

## RESEARCH ARTICLE

# Impact of Intracanal Calcium Hydroxide or Triple Antibiotic Paste on Bond Strength of Root Canal Sealers: An *In Vitro* Study

Mustafa GUNDOGAR<sup>1</sup>, Erhan ERKAN<sup>1</sup>, Ozgun Yusuf OZYILMAZ<sup>2</sup>

<sup>1</sup>Faculty of Dentistry, Department of Endodontics, University of Medipol, Istanbul, Turkey

<sup>2</sup>Faculty of Dentistry, Department of Prosthodontics, University of Medipol, Istanbul, Turkey

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### ABSTRACT

**Objectives:** The aim of this study is to compare the effects of intracanal treatments with calcium hydroxide (CH) or triple antibiotic paste (TAP) on bond strength of a calcium silicate-based sealer (MTA Fillapex) and an epoxy resin-based sealer (MM Seal).

**Materials and Methods:** Sixty extracted maxillary central incisors were prepared with a rotary system to size 40. The specimens were randomly divided into two groups, which received either intracanal CH or TAP. After rinsing, the teeth in each group were further divided into two additional groups, which were obturated with gutta percha and either the MTA Fillapex or MM Seal root canal sealer. Then, slices were obtained from three sections of the canal (coronal, middle, and apical). A push-out test was performed to measure the bond strength between the root canal dentin and the sealer.

**Results:** The push-out bond strengths of the root canal sealers were significantly affected by the type of intracanal medicament. Both the sealers showed bond strengths that were nearly two-fold higher after TAP application than after CH application, and these results were independent of the position along the canal.

**Conclusions:** The use of TAP rather than CH improves the bond strength of calcium silicate- and epoxy resin-based sealers throughout the root canal.

**Keywords:** Canal medicaments, canal sealers, push out test

## INTRODUCTION

The primary aim of endodontic treatments is to completely remove bacteria and pulpal remnants from an infected root canal and to seal the root canal once it has been disinfected.<sup>1-3</sup> The combined use of instruments, various irrigation solutions, and intracanal medications has been suggested as the best method for disinfecting an infected root canal system.<sup>4</sup> The microenvironment of a root canal is ideal for establishing microbial growth. If the root canal treatment is not completed in a single appointment, intracanal medicaments are recommended for intracanal asepsis to prevent the growth of microorganisms between appointments.<sup>5</sup> Calcium hydroxide (CH) is an intracanal medicament that is widely used throughout the world and plays an important role in endodontics due to its antibacterial effects, organic tissue dissolution capabilities, and anti-inflammatory effects.<sup>6</sup> However, because infection of the root canal system is considered to be polymicrobial, it is not possible to sterilize root canals in necrotic teeth using only CH, thus antibiotic combinations have also been suggested for disinfecting root canals.<sup>7</sup>

Antibiotic pastes have been successfully used in regenerative endodontic treatments and in the healing of large periradicular lesions.<sup>3, 8-10</sup> These pastes must have a broad antibacterial spectrum and low toxicity.<sup>11,12</sup> For instance, triple antibiotic paste (TAP) is biocompatible and has antimicrobial properties.<sup>13,14</sup> It is composed of ciprofloxacin, metronidazole, and minocycline; metronidazole and ciprofloxacin have bactericidal properties, while minocycline has bacteriostatic properties.<sup>13</sup> This antibiotic regimen can successfully promote the

healing and repair of periapical tissue.<sup>15,16</sup> In addition, TAP can effectively treat dentin infected with *Escherichia coli*<sup>17</sup> and kill bacteria in immature dog teeth with apical periodontitis.<sup>18</sup> A case report showed that using TAP as an antibacterial dressing during root canal treatment was successful in healing periradicular lesions.<sup>7</sup> Bhagat and Singh initially attempted to treat a large periradicular lesion in the maxillary central incisors using CH, but the treatment protocol was changed to TAP because the patient showed no signs of pain relief. Only after applying TAP in the root canal, they noticed progressive healing of the periradicular lesion.<sup>19</sup>

Another prerequisite of intracanal medicines is that they must be easily removed from the root canal walls.<sup>20</sup> The problem with using intracanal medicines is that they cannot be completely removed from the root canal, thus affecting the adhesion of endodontic sealers to the root canal dentinal walls. Sahebi et al. showed that CH negatively affected various mechanical properties of the radicular dentin.<sup>4</sup> Furthermore, Yassen et al. showed that both CH and antibiotic pastes can cause degradation or demineralization of the radicular dentin.<sup>21</sup> On the other hand, Economides et al. reported that sealer penetration into the dentinal tubules increased the contact surface area between the sealer and the root canal walls, thus improving the quality of the apical seal and the mechanical retention of the root canal fillings.<sup>22</sup> Many studies have examined the adhesion of resin-based sealers to root dentin after using CH as an intracanal medicament.<sup>20,23-24</sup> However, there is limited data on the effects of antibiotic pastes on the adhesion of epoxy resin-

based sealers and calcium silicate-based sealers to root dentin. The calcium silicate-based sealer MTA Fillapex (Angelus, Londrina, Brazil) and the epoxy resin-based sealer MM Seal (Micro-Mega, Besançon, France) have recently been introduced as root canal sealers. Therefore, the aim of the present study was to investigate the effects of TAP and CH on the bond strength of MTA Fillapex and MM Seal.

## **MATERIALS AND METHODS**

### **Specimen selection**

This *in vitro* study was conducted with 60 single-root teeth that were previously stored in 0.1% thymol at 4°C and used within 6 months of extraction for reasons unrelated to this study. The teeth were verified radiographically as having a single root canal without calcification. The inclusion criteria were straight canals and completely formed apices. The exclusion criteria were teeth with root caries, cracks, resorption, incomplete apices, or a root length of <15 mm, and/or root canal curvature of more than 10°. This study was approved by the Research Ethics Committee of Medipol University.

### **Specimen preparation**

The specimens were stored in 1% Chloramine T solution (Ricca Chemical Company, Arlington, TX, USA) for 48 h for disinfection. Then, the external root surfaces were scaled with ultrasonic instruments and washed with distilled water to remove any calculus or soft tissue from the root surface. The crowns were sectioned transversely at the cemento-enamel junction and the root length was adjusted to 15 mm to acquire a standardized root length. After endodontic

access was established, the working length was determined directly by subtracting 1 mm from the real root length. Biomechanical preparation was performed by Protaper Next (Dentsply/ Maillefer, Ballaigues, Switzerland), with up to an X4 (size 40, .06 taper) master apical file size. During the preparation, the root canal was irrigated with 2 mL of a 2.5% NaOCl (ImidentMed, Konya, Turkey) solution after using each instrument. After instrumentation, a final flush was applied using 5 mL of 17% ethylenediaminetetraacetic acid (EDTA; MDChelCream, Meta Biomed, Chungbuk, Republic of Korea) for 1 min, followed by 5 mL of 2.5% NaOCl for 1 min.

The specimens were dried using paper points (Dentsply/Maillefer, Ballaigues, Switzerland), and randomly distributed into two groups that received an intracanal dressing with either CH or TAP (n = 30 each). In group 1, the root canals were treated with intracanal CH (Kalsin; Spot Dis Deposu AS, Izmir, Turkey), which was prepared in distilled water until it reached a creamy consistency (1:1.5, powder to liquid ratio). In group 2, the root canals were treated with TAP, which was prepared in distilled water with equal amounts of metronidazole (Eczacıbaşı, Istanbul, Turkey), ciprofloxacin (Biofarma, Istanbul, Turkey), and minocycline (Ratiopharm, Ulm, Germany), until a creamy consistency (3:1, powder to liquid ratio) was reached.

The prepared intracanal medication was introduced into the root canals using a #40 lentulo spiral. The coronal accesses to the root canals were sealed with small cotton pellets and temporary filling material (Cavit; ESPE America, Norristown, PA, USA) to avoid leakage.

All roots were stored at 37°C and 100% relative humidity for 21 days to simulate clinical conditions. After 21 days, a 27-gauge conventional irrigation needle was inserted as deep apically as possible without binding into the root canals, and irrigation was performed with 10 mL of 17% EDTA, followed by 10 mL of 2.5% NaOCl, and a final irrigation with 5 mL of distilled water. After the procedures, the root canals were dried using paper points. Then, each group was subdivided into two groups (n = 15 per group) that each received one of two different root canal sealers, which were manipulated in accordance with manufacturer instructions. The root canals were obturated with MTA Fillapex or MM Seal using the single cone technique with matching tapered X4 gutta percha cones (Dentsply/Maillefer, Ballaigues, Switzerland) to obtain standard specimens for the push-out test. After root obturation, the coronal accesses to the root canals were sealed with temporary filling material. Teeth were stored at 37°C and 100% relative humidity for 7 days to allow the sealer to set.

### **Push-out bond strength**

After the sealers had set, each specimen was cut perpendicular to the longitudinal axis of the root with a low-speed diamond saw (Isomet, Buehler, Illinois, USA) under cold water irrigation. Three slices (1.0 ± 0.1 mm thick) were obtained from the coronal, middle, and apical sections of each root at the depths of 3, 7, and 10 mm. The apical and coronal aspects of each slice were examined under a stereomicroscope (Imaging Systems, Leica Ltd., Cambridge, England) to measure the diameter of each hole.

The push-out bond strength was measured with a universal testing machine (TSTM02500, Elista Inc., Konya, Turkey) at a crosshead speed of 1 mm/min. With regard to the tapered design of the root canal, three different sizes of cylindrical pins were used for the push-out test to guarantee that the strength was applied as adequately as possible to the adhesion area during the loading process. The maximum load was applied until the filling material was dislodged, which was recorded in Newtons (N).

The bond strength was calculated in megapascals (MPa) by dividing the load ( $F$ , in N) by the adhesion area of the root filling ( $A$ , in mm<sup>2</sup>), with the equation:  $Mpa = F/A$ .<sup>28</sup> The parameter  $A$  was calculated with the equation:  $A = \pi (R + r) \times g$ , where  $R$  is the coronal radius,  $r$  is the apical radius, and  $g$  is the height relative to the tapered inverted cone (mm). The parameter  $g$  was calculated with the following equation:  $g^2 = (R - r)^2 + (2.0)^2$ .

### **Statistical analysis**

All data are expressed as the mean ± standard deviation (SD). The four groups were analysed by two-way analyses of variance followed by post-hoc Tukey's tests using the Statistical Package for the Social Sciences (SPSS), version 13.0 (SPSS for Windows, SPSS Inc., Chicago, IL, USA).

## **RESULTS**

The effects of the type of intracanal medication on the adherence of the root canal sealers are summarized in Tables 1, 2 and Figure 1. The push-out bond strength of MTA Fillapex was nearly

two-fold higher when used with TAP than used with CH as the intracanal medication at the root canal third ( $p < 0.05$ ). Likewise, the push-out bond strength of MM Seal was nearly two-fold higher when used with TAP than used with CH as the intracanal medication at the root canal third ( $p < 0.05$ ). There is a significant difference between CH and TAP when used as the intracanal medication on the push out bond strength of root canal sealers at the root canal third ( $p < 0.05$ ).

**Table 1.** Effect of the type of medication on the push-out bond strength of MTA Fillapex

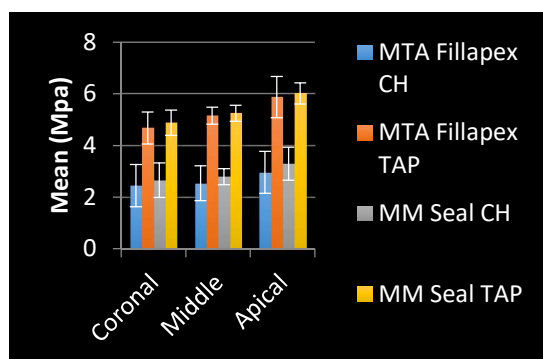
Root Canal Section	CH	TAP
Coronal	2.45 (0.82) <sup>a</sup>	4.68 (0.62) <sup>b</sup>
Middle	2.54 (0.67) <sup>a</sup>	5.16 (0.33) <sup>b</sup>
Apical	2.96 (0.81) <sup>a</sup>	5.88 (0.80) <sup>b</sup>

<sup>a,b</sup>Different letters indicate statistically significant differences ( $p < 0.05$ ).

**Table 2.** Effect of the type of medication on the push-out bond strength of MM Seal

Root Canal Section	CH	TAP
Coronal	2.66 (0.67) <sup>a</sup>	4.88 (0.49) <sup>b</sup>
Middle	2.80 (0.31) <sup>a</sup>	5.25 (0.31) <sup>b</sup>
Apical	3.30 (0.64) <sup>a</sup>	6.02 (0.41) <sup>b</sup>

<sup>a,b</sup>Different letters indicate statistically significant differences ( $p < 0.05$ ).



**Figure 1.** Mean push-out bond strength values (MPa) of sealer to root canal dentin according to the different medicaments and the root canal thirds.

## DISCUSSION

Endodontic treatments require various procedures, irrigation solutions, and intracanal medications in order to disinfect a root canal system.<sup>4, 25, 26</sup> Although CH is a widely used intracanal antibacterial medicament<sup>6</sup>, root canal infection is considered polymicrobial, thus CH is not entirely sufficient as a disinfectant. Accordingly, various broad-spectrum antibiotic pastes have been developed to provide more efficient antimicrobial treatments. However, the impact of TAP on the adhesion of sealers remains poorly documented. Therefore, the present study compared the effects of intracanal treatments with CH and TAP on the bond strengths of two newly introduced resin-based sealers, specifically the calcium silicate-based MTA Fillapex and the epoxy resin-based MM Seal. Our findings indicated that both MTA Fillapex and MM Seal showed higher bond strengths with TAP than with CH in the coronal, middle, and apical sections of the root canal.

Carvalho et al. found that treatment with CH for 14 days had a positive effect on the bond strength of AH Plus to root dentin.<sup>20</sup> Their protocol involved rinsing the CH with 1% NaOCl followed by 17% EDTA. In contrast, Guiotti et al. showed that the prior application of a CH dressing reduced the push-out bond strength of the resin-based AH Plus sealer after 21-day period.<sup>23</sup> On the other hand, Amin et al. found that treatment with intracanal CH for 7 days had no effect on the bond strength of AH Plus, even though the sealer was rinsed with 2.5% NaOCl and 17% EDTA.<sup>27</sup> These data suggest that the effect of CH on sealer adhesion may be influenced by the treatment duration, rinsing protocol, and

type of sealer. The optimal conditions for enhancing sealer adhesion with CH appear to be a 14- to 21-day treatment with a NaOCl/EDTA rinse protocol. The present study utilized these ideal conditions; thus it was expected that the CH treatment would not reduce the push-out bond strength of MTA Fillapex and MM Seal, but sealers showed lower bond strengths with CH application.

Various intracanal antibiotic treatments have been tested *in vitro* for their effects on resin-based sealer adhesion to root dentin. Üstün et al. demonstrated that the push-bond strength of AH Plus sealer (Dentsply/Maillefer, Ballaigues, Switzerland) was significantly higher in the apical section of the root canal after intracanal treatment with the natural antibiotic propolis than after treatment with CH.<sup>18</sup> Interestingly, Akcay et al. showed that a double antibiotic paste (metronidazole and ciprofloxacin) did not affect the bond strength of an epoxy resin-based sealer, whereas TAP (metronidazole, ciprofloxacin, and minocycline) improved the bond strength of the epoxy resin-based sealer in the middle and apical sections of the root canal.<sup>28</sup> This study corroborates our findings, as the bond strength in our study was two-fold higher with TAP treatment than with CH treatment for the epoxy resin-based MM Seal. In addition, we demonstrated that the beneficial effects of TAP on the bond strength also apply to another category of sealer, the calcium silicate-based MTA Fillapex. These findings demonstrate the versatility of TAP application for improving sealer adhesion.

The mechanisms behind the different responses to TAP and CH may reside in the ability to remove them after treatment

and their interactions with the dentin. Berkhoff et al. used different irrigation procedures to investigate the removal of CH and TAP, and showed that TAP appears to have high diffusion and retention within the dentin with more than 80% of the TAP being retained in the root canal regardless of the type of irrigation method used to remove it. On the other hand, most of the CH was adequately removed.<sup>26</sup> Arslan et al. also showed that it was difficult to remove TAP from the canals, even when using different irrigation solutions.<sup>29</sup> Tanase et al. reported that minocycline binds to calcium ions via chelation to form an insoluble complex in the tooth matrix.<sup>7</sup> Therefore, the high bond strength values we detected after TAP application may be related to the binding of residual minocycline to calcium ions via chelation. This could increase the bond strength when using TAP as an intracanal medicament. Moreover, in the present study, the highest bond strength values were observed in the apical sections of the root canal after TAP application. This may be related to the presence of residual minocycline, because, as mentioned above, it is difficult to remove TAP from the root canals and less of the irrigation solution reaches the apex.

However, despite its benefits, recent studies show that TAP should be used with caution. First, minocycline should be limited to the root canal because of the potential risk of tooth discoloration.<sup>30</sup> Second, TAP may be cytotoxic in excessive doses.<sup>11</sup> Therefore, additional studies should be performed to investigate the cytotoxicity of TAP.

## CONCLUSION

Based on the methods and conditions employed in the present study, these data suggest that applying TAP can produce superior retention of the root canal fillings with a variety of sealers. We conclude that TAP has the potential to improve the adhesion of sealers during endodontic treatments.

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