Effect of medicaments used in endodontic regeneration technique on push-out bond strength of MTA and Biodentine

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ABSTRACT
The aim of the present study was to evaluate the effect of the intracanal medicaments used in regenerative endodontic treatment on push-out bond strength (PBS) of mineral trioxide aggregate (MTA) and Biodentine (BD). The root canals of 102 maxillary incisors were enlarged to simulate immature roots and were randomly divided into three groups (n = 34): a control group (no intracanal medicament) and two test groups, subjected to calcium hydroxide (CH) or triple antibiotic paste (TAP) medication for two weeks. After the medication removal, each group was divided in two subgroups and the coronal portion of each canal was filled with MTA or BD. After one week of storage, the coronal region of each root was horizontally sectioned and push-out test was performed. Data were analyzed by two-way analysis of variance and the Tukey post hoc test (P = 0.05). PBS values were significantly affected by the type of material (P < 0.001) and the type of medication used (P = 0.049), but no interaction was found (P = 0.97). The BD group showed significantly higher push-out resistance values than those of the MTA group (P < 0.001). TAP showed the lowest PBS values, which were significantly lower than those of the control group (P = 0.043) but not than those of CH (P = 0.229). After a two-week application period, TAP seemed to decrease the PBS of both cements, while CH did not. BD appeared to have higher PBS compared to MTA, regardless of medication.

Introduction
The root canal treatment of immature teeth has been an issue of considerable debate for many years due to the wide apical foramen, large root canal lumen, weak root canal walls and tendency to fracture of the immature teeth.[¹] Regenerative endodontic treatment (RET) is a tissue engineering concept specific to pulp and dentin regeneration inside the root canal space of immature non-vital teeth in which the pulp tissue is completely removed due to previous irreversible inflammatory responses and microbial invasion.[²]

To avoid further weakening of immature teeth, minimal mechanical instrumentation is recommended, but, conversely, a sterile environment is necessary for pulp tissue regeneration in RET.[³] Thus, disinfection of root canals is attempted only by irrigation solutions and intracanal medications; antibiotic pastes (metronidazole and ciprofloxacin with or without minocycline) and calcium hydroxide (CH) are the medications commonly used for RET.[³,⁴]

To obtain the therapeutic effects of medicaments, medication periods, varying from weeks to several months, have been used.[⁵–⁸] However, alterations in the surface properties and deterioration of the mechanical properties of root canal dentin were previously demonstrated when endodontic regeneration medicaments were used.[⁹–¹²]

After a medication period, mineral trioxide aggregate (MTA) is placed to the coronal part of the root canal.[³,⁴] MTA is biocompatible, conductive and inductive calcium silicate-based cement (CSBC) that is able to bond dentin chemically.[¹³] Biodentine (BD) is a relatively newer CSBC, which resembles MTA and its endodontic indications. It has some additional modifications in its formulation to enhance its physical and chemical properties.[¹⁴–¹⁶] BD, compared with MTA, has been noted to have higher bond strength to dentin.[¹⁵–¹⁸]

Pretreatment of dentin with either irrigants or medicaments may influence the bond strength of silicate-based cements,[¹⁷,¹⁹] which is an important factor since the tooth is exposed to occlusal and procedural forces that might dislodge the cement after its placement. [¹⁵,²⁰,²¹] Recently, Topçuoğlu et al. [¹⁹] reported that a
three-week application of medicaments used in regenerative endodontics reduced the bond strength of MTA placed in root canals.

Therefore, the aim of the present study was to evaluate the effects of the intracanal medicaments used in RET on push-out bond strength (PBS) of MTA and BD.

Materials and methods

The study protocol was approved by the Ethics Committee of Ege University (Protocol No. 15-1/9). One hundred and two human maxillary incisor teeth with a single straight root canal, extracted for periodontal reasons, were collected for this study. The intact teeth were chosen and stored in thymol solution (0.1%) for up to three months.

Buccolingual and mesiodistal dimensions of teeth at the cemento-enamel junction were measured with a digital caliper (Mitutoyo, Tokyo, Japan) to provide standardization, and the mean mesiodistal and buccolingual dimensions were obtained. Thereafter, teeth presenting 20% deviation in dimensions compared with the standard values were excluded.

Sample preparation

Teeth were sectioned apically 12 mm below and coronally 2 mm above the cemento-enamel junction with a low-speed rotary saw (Isomet, Buehler, Lake Bluff, IL, USA). Tissue remnants were removed by means of a size 20 Hedström file (Dentsply Maillefer, Ballaigues, Switzerland); samples were instrumented with rotary files (ProTaper, Dentsply Maillefer) to standardize the master apical file to size 40 (F4). Next, Peeso reamers (Dentsply Maillefer), from #1 to #6, were used sequentially to enlarge canals.

Between using each file, root canals were irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl) (Sigma-Aldrich, St. Louis, MO, USA). NaOCl (2.5%, 5 mL, 1 min) and ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich) (17%, 5 mL, 1 min) were used to remove the smear layer. Finally, canals were rinsed with sterile distilled water and were dried with paper points (Meta, Metabiomed, Chungbuk, Korea). The teeth were then randomly divided into a control group (no intracanal medicament was used) and two experimental groups, in which each tooth was treated with triple antibiotic paste (TAP) or CH intracanal medicament (n = 34 for each group).

**TAP group:** TAP paste, 1:1:1 mixture of ciprofloxacin (Bayer, Leverkusen, Germany), metronidazole (Sanofi Aventis, Frankfurt, Germany) and minocycline (Ratiopharm, Ulm, Germany), was prepared with sterile distilled water (powder:liquid ratio of 3:1) and was delivered into the canal by means of a lentulo spiral (size 40, Dentsply Maillefer) which was introduced 2 mm short of the working length.

**CH group:** CH paste prepared by mixing CH powder (Merck, Darmstadt, Germany) with sterile distilled water (powder: liquid ratio of 2:1) was delivered into the canal by means of a lentulo spiral (size 40).

The root canal orifices were sealed with a temporary filling material (Cavit, 3M ESPE, Neuss, Germany). All samples were kept in a saline solution, which was replenished every seven days to avoid dehydration throughout the two-week medication period. At the end of the incubation period for each sample, TAP and CH were removed with 5 mL of 2.5% NaOCl and 5 mL of 17% EDTA, and the root canals were dried with paper points.

The samples of each group were then randomly divided into two subgroups (n = 17). In the first subgroup, MTA (batch #13102907) was used as a coronal plug (4 mm). MTA (Dentsply Tulsa Dental, Tulsa, OK, USA) was prepared in accordance with the manufacturer's instructions and was placed into the coronal third of the root with an amalgam carrier having a 4-mm-long chamber. Also, a saline-moistened cotton pellet was placed on top of the MTA to accelerate the setting of MTA. For the second subgroup, BD (Septodont, Saint Maur des Fossés, France, batch #B10981) was used. BD liquid from a single-dose container was emptied into a powder-containing capsule, mixed for 30 s at 4000–4200 rpm (Maecolux, Bettembourg, Luxembourg) and placed in the coronal thirds of the root canals. The root canal orifices were sealed with a temporary filling material. After the radiographic control of the coronal plug placement, the samples were stored for one week at 37 °C under 100% humidity.

Push-out test

Push-out test was used to measure the PBS of specimens. After the one-week storage period, the coronal region of each root was horizontally sectioned by using IsoMet saw (Buehler, Lake Bluff, IL, USA) under water cooling to obtain a slice that was 1 ± 0.1 mm in thickness, which was checked by means of a digital caliper (Mitutoyo, Tokyo, Japan).

A testing machine (AGS-X, Shimadzu Co., Kyoto, Japan) was used to measure the force in Newtons (N), needed to dislocate the cement. The diameter of the pin was 1.2 mm, whereas the canal diameter, the circular space which was filled with cement, was 1.5 mm. The pin was positioned so that it touched only the cement and did not stress the root canal walls. The crosshead speed of the testing machine was 1 mm/min. To express
the PBS (MPa), the load at failure (N) was divided by the area of adhesion surface (mm²), calculated by using the following equation: $2\pi r h$, where $\pi$ is the constant 3.14, $r$ (mm) is the root canal radius and $h$ is the thickness of the root slice in millimeters.

**Failure mode analysis**

Following the push-out test, the samples were viewed at 20× magnification so that the failure types could be determined. The failure type was classified into three categories, as previously done by Shokouhinejad et al. [21]: (1) adhesive failure between cement and dentin, (2) cohesive failure within cement or (3) mixed failure which includes cement and dentin together.

**Statistical analysis**

The two-way analysis of variance (ANOVA) was used to evaluate the PBS of filling material to dentin, exposed to both of the intracanal medicaments. The post hoc test with Tukey adjustment was performed for multiple comparisons. The significance level was set at $P < 0.05$. All statistical analyses were performed with IBM SPSS Statistics 20 software (IBM Corporation Software Group, Somers, NY, USA).

**Results and discussion**

The PBS values of the control and experimental groups are presented in Table 1 as means and standard deviations and 95% confidence interval (CI). The push-out resistance values were significantly affected by the type of material ($P < 0.001$) and the type of medication used ($P = 0.049$), but no interaction was found ($P = 0.97$). According to the type of material, the BD group showed significantly higher push-out resistance values than the MTA group ($P < .001$). According to the type of medication, TAP showed the lowest push-out resistance values, which were significantly lower than those of the control ($P = 0.043$) but not significantly lower than those of the CH ($P = 0.229$). CH medication group showed slightly lower push-out resistance values compared to the control group, but they were not significant ($P = 0.708$). In both the MTA and BD groups, pairwise comparisons revealed no significant differences in push-out resistance values ($P > 0.05$).

The modes of failure are listed in Table 2. Cohesive failure was the most frequent type of failure for BD, whereas no predominant failure mode was found for MTA.

Since CSBCs may be subjected to forces that arise during restorative procedures or mastication after RET procedures,[15,19,21–23] the dislocation resistance (DR) of cements should be adequate.[15,17,20,22] The push-out test has been shown to be a reliable method for the evaluation of the materials’ DR,[24] and it has been utilized in many studies.[17–19,21–23] In the present study, the push-out test was used to compare the bonding strength of MTA and BD to root dentin treated with CH or TAP. It has been reported that the DR of MTA might be influenced by its thickness, humidity of the surrounding tissues, evaluation time, pH of the environment and physical properties of the contacting dentin surface. [17–19,21–23] The evaluation of DR of MTA and BD has become crucial, since the dentin surface of the root canal is exposed to various irrigation solutions and intracanal medicaments during RET procedures, which may alter its chemical and mechanical properties. According to the results of the present study, the DR of MTA and BD was influenced by the type of medication used. For the two-week medication period, TAP reduced the DR of MTA and BD, whereas CH did not. Recently, the medicaments used in RET have been evaluated regarding their influence on the DR of MTA placed in the root canal dentin [19]; the application of a double antibiotic paste reduced the DR of MTA, whereas TAP and CH did not affect the DR after a three-week period.[19]

Effects of antibiotic pastes on dentin were evaluated by Yassen et al. [11] and were found to possess a demineralization effect due to their acidic nature. Demineralization of dentin may decrease the mechanical and chemical adhesion established between CSBC and dentin. El-Ma’aita et al. [17] revealed that the DR of MTA and BD to EDTA-treated dentin is lower compared with that

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**Table 1.** Push-out resistance values of the test groups.

|         | MTA (MPa) | 95% CI for means (MPa) | Biodentine (MPa) | 95% CI for means (MPa) |
|---------|-----------|------------------------|-----------------|------------------------|
| Control | 5.68 (0.81) | 5.27–6.09 | 6.43 (0.85) | 6.02–6.85 |
| TAP     | 5.19 (0.74) | 4.77–5.60 | 5.92 (0.77) | 5.51–6.33 |
| CH      | 5.48 (1.01) | 5.07–5.90 | 6.30 (0.92) | 5.89–6.71 |

Note: Mineral trioxide aggregate (MTA); standard deviation (SD); confidence interval (CI); triple antibiotic paste (TAP); calcium hydroxide (CH).

**Table 2.** Type of failure numbers for each group, evaluated by 20× optical magnification.

|         | MTA | Biodentine |          |          |          |          |
|---------|-----|------------|----------|----------|----------|----------|
|         | Adhesive | Cohesive | Mix | Adhesive | Cohesive | Mix |
| Control | 6    | 5          | 6      | 4        | 7         | 6      |
| TAP     | 7    | 4          | 6      | 5        | 7         | 5      |
| CH      | 5    | 5          | 7      | 3        | 8         | 6      |

Note: Mineral trioxide aggregate (MTA); triple antibiotic paste (TAP); calcium hydroxide (CH).
of untreated dentin, and the presence of a smear layer on the dentin surface has been reported to enhance the formation of the interfacial layer between the CSBC and radicular dentin.[17] During the physicochemical interaction of MTA with dentin, MTA is leaching ions, of which Ca\(^{2+}\) is the most abundant.[13] This leads to the formation of an interfacial layer of hydroxyapatite or carbonate apatite, which is important for such biomaterials, because it forms a chemical bond.[13,25] It has been speculated that facilitating the formation of an interfacial layer between MTA and dentin would result in a favorable sealing ability since it would enhance the adhesion and the DR of MTA.[14,22,25] In the present study, adhesion of cement to CH-treated dentin was greater than the adhesion of cement to TAP-treated dentin. Shokouhinejad et al. [21] suggested the application of non-setting CH to neutralize the pH and to increase the DR of MTA prior to the placement of MTA in a low-pH environment. The effect of one-week CH medication on the improved marginal adaptation of an MTA-apical plug has also been demonstrated.[26] This improvement was attributed to the conversion of CH to calcium carbonate or to the reaction of MTA with residual CH. Thus, in the present study, favourable results from the CH-treated group may be related to the Ca\(^{2+}\) ions supplied by the residual CH, which was previously suggested to improve the adhesion of MTA. Despite irrigation, complete removal of TAP and CH has been reported to be difficult.[27] Residues of CH were distributed rather superficially and contained less material, while TAP appeared to have greater retention and deeper penetration. Consequently, residual TAP may lead to the demineralization of dentin via decreasing the pH and chelating the calcium from dentin [11]; demineralized dentin has been reported to be significant in establishing the adhesion of MTA to dentin.[17,21]

In the present study, in all medication groups, the BD group displayed significantly higher DR than the MTA group \(P < 0.05\). This finding confirmed the results from previous studies that compared the bond strengths of MTA and BD in different experimental conditions. [15,17,18] Both materials were reported to form an interfacial layer and a tag-like mineral structure extending to the dentinal tubules.[13,14,16,25] However, some differences in interfacial layers formed by MTA and BD were reported.[14,16] Han and Okiji [14] reported that BD released more Ca\(^{2+}\) ions and formed more calcium phosphate precipitates and thicker Ca- and Si-rich dentin areas than MTA. In contrast, Kim et al. [16] found the thickness of the interfacial layer of MTA to be significantly higher than that of BD; however, they could not find significant differences in the Ca/P ratio of the interfacial layer of either MTA or BD. Thus, the Ca/P ratio of the interfacial layer may perhaps be more significant than the thickness of the layer.

It is known that differences in the particle sizes of the cements have a great effect on the push-out strength of materials.[22] Gunser et al. [15] reported that the smaller and more uniform particle size of BD may enhance the tubular penetration of BD and lead to a better mechanical retention. Along with this phenomenon, in the present study, cohesive failure was dominant for BD, whereas no predominant failure mode was found for MTA. It is noteworthy that, besides chemical bonding, mechanical retention of cements may enhance the push-out bonding strength of materials.

Conclusions

In the present study, antibiotic medications seemed to decrease the DR of MTA and BD after a two-week application period. Conversely, CH did not influence the DR of either cement. BD appeared to have higher PBS, when compared with MTA, regardless of the medication type. The types and the application times of medicaments used for RET should be optimized to provide maximum antimicrobial effects, while creating a favourable environment for both stem cell attachment and bioactive cement adhesion.

Disclosure statement

No potential conflict of interest was reported by the authors.

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