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ORIGINAL ARTICLE

Comparison of Medicaments Used in Regenerative Endodontics in Terms of Dentin Microhardness and Adhesion of Mineral Trioxide Aggregate

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ABSTRACT

Eliminating microorganisms in the root canal system is important for the success of regenerative endodontics. **Objective:** This study evaluated the effects of different antibiotic pastes used for regenerative endodontic procedures on dentin microhardness and the push-out bond strength of mineral trioxide aggregate (MTA) to root canal dentin. **Methods:** Sixty-four maxillary central incisors were instrumented and randomly divided into the following four groups (n = 16) for medicament treatment: triple antibiotic paste, amoxicillin+clavulanic acid, cefaclor, and control (no dressing). After 21 days, two root segments were obtained by sectioning the roots horizontally for push-out and microhardness evaluations. MTA was placed into the root canal of the sectioned segment for the push-out test. In the microhardness evaluation, three indentations were made at 500 and 1,000 μm from the canal lumen. The arithmetic mean was then calculated for each distance. ANOVA with post hoc Scheffe test and *t* test were used for the statistical analyses. The significance level was set at $p < 0.05$. **Results:** No significant difference was found between the groups in terms of push-out bond strength ($p > 0.05$). Cefaclor and amoxicillin+clavulanic acid reduced the microhardness values of the dentin at 500 μm ($p < 0.05$) while cefaclor had the lowest value at 1,000 μm ($p < 0.05$). **Conclusion:** Cefaclor reduced the microhardness value more than the other medicaments did at a depth of 1,000 μm. The pastes provided similar adhesion of MTA.

Key words: antibiotics, augmentin, cefaclor, mineral trioxide aggregate, regenerative endodontics

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INTRODUCTION

Immature teeth are at risk for pulp necrosis due to trauma, dental anomalies, or caries; specifically, necrosis causes incomplete root formation.[1,2] Regenerative endodontic treatment (RET) is a biological procedure used to provide the physiological functions of normal pulp, replace damaged structures such as the cells of the pulp–dentin complex, aid the completion of root development, and heal apical lesions.[3] Eliminating microorganisms in the root canal system is important for the success of regenerative endodontics.[4,5] Triple antibiotic paste (TAP, 1:1:1 mixture of ciprofloxacin, metronidazole, and minocycline) is a commonly used dressing material for regenerative endodontic procedures because of its protective effects against endodontic microorganisms.[6,7] As minimal instrumentation is advised to promote stem cell survival and avoid the weakening of thin root canals, antibiotic pastes cannot be removed from root canals except through irrigation procedures.[8,9] Therefore, remnant medicaments may have negative effects on the adhesion and penetration of barrier materials into the root canal dentin.[10]

Amoxicillin+clavulanic acid and cefaclor are known to have similar stem cell survival, antimicrobial efficacy, and discoloration outcomes to TAP when used in regenerative protocols.[11,12] Determining any differences in mechanical properties, such as microhardness

and adhesion to barrier materials, is important when selecting medicaments for regenerative endodontics.

Previous studies compared the microhardness levels of root canal dentin according to surface treatment with TAP, double antibiotic paste (a mixture of ciprofloxacin and metronidazole), or calcium hydroxide.[8,13-16] However, no study has explored the effects of amoxicillin+clavulanic acid or cefaclor on dentin microhardness. In the present study, TAP, amoxicillin+clavulanic acid, and cefaclor were compared in terms of dentin microhardness and adhesion of MTA, which has a high success rate when used in RET procedures.[17] The null hypothesis was that the three root canal medicaments used in endodontic regeneration techniques exert no significant effect on radicular dentin microhardness or MTA bonding to root dentin.

METHODS

Ethical approval for this study was obtained from the Health Ethics Committee of the University of Trakya (ID: 2018-361/19-24). A total of 64 single-rooted human maxillary central incisor teeth that were recently extracted for periodontal reasons were selected and stored in 0.1% thymol until the beginning of the experiment. Teeth with a root length of 16 mm (from the cemento-enamel junction to the apex) were included. Preoperative mesiodistal and buccolingual digital radiographs of each tooth were taken to confirm the presence of a single canal, full root development, and the absence of internal resorption or calcification. An endodontic access cavity was prepared using diamond burs. The apical parts of the roots were cut by a diamond disc such that the remaining root length was 12 mm.[18] Peeso reamers (Mani Inc, Tochigi, Japan) between #1 and #6 were introduced into the root canals. At each instrument change, 2 mL of 1.5% sodium hypochlorite (NaOCl) was used for irrigation. After finishing the instrumentation protocol, 20 mL of 1.5% NaOCl (5 mins), 5 mL of distilled water, and 20 mL of 17% EDTA (5 mins) were applied to the root canals.[19] The root canals were then dried using paper points (Dentsply Sirona, Vaughan, ON, Canada), and the teeth were randomly divided into four groups: control, TAP, amoxicillin+clavulanic acid, and cefaclor (n = 16 teeth per group).

Preparation of intracanal medicaments

No medicament was used in the control group. A 1:1:1 mixture of metronidazole (Eczacıbası, Istanbul, Turkey), ciprofloxacin (Biofarma, Istanbul, Turkey), and minocycline (Ratiopharm, Ulm, Germany) was prepared for the TAP group. For the amoxicillin+clavulanic acid group, the antibiotic paste was made using amoxicillin+clavulanic acid (GlaxoSmithKline, Istanbul, Turkey). For the cefaclor group, the antibiotic paste was prepared using cefaclor (Sanovel, Istanbul, Turkey).

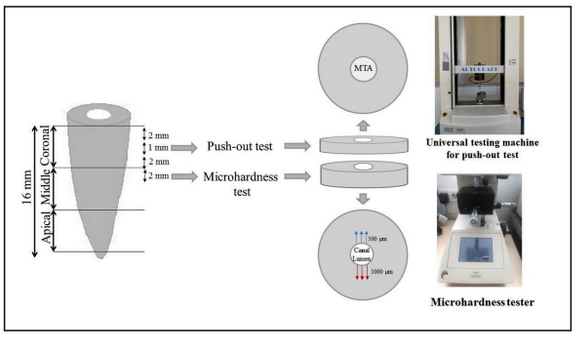


Figure 1. Microhardness and push-out testing procedure stages and testing devices. Blue arrows show the measurements at a depth of 500 µm, and red arrows show the measurements at a depth of 1,000 µm, with a distance of 100 µm between arrows, for microhardness evaluation.

The pH values of the pastes were measured with a pH meter (S220 SevenCompact; Mettler Toledo, Schwerzenbach, Switzerland). The pH values of the medicaments were as follows: TAP = 3.65, amoxicillin+clavulanic acid = 6.08, cefaclor = 5.47. The medicaments were prepared by mixing the powder with distilled water (powder-to-liquid ratio of 1 mg:1 mL). The medicaments were introduced to the canals with a Lentulo spiral. The access cavity was then temporarily sealed using CavitG (3M ESPE, Seefeld, Germany). The teeth were stored at 37°C under 100% humidity for 21 days. Thereafter, the intracanal medicaments were removed by 17% EDTA (20 mL applied over 5 mins) and distilled water (5 mL) irrigation.[19] The access cavity was then temporarily sealed using CavitG, and the apical portions of the canals were sealed with a flowable composite resin (Vertise Flow; Kerr, Orange, CA, USA). The roots were then embedded in acrylic resin blocks. For the microhardness evaluation, a 1 mm-thick root segment (from the coronal third of the tooth; 1 mm below the cemento-enamel junction) for the push-out test and a 2 mm-thick root segment (from the middle third of the tooth; 2 mm below the slice taken for the push-out test) were obtained by sectioning the roots horizontally under distilled water coolant using a low-speed saw (Micracut 200; Kemet, Kent, UK) (Figure 1).

Microhardness evaluation

A Vickers microhardness tester (HMV-700; Schimadzu Corporation, Tokyo, Japan) was used at a load of 100g for 10s. Three indentations were made at 500 and 1,000 µm from the canal lumen, with 100 µm between indentations (Figure 1). The indentations were observed under a stereomicroscope at 40x magnification. Vickers hardness values were provided using HMV-700 instrument. The arithmetic mean was then calculated for each distance.

MTA replacement
ProRoot MTA (Dentsply Sirona, Vaughan, ON, Canada) and saline solution were mixed according to the manufacturer's instructions. MTA was placed into the root canal of the sectioned segments on a clean glass surface by using an MTA gun. MTA was compressed with hand plugs (Dentsply Maillefer) and gently applied to the dentinal walls with a moistened cotton pellet. The dentin specimens were wrapped with wet gauze and incubated for 7 days at 37°C.

Push-out test
Push-out tests were performed at a crosshead speed of 0.5 mm min^{-1} by using a universal testing machine (AG-IS; Schimadzu) (Figure 1). Care was taken to center the push-out plugger (diameter = 1.2 mm) on the center of the MTA-filled surface. The maximum load applied before failure was recorded in Newtons (N) and converted to megapascals (MPa) by using the formula MPa= N/A[20], where A represents the adhesion area and was calculated as $2\pi rh$ (where π is the constant 3.14, r is the radius of the root canal spaces, and h is the thickness of the slice in millimeters). The radius of the root canal space and the thickness of the slice were measured using a digital caliper (Teknikel, Istanbul, Turkey).

Failure mode
After measuring the dislocation resistance, the specimens were photographed under a 7.8x magnification stereomicroscope (M205 C; Leica Microsystems, Wetzlar, Germany) to evaluate the failure type. Failure was categorized into the following three types:[21] adhesive failure at the MTA–dentin interface; cohesive fracture within the MTA; and mixed failure, i.e., in the MTA and in the dentin (Figure 2).

Statistical analysis
All statistical analyses were performed using SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p < 0.05$. The data were examined for normality of distribution by using the Shapiro–Wilk test ($p > 0.05$). ANOVA and post hoc Scheffe tests were used to evaluate the significance of the effects of the medicaments on the push-out bond strength and dentin microhardness. The microhardness values for different depths for each paste were compared with a *t* test. The statistical power of the study was estimated to be 80% ($p = 0.05$, ANOVA).

RESULTS

Push-out test
Table 1 presents the mean bond strength values and distribution of failure modes for each group. The results showed no significant difference between the groups ($p = 0.494$, ANOVA; Table 1). The TAP group showed the lowest push-out strength, whereas the control group showed the highest strength. The modes of failure are also presented in Table 1. The most common failure type in all paste groups was cohesive failure while that in the control group was mixed mode failure.

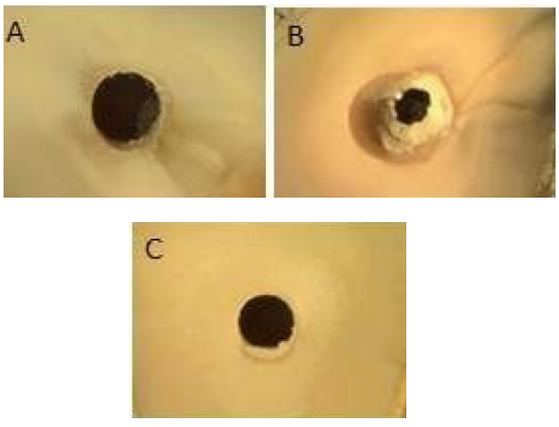


Figure 2. Failure modes: (A) adhesive, failure at the dentin–sealer interface; (B) cohesive, failure within the sealer; (C) mixed, adhesive and cohesive failure.

Microhardness test
At 500 μm from the canal lumen ($F = 7.4$, $p = 0.0001$, ANOVA; Table 2), amoxicillin+clavulanic acid and cefaclor decreased the microhardness more than the control and TAP groups did. At 1,000 μm from the canal lumen ($F = 7.3$, $p = 0.0001$, ANOVA; Table 2), the cefaclor group had a lower microhardness value than the other three groups. No significant difference was found between the other three groups ($p > 0.05$). The microhardness values for the same paste at 500 and 1,000 μm did not differ significantly ($p > 0.05$, ANOVA; Table 2).

DISCUSSION

This study evaluated the effects of TAP, amoxicillin+clavulanic acid, and cefaclor on dentin microhardness and adhesion of MTA. Cefaclor reduced the microhardness value more than the other medicaments did at a depth of 1,000 μm. Meanwhile, TAP provided the highest microhardness value among all pastes at 500 μm. Therefore, the null hypothesis for microhardness evaluation was rejected. All of the pastes provided a similar adhesion of MTA, and the null hypothesis regarding adhesion to MTA was accepted.

As RET requires little or no instrumentation to prevent the weakening of immature thin roots, chemical agents such as irrigation solutions and intracanal medicaments are used to eliminate microorganisms and provide the biological conditions needed for the treatment.[8] However, the long-term use of these chemicals may exert adverse effects on the chemical, physical, and mechanical properties of radicular dentin.[13-15,22,23] In the present study, the physical and

Table 1. Mean and standard deviation (SD) values for the push-out strength and the distribution of failure modes for each group

Group (n = 16)	Mean ± SD (MPa)	Test†	p	Failure modes		
				Adhesive	Cohesive	Mixed
Control	1.39 ± 0.91	0.808	0.494	3	4	9
TAP	1.00 ± 0.67			6	8	2
Amoxicillin+clavulanic acid	1.11 ± 0.64			5	8	3
Cefaclor	1.26 ± 0.79			5	7	4

† F value for ANOVA (normally distributed data), $p > 0.05$

Table 2. Comparison of mean microhardness values between and within groups at different depths.

Group (n = 16)	500 μm depth	1.000 μm depth	*t* value	p
	Mean ± SD	Mean ± SD		
Control	54.07 ± 7.79[a]	56.03 ± 5.19[a]	0.84	0.403
TAP	51.06 ± 7.12[a]	53.28 ± 6.92[a]	0.90	0.374
Amoxicillin+clavulanic acid	48.60 ± 6.41[b]	52.63 ± 6.56[a]	0.69	0.089
Cefaclor	42.89 ± 6.39[b]	46.41 ± 5.14[b]	1.70	0.095

SD: Standard deviation. Different letters represent significant difference among the groups within each column ($p < 0.05$; ANOVA).

mechanical properties of root dentin were evaluated through a microhardness test. This test was chosen as it is a well-standardized test that provides information about other mechanical important properties, such as tensile strength, compressive strength, and modulus of elasticity.[24]

Another important factor in the clinical success of RET is the bond strength between the barrier material and the root canal dentin.[25] The push-out test is considered the most reliable method to evaluate the bond strength between barrier materials and root canal walls.[25,26] In the current study, the adhesion of MTA was evaluated with a push-out test. For the push-out bond strength test, the coronal section was used to simulate clinical conditions as MTA was placed at coronal thirds of the roots in the RET procedure. MTA was replaced after sectioning the tooth to prevent its dislodgement during the cutting procedure.[25] MTA was chosen as the barrier material in this study because it is the most preferred material in RET given its biocompatibility, sealing ability, and marginal adaptation.[17] The antibiotic pastes used in this study were prepared by mixing drugs in distilled water at a concentration of 1 mg:1 mL, which has been reported to be ideal for stem cell survival.[11]

In this study, the application of antibiotic paste decreased the adhesion of MTA to dentin, but the difference between the control and the paste groups (TAP, amoxicillin+clavulanic acid, and cefaclor) was not statistically significant. As antibiotic pastes are removed using irrigation protocols in RET, they could not be completely removed from the root canal system, and residual antibiotic paste can disrupt the adhesion of MTA to dentin.[1,25,27] The remnant material also changes the chemical adhesion and penetration properties of barrier materials and may result in micromechanical locking at the surface between the barrier material and the dentin.[26,28]

The minocycline in TAP has been shown to bind calcium via chelation, thus forming an insoluble complex in the dentin. Hence, remnants on the dentin surface negatively affect the bond between dentin and MTA[1]. In the present study, the push-out strength of MTA in the TAP group was lower than that in the control group, but the difference was not significant. Similar to the present study, previous works showed no significant differences in the adhesion of MTA between the TAP and control groups.[29,30] In the present study and unlike that in previous research, cefaclor was used alone, i.e., it was not mixed with metronidazole and ciprofloxacin, to evaluate the effect of cefaclor singularly.[26,29] However, no significant difference was found.

The bond strength values in this study were similar to those reported by Nagas et al.[25] and Aydın and Buldur[26] but lower than those reported by Topçuoğlu et al. and Tulumbacı et al.[29,30] Similar to the protocols of Nagas et al.[25] and Aydın and Buldur[26], this study performed MTA placement after slicing as microfractures can occur because of fragility during the MTA setting period[26]. In contrast to the methodology of this study, previous

reports introduced MTA into the root canals prior to slicing so as to increase bond strength.[29,30] Differences in results among studies could be associated with methodological differences.

In this study, the most common failure type in all paste groups was cohesive failure while that in the control group was mixed failure. Remnant intracanal medicament may adversely affect the setting, adaptation, and penetration of sealers.[10] Residual antibiotic pastes inside the root canal space may interact chemically with MTA; this topic could be investigated in future studies.[26]

Significant decreases in microhardness were observed after the dentin was treated with the antibiotic pastes cefaclor and amoxicillin+clavulanic acid (500 μm); however, cefaclor had the lowest microhardness value at 1,000 μm. In previous studies, microhardness for TAP was lower than that in controls.[8, 13, 22, 23] Unfortunately, none of these studies compared TAP with amoxicillin+clavulanic acid and cefaclor. As mentioned in another previous study, an acidic pH may allow calcium ions to detach from the dentin surface.[16] In the present study, although the pH values of amoxicillin+clavulanic acid and cefaclor were higher than that of TAP (TAP = 3.65, amoxicillin+clavulanic acid = 6.08, cefaclor = 5.47), their microhardness was lower than that of the TAP and control groups. Changes in microhardness may be more associated with an erosive effect than with an acidic pH. A previous study showed that TAP with cefaclor causes the excessive erosion of dentinal tubule orifices.[31] The lower microhardness values of cefaclor could be associated with its increased erosion ability. As dentin hardness is believed to be correlated with mineral concentrations,[24] further studies could explore the relationship between microhardness and the demineralization or erosion of pastes.

In each paste group in this work, microhardness at 500 μm was lower than that 1,000 μm, but the microhardness values at different depths did not differ significantly. Thus, the pastes could be highly effective at the superficial dentin as they directly contact the surface.

CONCLUSION

Cefaclor and amoxicillin+clavulanic acid caused significant reductions in the microhardness of root canal dentin relative to the control and TAP groups at 500 μm while cefaclor caused greater reduction than the other groups at 1,000 μm. TAP, amoxicillin+clavulanic acid, and cefaclor provided similar MTA adhesion.

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