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Original Article

Measurement of pH and calcium ions release from different calcium hydroxide pastes at different intervals of time: Atomic spectrophotometric analysis

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ABSTRACT

Aim: To evaluate the effect of different vehicles on pH and release of calcium ions from calcium hydroxide (CH) paste from apical third of root canals.

Methods: 40 single rooted extracted human mandibular premolars were instrumented with RevoS files (MicroMega) up to ISO size 40. The teeth were divided into 4 groups on the basis of vehicle as follows: Group I – calcium hydroxide mixed with 2% chlorhexidine; Group II – calcium hydroxide mixed with propylene glycol; Group III – calcium hydroxide mixed with glycerine; and Control – calcium hydroxide mixed with double distilled water. Each group had two subgroups ($n = 5$) on the basis of the calcium hydroxide delivery. Subgroup A – calcium hydroxide paste placed with spiral filler (Lentulospiral) subgroup B – calcium hydroxide paste placed with flat wire filler (Paste Inject). pH and calcium ion release was evaluated at 24 h, 48 h, 7th day, 15th day and 30th day using a pH meter and atomic absorption spectrophotometer respectively.

Results: The calcium release from various groups was highest for CH+glycerine (Group III) followed by CH+chlorhexidine (Group I), CH+propylene glycol (Group II) and CH+double distilled water (control). At all the intervals the differences in calcium ion release among the groups were statistically significant ($p < 0.05$), except on day 7. Delivery technique did not have a significant effect on calcium ion release. Highest pH values were recorded from CH +glycerine group at day 30 for both the delivery systems, however rise in pH from day 1 to day 30 were non-significant in all groups with both delivery systems.

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Conclusion: Demonstrable changes in calcium ion release occurred from the calcium hydroxide mixed with various vehicles and CH+glycerine group showed the maximum calcium ion release at all intervals and highest pH day 30.

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1. Introduction

Periapical repair following endodontic treatment is a much sought after goal, especially in cases with periapical lesions.¹ Even a thorough chemo-mechanical preparation of the canal is sometimes not enough to sterilise the root canal. Owing to anatomical complexities and niches inaccessible to instrumentation and irrigation, there may still be a need for an intra-canal medicament for better treatment outcomes. Intra-canal medicaments prevent the penetration of bacteria into the root canal by mechanically preventing their ingress and by their antimicrobial action.² Calcium hydroxide (CH) finds use as an intra-canal medicament owing to its antimicrobial action, high pH and ability to induce hard tissue formation. Also, CH has a low solubility in saliva, thus can remain in the canal for a longer period, thus delaying the bacterial progression towards apical foramen.^{3,4}

The efficacy of CH as intra-canal medicament depends largely on its dissociation into calcium (Ca) and hydroxyl ions (OH). The vehicle which carries CH affects its speed of ionic dissociation and its ability to stay in the canal for longer time.⁵ Different vehicles used to carry CH are grouped as aqueous, viscous or oil based. While the aqueous vehicles are more soluble and dissociate rapidly, the viscous ones dissociate slowly and remain in the root canal for a longer period.⁶ The OH ions released into the periapical area promotes healing because of its high pH and antimicrobial action. Ca ions also play a very important role in inducing hard tissue formation by activation of Ca-dependent ATP and the quantity of free calcium ions in the medicament determines its remineralization potential.⁷ Though most of the studies have been conducted to study the release of OH ions and their effect on periapical pH and repair, a very few discuss about the Ca ion release and its role in periapical healing.^{8,9}

Therefore, this study was conducted to determine the effect of various vehicles and delivery techniques on the pH of the paste and release of Ca ions from the apical one-third of the root canals using an atomic spectrophotometer.

2. Methodology

Forty freshly extracted, single-rooted human mandibular premolars with no visible fractures or cracks were selected for the study. After debridement and disinfection of the teeth, they were decoronated at the cemento-enamel junction. The working length was determined by inserting a No. 10 K-file (Dentsply, Maillefer) until it was seen at the apex and then subtracting 0.5 mm from its length. The root canal of each tooth was instrumented with RevoS files (MicroMega) using an endo motor (X Smart, Dentsply) up to size 40 apically using

17% ethylenediaminetetraacetic acid (EDTA) gel (Glyde, Dentsply), at 500 rpm and 0.8 torque. After each instrument, the canal was passively irrigated with 5 ml of 5% sodium hypochlorite (Vishal Dentocare Pvt Ltd) the needle being placed 1–2 mm short of working length. Final irrigation was performed with 3 ml of 17% EDTA solution for 1 min and finally the root canal was flushed with 5 ml of double distilled water to remove any precipitate of EDTA. Each tooth was stored in 5 ml of saline in an airtight bottle.

The teeth were divided into four groups on the basis of the vehicle. **Group I** – calcium hydroxide mixed with 2% chlorhexidine; **Group II** – calcium hydroxide mixed with propylene glycol; **Group III** – calcium hydroxide mixed with glycerine; and **Control** – calcium hydroxide mixed with double distilled water. In all the groups the CH powder was mixed with the vehicles to obtain a uniform paste like consistency. Each group had two subgroups ($n = 5$) on the basis of the calcium hydroxide delivery: Subgroup A – calcium hydroxide paste placed with spiral-filler 800 rpm and torque 1, until canal was fully filled (Lentulospiral, Dentsply, Maillefer, Switzerland); and Subgroup B – calcium hydroxide paste placed with flat-wire filler 800 rpm and torque 1, until canal was fully filled (Paste Inject, MicroMega). The teeth were coated with nail paint except in the apical third region to seal lateral canals present in coronal and middle third region of the root and immersed into 10 ml of double distilled water in test tubes tightly sealed with paraffin wax films to avoid exposure to atmosphere.

After 24 h, the test tubes were stirred with a stirrer; the leachate was removed from the test tubes, and samples were moved to the fresh test tubes. pH measurement of the leachate was done with pH meter and calcium ion analysis was done using atomic absorption spectrophotometer. The operational conditions were: Light current three mA; fuel: acetylene; support: oxygen; and stoichiometry reductor. Before measurement, the solution was homogenized by a shaker. Readings were measured in ppm and data obtained was statistically analysed. Same procedure was followed at following time intervals: 24 h, 48 h, 7th day, 15th day and 30th day and the test tubes containing the teeth were replenished with a fresh 10 ml of double distilled water at each time interval.

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 Statistical Analysis Software. ANOVA-test was used to compare the within the group and between group variances among the study groups, i.e. the three different sealers. Analysis of variance of these three sealers at a particular time interval revealed the differences among them. ANOVA provided “F” ratio, where a higher “F” value depicted a higher inter-group difference. To test the significance of two means, the ‘Student-t’ test was used. Level of significance “p” was considered not significant if $p > 0.05$, significant if $p < 0.05$, highly significant if $p < 0.01$ and very highly significant if $p < 0.001$.

3. Results

For the spiral filler group, on day 1 and 7 maximum calcium ion release was recorded from CH+glycerine (6213.0 ± 546 and $17,688.8 \pm 458.4$ respectively), while it was maximum for CH+chlorhexidine on day 15 and 30 (6791.0 ± 286.5 and 1656.4 ± 250.0 respectively) while minimum calcium ion release was found from CH+double distilled water day 1, 15 and 30 (3063.0 ± 137.1 , 4863.0 ± 158.6 and 982.0 ± 19.2 respectively). At all the intervals the differences in calcium ion release among the groups were statistically significant (Table 1). For the flat-wire filler group, maximum Ca ion release was recorded in CH+glycerine group at 1st, 7th and 30th day (7004.8 ± 1504.0 , 6770.0 ± 853.6 and 1798.2 ± 46.5 respectively), while it was highest for CH+chlorhexidine on 15th day (7271.0 ± 323.1) (Table 2). The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in number (%) and mean $\pm$ SD. In a model with calcium release as a dependent variable on independent variables group, time and delivery system, only Group and time had an independent association as well as interaction of group and time with the outcome variable calcium release ($p < 0.001$). Delivery system

did not show a significant independent association as well as in interaction with other variables ($p > 0.05$).

The results for the pH analysis showed that in samples placed with spiral filler the difference in pH values in between various groups was not significant on day 1, 7 and 15. On day 30, pH values of CH+glycerine were found to be highest (7.1 ± 0.2) and that of CH+propylene glycol were found to be lowest (6.7 ± 0.1). The difference in pH values among the groups was found to be statistically significant ($p = 0.000$) (Table 3). For the flat wire group, the difference in pH values between the various groups was non-significant on day 1. On day 7 and 15, CH+propylene glycol group was recorded the highest pH (6.8 ± 0.1 and 7.3 ± 0.2 respectively) while on day 30, pH values of CH+glycerine group were found to be highest (7.1 ± 0.2) (Table 4). In a model with pH as a dependent variable on independent variables group, time and delivery system, only time had an independent association as with the outcome variable pH ($p < 0.001$). The rise in pH from day 1 to day 30 was non-significant.

4. Discussion

The efficacy of CH as an intra-canal medicament depends largely on its ability to dissociate from particle form into an

Table 1 – Mean calcium ion release (in ppm) at different intervals in samples placed with spiral-filler.

Groups	Day 1 Mean $\pm$ SD	Day 2 Mean $\pm$ SD	Day 7 Mean $\pm$ SD	Day 15 Mean $\pm$ SD	Day 30 Mean $\pm$ SD
Group I (glycerine)	3167.2 ± 328.6	2868.8 ± 308.6	5185.6 ± 227.5	6791.0 ± 286.5	1656.4 ± 250.0
Group II (chlorhexidine)	3362.4 ± 339.2	2947.0 ± 712.3	6454.0 ± 979.2	5289.6 ± 1230.4	1295.0 ± 116.4
Group III (propylene glycol)	6213.0 ± 546.1	6707.4 ± 1143.3	7688.8 ± 458.4	5227.6 ± 183.3	1626.4 ± 81.5
Control (double distilled water)	3063.0 ± 137.1	5077.0 ± 153.7	6219.4 ± 209.1	4863.0 ± 158.6	982.0 ± 19.2
ANOVA (F)	84.74	35.30	16.72	8.80	24.27
"p"	<0.001	<0.001	<0.001	0.001	<0.001

Table 2 – Mean calcium ion release (in ppm) at different intervals in samples placed with flat-wire filler.

Groups	Day 1 Mean $\pm$ SD	Day 2 Mean $\pm$ SD	Day 7 Mean $\pm$ SD	Day 15 Mean $\pm$ SD	Day 30 Mean $\pm$ SD
Group I (glycerine)	3156.2 ± 260.7	3033.6 ± 177.2	5411.4 ± 467.6	7271.0 ± 323.1	1783.8 ± 171.0
Group II (chlorhexidine)	3223.0 ± 413.4	3190.4 ± 497.4	6223.6 ± 1098.7	5481.4 ± 712.0	1300.8 ± 128.4
Group III (propylene glycol)	7004.8 ± 1504.0	6684.0 ± 513.3	6770.0 ± 853.6	4598.4 ± 460.5	1798.2 ± 46.5
Control (double distilled water)	2922.8 ± 251.3	4922.8 ± 249.9	6023.0 ± 92.8	4832.0 ± 139.0	996.2 ± 37.3
ANOVA (F)	29.85	97.09	2.91	34.71	62.12
"p"	<0.001	<0.001	0.067	<0.001	<0.001

Table 3 – Mean pH at different intervals in samples placed with spiral filler.

Groups	Day 1		Day 2		Day 7		Day 15		Day 30	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group I (glycerine)	6.6	0.1	6.7	0.1	6.8	0.1	7.0	0.2	7.0	0.1
Group II (chlorhexidine)	6.3	0.7	6.8	0.3	6.6	0.3	7.1	0.1	6.5	0.1
Group III (propylene glycol)	6.5	0.0	6.5	0.1	6.8	0.2	7.1	0.1	7.2	0.1
Control (double distilled water)	6.7	0.1	6.5	0.2	6.5	0.1	7.1	0.2	7.0	0.1
ANOVA (F)	0.80		2.61		2.22		0.75		52.99	
"p"	0.512		0.088		0.126		0.540		0.000	

Table 4 – Mean pH at different intervals in samples placed with Flat-Wire filler.

Groups	Day 1		Day 2		Day 7		Day 15		Day 30	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group I (glycerine)	6.6	0.1	6.6	0.1	6.8	0.1	6.9	0.0	7.0	0.1
Group II (chlorhexidine)	6.4	0.6	7.1	0.6	6.8	0.2	7.3	0.2	6.7	0.1
Group III (propylene glycol)	6.5	0.1	6.4	0.1	6.5	0.1	6.9	0.1	7.1	0.2
Control (double distilled water)	6.6	0.1	6.5	0.1	6.5	0.1	6.8	0.1	7.0	0.1
ANOVA (F)	0.23		5.73		13.39		16.85		7.11	
“p”	0.875		0.007		0.000		0.000		0.003	

ionic form. The ionic dissociation causes diffusion of Ca and OH ions through the dentinal tubules of the root canal walls. The vehicle that carries the CH particles is important as its quality and consistency determine the velocity of ionic dissociation and is responsible for it being dissolved and adsorbed into the periapical area as well as from within the root canal. Methods used to measure the ionic release are atomic absorption spectrophotometry, fluorometry, flame photometry, Ca ion selective electrode, etc.^{10,11} Most of the earlier experiments have utilized a plastic tube model simulating a root canal or from whole length of root canal, unlike the present study which is based on an extracted human tooth model for evaluating release of calcium only from the apical one third.^{12,13}

The usage of intra-canal medicament is now established for routine chemo-mechanical preparation of root canal. The coronal seal may break when the temporary restoration is dislodged or fractured during the root canal therapy, leaving the root canal system open to contamination from the oral cavity. Even in the presence of temporary restoration the possibility of leakage via the margins remains, thus, the intra-canal medicament used should protect the root canal system from the invading microorganisms for the desired period.¹⁴

In the present study, the CH+glycerine group showed a consistent and highest Ca release among all the groups whereas the CH+double distilled water (control group) showed a high initial release for a short duration, followed by a decline. Steiner et al. first used glycerine with CH in a paste that he used for root-end closure of immature non-vital teeth.¹⁵ Studies have also shown that to get more free ions, coating the root canal walls with glycerine and CH paste is superior to water.¹⁶ The amount of Ca release from glycerine group was in accordance with earlier studies done for ionic release from medicaments.^{17,18}

The differences in the velocity of ionic dissociation are in a direct proportion to the vehicle employed. Viscosity is a measurement of the inner friction of a fluid. Thus, if a solution flows easily and the inter-particle interactions are very less, it has a lower viscosity and a higher ionic dissociation. This type of vehicle has a higher solubility when the paste remains in direct contact with the tissue and tissue fluids and resorbed by macrophages in a shorter time. The root canal may become empty rapidly, delaying healing, and the root canal must be redressed multiple times to achieve the desired effect. The viscous vehicles, owing to their high molecular weight, reduce the dispersion of calcium hydroxide into the tissue and maintain the paste in the desired area for longer interval. They can remain in the root canal for a longer time interval up to 2 ± 4 months, thus reducing the number of appointments for re-dressing the root canal.^{18,19}

The other viscous vehicles groups, i.e. the Propylene glycol (Group II) and Chlorhexidine group (Group I) also showed a comparable Ca ion release at all intervals. 2% Chlorhexidine gel has a wide spectrum of antimicrobial activity with prolonged action (substantivity). It is biocompatible with periapical tissues, stays longer in contact with the microorganisms, diffuses through the dentin tubules, and has proven to be effective against microorganisms resistant to calcium hydroxide alone viz. *Enterococcus faecalis*.^{20,21} Chlorhexidine gel is a viscous formulation that makes instrumentation easier thus increasing the mechanical removal of organic tissues, compensating for its inability to dissolve them. Furthermore, it also decreases the smear layer formation. However, the Ca release from CHX group was contrary to a study by Durate et al. where 2% chlorhexidine gel+calcium hydroxide showed a decrease in calcium ion release for the study period of 30 action against common endodontic pathogens. Its hygroscopic property allows the absorption of water, causing a sustained release of calcium hydroxide for a longer period.²² The ionic release in this group were consistent with similar studies performed in the past.^{10,12}

The difference in pH values among all the four vehicles was statistically insignificant. All the four groups of vehicles maintained the pH for the study period of thirty days, which is similar as the study conducted by Pacios et al. in 2003 study, where all the vehicles used along with calcium hydroxide for the study period maintain the alkalinity.¹⁸ However, there was no significant rise in pH of the surrounding media and a non-significant reduction during the test period. The result of the study is in accordance with previous study done by Çalt et al. in 1999.²³ The non-setting calcium hydroxide paste released calcium ions through the dentin but did not increase the pH of the media, which indicate that the hydroxyl ion showed less diffusion out of the dentin. The permeability and buffering capacity of the dentin are the key factors affecting the diffusion of hydroxyl ion through dentin and could explain these findings. Calcium hydroxide intracanal dressing diffuses in a matter of hours into inner root dentin but requires 1–7 days to reach outer dentin and 2–3 weeks to reach peak levels.²⁴ The diffusion is faster and reaches higher level cervically than apically. So in the present study the factors that might have contributed to the insignificant rise in pH of the media may be the intact cementum layer of the teeth used in the study, glass ionomer cement used to close the orifice of the root and nail paint barrier used in the cervical and middle third which covered any exposed dentin, if present. In addition, the exposed apical third has irregular and a reduced number of dentinal tubules, which may further reduce the diffusion of hydroxyl ions.

The role of Ca ions in periapical healing and repair is lesser discussed than the role of OH ions. Calcium ions act by clearing the carbon dioxide used by the bacteria for anaerobic respiration. Also, calcium ions play an essential role on tissue mineralization by stimulating the fibronectin gene expression.¹³ Calcium ions are necessary for cell migration, differentiation and mineralization.²⁵ Their role in the stimulation of cell proliferation was established much earlier, and presence of large quantities of calcium ions in vivo could activate ATP, which played a significant role in the mineralization process. The calcium ions also are mitogenic to pulp fibroblasts.²⁶ Though differences of opinion exist about the origin of calcium ions incorporated in tertiary dentine, and it has been shown that calcium ions from medicaments do not become a part of mineralized repaired tissue but, the fact that at the initiation of mineralization, calcium came from the applied calcium hydroxide and not from pulp tissue cannot be ignored.²⁷

The delivery system for the placement of the calcium hydroxide in the root canal is an important factor contributing to the quality of fill and bulk of calcium hydroxide placed in the canal, as the release of calcium and hydroxyl ions depend on quantity of calcium hydroxide in the canal, especially till the apical third of the canal.²⁸ Lentulospiral can be an adequate instrument to place calcium hydroxide paste even in minimally instrumented canals to full working length. Paste Inject are tapered flat wire fillers that have been designed with greater capacity for holding canal sealer and introducing it into curved canals. These wires are capable of applying paste evenly, coating the smallest and deepest discrepancies.²⁹ The quantity of CH loaded into the canal might effect the quantity of ions released, however, according to the results of the present study, there was no significant difference in the amount of Ca ions released using the two delivery techniques.

5. Clinical implications

The results of the present study showed that the choice of vehicle does effect the ionic dissociation and release from the medicament. When an aqueous vehicle is used (double distilled water in this study), the initial ionic dissociation is very rapid, but decreases consequently. Thus, use of aqueous vehicle seems appropriate when a clinician encounters a wet or weeping canal (moisture in the canal will enhance dissociation), or situations which do not need a long duration dressing, as decline in ionization is observed after high initial release. The viscous vehicles (glycerine in this study) on the other hand exhibit a sustained release of ions, and may remain in the canal for a period of 2–4 months, thus are more suitable for cases, which require a long-term dressing, such as in non-surgical treatment of teeth with periapical lesions. Chlorhexidine can be considered a good vehicle of choice in refractory or failure cases when an antimicrobial action is required along with ionic release, against micro-organisms like *E. faecalis* and *Candida albicans*.

6. Conclusion

The choice of vehicle definitely seems to effect the efficacy of the medicaments, and although many studies are done

to evaluate the release of OH ions, the release and contribution of calcium ions from calcium hydroxide pastes in healing and repair of the periapical pathologies needs to be explored more.

Conflicts of interest

The authors have none to declare.

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