RESEARCH

Effect of tannic acid irrigation on microhardness of root canal dentin and bond strength of epoxy resin based sealer

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ABSTRACT

Effect of tannic acid irrigation on microhardness of root canal dentin and bond strength of epoxy resin based sealer

Background: To investigate the effects of final irrigation protocols with tannic acid (TA) on dentin microhardness and push-out bond strength of an epoxy resin based sealer to root canal dentin.

Materials and Methods: The 50 root halves were embedded in an acrylic resin for the microhardness measurement. After the initial baseline microhardness measurement the root samples were immersed in 2.5% NaOCl and 17% ethylenediaminetetraacetic acid (EDTA) for 2 min, respectively. Then, the root halves were randomly divided into 5 groups according to the final irrigation protocol used (n=10): distilled water (control), 10% TA for 2 min, 10% TA for 5 min, 20% TA for 2 min, and 20% TA for 5 min. After surface treatment, dentin microhardness values were recorded at close proximity to the initial indentation areas. For push-out bond strength test, the root canals of 75 single-rooted mandibular premolars were instrumented and the irrigation protocols were applied as described above (n = 15). Following root canal obturation with single cone technique using matched-taper gutta percha cones and a epoxy resin based root canal sealer, 1 mm-thick slices were obtained from the middle third of the root canals. Push-out bond strength test was applied. Data were analyzed using one way analysis of variance and Tukey HSD tests (p=0.05).

Results: In TA-treated groups, there was a significant increase in the microhardness values compared with the control group (p<0.05) while no significant difference was found among the microhardness values of TA-treated groups (p>0.05). Regarding the push-out bond strength test, there was no significant difference between the bond strength values of 10% TA for 2 min and the control group (p>0.05). The remaining groups presented higher bond strength values than the control group (p<0.05).

Conclusion: TA increased dentin microhardness and the bond strength of the epoxy resin based sealer to root canal dentin. The effect on the bond strength was time and concentration dependent.

KEYWORDS

Bond strength, dentin, epoxy resin based sealer, microhardness, tannic acid

ÖZ

Tannik asitle irrigasyonun kök kanal dentininin mikrosertliğine ve epoksi rezin esaslı patın bağlanma dayanımına etkisi

Amaç: Tannik asit (TA) içeren final irrigasyon protokollerinin dentin mikrosertliğine ve epoksi rezin içerikli patın kök kanal dentinine olan itme bağlanma dayanımına etksinin incelenmesi.

Gereç ve Yöntemler: 50 adet yarım kök mikrosertlik ölçümü için akrilik rezine gömüldü. İşlem öncesi miksrosertlik değerleri elde edildikten sonra, dentin yüzeyleri % 2,5 sodyum hipoklorit (NaOCl) ve %17 etilendiamintetraasetik asit (EDTA) solusyonları ile ikişer dakika muamele edildi. Sonrasında, örnekler kullanılacak olan final irrigasyon protokolüne göre rastgele 5 gruba dağıtıldı (n=10): distile su (kontrol), % 10 TA 2 dk, %10 TA 5dk, % 20 TA 2 dk, ve %20 TA 5 dk. Başlangıç ölçümlerinin yapıldığı noktalara yakın olacak şekilde mikrosertlik değerleri tekrar ölçülüp kaydedildi. İtme bağlanma dayanımı testi için 75 adet tek köklü alt premolar dişin kanalı genişletildi ve irrigasyon protokolü yukarıda bahsedildiği şekilde uygulandı (n = 15). Örneklerin kanalları tek kon yöntemi kullanılarak uyumlu gutaperka konlar ve epoksi rezin içerikli patla doldurulduktan sonra köklerin orta üçlülerinden 1-mm kalınlığında kesitler elde edildi ve itme bağlanma testi uygulandı. Veriler tek yönlü varyans analizi ve Tukey HSD testleri kullanılarak değerlendirildi (p=0,05).

Bulgular: TA uygulanmış gruplarda mikrosertlik değerleri kontrol grubuna göre anlamlı artış gösterirken (p<0,05), TA uygulanmış grupların mikrosertlik değerleri kendi aralarında anlamlı bir fark göstermedi (p>0.05). İtme bağlanma dayanımı testi sonucunda % 10 TA 2 dak. grubu ile kontrol grubu arasında anlamlı fark bulunmadı (p>0,05). Diğer TA grupları kontrol grubuna göre daha yüksek bağlanma dayanımı değerleri gösterirken (p<0,05), aralarında anlamlı fark bulunmadı (p>0,05).

Sonuç: TA ile final irrigasyon yapılması dentin mikrosertliğini ve epoksi rezin içerikli kanal dolgu patının kök kanal dentinine bağlanma dayanımını arttırdı. TA'nın bağlanma dayanımı üzerindeki etkisi zaman ve konsantrasyon bağımlıdır.

ANAHTAR KELİMELER

Bağlanma dayanımı, dentin, epoksi rezin esaslı pat, mikrosertlik, tannik asit

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The main goal of root canal treatment is to prevent or treat apical periodontitis which is achieved by the chemo-mechanical disinfection and three-dimensional obturation of the root canal system.[1] In this regard, the adhesion of root canal sealer to both gutta percha and root dentin is an important property in obtaining long term endodontic success since inadequate adhesion leads to microleakeage.[2,3] Dentin is composed of 22% organic material consisting mostly type I collagen which plays an important role in the mechanical and adhesive properties.[4,5] Epoxy resin based sealers can bond chemically to the amino groups of dentinal collagen owing to the epoxide rings in their structure.[6] However, resin-dentin bonding interface fails due to collagen degradation by matrix metalloproteinases released from dentin.[7] Intermolecular crosslinks are responsible for the structural integrity, stability and tensile strength of collagen fibrils in dentin structure. For this purpose, several crosslinking agents have been evaluated in order to modify dentin structure to enhance and prolong resin-dentin integrity.[7-9]

The cleaning and shaping procedures of root canal treatment comprises mechanical preparation and irrigation of the root canal system with different chemical irrigants.[10] It is well-established that the root canal irrigants may affect the physical properties of dentin by changing its structure.[11] Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are the most common irrigating solutions used during endodontic treatment and it has been shown that the use of these irrigants causes a significant decrease in the mechanical properties of dentin such as flexural strength, elastic modulus, and microhardness.[12,13] Furthermore, the sequential irrigation of NaOCl and EDTA results in dentinal erosion[14], which may lead to an adverse effect on resin-dentin interface[15] and the adhesion of root canal sealer to root dentin.[16]

Tannic acid (TA) is a natural polyphenol with crosslinking ability that leads to modification of chemical structure of collagen.[17] TA is found in a wide variety of vegetables, fruits, and plants[18] and has been investigated in the fields of health and medicine owing to its antioxidant, antimicrobial, antiviral activity and collagen crosslinking ability.[17,18] Furthermore, TA was found to have a positive effect on the mechanical properties of crown dentin due to its cross-linking ability of dentin collagen.[19]

To the best of our knowledge, no information exists regarding the effect of TA on the microhardness of root canal dentin and resin based sealer-dentin bonding when used as a final endodontic irrigant. Therefore, the aim of the present study was to evaluate the effects of final irrigation protocols with TA on dentin microhardness and the bond strength of an epoxy resin based sealer to root canal dentin. The null hypotheses of this study were that irrigation protocols with TA do not affect dentin microhardness and the bond strength of an epoxy resin based sealer to root canal dentin.

MATERIALS AND METHODS

The study protocol was approved by the Ethical Committee of Hacettepe University, Faculty of Medicine (Protocol no: GO/16704).

Microhardness measurement

Freshly extracted, 50 human mandibular third molars from 30-50 years old patients were collected for microhardness measurement and thoroughly cleaned by removing the hard and soft tissues with periodontal curettes. The crowns were removed at the cemento-enamel junction under water cooling. Then, the distal roots were split longitudinally into the buccal and lingual segments. The obtained root halves were embedded in an acrylic resin, leaving the dentin surface exposed (Figure 1A). The dentin surface of the roots were ground and polished with silicone carbide abrasive papers (180, 320, and 600 grit) (Buehler, Lake Bluff, IL, USA) using a polishing device (Mecapol P230, Presi, France) under running water to remove any surface scratches. The initial dentin microhardness of the root samples was measured using HMV microhardness tester (Shimadzu Corporation, Japan) in Vickers hardness units (VHU) before the irrigation procedures (Figure 1B). The microhardness values were measured at 3 different points parallel to the edge of the root canal lumen at a depth of 100 μm from the pulp-dentin interface from the middle root level using a 300-g load and a 20-second dwell time oriented perpendicularly to the surface. The representative microhardness values were obtained as the average of the results for 3 indentations.

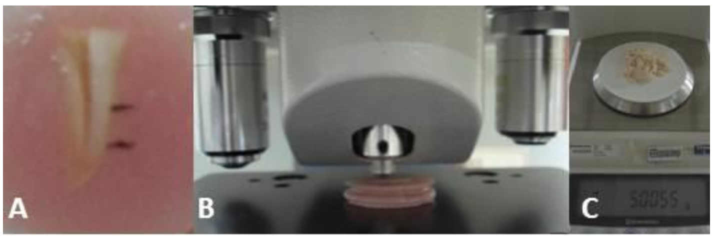

Figure 1.

A) Root halve embedded in an acrylic resin for microhardness measurement
B) Dentin microhardness of the root sample measured using HMV microhardness tester
C) Tannic acid powder

The root samples were immersed in 5 ml of 2.5% NaOCl for 2 min. Following the treatment with NaOCl, the samples were immersed in 5 ml of 17% EDTA for 2 min. Then, the samples were randomly divided into 5 groups according to the final irrigation protocol (n=10): distilled water (control), 10% TA for 2 min, 10% TA for 5 min, 20% TA for 2 min, and 20% TA for 5 min by delivering 5 ml of each solution. Distilled water was used to rinse the samples between different solutions to avoid prolonged effects. Two concentrations of TA solution (10% and 20%) (Sigma-Aldrich, St Louis, MO) (w/v) in saline were freshly prepared for the experiments (Figure 1C). The freshly prepared TA solutions had a initial pH of 6 and the pH of all TA solutions was adjusted to 7.4 using sodium hydroxide. The pH levels were confirmed using a pH meter (Sentron Europe BV, Roden, Netherlands). After surface treatment, the samples were dried, and dentin microhardness of each sample was measured again as described above.

Push-out bond strength test

Freshly extracted 75 single-rooted human mandibular premolars from 30-50 years old patients with no resorptions, caries, cracks, or deformities were collected and thoroughly cleaned by removing the hard and soft tissues with periodontal curettes. The teeth were stored in saline before the experiment. The crown of each tooth was removed to obtain a standardized length of 18 mm. The root apices were sealed with composite resin to simulate a closed root apex. The root canals were instrumented up to F4 file using ProTaper Universal rotary system (Dentsply Maillefer, Ballaigues, Switzerland) and 1 ml of 2.5% NaOCl was used as an irrigant between each file. After the preparation of the root canals, irrigation was performed with 5 ml of 2.5% NaOCl, and 5 ml of 17% EDTA for 2 min. The final irrigation solutions were then applied as described previously in the microhardness measurement using a 30-G side-vented needle (Maxi-i-probe, Dentsply, Rinn, Elgin, IL) (n = 15). The root canals were dried with paper points (Dentsply Maillefer) and then filled with matched-taper gutta percha cones (Dentsply, Maillefer) and an epoxy resin based root canal sealer (AH Plus; Dentsply DeTrey, Konstaz, Germany). The sealer was placed into the root canal using a lentulo spiral (Dentsply Maillefer). The teeth were then radiographed at different angulations to verify the quality of filling procedure and the absence of voids. The specimens were stored in 100% humidity for 48 h to ensure the complete setting of the sealer. Each root was then embedded in acrylic resin placed in a cylindrical plastic mold. After setting of the acrylic resin, 1 mm-thick transverse sections were obtained from the middle third of each root canal using a low-speed saw (IsoMet, Buehler Lake Bluff, IL, USA) under deionized running water. First root section from middle third of each tooth was used for the bond strength test (Figure 2A).

The bond strength of the root canal sealer to dentin was determined using push-out bond strength test via universal testing machine (Lloyd LRX-plus; Lloyd Instruments Ltd, Fareham, UK) (Figure 2B). The specimens were placed on a metal slab with a 1.5 mm central hole. A cylindrical stainless steel plunger of 0.8 mm diameter and operating at a speed of 1 mm/min was used to apply force on materials inside the root slices. The load applied to the material at the time of displacement was recorded in Newton. The apical and coronal diameters of the selected section were determined using digital caliper. The recorded values were then converted to megapascals (MPa) using the following formula: Load/(2 prh), where p is the constant, r is the mean root canal radius, and h is the thickness of the root slice in millimeters.

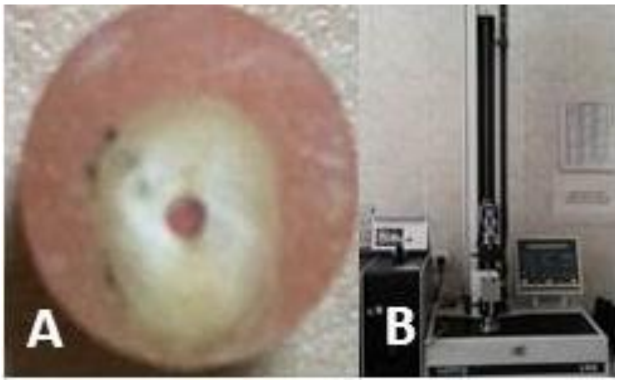

Figure 2.

A) 1 mm-thick transverse section was obtained from the middle third of each root canal
B) Push-out bond strength test via universal testing

Statistical analysis

Differences between pre-treatment and post-treatment microhardness values as a percentage were statistically analyzed by using the t test. The results of the microhardness and push-out bond strength analyses were statistically analyzed using one-way analysis of variance (ANOVA) and Tukey HSD test ($p=0.05$).

RESULTS

The statistical comparison of pre-treatment and post-treatment microhardness values demonstrated that in TA-treated groups there was a significant increase in the microhardness values compared with the control group ($p<0.05$), while no significant difference was found between the microhardness values of TA-treated groups ($p>0.05$) (Table 1). The bond strength values are summarized in Table 2. Accordingly, there was no significant difference between 2 min application of

10% TA and the control group (p>0.05), while 2 min application of 20% TA and 5 min applications of both concentrations showed significant increase in the bond strength values (p<0.05). No significant difference was found among the bond strength values of 2 min application of 20% TA, 5 min applications of 10% TA and 20% TA (p>0.05).

Table 1.

The initial and post-treatment microhardness values (mean±standard deviation) and the percentage changes with respect to the final irrigation protocols

Groups	Initial microhardness values	Post-treatment microhardness values	Microhardness Change (%)
Control	60.62±5.38	57.66±5.39	-2.96[a]
10% TA-2 min	59.01±6.92	63.13±5.01	4.13[b]
10% TA- 5min	57.46±4.78	60.97±8.51	3.51[b]
20% TA-2 min	59.95±6.73	63.02±5.14	3.06[b]
20% TA- 5 min	62.89±4.34	65.30±2.30	2.41[b]

Different letters indicate significant differences (p < 0.05)

Table 2.

The bond strength values (mean±standard deviation) with respect to the final irrigation protocols

Groups	Push-Out Bond Strength Values (MPa)
Control	2.47 ± 1.43[a]
10% TA-2 min	2.93 ± 1.04 [a]
10% TA- 5min	4.31 ± 1.44 [b]
20% TA-2 min	3.90 ± 1.55 [b]
20% TA- 5 min	3.63 ± 1.39 [b]

Different letters indicate significant differences (p < 0.05)

DISCUSSION

The degradation of collagen in dentin matrix affects the mechanical and bonding properties of the dentin.[20] TA can inhibit collagen degradation and therefore increase resin-dentin bond strength.[9,17] TA may also increase root dentin microhardness due to its collagen crosslinking property. The present study investigated the effect of TA as a final irrigant on root dentin microhardness and bond strength of resin sealer to root dentin.

The results of the present study demonstrated that the final irrigation with TA significantly increased the microhardness of root canal dentin compared with the control group regardless of the concentration and irrigation time. Thus the null hypotheses of this study must be rejected. The increased microhardness values after TA irrigation could be attributed to its effect on collagen stability due to an increase in the number of cross-links within and between the collagen fibrils.[19] In this way, the increased number of collagen cross-links can contribute to improvement of physical properties of dentin.[19] Xie et al. (2008) evaluated the effect of grape seed (a polyphenolic collagen crosslinking agent) on remineralization of artificial caries and demonstrated that grape seed extract significantly increased microhardness values which is in line with the current results.[21] Similarly, in another study, grape seed extract was shown to promote remineralization on the surface of demineralized dentin.[22] Moreover, a previous study showed that 20% TA treatment for 1 h contributes to enhanced collagen matrix properties and improved dentin bond strength.[9] However, this treatment time is not clinically applicable. For this reason, shorter applications of TA (2 and 5 min) during root canal treatment were evaluated in the present study. The concentration of 10% and 20% were used as final irrigation as suggested in previous studies.[9,23] The pH was also adjusted to neutral to prevent undesired effect of TA on dentin and TA-collagen interaction.[9]

The adhesion of the epoxy resin based sealer to TA treated root canal dentin was evaluated in the second part of this study using a push-out bond strength test. The adhesion of filling materials to dentin have been examined using various methodologies including microleakage tests[24], adaptation analysis[25], and bond strength evaluation.[26] The push-out bond strength test has been accepted as reliable and efficient method for evaluation of the adhesion of different root canal filling materials as testing conditions simulate clinical stresses and loading is perpendicular to dentinal tubules.[27] However, different root canal diameters and different plunger sizes can affect the outcomes of a push-out test. To avoid these limitations, the root canals were shaped with the same endodontic files and sections from the middle of the roots with similar dimensions were used in the present study. In addition, the punch diameter corresponding to 90% of the canal diameter was chosen to ensure that the dislocation force is not influenced by the punch diameter.

Based on the present findings, the protocols in which dentin was treated with 10% TA for 5 min or 20% TA for 2 and 5 min enhanced the bond strength of the epoxy resin based sealer to dentin. Therefore the second null hypothesis of the study must be rejected. This result is in agreement with previous studies that showed surface treatment with tannic acid, proanthocyanidins, epigallocatechin-3-gallate as cross-linking agents resulted in increase of resin bond strength to dentin.[7-9] Collagen cross-linking ability of TA is most likely to be responsible for higher bond strength values, considering the bonding ability of the epoxy resin based sealers to dentin collagen.[20] Degradation of dentin collagen is caused by endogenous collagenolytic matrix metalloproteinases (MMPs) that act in specific sites of the collagen molecule. In TA treated dentin matrices, hydroxyl groups of TA bond with amide NH groups of dentin collagen[17] and thus, these hydrogen bonds inhibit action of collagenase and MMPs enzymes by reducing possible cleavage site.[9] This mechanism may explain why TA treatment groups presented higher bond strength values in the present study. On the other hand, it was found that the effect of TA on the bond strength was time and concentration dependent. The application of 10% TA for 2 min did not exhibit a significant effect compared with the control group. For this reason, 5 min application of 10% TA or 2 and 5 min application of 20% TA as a final irrigation solution can be recommended to increase the bond strength of epoxy resin based sealer to dentin. The positive effect of TA on the root canal dentin might be increased by the activation of the solution, however, further research is required to obtain information on this issue.

CONCLUSION

Within the limitations of the present study, the final irrigation with TA may compensate decrease in dentin microhardness caused by irrigation protocol and may enhance bond strength of the epoxy resin based sealer to root canal dentin.

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