rather than NaOCl-EDTA or distilled water. The push-out bond strength of Endoseal MTA and Tech Biosealer Endo to the root dentin were not affected by final irrigant. Therefore, the null hypothesis was partially rejected.

Different from the previous studies, there is a methodological aspect of this study to be considered. Standard canal-like holes were opened on dentin slices obtained from extracted human teeth in order to establish a more standardized groundwork. Moreover, sealers and irrigants were compared in the same dental samples which allow for better control on confounding factors such as dental age, sclerosis, micro-stiffness of dentine, canal shape, etc. Thus, this experimental setup overcomes the effects of different dentine sources on study design by allowing for placing three different sealers in the same slice (22, 24, 25). All of these biological-chemical-physical variances of the root dentine can increase sealer retention in the undercut nonprepared areas of natural root canals. Also, all the canal-like cavities were opened as a cylinder-shape with 1mm in diameter in order to maintain a standard root canal anatomy between the groups (25). In addition, the holes were filled only with sealers so that the load is fully applied to this material. Thus, erroneous interpretations of the performance of the gutta-percha are avoided (26). Although this method does not exactly reflect clinical practice is very useful for standardizing examples.

After discovering that the precipitate produced after successive usage of CHX and NaOCl does not contain a carcinogenic PCA, CHX solution, which has a potential to contribute to the antimicrobial activity of NaOCl, can be added to the standard irrigation protocol more easily (6, 19, 20). As it is planned to examine the effects of the precipitates, which emerge upon mixing the two solutions, on the dentinal adhesions of root canal sealers, neutralizing solutions were not used between the successive irrigants in the CHX groups of the present study. Push-out bond strength of calcium silicate-based sealers was not affected by irrigant combinations, while the push-out bond strength of AH Plus sealer to the dentine surprisingly increased compared to NaOCl-EDTA combination in dentinal samples that were immersed in NaOCl and CHX solutions successively. In a previous study, different CHX formulations used in final irrigant caused a higher chemical precipitate and smear layer on radicular dentin compared to irrigation protocol that contains 17% EDTA and 2.5% NaOCl (14). However, these residuals did not change the push-out bond strength of epoxy resin-based sealer (AH Plus) on radicular dentin (14, 27). The reason for this difference between the result of the present study and the results of previous studies may be due to the fact that the push-out bond strength experiments in those studies are performed on the main root canals. AH Plus can chemically bond to dentinal collagen amino groups. In order to establish a good bonding, it is necessary to bring out and more importantly to protect the collagen network (28, 29). From this perspective, amino groups that were dwelled on dentine depending on the irrigation solution can affect the adhesion of resin-based sealer to the canal walls (30).

There is a limited data in the literature about the dentinal push-out bond strength of both calcium silicate-based sealers, Endoseal MTA and Tech Biosealer Endo. In the present study, when final irrigant is not taken into account, Endoseal MTA and Tech Biosealer Endo root canal sealers showed weak dentinal push-out bond strength compared to AH Plus. Similarly, Oliveira *et al.* reported that AH Plus had significantly higher bond strength than both MTA Fillapex and iRoot SP (31). Furthermore, in a recent systematic review and meta-analysis, it has been reported that bioceramic sealers do not perform better than traditional epoxy sealers in terms of dislodgement resistance (32). *Silva et al.* used artificial canals opened in dentinal slices for push-out strength experiment, as the present study, and found that push-out strength of Endoseal MTA is weaker than of AH Plus but stronger than of MTA Fillapex (25). The push-out bond strength of Tech Biosealer Endo did not improve with the irrigation protocols in the present study. These results are in accordance with an earlier report that presented the push-out strength of Tech Biosealer Endo when root canals were irrigated with NaOCl-EDTA or NaOCl-CHX (33).

Conclusion

Within the limitations of this *in vitro* study, using CHX after NaOCl in final irrigation leads to increase in push-out bond strength of epoxy resin-based sealers, whereas it does not affect the dentinal bond strength of calcium silicate-based sealers.

Türkçe Özet: *Final irrigantların iki kalsiyum silikat esaslı kök kanal patının push-out bağlanma dayanımına etkileri: in vitro çalışma. Amaç: Bu çalışmanın amacı, final irrigasyonda kullanılan farklı irrigant kombinasyonlarının, farklı içeriklere sahip kök kanal patlarının push-out bağlanma dayanımları üzerine etkilerini incelemektir. Gereç ve Yöntem: 15 adet alt premolar dişin her birinden 1 mm kalınlığında 4 dilim olacak şekilde, toplam 60 dentin dilimi toplandı. Her dentin dilimi üzerinde 3 adet kanal benzeri yapay kavite açıldı. Örnekler her biri 15 örnekten oluşan 4 deney grubuna ayrıldı. Örnekler grup 1'de sırasıyla %5.25 NaOCl ve %17 EDTA solüsyonlarına; grup 2'de sırasıyla %5.25 NaOCl ve %2 CHX solüsyonlarına; grup 3'te sırasıyla %5.25 NaOCl, %17 EDTA ve %2 CHX solüsyonlarına; ve grup 4'de distile su içine daldırıldılar. Dentin dilimlerindeki her bir yapay kavite emici kâğıtlarla kurutulduktan sonra, farklı kanal patlarıyla (Endoseal MTA, Tech Biosealer Endo veya AH Plus) dolduruldu. İki gün sonra, push-out bağlanma dayanımı testi yapıldı. Bulgular: CHX içeren iki final irrigant kombinasyonunda da (grup 2 ve 3) AH Plus daha yüksek push-out bağlanma dayanımı gösterdi (p <0,001). Endoseal MTA ve Tech Biosealer Endo kök kanal patlarının push-out bağlanma dayanımları final irriganttan etkilenmedi (p=0,965). Sonuç: Final irrigasyonda NaOCl'den sonra CHX kullanılması epoksi rezin bazlı kanal patının push-out bağlanma dayanımını arttırırken, kalsiyum silikat esaslı kanal patlarının*

bağlanma dayanımında herhangi bir fark oluşturmadı. Anahtar kelimeler: Kalsiyum silikat esaslı kanal patı, klorheksidin glukonat, çökelti, push-out bağlanma dayanımı, kök kanal irrigantları.

Ethics Committee Approval: This study was reviewed and approved by the Research Ethics Committee of Karadeniz Technical University (Protocol Number: 2021/75).

Informed Consent: Participants provided informed consent.

Peer-review: Externally peer-reviewed.

Author contributions: DC, TT participated in designing the study. ATOK, TK participated in generating the data for the study. ATOK, TK participated in gathering the data for the study. DC participated in the analysis of the data. DC, TT wrote the majority of the original draft of the paper. DC, TT participated in writing the paper. ATOK has had access to all of the raw data of the study. DC, TT have reviewed the pertinent raw data on which the results and conclusions of this study are based. DC, ATOK, TK, TT have approved the final version of this paper. DC, ATOK, TK, TT guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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