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## **ORIGINAL ARTICLE**

# **Acidic Environment Effect on the Push-out Bond Strength of Mineral Trioxide Aggregate Mixed with Different Liquids**

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## **ABSTRACT**

Mineral trioxide aggregate (MTA) as retrograde filling is always in contact to inflamed tissues in periradicular area.

**Objective:** To investigate the effect of acidic environment on push-out bond strength of MTA mixed with sterile water, local anesthetic, and 5% CaCl<sub>2</sub>. **Methods:** Thirty middle third of mandibular premolar roots were randomly assigned into 3 groups of 10 each. MTA mixed with sterile water (Group 1), local anesthetic (group 2), 5% CaCl<sub>2</sub> (group 3). Each group was then divided into group A: soaked in synthetic tissue fluid with pH 5, and group B: pH 7.4. Specimens were stored in an incubator with a temperature of 37°C for 72 hours, undertaken a push-out test, and observed under a stereo-microscope. **Results:** A two-way ANOVA showed that acidic environment reduced the push-out bond strength of MTA mixed with either sterile water, local anesthetic or 5% CaCl<sub>2</sub> (p <0.05). The predominantly failure was a mixture of adhesive and cohesive type. **Conclusion:** The acidic environment reduced the push-out bond strength of MTA mixed with either sterile water, local anesthetic or 5% CaCl<sub>2</sub>. MTA mixed with 5% CaCl<sub>2</sub> produced the greatest push-out bond strength, whereas MTA mixed with local anesthetic had the lowest push-out bond strength.

**Key words:** acidic environment, mineral trioxide aggregate, push-out bond strength.

## **INTRODUCTION**

Apical surgery aims to remove irritant from the root canal and to eliminate periradicular inflammation.<sup>1</sup> Apical surgery is usually followed by retrograde filling to close the entrance of irritant to the apical of root canal. Selection of appropriate retrograde filling material is crucial to the success of apical surgery.<sup>2</sup> The material used to seal the external surface of roots should both be able to prevent bacterial leakage between the pulp and the periradicular tissues, and remain in place under dislodging forces. An ideal retrograde filling material should be biocompatible, dimensionally stable, adhere to the root-end cavity walls, resist dislocating forces, prevent the passage of the bacteria, and unaffected by the presence of tissue fluid that may be acidic in an infected area.<sup>3,4</sup>

Mineral trioxide aggregate (MTA) was first introduced in 1993 is a potential alternative to previous materials for retrograde filling such as amalgam, Super EBA, IRM, composite resin, and glass ionomer cement.<sup>5</sup> The original formulation of MTA is gray (Gray ProRoot MTA, Dentsply, Tulsa Dental, Tulsa, OK). The newer tooth-colored material, commonly referred to as white MTA (White ProRoot MTA, Dentsply, Tulsa Dental, Tulsa, OK), was developed for its application in esthetically sensitive areas.<sup>6,7</sup> Mineral trioxide aggregate, which is a mixture of tricalcium aluminate, dicalcium silicate, tricalcium silicate, tetracalcium aluminoferrite, and bismuth oxide, has been widely used for retrograde filling during apical surgery due to several advantages namely good sealing ability and biocompatibility.<sup>3,4</sup> Although MTA has many favorable properties that support its clinical use, there are several

drawbacks. The setting time of MTA has been reported to be about 2 h and 45 min<sup>8</sup>, which may mean less shrinkage and better marginal adaptation.<sup>9</sup> However, when MTA is used as a retrograde filling material, it may wash out of the preparation if special care is not taken due to long setting time.<sup>7</sup> Another less than ideal property of MTA is its handling characteristic. The manufacturer recommends mixing MTA with sterile water, which sold in pack with MTA powder. This produces a grainy, sandy mixture which is typically difficult to deliver to the required site and hard to compact adequately.<sup>10,11</sup>

Several studies have been demonstrated that MTA mixed with other liquids instead of sterile water in order to change some properties of MTA and accelerate the setting time of MTA, such as 2% chlorhexidine gluconate, glycerin, saline and NaOCl gel.<sup>7,10,12</sup> In clinical use, ampoules of sterile water are often exhausted before the powder, hence it has been suggested that local anesthetic be used as a sterile, convenient, and readily available substitute.<sup>7</sup> Recently, 5% CaCl<sub>2</sub> has been used as a mixing liquid of MTA. Previous studies reported that 5% CaCl<sub>2</sub> can accelerate the setting time of MTA is between 25 to 35 min.<sup>12,13,14</sup> Rapid setting time is likely to affect the physical properties of the MTA. However, it is not known whether accelerating its setting time would change the physical properties of MTA. Therefore, it is crucial to select a liquid, which to be mixed with MTA that produces a fast setting time but not influence on the physical properties of MTA in order to achieve the success of treatment in clinical condition.

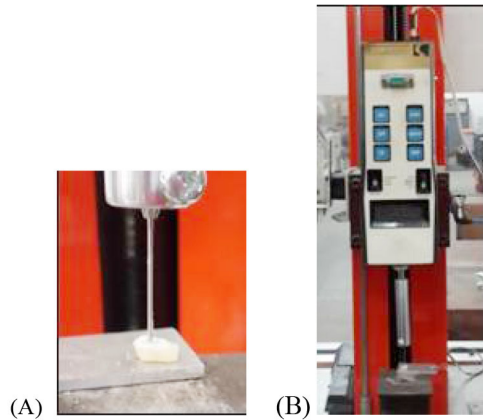
During clinical applications, MTA as retrograde filling material may be exposed to an inflammation environment in which the pH value has been measured as low as 5.<sup>15</sup> A low pH could potentially inhibit setting reactions, affect adhesion, or increase the solubility of MTA. Additionally, retrograde filling material should withstand with the forces that lead to discharge of material from the root canal dentin when the operative procedures are carried out.<sup>16,17</sup> Impeded MTA setting as well as reduced strength and hardness has been reported in an acidic environment.<sup>18</sup> The bond strength of a material with dentin is an extremely important factor for the success of the bond strength of restorative material to dentin. To assess bond strength, the push-out test has been shown to be efficient and reliable, since investigation of the bond strength between MTA and dentin wall will reveal the value of adhesion between them.<sup>19,20</sup> However, the effect of an acidic environment on the push-out bond strength of dentin to MTA mixed with different mixing liquids has not been studied. The purpose of this study was to investigate the effect of the acidic environment on push-out bond strength of MTA mixed with different liquids, namely sterile water, a local anesthetic and 5% CaCl<sub>2</sub>.

## METHODS

A total of thirty single-rooted, extracted human mandibular premolars were used in this study. The teeth were cleaned of any tissue remnants and stored in 0.1% Thymol in distilled water until the study was conducted. The crowns were removed and the middle third of roots were sectioned transversally by using a water-cooled diamond saw microtome (SP 1600 microtome, Leica, Nubloh, Germany) to obtain 3 mm thick root sections. The lumens of root slices were drilled with # 2 to # 5 Gates Glidden burs (Dentsply, Maillefer, Ballaigues, Switzerland) to achieve 1.3 mm diameter standardized cavities. The roots were randomly assigned into 3 groups of 10 each. Group 1: MTA (White ProRoot MTA, Dentsply Tulsa Dental, Tulsa, OK, USA) was mixed with sterile water, group 2: MTA was mixed with local anesthetic (2% Lidocain HCl, Pehacain, Phaphros, Semarang, Indonesia), group 3: MTA was mixed with 5% CaCl<sub>2</sub> (Faculty of Pharmacy, UGM, Yogyakarta, Indonesia). As manufacturer's recommendation for MTA, a 3:1 powder to liquid ratio was used for all specimens to achieve putty consistency. MTA was placed inside the lumens of the root slices using micro apical placement (MAP, Dentsply, Mailefer, Ballaigues, Switzerland). Saline-moistened Gelatamp (Roeko-Coltene/Whaledent, Langenau, Germany) was used as a matrix to prevent extrusion of the mixed MTA below the inferior surface of the specimens.

Each group was further divided randomly into 2 subgroups of 5 each, namely group A: specimens were soaked in synthetic tissue fluid with adjusted to pH 5 to mimic inflamed tissue, and group B: specimens were soaked in synthetic tissue fluid with pH 7.4 to simulate neutral pH of tissue fluid. The fluid was prepared as follows<sup>17</sup>: 0.400 g NaCl, 0.690 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, 0.400 g KCl, 0.005 g Na<sub>2</sub>S·9H<sub>2</sub>O, 0.795 g CaCl<sub>2</sub>·H<sub>2</sub>O, 0.300 g KSCN and 1.000 urea dissolved in 1.000 mL distilled H<sub>2</sub>O. This solution was adjusted to pH 7.4 and pH 5 using lactic acid (Faculty of Mathematics & Natural Science, UGM). Specimens were kept in an incubator for 72 hours at 37°C.

The push-out test was carried with a 1 mm diameter stainless plugger (Dentsply, Mailefer, Ballaigues, Switzerland) (Figure 1A) that was attached to universal testing machine (Pearson Parke Equipment Ltd., London, UK) (Figure 1B). The specimens were placed on a metal slab with a central hole to allow the free motion of the plugger. The compressive load was applied by exerting a downward pressure on the surface of MTA by using a 1 mm diameter cylindrical stainless steel plugger at a crosshead speed of 1 mm/min. The plugger had a clearance of approximately 0.2 mm from the margin of the dentinal wall to ensure contact with MTA only. The maximum load applied to MTA at the time of dislodgement was recorded in newtons.



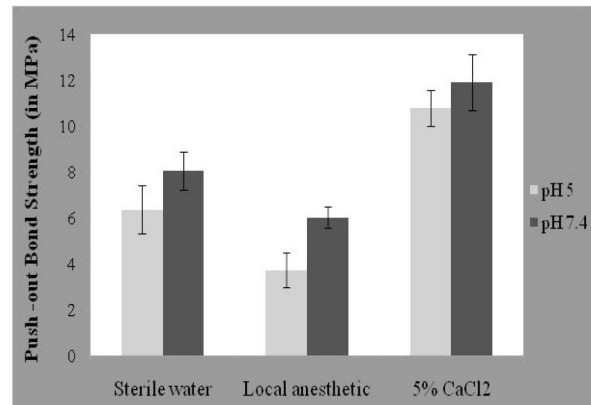
**Figure 1.** A cylindrical stainless steel plugger (A) attached to the load cell of the universal testing machine (B) loading on MTA inside a root section

In order to express the bond strength in megapascals (MPa), the recorded value was divided by the adhesion surface area of root canal filling calculated by the following formula:<sup>11</sup>  $2 \pi r \times h$ , where  $\pi$  is the constant 3.14,  $r$  is the root canal radius,  $h$  is the thickness of the root dentin slice in millimeters. After push-out test had been done, each slice was then examined under a stereomicroscope (SMZ 800, Nikon, Tokyo, Jepang) at x 40 magnification to determine the nature of the bond failure. Each specimen was categorized into 1 of the 3 failure modes: adhesive failure at the MTA and dentin interface, cohesive failure within MTA, or mixed failure.<sup>21</sup> Data obtained were analysis using a two-way ANOVA followed by Tukey post hoc test with a 5% level of significant.

## RESULTS

The results of the study are summarized in Figure 2, which showed that the push-out bond strength of groups which were immersed in pH 5 was lower than in the groups of pH 7.4. The results also revealed that the greatest push-out bond strength occurred in the group of MTA mixed with 5% CaCl<sub>2</sub> in pH 7.4 (11.89 ± 1.24), followed by MTA mixed with 5% CaCl<sub>2</sub> in pH 5 (10.77 ± 0.77), MTA mixed with sterile water in pH 7.4 (8.05 ± 0.82), MTA mixed with sterile water in pH 5 (6.35 ± 1.04), MTA mixed with local anesthetic in pH 7.4 (6.01 ± 0.47), while the lowest occurred in the group of MTA mixed with local anesthetic in pH 5 (3.72 ± 0.76 ) (Figure 2).

Statically analysis using a two-way ANOVA demonstrated that MTA which mixed with sterile water, local anesthetic and 5% CaCl<sub>2</sub> and immersed in pH 5 had significantly lower push-out bond strength than those immersed in pH 7.4 ( $p < 0.05$ ). Tukey post hoc test revealed that the significant difference among mean push-out bond strength values occurred in all



**Figure 2.** The mean and standard deviation of push-out bond strength of MTA mixed with sterile water, local anesthetic, and 5% CaCl<sub>2</sub>

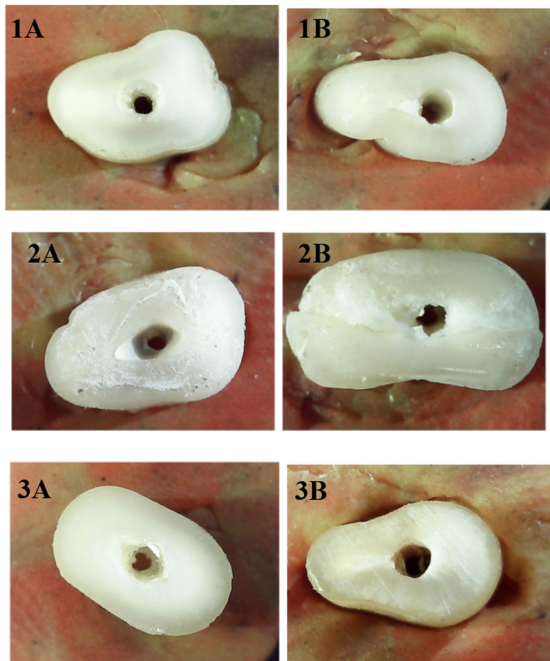
groups ( $p < 0.05$ ). Inspection of the specimens revealed the bond failure was the mixed between adhesive and cohesive type in the majority of the specimens (Figure 3).

## DISCUSSION

Since it has been introduced to the market, MTA has been widely used in Dentistry, one of which is as retrograde filling material that is applied to the root apex to produce a good seal of apex and surrounding tissues during apical surgery.<sup>2</sup> There are several methods to evaluate the adhesion strength, but the push-out bond strength test reveals the most reliable technique for the evaluation of resistance to dislodgement of materials since this method is capable to evaluate the magnitude of adhesion between the MTA and the dentin walls of root canals.<sup>18</sup>

To simulate inflamed tissues as in clinical conditions, in this present study, the MTA was immersed in a synthetic tissues fluid with a pH of 5 for 72 hours. A pH 5 was selected since pulpal and periapical inflammation typically lowers the tissue pH near the involved tooth to approximately 5, while a pH 7.4 was chosen to simulate the neutral environment as comparison to acidic environment.<sup>9,15</sup> The period of 72 hours was selected, since in the clinical condition, the initiating and perpetuating factors of inflammatory process are removed by appropriate treatment. In the other words, after apical surgery, the acidic environment due to inflammation would be less neutral within 3 days (72 hours). This is because the inflammation will be subsided within 3 days.<sup>16,21</sup>

Mineral trioxide aggregate is a type of mineral cement which solidifies as a hard structure upon hydration, a process that occurs with the dissolution of the anhydrous phases of MTA followed by the



**Figure 3.** Mixed failure modes, note the MTA residual inside the all canals. (Group 1A: MTA mixed with sterile water in pH 5; 1B: MTA mixed with sterile water in pH 7.4; 2A: MTA mixed with local anesthetic in pH 5; 2B: MTA mixed with local anesthetic in pH 7.4; 3A: MTA mixed with 5% CaCl<sub>2</sub> in pH 5; 3B: MTA mixed with 5% CaCl<sub>2</sub> in pH 7.4)

crystallization of hydrates in an interlocking mass.<sup>3</sup> This crystallization consists of the formation of cubic and needle-like crystals. The cubic crystal formed the principal structure of MTA, while the needle like crystals formed between the cubic crystals and were less prominent. The needle-like crystals are important in interlocking the entire mass of material, and their disappearance caused the material hardness to decrease and lead to dissolve easily.<sup>4,19</sup> The acidic environment with pH 5 might be able to affect the setting of MTA, by interfering the crystallization of the cubic and needle-like crystals. More specifically, pH 5 environments does indeed affect MTA hydration by disrupting the acid crystallization processes that influence the hydration of MTA, causing only a cubic crystals were found in the MTA hydrated in acidic pH, whereas no needle-like crystals occurred.<sup>17</sup> The absence of needle-like crystals may be because the large surface areas of such crystals provide numerous reaction sites for fast dissolution in an acidic environment.<sup>21</sup> In addition, study it was reported that in the pH 5, erosion of the cubic crystal surfaces was noted.<sup>7</sup>

This study supports the previous study showed that an acidic environment affects the setting of MTA by interfering the crystallization of the cubic and needle-like crystals.<sup>11</sup> In the other words, acidic environment *i.e.*, pH 5 is detrimental to physical properties and hydration behavior of MTA. The absence of needle-

like crystals caused the increased speed of the crystal MTA soluble in an acidic environment, and may result in reducing push-out bond strength.<sup>11,19</sup> Furthermore, the acidic environment might affect the formation of hydroxyapatite crystals and hybrid layer at the interface between the MTA and dentin, resulting in a gap formation. Gap formation would produce lower the push-out bond strength of MTA.<sup>21</sup> Previous study reported that to treat the inflammation with an alkaline medication, such as Ca(OH)<sub>2</sub>, may neutralize the environmental pH before applying MTA on an inflamed area.<sup>9</sup> However, this method needs to further investigation.

The hydration of MTA undergoes four stages namely the pre-induction, the induction (dormant), acceleration, and post-acceleration phase.<sup>22</sup> Pre-induction occurred in the first few minutes after mixing the powder of MTA and the liquid. At this stage, a rapid dissolution of ionic materials occurs. Induction phase (dormant) occurs in the first few hours after mixing the powder of MTA with the liquid. At this stage the hydration of all the clinker minerals progress very slowly. The silicate hydrate coating on the unreacted cement grains retards further hydration and leads to the dormant period, *i.e.*, a period of 1-2 h relative inactivity where the cement is plastic and can be manipulated. The next stage is acceleration (3- 12 h after mixing). This stage reveals the progress of hydration accelerates again and is controlled by the nucleation and growth of resultant of hydration products. The last stage is the post-acceleration phase, which is the last stage of the hydration mechanism MTA. At this stage the hydration rate slows down gradually as the amount of non-reacted material declines and the rate of hydration process becomes diffusion controlled. Thus, the mixing liquids of MTA powder, which generated a faster setting time, may merely affect the pre-induction and induction phases. On the contrary, mixing liquids, which caused a longer setting time, may affect the four stages of hydration mechanism of MTA, hence, the longer setting times the greater effect of acidic environment on MTA. Local anesthetic as mixing liquid of MTA has a long setting time (approximately 3 hours), hence the possibility of the four stages of the hydration of MTA will be affected, which in turns, could considerably influence the push-out bond strength of MTA.<sup>7,12,13</sup> Additionally, a study revealed that mixing MTA with an acidic solution like 2% lidocaine HCl, which used as local anesthetic in this study, reduced the physical and mechanical properties of MTA in an acidic environment.<sup>9</sup> This phenomenon may explain why the MTA mixed with local anesthetic had the lowest bond strength than other mixing liquids in this study.

The results of this study also revealed that MTA mixed with sterile water had the higher push-out bond strength than mixed with local anesthetic. White MTA, which used in the present study, mixed with the sterile water has setting time of 50 min, which was significantly

shorter than 2 h and 45 min.<sup>8,14</sup> This difference can be attributed to change that may have been incorporated into the MTA powder since it was introduced. It was found that the main ingredients of MTA were calcium and phosphorus, while the white MTA introduced recently containing phosphorus concentrations are very minimal, hence accelerate the setting time of MTA.<sup>8,23</sup> Acceleration of setting time cause the MTA to withstand to acidic environment and only two stages of MTA hydration affected, as a result might produce greater push-out bond strength.

The push-out bond strength of MTA mixed with 5% CaCl<sub>2</sub> was the greatest compared to other mixing liquids. This is because the setting time of MTA, which mixed with 5% CaCl<sub>2</sub> was faster (between 25 to 35 min) compared to other mixing liquids, hence early setting of MTA enhances its anti-washout properties.<sup>7,10,13</sup> In addition, early solidification increases the push-out bond strength of MTA. This phenomenon occurred since 5% CaCl<sub>2</sub> is able to accelerate the setting reaction of MTA by increasing the rate of hydration. This phenomenon occurred since CaCl<sub>2</sub> is partially consumed during hydration, thereby reacting with tricalcium aluminate and forming chloroaluminate.<sup>12</sup> Previous study confirmed that MTA mixed with CaCl<sub>2</sub> is non-toxic to human cells *in vitro* as well.<sup>13</sup> Mineral trioxide aggregate is a bioactive material that can form a layer of hydroxyapatite or carbonate apatite on its surface when it comes in contact with phosphate-containing fluid. Formation of this interfacial layer develops a chemical bond between MTA and dentinal walls.<sup>19</sup> Stereo microscope observation of the specimens confirmed that almost entire material was displaced from the dentin surface (adhesive failure), although in few areas of all specimens showing a cement-cement failure (cohesive) (figure 3). Therefore, the predominantly failure in this study was a mixture of adhesive and cohesive type. This condition might be attributed to the duration of storage time before testing the push-out bond strength, which was 72 hours.<sup>20</sup> This result is in accordance with previous report showing that reported that after 72 hours the failure mostly is mix between adhesive and cohesive.<sup>19</sup> If the push-out test was performed at an earlier stage (24 hours) after mixing the liquid and powder of MTA.<sup>19</sup> It seems mostly adhesive failure between the walls of the root canal dentin with MTA would occur.<sup>21</sup> In the early stages, the bond between MTA and the dentin is mechanical. With the passage of time, the bond between them is chemical since the retention characteristics increase from 24 to 72 hours. This means that the failure modes of chemical bond is cohesive, in contrast the failure of mechanical bond is adhesive.<sup>20,21</sup> Additionally, in the presence of tissue fluid, hydration of MTA powder results in the development of hydroxyapatite crystals and formation of a hybrid layer between dentin and MTA. The ensuing hydroxyapatite crystals cover MTA, fill the microscopic gap between MTA and dentin, and create a chemical bond.<sup>13,21</sup>

## CONCLUSION

Based on the results of this study, it can be concluded that the acidic environment reduced the push-out bond strength of MTA mixed with either sterile water, local anesthetic or 5% CaCl<sub>2</sub>. The MTA mixed with 5% CaCl<sub>2</sub> produced the greatest push-out bond strength, whereas MTA mixed with local anesthetic had the lowest push-out bond strength.

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