# The Effect of Chlorhexidine on Push-out Bond Strength of Mineral Trioxide Aggregate

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**Background:** Trioxide Aggregate (MTA) has been widely used in root canal therapy. MTA has been mixed with chlorhexidine to increase its antimicrobial effect.

**Objectives:** The aim of this study was to evaluate the effect of chlorhexidine (2%) on push-out bond strength of Mineral Trioxide Aggregate (MTA).

**Materials and Methods:** Sixty dentin disks with a thickness of 1.5 ± 0.2 mm and lumen size of 1.3 mm were prepared. Dentin disks were randomly divided into four groups (n = 15), and their lumens were filled with MTA mixed with distilled water (groups 1 and 3) or with chlorhexidine 2% (groups 2 and 4). Specimens were incubated at 37°C for 3 days (groups 1 and 2) or 21 days (groups 3 and 4). Bond strengths of the MTA-treated dentin surfaces were evaluated using a universal testing machine, and bond failure on the disks was examined by light microscope. Data was analyzed using Kruskal-Wallis H test (P = 0.976).

**Results:** There were no statistically significant differences between all the experimental groups. The mode of bond failure was predominantly mixed for distilled water groups and cohesive for CHX groups.

**Conclusions:** This study suggested that chlorhexidine had no negative effect on the bond strengths of MTA-treated dentin.

*Keywords:* Mineral Trioxide Aggregate; Chlorhexidine; Push-Out Bond Strength

## 1. Background

Since its introduction in 1993 (1), Mineral Trioxide Aggregate (MTA) has been widely used in clinical procedures such as pulp capping (2, 3), sealing of root and furcal perforations (4, 5), periradicular surgeries (6), and one-visit apexification (7). MTA apears an acceptable material for these procedures because of its sealing ability, biocompatibility, dimension stability, and radiopacity (8). Antibacterial and antifungal properties of MTA have been extensively evaluated with conflicting reports (9). MTA has been mixed with chlorhexidine to increase its antimicrobial effects. Stowe et al. showed that mixing MTA with 0.12% chlorhexidine rather than water enhanced its antimicrobial activity (10). In another study, chlorhexidine 2% (CHX) was mixed with MTA leading to a significant increase in antibacterial effects of White MTA (WMTA) and Gray MTA (GMTA) against *Enterococcus faecalis* (11). Another study confirmed that using CHX with MTA mixtures led to an antibacterial effect against *E. faecalis*; this effect was significantly higher in combination with WMTA compared to GMTA (12). Regarding biocompatibility, it has been reported that mixing MTA with CHX stimulates an apoptotic response in mouse fibroblasts and macrophages (13). Nevertheless, a study in rats showed that MTA mixed with CHX caused only a weak inflammatory response on subcutaneous connective tissue, which subsided continuously over time; therefore, the set mixture has been considered biocompatible (14). The question is whether chlorhexidine affects the physical properties of the cement. One study reported that MTA mixed with chlorhexidine gel did not set even after seven days (15). In contrast, in the study by Holt et al., MTA was mixed with 2% CHX liquid, and most samples had set adequately after 72 hours to allow performing compressive strength test. Their results revealed that MTA mixed with sterile water had compressive strength higher than that of MTA mixed with CHX (11). In an in vitro study, immersion of dentin disks filled with MTA in CHX had no significant effect on bond strength of MTA-treated dentin (16).

## 2. Objectives

With these inconsistent results in the literature, further research is necessary to evaluate the effect of CHX on the physical properties of MTA. Therefore, this in vitro study was designed to assess the effect of CHX on the push-out bond strength of MTA.

## 3. Materials and Methods

Freshly extracted, single-rooted human teeth, includ-

ing maxillary incisors and mandibular premolars were selected and stored in 0.5% chloramine-T before use. All of the teeth had mature apices and intact roots. Teeth with cracks or internal resorption were excluded from the study. The crowns of all teeth were removed, and the middle thirds of the roots were sectioned perpendicular to the long axis to obtain 60 dentin disks with a thickness of 1.5 ± 0.2 mm. The lumens of the dentin disks were enlarged with Gates Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland), sizes 2 to 5, to achieve a standardized diameter of 1.3 mm after instrumentation. To remove the smear layer, we immersed the disks in 17% ethylenediaminetetraacetic acid, and then in 2.5% sodium hypochlorite for three minutes each. The samples were then immediately washed in distilled water and dried. The dentin disks were randomly divided into four groups (n = 15), and their lumens were filled with MTA Angelus (Angelus, Londrnia, PR, Brazil) as described: groups 1 and 3, MTA mixed with distilled water; groups 2 and 4, MTA mixed with chlorhexidine 2% (Ultradent Products, Inc., Logan, UT, USA). MTA cements were mixed according to the manufacturer's recommendations. Specimens were wrapped in pieces of gauze soaked in normal saline and kept in sealed plastic containers. Specimens were incubated at 37°C for three days (groups 1 and 2) or 21 days (groups 3 and 4).

### 3.1. Push-out Bond Strength Test

After the experimental periods, push-out bond strength of each specimen was measured using a universal testing machine (Zwick/Roell, Z050; Zwick/Roell, Ulm, Germany). Dentin disks were placed on a metal slab with a central hole to allow free motion of the plunger. The MTA was loaded with a 0.7 mm diameter cylindrical stainless steel plunger at a speed of 1 mm/min (Figure 1). The maximum load applied to MTA was recorded in Newton's before dislodgement occurred. To express the bond strength in megapascals (MPa), recorded values in Newton's were divided by the adhesion surface area of MTA in mm² calculated according to the following formula:

$2\pi r \times h$, where $\pi$ is the constant 3.14, r is the root canal radius, and h is the thickness of the root slice in millimeters.

The slices were then examined by light microscope at 40 × magnification to determine the nature of the bond failure. Each sample was categorized into 1 of 3 failure modes: adhesive failure at the MTA and dentin interface, cohesive failure within MTA, and mixed failure mode. Shapiro-Wilk test was used to assess the normality of data within groups. Some data deviated from normality assumptions. Therefore, nonparametric Kruskal-Wallis H test was used to compare the four groups ($P < 0.05$).

## 4. Results

The mean push-out bond strength and standard deviations of the four experimental groups were shown in Table 1. Kruskal-Wallis test showed that there were no



**Figure 1.** Cylindrical Stainless Steel Plunger Attached to the Load cell of the Universal Testing Machine

**Table 1.** The Means and Standard Deviations of Push-out Bond Strength of Experimental Groups [a]

| Experimental Groups | Mean ± SD, MPa |
|---|---|
| MTA + NS, 3 days | 2.4534 ± 0.88923 |
| MTA + CHX, 3 days | 2.1986 ± 0.66279 |
| MTA + NS, 21 days | 2.1494 ± 0.68824 |
| MTA + CHX, 21 days | 2.2466 ± 0.71347 |

[a] Abbreviations: CHX, chlorhexidine; MTA, mineral trioxide aggregate; NS, normal saline.
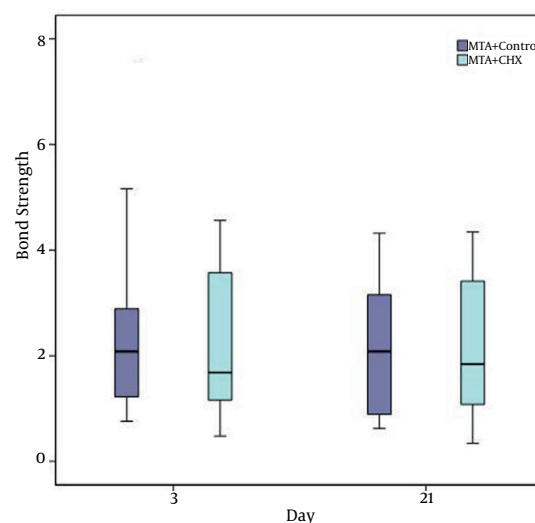


**Figure 2.** Push-out Bond Strength of the Experimental Groups

statistically significant differences between all the experimental groups (P = 0.976).

Figure 2 demonstrates the push-out bond strengths of the four experimental groups. Inspection of the samples revealed that failure mode was predominantly mixed for distilled water groups and cohesive for CHX groups.

## 5. Discussion

An ideal endodontic material should bond to dentin and remain in place under dislocating forces such as mechanical stresses caused by tooth function or operative procedures (17-19). Push-out test is efficient and reliable to evaluate the bond strength of materials to dentin (20). MTA is a bioactive material and bonds chemically via a diffusion-controlled reaction between its apatitic surface and dentin (21). Substituting CHX for water to achieve a greater antibacterial effect would be irrelevant if MTA failed to bond to dentin. In this study, after three days, samples mixed with CHX were set sufficiently to allow performing push-out test. A previous study reported that MTA mixed with 2% CHX did set after 72 hours (12). Our findings do not agree with those of Kogan, who found that MTA mixed with 2% CHX gel did not set even after 7 days (15). It seems that CHX in gel, not in liquid form, interferes with the setting of MTA. The results of the present study showed that there was no statistically significant difference between the push-out bond strengths of the MTA/distilled water mixture and the MTA/chlorhexidine mixture at both time intervals. This finding is in accordance with those of Yan et al. (16), who found that immersion in CHX, did not adversely affect MTA-dentin bond strength. They also found that CHX caused a statistically significant increase in quantity of the needle-shaped and the amount of floc was reduced. Therefore, it seems that morphological alteration of interfacial layer caused by CHX has no adverse effect on the retention of MTA to dentin. While according to results of the present study, mixing of MTA with 2% CHX had no adverse effect on the bond of MTA to the dentinal walls, Holt et al. (11) did not consider this combination suitable in clinical situations that may be subjected to compressive forces such as an artificial apical barrier, pulp capping, or perforation repairs. Discrepancy between the conclusions of these two studies can be attributed to the different nature of the push-out and compressive tests. Meanwhile, in different studies, different types of material, with different chemical formulations were used, and they should not be expected to behave identically. In the current study, MTA Angelus was used, which contains 80% Portland cement and 20% bismuth oxide; however, ProRoot MTA consists of 75% Portland cement, 20% bismuth oxide, and 5% calcium sulfate dehydrate (21). In a recent study by Kayahan et al. (22), compressive strength of MTA Angelus was significantly lower than ProRoot MTA, and differences in the composition of the two cements was suggested as the reason for the differences in their compressive strengths. According to our results, increasing the curing time from 3 to 21 days did not increase the push-out strength of MTA. Gancedo-Caravia et al. (23) similarly evaluated the effect of curing time on push-out strength of MTA. While they did not find any significant increase in the push-out strength of MTA in dry conditions, under wet condition, the push-out strength of MTA showed a statistically significant increase when curing time was increased from 3 to 21 days. One reason for such discrepancies could be due to different experimental set-ups, particularly different humid environments in which the samples were cured. Another explanation is that Gancedo-Caravia et al. used gray ProRoot MTA, but we used tooth-colored MTA Angelus (23). In the present study, bond failure in groups that MTA was mixed with distilled water was predominantly of the mixed type, although some samples exhibited cohesive and adhesive failure patterns. This result is in accordance with those of Rahimi et al. (24) who reported that MTA-dentin bond failure was of the mixed type. In contrast, when MTA was mixed with CHX, most samples showed failure of the cohesive bond. This can be attributed to the fact that CHX can be adsorbed onto hydroxyapatite and teeth (24) and improve the bond strength of MTA to dentinal walls. In the study by Stowe et al. (10), the operator observed that the MTA/CHX mixture seemed to set more rapidly (1-2 minutes) than the MTA/water mixture (5-6 minutes) and took on a more crumbly texture at placement. This finding is not compatible with the observation of the operator in the present study. Therefore, further studies are needed to assess physical properties, e.g. setting and working times, of MTA/CHX mixtures. In conclusion, the push-out bond strengths of MTA/water and MTA/CHX were similar. Increasing the curing time from 3 to 21 days did not increase the push-out strength of MTA mixed with water or CHX.

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## Author's Contributions

Study concept and design: Alireza Adl, Nooshin Sadat Shojaee, Fereshte Sobhnamayan and Neda Sadat Shojaee; acquisition of data: Alireza Adl, Nooshin Sadat Shojaee, Fereshte Sobhnamayan and Neda Sadat Shojaee; drafting of the manuscript: Nooshin Sadat Shojaee; critical revision of the manuscript for important intellectual content: Alireza Adl and Nooshin Sadat Shojaee; administrative, technical, and material support: Alireza Adl, Nooshin Sadat Shojaee, Fereshte Sobhnamayan and Neda Sadat Shojaee; study supervision: Nooshin Sadat Shojaee,

Alireza Adl and Feresete Sobhnamayan.

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