Assessment of calcium and hydroxyl ions release from mineral trioxide aggregate mixed with four different vehicles (In Vitro Study)

Aseel Isam Al-Atar B.D.S.,M.Sc.*
Zainab A A Al-Dahan B.D.S.,M.Sc.**

Abstract:

This study was carried out to determine whether the dissociation of mineral trioxide aggregate (MTA) depends on the vehicle used with it by evaluating the release of calcium ions (Ca^{+2}) and hydroxyl ions (OH^-) from mixing of MTA with four types of vehicles: distilled water, camphorated monochlorophenol (CMCP), plain local anesthetic solution, and chlorhexidine gluconate, using Atomic Absorption Spectrophotometer (AAS) and digital pH meter respectively.

The results demonstrated that the distilled water and plain local anesthetic solution are the most suitable vehicles to be mixed with MTA and can be routinely used in preference to all the other vehicles.

Keywords:

Mineral trioxide aggregate (MTA), distilled water, camphorated monochlorophenol (CMCP), plain local anesthetic solution, chlorhexidine gluconate.

Introduction:

One of the most difficult endodontic problems is the management of necrotic immature tooth which present a clinical challenge due to the blunderbuss apex and due to the difficulty in achieving a tight seal between the root canal system and the external surface of the tooth [1, 2].

Apexification refers to that method of treatment aimed to induce apical repair as a hard tissue barrier across an open apex [3].

Mineral Trioxide Aggregate (MTA) is a special purpose dental material with numerous exciting clinical applications in endodontics. Several in vitro and in vivo studies have shown that MTA prevents micro leakage, biocompatible, and stimulating hard tissue formation [4, 5].

The mechanism of action of MTA has some similarity with that of calcium hydroxide Ca $(OH)_2$.Although MTA doesn't have Ca $(OH)_2$ in its composition but it has calcium oxide (CaO) that could react with tissue fluid to form Ca $(OH)_2$ [6, 7].

The manufacturer recommends mixing of MTA with sterile water. Some clinicians report success in mixing MTA with anesthetic or other sterile liquids, but the other liquids may have an effect on MTA physical, chemical and biological properties [8].

MTA is a promising material with an expanding foundation of research. To date, there have been no published studies about the effect of

*Assistant Lecturer in the Department of Pedodontics, Orthodontics and Preventive Dentistry, College of Dentistry, Al-Mustansiria University
*Professor in the Department of Pedodontics, Orthodontics and Preventive Dentistry, College of Dentistry, Baghdad University

mixing different vehicles with MTA. Therefore, this study is the first study that focuses on the effect of mixing different vehicles with MTA by determining the release of Ca^{+2} and OH^- from MTA powder mixed with distilled water, CMCP, plain local anesthetic solution, and chlorhexidine gluconate, to identify the most suitable vehicle to be used with MTA in dentistry.

Materials and Methods:

Forty freshly extracted human premolar teeth with single and straight canals were used in this study. The crown of each tooth was sectioned using straight hand piece and diamond disk and their root canals were cleaned and instrumented conventionally to a standard size #90 to create an open apex. The roots were sealed cervically with amalgam filling, and all the external surfaces except the apical area were sealed with two layers of clear nail polish. The roots were divided into four groups, each group consists of ten roots and each root was placed in a polyethylene vial containing (25 ml) of synthetic tissue fluid (STF). The release of Ca^{+2} and OH^- were assessed by measuring Ca^{+2} concentration and pH of the surrounding STF media(using AAS and digital pH meter respectively) after three days as a control period. Then the roots were removed from the STF and the cervical seals were displaced, then using the messing gun the canals were packed with (4 mm) apical plugs of MTA mixed with four different vehicles: - distilled water, CMCP, plain local anesthetic solution, and chlorhexidine gluconate.

The root canals were filled with gutta-percha and zinc oxide eugenol (ZOE) sealer to the coronal end of the apical plug. Then they were kept in the same solutions in which they were immersed at the control period after replacing the cervical seals. Ca^{+2} concentrations and pH values of the surrounding STF media were measured at 1, 3, 7, 14 and 21 days of the test period. The collected data were analyzed by descriptive statistics including means and standard deviations of the mean, analysis of variance (ANOVA) test to determine if there were a significant differences in Ca^{+2} concentrations and pH values among and within the groups, and t-test to compare the Ca^{+2} concentrations and pH values between each pair of groups.

Results:

Calcium ion readings

At the end of the three days of the control period, all the studied groups released a minute amount of Ca^{+2} in the surrounding STF media. The mean values of Ca^{+2} concentrations were (3.47±0.37, 3.22±0.41, 3.44±0.46, and 3.05±0.35) for group I (MTA + distilled water), group II (MTA + CMCP), group III (MTA + plain local anesthetic solution), and group IV (MTA + chlorhexidine gluconate) respectively (table 1).

At the test period and after placement of MTA paste ‹group I (MTA + distilled water) showed the highest recorded values at all time intervals. The mean values of Ca^{+2} concentrations were (8.49±0.57) PPM at day 1, and (43.15±2.74) PPM at day 21. Group III (MTA + plain local anesthetic solution) came next with mean values of (7.32±0.56) PPM at day 1, and (41.25±2.43) PPM at day 21, whereas group II (MTA + CMCP) showed mean values of (6.18±0.70) PPM, at day 1, and

(29.06±3.33) PPM at day 21. Finally, group IV (MTA + chlorhexidine gluconate) showed the lowest recorded values of the mean of Ca^{+2} concentrations that were (4.98±0.69) PPM at day 1, and (25.75±3.04) PPM at day 21 (table 1, figure 1).

Statistical analysis of results using (ANOVA) test demonstrated that no significant difference was found at the end of the control period. Whereas very high significant differences (P<0.001) were found among the four studied groups at all time intervals. Also, a very high significant difference (P<0.001) were seen within each group among studied intervals for the four studied groups (table 1).

Table (1): The differences in Ca^{+2} concentrations among and within the four studied groups at all time intervals.

	Mean	±S.D	Mean	±S.D	Mean	±S.D	Mean	±S.D	P.ANOVA	
Control	3.47	0.37	3.22	0.41	3.44	0.46	3.05	0.35	0.082	N.S
	MTA+ distilled water		MTA+ CMCP		MTA+ plain anesthesia		MTA+ chlorhexidine			
Day 1	8.49	0.57	6.18	0.70	7.32	0.56	4.98	0.69	0.001>	V.H.S
Day 3	16.34	1.32	8.37	0.63	12.16	0.94	7.56	0.61	0.001>	V.H.S
Day 7	23.56	3.07	12.53	1.87	18.62	1.55	10.75	1.26	0.001>	V.H.S
Day 14	31.36	3.83	17.66	1.17	29.47	2.29	16.34	1.12	0.001>	V.H.S
Day 21	43.15	2.74	29.06	3.33	41.25	2.43	25.75	3.04	0.001>	V.H.S
P-value	0.001>		0.001>		0.001>		0.001>			
Sig.	V.H.S		V.H.S		V.H.S		V.H.S			

$p < 0.001$ Very high significant difference (V.H.S)
$P > 0.05$ Non significant (N.S)

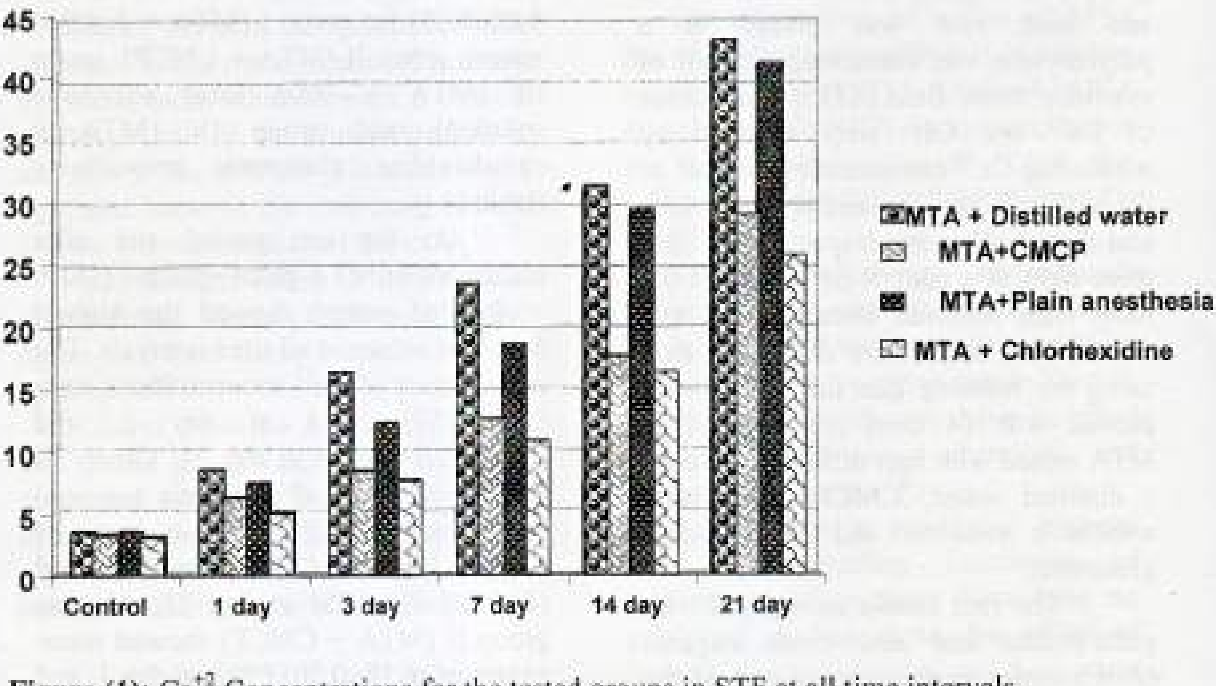

Figure (1): Ca^{+2} Concentrations for the tested groups in STF at all time intervals.

pH values

All the studied groups showed approximately the same pH values in the surrounding STF media at the end of the three days of the control period. The mean pH values were (7.353±0.003, 7.357±0.004, 7.355±0.005, and 7.357±0.006) for group I (MTA + distilled water), group II (MTA + CMCP), group III (MTA + plain local anesthetic solution), and group IV (MTA + chlorhexidine gluconate) respectively (table 2).

At the test period, group II (MTA + CMCP (showed the highest recorded values at all time intervals. The means of pH values were (7.447±0.07) at day 1, and (8.320±0.15) at day 21. Group I (MTA+ distilled water) and group III (MTA + plain local anesthetic solution) came next with mean values of (7.42±0.05) and (7.407±0.067) at day 1, and (7.922±0.17) and (7.931±0.09) at day 21 respectively. Group IV (MTA chlorhexidine gluconate) showed the lowest recorded pH values that were (7.398±0.055) at day 1, and (7.786±0.082) at day 21(table 2, figure 2).

Statistical analysis of the results using (ANOVA) test to compare the differences in pH values between groups and within each group demonstrated that no significant differences were found between groups at the control period, day 1, 3, and 7 respectively, whereas at day 14 and 21 very high significant differences were noticed (P<0.001). A high significant difference were found within each group among studied intervals for the four studied groups (P<0.001) (table 2).

Table (2): The differences in pH values among and within the four studied groups at all time intervals.

	Mean	±S.D	Mean	±S.D	Mean	±S.D	Mean	±S.D	P-value	Sig
Control	7.353	0.003	7.357	0.004	7.355	0.005	7.357	0.006	0.313	N.S
	MTA +distilled water		MTA+ CMCP		MTA+ plain anesthesia		MTA + chlorhexidine			
Day 1	7.42	0.05	7.447	0.07	7.407	0.067	7.398	0.055	0.294	N.S
Day 3	7.500	0.07	7.501	0.07	7.475	0.07	7.441	0.054	0.157	N.S
Day 7	7.542	0.07	7.593	0.08	7.563	0.07	7.515	0.059	0.104	N.S
Day 14	7.789	0.12	7.867	0.10	7.686	0.11	7.676	0.09	0.001>	V.H.S
Day 21	7.922	0.17	8.320	0.15	7.931	0.09	7.786	0.082	0.001>	V.H.S
P-value	0.001>		0.001>		0.001>		0.001>			
Sig.	V.H.S		V.H.S		V.H.S		V.H.S			

P<0.001 Very high significant difference (V.H.S).
P> 0.05 Non significant (N.S).

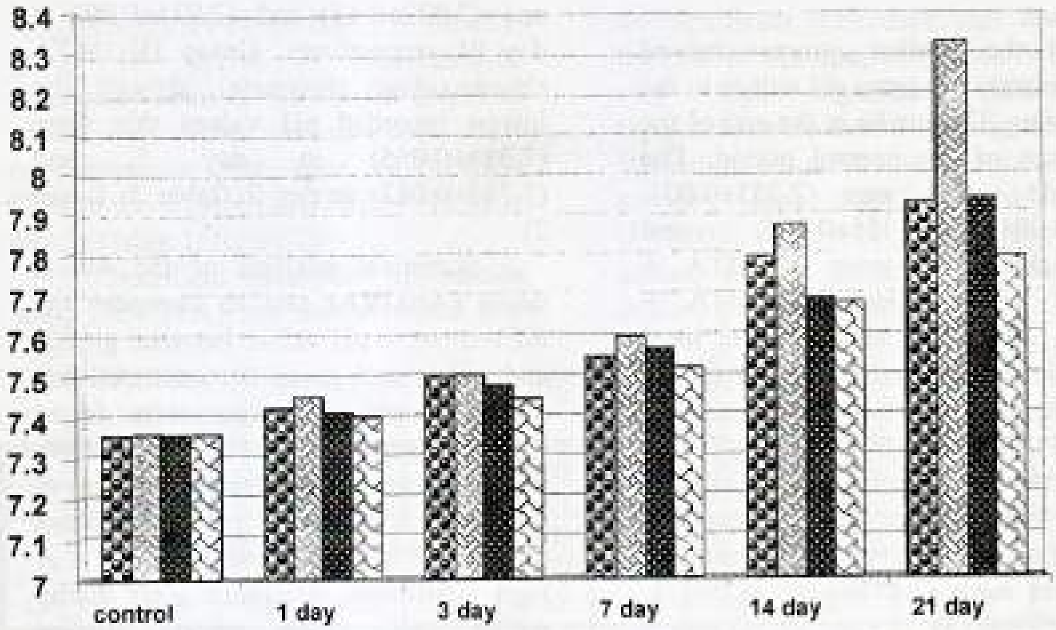

Figure (2): pH Values of the four tested groups in STF at all time intervals

Discussion:

A minute amount of Ca^{+2} were released into the surrounding STF media at the end of the three days of the control period; the Ca^{+2} came from hydroxyapatite crystals. There were differences in the Ca^{+2} concentration from one tooth to another; therefore, each tooth has an individual dissolution rate different from the other, so, the dissolution rate for each group were different from the other groups. These differences were not statistically significant [9].

Regarding the Ca^{+2} concentrations, group I (MTA+ distilled water) had the highest Ca^{+2} concentrations at all time intervals than the remaining three groups, these results emphasizes the fact that calcium oxides (CaO) which present within the composition of MTA can react with distilled water and give $Ca(OH)_2$ which can dissociate into Ca^{+2} and OH^- and diffuse into the surrounding aqueous medium. Also, distilled water had been proved to have the best chemical characteristics that speed the ionic dissociation and diffusion ability of the dressing material that mixed with it [10].

A delay of seven days was observed in the release of Ca^{+2} from group III (MTA+ plain local anesthetic solution) showed very highly significant differences between group III (MTA+ plain local anesthetic solution) and group I (MTA+ distilled water) at days 1, 3, and 7, thereafter, no significant differences could be seen between them at day 14 and 21. The delay could be due to the chemical reaction between MTA and sodium chloride (NaCl), which is present in the local anesthetic solution leading to the formation of calcium chloride ($CaCl_2$), and the release of Ca^{+2} commencing after the NaCl is completely removed by chemical action. Then after day 7, no actual chemical reaction occurred between MTA and plain anesthesia and it only represent a suitable vehicle for the dissociation of MTA and the release of Ca^{+2} to the surrounding media [11].

Group IV (MTA+ chlorhexidine gluconate) scored the lowest Ca^{+2} concentrations values among the four studied groups due to the chemical reaction that occurs between MTA and chlorhexidine which leads to incorporation of Ca^{+2} within the chemical structure of chlorhexidine and formation of a complex compound with strong chemical bond and limitation of the diffusion of Ca^{+2} into the aqueous medium [12].

The pH values of the four studied groups indicated that a minute amount of OH^- was released into the surrounding medium throughout the days 1, 3, and 7 of the test periods; however, Group II (MTA+ CMCP) had pH values higher than the remaining three groups at all recorded intervals of the test period.

CMCP react with MTA to form calcium monochlorophenolate, which is a weak salt that may release OH^- readily and increase pH values. Also the surface tension of CMCP influences the diffusion and spreading of OH^-. Phenolic compounds like CMCP have lower surface tension than the remaining three vehicles and this would increase the penetrability of OH^- [13,14].

Group I (MTA+ distilled water) and Group III (MTA+ plain local anesthetic solution) showed the next higher pH values at all time intervals of the test period. Hydrosoluble vehicles like distilled water and plain anesthesia enhance the chemical characteristics of dissociation and diffusibility which is decisive for the biological behavior, i.e., antimicrobial qualities and induction of tissue repair of the material mixed with it. They only represent the aqueous medium that allows the favorable dissociation of MTA into Ca^{+2} and OH^-.

Regarding the use of plain anesthesia as a vehicle, although its pH is 4.5-6.5 which is slightly acidic [15], but the pH of MTA is 12.5 after setting, so it is a very strong base that can not be affected by acids easily.

The lowest pH values were recorded with group IV (MTA+ chlorhexidine gluconate) compared with the other studied groups. This may be due to the chemical reaction between OH^- of MTA and chlorhexidine gluconate, which leads to the formation of a weak acid that will dissociate to give hydrogen ions (H^+) and decreasing the pH. Also chlorhexidine gluconate has a high surface tension, which hinders the diffusion of OH^- to the surrounding media.

There were no significant differences in the pH values among the four studied groups until day 7 of the test period where the pH values were not more than (7.5) for the four studied groups. Mean while there were very highly significant differences could be seen at days 14 and 21. This may be explained by the fact that the STF in which the teeth were immersed is a buffer solution (PBS) which has the ability to bind or release H+ in solution, thus keeping the pH of the solution relatively constant despite the addition of considerable quantities of acid or base. The amount of acid or base that can be neutralized by a chemical buffer system depends on two factors: the concentration of the buffer and the optimum pH at which it can function, therefore, its effectiveness is greatly reduced if the pH of its environment deviates too far from this [16].

Also hydroxyapatite crystals is a highly effective buffer that surprisingly prevent the movement of OH^- in vitro across a layer of dentin when a paste of $Ca(OH)_2$ was placed inside the root canal. OH^- adsorb into the hydrated layer of hydroxyapatite crystals thus preventing their diffusion along the dentinal tubules [9], therefore, after 7 days the effectiveness of PBS and hydroxyapatite buffer decreased, also, after saturation of teeth structure with PBS, the dissolution of OH^- had increased and gave very highly significant differences in the pH values among the four studied groups.

Conclusions:

1. Very highly significant effect of time was found on ions concentration in the four studied groups. The diffusion of Ca^{+2} and OH^- from MTA through apical area continued through out the 21 days of the study period and the increase in ions concentration were directly related to the duration of the test period.
2. Group I (MTA+ distilled water) demonstrated the highest Ca^{+2} concentrations at all recorded intervals of the test period in comparison with the remaining three groups.
3. Group III (MTA+ plain local anesthetic solution) showed the next higher values in Ca^{+2} concentrations, which was not significantly different from group I at days 14 and 21 of the test period. Group II (MTA+ CMCP) came next.
4. Group II (MTA + CMCP) showed the highest pH values at all time intervals of the test period compared with the remaining three groups.
5. Distilled water and plain local anesthetic solution allow for a gradual release of Ca^{+2} and controlled pH level.

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