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 properties:10-12

• 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
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 and 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-liquid 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...
100% ethanol.21 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/min. 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:

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

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.20 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.13 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.22 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 al13 and Lotfi et al.14 Also, Yavari et al20 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

<table>
<thead>
<tr>
<th>Medication</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAP</td>
<td>MTA</td>
<td>56.6500</td>
<td>9.43637</td>
</tr>
<tr>
<td></td>
<td>CEM</td>
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<td>11.39131</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<tr>
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<tr>
<td></td>
<td>CEM</td>
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<td>Total</td>
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<tr>
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</tbody>
</table>
hydroxide in combination with interstitial liquid phosphate forms a hydroxyapatite layer that forms the interstitial layer in combination with dentinal hydroxyapatite. This layer is responsible for the chemical bonding with the dentin.\cite{15} It has been shown in previous studies on MTA that DAP and TAP lead to collagen regeneration, and considering the destruction of the scaffold, they probably have a negative effect on displacement resistance;\cite{16,17} however, we did not obtain such a result, and there was no difference between the intracanal medicament-treated groups and the control group in terms of the bond strength to dentin.

Simvastatin was also another compound used as an intracanal medicament in the present study. This medicament belongs to the statin family. This material has previously been used in various studies in the regeneration field. These studies have shown that it inhibits the production of inflammatory cytokines and increases the regeneration induction ability of the pulp cells and dentin formation. It also increases the activity of alkaline phosphatase.\cite{18,19,20,21} In order to be used as an intracanal medicament, a medicament must have a suitable antimicrobial property. Previous studies indicate the inhibitory effect of simvastatin on the biofilm of *Candida albicans* and *Staphylococcus aureus* and other species such as *Streptococcus sanguis*, *S. mutans*, *S. salivarius*, *S. sanguinis*, and *Porphyromonas gingivalis*.\cite{22,23}

It did not have any adverse effect on the bond strength of the biomaterials studied. It also seems that after investigating its other properties, such as lack of any adverse effects on the physical properties of dentin and proper effect on *Enterococcus faecalis*, as one of the most resistant intracanal bacteria, it can be used as an intracanal medicament in regeneration processes. The simvastatin + TAP combination has also been evaluated in some studies under the title of 3 mixtatin\cite{24,25,26} and can be evaluated as an intracanal medicament in other studies.

Attempts were made in the present study to minimize the effect of confounding factors on the push-out test. The specimens were kept under identical temperature and moisture. Materials used as a cervical barrier were placed and packed in one region by one person. Also, after cutting the discs into the same thickness of 2 mm, attempts were made to ensure that the materials were fully set in the disks. The diameter of the disks and the universal machine rod were 1.3 and 1.1 mm, respectively, and this ratio of diameters (90%) led to the least confounding effect on the reported bond strength.\cite{27} Attempts were made to ensure that the rod was perpendicular to the specimens and did not collide with the root canal walls during force application. The discs were checked for cracks after cutting. The cracked specimens and those that had not been tested were removed from the specimens and replaced by suitable specimens. Before placing the materials, the smear layer was removed to reconstruct the conditions of the clinical work. The intracanal medicaments were placed within the root canal for three weeks to comply with the guidelines recommended for clinical practice. Glass-ionomer was 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 \textit{et al}\cite{28} 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,\cite{29} 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.

**Competing Interests**

There are no competing interests.

**References**


