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Influence of Different Vehicles on Penetration of Calcium Hydroxide Pastes in Radicular Dentin - Evaluated Using Anthocyanin for the Colorimetric Analysis

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Abstract

Background: Antimicrobial activity of calcium hydroxide is due to the release of hydroxyl ions in an aqueous environment. Hydroxyl ions can penetrate biofilms, inhibit the inflammatory actions of endotoxins and help to dissolve organic tissue.

Aim: This in vitro study evaluated hydroxyl ion diffusion from calcium hydroxide pastes with different vehicles using a novel colorimetric method. In this study, anthocyanin dye was used to stain human tooth roots which changes color according to the pH over the alkaline range.

Materials and methods: Extracted single-rooted human permanent teeth were decoronated at the Cemento - enamel junction and root surface smoothened with abrasive discs. After achieving patency of the root canals biomechanical preparation was done using protaper rotary instruments. The roots were submerged in the dye for 48 hours to achieve a uniform stain followed by drying with paper points. The prepared canals were filled with Calcium Hydroxide pastes formulated with the vehicle of water (RC Cal), silicone oil (CalPlus) and Polyethylene Glycol

(ApexCal). The changes in the dye color from the canal wall were monitored using standardized digital

photography over a period of 3 weeks. All samples were subjected to statistical analysis and results obtained.

Results: APEXCAL showed highest change in dye color after 3 weeks (2.096 ± 0.54), followed by RC CAL and CALPLUS.

Conclusions: Hence, it was concluded that Polyethylene glycol as the vehicle may be used to achieve enhanced alkaline effects for inactivating microorganisms in the root canal.

Keywords: anthocyanin dye, calcium hydroxide, hydroxyl ions, intracanal medicaments, vehicle.

Introduction

The success of endodontic therapy depends on the extermination of microorganisms and disinfection of root canals by chemo mechanical preparation and interappointment intracanal medicament.[1] The need for intracanal medication is larger in those cases where microorganisms are resistant to routine therapy and in cases where the presence of pain or continuing exudate is evident.[2] Calcium hydroxide is considered as the universal intracanal medicament with a high pH (12.5-12.8).[3] Its antimicrobial activity is attributed to the diffusion of hydroxyl ions in an aqueous environment.[4]

The fatal effects of hydroxyl ions are mediated by various mechanisms, such as injury to the microbial cytoplasmic membranes, damage to DNA, constraining DNA replication, and restraining enzymatic activity, and disruption of cellular metabolism.[5] Various vehicles improve and influence the diffusion and dissociation of calcium hydroxide. Thus the aim of the study was to assess the influence of vehicles on penetration and availability of hydroxyl ions in calcium hydroxide.

Materials and Methods

Preparation of Roots

Thirty extracted single rooted teeth with completely formed apices and a single canal were collected from the Department of Oral And Maxillofacial Surgery. Teeth with resorption, intrinsic discolourations, root caries, fractures and developmental anomalies were excluded. The teeth were decoronated at the CEJ and root face smoothened with abrasive discs to obtain a flat coronal surface. Working length was determined with a #10 K-file and biomechanical preparation was done using Protaper Gold rotary instruments till F3. Irrigation with 2ml of 5.25% sodium hypochlorite was done between each instrument, followed by a flush of 5ml saline. 5ml of 17%EDTA was used as a final irrigant to remove the smear layer.

Preparation of Anthocyanin Dye

The anthocyanin dye was formulated by concocting red cabbage extract based on a procedure formulated by Pereira et al (2015) with few variations. 150g of red cabbage was cleaned, finely chopped and pureed along with 80ml of ethanol and water in the ratio 7:3 (volume). The pH was adjusted to 2 with a slow trickle of hydrochloric acid (1mol L^{-1}).This solution was stored at 4^{o}C in a dark place for 24 hours and then strained using a clean muslin cloth followed by centrifugation at 2000 rpm for 10 minutes.[6] The supernatant liquid procured was filtered through a #1 Whatman filter paper and this extract was neutralized by NaOH (2.5mol L^{-1}) to a pH of 7. The resultant filtrate was stored in a dark place at 4^{o}C until further use.

Root Staining With Anthocyanin Dye

The canals were dried with paper points and irrigated with 5ml of anthocyanin dye and all the roots were immersed in the dye for 48 hours to achieve a consistent purple stain.Each root was dried with paper towels and mounted on a glass surface with sticky wax to exhibit the coronal surface of the root for photographic analysis using a digital camera under constant conditions (uniform light, distance and exposure environment).

Placement of Intracanal Medicaments

The experimental samples were randomly divided into 3 groups (n=10)

GROUP I: received RC Cal (vehicle – water) as the intracanal medicament.

GROUP II: APEX CAL (PEG 400 as vehicle) was placed in the canals

GROUP III: CALPLUS (silicone oil as vehicle) was placed.

The medicaments were injected into the canals until excess extruded through the apex. The root apex was sealed with modeling wax. Each root was placed in individual vials containing anthocyanin dye to a level just below the coronal surface of the root. All the vials were placed in an incubator at 37^{o}C and 100% humidity for 3 weeks.

Recording Colour Changes Over Time

Digital photographs of the coronal surface of the tooth were clicked after 1, 7, 14 and 21 days under standardized conditions. The subsequent images were analyzed using Adobe Photoshop software to record the colour changes in the roots. The selected areas of interest were marked by a select tool and were superimposed on each sequential image of the same root. This enabled tracking the changes in colour of a particular area on the root in every image.

Statistical Analysis

One-way ANOVA test followed by Tukey's HSD Post hoc Analysis was used to compare the mean change in dye color.

Results

The mean distance from canal wall to change in dye color after 1, 2 and 3 weeks for all the groups are shown in table no 1 and represented in graph 1. Group II APEXCAL showed highest change in dye colour after 3 weeks (2.096 ± 0.54), Group I RC CAL showed a mean distance of 1.556 ± 0.115 and Group III CALPLUS showed the least distance of colour change at 1.290 ± 0.121.

Multiple comparison between different time intervals for APEXCAL group as shown in table 2 indicated that mean distance at 3rd week was significantly highest as compared to 1st & 2nd week at P=0.006 & P=0.04 respectively. This was followed by 2nd week where the mean distance was significantly higher as compared to 1st week at P=0.007. This infers that the mean distance from canal wall to change in dye color in APEXCAL group significantly increased from 1st week to 3rd week, with 3rd week having the highest and 1st week having lowest mean distance.

Comparison of mean distance (in mm) from canal wall to change in dye color b/w 3 groups at different time intervals using One-way ANOVA Test

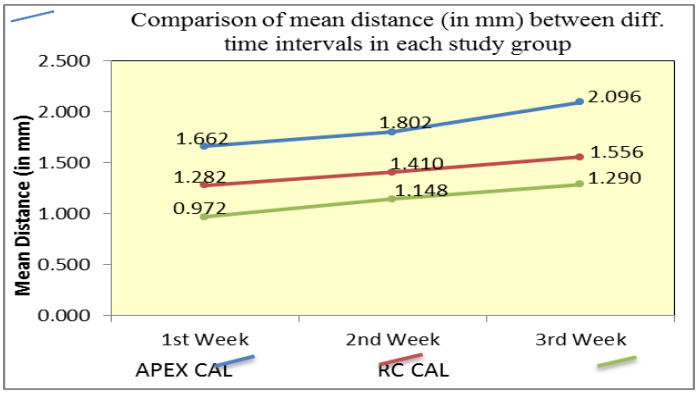
Time	Groups	N	Mean	SD	Min	Max	P-Value
1st Week	RC CAL	10	1.282	0.144	1.08	1.45	<0.001*
	APEXCAL	10	1.662	0.129	1.45	1.80	
	CALPLUS	10	0.972	0.233	0.67	1.24	
2nd Week	RC CAL	10	1.410	0.121	1.24	1.55	<0.001*
	APEXCAL	10	1.802	0.147	1.56	1.93	
	CALPLUS	10	1.148	0.178	0.95	1.35	
3rd Week	RC CAL	10	1.556	0.115	1.36	1.65	<0.001*
	APEXCAL	10	2.096	0.154	1.89	2.32	
	CALPLUS	10	1.290	0.121	1.16	1.44	

Table 1: Mean Distance of Change in Dye Colour From Canal Wall.

Multiple comparison of mean difference in distance (in mm) between different time intervals using Bonferroni's Post hoc Test

Groups	(I) Time	(J) Times	Mean Diff. (I-J)	95% CI of the Diff.		P-Value
				Lower	Upper	
APEXCAL	1st Week	2nd Week	-0.140	-0.219	-0.061	0.007*
		3rd Week	-0.434	-0.674	-0.194	0.006*
	2nd Week	3rd Week	-0.294	-0.576	-0.012	0.04*

Table 2: Mean difference in change in dye color between different time intervals in Group II.



Graph 1: Mean Distance of Colour Change from Canal Wall Between Different Time Intervals.

Discussion

Dissociation of calcium hydroxide is influenced by the vehicle used. It determines the ionic dissociation kinetics by bringing about solubilization, resorption or absorption of calcium hydroxide at various rates in the periapical tissues and within the root canal.[7]Many commercial calcium hydroxide pastes use water as the solvent, resembling the original formulation back in 1920. Other agents have been mixed with distilled water, saline solution, glycerin, polyethylene glycol (PEG), silicone oil, propolis, anesthetic solution, chlorhexidine, camphorated monochlorophenol which perform as thickeners and increase the viscosity.[8]

The high molecular weight of common vehicles minimizes the dispersion of Ca(OH)2 into the tissues and maintains the paste in the desired area for longer periods of time.[9]

Anthocyanins are a group of phenolic compounds that belong to the flavonoid family and are responsible for red, purple and blue hues of plant fruits, flowers and leaves.[10] Red cabbage (Brassica oleraceae) is one of the sources of anthocyanins exhibiting color over a very broad pH range. The colors vary from red at low pH to blue and green at high pH. Thus, this broad color change makes it attractive for application as natural pH indicators.[11]

The results of this study elucidated significant difference in the release of hydroxyl ions from different calcium hydroxide pastes with different vehicles.

In the current study, APEXCAL with PEG 400 as the vehicle showed the maximum penetration through the dentinal tubules and for a longer period of time with substantial increase in availability of hydroxyl ions deeper in the dentin. There was a sustained release and dissociation of hydroxyl ions when compared to other calcium hydroxide formulations tested.

Chemically, this is due to the large number of ethylene oxide groups along its backbone, which allows PEG to form complexes with metal cations, including calcium ions. [12]

Such binding of calcium ions drives the dissociation of calcium hydroxide, thus releasing more free hydroxyl ions.

CALPLUS is a premixed oil based calcium hydroxide with iodoform. In the present study CALPLUS showed the least ability to dissociate deep into the dentin.

Conclusion

Within the limitations of the study calcium hydroxide with PEG as the vehicle may be used to achieve enhanced alkaline effects for inactivating microorganisms in the root canal. The results of the present study also show the potential of anthocyanin staining as a method which can show pH fluctuations in root dentine caused by endodontic medicaments.

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