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Original Article

Do the intracanal medicaments affect the marginal adaptation of calcium silicate-based materials to dentin?

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Abstract *Background/purpose:* In order to prevent reinfection of the pulp canal space and dressing for regenerative purpose, the coronal seal should have a perfect marginal adaptation. Mineral trioxide aggregate (MTA) and Biodentine are among the most popular sealing materials. These are commonly used in combination with antibiotic medicaments, to ensure disinfection. Aim of the present study was to evaluate the effect of 3 different medicaments on the marginal adaptation of MTA and Biodentine to the dentin.

Materials and methods: Teeth were divided into 4 groups (n = 20) that were treated with the following medicaments; triple antibiotic paste (TAP), double antibiotic paste (DAP), a calcium hydroxide (CH) and a control group. The specimens were then assigned into two subgroups (n = 10), which received a coronal barrier of MTA or Biodentine. The specimens were scanned using an *ex vivo* micro-CT scanner. The data were statistically analysed using one-way ANOVA and the unpaired Student's t-test ($P < 0.05$).

Results: Percentage volume of external voids in the MTA group was as follows: DAP > TAP > Control > CH. In the Biodentine group, the percentage of voids was determined in the following order: TAP ≥ DAP > CH > Control. Significantly lower percentage of voids was observed in the CH-medicated specimens in the MTA group when compared to all test groups ($P = 0.04$).

Conclusion: The application of CH as an intracanal medicament reduced the void occurrence between the ProRoot MTA and root dentin. However, TAP and or DAP decreased the marginal adaptation in both ProRoot MTA and Biodentine.

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Introduction

Caries, dental trauma or developmental aberrations in immature teeth could result in pulp necrosis and arrest further development of the root.[1] In such cases, achieving an ideal conventional root canal treatment may be more challenging due to the thin, fracture-prone dentinal walls and short roots.[2] Regenerative techniques help the thickening and lengthening of root canal walls, healing of apical periodontitis and restitution a positive response to pulp vitality tests.[3] Revitalization procedures in immature teeth aim to regenerate the pulp-like tissue inside the root canal with an influx of stem cells from the apical papilla.[4] The most important step in regenerative endodontic therapy is the elimination of intracanal microorganisms and by-products. Moreover, the long-term success of regenerative endodontic therapy depends on preventing the reinfection of the root canal system.[5,6]

Although sodium hypochlorite (NaOCl) is the most popular endodontic irrigant for the disinfection of the root canal walls, previous studies have concluded that NaOCl does not adequately provide the conditions necessary for revascularization of the necrotic tooth.[4,7] For this purpose, antibiotic pastes are often used to disinfect the root canal system.[8,10] Previous studies advocated the use of the triple antibiotic paste (TAP) consisting of equal portions of ciprofloxacin, metronidazole, and minocycline developed by Hoshino et al.[8,11,12] Furthermore, it was shown that TAP has biocompatibility with good antimicrobial characteristics.[5] However, double antibiotic paste (DAP) containing metronidazole and ciprofloxacin has been used as an alternative to TAP due to the risk of visible teeth discoloration caused by the minocycline.[9,13] Additionally, calcium hydroxide (CH) is another alternative intracanal dressing used in regenerative procedures for root canal disinfection.[14,15]

The ability to prevent reinfection of the pulp canal space by the placement of the coronal seal is one of the important factors affecting the success of regenerative endodontic treatment. Ideally, the coronal seal material should have a perfect marginal adaptation to the root canal walls to prevent the leakage of microorganisms and their by-products into the pulp canal space.[3,16] Mineral trioxide aggregate (MTA) is a calcium silicate-based material, which was developed at Loma Linda University in the 1990s.[17] As a non-cytotoxic, biocompatible and bioactive material, MTA has long-term success in sealing ability. However, MTA does not show good handling properties. Besides, it has a long setting time and potential for discoloration.[16,18] Biodentine (Septodont, Saint-Maur-des-Fosses, France) is a bioactive dentin substitute which has mechanical properties similar to the sound dentin.[19,20] Furthermore, Biodentine has a positive effect on vital pulp cells and supports the formation of reparative dentin when in direct contact with vital pulp tissue.[21]

The aim of this *in vitro* study was to evaluate the effect of 3 different intracanal medicaments (TAP, DAP and CH) on the marginal adaptation of ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK) and Biodentine to the dentin surface by using micro-computed tomography (micro-CT).

Materials and methods

The study was approved by the Bezmialem Vakif University Research Ethics Committee. (No:13/25). Eighty single-rooted human teeth, recently extracted for periodontal reasons, were selected for the present study. The inclusion criteria were existence of well-developed roots, direct root canals and no root caries, root resorption, cavity, fractures and cracks. All soft tissue residue and calculus were removed and the teeth were stored in distilled water until used.

Specimen preparation

The teeth were partially decoronated 4 mm above the cement-enamel junction to standardize the root length. After the removal of the 3 mm of apical part of the roots, a standard root canal access cavity was performed. The working length was measured using a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until its tip was visible at the apical foramen. The root canal was instrumented by using ProTaper rotary nickel-titanium files (Dentsply Maillefer, Ballaigues, Switzerland) to a size F5. Then, the root canals were enlarged with #1 through #5 peeso reamers (Dentsply Maillefer, Ballaigues, Switzerland) to mimic open apex teeth. The root canals were rinsed with 2.5% NaOCl (Wizard, Rehber Kimya, Istanbul, Turkey) solution between each file. The final irrigation was performed with 20 ml 2.5% NaOCl (5 min), 20 ml 17% EDTA (Meta Biomed, Cheongwon-gun, Korea) and 5 ml distilled water. The irrigation was performed with a 27 gauge side-vented needle (Calasept, Nordiska Dental, Sweden), placing 2 mm from the apex described previously.[13] Subsequently, the canals were dried with paper points (Dentsply Maillefer, Ballaigues, Switzerland), and the apical openings were blocked using composite resin (Charisma, Heraeus Kulzer GmbH, Germany).

Intracanal medicaments

The teeth were then randomly divided into 3 experimental groups (TAP, DAP and CH) and a control group without any medicament (n = 20 for each group).

TAP group TAP paste was prepared by mixing equal portions of metronidazole (Flagyl, Eczacıbaşı, Turkey), ciprofloxacin (Cipro, Biofarma, Turkey) and minocycline

(Minocin, Teofarma, Italy) with macrogol and propylene glycol. The powder to liquid ratio of pastes was 7:1.

DAP group DAP paste was prepared by mixing equal portions of metronidazole (Flagyl, Eczacibasi, Turkey) and ciprofloxacin (Cipro, Biofarma, Turkey) powder with macrogol and propylene glycol. The powder to liquid ratio of paste was 7:1.

CH group CH paste was prepared by mixing CH powder and distilled water using a metal spatula on the glass slab at a powder liquid ratio 1:1.5.

All pastes were placed into canals by using a lentulo spiral (Mani, Japan). A small cotton pellet was then placed over the paste, and the coronal openings of the root canals were sealed with a temporary filling material (Cavit, 3M ESPE, AG, Germany). The specimens were incubated for 3 weeks at 37 °C in 100% humidity.

After a 3-week incubation period, the temporary filling material was removed under the dental operative microscobe (Carl Zeiss, Oberkochen, Germany) with X10 magnification to confirm the complete removal. The roots were flushed with 20 ml 17% EDTA (5 min) and 5 ml distilled water to remove the paste from the canal. Subsequently, the root canals were dried with paper points. The spongostans (Cutanplast, Mascia Brunelli, Italy) were cut into equal slices and embedded into fresh human blood, which was collected from a volunteer member of this research group. The spongostans were placed into the root canals and condensed up to 3 mm below the cementoenamel junction to simulate a clinical scenario.

Barrier materials

The groups were then divided into two subgroups (n = 10), according to the coroner barrier materials used. The ProRoot MTA and Biodentine were mixed according to the instructions provided by the manufacturer. Five drops of Biodentine liquid from a single-dose container was added into a powder-containing capsule and mixed for 30 seconds at 4,000—4,200 rpm. One gram of MTA was mixed with 0.34 g distilled water for 30 seconds using a metal spatula on the glass slab, in accordance with the manufacturer's instructions.

Approximately 3 mm of barrier material was placed over the blood-embedded spongostans by using a sterile amalgam carrier and gently adapted with a moistened cotton pellet to the dentinal walls. A moistened cotton pellet was placed over the barrier material. The specimens were then wrapped in the moistened sponge and incubated at 37 °C for 4 days. Finally, after the removal of the cotton pellet, the glass ionomer cement (Glass Liner, Willmann & Pein, Germany) and composite resin material were placed over the set barrier material.

Micro-CT

All teeth were scanned, and three-dimensional micro-CT images were constructed using an *ex vivo* micro-CT scanner. This procedure was done to analyse the marginal adaptation of barrier materials with respect to void occurrence along the previously medicated root canal walls.

The samples were scanned with a micro-CT scanner (Skyscan 1176, Bruker, Kontich, Belgium) with the following parameters: Cu 0.1 mm filter with a resolution of 9 μm, voltage of 90 kV, current of 270 μA, a rotational step of 0.30°, a rotational angle of 360° and an exposure time of 1150 ms. The projection data were corrected for distortion and reconstructed by adjusting the smoothing and correction of the ring artefact and beam hardening with scanner software (NRecon 1.6.10.4, Skyscan, Bruker, Kontich, Belgium).

The region of interest (ROI) was particularized as an annular area of the tooth over a length of 2 mm for measuring the volume of the gap between the dentin surface and the barrier materials. The density between 80 and 255 was determined to be the volume of the barrier material and the density between 0 and 63 was determined to be the volume of gap. Then, the percentage volume of material which is not in contact with dentin was calculated.

Statistical analysis

The marginal adaptation values were expressed as a percentage volume of external voids between the dentin surface and the MTA and Biodentine coronal plugs. The D'Agostino & Pearson omnibus normality test showed that the data were normally distributed. Thus, the comparisons between the experimental groups within the MTA and Biodentine groups were performed using the 1-way analysis of variance. The unpaired Student's t-test was used for the pairwise comparisons of percentage volume of external voids between the MTA and the Biodentine groups. The significance level was set at $P = 0.05$.

Results

1-way analysis showed that medication with DAP caused more external voids between the dentin surface and the coronal barrier materials in the MTA groups when compared to other experimental groups ($P < 0.05$). The statistical ranking of the percentage volume of external voids in the MTA group was as follows: DAP $>$ TAP $>$ Control $>$ CH. In the Biodentine group, the TAP, DAP and CH groups showed significantly lower marginal adaptation values than the control group ($P < 0.05$). The percentage volume of external voids between the dentin walls and coronal barrier materials in the Biodentine groups was determined in the following order: TAP $\geq$ DAP $>$ CH $>$ Control.

In both MTA and Biodentine groups, pairwise comparisons between the TAP, DAP and control subgroups showed similar external voids ($P = 0.10$, $P = 0.31$, and $P = 0.53$, respectively). However, the MTA group demonstrated a significantly superior marginal adaptation to the Biodentine group when premedicated with CH ($P < 0.0001$).

The representative axial cross sections obtained from each experimental group are shown in Fig. 1A—H. The minimum and maximum values (percentage of volume) of the external voids between the dentin walls and the MTA and Biodentine coronal barrier materials for each group are shown in Table 1 (see Fig. 2).

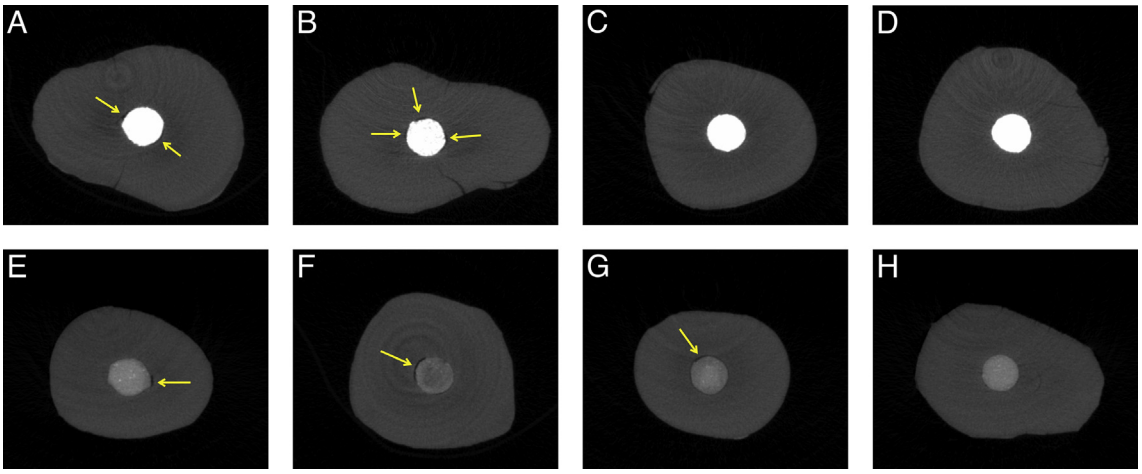

Figure 1 Representative micro-CT photos of coronal root dentin showing the external voids between the dentin walls and coronal with yellow arrows in axial view. (A) TAP-ProRoot MTA; significant differences in the gap volumes were observed between the TAP and the control group (B) DAP-ProRoot MTA; DAP decreased the marginal adaptation more than other test groups (C) CH-ProRoot MTA; CH caused less external voids when compared to the TAP and DAP groups (D) Control ProRoot MTA; (E) TAP-Biodentine; there were no significant differences in the gap volumes between the TAP and the DAP groups. (F) DAP-Biodentine; (G) CH-Biodentine; medication with CH showed more marginal adaptation in comparison with the TAP and the DAP groups (H) Control-Biodentine.

Discussion

Micro-CT analysis was performed to compare the marginal adaptation of MTA and Biodentine to the root dentin in the present study. Although the scanning electron microscopy (SEM) can evaluate the marginal adaptation, this technique has some disadvantages, such as its two-dimensional nature, the potential separation of the material from the root dentin and the possibility of crack formation in the hard tissue while sectioning the specimens before observation.[22] Conversely, micro-CT analysis provides accurate and rapid observation with high-resolution images due to its non-destructive 3D nature without the need to section the roots.[23] Hence, we preferred to determine volume of external voids between the material and dentin surface by micro-CT analysis.

Several studies have evaluated the effect of CH medication to the marginal adaptation of calcium silicate-based materials as an apical barrier material.[24,25] However, to the best of our knowledge, in the literature, there are no data about the effects of the intracanal medicaments on the marginal adaptation of calcium silicate-based materials when used as a coronal barrier material to the root dentin. Therefore, the aim of this study was to assess the effects of TAP, DAP and CH on the marginal adaptation of ProRoot MTA and Biodentine to the root canal dentin.

In the present study, TAP and DAP medicaments were associated with an increase in volume of voids for both the ProRoot MTA and Biodentine when compared with the control group. This result may be due to the effect of residual medicament despite the removal procedures. Berkhoff et al.[12] showed that CH was almost entirely removed with irrigation alone and that the penetration of the remaining medicament to the dentin was minimal. In addition, the TAP was not adequately removed from the root canal walls, and more than 80% of the TAP medicament was localised within the dentin, despite the use of copious irrigation. In another recent study by Arslan et al.[11], it has been shown that it was difficult to achieve complete removal of the TAP and DAP medicaments using irrigation activation procedures. Besides, there were no significant differences between the two antibiotic medicaments in their removal from the artificially created grooves. Therefore, based on the results of the present study, one may suggest that the marginal adaptation of the calcium silicate-based cements to dentin can be improved by using more effective removal procedures from the canals when using TAP and DAP medicaments for the root canal disinfection.

The findings of this study can only be compared with the studies in which the effects of various medicaments on the marginal adaptation of cements as a root end filling material were evaluated. Because there is no data in the literature about the effects of medicaments on the marginal adaptation of calcium silicate-based cements as a

Table 1 Mean and standard deviation values (Percentage of volume) of the external voids between the dentin walls and barrier materials.

Intracanal medicaments	ProRoot MTA	Biodentine
TAP	$4.02 \pm 1.60^{B,a}$	$5.65 \pm 2.50^{A,a}$
DAP	$6.47 \pm 2.25^{A,a}$	$5.18 \pm 3.17^{A,a}$
CH	$0.15 \pm 0.19^{D,a}$	$1.43 \pm 0.47^{B,b}$
Control	$0.69 \pm 0.73^{C,a}$	$0.86 \pm 0.45^{C,a}$

Uppercase letters show the statistical difference between the medicament groups in each barrier material test group. Lowercase letters show the statistical differences between barrier materials in each medicament group. Groups identified by different uppercase or lowercase letters are significantly different ($P < 0.05$).
TAP Triple antibiotic paste, DAP Double antibiotic paste, CH Calcium hydroxide.

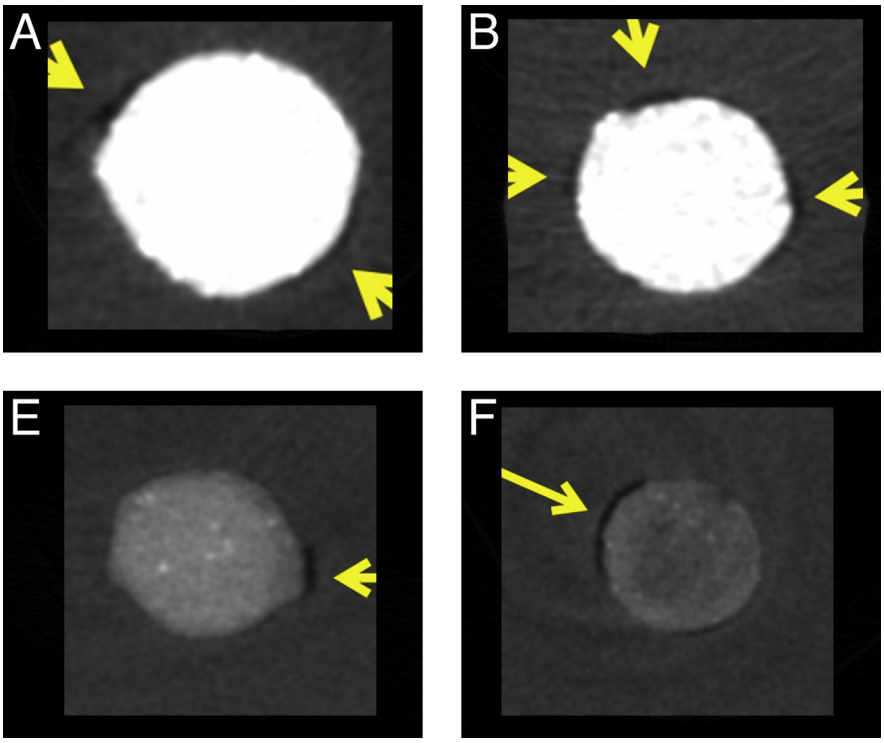

Figure 2 Details of some images (A-B-F-G) from Fig. 1 highlighting the voids between material and dentin.

coronal barrier used in regeneration procedures. In the present study, CH medication decreased the volume of voids between the ProRoot MTA and dentinal surface when compared to the control group. Bidar et al.[25] examined the effects of residual calcium hydroxide on the marginal adaptation of the MTA apical barrier by using the SEM technique. Similarly to our results, they found that the CH medication improved the marginal adaptation of the MTA apical plug. Nagas et al.[26] compared the effects of TAP, DAP and CH on the bond strength of ProRoot MTA and Biodentine, and they showed that medication with CH significantly improved the bond strength of both materials to the root dentin. Hachmeister et al.[24] also reported that the CH medication for 1 week did not influence the sealing ability of MTA for 70 days. Besides, the presence of residual CH on the root walls in an open apex despite the removal procedures did not affect the MTA properties. Furthermore, Porkaew et al.[27] concluded that the CH reacts and forms calcium carbonate, providing an initial decrease in the dye leakage in root canals obturated with gutta-percha. Hence, the conversion of CH to calcium carbonate at the coronal dentin or the reaction of MTA with residual CH could be attributed to the observed improvement of the marginal adaptation of ProRoot MTA in this study. However, future studies should be conducted to understand the mechanisms between the premedicated dentin and calcium silicate-based cements.

Banchs & Trope[28] used TAP in a regenerative endodontic procedure for the first time in 2004, and it became the most popular intracanal medicament for this purpose. However, due to its severe and unfavourable discolorations caused by minocycline in TAP, the use of DAP was recommended to overcome the esthetical problems.[13] Up to date, recommendations tend to support the use of CH, which do not have a risk of the development of bacterial resistance and sensitization. It has been shown that both TAP and DAP at the paste-like concentrations typically used in regeneration procedures are lethal in direct contact with human stem cells of the apical papilla (SCAP), while the application of CH had no detrimental effect on the SCAP.[15] We conclude that CH premedication promotes the decreased volume of external voids between the ProRoot MTA and coronal dentin surface. Conversely, one should keep in mind that CH could weaken the root canal dentin and cause an increased risk of root fractures.[1]

Results obtained within the experimental conditions of the present study indicate that all groups except the CH group revealed similar marginal adaptation. This result is in line with a previous study,[29] which compared the sealing ability and marginal adaptation of MTA and Biodentine in root-end cavities by using the SEM technique and found no significant difference between materials. According to the authors, this could be due to the similar composition and characteristics of these materials, such as dimensional stability, porosity and particle size. Alternatively, Kim et al.[30] reported multiple gaps in the Biodentine-mid root dentin interface when compared to the MTA group in SEM pictures. However, the differences in the results can be explained by the different methodologies used.

In the present study, an extreme care was taken to perform all the procedures strictly following the same protocol, just changing the materials. However, it is impossible to exclude that the operator's hand was 100% reproducible as happens in *in vivo* conditions.

A possible bias of this study is given by the fact that the radiopacity of most types of CH is very similar to that of the dentin, and this may cause a misinterpretation of the results. The CH used in this study had a radiopacity of 60 HU

(Hounsfield Units), which is similar but slightly higher then dentin (48 HU). So, they can be distinguished by a well-trained evaluator with a careful assessment.

Within the limitations of this *in vitro* study, it can be concluded that the medication with CH significantly improved the marginal adaptation of ProRoot MTA to the coronal third of root dentin walls. However, the use of TAP or DAP decreased the marginal adaptation of both ProRoot MTA and Biodentine. It should be emphasized that the present study was carried out using extracted human teeth and *in vivo* studies are recommended to obtain clinically relevant results.

Conflicts of interest

All authors disclose any potential sources of conflict of interest.

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