The Effect of Boric Acid on Root Dentin Mineral Content and Bond Strength of AH-Plus: A SEM-EDX Study

Kök Dentininin Mineral İçeriğine ve AH-Plus'ın Bağlanma Dayanımına Borik Asitin Etkisi: SEM-EDX Çalışması

Melek AKMAN,[a]
Sema BELLİ,[b]
Keziban OLCAY,[c]
Betül ÖZÇOPUR[d]

[a]Department of Endodontics, Konya Necmettin Erbakan University Faculty of Dentistry,
[b]Department of Endodontics, Selçuk University Faculty of Dentistry, Konya
[c]Department of Endodontics, Medipol University Faculty of Dentistry, İstanbul
[d]Private Medical Doctor, Antalya

Geliş Tarihi/*Received:* 14.06.2015
Kabul Tarihi/*Accepted:* 14.10.2015

This study was presented as a poster at PER/IADR 2012 Congress, 12-15 September 2012, Helsinki, Finland.

Yazışma Adresi/*Correspondence:*
Melek AKMAN
Konya Necmettin Erbakan University Faculty of Dentistry,
Department of Endodontics, Konya,
TÜRKİYE/TURKEY
melek_nas@hotmail.com

ABSTRACT Objective: To investigate the effects of boric acid, EDTA and citric acid on root-dentin mineral content and on shear-bond strength of AH Plus. **Material and Methods:** Eighteen extracted mandibular premolars were used. The crowns were removed. The roots were then longitudinally sectioned under water cooling to obtain two root halves from each tooth. Element levels were examined by SEM-EDX and build-ups were created using AH Plus. The element levels and shear-bond strength data (MPa) were recorded. After shear-test, the root surfaces were re-grounded, and subjected to 5,25% NaOCl followed by 10% Boric acid, 17% EDTA and 10% Citric acid. The root surfaces were re-examined by SEM-EDX. Shear-test was repeated. The element levels and shear-bond strength data (MPa) were recorded and analyzed by using paired samples t test. **Results:** Boric acid increased O, Na, K and Ca/P ratio ($p<0.001$), did not change Ca, C, Mg contents of root-dentin ($p>0.05$). EDTA increased O, Na, C, Ca/P ratio ($p<0.001$), decreased the Ca, P, S ($p<0.001$). Citric acid increased C, Na, Ca/P ratio ($p<0.001$), decreased Ca, P, S contents ($p<0.05$). Citric acid did not change shear-bond strength of AH Plus ($p=0.218$). Both Boric acid and EDTA treatment decreased shear bond strength of the sealer ($p=0.000$). **Conclusion:** All the tested materials changed the mineral contents of root dentin. Boric acid and EDTA treatment decreased shear bond strength of AH Plus. Boric acid might be considered as an alternative chelating agent however further experimental and clinical evaluations are needed to use the material safely.

Key Words: Boric acids; chelating agents; shear strength

ÖZET Amaç: Borik asit, EDTA ve sitrik asitin kök dentininin mineral içeriğine ve AH Plus'ın makaslama bağlanma dayanımına etkisini araştırmaktır. **Gereç ve Yöntemler:** 18 adet çekilmiş premolar dişler kullanıldı. Dişlerin kuron kısımları kaldırıldı. Herbir dişten iki kök yarısı elde etmek için su soğutması altında uzunlamasına kesit alındı. SEM EDX kullanılarak element seviyeleri ölçüldü. AH Plus ile build-up oluşturuldu. Element seviyeleri ve makaslama bağlanma dayanım değerleri (MPa) kaydedildi. Shear testi yapıldıktan sonra kök yüzeyleri tekrar düzleştirildi ve %5,25 NaOCl'u takiben %10 Borik asit, %17 EDTA and %10 Sitrik asit uygulandı. Kök yüzeyleri tekrar SEM EDX ile ölçüldü. Element seviyeleri ve makaslama bağlanma dayanım değerleri kaydedildi (MPa) ve paired t testi kullanılarak analiz edildi. **Bulgular:** Borik asit uygulanımı kök dentininin O, Na, K ve Ca/P oranını ($p<0.001$) artırdı, Ca, C, Mg mineral içeriğini ($p>0.05$) değiştirmedi. EDTA uygulanımı O, Na, C, Ca/P oranını artırdı ($p<0.001$), Ca, P, S mineral içeriğini azalttı ($p<0.001$). Sitrik asit uygulanımı C, Na, Ca/P oranını artırdı ($p<0.001$), Ca, P, S mineral içeriğini azalttı ($p<0.05$). Sitrik asit uygulanımı AH Plus'ın makaslama bağlanma dayanım değerini değiştirmedi ($p=0.218$). Hem Borik asit hem de EDTA uygulanımı AH Plus'ın makaslama bağlanma dayanım değerini azalttı ($p=0.000$). **Sonuç:** Tüm test edilen materyaller kök dentininin mineral içeriğini değiştirdi. Borik asit ve EDTA AH Plus'ın makaslama bağlanma dayanımını azalttı. Borik asit alternatif şelasyon ajanı olarak düşünülebilir ancak materyalin kullanım güvenilirliği için daha ileri çalışmalara ve klinik uygulamalara ihtiyaç vardır.

Anahtar Kelimeler: Borik asitler; şelasyon ajanları; kayma mukavemeti

doi: 10.5336/dentalsci.2015-46776

Turkiye Klinikleri J Dental Sci 2016;22(1):14-20

Dentin has been described basing on organic and inorganic components. Calcium (Ca) and phosphorus (P) present in hydroxyapapite crystals are the major inorganic components of dental hard tissues.[1] Researchers reported that some chemical agents cause alterations in the chemical structure of human dentin.[1,2] The alterations in Ca/P ratio may change the original proportion of organic and inorganic components that in turn change the permeability and solubility characteristics of dentin.[2,3] This change may affect the adhesion of dental materials such as resin-based cements and root canal sealers to dentin as dentin adhesion depends on the presence of residual Ca^{2+} on the bonding area.[2,4]

Mechanical instrumentation is one of the important steps of root canal treatment. An irregular layer of debris known as the smear layer is invariably formed after mechanical instrumentation of the root canal.[5] Some studies have shown that removal of the smear layer enhances adhesion of sealers to the root canal wall.[6,7] Smear layer includes both organic and inorganic components; therefore, chemical irrigants are necessary for debridement of root canals during shaping and cleaning procedures.[8] Sodium hypochlorite (NaOCl) is the most commonly used irrigation solution for chemomechanical debridement of root canals.[9,10] In addition to NaOCl, the use of a chelating agent to remove the smear layer has been proved to be essential.[5]

Decalcifying solutions have been reported to be suitable to remove the smear layer.[11] Ethylenediaminetetraacetic acid (EDTA) can be used as a final flush to open up the dentinal tubules thus allowing an increasing number of lateral canals to be filled.[12] On the other hand, an excessively aggressive effect on the canal walls and an alteration of root dentins mechanical properties with EDTA has been reported.[13,14]

Citric acid is a chelating agent that reacts with metals to form a nonionic soluble chelate.[15] In operative dentistry, citric acid has been proposed as a mild etchant for dental hard tissue, particularly for dentinal conditioning and enhanced smear layer and plug removal.[16] Goldman et al reported that the effects on the removal of the smear layer obtained with citric acid were similar to those by EDTA.[17]

Recently researchers have regarded boron as an antioxidant agent.[18] Boron is the chemical element with the atomic number 5. Boron is known as boric acid and borate is frequently found in diets plentiful in foods like fruits, vegetables and legumes.[18] According to the literature boron can regulate the oxidant antioxidant level of the affected tissues.[19] Boric acid is used in many cosmetics and personal care products such as shampoos, soaps and detergents, underarm deodorants, moisturizing creams, shaving lotions, and breath fresheners.[20] Although boric acid and sodium borates have been considered as being 'toxic to reproduction and development', following the results of animal studies with high doses, the findings to date suggest that exposure to boric acid and sodium borates under normal handling and use conditions are not toxic for reproduction in men.[21]

The purposes of this study were:

- to investigate the effects of boric acid, EDTA and citric acid on root dentin mineral content,
- to compare the effects of these chelating agents on shear bond strength of a well studied epoxy resin based sealer (AH Plus) with good physicochemical properties to root dentin.[22]

The null hypothesis tested was that there was no significant difference among the effects of tested solutions on the mineral content of root dentin and shear bond strength of AH Plus sealer.

MATERIAL AND METHODS

Eighteen intact human caries-free mandibular premolars scheduled for extraction for orthodontic reasons were selected. Tissue samples were obtained from different 18 healthy patients (10 women and 8 men). The mean age was 21 years, and the age range was 18-25 years. Informed consent was obtained from patients. Eighteen extracted mandibular premolars that had been stored in distilled water for a maximum of 2 months were scaled using a hand scaler (Gracey Curetta SG 17/18; Hu-Friedy, Chicago, IL, USA) to remove the soft-tissue covering surfaces. The crowns of all teeth were removed at the cement-enamel junction with a high speed diamond saw (Buehler, Lake Bluff, IL, USA) under water cooling. The pulp tissues were removed using barbed broaches (MANI Inc., Tochigi, Japan) and the roots were then longitudinally sectioned under water cooling to obtain two root halves from each tooth (n= 36). Each half was embedded horizontally in a self-curing polymethyl-methacrylate resin (Vertex, Dentimex Dental, Zeist, Netherlands) keeping the root surfaces exposed. Bonding surfaces were polished with 800-grit silicon carbide paper (E.A.C English Abrasives & Chemicals Ltd. England) using distilled water.

SCANNING ELECTRON MICROSCOPE-ENERGY DISPERSIVE X-RAY SPECTROSCOPY (SEM-EDX) ANALYSIS

SEM-EDX Analysis (SEM-ZEİSS, LS-10, Germany) was done at Selcuk University, Advanced Technology Research and Application Center, Konya, Turkey. The samples were randomly numbered from 1 to 36 and the mineral contents of each sample were evaluated. A surface analysis was performed during this procedure and the coronal 1/3 of the roots were chosen for examination as that was the most widened part of the root and provided an adequate surface for shear bond strength test. The ingredients and the distribution of the elements Calcium (Ca), Oxygen (O), Phosphorus (P), Carbon (C), Magnesium (Mg), Sodium (Na), Sulfur (S), Chlorine (Cl) levels were examined for each sample and recorded as tables and graphics (EHT:20.00kV).

SHEAR-BOND STRENGTH TEST

Three x 3mm high build-ups with a constant surface area of 3.45 mm^2 were created in the coronal 1/3 of the roots using AH Plus sealer (Dentsply, De Trey, Konstanz, Germany) that was manipulated according to the manufacturer's instructions at room temperature. Polyethylene tubes were used to create the build-ups and allowed to set (37ºC, 100% humid, 72 hrs). The samples were retained in a universal testing machine (Instron, Canton, MA) to apply shear stress at a crosshead speed of 1 mm/min. The data at the failure point was calculated as MegaPascal (MPa). Fractured specimens were examined with a stereomicroscope (Olympus SZ-CTV, Olympus, Tokyo, Japan) at X22 magnification to determine mode of failure. Failure modes were classified as adhesive, cohesive or mixed.

IRRIGATION REGIMEN

The root surfaces were re-ground with 800 silicon carbide paper after SEM-EDX analysis and then placed in 30 mL of 5.25% NaOCl for 5 minutes. Three test groups (n=12) were created according to the final rinse with the following chelating agents:

G1: 10% Boric acid (Isolab GmbH, Wertheim, Germany); G2: 17% EDTA (Imicryl, Konya, Turkey) and G3: 10% Citric acid (Sigma-Aldrich, Steinheim, Germany). All the samples were placed in 30 mL of chelating agents for 1 min. Figure 1 shows the root dentin surfaces after treatment with boric acid (Figure 1(a)); EDTA (Figure 1(b)) and citric acid (Figure 1(c)). The canals were dried with paper points until complete dryness of the last point was confirmed visually.[23]

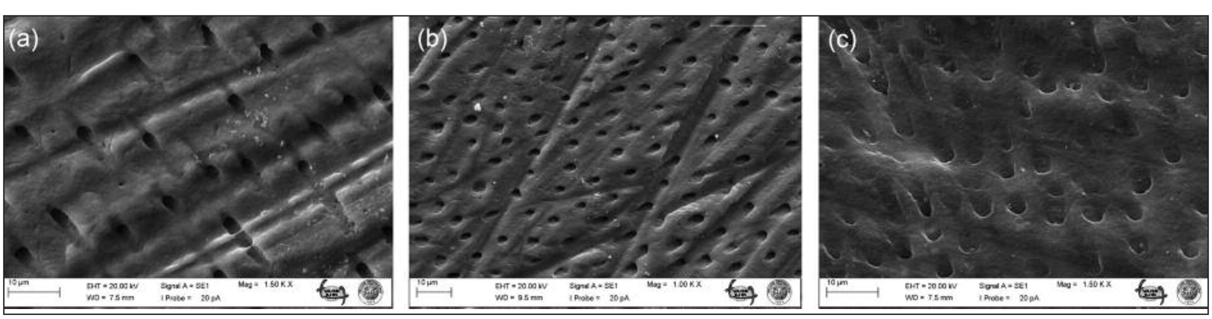

FIGURE 1: Exposed root dentin surfaces after treatment with (a) Boric acid; (b) EDTA and (c) Citric acid.

The root surfaces were then re-examined by SEM-EDX and the element levels were recorded. AH Plus sealer build-ups were re-created on the treated surfaces following the same procedures described above, allowed to set (37°C, 100% humid, 72 hrs) and tested to failure for shear bond strength.

STATISTICAL ANALYSIS

The initial SEM-EDX analysis and shear bond strength test of each sample was assumed as control of that sample. The mineral contents of the root surfaces and shear bond strength results before and after irrigation regimen were statistically analyzed using paired-t test with a significance set at $p<0.05$.

RESULTS

Table 1 presents the mineral contents of the experimental groups. Boric acid irrigation increased O, Na, K and Ca/P ratio ($p<0.001$), decreased P and S ($p=0.000$) but did not change Ca, C, Mg contents ($p>0.05$) while EDTA treatment increased O, Na, C, Ca/P ratio ($p<0.001$), decreased the Ca, P, S ratio of the root dentin ($p<0.001$). The Mg and K contents did not change with EDTA ($p>0.05$). Citric acid treatment increased C, Na, Ca/P ratio of the root dentin ($p<0.001$) but decreased Ca, P, S contents ($p<0.05$). O, Mg and K content did not change ($p>0.05$).

The mean shear bond strength measurements and failure patterns of the tested samples are shown in Table 2. Citric acid treatment did not change shear-bond strength of AH Plus ($p=0.218$). Both boric acid and EDTA treatment decreased shear bond strength of the sealer ($p=0.000$). All the groups showed mostly mixed failure pattern. Figure 2(a-c) show representative SEM photomicrographs with different magnifications after failure.

TABLE 1: Mineral content of the samples in the experimental groups.

		Ca	O	P	C	Mg
Boric Acid	Pretreatment	17,68±4,56	27,88±12,07	23,30±6,35	11,85±4,72	0,75±0,44
	Post-treatment	17,88±3,81	60,54±4,92	8,49±2,09	9,15±2,28	0,83±0,52
EDTA	Pretreatment	23,07±4,99	36,46±9,51	18,79±2,83	9,42±3,31	0,65±0,49
	Post-treatment	12,09±4,98	60,89±4,30	4,94±1,61	19,70±4,20	0,45±0,32
Citric acid	Pretreatment	24,78±4,73	40,36±6,65	18,05±4,05	11,63±6,16	0,45±0,24
	Post-treatment	1,21±1,076	46,46±8,01	0,34±0,38	33,44±5,52	0,68±0,80
		Na	**K**	**Ca/p**	**S**	
Boric acid	Pretreatment	0,14±0,07	0±0	0,81±0,27	5,02±2,09	
	Post-treatment	1,83±1,05	0,10±0,09	2,21±0,59	0,06±0,07	
EDTA	Pretreatment	0,57±0,62	0,04±0,09	1,29±0,46	2,58±1,33	
	Post-treatment	1,30±0,54	0±0	2,38±0,45	0±0	
Citric acid	Pretreatment	0,45±0,27	0,06±0,21	1,42±0,33	2,50±1,56	
	Post-treatment	1,59±1,71	0,14±0,18	5,71±5,90	0±0	

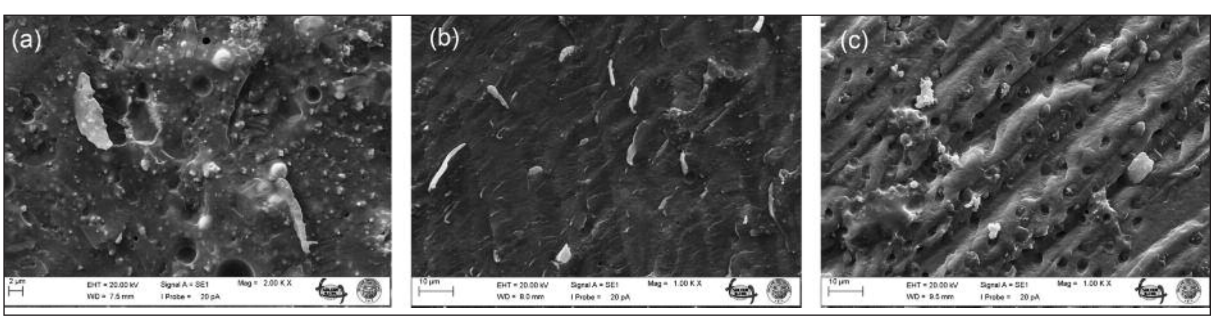

FIGURE 2: Representative scanning electron microscopy photomicrographs **(a)** at x2000 magnification of a fractured sample in boric acid treated group; **(b)** x1000 magnification of a fractured sample treated using by EDTA; **(c)** a fractured specimen treated using by citric acid (x1000).

TABLE 2: Shear bond strength values (Mean MPa ± SD) and failure patterns of the tested samples.

Groups	Citric acid	Failure pattern %	EDTA	Failure pattern %	Boric acid	Failure pattern %
Pre-treatment	4,26 ± 0,57[a]	17 A	6,22 ± 0,66[a]	17 A	4,00 ± 0,53[a]	9 A
		8 C		33 C		33 C
		75 M		50 M		58 M
Post-treatment	4,17 ± 0,72[a]	16 A	4,42 ± 0,90[b]	16 A	2,77 ± 0,42[b]	25 A
		8 C		25 C		25 C
		76 M		59 M		50 M

The same superscript letters are not significantly different (p>0,05). Different letters identify significantly different (p<0,05).
A: Adhesive failure; C: Cohesive failure; M: Mixed failure.

DISCUSSION

Measuring the bond strength after treating the samples with test solutions is an appropriate method.[24] However, this study focused on the alterations in root dentin with different chelating agents and the effect of these alterations on the bond between an epoxy resin based sealer and root dentin. Therefore both the surface analysis and bond strength tests were done before and after irrigation regimen.

Shear bond strength test method was used although a push-out test modified from the shear punch test has been advocated as a more suitable test for evaluating the bond strengths of intracanal filling materials.[25] Rahimi et al reported high standard deviations with push-out test compared to shear bond values. The type of dentin, number of tubules, presence or absence of sclerotic dentin were listed as high variables besides the great variability in canal shape and size with push-out test.[26] To avoid frictional stresses on the canal walls and to control the testing conditions shear bond strength test was chosen.[27]

Chelating solutions might play a part in influencing the mineral contents of dentin.[28] Earlier studies reported that irrigating solutions significantly changed the mineral contents of the root dentin.[29-31] Confirming these studies, SEM-EDX results of this in vitro study indicated that different chelating agents altered the mineral contents of root dentin. Therefore, the null hypothesis that there is no difference among the effects of tested solutions on the mineral content of root dentin can be rejected.

SEM-EDX is ideally suited for detailing surface morphology and identifying surface composition.[32] As the porosity of tissues may produce secondary diffractions, this instrument requires perfectly polished surfaces.[30] SEM-EDX can be used to evaluate the mineral content of dentin.[2] A variation of the EDX results in different sample areas may exist and this is a typical behavior of the SEM-EDX as this is a surface analysis method and shows only a part of the whole analytical information. One of the aims of this study was to evaluate a possible correlation between bond strength and root dentin mineral contents. Therefore, surface analysis of a specific area was needed. And that was possible with SEM-EDX technique.

Arı and Erdemir found that the Ca and P levels in dentin decreased after treatment with NaOCl and EDTA.[29] Similarly, the results of this study showed that Ca and P levels decreased when treated with EDTA and Citric acid following NaOCl. Furthermore, Ca/P ratio increased with all the tested chelating agents (Table 1). Rotstein et al and Perdigao et al reported that alterations in Ca/P ratio may affect the adhesion of resin-based cements and root canal sealers to dentin.[2,4]

In the present study, citric acid did not change shear-bond strength of AH Plus. Both boric acid and EDTA treatment decreased shear bond strength of the sealer. According to the results of this study we should accept the null hypothesis of the study in part. The previous studies, bond strength of AH Plus sealer was the highest when EDTA was used as a final rinse.[8,24] Pretreatment of dentin with 17% EDTA is known to remove the smear layer therefore, a stronger bond might be expected as it has been reported that the exposure of the dentinal tubules creates more irregular surface allowing penetration of resin tags.[33,34] Furthermore, AH Plus is able to bond to the organic phase of dentin, mostly to collagen network and a final rinse with 17% EDTA may make the substrate more suitable for bonding by causing exposure of the collagen network.[35] However, in the present study 17% EDTA treatment decreased shear bond strength of AH Plus. Similar findings have been reported by Saleh et al. and Wennberg&Orstavik.[36,37] These authors explained the decreased bond strength by the weak demineralization created by EDTA. According to Saleh et al, EDTA leaves a relatively smooth surface of the organic dentin structure which does not offer an increased area for adhesion.[36] Confirming these findings, Buzoğlu et al reported that single or combined use of EDTA significantly decreased the surface free energy of root dentin and according to Milosevic the lower the surface free energy, the lower the adhesion.[38,39]

Garcia-Godoy et al reported that 17% EDTA caused a collapse of the dentin matrix structure which impeded sealer infiltration.[40] Although there is little correlation between the AH Plus penetration and its push-out bond strength, this might be another reason for the decreased bond strength.[41]

The results of previous investigations have shown that the combined use of irrigating solutions has been found to decrease the effectiveness of chelating agents, might enhance the destruction of the dentinal surface, and has negative effects on the bond strength of adhesive cement to root canal dentin.[30,42] In the present study, citric acid treatment did not change shear-bond strength of AH Plus (p=0.218). According to Machado-Silveiro et al., the most effective decalcifying substance was 10% citric acid, followed by 1% citric acid, 17% EDTA and 10% sodium citrate.[43] Confirming this, citric acid treatment increased Ca/P ratio of the root dentin (p<0.001) and decreased Ca, P, S content (p<0.05) in the present study.

Boric acid solutions have not been tested yet in the complex root canal system. A recent study indicated that 2.5% citric acid solution was the most effective solution in removal of smear layer, 5 % boric acid showed the least effective.[44] Turk et al., has recommended that a combination of boric and citric acid solutions can be mixed for clinical use in order to achieve antimicrobial effect and smear layer removal capacity at the same time.[44]

When compared to citric acid and EDTA, boric acid might be considered as alternative chelating agent on the other hand previous studies indicated that very high intakes of boron induced reproductive and developmental toxicity in mice and rats.[45] Consequently, further experimental and clinical evaluations are needed to use the material safely.

CONCLUSION

Within the limitations of this in vitro study, the following results were drawn:

All the tested materials changed the mineral contents of root dentin. Citric acid treatment did not change shear-bond strength of AH Plus while boric acid and EDTA treatment decreased shear bond strength of the sealer.

REFERENCES

1. Hennequin M, Douillard Y. Effects of citric acid treatment on the Ca, P and Mg contents of human dental roots. J Clin Periodontol 1995;22(7):550-7.
2. Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A, Zalkind M. Histochemical analysis of dental hard tissues following bleaching. J Endod 1996;22(1):23-6.
3. De-Deus G, Paciornik S, Pinho Mauricio MH, Prioli R. Real-time atomic force microscopy of root dentin during demineralization when subjected to chelating agents. Int Endod J 2006;39(9):683-92.
4. Perdigão J, Eiriksson S, Rosa BT, Lopes M, Gomes G. Effect of calcium removal on dentin bond strengths. Quintessence Int 2001;32(7):142-6.
5. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94(6):658-66.
6. Tagger M, Tagger E, Tjan AH, Bakland LK. Measurement of adhesion of endodontic sealers to dentin. J Endod 2002;28(5):351-4.
7. Saleh IM, Ruyter IE, Haapasalo MP, Orstavik D. Adhesion of endodontic sealers: scanning electron microscopy and energy dispersive spectroscopy. J Endod 2003;29(9):595-601.

8. Hashem AA, Ghoneim AG, Lutfy RA, Fouda MY. The effect of different irrigating solutions on bond strength of two root canal-filling systems. J Endod 2009;35(4):537-40.

9. Rosenfeld EF, James GA, Burch BS. Vital pulp tissue response to sodium hypochlorite. J Endod 1978;4(5):140-6.

10. Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endod 1978;4(2):60-4.

11. Aktener BO, Bilkay U. Smear layer removal with different concentrations of EDTA-ethylenediamine mixtures. J Endod 1993;19(5): 228-31.

12. Villegas JC, Yoshioka T, Kobayashi C, Suda H. Obturation of accessory canals after four different final irrigation regimes. J Endod 2002;28(7):534-6.

13. Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. Int Endod J 2002;35(11): 934-9.

14. Calt S, Serper A. Time dependent effects of EDTA on dentin structures. J Endod 2002; 28(1):17-9.

15. Yamaguchi M, Yoshida K, Suzuki R, Nakamura H. Root canal irrigation with citric acid solution. J Endod 1996;22(1):27-9.

16. Salama FS, Abdelmegid FY. Six percent citric acid better than hydrogen peroxide in removing smear layer: an in vitro pilot study. Pediatr Dent 1994;16(6):424-6.

17. Goldman LB, Goldman M, Kronman JH, Lin PS. The efficacy of several irrigating solutions for endodontics: a scanning electron microscopic study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1981;52(2):197-204.

18. Uysal T, Ustdal A, Sonmez MF, Ozturk F. Stimulation of bone formation by dietary boron in an orthopedically expanded suture in rabbits. Angle Orthod 2009;79(5):984-90.

19. Ince S, Kucukkurt I, Cigerci IH, Fatih Fidan A, Eryavuz A. The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. J Trace Elem Med Biol 2010;24(3):161-4.

20. Hunt CD, Schuler TR, Mullen LM. Concentration of boron and other elements in human food and personal-care products. J Am Diet Assoc 1991;91(5):558-68.

21. Başaran N, Duydu Y, Bolt HM. Reproductive toxicity in boron exposed workers in Bandirma, Turkey. J Trace Elem Med Biol 2012;26(2-3):165-7.

22. Marin-Bauza GA, Rached-Junior FJ, Souza-Gabriel AE, Sousa-Neto MD, Miranda CE, Silva-Sousa YT. Physicochemical properties of methacrylate resin-based root canal sealers. J Endod 2010;36(9):1531-6.

23. Nagas E, Uyanik MO, Eymirli A, Cehreli ZC, Vallittu PK, Lassila LV, et al. Dentin moisture conditions affect the adhesion of root canal sealers. J Endod 2012;38(2):240-4.

24. De-Deus G, Namen F, Galan J Jr, Zehnder M. Soft chelating irrigation protocol optimizes bonding quality of Resilon/Epiphany root fillings. J Endod 2008;34(6):703-5.

25. Goracci C, Tavares AU, Fabianelli A, Monticelli F, Raffaelli O, Cardoso PC, et al. The adhesion between fiber posts and root canal walls: comparison between microtensile and push-out bond strength measurements. Eur J Oral Sci 2004;112(4):353-61.

26. Rahimi M, Jainaen A, Parashos P, Palamara J, Messer HH. Enhancing the bond of a resin-based sealer to root dentine. Int Endod J 2012;45(12):1141-7.

27. Ureyen Kaya B, Keçeci AD, Orhan H, Belli S. Micropush-out bond strengths of gutta-percha versus thermoplastic synthetic polymer-based systems - an ex vivo study. Int Endod J 2008;41(3):211-8.

28. Cobankara FK, Erdogan H, Hamurcu M. Effects of chelating agents on the mineral content of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112(6): e149-54.

29. Ari H, Erdemir A. Effects of endodontic irrigation solutions on mineral content of root canal dentin using ICP-AES technique. J Endod 2005;31(3):187-9.

30. Doğan H, Qalt S. Effects of chelating agents and sodium hypochlorite on mineral content of root dentin. J Endod 2001;27(9):578-80.

31. Gurbuz T, Ozdemir Y, Kara N, Zehir C, Kurudirek M. Evaluation of root canal dentin after Nd:YAG laser irradiation and treatment with five different irrigation solutions: a preliminary study. J Endod 2008;34(3):318-21.

32. Gwinnett AJ. Smear layer: morphological considerations. Oper Dent Suppl 1984;3:2-12.

33. Yamada RS, Armas A, Goldman M, Lin PS. A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: Part 3. J Endod 1983;9(4):137-42.

34. Gettleman BH, Messer HH, ElDeeb ME. Adhesion of sealer cements to dentin with and without smear layer. J Endod 1991;17(1):15-20.

35. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentin conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. Int Endod J 2011;44(6):491-8.

36. Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. The effects of dentin pretreatment on the adhesion of root-canal sealers. Int Endod J 2002;35(10):859-66.

37. Wennberg A, Orstavik D. Adhesion of root canal sealers to bovine dentin and gutta-percha. Int Endod J 1990;23(1):13-9.

38. Dogan Buzoglu H, Calt S, Gümüsderelioglu M. Evaluation of the surface free energy on root canal dentin walls treated with chelating agents and NaOCl. Int Endod J 2007;40(1): 18-24.

39. Milosevic A. The influence of surface finish and in-vitro pellicle on contact-angle measurement and surface morphology of three commercially available composite restoratives. J Oral Rehabil 1992;19(1):85-97.

40. García-Godoy F, Loushine RJ, Itthagarun A, Weller RN, Murray PE, Feilzer AJ, et al. Application of biologically-oriented dentin bonding principles to the use of endodontic irrigants. Am J Dent 2005;18(4):281-90.

41. De-Deus G, Brandão MC, Leal F, Reis C, Souza EM, Luna ES, et al. Lack of correlation between sealer penetration into dentinal tubules and sealability in nonbonded root fillings. Int Endod J 2012;45(7):642-51.

42. Erdemir A, Ari H, Güngünes H, Belli S. Effect of medications for root canal treatment on bonding to root canal dentin. J Endod 2004;30(2):113-6.

43. Machado-Silveiro LF, González-López S, González-Rodríguez MP. Decalcification of root canal dentin by citric acid, EDTA and sodium citrate. Int Endod J 2004;37(6):365-9.

44. Turk T, Kaval ME, Şen BH. Evaluation of the smear layer removal and erosive capacity of EDTA, boric acid, citric acid and desy clean solutions: an in vitro study. BMC Oral Health 2015;15:104.

45. Demirer S, Kara MI, Erciyas K, Ozdemir H, Ozer H, Ay S. Effects of boric acid on experimental periodontitis and alveolar bone loss in rats. Arch Oral Biol 2012;57(1):60-5.