Effect of three intracanal medicaments used in pulp regeneration on the push-out bond strength of mineral trioxide aggregate and calcium-enriched mixture: An in vitro study

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Abstract
Background. The bond strength of the materials used as a cervical barrier in the pulp regeneration is essential for the success of treatment. This study aimed to evaluate the effects of triple antibiotic paste (TAP), double antibiotic paste (DAP), and simvastatin as intracanal medicaments on the dislodgement resistance of mineral trioxide aggregate (MTA) and calcium-enriched mixture (CEM).

Methods. A total of 160 extracted human single-rooted teeth were selected, and root canal preparation was carried out. The teeth in each group were randomly divided into four subgroups: TAP, DAP, simvastatin, and the control group (without intracanal medicament). Four weeks after placing the medicaments, it was removed by sodium hypochlorite, and MTA and CEM were placed in the coronal third of the root canals. After a week, 2-mm-thick dentin disks were prepared from the coronal third of the roots, and the push-out test was performed using a universal testing machine. The data were analyzed using two-way ANOVA and independent t-test at a significance level of 0.05.

Results. Regardless of the intracanal medicament, there was no significant difference between the overall bond strength of MTA (59.3 ± 10 MPa) and CEM (55.8 ± 11 MPa) (P = 0.6). Furthermore, there were no significant differences in bond strength between the two intracanal medicament groups and the control group (P > 0.05).

Conclusion. Under the limitations of the current study, DAP, simvastatin, and TAP, as intracanal medicaments, did not adversely affect the push-out bond strength of CEM and MTA.

Introduction
The treatment of immature teeth with necrotic pulp with an open apex is one of the challenges in the endodontic field. Pulp regeneration is one of the methods used to treat these teeth to restore the physiological function of the tooth, considering the resulting continued longitudinal development of the root, the apical closure, and the increase in the thickness of the dentinal walls.1,2

One of the most important steps in the regeneration treatment is root canal disinfection. Compared to mature teeth, these teeth cannot be cleaned and shaped like adult teeth due to their open apex and thin dentinal walls. The antimicrobial process relies more on intracanal medicaments and irrigation.3 In this context, the mixture of three antibiotics consisting of metronidazole, minocycline, and ciprofloxacin, known as the triple antibiotic paste (TAP), has acceptable root canal disinfection efficacy.4-6 The double antibiotic paste (DAP) is obtained by removing minocycline.7 Simvastatin is an antimicrobial compound8,9 that has recently been considered in the endodontic field. This medicament belongs to the statin category and is used routinely to reduce blood fat. In addition to the therapeutic properties, this compound has the following properties10-12:

- Antimicrobial property against oral and periodontal pathogens, including bacteria, fungi, and viruses
- The potential to induce angiogenesis in pulp stem cells
- Affects the survival of osteoblasts and interferes with bone regeneration.

After root canal disinfection in the regeneration...
treatment, the next step is to fill the root canal with a resorbable matrix. Finally, a cervical barrier is placed in the coronal third of the root canal to prevent reinfection. Mineral trioxide aggregate (MTA) is a calcium silicate-based cement and is used as a cervical barrier. Its desirable properties include bioinductivity, biocompatibility, and the ability to induce pulp regeneration. Despite all its desirable characteristics, MTA has a long setting time; on the other hand, handling problems are always considered one of its disadvantages. Calcium-enriched mixture (CEM) is also an endodontic biomaterial with a calcium base that contains various calcium compounds. The clinical applications of this material are similar to those of MTA; however, it has a shorter setting time, better flow, and easier handling compared with MTA. The sealing ability of materials used as cervical barriers in pulp regeneration is critical because bacterial leakage is a cause of the treatment failure, and a bacteria-free root canal is needed for successful regeneration. The bond strength of the materials to the root dentin is one of the factors indicating their sealing ability. These materials should be resistant to displacement forces caused by physiological function and the placement of restorative materials since their displacement will disrupt the sealing process and cause bacterial leakage. According to previous studies, the push-out test is a suitable method for evaluating the bond strength of materials.

The effect of intracanal antimicrobial materials such as TAP and DAP on the bond strength of the cervical barrier materials should be considered in the regeneration. Previous studies have shown that antibiotic pastes have acidic properties that cause dentinal erosion and collagen degeneration, and the mechanical bond of MTA affects the dentinal walls of the root canal. At present, two published studies are available on the effect of TAP and DAP on the bond strength of the ProRoot MTA. According to the results, they exert their negative effect on the bond strength of the MTA 4 weeks after being placed in the root canal. DAP decreases the bond strength compared with TAP, which has been rejected in another study, and there are contradictory reports in this regard. The innovations of the current study, compared to the previous studies, are as follows:

1. Investigating the effect of DAP and TAP on CEM bond strength, which has not been studied by previous studies.

2. Evaluating the effect of simvastatin as an intracanal medicament on bond strength of MTA and CEM

This study aimed to investigate the effect of DAP, TAP, and simvastatin as intracanal medicaments on the push-out bond strength of MTA and CEM.

**Methods**

**Selection of teeth and preparation of the samples**

A total of 160 human teeth extracted for orthodontic reasons or periodontal disease were collected. The inclusion criteria for the study included:

1. Human tooth with a single root and a single canal [The single-channel characteristic was confirmed by taking radiographs from mesiodistal and buccolingual dimensions]

2. without previous root canal treatment

3. closed apex

4. no root caries

5. no calcification and erosion [Radiography examination]

6. no cracks and fractures in the root with visual examination and observation under an optical microscope at a magnification of x40.

To equalize the length of the roots, the crown of each tooth was sectioned 2 mm above the cementoenamel junction using a diamond disk (SP 1600 Microtome, Leica, Nu Block, Germany) under water coolant so that all the roots measured about 14 mm [the crown of the teeth was not sliced completely so that temporary dressing can be placed during the study].

**Root canal preparation and placement of intracanal medicaments**

The root canals were cleaned and shaped using the RaCe rotary system (FKG, Lachaux-de-Fonds, Switzerland) and the crown-down technique. The working length was determined by K-file #15 (Dentsply, Maillefer) so that when the file tip was observed at the apex, the final working length was obtained by subtracting 1 mm from this length. Master apical file (MAF) was set at #40 in all the specimens. To obtain the same standard diameter for each canal, Gates-Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland) were used, with 6, 5, 4, and 3 drills penetrating the root canal at depths of 3, 6, 9, and 12 mm, respectively. Sodium hypochlorite (2.5%) was used as an irrigation solution during instrumentation. To remove the smear layer at the end of the root canal preparation, 5 mL of 2.5% sodium hypochlorite and 17% EDTA were used for 1 minute. The final irrigation was performed using a normal saline solution.

The specimens were divided into two groups (n=80) based on the type of material used as a cervical barrier; group A: MTA, group B: CEM. Each group was divided into four subgroups based on the intracanal medicament: subgroup 1: TAP, subgroup 2: DAP, subgroup 3: simvastatin, and subgroup 4: control group (without intracanal medicament). These medicaments were placed using a lentulo spiral.

**TAP**: Equal proportions of metronidazole (Tehranshimi, Tehran, Iran), minocycline (Tehranshimi, Tehran, Iran), and ciprofloxacin (Ruzdarou, Tehran, Iran) were prepared as powder. These three powders were mixed with distilled water with a powder-to-liquid ratio of 3 to 1.

**DAP**: An equivalent combination of prepared ciprofloxacin and metronidazole powder was mixed with distilled water with a powder-to-fluid ratio of 2.5 to 1.

**Simvastatin**: 50 mg of the powder of this medicament, provided by the Pharmacy Co., was mixed with 1 mL of

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**J Dent Res Dent Clin Dent Prospects, 2022, Volume 16, Issue 1**

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After placing the intracanal medicaments up to the level of CEJ, the teeth were dressed with Cavit (ESPE, Norristown, PA). The specimens were incubated at 37°C under 100% moisture for four weeks. The root canal was empty in the control group and was dressed like the previous groups. After this period, the temporary dressing of the teeth was removed, and the antimicrobial materials were removed by 10 mL of 2.5% sodium hypochlorite and normal saline solution. The canals were dried with paper points.

Placement of cervical barriers and evaluation of the bond strength

The biomaterials used in the study were mixed according to the manufacturer’s instructions:

- MTA (Angelus, Londrina, Brazil): A powder-to-fluid ratio of 3:1 and hand mixing
- CEM (BioniqueDent, Tehran, Iran): Mixing the fluid with powder until achieving a thick consistency according to the manufacturer’s instructions
- MTA (A Group) and CEM (B Group) were transferred to the root canals by an MTA carrier and packed with a condenser with a suitable size. Parallel digital radiography confirmed that at least 4 mm of the coronal canal below CEJ was filled with the materials. A wet cotton pellet was placed on the materials, and the teeth were dressed with glass-ionomer (Fuji II LC; GC Corp.; Tokyo, Japan).

The specimens were incubated at 37°C for one week. To do the push-out test, the 2-mm discs were prepared from the coronal third of each root. The push-out test was performed by a universal testing machine (Hounsfield Test Equipment, Model HSK-S, Surrey, UK), which applied force to the specimens in the apico-coronal direction with a crosshead speed of 1 mm/minute. The highest force that led to the bond failure was recorded in Newton (N).

The push-out bond strength was calculated in MPa by dividing the recorded values in N by the surface area of the experimental specimen in mm² calculated from the following formula:

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\text{Surface area} = 2\pi r \times h \quad (\pi = 3.14, r \text{ is the radius of the root canal, and } h \text{ is the disk thickness in mm}).
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Statistical analysis

After performing descriptive statistical analyses (means and standard deviations of the study data), the normal distribution of data was confirmed by the Kolmogorov-Smirnov test \((P=0.9)\). Two-way ANOVA was used to examine the significance of the effect of intracanal medicaments on the bond strength of the biomaterials, and the bond strength of CEM and MTA was compared using the independent t-test. \(P < 0.05\) was considered the level of significance.

Results

Table 1 presents the means and standard deviations of the study data. In both biomaterials, there were no significant differences between the bond strength of the subgroups treated with the intracanal medicaments and the control group \((P > 0.05)\). The overall bond strengths in the MTA and CEM groups were not significantly different regardless of the intracanal medicament used \((P = 0.6)\).

**Discussion**

This study evaluated the effects of simvastatin, DAP, and TAP as intracanal medicaments on the displacement resistance of CEM and MTA as cervical barriers. The results indicated no adverse effects of these three compounds on the bond strength of the biomaterials.

The pulp regeneration as an endodontic treatment for the root of immature teeth with necrotic pulp is based on the use of intracanal medicaments instead of conventional root canal cleaning and shaping. Another important treatment step is the placement of a biomaterial as a cervical barrier to activate the sealing property to prevent leakage of bacteria and their products, which are considered the most important factors for treatment failure. These two factors, i.e., placing the intracanal medicament and the cervical barrier (biomaterials), were considered two independent variables of this study. The dependent variable of this study was the displacement resistance of the material placed in the cervical region of the root, which indirectly indicates the sealing ability of the material and is measured using the push-out test.

MTA and CEM are two endodontic biomaterials with a calcium silicate base used for various purposes such as regeneration of permanent immature teeth. Considering their physicochemical properties, these two materials were used as a cervical barrier in the present study. According to the results of the present study, regardless of the use of the intracanal medicament, the displacement resistance of these two materials was not significantly different, which is consistent with the results of studies conducted by Rahimi et al. and Lotfi et al. Also, Yavari et al. showed that the salivary microleakage was not significantly different in these two materials, used as a cervical barrier.

Considering the release of calcium hydroxide during the setting reaction, both materials have the potential for chemical bonding with dentin because the released calcium

| Medication | Group | Mean   | SD    |
|------------|-------|--------|-------|
| TAP        | MTA   | 56.6500| 9.43637|
|            | CEM   | 56.3000| 13.59518|
|            | Total | 56.3750| 11.39131|
| DAP        | MTA   | 47.2800| 9.08647|
|            | CEM   | 53.9600| 15.00572|
|            | Total | 50.6200| 12.55039|
| Simvastatin| MTA   | 58.7000| 14.98095|
|            | CEM   | 62.2700| 14.80338|
|            | Total | 60.4850| 14.61046|

Table 1. Means ± SD of the bond strength for studied groups
The temporary dressing used after the insertion of cervical materials instead of Cavit because Cavit absorbs the moisture of the area, interfering with the setting reaction of the hydration-based hydrophilic biomaterials.

The numerical range obtained in the present study was similar to those reported by Topçuoğlu et al. for MTA using calcium hydroxide, TAP, and DAP. The difference between the present study and the above study was that simvastatin and CEM were used in the present study. In addition, none of the previous studies and the present study did take into account the adverse effects of blood contamination, and this should be evaluated in future studies.

Conclusion
Under the limitations of the present study and according to the results of previous studies that have highlighted the properties of CEM, it can be concluded that this material, like MTA, can be used as a cervical barrier in the regeneration process. Considering its acceptable characteristics shown in previous studies, simvastatin can also be regarded as an intracanal medicament, provided that it exhibits other required characteristics in further studies.

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Authors’ Contribution
Study concept and design by NG; study supervision by MS; critical revision of the manuscript for important intellectual content by NAA; statistical analysis by FDT; acquisition of data by ST; and drafting of the manuscript by NG and PD.

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Ethics Approval
This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (ethics code: IR.TBZMED.REC.1396.1056).

Competing Interests
There are no competing interests.

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Ghasemi et al
J Dent Res Dent Clin Dent Prospects, 2022, Volume 16, Issue 1

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