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Acid Challenge on Push-Out Bond Strength of Three Different Tricalcium Silicate Cements: An *In-vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MBB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors TBVGR and NMV managed the analyses of the study. Authors GDD, SP and TS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the impact of environmental pH on intra-radicular dentin push-out bond strengths of MTA, MTA HP, and Biodentine.

Materials and Methodology: Freshly extracted human mandibular single-rooted premolars or maxillary anterior incisors that were either intact or contained only small carious lesions were selected.120 mid root dentins is horizontally divided into 1.0 mm thick slices and divided into 3 MTA, MTA HP, BIODENTINE groups. The compressive load is applied at a speed of 0.5 mm/min by exerting a downward pressure on the outer surface of MTA using a 1.00 mm diameter cylindrical stainless-steel plunger. Maximum load to MTA was reported in newtons at the time of dislodgement and converted to megapascals. The 1-way analysis of variance test was used to compare the push-out bond strength of the groups with the same storage time (4 or 34 days), followed by the pair-wise comparison of the Tukey post hoc test. The Student's t-test was used to evaluate 3-group

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means. At P = .005, the degree of significance was set. Thus the clinical importance of the present study states that considering several factors like microhardness, composition by products, particle sizes and the environmental pH plays a critical role in selection of root end filling material.
Results: There was significant difference between groups (P = .001) after 4 days of PBS and Acid condition, where Bio dentine had significantly the highest bond strength.
Conclusion: The strength of MTA HP, BIODENTINE, MTA materials at dentine interface increases over 30 days in the storage of PBS solution at pH 7.4, after an initial acid challenge by acetic acid of pH 5.4, which decreases initial bond strength.

Keywords: MTA angelus; biodentine; MTA- HP; acid challenge; endodontics.

1. INTRODUCTION

In the presence of tissue fluids that may be acidic in a contaminated environment, the ideal root-end filling material must be biocompatible, dimensionally stable, adhere to dentinal walls with excellent marginal adaptation to prevent the passage of bacteria, and be unaffected.

Most of these critical properties are of mineral trioxide aggregate (MTA) and are highly common as a root-end filling material. A new material based on tricalcium silicate, known as Biodentine, has recently been introduced, with applications close to those of MTA.

There are some disadvantages to the conventional MTA formulations, such as a long setting time, tooth and marginal gingiva discoloration, and difficult handling [1,2]. In processes such as filling root-end cavities and furcation or root perforation, the problematic handling of MTA frequently mentioned by clinicians seems to be aggravated [3,4,5]. MTA HP (Angelus, Londrina, Brazil), a more recent modified tri-calcium silicate based material, was developed based on the biological and physical properties of calcium-silicate cements, claiming improved performance compared to conventional MTA. MTA HP powder consists mainly of tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, calcium carbonate (filler material) and tungstate calcium (radiopacifier), while the liquid supplied for mixing with the cement powder is water and a plasticizing agent. Compared to White MTA [6], this new material has high-plasticity and improved physical properties, according to the manufacturer. A good seal is formed, and the bond with radicular dentin is directly dependent on the type of material used, one of the essential requirements of both root-end cavities and root perforation materials.

After an acid challenge of the bond strength of Bio dentine and MTA HP and evaluating the effect of environmental pH on the push-out bond strength of MTA, MTA HP and bio dentine to intra-radicular dentin, there is a lack of literature on the reversal.

1.1 Aim

To evaluate the impact of environmental pH on intra- radicular dentin push-out bond strengths of MTA, MTA HP, and Biodentine.

- Effect of acidic and alkaline pH for 4 days on experimental materials
- Effect of alkaline environment for 34 days
- Effect of acidic pH for 4 days followed by 30 days of an alkaline environment.

2. MATERIALS AND METHODOLOGY

Freshly extracted human mandibular single-rooted premolars or maxillary anterior incisors that were intact were selected.120 mid root dentins were sectioned horizontally into slices with a thickness of 1.0 mm. (Fig. 1).

The lumen of the root dentin disks was instrumented with Gates Glidden drills (Dentsply Maillefer, Switzerland), sizes 2 to 5, to achieve a standard diameter of 1.3 mm. All the materials were mixed according to the manufacturer's instructions. A collagen sponge was used as a matrix to prevent extrusion of the material below the specimens' inferior surface. The mixed material was then introduced incrementally without any pressure into the lumens of the root-dentin slices.

- All the experimental samples were then randomly divided into three groups (n = 40).
- Group I – MTA HP
- Group II – MTA
- Group III– BIODENTINE

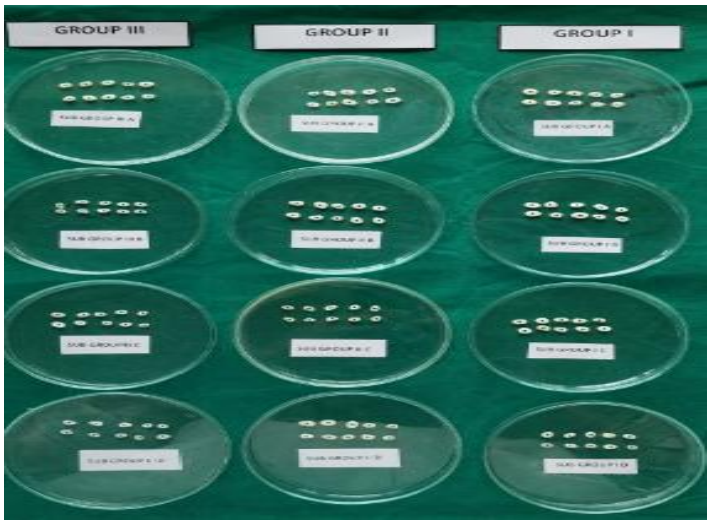

Fig. 1. Treatment group

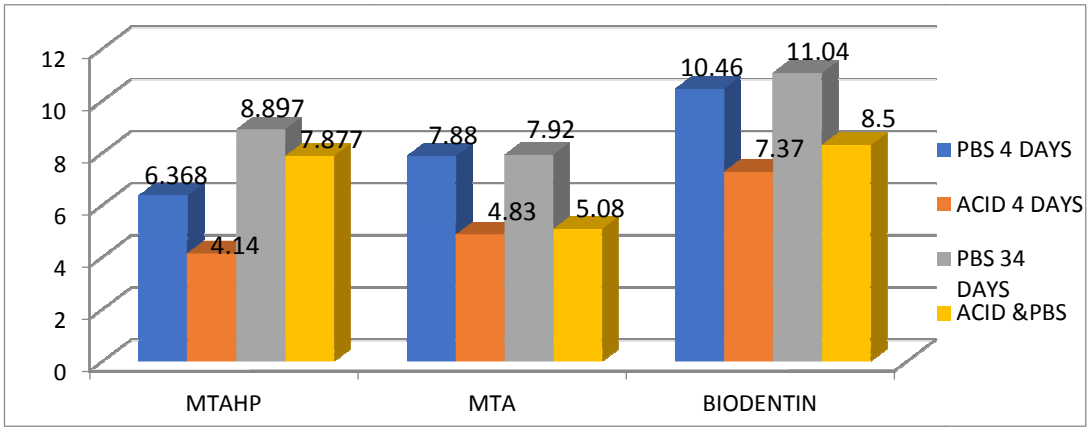

Fig. 2. Variation in different concentration of MTAHP, MTA and biodentin

In the respective groups, the specimens were wrapped in pieces of gauze soaked in acetic acid buffered at pH 5.4 and PBS buffered at pH 7.4 and then incubated at 37 ° c for 4 days and 30 days, respectively. The strength of the push-out bond was measured using a universal measuring machine (INSTRON UK). The samples were placed on an acrylic slab with a central hole, to allow the free motion of the plunger,. Using a 1.00-mm diameter cylindrical stainless steel plunger at a speed of 0.5mm/min, compressive load was applied by exerting a downward pressure on the surface of the MTA. The plunger was positioned to ensure contact with MTA with a clearance of approximately 0.2 mm from the dentinal wall margin. The maximum load applied to MTA was reported in Newtons at the time of dislodgement and converted to megapascals.

2.1 Statistical Analysis

The 1-way variance test study was used to compare the push-out bond strength of the groups with the same storage time (4 or 34 days), followed by the pair-wise comparison of the Tukey post hoc test. The Student's t-test was used to evaluate three-group means. At P = .005, the degree of significance was set.

3. RESULTS

- There was a statistically significant difference between all groups (P= .001) after 4 days of PBS and Acid exposure, where Bio dentine had significantly the highest bond strength.

- After 34 days, PBS 4 days Acid plus 30 days PBS there was also a statistically significant difference among groups (P = .021); the Bio dentine group had significantly the highest bond strength and followed by MTA HP and MTA had the lowest (Table 1).
- A statistically significant difference was found between all groups (p<0.001) in bond strength of 10.46 Mpa for bio dentine after 4 days in PBS.
- There are statistically significant differences among groups (p<0.001) in bond strength for bio dentine 7.375 Mpa after 4 days of Acid challenge.

4. DISCUSSION

Sorenson (1909) first introduced the pH concept and defined it as the hydrogen ion concentration in a solution [7]. Thus, a decrease in one pH unit is equivalent to a 10-fold increase in the hydrogen ion concentration [8].

The pH of the local anesthetic solution and the tissues it is injected affect its nerve blocking action [9]. Acidification of the tissues resulting from inflammatory products is believed to decrease local anesthetics' effectiveness [10].

The increased concentration of hydrogen ions results in a more significant proportion of the anesthetic agent in its cationic form than its basic form. This equilibrium change implies that the anesthetic might be less capable of diffusing through contaminated tissues than through normal tissues, resulting in a delayed onset and decreased anesthesia intensity [11,9].

Furthermore, a low pH environment may affect dental materials [12,13]. For example, Silva et al. [14] reported in a laboratory study that an acidic environment altered the surface characteristics and microhardness of glass ionomer cements.

According to the results of the present study, at 34 days Biodentine showed greatest resultsfollowed by MTA HP and MTA.

Torabinejad et al. (1995) proposed that the setting process of Mineral Trioxide Aggregate [15] is impeded by acidic pH. Therefore, changes in host tissue pH due to pre-existing disease at the time of MTA placement may affect its physical and chemical properties [16,17].

Various investigations have suggested that the pH of infected tissue and pus is likely to be lower than that of healthy tissue [9,10]. Acidic pH may also affect the properties of dental materials, which are routinely placed in environments that may be inflamed or infected. A laboratory study [17] demonstrated that adverse effects on the surface microhardness of Mineral Trioxide Aggregate occurred at pH 4.4.The mean Vickers surface microhardness value at this pH was 14.34 compared to 53.19 when the material was exposed to pH 7.4.At pH 6.4, the mean microhardness value of Vickers was 40.73.

There are different techniques for measuring the adhesion of a dental material to dentin, including tensile, shear and pushout strength tests. In the presence of tissue fluid, hydration of the MTA powder results in the formation of hydroxyapatite crystals and the development of a hybrid layer between dentin and MTA [18].

This reaction is mimicked by combining MTA powder with disodium hydrogen phosphate, a phosphate-containing solution [19]. The structure and morphology of the hydroxyapatite crystals are related to various factors including the environmental Ph [20]. The hydration reaction of MTA occurs best at neutral pH [18]. The MTA's pH value was stated by Torabinejad et al. [15] as between 10.5 and 12.9. MTA can release calcium hydroxide [21] upon hydration. Calcium hydroxide formation and calcium phosphate precipitation may clarify MTA's ability to retain the pH of the surrounding environment at high levels [21,22].

But these materials may come in contact with tissues of different pH, based on the surrounding tissue's condition. According to MALAMED SF, mostly the pH of inflamed tissue was about 5. The clinical situations where we can encounter

Table 1. Treatment subgroups

Subgroups (n=10)	Storage medium	Time
IA, IIA, IIIA	Acidic pH of 5.4 in Acetic acid	4 days
IB, IIB, IIIB	Alkaline pH of 7.4 in PBS	4 days
IC, IIC, IIIC	Alkaline pH of 7.4 in PBS	34 days
ID, IID, IIID	Acidic pH of 5.4 and alkaline pH of 7.4 in PBS	4 days and 30 days, respectively

acidic pH include non-vital teeth, immature teeth with periapical lesions, lateral perforations or furcal perforations, radiolucent lesions, retrograde filling [17,23].

In order to determine the degradation of root-end filling materials due to acidic conditions, Roy et al. [24] set experimental factors at pH 5.0 and 7.4. Only cubic crystals hydrated in acidic Ph were found in the MTA. The absence of needle-like crystals in an acidic environment is due to the wide surface areas of such crystals providing multiple reaction sites for rapid dissolution [25].

An analysis of the tricalcium silicate complex, the main component of MTA, showed that the complex's silicate could be dissolved in 20% salicylic acid or 20% maleic acid [20]. Besides, Nilforoushan and Sharp have stated that environmental lithium salts, including sodium chloride, havea particularly notable effect on the chemical reactions of the cement complex calcium [26].

An MTA microstructure analysis showed that both the surfaces of the crystal were dissolved, suggesting structural weakening. In interlocking the entire mass of material, the needle-like crystals are central, and their absence has caused the material hardness to decrease. In cases of incomplete reaction, the strength, stiffness, and other associated physical properties of the MTA have also been adversely affected. The use of MTA as a filling material in a highly inflamed environment appears to be harmful to the microstructure of the material and other physical properties [3,27].

MTA Repair HP, NeoMTA Plus, and Bio dentine, the new pulp capping materials, showed a fair degree of cytocompatibility with hDPSCs and reasonable cell migration rates, while bio dentine showed higher time-dependent proliferation rates. Based on the biological and physical properties of calcium-silicate cements, MTA HP (Angelus, Brazil), a more recent cement material based on silicate, was developed, claiming improved performance compared to traditional MTA. Tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, calcium carbonate (filler material) and calcium tungstate (radio pacifier) are comprised of MTA HP powder, whereas the liquid needed for strength is supplied. MTA HP has stronger push-out bond strength than White MTA, its predecessor; however, BD had greater resistance to dislodgment than both MTA formulations.

The final result of hydration of Biodentine setting reaction includes: unreacted particles of cement (slowing down the effect of further reactions), CSH gel, Ca $(OH)_2$.

$$2(3CaO.SiO_2) + 6H_2O \rightarrow 3CaO.2SiO_2.2H_2O + 3Ca(OH)_2$$

C3S CSH

Han and Okiji et al. [28] showed that calcium and silicon ion uptake into dentin, leading to tag-like structures of Biodentine, was greater than MTA and may play a role in overall root dentin adaptation. In addition, the various particle sizes of MTA and Biodentine can influence their penetration into the dentinal tubules, with implications for displacement resistance. Atmeh et al.[29] proved that Ca-Si cements facilitate the permeation of Ca and OH ions into the dentine due to their caustic effect. It can be concluded that Biodentine's improved ability to release remineralizing Ca and OH ions is responsible for improved apatite formation at interface [30,31] and micromechanical anchorage [32]. Biomineralization at MTA- Dentine interface occurs through apatite formation by the cement-PBS system promoting controlled nucleation and triggering the appearance of interfacial tag-like structures at the cement-dentin interface [18,33] and could be responsible for chemical bonding [34]. Biomineralization at Bio dentin- Dentine interface is much more with greater uptake of calcium and silicate ions into dentine – BIODENTINE interface. Repair materials may come in contact with different environmental pH based on clinical situations, i.e., pH 5.This low pH may affect the properties of the repair materials.

The pushout bond strength of MTA, BIODENTINE, and MTA HP to dentine interface showed reduced bond strength after storage in acetic acid at pH 5 for four days compared to the same materials stored in PBS at pH 7.4 for four days in an incubator at 37^0C.These results could be by the alterations in the materials' physical and chemical properties in such a low pH environment. Moreover, the formation of hydroxyapatite crystals and forming a hybrid layer at the MTA-dentin interfacial gap is likely to be disturbed in an acidic environment. (Lee et al. JOE, 2004).

Scanning electron microscopy evidence also suggests developing a porous surface and lack of needle-like crystals in a more acidic solution.

In addition, Saghiri et al. stated that in samples stored at lower pH values, the time required for leakage to occur was significantly shorter. In these studies, specimens were exposed to butyric acid with pH values of 4.4, 5.4, 6.4, and 7.4.

MTA's slow setting with ettringite and calcium silicate hydrate formation over some time in simulated tissue fluid greatly enhances the material's strength according to Hann et al. The lack of tricalcium aluminate and the presence of calcium chlorite serve as an accelerator, and calcium carbonate leads to the early formation of calcium silicate hydrate crystals, which contributes to the rapid setting of Biodentine.

Biodentine reached 67.5 ± 4.1 HV at pH 7.4. And the surface microhardness was 46.3 ± 5.0 HV, also at a pH as low as 4.4. Based on calcium chloride (CaCl2) present in the liquid supplied by the manufacturer, higher values of the microhardness of Biodentine than MTA can be clarified. The addition of CaCl2 is intended to reduce the setting time of Portland cement in civil construction and to boost its physicochemical properties [35,36]. A potential reason for improving physical properties behind CaCl2 is that CaCl2 penetrates cement pores, significantly accelerates silicate hydration, leading to faster crystallization and reducing setting time [37,38]. It can also alter the chemical composition, surface area and characteristics of cement pores, offering the benefits of improved compression resistance and decreased permeability.

Bruno Martini GUIMARAES et al. concluded that MTA Repair HP and MTA had prolonged alkanizing activity and calcium release favoring calcium phosphate nucleation and that MTA HP has less strength at pH 5.4 for 4 days could be due to more solubility and increased porosity in an acidic environment and increased bond strength over 30 days in PBS. The inclusion of the plasticizer in MTA HP may increase its solubility and porosity. The radiopacifier calcium tungstate can be used for the replacement of bismuth oxide.The average particle size of MTA was 15.8 micrometers; for MTA HP, about 12.48 micrometers, for BIODENTINE was approximately 10.24 micrometers.

The average particle size of BIODENTINE is around 10.24 micrometers, which helps decrease surface roughness and decreases porosity with more calcium hydroxide release initially in BIODENTINE, compared to MTA with a mean particle size of 15.8 micrometers and MTA HP with 12.48 micrometers, which may be the reasons for increased porosity and decreased bond strength of the related materials. Compared with White MTA ($p<0.05$), better push-out bond strength values were shown by MTA HP. In the MTA HP for calcium tungstate, the substitution of bismuth oxide as a radiopacifier agent could explain the better effects of this cement than White MTA. Calcium tungstate contributes to higher release of calcium, enabling greater biomineralization [39]. In addition, the high-plasticity of MTA HP will positively influence the marginal adaptation of the cement to the root walls, correlated with higher bond strength.

The moistening of calcium silicate cements during setting is particularly important as these cements have higher compressive strength when held in a moist environment [40]. In addition, retention characteristics and push-out strength of calcium silicate cements are improved over time if maintained under wet conditions [33].

Calcium-silicate-based materials are known to interact with dentin in order to promote intratubular Ca and Si incorporation [28], as well as dentin remineralization [41], intrafibrillary apatite deposition [42,43], and the development of tag-like structures [28,33] in the presence of PBS. The nucleation of apatite at the interface increases the sealing potential by reducing the interface voids [31] and improving the dislocation resistance [32]. Through the use of PBS, a condition closer to a clinical condition is promoted.

Thus the clinical significance of the present study states that considering several factors like microhardness, composition, byproducts, particle sizes and the environmental pH plays a critical role in selection of root end filling material.

5. CONCLUSION

The strength of MTA HP, BIODENTINE, MTA materials at dentine interface increases over 30 days in the storage of PBS solution at pH 7.4, after an initial acid challenge by acetic acid of pH 5.4, which decreases initial bond strength.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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