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Effects of Intracanal Medicaments and Coronal Barrier Materials Used in Regenerative Endodontics on the Fracture Resistance of Simulated Immature Teeth

Rejeneratif Endodontide Kullanılan Kanal İçi Medikamanların ve Koronal Bariyer Materyallerinin Simüle İmmatür Dişlerin Kırılma Direncine Etkisi

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Abstract

Objective: Immature teeth are more susceptible to fracture because of their fragile roots. Intracanal medicaments and coronal filling materials used in regenerative endodontics affect the fracture resistance of immature teeth. This study evaluated the fracture resistance of simulated immature teeth coronally filled with mineral trioxide aggregate (MTA) and EndoSequence (ES) BC Root Repair Material following the intracanal placement of several antibiotic pastes.

Materials and Methods: Sixty maxillary central incisors simulating immature teeth were divided into six randomized groups (n=10/group) according to the type of antibiotic pastes [triple antibiotic paste (TAP; a mixture of metronidazole, ciprofloxacin and minocycline); modified triple antibiotic paste (mTAP: metronidazole, ciprofloxacin and cefaclor); or augmentin] and used coronal barrier materials (MTA; or ES). After 21 days of storage, the antibiotic pastes were removed, and the coronal-barrier materials were placed. The samples were submitted to the fracture tests. The data were analyzed using t-test and one-way ANOVA tests. The significance level was set at $\alpha<0.05$.

Results: Fracture resistance was significantly lower for the mTAP-MTA group than the other MTA groups ($p<0.05$) and for the mTAP-ES group ($p<0.05$). No significant differences were observed for the other pair-wise comparisons ($p>0.05$).

Conclusion: Immature teeth coronally filled with MTA were more prone to fracture than ES after intracanal placement of mTAP. Before coronal MTA filling, the placement of mTAP decreased the fracture resistance of immature teeth compared with other medicaments. The teeth coronally filled with ES presented with similar fracture strength regardless of the application of intracanal medicament.

Keywords: Antibiotics, biomaterials, fracture strength, regenerative endodontics

Öz

Amaç: İmmatür dişler, ince kökleri nedeniyle kırılmaya yatkındır. Rejeneratif endodontide kullanılan kanal içi medikamanlar ve koronal dolgu materyalleri, immatür dişlerin kırılma direncini etkilemektedir. Bu çalışmanın amacı, farklı antibiyotik patların kanal içi uygulaması sonrası mineral trioksit agregat (MTA) ve EndoSequence (ES) BC Root Repair Material ile koronal olarak dolumu yapılmış simüle immatür dişlerin kırılma direncini değerlendirmektir.

Gereç ve Yöntemler: İmmatür dişleri simüle eden altmış adet maksiller santral diş, uygulanan kanal içi antibiyotik patı [üçlü antibiyotik patı (ÜAP: metronidazol, siprofloksasin ve minosiklin); modifiye üçlü antibiyotik patı (mÜAP: Metronidazol, siprofloksasin ve sefaklor); ve augmentin], ve koronal bariyer materyaline göre (MTA ve ES) rastgele altı gruba ayrıldı (n=10/grup). Antibiyotik patları, 21 gün sonra kanaldan uzaklaştırıldı ve koronal bariyer materyalleri yerleştirildi. Numuneler kırılma testine tabi tutuldu. Veriler t-testi ve tek yönlü ANOVA testleri kullanılarak analiz edildi. Anlamlılık düzeyi $\alpha<0,05$ olarak belirlendi.

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Bulgular: Kırılma direnci mTAP-MTA grubunda, diğer MTA gruplarına göre (p<0,05) ve mTAP-ES grubuna göre (p<0,05) anlamlı derecede düşüktü. Diğer ikili karşılaştırmalar arasında anlamlı bir farklılık gözlenmedi (p>0,05).

Sonuç: Rejeneratif endodontik tedavi prosedüründe, mTAP'nin kanal içi uygulamasından sonra, koronal olarak MTA ile doldurulmuş immatur dişler, ES ile doldurulmuş dişlere göre kırılmaya daha yatkındı. Koronal MTA dolgusu öncesi kök kanallarına mTAP yerleştirilmesi, olgunlaşmamış kök anatomisi taklit edilmiş dişlerde diğer medikamanlara kıyasla kırılma direncini azalttı. Koronal olarak ES ile doldurulmuş dişler, uygulanan kanal içi medikamana bağlı olmaksızın benzer kırılma direnci sergiledi.

Anahtar Kelimeler: Antibiyotik, biyomateryaller, kırılma direnci, rejeneratif endodonti

Introduction

Endodontic treatment of non-vital immature teeth is challanging due to their wide-open apices and thin root canal walls. The loss of tooth structure diminishes fracture strength against traumatic and excessive occlusal forces (1). Regenerative endodontic treatment (RET) may be used to replace functional pulpal tissue and eventually maintain root development by increasing the lengths and widths of immature roots. RET is a biology-based approach that contains three primary elements of tissue engineering: stem cells, growth factors, and scaffolds (2).

The clinical steps of the RET procedure may be summarized as follows: minimal/no preparation and disinfection of the root canals, intracanal bleeding formation, and coronal sealing (3). Disinfection of the root canals is mostly achieved using intracanal medicaments in the RET procedure (3). Therefore in regeneration-based approaches, calcium hydroxide and triple antibiotic paste (TAP; a mixture of metronidazole, ciprofloxacin and minocycline) are generally preferred as intracanal dressing materials (4). Since calcium hydroxide increases the risk of future cervical root fractures by increasing the brittleness of root dentin, antibiotic pastes appear to be the preferred choice (5). Several antibiotic mixtures, such as modified triple antibiotic paste (mTAP: metronidazole, ciprofloxacin and cefaclor), double antibiotic paste (DAP: metronidazole, ciprofloxacin) and augmentin have also been recommended for this purpose (6). The impact of antibiotic pastes on chemical and physical compositions of dentin has been proven in previous studies (7). The long-term application of intracanal medicament has a negative effect on dentin structure and generally results in a reduction of fracture resistance (8). However, the possible negative effects of antibiotic pastes have not yet been clarified yet.

During the RET procedure, below the cemento-enamel junction, a 2 mm thickness of coronal filling material is applied directly onto the blood clot, which consists of stem cells (3,9). Mineral trioxide aggregate (MTA) is the most commonly used material for providing intra-orifice barriers because of its perfect sealing ability and stimulation effect on stem cells (4,9). However, even with white MTA some degree of tooth discoloration has been reported (10). A calcium silicate-based material, EndoSequence (ES) BC Root Repair Material (Brasseler, Savannah, GA, USA), has stood out with stem cell differentiation capacity and has caused less tooth discoloration in RET procedures (9,11).

The effect of MTA and ES placements have already been investigated with respect to the fracture strength of immature teeth (12,13). However, in contrast to the recommendations of European Society of Endodontology (ESE) that "coronal barrier material should be placed 2 mm below the cement-enamel junction" (3), the root canals were completely filled in these aforementioned studies (12,13). A review of the literature reveals that, no study has evaluated fracture resistance effects in simulated immature teeth treated with a combination of several antibiotic pastes and coronal sealing materials. Therefore, this study aimed to evaluate the fracture resistance of simulated immature teeth filled with MTA and ES as coronal barrier materials following the intracanal placement of TAP, mTAP, and augmentin pastes. The null hypothesis was that no difference would be detected between coronal sealing materials and among antibiotic pastes with respect to the fracture resistance of immature teeth.

Materials and Methods

The study was approved by the Ethics Committee of Ankara Yıldırım Beyazıt University (decision no: 51, date: 27.12.2019). Sixty non-decayed maxillary central incisor teeth that extracted for orthodontic or periodontal reasons were selected. Single-rooted upper centrals with one root canal were included in this study also it was confirmed radiographically and visually that there were no cracks or resorption in the teeth.

Sample Preparation

To simulate immature teeth, the teeth's apical parts were cut using a diamond disc to standardize the remaining root length at 9 mm. Endodontic access cavities were prepared using diamond burs. The root canals were prepared with #1 to #6 Peeso reamer drills and carbid burs by passing beyond apex to obtain a 2.1 mm internal diameter for simulating the Cvek's stage #2 of root development (14,15). Between each instrument change, 2 mL of 1.5% sodium hypochlorite (NaOCl, Werax, İzmir, Turkey) was used for irrigation. Finally, the root canals were irrigated with NaOCl (1.5%, 20 mL, 5 min), followed by distilled water (20 mL, 5 min) and ethylenediaminetetraacetic acid (EDTA; Werax, İzmir, Turkey) (17%, 20 mL, 5 min) solutions. The root canals were dried with paper points and then the apical parts of the teeth were sealed with composite resin. After that, the samples were divided into six groups according to type of antibiotic paste and type of intra-orifice filling material (n=10), as presented in Table 1.

RET Procedures

The antibiotic pastes used in this study were prepared as follows:

TAP: Metronidazole (Eczacıbaşı, İstanbul, Turkey), ciprofloxacin (Biofarma, İstanbul, Turkey), and minocycline (Ratiopharm, Ulm, Germany) were mixed to at a ratio of 1:1:1.

mTAP: Metronidazole (Eczacıbaşı, İstanbul, Turkey), ciprofloxacin (Biofarma, İstanbul, Turkey), and cefaclor (Sanovel, İstanbul, Turkey) was mixed to at a ratio of 1:1:1.

Augmentin: The antibiotic paste was made using augmentin (GlaxoSmithKline, İstanbul, Turkey).

All antibiotic pastes were prepared by mixing the powders with distilled water (powder-to-liquid ratio 1 mg: 1 mL). The antibiotic pastes were placed into the root canals with the aid of a Lentulo spiral. The access cavities were then temporarily sealed using CavitG (3M ESPE, Seefeld, Germany). The teeth were stored at 37 ºC under 100% humidity for 21 days. Removal of the antibiotic pastes was performed using EDTA (17%, 20 mL, 5 min) and distilled water (5 mL) rinse (3). The root canals were then dried with paper points.

The coronal thirds of the root canals were obturated below the cemento-enamel junction (with 2 mm thickness) using the MTA (ProRoot MTA; Dentsply Sirona, Ontario, Canada) and ES (Brasseler; Savannah, Georgia, USA), in accordance with the manufacturer's instructions. The teeth were restored with a light-cured composite after the 12 hour setting period of both MTA and ES. The teeth were incubated at 37 ˚C and 100% humidity for 7 days until measuring their resistance for fracture.

Table 1. Experimental groups according to antibiotic paste and barrier material application

Groups (n=10)	Antibiotic paste	Barrier material
I	TAP	MTA
II	TAP	ES
III	mTAP	MTA
IV	mTAP	ES
V	Augmentin	MTA
IV	Augmentin	ES

TAP: Triple antibiotic paste, mTAP: Modified triple antibiotic paste, MTA: Mineral trioxide aggregate, ES: Endosequence root repair material

Fracture Strength Evaluation

The roots were embedded at the level of cemento-enamel junction into cylindrical acrylic resin blocks. The fracture strength was tested using a universal testing machine (Lloyd, Lloyd Instruments Ltd, West Sussex, United Kingdom) and the compression loads were applied at a crosshead speed of 1 mm/min. The occlusal load was applied to the cingulum at an angle of 135 degrees. The maximum force required to fracture was recorded in Newtons.

Statistical Analysis

Descriptive and analytical statistics of the data were analyzed using SPSS software (ver. 21; Chicago, IL, USA) at the significance level of α=0.05. Normal distribution of the data was evaluated using the Shapiro-Wilk test. Statistical analysis was carried out using one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons. A t-test was used to evaluate statistical differences between the means of the two groups.

Results

Table 2 represents the mean force values that generated fracture in the experimental groups.

Analysis of Antibiotic Pastes

No significant difference was observed among the groups in which ES repair material was used as coronal plug in terms of fracture strength reduction triggered by different antibiotic pastes ($p>0.05$). In MTA filling groups, intracanal placement of mTAP exhibited lower fracture resistance compared to TAP and augmentin, while no significant difference was observed between TAP and augmentin ($p>0.05$).

Analysis of Coronal Barrier Materials

Coronal MTA filling caused statistically lower fracture resistance when compared to ES filling after the intracanal

Table 2. Mean and standard deviation values (N) of fracture strength for the experimental groups (n=10)

Antibiotic pastes	MTA		ES		t value	P
	Groups	Mean ± SD	Groups	Mean ± SD		
TAP	I	287.19±38.27[a]	II	223.1±49.25[a]	1.027	0.318
mTAP	III	113.31±15.63[b]	IV	282.49±41.74[a]	3.796	0.003*
Augmentin	V	278.54±36.9[a]	VI	237.41±43.53[a]	0.721	0.480

SD: Standard deviation. TAP: Triple antibiotic paste, mTAP: Modified triple antibiotic paste, MTA: Mineral trioxide aggregate, ES: Endosequence root repair material. Different lowercases mean statistically significant differences between the groups within the same column (p<0.05, ANOVA; Tukey) placement of mTAP ($p<0.05$). However, no significant difference was observed between MTA and ES fillings ($p<0.05$), when TAP or augmentin was used as intracanal medicament.

Discussion

This study investigated the effects of MTA and ES on the fracture resistance of immature teeth following intracanal medicament placement of TAP, mTAP, and augmentin. The fracture resistance scores was lower for the mTAP-MTA group than for the other MTA groups and also lower than the mTAP-ES group. Therefore, the null hypothesis of the present study could not be accepted.

Prior to the blood clot formation, a final irrigation with 17% EDTA is advised as it stimulates growth factor release and stem cell formation in RET procedures (16). Therefore, in the present study, the root canal medicaments were removed using 17% EDTA. The irrigation procedure was performed according to the RET protocol of ESE to mimic possible structural changes in the dentin. It has been claimed that prolonged exposure of root canals to antibiotic pastes diminishes the fracture resistance of the immature teeth depending upon the demineralization of dentin (17).

Several studies evaluated MTA and other barrier materials with respect to their influence on fracture resistance of immature teeth filled in two scenarios one with using repair materials as apical plugs (18) and other with using as the obturation material of the entire root canal system (12,13). A review of the existing literature reveals that one study evaluated the fracture resistance of MTA as a coronal filling of 3 mm thickness below the cemento-enamel junction (19). In contrast to our study which MTA was used in the coronal parts of the roots, the roots were apically filled with MTA and restored with fiber-posts were evaluated in that study. Based on our research, no other study has compared the effect of MTA and other intra-orifice barrier materials on the fracture resistance of immature teeth when performed exactly as recommended by ESE (with 2 mm thickness just below the cemento-enamel junction) (3). In this regard, the present study compared, the fracture strength of teeth with MTA coronal plug and teeth with ES coronal plug with 2 mm thickness just below the cement-enamel junction. The results of this study showed that MTA and ES provided similar fracture resistance following the application of TAP and augmentin, while ES was superior after mTAP placement. An earlier study has compared simulated immature teeth which were filled with MTA, ES, and BioAggregate with respect to fracture resistance (13) and found that fracture resistance scores of ES was superior to MTA. Contrary to our study, in the aforementioned study (13), the root canals were completely filled with repair material and no medicament was applied to the root canals, and were used different brand of MTA than in our present study.

These changes in physical and chemical composition of the root dentin the could affect the fracture resistance of the immature teeth that are already prone to fracture (20). In previous studies, the intracanal usage of TAP, DAP, and calcium hydroxide have previously been associated with collogen degradation from dentin (17) and it has been reported to decrease on the fracture resistance of immature teeth (20). In this study, in MTA groups, mTAP reduced the fracture strength of the teeth compared to the TAP and augmentin. The medicament on dentin walls could only be removed through 17% EDTA irrigation, as minimal preparation is advised. Therefore, antibiotic pastes could not be completely eliminated from the dentin walls (21). The coronal barrier material reacts chemically with the dentin (22) and probably with the residual medicaments. It has been found that mTAP has induced a higher increase in pH than TAP by raising the Ca/P ratio on the dentin surface (23). It was also observed that MTA caused a higher pH value than ES did (24). The additional effect between MTA and mTAP might have created a more alkaline environment compared to other groups. It has been determined that alkaline pH can make the root structure more susceptible to fracture (25). The lowest fracture resistance value observed in the mTAP-MTA group may be related to alkaline pH. Further studies are required to evaluate the reaction between antibiotic pastes and coronal sealing materials.

Conclusion

The immature teeth coronally filled with MTA were more prone to fracture compared to ES after intracanal placement of mTAP. Prior to coronal MTA filling, placement of mTAP decreased the fracture resistance of immature teeth when compared to other medicaments. The teeth coronally filled with ES presented similar fracture strength regardless of the applied intracanal medicament.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Ankara Yıldırım Beyazıt University (decision no: 51, date: 27.12.2019).

Informed Consent: This study does not require informed consent.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.S., S.İ.Y., M.A., Concept: E.S., Design: E.S., Data Collection or Processing: E.S., S.İ.Y., M.A., Analysis or Interpretation: E.S., S.İ.Y., Literature Search: E.S., Writing: E.S.

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