

Assessment of calcium ions diffusion and pH measurements of three intracanal medicaments through dentinal tubules (in vitro comparative study)



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Chrakhan A-Latif A-Qadir^aSalam D. Al-Qaisi^b

Abstract

Objectives: The purpose of this study was to evaluate the release of Calcium ions from three intracanal medicaments [Ca(OH)₂, White Proroot MTA, Biodentine], and to measure the PH of these three materials.

Materials and Methods: Forty-two single rooted permanent teeth were prepared using crown down technique with Protaper rotary files to a master apical file size 40. The canals were irrigated by using 2 ml 5.25% NaOCl for 60 seconds after each instrument change, final irrigation of canals using 5 ml of 17% EDTA for 3 minutes and 5 ml of 5.25% NaOCl for 60 seconds and finally 10 ml of saline solution. The canals were dried and the intracanal medicaments were applied according to manufacturer instruction after setting of materials, both apical and coronal openings were sealed by Cavit and a layer of Epoxy resin on it. Each root was immersed in 30 ml of deionized water. The digital pH meter was used for measuring the pH values for each root 10 times per each studied period (1, 2, 7, 15, 30, 45, 60) days; The Calcium ions release measurements were taken for each studied periods using Atomic Absorption Spectrophotometer device. The results were analyzed statistically using ANOVA (F test), a p-value < 0.05 was considered as a significant.

Results: The pH values for Ca (OH)₂ and PMTA groups were gradually increased and need more time for increasing than the Biodentine. The pH values for Biodentine were the highest at the first day then decreased in the 2nd and 7th days, then increased over time until sixty days. The calcium ions release values were gradually increase for three medicament and its values were highest for Biodentine followed by Ca(OH)₂ and finally PMTA. The differences between groups were statistically highly significant.

Conclusions: Biodentine exhibited highest mean values in the calcium ions release and pH measurements than other groups and PMTA showed lowest mean values in the calcium ion release and pH measurement.

Keywords: Calcium hydroxide, Proroot MTA, Biodentine, deionized water

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Introduction:

Cleaning and shaping of the root canal system are among the important phases in endodontic therapy. Nevertheless, it is recognized that chemo-mechanical instrumentation alone is unable to completely disinfect root canal systems. The remaining bacteria in the root canal after instrumentation have been shown to proliferate between appointments. Intra canal medicament is also well-established method for inhibition of external root resorption and stimulation of periapical healing in traumatized teeth⁽¹⁾.

Microorganisms are not only in the main root canal, but also disseminated throughout the root canal system therefore, the use of an intracanal dressing to eliminate the microorganisms is indicated⁽²⁾.

The selection of intracanal medicament depends on the root canal microbiota and on the mechanism of action of the medication. Calcium hydroxide is the most used intracanal dressing due to its antimicrobial effect within root canal, its indirect and direct actions in the dentinal tubules⁽³⁾.

Calcium hydroxide dissociate to hydroxyl ions and calcium ions & create an alkaline environment inside dentinal tubules, and as a result it shows antibacterial properties and stimulates hard tissue barrier formation in aqueous solution. Various biological properties have been attributed to this substance, such as inhibition of tooth resorption and induction of repair by hard tissue formation^(4,5).

The new intracanal medicaments has been introduced like mineral trioxide aggregate (MTA) first introduced as a root end filling material and for repair of lateral root perforations since then it has been used for many clinical applications like pulp capping, pulpotomy & for treatment of root resorption & root filling material; similar to calcium hydroxide it has high alkaline pH & the same mechanism⁽⁶⁾.

Recently calcium – silicate based material (Biodentine) has been introduced with the same clinical application as MTA but with a different chemical composition. It exhibits high alkaline pH and

^a M.Sc. Department of Conservative Dentistry, School of Dentistry, Faculty of Medical Sciences, University of Sulaimani.

^b Professor. Department of Conservative Dentistry, School of Dentistry, Faculty of Medical Sciences, University of Sulaimani.

release of calcium ions & also has good antimicrobial properties⁽⁷⁾.

The pH measurement and calcium ions release measurement are determined by using digital pH meter and Atomic Absorption Spectrophotometer device respectively.

Materials and Methods:

Forty-two extracted permanent maxillary central and maxillary second premolar human teeth with single and straight root were selected for present study. The soft tissues and hard deposits were removed using the ultrasonic scalar, the teeth were stored in 10% Formalin at room temperature for two weeks before instrumentation for sterilization, and then teeth were rinsed with distilled water to remove formalin and stored in normal saline until the time of instrumentation.

The length of teeth was measured by a vernier, then the teeth were decoronated transversally with a water-cooled diamond disc with straight handpiece at the level of (12 mm) from the apex perpendicular to the long axis of the root to obtain a relatively standard root length. The pulp tissues removed by barbed broach, the root canals were measured by insertion of a K file #10 with a rubber stop. The root canals were prepared with a rubber dam in place; using a crown-down technique with Protaper rotary files to a master apical file size of 40. The canals were irrigated by using 2 ml of 5.25% sodium hypochlorite for 60 sec after each instrument changed, After finishing the instrumentation process, the canal was irrigated with 5 ml of (17% EDTA) for 3 minutes, followed by 5 ml of 5.25% NaOCl for 60 sec to remove smear layer, Finally, the canal was irrigated with 10 ml saline solution Then the canal dried with paper point size 40.

Sample grouping:

The prepared (42) roots were divided randomly in to three main groups of fourteen roots, which were calcium hydroxide, PMTA, and Biodentine as shown in figure1.

The powder of materials was mixed with distilled water according to manufacturer instruction. The calcium Hydroxide (Bio Dinamica Hydroxide Decalcium, France) and PMTA (Dentsply, Tulsa Dental, USA) were applied by lentulo spiral filler size 40 to 1 mm shorter than full working length using slow speed handpiece. While Biodentine (Septodont) was applied to the canal by amalgam carrier. Then materials condensed using finger plugger and endodontic condenser until 2 mm remain coronaly for sealing materials. Complete filling of the root canals for all three groups were checked by the over flow of material through the root canal opening and radiographic documentation through taking periapical radiograph for each root in buccolingual and mesiodistal aspects.

When the filling material set, both opening of roots were sealed by Cavit and when become hard, a layer of epoxy resin used on it for better sealing. After self drying of the epoxy resin, each root immersed in a separated plastic tubes containing 30 ml of Deionized water and transferred to an incubator at 37°C with humidify environment. The digital pH meter (pH meter 765 Calimatic, Knick, Germany) was used for measuring the pH values for each root 10 times per each studied period (1, 2, 7, 15, 30, 45, 60) days; The calcium ions release measurements were taken for each studied periods using Atomic Absorption Spectrophotometer device (PYE uniCAM Spg; Philips, England), figure 2. The results were analyzed statistically using ANOVA (F test), a P-value < 0.05 was considered as a significant.

Results:

The changes in pH and calcium ion release within the immersion medium are presented in table 1 and figure 3, table 2 and figure 4 respectively.

The pH values of the immersion media for three groups were approximately between 6.166 and 8.634.

Group1 which is Ca (OH)₂ and Group2 (Pro root MTA) show positive increase of pH over time while Group3 (Biodentine) shows very high pH values in the first day then decreases in the 2nd and 7th days

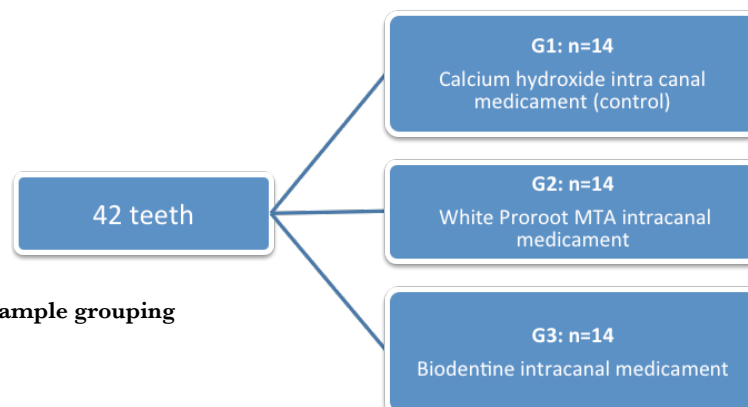


Figure 1: Sample grouping



Figure 2: Atomic Absorption Spectrophotometer

Table 1: The Comparison between Mean and Standard Deviation of PH Measurement of Three Intra Canal Medicament at Different Studied Periods

Period of measurements (Days)	Number of teeth	Group 1 calcium hydroxide Ca(OH) ₂ Mean±S.D	Group 2 Mineral Trioxide Aggregate (PMTA) Mean±S.D	Group 3 Biodentine Mean±S.D	P- value	Significant degree
1 st day	14	7.423±0.520	6.166±0.289	8.634±0.877	< 0.001	S
2 nd day	14	8.190±0.513	7.097±0.256	8.461±0.513	< 0.001	S
7 th day	14	8.163±0.500	7.432±0.195	8.134±0.458	< 0.001	S
15 th day	14	8.314±0.172	7.718±0.221	8.274±0.212	< 0.001	S
30 th day	14	8.334±0.208	7.960±0.196	8.248±0.153	< 0.001	S
45 th day	14	8.464±0.231	8.034±0.213	8.418±0.207	< 0.001	S
60 th day	14	8.482±0.180	8.054±0.177	8.400±0.176	< 0.001	S

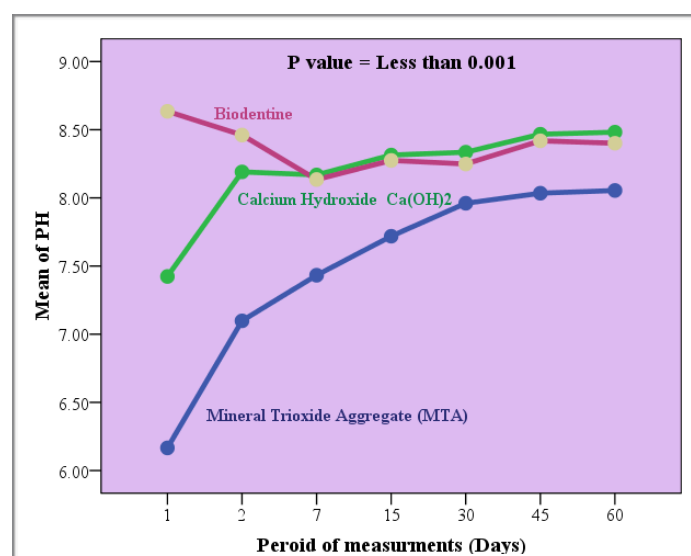
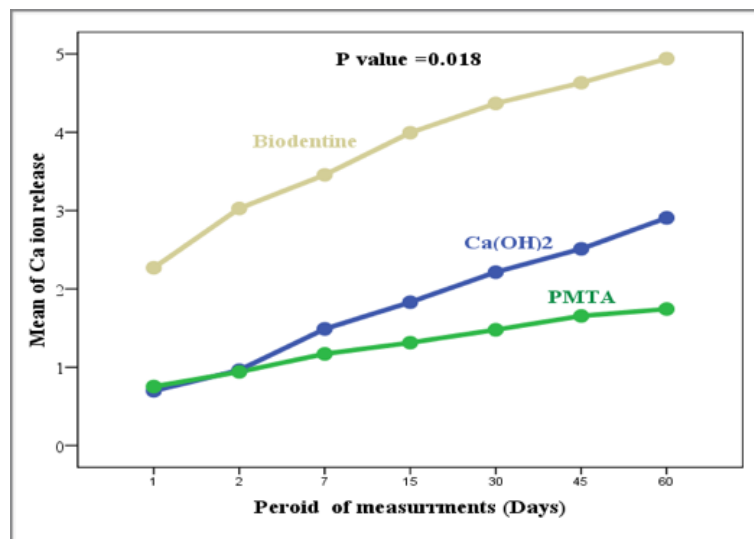


Figure 3: The Comparison between Mean pH at Different Period of Measurements among Three Studied Groups

Table2: The Comparison between Mean Values of Calcium ions Measurement of Three Studied Groups at Different Studied Periods

Periods of measurement (days)	Number of teeth	G1: Ca(OH) ₂ (Control group) Mean	G2: PMTA Mean	G3: Biodentine Mean	P-values	Significant degree
1 st day	14	0.698	0.753	2.269	< 0.001	S
2 nd day	14	0.962	0.94	3.027	< 0.001	S
7 th day	14	1.489	1.17	3.456	< 0.001	S
15 th day	14	1.83	1.313	3.994	< 0.001	S
30 th day	14	2.214	1.478	4.368	< 0.001	S
45 th day	14	2.511	1.654	4.632	< 0.001	S
60 th day	14	2.907	1.742	4.94	< 0.001	S

**Figure 4: The Comparison between Mean Values of Calcium Ions Release at Different Period of Measurements among Three Studied Groups**

then increases over time from 15th day to the 60th day. The results show that Group2 (PMTA) exhibit lowest mean value in the pH measurement (6.166) in the first day than other groups while the highest mean value of pH measurement is observed in Group3 Biodentine (8.634) in the first day. The difference between the pH measurements of three intracanal medicaments groups for mean values is statistically highly significant (P value <0.001). The three groups of intracanal medicaments show positive increase of mean value of calcium ions release at different studied periods. The results showed that Group1 calcium hydroxide medicament exhibits the lowest mean value in the calcium ions release (0.698) in the first day while the highest mean value of calcium ions release measurement is observed in Group3 Biodentine (4.94) in the sixty days. The difference between the three intracanal medicament groups for mean values of

calcium ions release measurement is statistically highly significant (P value <0.001).

Discussion:

This study sheds light on the comparison of pH measurement & calcium ions release measurement of three intracanal medicaments Ca(OH)₂, Proroot Mineral Trioxide Aggregate and Biodentine at different studied periods (1, 2, 7, 15, 30, 45 and 60) days.

1. pH measurement:

The diffusion through dentinal tubules, apical foramen, secondary and accessory canals allows the calcium hydroxide to reach regions contaminated by

microorganisms, areas of root resorption and surrounding tissues, promoting its antimicrobial and anti-resorptive action⁽⁷⁾.

PH measurement method in immersion media applied in this study is to allow the measuring of pH at periods longer than the setting time, not representing the pH of material before setting, but rather its ability of alkanization⁽⁸⁾.

The pH value of calcium hydroxide in this study came in agreement with the results of Mori et al⁽⁷⁾. The pH values of calcium hydroxide obtained in 2nd, 7th, 15th days came in agreement with Sevimay et al⁽⁹⁾, Barekatin et al⁽¹⁰⁾ which could be due to irrigation materials, and pattern of hand instrumentation which affects the amount of dentine removal and the diffusion of calcium ion by its buffering effect.

This study showed that the pH values of PMTA were low in the 1st, 2nd, 7th days and close to the results of Ozdemir et al⁽¹¹⁾ who concluded that MTA didn't produce enough alkaline shift in the immersion media during entire test period, which continued until 28th day, and this is due to inactivation of materials by dentin because of its buffering capabilities.

The pH values of Biodentine in this study showed highest values in the first day, then decreased similar to the results of Khan et al⁽⁸⁾ study during the 1st day, who found slightly higher pH values for Biodentine, but with the same pattern in that it obtained higher results in the first day then decrease.

The results of calcium hydroxide are not in line with studies of Zmener et al⁽¹²⁾ since their results are higher than the results of this study, because of the use of saline solution which has higher pH than deionized water.

The results of PMTA disagree with Bortoluzzi et al⁽¹³⁾ results which are higher, the reason behind this could be due the use of polyethylene tubes instead of natural roots.

2. Calcium ions measurement:

Intracanal medicaments should also release calcium ions in order to stimulate mineralization, the calcium ions react with tissue carbonic gas, forming calcium carbonate that favors mineralization⁽¹¹⁾.

In the present study there was remarkable amount of calcium ion released and it's gradually increased with time and also came in agreement with Ghazvini et al⁽¹⁴⁾ study showed that all tested materials released remarkable amounts of calcium ions.

The results of calcium hydroxide in this study were close to the result of Uzunoğlu et al⁽¹⁵⁾ who showed that calcium ion release from different calcium hydroxide pastes have continued up to 30

days; The results of PMTA were identical to the result of Ozdemir et al⁽¹¹⁾ especially in the 15 days which reached 1.3mg/dl.

Calcium ions release values of the present study are in agreement with Bortoluzzi et al⁽¹³⁾ for PMTA and with Khan et al⁽⁸⁾ for both PMTA and Biodentine which are as follows; in the first day calcium ions release values are nearly the same but with time the present study shows increase in values in contrast to both Bortoluzzi et al⁽¹³⁾ and Khan et al⁽⁸⁾ studies who showed decrease in the values and the reason behind this could be due to using poly ethylene tube instead of roots and changing deionized water with each measurements periods.

Also the result is not in line with Camargo et al⁽¹⁶⁾ who showed higher values of calcium ions release from calcium hydroxide than in the present study the reason could be the type of atomic absorption Spectrophotometer device which was the UV type and the sample model which was the outer root surface cavity. The results of calcium hydroxide do not come in agreement with study of Fulzele et al⁽⁴⁾ since their results were higher than the results of this study because of the use of polyethylene tubes and the use of smaller quantity of deionized water in the test tubes than present study leading to higher result in their study.

The results of PMTA in the first day calcium ions release values are nearly the same but with time the present study shows increase in values in contrast to the both Bortoluzzi et al⁽¹³⁾ who showed decrease in the values and the reason behind this could be due to using poly ethylene tube instead of roots and changing deionized water with each measurements periods.

Conclusions:

The pH values for Ca (OH)₂ and PMTA groups were gradually increased from first day to the sixty days. The pH values for Biodentine were the highest at the first day then decreased in the 2nd and 7th days, then increased over time until sixty days.

According to the results, the calcium hydroxide and PMTA need more time for increasing the pH values than the Biodentine. The calcium ions release values were gradually increase for three intracanal medicaments Ca(OH)₂, PMTA, Biodentine. The calcium ions release values were highest for Biodentine followed by Ca(OH)₂ and finally PMTA.

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