OPEN ACCESS Saudi Journal of Oral and Dental Research Abbreviated Key Title: Saudi J Oral Dent Res ISSN 2518-1300 (Print) |ISSN 2518-1297 (Online) Scholars Middle East Publishers, Dubai, United Arab Emirates

Scholars Middle East Publishers, Dubai, United Arab Emirates Journal homepage: <u>http://scholarsmepub.com/sjodr/</u>

Original Research Article

Comparative Evaluation of Effect of Different PH and Mixing Agents on Compressive Strength of Mineral Trioxide Aggregate – An In Vitro Study

Dr. Tarun Kumar Singh, MDS¹, Dr. Manish Kumar, MDS^{2*}

¹Ex Assistant Professor, Department of Dentistry, Army College of Medical Sciences, New Delhi, India ²Assistant Professor, Department of Dentistry, Government Medical College & Hospital, Ratlam, Madhya Pradesh, India

DOI:10.21276/sjodr.2019.4.7.10

| Received: 03.05.2019 | Accepted: 06.06.2019 | Published: 30.07.2019

*Corresponding author: Dr. Manish Kumar

Abstract

Background and Objectives: MTA is the material of choice for Pulp capping, Apexogenesis, Apexification, Perforation repair and as well as for Root end filling. In this in vitro study, different mixing reagents were tested and their effect on the compressive strength, at different pH, was evaluated when MTA was mixed, individually with each of them. Materials and Method: A total 40 samples were prepared, 10 samples each for four mixing reagents. MTA (white) was mixed with four mixing reagents and condensed into split thickness molds after which it was allowed to set for 72 hours. After removing the samples from mold, samples were kept in freshly prepared Phosphate buffer saline solution at different pH (5.0 and 7.0) for 7 days after which they were removed from PBS and dried overnight before testing with Universal Testing Machine for compressive strength. Results were statistically analysed. Results: There was no significant difference found in compressive strength when comparison was done between sterile water and 1% Sodium hypochlorite at pH 7.0 and at pH 5.0. Within group comparison revealed, minimum changes in compressive strength with change in pH from 5 to 7 in Group I (MTA + Sterile water) while maximum was obtained in Group II (MTA + Metrogyl). In Group III (MTA + Sodium hypochlorite), a decrease in compressive strength was seen as pH decreased; however, the difference between pH 5 and 7 was not statistically different. Conclusion: All the groups tested showed a good compressive strength except MTA + Chlorhexidine group which did not set even after 7 days and amongst the group, MTA + Metrogyl exhibited better compressive strength. No significant difference was found at pH 5.0 and 7.0 among the group.

Keywords: Mineral Trioxide Aggregate, Chlorhexidine, Sterile water, Metrogyl, Sodium hypochlorite, Phosphate Buffer Saline.

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INTRODUCTION

The greatest threats to developing teeth are dental caries and traumatic injuries. The primary goal of all restorative treatment is to maintain pulp vitality so that normal root development can occur. Apexogenesis is done in immature teeth when part of the pulp tissue remain vital and uninflamed, as in carious exposures or in some trauma cases in which pulp exposure occurred and treatment was delayed and it become necessary to extend further into canal to reach healthier tissue [1].

A major complication of endodontic and restorative treatments is accidental perforation of roots or floor of the pulp chamber. Such perforations can occur during root canal treatment or during preparation for a variety of restorative procedures. The result is a chronic inflammatory reaction of periodontium (characterized by formation of granulation tissue) that can lead to irreversible loss of attachment or loss of tooth. Such perforations are managed surgically or nonsurgically, depending on particular characteristic of the case [2]. Various materials have been used in managing perforations, including Zinc oxide eugenol, amalgam, Calcium hydroxide, Composite resin, GIC, and Resin modified GIC [3].

Recently a new material, Mineral Trioxide Aggregate, has been developed which is useful in managing perforations. The other indications of MTA include, Apexification, Pulp capping, Root resorption, and as well as for Root end filling [2].

Proroot MTA (Dentsply Tulsa Denta, OK) and MTA-Angelus (Angelus) are the commercial version of MTA introduced in 1998 that consist of 75% Portland cement, 20% Bismuth oxide, and 5% Gypsum by weight. It is available in two different formulations, Tooth coloured MTA and Gray MTA. MTA is composed of Dicalcium silicate, Tricalcium silicate, Tricalcium aluminate, Tricalcium aluminoferrite, Calcium sulphate, and Bismuth oxide. Hydration of powder results in a colloidal gel composed of calcium oxide crystals in an amorphous structure: 33% Calcium, 49% Phosphate, 6% Silica, 3% Chloride, and 2% Carbon [1]. The 1000% increase in FeO concentration in Gray MTA is thought to be responsible for variation in colour between gray and tooth coloured MTA [4]. Several investigators have altered the mixing regimen of MTA and experimented with other liquids such as sterile saline, Local anesthetics, 0.12% Chlorhexidine liquid and 2% Chlorhexidine gel. The purpose of the present investigation was to compare the Compressive strength of MTA mixed with sterile water, Metrogyl, Sodium hypochlorite, and 2% Chlorhexidine.

MATERIAL AND METHOD

The present study was carried out in Department of Pedodontics and Preventive Dentistry, Kothiwal Dental College and Research Centre, Moradabad along with the collaboration of I.T.S Engineering Institute, Greater Noida.

A total of 40 samples were prepared, 10 samples each for four mixing reagents: sterile water, metrogy 1,1% sodium hypochlorite,2% chlorohexidine. After mixing, mixed MTA was condensed with moderate force by using sterile plugger into custom made 4x6mm split thickness moulds. Split thickness

mold was made up of brass as it is an inert material and did not react with any of the constituents of MTA. Over the condensed MTA, moist cotton was placed and it was allowed to set for 72 hours at room temperature in 100% humidity. After removing the samples from the mould, samples were kept in freshly prepared phosphate buffer saline solution at different ph(5.0 and 7.0). 10 samples which were mixed with 2% chlorhexidine were excluded from further procedure because it was evident that MTA did not set even after 72 hours.

Half of the samples of each group were kept at ph 7.0 and half were kept at solution of ph 5 in a container for 7 days. After that, this container was kept in incubator for 7 days.

After 7 days, samples were taken out from the buffer solution and edges were polished by sand paper to achieve a flat surface. The samples were placed lengthwise between the platens of universal testing machine. The samples were compressed at rate of 1mm/min and the compressive strength was recorded in megapascals. Obtained data was statistically analyzed by student t-test and ANOVA.

RESULTS

The mean compressive strength at ph 7.0 was maximum in Group II (MTA+Metrogyl) (52.946±8.447) followed by Group I (MTA + Sterile water) (34.652±11.984) while it was minimum in Group III (MTA+NaOCI) (23.420±7.024 MPa) (Table-1).

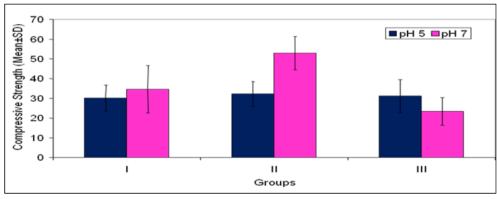
S.NO.	MTA + STERILE WATER	MTA + METROGYL	MTA + 1% SODIUM HYPO
1.	40.25	54.57	12.73
2.	26.89	42.20	27.05
3.	51.71	65.61	31.50
4.	33.57	51.75	21.48
5.	20.84	50.60	24.34
Mean	34.652	52.946	23.420
SD	11.984	8.447	7.024
Min	20.84	42.20	12.73
Max	51.71	65.61	31.50

 Table-1: Mean compressive strength at pH 7.0.

Mean compressive strength at ph 5 was maximum in Group III (MTA + NaOCl) (34.204±6.37) followed by Group II (MTA+Metrogyl) (33.296±6.24) while it was minimum in Group I (MTA+Sterile water) (30.144±6.55 MPa) (Table 2).

Table-2: Mean compressive strength at pH 5				
S.NO.	MTA + STERILE WATER	MTA + METROGYL	MTA + 1% SODIUM HYPO	
1.	20.87	28.39	25.47	
2.	30.25	32.29	45.56	
3.	38.29	30.17	25.89	
4.	27.56	31.48	31.45	
5.	33.75	44.15	27.65	
Mean	30.144	33.296	34.204	
SD	6.559	6.244	6.37	
Min	20.87	28.39	25.47	
Max	38.29	44.15	45.56	

Within group comparison revealed, minimum change in compressive strength with change in pH from 5 to 7 in Group I while maximum change was observed in Group II. In Group III, a decrease in compressive strength was seen as the pH increased; however, the difference between two pH was not significant statistically. Changes are significant only for group II (Graph-1).



Graph-1: Within group comparison

DISCUSSION

Retrograde root canal therapy is the preferred approach in teeth with persistent periapical infections or when conventional therapy fails or it is not feasible [3]. Root canal perforation is the second most common cause of endodontic failure, accounting for 9.6% of all unsuccessful cases [5]. A number of materials have been advocated for use as root end filling materials and for perforation repair and these include Amalgam, Composite resins, Ethoxybenzoic acid cement, Cavit, Glass ionomer cement, Gutta percha, Zinc oxide eugenol cements, and Polycarboxylate cements [3]. Properties of good root end filling material include the ability to adhere and seal the root canal system. The material also should be easy to manipulate, radiopaque, dimensionally stable and biocompatible with the periradicular tissue [4].

Mineral Trioxide Aggregate (MTA) is a cement type material that was introduced into the dental field in mid 1990's and has gained much popularity ever since [6]. Its use was pioneered by Dr.Torabinejad of the University of Loma Linda in California in 1993 and its clinical use was approved by U.S Food and Drug Administration in 1998. MTA was originally advocated to be used for perforation repair and as root end filling material [7]. Additional applications have subsequently proposed including direct pulp capping, been apexification [8], external root resorption repair [6] and obturation of retained primary teeth [6]. Torabinejad M et al., found that MTA leaked significantly less than amalgam and super EBA [9]. When studied as root end filling material, MTA has shown to be better than amalgam. ProRoot MTA (Dentsply Tulsa Denta, Tulsa OK) is the commercial version of MTA introduced in 1998 that consist of 75% Portland cement, 20% bismuth oxide, and 5% gypsum by weight. It is available as White and Gray MTA. Composition of both the MTA is same but the difference is presence of 1000% iron oxide

in gray MTA which imparts gray color to cement. ProRoot MTA is packaged in single use containers and each box contains 1gm pouch of powder and 0.35gm ampule of sterile water [4].

Several investigators have altered the mixing regimen of MTA and experimented with other liquids such as sterilewater [4] saline, local anesthetic, 0.12% chlorhexidine liquid and 2% chlorhexidine gel [4]. There are only two studies in which MTA is mixed with 2% chlorhexidine liquid and these are conducted to evaluate bond strength and compressive strength.

In the present study, four different liquids (Sterile water, Metrogyl, 1% Sodium hypochlorite, and 2% Chlorhexidine) were chosen to mix MTA. We chose sterile water because ProRoot MTA is packaged in single use containers. Each contains 1gm pouches of powder and 0.35gm ampule of sterile water [4], hence used by most of the clinicians. Metrogyl was chosen because it is a highly active amoebicide and has broad spectrum cidal activity against protozoa and anaerobic bacteria [10]. Sodium hypochloride is widely used as an irrigant during endodontic procedure because of its wide spectrum antimicrobial properties [10]. Sodium hypochloride can be used in various concentrations; but we selected it in 1% concentration which is similar to Zender M, who reported that 1% sodium hypochlorite solution should suffice to dissolve the entire pulp tissue in the course of an endodontic treatment session [10]. 1% sodium hypochlorite was prepared freshly by mixing 3 pats of distilled water in 1 part of 4% sodium hypochlorite.

All these four liquids have the potential to improve the working properties of MTA. The reagents may improve the handling characteristics of the mixture and also provide antimicrobial action [6].

MTA was mixed with each of the four mixing agents by sterile spatula on a sterile glass slab in a ratio of 3:1, according to manufacturer instruction which is similar to the study by Holt DM et al., [4] After mixing, mixed MTA was carried by amalgam carrier and condensor with moderate force into custom made 4x6 mm split thickness mold, by using sterile plugger. Moderate force was applied to reduce internal porosities. Custom made split thickness mold was made by brass because brass does not react with any constituent of MTA. These split thickness molds had an inner diameter of 4mm and a thickness of 6mm in accordance to study by Holt DM et al., [4] after pouring the material into mold, moist cotton was wrapped onto the samples and allowed to set for 72 hours at room temperature. After 72 hours samples were removed from the mold and visually assessed for lack of voids and chips. The edges of samples were polished with fine sand paper to achieve a flat surface.

pH factor

Before testing the samples, they were kept in freshly prepared phosphate buffer saline solutions A&B at different pH i.e 5.0 and 7.0 for 7 days. Solution A was prepared by mixing 3.12gm sodium dihydrogen orthophosphate (mW=156) in 100 cm³ of distilled water while solution B was prepared by mixing 2.83gm sodium dihydrogen orthophosphate (mW=142) in 100cm³ of distilled water.¹¹For making PBS at pH 5.0, 46ml of solution A and 4ml of solution B were mixed and for pH 7.0, 9.5ml of solution A and 40.5ml of solution B were mixed [11]. pH was measured by pH indicator. Half of the samples were kept in incubator at 37 C for 7 days because this temperature simulates the oral cavity. After 7 days the sample were taken out of the incubator. Before testing, samples were dried overnight at room temperature.

MTA is a type of mineral cement which solidifies as a hard structure upon hydration, a process that occurs with the dissolution of anhydrous phases of MTA followed by the crystallization of hydrates in an interlocking mass. ¹²This crystallization consists of the formation of cubic and needle like crystals and can be explained in terms of crystal kinetics. Before hydration, MTA is a powder with particles, ranging in size from 1-10 micrometer. Morphological observation revealed that the hydrated MTA stored in distilled water possessed a microstructure resembling an epitaxially growth pattern which consist of cubic and needle like crystals. At pH 5, microstructure of MTA consisted of relatively undeveloped cubic crystals. The crystal boundaries were less distinct and the grains less defined. Scanning Electron Microscope results of hydrated MTA stored in pH 5 revealed that no needlelike crystals were found. At pH 7, it exhibited epitaxial growth patterns. The cubic crystals contributed to framework of resulting structure, and the needle like crystals filled in intergrain space. The size of the

crystallized formation appeared layer, and the overall structure seemed less intricate [13].

Fridland et al., determined that MTA was able to maintain a high pH in the range of 11-12 for 78 days [14]. Torabinejad and Chivin suggested that MTA might remain soft when placed in perforation with a high degree of inflammation, and the inflamed area surrounding the involved tooth may have an acidic pH as low as 5.5-5.6 [13]. Roy et al., set experimental factors at pH 5 and 7.4 to evaluate how an acidic environment affects the leakage of root end filling materials. In their study, only cubic crystals were found in the MTA hydrated in acidic pH. The absence of needle like crystals may be because the larger surface areas of such crystals provide numerous reaction sites for fast dissolution in an acidic environment. At pH 5, environment inhibited the reaction to hydration of MTA. Before applying MTA on an inflamed area, treating the inflammation with an alkaline medication such as calcium hydroxide may be advisable to neutralize the environmental pH and allow the material to perform optimally [15].

Lee *et al.*, reported that hardness of MTA and its hydration behavior had been adversely affected with exposure to the pH range of inflammatory environment (pH=5) as compared to physiologic condition (pH=7) [13].

MTA with 2% CHLORHEXIDINE

Chlorhexidine was developed in the late 1940's in the research laboratories of Imperial Chemical Industries Limited (Macclesfield, England). Initially, a series of polybisguanides were synthesized to obtain anti viral substances. However, they had little antiviral efficacy and were put aside, only to be rediscovered some years later as anti-bacterial agents. Chlorhexidine was the most potent of the tested bisguanides. Chlorhexidine is a strong base and is most stable in the form of its salts. The original salts were chlorhexidine acetate and hydrochloride, both of which are relatively poorly soluble in water. Hence, they have been replaced by chlorhexidine digluconate. Aqueous solutions of 0.1 to 0.2% are recommended for chemical plaque control in the oral cavity, while 2% is the concentration of root canal irrigating solution usually found in the endodontic literature [10].

In our study, 2% Chlorhexidine was tried as solution for mixing MTA and evaluation of compressive strength was done.

Holt DM *et al.*, indicated that MTA with 2% chlorhexidine does set but is extremely brittle and exhibits extremely low compressive strength when compared to MTA/water. Brittleness was so much that most of the samples were fractures on removal. Compressive strength of MTA/chlorhexidine was so low that clinician might not be confident in its usage.

Authors do feel confident that mixing MTA with 2% chlorhexidine would not be suitable in clinical situation that might be subjected to additional compressive forces such as an artificial apical barrier, pulp capping, and perforation repair [4].

In our study, the results have shown that the compressive strength of MTA when mixed with 2% chlorhexidine was not obtainable as the samples did not set even after 7 days. Kogan P *et al.*, also found that MTA/chlorhexidine specimens had areas that did not completely set even after 7 days and hence a compressive strength measurement was not obtainable for this material.

MTA with STERILE WATER

When MTA powder is mixed with sterile water, a colloidal gel is obtained which hardens in approximately in 3 hours in clinical conditions [1]. Although slow setting may mean better adaptation and less shrinkage, special care should be taken to protect the integrity of material as it may be washed out with oral fluid before setting is accomplished [6].

In our study, MTA was mixed with sterile water and it showed a mean compressive strength of 34.65+_ 11.98 MPa at 7.0 pH and a significant decrease at 5 pH i.e, 30.14+_ 6.55 MPa after 7 days. Kogan P et al showed compressive strength for MTA mixed with sterile water after 7 days was 28.4 MPa [6]. Torabinejad et al., showed that compressive strength of MTA after 24 hours was 40 MPa and it was increased to 67.3 MPa after 21 days [16]. Islam I et al., reported mean compressive strength of MTA was 40+ 4.4 MPa at 24 hours and increased to 67.3+ 6.6 MPa after 21 days [3]. These discrepancies can be attributed either to differences in protocols that were followed in testing for the compressive strengths and/or changes in composition of MTA since it was introduced. Torabinejad found that the main ingredients of MTA were calcium and phosphorous [16]. After evaluating the chemical composition of MTA, Asgray et al., concluded that a significant change in composition had occurred since MTA was first introduced. The observed concentration for phosphorous in MTA was very minimal in their study [17].

MTA with SODIUM HYPOCHLORITE

Potassium hypochlorite was the first chemically produced aqueous chlorine solution, invented in France by Berthollet (1748-1822). Starting in the late 18th century, this solution was industrially produced by Percy in Javel near Paris, hence the name "Eau de javel". First hypochlorite solutions were used as bleaching agent. In 1777-1850, sodium hypochlorite was recommended by Labarraque to prevent childhood fever and other infectious disease. Based on the controlled laboratory studies by Koch and Pasteur, hypochlorite then gained wide acceptance as a distribution by the end of the 19th century. ¹⁰The strength of sodium hypochlorite is low. It was related to high pH value and lack of ability to dissolve inorganic material of sodium hypochlorite [6].

In our study, sodium hypochlorite was used in its lowest concentration i.e, 1% rather than 5.25%. Slim TP *et al.*, suggested that 5.25% solution significantly decreases the elastic modulus and flexural strength of human dentin compared to physiologic saline. This is more likely because of the proteolytic action of concentrated hypochlorite on collagen matrix of dentin. ¹⁸ Ari et al indicated that 5.25% sodium hypochlorite solution significantly decreased the microhardness of root canal dentin [18]. Sirtes G *et al.*, observed that 1% Sodium hypochlorite solution should suffice to dissolve the entire pulp tissue in course of an endodontic treatment [10].

In our study, the results have shown that compressive strength of MTA when mixed with 1% sodium hypochlorite decreased at pH 7.0 but it was evident that when pH decreased from 7.0 to 5, the compressive strength significantly increased. Zehnder M concluded that the way to increase the efficacy of hypochlorite solution could thus be to lower their pH i.e, the efficacy of hypochlorite is inversely proportional to pH [10]. Kogan P et al., found that sodium hypochlorite improved setting time, but had reduced compressive strength. Depending upon application of MTA, this decreased may affect its usefulness. Based on their results, MTA with sodium hypochlorite can be recommended for single visit procedure where compressive strength is not a major concern [6].

MTA with METROGYL

Metrogyl is the prototype nitroimidazole introduced in 1959 for trichomoniasis and later found to be highly active amoebicide. It has broad spectrum cidal activity against protozoa and anaerobic bacteria. ¹⁹Known for its strong antibacterial activity against anaerobic cocci as well as Gram negative and Gram positive bacilli, it has been used both systemically and topically in the treatment of periodontal disease [20]. Windley et al., reported that Metronidazole readily permeates bacterial cell membranes and it then binds to DNA, disrupting its helical structure, which leads to rapid cell death [20]. Wang et al., evaluated the effect of a metronidazole-chlorhexidine solution on the treatment of chronic apical periodontitis and reported that 97.6% of the cases healed [20]. Krithikadatta et al., evaluated the disinfection of dentinal tubules using 2% chlorhexidine gel, 2% metronidazole gel, bioactive glass (S53P4) and calcium hydroxide. Thev demonstrated that the overall percentage inhibition of bacterial growth (at depths of 200 micron and 400 micron into the dentin) was 100% with the chlorhexidine gel whereas metronidazole gel (86.5%), bioactive glass (62.8%) and calcium hydroxide (58.5%) were less effective [20].

We had tried metrogyl to mix with MTA for evaluating its compressive strength at different pH. MTA/metrogyl samples showed a mean compressive strength of 52.94+_8.44 MPa at pH 7.0 and 33.29+_6.24 at pH 5, after 7 days. <u>PH</u> affected the compressive strength of MTA/metrogyl. As pH decreased from 7 to 5, the compressive strength of MTA/metrogyl was also decreased. There are no reports present related to mixing of MTA with metrogyl.

CONCLUSION

Within the limitation of this study and the results obtained, it can be concluded that Mineral Trioxide Aggregate had the higher compressive strength when mixed with Metrogyl at pH 7.0 while 1% Sodium hypochlorite had higher compressive strength at pH 5.0 among all mixing reagents.

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