

THE EFFECT OF FINAL IRRIGATION SOLUTIONS USED DURING REGENERATIVE ENDODONTIC TREATMENT ON THE PUSH-OUT BOND STRENGTH OF ENDODONTIC CEMENTS: AN IN VITRO STUDY

Tan Fırat Eyüboğlu, DDS PhD

Assistant Professor, Department of Endodontics,
School of Dentistry, Istanbul Medipol University
Istanbul, Turkey
ORCID: 0000-0002-0308-9579

Keziban Olcay, DDS, PhD

Assistant Professor, Department of Endodontics,
School of Dentistry, Istanbul University-Cerrahpaşa
Istanbul, Turkey
ORCID: 0000-0002-2168-710X

Correspondence

Keziban Olcay, DDS PhD

Istanbul University-Cerrahpaşa,
Faculty of Dentistry,
Department of Endodontics, Kocamustafapaşa,
Cad. 34/E, Cerrahpaşa, Fatih, Istanbul, Turkey
ORCID: 0000-0002-2168-710X
Phone: +90 212 453 48 00
Fax: +90 212 521 04 26
E-mail: kolcay@medipol.edu.tr

ABSTRACT

Background and Aim: Study's aim was to compare the effect of boric acid versus ethylene-diamine-tetra-acetic acid (EDTA) in regenerative endodontic treatment on the push-out bond strength of endodontic cements.

Materials and Methods: Crowns and apical parts of 72 human straight lateral teeth were removed under water cooling; length was fixed at 10 mm. Canals were prepared up to file F4, standardized using a peeso reamer 1-4 (Mani). All teeth were irrigated with 20 ml 1.5% sodium hypochlorite, divided into following groups: 1) 20 ml 17% EDTA, 2) 20 ml 5% boric acid, and 3) 20 ml saline for final irrigation. Canals were dried, filled with calcium hydroxide (CaOH₂) for 3 weeks. CaOH₂ was removed using 20 ml of respective final irrigation solution. Each group was divided into two subgroups: Either ProRoot MTA or Biodentine was used as the coronal barrier (h=6 mm; n=12). After 7 days, 1 mm thickness 3-4 dentin sections were taken from each sample. Push-out test was performed at a rate of 1 mm/min. Two-way ANOVA and Tukey's post hoc tests were used for statistical analyzing.

Results: Biodentine-EDTA, Biodentine-saline, and ProRoot MTA-saline groups had highest bond strength ($p < 0.05$). Boric acid groups showed lower bond strength ($p < 0.05$). ProRoot MTA-EDTA group had higher bond strength than boric acid groups ($p < 0.05$). Biodentine was found to higher than ProRoot MTA ($p < 0.05$).

Conclusion: In terms of bonding to dentin, 17% EDTA was more successful than 5% boric acid; Biodentine was more successful than ProRoot MTA.

Clin Dent Res 2020; 44(3): 96-104

Keywords: Regenerative Medicine, Regenerative Endodontics, Mineral Trioxide Aggregate.

Submitted for Publication: 11.15.2019

Accepted for Publication : 08.24.2020

REJENERATİF ENDODONTİK TEDAVİ SIRASINDA KULLANILAN FİNAL İRRİGASYON SOLÜSYONLARININ ENDODONTİK SİMANLARIN PUSH-OUT BAĞLANMA DAYANIMI ÜZERİNE ETKİSİ: BİR İN VİTRO ÇALIŞMA

Tan Fırat Eyüboğlu

Dr. Öğr. Üyesi, İstanbul Medipol Üniversitesi,
Diş Hekimliği Fakültesi, Endodonti Ana Bilim Dalı,
İstanbul, Türkiye
ORCID: 0000-0002-0308-9579

Keziban Olcay

Dr. Öğr. Üyesi, İstanbul Üniversitesi-Cerrahpaşa,
Diş Hekimliği Fakültesi, Endodonti Ana Bilim Dalı,
İstanbul, Türkiye
ORCID: 0000-0002-2168-710X

Sorumlu Yazar

Keziban Olcay

İstanbul Üniversitesi-Cerrahpaşa, Diş Hekimliği Fakültesi,
Endodonti Ana Bilim Dalı,
Kocamustafapaşa Cad. No:34/E Cerrahpaşa, Fatih, İstanbul, Türkiye
ORCID: 0000-0002-2168-710X
Telefon: +90 212 453 48 00
Faks:+90 212 521 04 26
E-mail: kolcay@medipol.edu.tr

ÖZ

Amaç: Çalışmanın amacı, rejeneratif endodontik tedavide borik asit ile etilen-diamin-tetra-asetik asitin (EDTA) endodontik simanların push-out bağlanma dayanımı üzerindeki etkisini karşılaştırmaktır.

Gereç ve Yöntem: Yetmiş iki adet sağlam, insan lateral dişinin kronları ve apikal kısımları su soğutması altında kesilerek, boyları 10 mm'ye sabitlendi. Kanallar F4 nolu eğeye kadar genişletildi; 1-4 nolu peeso reamer kullanılarak standardize edildi. Tüm dişler 20 ml %1.5 sodyum hipoklorit ile irrig edildi, şu gruplara ayrıldı: Final irrigasyon için; 1) 20 ml %17 EDTA; 2) 20 ml %5 Borik asit; 3) 20 ml Serum fizyolojik. Kanallar kurulandı, kalsiyum hidroksit (CaOH₂) ile 3 hafta süresince dolduruldu. CaOH₂, ait olduğu grupta kullanılan final irrigasyon solüsyonundan 20 ml kullanılarak uzaklaştırıldı. Her grup iki alt gruba ayrıldı: Koronal bariyer olarak ProRoot MTA ve Biodentine kullanıldı (h=6 mm; n=12). Yedi gün sonra, her örnekten 1 mm kalınlığında 3-4 dentin kesiti alındı. Push-out testi 1 mm/dk hızda gerçekleştirildi. İki Yönlü ANOVA testi ve Tukey's Post Hoc testleri istatistiksel analiz için kullanıldı.

Bulgular: Biodentine-EDTA, Biodentine-serum fizyolojik, ProRoot MTA-serum fizyolojik grupları en yüksek bağlanma dayanımına sahipti (p<0.05). Borik asit grupları daha düşük bağlanma dayanımı gösterdi (p<0.05). ProRoot MTA-EDTA grubu, Borik asit gruplarından daha yüksek bağlanma dayanımına sahipti (p<0.05). Biodentine, ProRoot MTA'dan daha iyi bulunmuştur (p<0.05).

Sonuç: Dentine bağlanma açısından, %17 EDTA, %5 borik asitten daha başarılıdır; Biodentine ise ProRoot MTA'dan daha başarılıdır.

Clin Dent Res 2020; 44(3): 96-104

Anahtar Kelimeler: Rejeneratif tıp, Rejeneratif Endodonti, Mineral Trioksit Agregat.

Yayın Başvuru Tarihi : 15.11.2019

Yayına Kabul Tarihi : 24.08.2020

INTRODUCTION

Rapid development in the field of regenerative medicine and tissue engineering in recent years has led to the emergence of regenerative applications in all branches of medicine, including in dentistry. Regenerative treatments in the field of endodontics encompass all of the methods used to restore the integrity of the pulp-dentin complex by regenerating the damaged pulp tissue due to caries or trauma and physiologically restoring root dentin for apical foramen formation in immature teeth due to a disruption during root development.

Particularly in immature permanent teeth of open apices, difficulties are encountered due to failure of root canal treatment.^{1,2} The most suitable method for treating such teeth should maintain the physiological root development by regenerating the pulp tissue via regenerative endodontic treatment.³ Thus, the aim is to restore the tooth vitality, to complete the root canal dentin formation, to develop the root length, and ensure the physiological closure of the apical foramen.⁴

Mechanical instrumentation is not recommended for regenerative endodontic treatment, as it negatively affects the amount of the growth factors released from the dentin.⁵ In this respect, the type of irrigation solution used to disinfect the root canal is immensely important.⁶⁻⁸ Sodium hypochlorite (NaOCl) is preferred in root canal treatment because of its effective antibacterial properties and the ability to dissolve organic tissues.^{9,10} Ethylene diamine tetra acetic acid (EDTA) is also routinely used because it removes the smear layer, exposing the dentinal tubules to further disinfection.^{9,10} However, according to the results of a recent review of 60 published studies on the regenerative endodontic treatment, 65% of these studies used only NaOCl in a concentration ranging from 1–6% as an irrigation solution, whereas in other studies various solutions were used in addition to NaOCl. As a final irrigation solution, 75% of these studies used NaOCl, whereas 4% used chlorhexidine, and 13% used EDTA.¹¹ Overall, these results reveal that more research is needed to achieve a standardized and accepted clinical protocol for this treatment.¹²

EDTA has a high cytotoxic effect compared to the other acidic solutions used in root canal treatment in the literature;¹³⁻¹⁵ this may represent a significant disadvantage in terms of using this solution in regenerative treatments. In addition, the combined use of NaOCl and EDTA decreases

the free chlorine content of NaOCl, weakening its organic tissue-dissolving capacity and antimicrobial properties.^{16,17} This may compromise the optimal disinfection of the root canal, especially in regenerative endodontic treatments, where infection control is only achieved by the antibacterial efficacy of the irrigation solutions.

Several studies have reported that boric acid has antiseptic, antibacterial, and antifungal properties¹⁸ as well as a regulatory effect on immune response.^{19,20} Turk et al.¹⁰ compared boric acid, EDTA, and other chelating agents to determine their removal of the smear layer during endodontic treatment. The practice of using boric acid in the root canal system is new, and to the best of the authors' knowledge, a study of its clinical use in regenerative endodontic treatment has not been reported.

The aim of this study is to compare the effect of using boric acid as an irrigation solution in regenerative endodontic treatment on the bonding of tricalcium silicate cements to dentin. It also investigates the potential usefulness of boric acid in regenerative endodontic treatments as a final irrigation solution. The HO hypothesis of this study is as follows: Using different irrigation solutions does not affect tricalcium silicate cements' bonding to dentin. Hopefully, the data obtained here will shed light on the formation of a more successful and reliable irrigation protocol in regenerative endodontic treatment.

MATERIALS AND METHODS

Ethics statement: Necessary ethics committee approval was obtained from the University's Non-Interventional Clinical Research Ethics Committee (Approval Number: 10840098-604.01.01-E.56349/768). The present study was based on the current regenerative endodontic considerations of the American Association of Endodontists.²¹ This study used 72 sound human maxillary lateral teeth. The extracted teeth were kept in 0.5% chloramine at room temperature until the samples were prepared. Single root and single root canal formation as well as the absence of structural deformation were checked in all the samples using a stereomicroscope (Zeiss AxioZoom V16, Carl Zeiss, Jena, Germany) at a x25 magnification. Teeth with caries, cracks, or fractures in the root structure, and teeth with curved root structures, were excluded from the study.

The residual tissues on the teeth were removed using a periodontal curette. The teeth were then cut using diamond discs (Komet, Gebr Brasseler GmbH & Co. Lemgo, Germany) under water cooling at the cemento-enamel junction. Apical

2–3 mm portions of the samples were removed to obtain a standardized root length of 10 mm. The root canals were first shaped up to file F4 using the Protaper Rotary System (Dentsply Maillefer, Ballaigues, Switzerland). Then, peeso reamer 1–4 (Mani, Inc. Tochigi, Japan) was used to standardize the root canal diameter in all the samples. During mechanical instrumentation, 2 ml sodium hypochlorite (NaOCl; Promida Tic. Eskisehir, Turkey) in a concentration of 1.5% was applied at 1 mm less than the working length using a 27-gauge endodontic irrigation needle (KerrHawe SA, Bioggio, Switzerland) between each file for each canal for 30 s. In total, the amount of NaOCl applied to each tooth during enlargement and shaping was 22 ml. The teeth were randomly divided into three main groups (n=24 in each group). The following irrigation protocols were applied with a continuous gentle and slow in-and-out motion of the needle tip for 5 minutes for each solution:

- Group 1: 20 ml of 1.5% NaOCl + 20 ml 17% EDTA (Endo-Solution, Cerkamed, Stalowa Wola, Poland).
- Group 2: 20 ml of 1.5% NaOCl + 20 ml of 5% boric acid (Tekkim Kimya Tic., Istanbul, Turkey).
- Group 3: 20 ml of 1.5% NaOCl + 20 ml of saline (Osel İlaç Tic., İstanbul, Turkey).

All the samples were then irrigated with 2 ml of saline solution for 30 s to eliminate any long-term effects caused by the irrigation solutions. Following the completion of irrigation protocols, the canals were gently dried with paper points ISO #130 (DiaDent Europe B.V., Almere, Netherlands) and calcium hydroxide (Vision; ADD, İstanbul, Turkey) were mixed with saline (in a ratio of 1:1) and applied to the root canals using a Lentulo spiral (Mani, Inc. Tochigi, Japan). The coronal parts of the samples were sealed with a temporary restorative filling material (Coltosol F, Coltene, Altstätten, Switzerland). The samples were stored at 37°C and 100% humidity for three weeks.

In the second session, the temporary restorative filling was gently removed with the aid of a dental probe. Then, 20 ml of the respective irrigation solutions used in the first session was slowly applied to each canal for 5 min to remove the calcium hydroxide from the root canal. Each main group was then divided into two subgroups related to the endodontic cement that was applied (n=12 in each group):

- Subgroup 1: Biodentine (Septodont, Saint-Maur-des-Fosses, France)

- Subgroup 2: ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA)

The ProRoot MTA was mixed in a 3:1 powder-to-liquid ratio; the Biodentine was mixed in an amalgamator for 30 s according to the manufacturers' recommendations. Both cements were placed in the coronal 6 mm section of the prepared canals with the aid of an MTA carrier (Dentsply Tulsa Dental, Tulsa, OK, USA). The samples were then stored at 37°C at 100% humidity for seven days to ensure optimal setting of the tricalcium silicate-based endodontic cements. One week later, the specimens were vertically embedded in acrylic blocks and then cut using water-cooled diamond discs in a cutting device (Mecatome T180, Presi SA, Briet-Angonnes, France). Next, 3–4 horizontal cross sections 1 mm in thickness were obtained from the coronal section perpendicular to the long axis of each root (Figure 1). The sections' thickness was checked using a digital caliper (Mitutoyo Corp., Kanogawa, Japan). The effect of the final irrigation solution on the bonding strength of the tricalcium silicate cements to the dentin was measured via push-out method using a universal testing machine (Instron, Canton, MA, USA) (Figure 2). For the push-out test, a stainless steel cylindrical tip with a diameter of 1 mm was applied in the coronal-apical direction. Force was applied at a rate of 1 mm/min until failure of the cement connection to the dentin (Figures 3 and 4). The data in Newton were divided into area and converted into Mpa.

IBM SPSS Statistics 22 (IBM Corporation, NY, USA) program was used for the statistical analysis of the data. The data were evaluated using the Shapiro-Wilks test; the parameters were found to be suitable for normal distribution. Two-way ANOVA and Tukey's post hoc test were used to determine the difference between the groups. The significance level was set at 5% ($p < 0.05$).

RESULTS

The results of the statistical analysis are summarized in Table 1. The findings reveal significant differences both in terms of the tricalcium silicate cements and the final irrigation solutions used in the regenerative endodontic treatment ($p=0.00$) (Figure 5).

The boric acid group showed significantly lower bond strength values than the EDTA and saline groups ($p=0.00$). There was no significant difference between the EDTA and saline groups in terms of bond strength ($p=0.06$). The Biodentine group showed significantly higher bond strength values than the ProRoot MTA group ($p=0.00$) (Table 1).



Figure 1. Photograph of the horizontal cross sections of dentine.

In the pairwise comparisons, the bond strength values were significantly lower in the ProRoot MTA & boric acid and Biodentine & boric acid groups compared to all the other groups ($p=0.51$). The ProRoot MTA & EDTA group had significantly higher bond strength values than all of the boric acid groups ($p=0.00$). There was no significant difference between the ProRoot MTA & saline, the Biodentine & saline, and the Biodentine & EDTA groups in terms of bond strength ($p=0.99$ and $p=0.22$, respectively). These groups showed significantly higher bond strength values than all the other groups ($p=0.00$). Although not statistically significant, the highest bond strength values were observed in the Biodentine & EDTA group (Table 1).

DISCUSSION

In this study, a regenerative endodontic treatment protocol was performed on human teeth *in vitro* in order to reflect actual clinical conditions. To the best of the authors' knowledge, this is the first time that the efficacy of boric acid in regenerative endodontic treatment was compared with EDTA, which is the golden standard in this treatment. A saline group was used as a negative control group. Because fractures between the root canal filling materials and dentin occur parallel to the dentin-resin bonding surface, a widely used, efficient and applicable method -the push-



Figure 2. Photograph of the Universal test device used in the study.

out test- was selected to test the bond strength between tricalcium silicate cement plug and dentin in order to mimic clinical conditions.²²⁻²⁵ All the dentin sections were taken from the coronal region of the samples in order to perform regenerative endodontic treatments in accordance with clinical conditions. The section thickness was limited to 1 mm in order to increase the reliability of the results and to reduce the non-homogeneous stress distribution caused by

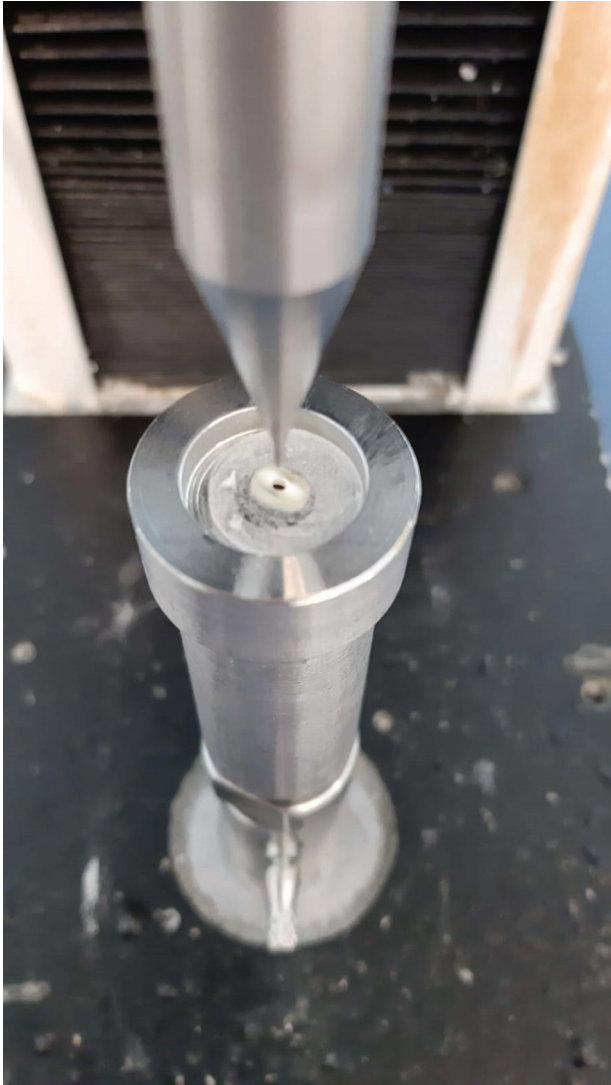


Figure 3. Photograph of the failure of the cement connection to the dentin following application of force.

friction.²⁶ A single researcher carried out sample preparation to ensure standardization. The methods used in this study aimed to fully reflect actual clinical conditions, and the protocols used were based on American Association of Endodontists' recommendations for dental regenerative procedures²¹. Based on the results of this study, the H0 hypothesis was rejected. The boric acid groups presented significantly lower bond strength values than the EDTA and saline groups. Further, Biodentine had significantly higher bond strength than ProRoot MTA.

Disinfection of the root canals is a crucial step in regenerative endodontic treatment.⁵ Aim in the minimal shaping of the root canals in regenerative endodontic treatment, the disinfection of the root canals can only be acquired using

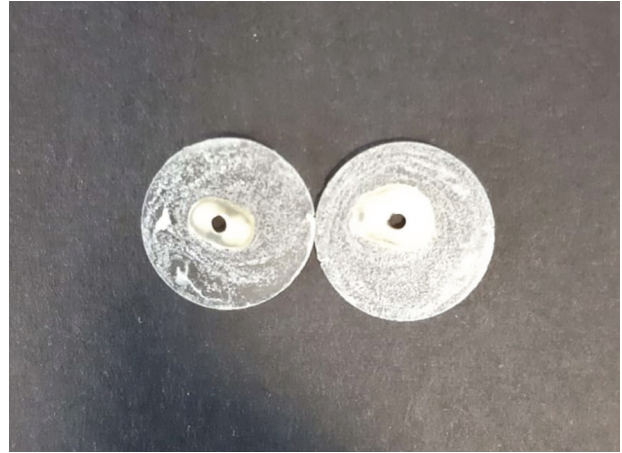


Figure 4. Photograph of the horizontal cross sections of dentine after the push-out test.

irrigation solutions and intracanal medicaments.⁶⁻⁸ Although the use of boric acid (5%) in root canal treatment was reported to have lower smear dissolution efficiency than the combination of citric acid (2.5%) and EDTA (17%),¹⁰ it is well-known that the erosion caused by EDTA and citric acid in dentin is far greater than that caused by boric acid.²⁷⁻²⁹ Furthermore, the anti-inflammatory, antimicrobial, antifungal, and immune-response-regulating effects of boric acid^{10,18-20,30} make this material a promising one for regenerative endodontic purposes.

According to the results of this study, the probable cause of the lower push-out bond strength value of the boric acid compared to the EDTA and saline solutions may be related to the negative effect on the adhesion, as a 5% boric acid application may not completely dissolve the smear layer in dentin tubules.¹⁰ Another possible reason why the boric acid showed a lower push-out value than the EDTA may be related to the calcium hydroxide removal capabilities of the two materials. Also previous studies reported that the physical properties of endodontic cements can change after irrigation with chelating agents.^{23,25} These studies found that ProRoot MTA had a lower push-out bond strength, which is in accordance with the results of the present study. Here, the higher bond strength value of the Biodentine & EDTA group may be because Biodentine supports dentin tissue as a result of its dentin-like mechanical properties.^{31,32} Moreover, Biodentine is more adaptable to dentin tubules than ProRoot MTA due to its uniform structure, smaller particle size,³³ and superior plastic consistency.³⁴ That Biodentine is less brittle than ProRoot MTA due to the

Table 1. Mean±Std. Dev. and p values about irrigation solutions and endodontic cements used in the study.

	Biodentine	ProRoot MTA	P value
	Mean±SD	Mean±SD	
EDTA	10.51±1.43 ^{a,x}	7.80±1.46 ^{b,x}	p<0.05
Boric Acid	6.65±1.25 ^{a,y}	5.99±1.29 ^{a,y}	p>0.05
Saline	9.81±1.49 ^{a,x}	9.67±1.97 ^{a,z}	p>0.05
P value	p<0.05	p<0.05	

a,b Different superscript letters show statistically significant groups in the same line.

x,y,z Different superscript letters show statistically significant groups in the same column.

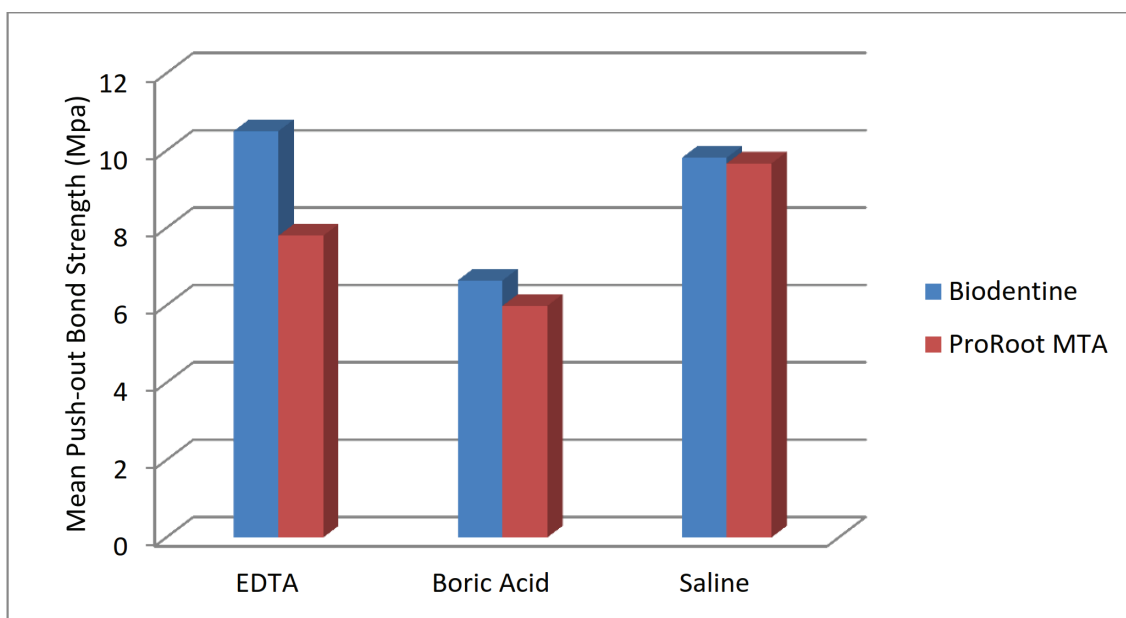


Figure 5. Graph for the results of push-out test.

absence of alumina crystals in its structure³⁵ may support the material's high bond strength. Applying EDTA reduces the surface hardness and biocompatibility of ProRoot MTA;³⁶ this might be a possible cause of the low bond strength values observed in the ProRoot MTA & EDTA group compared to the saline groups.

CONCLUSION

Within the limitations of this *in vitro* study, Biodentine had higher bond strength values than ProRoot MTA. Boric acid (5%) had a negative effect on bond strength values compared to other solutions. Future studies are needed to investigate the effectiveness of boric acid solutions

at different concentrations in order to improve the bond strength of tricalcium silicate cements to dentin.

Conflict of Interest Statement: The authors declare that there is no conflict of interest.

REFERENCES

1. Al Ansary MA, Day PF, Duggal MS, Brunton PA. Interventions for treating traumatized necrotic immature permanent anterior teeth: inducing a calcific barrier & root strengthening. *Dent Traumatol* 2009; 25: 367-379.
2. Mente J, Hage N, Pfefferle T, Koch MJ, Dreyhaupt J, Staehle HJ et al. Mineral trioxide aggregate apical plugs in teeth with open apical foramina: a retrospective analysis of treatment outcome. *J Endod* 2009; 35: 1354-1358.

3. Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM. Regenerative endodontics: A way forward. *J Am Dent Assoc* 2016; 147: 372-380.
4. Hargreaves KM, Giesler T, Henry M, Wang Y. Regeneration potential of the young permanent tooth: what does the future hold? *J Endod* 2008; 34: 51-56.
5. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? *J Endod* 2004; 30: 196-200.
6. Galler KM, Buchalla W, Hiller KA, Federlin M, Eidt A, Schiefersteiner M et al. Influence of root canal disinfectants on growth factor release from dentin. *J Endod* 2015; 41: 363-368.
7. Aktemur Turker S, Aslan MH, Uzunoglu E, Ozcelik B. Antimicrobial and structural effects of different irrigation solutions on gutta-percha cones. *Eur Oral Res* 2015; 49: 27-32.
8. Ferrer-Luque CM, González-Castillo S, Ruiz-Linares M, Arias-Moliz MT, Rodríguez-Archilla A, Baca P. Antimicrobial residual effects of irrigation regimens with maleic acid in infected root canals. *J Biol Res (Thessalon)* 2015; 15: 22:1.
9. Zehnder M. Root canal irrigants. *J Endod* 2006; 32: 389-398.
10. 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.
11. Kontakiotis EG, Filippatos CG, Tzanetakos GN, Agrafioti A. Regenerative endodontic therapy: a data analysis of clinical protocols. *J Endod* 2015; 41: 146-154.
12. Zeng Q, Nguyen S, Zhang H, Chebrolu HP, Alzebeid D, Badi MA et al. Release of Growth Factors into Root Canal by Irrigations in Regenerative Endodontics. *J Endod* 2016; 42: 1760-1766.
13. Malheiros CF, Marques MM, Gavini G. In vitro evaluation of the cytotoxic effects of acid solutions used as canal irrigants. *J Endod* 2005; 31: 746-748.
14. Ballal NV, Kundabala M, Bhat S, Rao N, Rao BS. A comparative in vitro evaluation of cytotoxic effects of EDTA and maleic acid: root canal irrigants. *Oral Surg Oral Med Oral Pathol Oral Radiol and Endod* 2009; 108: 633-638.
15. Karkehabadi H, Yousefifakhr H, Zadsirjan S. Cytotoxicity of Endodontic Irrigants on Human Periodontal Ligament Cells. *Iran Endod J* 2018; 3: 390-394.
16. Atasoy Ulusoy Öİ, Savur İG, Çelik B. Etilendiamin tetraasetik asit, perasetik asit ve etidronik asitin sodyum hipokloritin doku çözme kapasitesi üzerine etkisi: in vitro. *Acta Odontologica Turcica* 2017; 34: 50-54.
17. Grawehr M, Sener B, Waltimo T, Zehnder M. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. *Int Endod J* 2003; 36: 411-415.
18. Meers PD, Chow CK. Bacteriostatic and bactericidal actions of boric acid against bacteria and fungi commonly found in urine. *J Clin Pathol* 1990; 43: 484-487.
19. Benkovic SJ, Baker SJ, Alley MR, Woo YH, Zhang YK, Akama T et al. Identification of borinic esters as inhibitors of bacterial cell growth and bacterial methyltransferases, CcrM and MenH. *J Med Chem* 2005; 48: 468-476.
20. Nielsen FH. Is boron nutritionally relevant? *Nutr Rev* 2008; 66: 183-191.
21. AAE Protokol: <https://www.aae.org/specialty/wp-content/uploads/sites/2/2017/06/currentregenerativeendodonticconsiderations.pdf>
22. Drummond JL, Sakaguchi RL, Racean DC, Wozny J, Steinberg AD. Testing mode and surface treatment effects on dentin bonding. *J Biomed Mater Res* 1996; 32: 533-541.
23. Buldur B, Oznurhan F, Kaptan A. The effect of different chelating agents on the push-out bond strength of proroot mta and endosequence root repair material. *Eur Oral Res* 2019; 53: 88-93.
24. 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: 353-361.
25. Guneser MB, Akbulut MB, Eldeniz AU. Effect of various endodontic irrigants on the push-out bond strength of biodentine and conventional root perforation repair materials. *J Endod* 2013; 39: 380-384.
26. Goracci C, Grandini S, Bossù M, Bertelli E, Ferrari M. Laboratory assessment of the retentive potential of adhesive posts: a review. *J Dent* 2007; 35: 827-835.
27. Çalt S, Serper A. Time- dependent effects of EDTA on dentin structures. *J Endod* 2002; 28: 17-19.
28. Sen BH, Ertürk O, Pişkin B. The effect of different concentrations of EDTA on instrumented root canal walls. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 108: 622-627.
29. Poggio C, Dagna A, Colombo M, Rizzardi F, Chiesa M, Scribante A et al. Decalcifying effect of different ethylenediaminetetraacetic acid irrigating solutions and tetraclean on root canal dentin. *J Endod* 2012; 38: 1239-1243.

CLINICAL DENTISTRY AND RESEARCH

30. Luan Q, Desta T, Chehab L, Sanders VJ, Plattner J, Graves DT. Inhibition of experimental periodontitis by a topical boron-based antimicrobial. *J Dent Res* 2008; 87: 148-152.
31. Koubi G, Colon P, Franquin JC, Hartmann A, Richard G, Faure MO et al. Clinical evaluation of the performance and safety of a new dentine substitute, Biodentine, in the restoration of posterior teeth - a prospective study. *Clin Oral Invest* 2013; 17: 243-249.
32. Raskin A, Eschrich G, Dejou J, About I. In vitro microleakage of Biodentine as a dentin substitute compared to Fuji II LC in cervical lining restorations. *J Adhes Dent* 2012; 14: 535-542.
33. Rajasekharan S, Martens LC, Cauwels RGEC, Anthonappa RP, Verbeeck RMH. Biodentine™ material characteristics and clinical applications: a 3 year literature review and update. *Eur Arch Paediatr Dent* 2018; 19: 1-22.
34. Aguiar BA, Frota LMA, Taguatinga DT, Vivan RR, Camilleri J, Duarte MAH et al. Influence of ultrasonic agitation on bond strength, marginal adaptation, and tooth discoloration provided by three coronary barrier endodontic materials. *Clin Oral Investig* 2019; 23: 4113-4122.
35. Butt N, Talwar S, Chaudhry S, Nawal RR, Yadav S, Bali A. Comparison of physical and mechanical properties of mineral trioxide aggregate and Biodentine. *Indian J Dent Res* 2014; 25: 692-697.
36. Lee YL, Lin FH, Wang WH, Ritchie HH, Lan WH, Lin CP. Effects of EDTA on the hydration mechanism of mineral trioxide aggregate. *J Dent Res* 2007; 86: 534-548.