Original Article

An in-vitro Evaluation of pH and Calcium Ion Release from Calcium Hydroxide Root Canal Sealers

Kavita Verma[1], Vipul Srivastava[2], Shrish Srivastava[3], Shally Mahajan[4], Ramesh Chandra[5], AfreenA Nasir[6]

[1]Senior lecturer, [2,6]Professor, [3,4]Reader, [5]Professor and Head, Dept of conservative dentistry and endodontics
[1,2,3,4]Saraswati Dental College
[5,6]Career Post Graduate Institute of Dental College and hospital, Lucknow

ABSTRACT

Objective: An in vitro evaluation of the pH and calcium ion release exhibited by different calcium hydroxide based sealers, Sealapex, Acroseal and Endoflas F.S. from simulated root canal apical openings.
Study Design: The sealers were mixed according to the manufacturer's instructions and placed in the eppendorf tubes of 0.3 and 0.5 mm diameter aperture. Tubes were then immersed in flasks containing double distilled water. The solution was tested for pH by pH meter and calcium ion release by spectrophotometer at 24hrs, 48hrs, 5th day, 7th day, 15th day and 28th day intervals. The data obtained was statistically analysed using ANOVA.
Results: Seal apex showed highest pH and calcium ion release followed by Acroseal and then Endoflas F.S.in both 0.3 and 0.5 diameter aperture tubes.
Conclusion: Sealapex showed highest pH and calcium ion release followed by Acroseal and then Endoflas F.S.

Keywords: acroseal , atomic absorption spectrophotometer, calcium hydroxide, sealapex, endoflas f.s.

INTRODUCTION

The success of root canal treatment depends upon proper cleaning and shaping of root canal as well as hermetic sealing of the canal spaces with an inert, dimensionally stable, and biocompatible material [1], therefore a sealer with ideal physiochemical and biologic properties is yet to be found.A sealer performs the role of bridging the gap between core obturating material and radicular dentin. Many root canal sealers have been advocated through the years. They can be grouped according to their basic components such as zinc oxide eugenol, resins, glass ionomers, iodoform or silicon and calcium hydroxide sealers,[2] because these sealers may be in contact with the periapical tissues for a prolonged period of time, their biocompatibility is of prime concern.Calcium hydroxide sealers have been suggested for permanent sealing of root canals [3] and as replacement for traditional zinc oxide eugenol sealers because calcium hydroxide is heralded as having osteogenic potential as well as being effective in helping to eliminate bacteria.[4]

Calcium hydroxide based sealers have been found to be least cytotoxic against human gingival fibroblasts.[5] The calcium hydroxide based sealers like sealapex have also been shown to produce no inflammatory infiltrate when compared to zinc oxide eugenol based sealers.[6]

Correspondence: ***Dr Vipul Srivastava****; e-mail*: ***vipul13@gmail.com***

Physiochemical properties of a sealer like pH and calcium ion release is essential for suitable biologic behaviour.[7] Tronstad in 1981[8] suggested that calcium hydroxide based sealer in root canals elevates the pH, producing an alkaline environment in the surrounding tissues by diffusion through the dentinal tubules, this increased pH at the surface of the root adjacent to the periodontal tissues interferes with the osteoclastic activity.[8]Increase of hydroxyl ions also promotes the enzymatic inhibition of microorganisms.[9,10] Siqueira et al[11] reported in 1999 that hydroxyl ions act on molecules or structures that are essential for microbial metabolism or reproduction. The bactericidal activity of calcium hydroxide has been investigated by numerous authors .[12, 13, 14, 15, 16, 17] In addition to alkaline pH, calcium ion release is important because abundance of calcium ions in the tissue participates in the activation of calcium dependant Adenosine tri phosphatase.[18] Calcium reacts with tissue carbonic gas to form calcium carbonate [19], which favours dental pulp cell proliferation[20] and differentiation[21] serves as a nucleus for calcification and thus favours mineralization.[22,23] It has also been demonstrated that calcium ions react with carbon dioxide to reduce the source of respiration for anaerobic bacteria and inhibit the bacterial enzyme system.[24]The therapeutic action of calcium hydroxide sealers i.e. biologic and microbiologic action will depend upon the dissociation of calcium and hydroxyl ion and their release.[25, 9] In the present study the release of calcium from the aperture nearly simulating the root canal apical openings has been studied. No study as per available literature, till date, has taken this criterion which has been undertaken in this present study, to re-specify, simulating the openings of the apical foramen and uniformity of the apical opening diameter. This criterion is important as the results obtained would present more realistic picture of the clinical scenario in the somatognathic system. The release of calcium ion was detected by spectrophotometer. The aim of this study was thus, to evaluate in vitro, the pH and calcium ion release exhibited by different calcium hydroxide based root canal sealers which is essential for their bactericidal and osteogenic effect from simulated root canal apical openings.

MATERIALS AND METHOD

Sealers used in the experiment were

Acroseal; Septodont, Sealapex; Kerr, Endoflas F.S.; Sanlor

A total of 6 Eppendorf tubes were taken for each group. In three tubes an aperture of 0.3 mm was made (subgroup a) and in other three aperture of 0.5mm diameter was made (subgroup b) with the help of needles of their respective diameters.

Group I consisted of tubes with Sealapex sealer, Group II with Acroseal and Group III with Endoflas F.S.

The root canal sealers were mixed according to the manufacturer's instructions, with the help of 5ml syringe, the sealers were syringed into the tubes. Eppendorf tubes were sealed using surgical tape after placing the cap in position.

10 ml of double distilled water was then measured using a measuring cylinder, the water was poured into the flasks. The tubes were then placed into the flasks and the flasks were hermetically sealed. The control group contained double distilled water with empty tubes. The flasks were then kept in the incubator. At 24hrs, the solution from the flasks were obtained and tested for pH and calcium ion release. The tubes were moved to fresh double distilled water for next measurement. Same procedure was repeated at 48hrs, 5th day, 7th day, 15th day and 28th day.

Determination Of pH

pH meter was used in the study for the measurement of pH it was also used in various other studies .[8,23,26,27,28,29,30,31,32,33,34] The pH probe measures pH as the activity of hydrogen ions surrounding a thin-walled glass bulb at its tip. The probe produces a small voltage (about 0.06 volt per pH unit) that is measured and displayed as pH units by the meter. A pH meter was calibrated with known standard pH solution of 7. The pH level of the solution was determined. The readings were taken in triplicate to remove bias.

Analysis of Calcium Ion Release

Spectrophotometer with calcium measurement reagent was used for measurement of calcium ion release. The amount of calcium released was measured using a calcium measurement reagent and spectrophotometer. Ten microliters of solution was obtained from the flask and mixed with 1 ml of a calcium measurement reagent in P.V.C. tubes. The calcium measurement reagent changes the colour of the solution according to the calcium content (purplish red colour). The colour changes of the samples were measured with a spectrophotometer at 650 nm. Data was obtained in mg/dl. The values obtained were statistically analysed using analysis of variance.

RESULTS

The intergroup comparison of pH of the groups is shown in the figure 1 and 2 and table 1 and 2 for 0.3 and 0.5 mm diameter aperture tubes respectively.

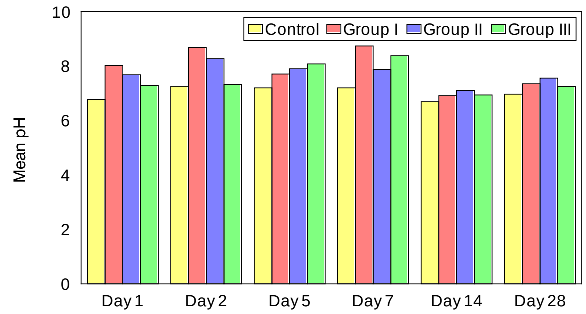

Figure 1. Mean pH of different groups at different time intervals for 0.3mm

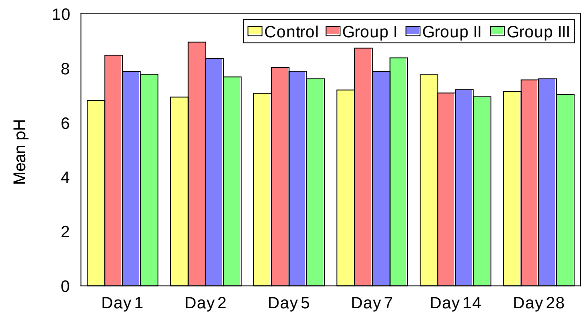

Figure 2. Mean pH of different groups at different time intervals for 0.5mm diameter aperture tube

Table 1. Intergroup Comparison of mean pH in different groups at different time intervals (0.3 mm)

SN	Time interval	Group I		Group II		Group III		Statistical Significance	
		Mean	SD	Mean	SD	Mean	SD	"F"	"p"
1	1st day	8.02	0.04	7.68	0.02	7.29	0.01	1696.20	<0.001
2	2nd day	8.68	0.09	8.27	0.11	7.33	0.07	212.84	<0.001
3	5th day	7.71	0.20	7.90	0.01	8.08	0.03	42.77	<0.001
4	7th day	8.74	0.01	7.88	0.01	8.38	1.16	3.98	0.052
5	15th day	6.91	0.01	7.11	0.01	6.94	0.01	891.56	<0.001
6	28th day	7.35	0.04	7.56	0.01	7.25	0.02	432.51	<0.001

The intergroup comparison of calcium ion release of the groups is shown in the figure 3 and 4 and table 3 and 4 for 0.3 and 0.5 mm diameter aperture tubes respectively.

It was observed that Sealapex has shown highest pH and calcium ion release, which was followed by Acroseal and then Endoflas F.S.

Table 2. Intergroup Comparison of mean pH in different groups at different time intervals (0.5 mm)

	Time interval	Group I		Group II		Group III		Statistical Significance	
		Mean	SD	Mean	SD	Mean	SD	"F"	"p"
1	1st day	8.48	0.13	7.88	0.01	7.78	0.11	181.71	<0.001
2	2nd day	8.96	0.06	8.36	0.02	7.68	0.06	411.38	<0.001
3	5th day	8.02	0.13	7.89	0.10	7.61	0.10	40.068	<0.001
4	7th day	8.74	0.01	7.88	0.01	8.38	1.16	1.798	0.225
5	15th day	7.09	0.01	7.21	0.01	6.95	0.01	1206.15	<0.001
6	28th day	7.57	0.02	7.61	0.01	7.04	0.02	1067.91	<0.001

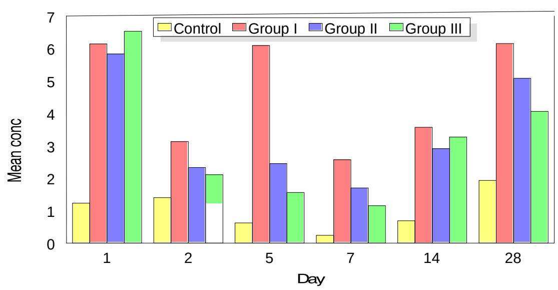

Figure 3. Mean calcium ion release of different groups at different time intervals for 0.3mm diameter aperture tubes

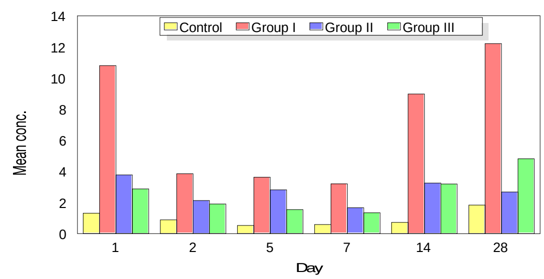

Figure 4. Mean calcium ion release of different groups at different time intervals for 0.5mm diameter aperture tubes

Table 3. Intergroup Comparison of mean release of calcium ions in different groups at different time intervals (0.3 mm)

SN	Time interval	Group I		Group II		Group III		Statistical Significance	
		Mean	SD	Mean	SD	Mean	SD	"F"	"p"
1	1st day	6.14	0.06	5.83	0.06	6.53	0.18	1661.61	<0.001
2	2nd day	3.13	0.06	2.33	0.03	2.11	0.04	710.42	<0.001
3	5th day	6.09	0.03	2.45	0.03	1.56	0.05	12501.4	<0.001
4	7th day	2.57	0.04	1.70	0.03	1.15	0.07	1499.27	<0.001
5	15th day	3.57	0.01	2.91	0.02	3.27	0.08	1389.72	<0.001
6	28th day	6.15	0.16	5.08	0.09	4.06	0.07	842.82	<0.001

Table 4. Intergroup Comparison of mean calcium ion release in different groups at different time intervals (0.5 mm)

	Time interval	Group I		Group II		Group III		Statistical Significance	
		Mean	SD	Mean	SD	Mean	SD	"F"	"p"
1	1st day	10.79	0.15	3.77	0.02	2.87	0.08	5057.14	<0.001
2	2nd day	3.85	0.03	2.13	0.11	1.91	0.05	1077.77	<0.001
3	5th day	3.63	0.04	2.81	0.06	1.55	0.06	2005.80	<0.001
4	7th day	3.20	0.01	1.67	0.08	1.34	0.03	1776.51	<0.001
5	15th day	8.97	0.20	3.25	0.07	3.19	0.04	1784.24	<0.001
6	28th day	12.21	0.11	2.68	0.04	4.81	0.06	12528.5	<0.001

DISCUSSION

The role of calcium hydroxide in endodontics cannot be overemphasized. Since the introduction of calcium hydroxide to Dentistry by Hermann[35], this medication has been used in dentistry to promote healing in many clinical situations.

Root canal sealers remain in contact with the periapical tissues for a longer period of time, therefore, their biocompatibility is of prime concern [23] also sealers play an important role in sealing the root canal system with entombment of remaining microorganisms and filling of inaccessible areas of prepared canals.[24] Therefore the use of calcium hydroxide as root canal sealers was proposed.[3] The first clinical use of calcium hydroxide as a root canal–filling material was probably by Rhoner in 1940[36]. The calcium hydroxide containing pulp-capping agent, Dycal (Dentsply-Caulk, Milford, DE), also became popular as a sealer among some clinicians in late 1970s.[37] The zinc oxide–eugenol based root canal sealers, long employed for root canal filling, display quite satisfactory chemical outcomes however, the biocompatibility of these materials is impaired by the presence of eugenol, which has cytotoxic properties[38] calcium hydroxide based root canal sealers were therefore introduced to meet the ideal requirements as regards the biological and physiochemical properties.

The biologic properties of calcium hydroxide root canal sealers can be explained by two mechanisms they are Bactericidal action due to high pH and Calcium hydroxide induces tissue healing by hard tissue formation or mineralisation. The antibacterial action of calcium hydroxide can be explained by the control of bacterial enzymatic activity.[9] The release of hydroxyl ions causes increase in pH of the medium, which inactivates the essential enzyme system of bacteria located in their cytoplasmic membrane responsible for metabolism and cellular growth. Loss of integrity of cell membrane of bacteria due to destruction of unsaturated fatty acids on phospholipids by the process of lipid peroxidation by hydroxyl ions. The mineralizing effect of calcium hydroxide can be explained by the Activation of Alkaline phosphatase: Calcium hydroxide activates tissue enzymes which favor mineralisation. Due to its elevated pH it activates alkaline phosphatase enzyme.[9] The abundance of calcium ions in the tissue participates in the activation of calcium dependant adenosine tri phosphatise.[23] Calcium reacts with tissue carbonic gas to form calcium carbonate crystals which serves as a nucleus for calcification and thus favours mineralization.[9]

Sealers containing calcium hydroxide will only perform these biologic and microbiologic actions if calcium and hydroxyl ion release occurs .[9] Thus the study was done to evaluate the pH and calcium ion release of calcium hydroxide based root canal sealers.

It is believed that the property of solubility is beneficial for $Ca(OH)_2$ based root canal sealer as both from a physiochemical and biological view point, since release of calcium ions into the tissue as well as high pH may lead to the elimination of bacteria remaining after instrumentation and to biological sealing of the root apex by the formation of mineralized tissue.[39] The property of solubility to liberate hydroxyl and calcium ions to bring about their biological effects can bring to mind that it decreases their sealing ability with time, as compared to other sealers and result in microleakage but it is believed to be minimal.[23] $Ca(OH)_2$ based root canal sealers have shown to provided a seal equal to or they leaked comparatively less as compared to zinc-oxide eugenol Sealer .[40] It has also been found that in a study of various combinations of intra canal medicaments and root canal sealers, that the sealing ability of sealapex Improved with calcium hydroxide intracanal medicaments .[41] One of the first commercially available calcium hydroxide root canal sealer is Sealapex. Several studies have reported the biologic advantages of sealapex. It has been found to be least cytotoxic when compared to N_2, Endomthesone, AH26, AH plus.[42] Sealapex was found to produce rapid healing of apical periodontitis as compared to CRCS and procosal sealer.[43]

Several root filling materials containing calcium hydroxide have been advocated and marketed. They are Endoflas F.S., CRCS, Apexit and Sealer 26. A comparatively new sealer has been introduced called Acroseal.

Thus the study was done to evaluate the pH and calcium ion release of Acroseal, Sealapex and Endoflas F.S.

The sealers were placed in eppendorf tubes for the uniformity of size of the samples as eppendorf tubes comes in a standardized size. The aperture of diameter 0.5 mm and 0.3 mm were made at the end of eppendorf tubes. This was done to simulate approximately the diameter of apical foramen of anterior teeth i.e.0.3mm and the total diameter of both the roots of posterior teeth will be approximately 0.5 mm.[44]

Eppendorf tubes were preferred over natural teeth because the difference in size of apical foramina and anatomic variations like cul-de-sacs fins and lateral canals etc. could have lead to various dentinal tubules opening onto the root surface and thus variable results could have achieved.[45]

To measure preciously the amount of calcium, the technique employed, has a great role. There are various methods that have been used in the past for the measurement of calcium. They are atomic absorption spectrophotometer (AAS).[26,46] fluometry, flame photometry, calcium ion selective electrode, complexometric titration[34] with EDTA, colorimetric method [31] and calcium measurement regent & spectrophotometer [23] In our study atomic absorption spectrophotometer was used for measuring Ca^{++} diffusion because of accuracy and ease of use. pH meter was chosen for our study for ease of experimental procedure and high accuracy.

The time intervals taken in our study to evaluate the release of calcium and hydroxyl ions, were 24 hours, 48 hours, 5th day, 7th day, 15th day and 28th day. They were taken to determine the pH and calcium ion release during setting and after setting. Sealapex has the setting time of 2-3 weeks which is more than the other two sealers, no changes were made to modify the experimental procedure to simulate clinical use. The weight of the sealer tube were not considered as it has been shown in previous studies that amount of leachable calcium is important for the biology effect as compared to the calcium content.[23]

The analysis of pH in different groups at different time intervals showed that mean pH of group I (sealapex) had highest pH, followed by Grp II acroseal, which was followed by (endoflas F.S.) in case of both the aperture 0.3 and 0.5.This result is in accordance with previous studies. [30, 39, 23,27, 29,47] On evaluating the calcium ion release again the mean calcium ion release of sealapex was seen to be significantly higher as compared to acroseal and endoflas F.S. in both the diameter tubes. This is again in accordance with the previous studies data. [39, 23, 27, 29, 47]

The excipient and additional ingredients even in micrograms at times do create some constraints that may even lead to differing results.

High release of calcium from sealapex can be explained by the intrinsic properties of the sealer. Sealapex has a poorly formed matrix and low dimensional liability. It also exhibits very high water absorption. Thus it becomes a porous material and permits a marked ingress of water. This water will facilitate continuous reaction between powder and binder. This high solubility of sealapex supports our finding of highest release of calcium and hydrogen ions.

Similarly, relatively lower amount of Ca^{++} and hydroxyl ion release of Acroseal can be explained by its relative insolubility as it has an epoxy matrix containing diglycidyle ether in bisphenol A with methanamine.

In case of Endoflas F.S. the free eugenol would have captured the calcium ions and made them unavailable to the surrounding medium. It also hampers the release of hydroxyl ions.[27] this explains to lowest calcium ion release and pH .

This study has evaluated the release and pH analysis by taking 0.3 and 0.5 mm apertures, it is important and relevant to state here that no study as per available literature, till date has taken this criterion which has been undertaken in this present study to simulate the openings of the apical foramen.

Hence a judicious comparison was tried to be done utilizing this criterion. The results definitely need long term research by increasing the number of samples. Yet they are important and relevant.

CONCLUSION

Thus in conclusion, evaluation of the results clearly depicts that highest pH and calcium ion release was shown by Sealapex followed by Acroseal which was followed by Endoflas F.S.This encourages the clinical usage of sealapex as it shows the highest pH and calcium ion release. This high pH and calcium ions concentration would result in higher antimicrobial activity and would help in the formation of biologic seal.

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