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Effect of alexidine dihydrochloride on the bond strength of resin-based sealer to dentin

Aleksidin dihidroklorürün rezin esaslı kanal patının bağlanmasına etkisi

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ABSTRACT

Objective: To evaluate the bond strength of resin-based sealer to dentin after using different final irrigation solutions, one of which is alexidine dihydrochloride (ALX).

Methods: A total of 90 human maxillary central teeth were used. The groups were designed according to the final irrigation solution applied, as follows: Group 1: 17% ethylenediamine tetra-acetic acid (EDTA), Group 2: QMix, Group 3: 2% chlorhexidine gluconate (CHX), Group 4: 1% ALX, and Group 5: Distilled water (Control group). All canals were obturated with gutta-percha and AH Plus. For bonding strength measurement, three horizontal sections of 2 mm thickness were obtained from each sample towards the coronal to apical. Push-out force was applied until bond failure occurred. Modes of failure were classified by examining each sample using a stereomicroscope as follows: adhesive (sealer-dentin or sealer-gutta-percha interface), cohesive (failure within sealer or dentin), mixed (failure in both the sealer and dentin). The Kruskal-Wallis *H* test was used for comparison among the groups ($P < .05$).

Results: The mean highest values were obtained from the coronal, middle and apical regions, respectively ($P < .05$). The highest significant values for bonding strength was obtained in the EDTA group and the lowest in the control group ($P < .001$). There are no statistical differences among QMix, CHX and ALX groups, except for the coronal third, where the bond strength for the QMix was higher compared to the other two groups ($P = .001$). All samples showed the cohesive failure type in most.

Conclusion: Bonding strength is high when EDTA is used in any of the three parts of the root canal. ALX showed similar bond strengths to CHX.

Key Words: AH Plus, alexidine, bond strength, chlorhexidine, EDTA, Qmix

ÖZ

Amaç: Bu çalışmanın amacı aralarında aleksidin dihidroklorürün (ALX) de bulunduğu farklı final irrigasyon solüsyonları kullanımı sonrası rezin esaslı kök kanal patının dentine bağlanma dayanımını değerlendirmektir.

Yöntemler: 90 adet insan üst birinci keser dişi kullanıldı. Uygulanan son yıkama solüsyonuna göre gruplar şu şekilde oluşturuldu: Grup 1: 17% EDTA, Grup 2: QMix, Grup 3: %2 CHX, Grup 4: %1 ALX, Grup 5: Distile su (kontrol grubu). Tüm kanallar güta-perka ve AH Plus ile dolduruldu. Bağlanma dayanımı ölçümü için her bir örnekten koronalden apikale doğru 2 mm kalınlıkta üç yatay kesit elde edildi. Bağlantıda kopma oluşana kadar push-out kuvveti uygulandı. Bağlantı başarısızlığı, her bir örnek stereomikroskop ile değerlendirilerek şu şekilde sınıflandı: Adeziv (pat-dentin ya da pat-güta-perka arayüzünde), koheziv (pat ya da dentin arayüzünde), karma (hem pat hem dentinde). Gruplar arası karşılaştırmalar Kruskal-Wallis *H* testi ile yapıldı ($P < .05$).

Bulgular: En yüksek ortalama bağlanma dayanımı değerleri sırasıyla koronal, orta ve apikal bölgede görüldü ($P < .05$). En yüksek ortalama bağlanma dayanımı değeri etilendiamin tetra-asetik asit (EDTA), en düşük kontrol grubundaydı ($P < .001$). Qmix'in, CHX ve ALX'le kıyaslandığında daha yüksek değer gösterdiği koronal bölge dışında, bu üç grup arasında farklılık yoktu. Tüm örneklerde en fazla koheziv başarısızlık görüldü.

Sonuç: EDTA kullanıldığında kök kanalının her üç bölümünde de bağlanma dayanımı en yüksekti. ALX, CHX ile benzer bağlanma dayanımları gösterdi.

Anahtar Kelimeler: AH Plus, aleksidin, bağlanma dayanımı, klorheksidin, EDTA, Qmix

INTRODUCTION

Endodontic success depends on cleanse of entire root canal system with antimicrobial solutions and sealing using a core material in combination with root canal sealers three-dimensionally.[1] To accomplish this, irrigation solutions should exhibit high antimicrobial activity and substantivity in the root canals.[2] Chlorhexidine gluconate (CHX) is a bisbiguanide having these properties. It exhibits a slow, long-term release at therapeutic levels.[3] However, the interaction of CHX with sodium hypochlorite (NaOCl) results in para-chloroaniline (PCA)[4], a toxic compound causing human-associated methemoglobinemia.[5] Therefore, caution is required when using CHX.[4]

QMix® 2in1 (Qmix) (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) is a ready-to-use, transparent, combined solution of CHX, ethylenediamine tetra-acetic acid (EDTA), and detergent. It exerts anti-

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microbial activity on *Enterococcus faecalis* and eliminates the smear layer.[6] Because of QMix's chemical formula, the formation of PCA from interactions with CHX and NaOCl is inhibited.

The search for the ideal irrigation solution continues. Alexidine dihydrochloride (ALX), which is chemically similar to CHX[7] and has positive effects on cancer[8], aging, and age related disorders[9], is considered as a potential endodontic irrigation solution.[10-14] CHX and ALX bind to lipopolysaccharide and lipoteichoic acid; however, ALX shows more affinity for them than CHX.[15, 16] While the bactericidal activity and minimum inhibitory concentration values of CHX and ALX are similar, ALX reaches these values much more quickly, and it shows a more rapid antibacterial effect, more bacterial permeability than CHX and more antimicrobial substantivity.[7] The 1% and 2% ALX solutions used for 1 min showed longer antimicrobial activity against *E. faecalis* than the 0.5% and 2% CHX solutions.[7] Silva *et al* [10] reported that the use of NaOCl together with ALX is effective in biofilm eradication. Surender *et al* [17] reported that the same concentrations of ALX were more effective than CHX on *E. faecalis*. Silveira *et al* [18] reported that low concentrations of ALX also showed antimicrobial activity on *E. faecalis* biofilm.

The interaction of NaOCl and CHX results in the formation of PCA[5], whereas not with ALX.[19-21] Sharp *et al*. [22] reported allergic reactions resulting in anaphylactic shock with CHX while no such notification has been made for ALX to date. Also ALX is less toxic than CHX[23] and has low toxicity on mammalian cells.[21]

If ALX is combined with NaOCl, it can be a potential endodontic final irrigation solution.[10-14] Moreover, it may be an alternative to CHX due to its activity on *E. faecalis* and *Candida albicans*.[24]

Adhesion of the root canal filling is one of the important clinical factor in endodontic treatment.[25] After disinfecting the root canals with irrigation solutions, a tight connection between the root canal filling and the root dentin contributes to the positive prognosis of root canal treatment. This is necessary to prevent reinfection.The final irrigation protocol affects the adhesion of the root canal filling to the root canal dentin.[26, 27]

The impact of various irrigation solutions on the bond strength of root canal sealers has been investigated in the literature.[26, 28, 29] However, no study using ALX as final irrigation solution has been found. In this study, we aimed to evaluate the effect of ALX as an endodontic final irrigation solution, which is a potential alternative to CHX, on the connection between the resin-based sealer AH Plus and intradicular dentin and compared these values with the values of other final irrigation solutions commonly used clinically. The null hypothesis was that there is no difference in bonding strength values among the tested irrigation solution.

MATERIAL AND METHODS

This study was accepted by the Ethics Committee of Ankara University (protocol no: 36290600/13). Based on the results obtained by searching similar studies to our research[25, 28, 29], a total of 90 teeth, at least 18 for each group, at 5% type-I error and 80% power levels, were included in the study using GPower 3.1.9.2 package program. Ninety recently extracted human maxillary central teeth were used in this study. Non-restorative teeth without root fractures, cracks, or closed root apices were used. To ensure root length standardization, the teeth were cut with a diamond fissure bur (ISO 806314, 014, Meisinger, Germany) to a distance of 13 mm of the apex. The working length was determined 1mm away from the apical foramen with a #15 K-file. (Dentsply Maillefer, Ballaigues, Switzerland). Then apex covered with melted wax (Base Plate Wax; Kerr, Brea, CA) to mimic the clinical situation. The roots were randomly assigned to five groups (n = 18) according to the final rinse protocol.

Specimen Preparation

Group 1 (EDTA): After each instrumentation, 2 mL of 5.25% NaOCl (Sultan Chemists Inc., Englewood, New Jersey, USA) were used for irrigation, and final rinse was made with 5 mL of 17% EDTA (Werax, Spot Dental Industry, Izmir, Turkey) for 1 min.

Group 2 (QMix): According to the manufacturer's instructions, 2 mL of 6% NaOCl (Vista Dental Products, Racine, USA) were used after each instrumentation, and 5 mL of QMix (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) were used for 90 s as the final irrigation.

Group 3 (CHX): After each instrumentation, 5.25% NaOCl was used, and, after completion of the preparation, 5 mL of 17% EDTA were maintained in the root canal for 1 min. Then the specimens were irrigated with 5 ml of distilled water. Finally, 2% CHX solution (Klorhex, Drogsan Medicine, Ankara, Turkey) was used for 1 minute.

Group 4 (ALX): The same irrigation protocol was used as for the CHX group; however, 5 mL of 1% ALX (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA) were maintained in the root canal for 1 min as the final irrigant.

Group 5 (Distilled water) (Control group): After each instrumentation, 2 mL of 5.25% NaOCl were used for irrigation, and the final rinse was made with 5 mL distilled water.

The irrigation procedure was applied for each sample, with a syringe and 29-G needle (NaviTip; Ultradent, South Jordan, UT) 1 mm shorter than the working len hemomechanical preparation was performed using ProTaper Universal rotary (Dentsply Maillefer, Ballaigues, Stwizerland) instruments up to F4.

After chemomechanical preparation, the root canals were dried using paper points. Next, using the single-cone technique, the root canals were obturated with AH Plus sealer (Dentsply-Tulsa Dental, Tulsa, OK) and F4 gutta-percha cones (Dentsply Maillefer, Ballaigues, Switzerland). Radiographs of the specimens were taken to see if there was a void in the root canal filling. The samples were sealed with temporary filling (Cavit; 3M ESPE, Seefeld, Germany). The samples were stored at 37°C for 2 weeks 100% humidity in a dark environment to completely harden the root canal filling. Later, the roots were embedded in acrylic resin using plastic moulds.

Horizontal sections of approximately 2 mm in thickness were taken from the coronal to the apical third in each sample, under water cooling with 0.3 mm thick diamond disks rotating at a low speed by using the precision saw instrument (IsoMet, Metkon, Microcut precisioncutter, Bursa, Turkey). Coronal faces of the samples were labelled and 54 samples were obtained for each test group.

Three different sizes of plungers (0.3, 0.6 and 0.8 mm) were used to be dimensionally compatible with different root canal fillings. At this stage, force was applied from the apical face of the canal to the coronal face due to the apical to coronal elevation of the root sections. At the connection between the universal testing machine (Lloyd; Fareham, Hants, England) and the root canal filling and dentin, the rupture was applied at a constant speed of up to 1.0 mm/min.

Table 1. Push-out Bond Strength (MPa, Mean ± Standard Deviation [SD]) of AH Plus after 4 Irrigation Protocols According to Root Third (n = 18)

Groups	Coronal	Middle	Apical
1-EDTA	$2.09 \pm .09^{aA}$	$1.52 \pm .02^{bA}$	$1.11 \pm .01^{cA}$
2-QMix	$1.94 \pm .03^{aB}$	$1.44 \pm .03^{bB}$	$1.09 \pm .01^{cB*}$
3-CHX	$1.84 \pm .02^{aC}$	$1.43 \pm .02^{bB}$	$1.08 \pm .04^{cB}$
4-ALX	$1.83 \pm .03^{aC}$	$1.42 \pm .03^{bB}$	$1.06 \pm .03^{cB*}$
5-Control	$1.02 \pm .01^{aD}$	$0.66 \pm .08^{bC}$	$0.55 \pm .09^{cB}$

Within each group, values with identical lowercase superscript letters indicate no significant difference ($P > .05$); among the groups for the same subgroup, values with identical uppercase superscript letters indicate no significant difference ($P > .05$). Also, there is a significant difference between two subgroups indicated by the B* symbol.

The breaking forces were recorded in Newtons (N) using a Nexygen data analysis program (Lloyd LRX, Fareham, UK), and the bonding strength was calculated by converting the megapascals (MPa), according to the following formula.[30]

Bonding Strength (MPa) = F / (2πr × h)

where MPa: Bond strength unit; F: Applied force (Newton); r: Radius of root canal section (mm); and h: Height of root section (mm); π value is taken as 3.14.

When the push out test was completed, types of failure were classified by examining each sample at 40 × magnification using a stereomicroscope (M3Z; Leica Microsystems, Wetzlar, Germany) as follows: adhesive (sealer-dentin or sealer-gutta-percha interface), cohesive (failure within sealer or dentin), mixed (failure in both the sealer and dentin).[31]

Statistical Analyses

Data were analysed by using IBM SPSS Statistics ver. 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) software. Normality tests were performed with the Shapiro-Wilk test, and the Kruskal-Wallis *H* test was used for comparison of the groups. The significance level was set at $P < .05$.

RESULTS

The mean bond strength values are presented in Table 1. Depending on the final irrigation solution applied and the cross-sections taken from the root canal, there was a significant difference in the values of the bonding strength of AH Plus to the root canal dentin. The samples showed the highest significant mean bonding strength values in the EDTA group (2.09 ± 0.09 MPa) and the lowest in the control group (1.02 ± 0.01 MPa) ($P < .001$, for all comparisons). In all three sections, the highest values were observed in the coronal, middle, and apical third regions, respectively, and the differences were significant ($P < .001$). Bonding strength values from high to low were the QMix, CHX and ALX groups, respectively, and differences from the middle third sections in these groups were not statistically significant ($P > .05$). There were no statistically significant differences between CHX and ALX for all root regions. All samples showed the cohesive failure type in most.

Within each group, values with identical lowercase superscript letters indicate no significant difference ($P > .05$); among the groups for the same subgroup, values with identical uppercase superscript letters indicate no significant difference ($P > .05$). Also, there is a significant difference between two subgroups indicated by the B* symbol.

DISCUSSION

In our study, significant differences were found among the groups. For this reason, null hypotheses were rejected based on the findings of the study.

Adhesion of the canal filling to root dentin is crucial to reduce the gaps that may cause leakage [32] and to prevent the movement of the root canal filling during dental procedures.[33]

The push-out test is dependable in determining the bond strength of root canal sealers.[34] In this test method, similar to the clinic, failures occur parallel to the dentin-resin bonding surface, and this method provides better evaluation than traditional shear testing.[35] Different irrigation solutions used in endodontic treatment may cause changes in dentin permeability, solubility or surface energy by affecting the structural and chemical composition of dentin surface[28] and hence affecting the adhesion of materials to dentin surfaces.[36] This study aimed to evaluate the effect of ALX, an experimental irrigation solution, on push out bond strength by comparing it with other irrigation solutions that are frequently used in the clinic.

Similar to the current study, AH plus has been used with single cone technique in various studies where the bond strength was evaluated before.[37-40] The impact of the removal of the smear layer on adhesion between sealer and dentin has been extensively discussed in the literature.[29, 41] When chelating agents are used, it may be easier for the root canal sealers to penetrate dentin tubules that are open, so that microretention, the mechanical lock between sealer and dentin, can be attained.[26] The use of inorganic tissue dissolvers, such as EDTA, following NaOCl irrigation results in an increase in the bonding strength values of resin-based sealers to root canal dentin.[25, 26]

Buzoğlu *et al.*[42] reported that EDTA reduced dentin wettability and surface energy. Ballal *et al.*[27] suggested that the increase in dentin wettability is an important factor in attaining better bonding of hydrophilic sealers, and they also noted that hydrophilic and hydrophobic root canal sealers exhibit different contact angles. Hashem *et al.*[28] reported that the decrease in dentin wettability increased the bonding of the hydrophobic sealers. Similar to those previous studies, in the current study, the highest bond strength values were obtained in the EDTA group.

Although the ratios and percentages of the ingredients are unknown because the manufacturer does not specify them, QMix contains polyamino-carboxylic acid as a chelating agent, bisbiguanide as an antimicrobial agent, surfactant, and deionized water. The surfactants in QMix are responsible for the low surface tension of the solution and increase its wettability.[43] In the current study, EDTA affected the bonding of AH Plus to root canal dentin more positively compared to the QMix. This may explain why the lower wettability with EDTA than with QMix provides favourable conditions for bonding of the hydrophobic structured sealer. Also, Aranda-Garcia *et al.*[29] reported that QMix does not increase bonding strength as effectively as EDTA. However, some studies reported that EDTA combined with surfactants does not change the properties of the solution.[44]

The impact of CHX on the bonding strength of root canal sealers is questionable.[25, 28] Carrilho *et al.*[45] reported that CHX positively contributed to the resin-dentin connection in the long-term. On the other hand, Nassar *et al.*[46] stated that CHX did not affect the bonding strength either positively or negatively. Similarly, in this study, CHX showed a significantly lower level of bonding values than EDTA and QMix, while showing a significant difference from the control group.

Barrios *et al*.[7] emphasized that 1% and 2% ALX solutions exert similar antimicrobial activity on *E. faecalis* and suggested that ALX may be an alternative to CHX for endodontic treatment. In addition, an ALX solution at concentrations of 1% or 2% can effectively eradicate the *E. faecalis* biofilm layer on root canal dentin. For this reason, a 1% concentration of solution was used in this study. The clinical advantages of using ALX as an endodontic irrigation solution are that the interaction with NaOCl does not alter the antibacterial efficacy.[47]

NaOCl and CHX interaction does not result in PCA formation.[19-21] However in the literature, there are different opinions about whether the combined use of NaOCl and ALX creates a precipitate. In some studies, it was reported that no precipitate formed and the solution became more transparent as the NaOCl concentration increased.[19, 20] On the contrary, in a recent study, it was reported that a yellowish precipitate was formed by the interaction of NaOCl and ALX, but PCA was not formed.[13] Unlike PCA[12], SEM (Scanning Electron Microscope) images in previous studies revealed that this precipitate did not occlude dentin tubules, so does not compromise the quality of the root canal filling in terms of microleakage.[11, 19] It was recommended that ALX and NaOCl should be used without mixing during endodontic irrigation since aliphatic amines which are the products of this reaction have neurotoxic properties.[13] Also in our study ALX and NaOCl did not used consecutively.

Because of these positive properties, the current study aimed to evaluate the effect of 1% ALX as a potential endodontic final irrigation solution on the dentin bonding strength of root canal sealer. In this study, ALX showed significantly higher bonding values than the control group. However, there was no statistically significant difference between ALX and CHX in all groups. In the literature, there were no investigations of the bonding strength of root canal sealer to the root canal dentin after irrigation with ALX. For this reason, there is no reference with which to compare the results of the current study.

ALX is a bisbiguanide containing two ethyl-hexyl groups, whereas CHX contains p-chlorophenyl end groups. ALX differs from CHX.[15, 19] However, because both compounds are chemically very similar, they may have produced similar effects on the dentin, and, therefore, similar bonding strength values were obtained in the study. ALX may cause changes on the dentin structure. There is a need to evaluate this with further studies.

In the study, the reason for the significant decrease in the bonding strength values in all groups, from coronal to apical, could be explained by the low tubular density because of sclerotic dentin in this region, which results in less tubular penetration of the root canal sealers in the apical area.[48]

Low demineralization with EDTA resulted in flat surfaces in the dentin.[41] In the control group, the chemical bond between AH Plus and dentin may have been adversely affected by the absence of EDTA and possibly occurred as a result of residual NaOCl in the deep layers of the rough dentin surface. The irrigation of root canal dentin alone with NaOCl may not be seen as favourable since it does not result in demineralisation and, therefore, does not release collagen for monomer infiltration.[49]

Consistent with previous studies, the highest failure was observed as cohesive failure.[37, 50] The observation of dominantly cohesive failure may be related to the highest adhesion to dentine.[50]

CONCLUSION

The present study suggests that epoxy resin-based sealers' adhesion is affected by the final irrigation procedure. It also demonstrates that the effect of ALX on the adhesion of root canal sealers to root dentin, which has the potential to be recognized in endodontic clinics due to its positive properties at this point, is comparable to CHX which is often preferred. Further studies on clinical use of ALX are needed in the future.

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