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SPECTROPHOTOMETRIC ANALYSIS OF HYDROXYL ION AND CALCIUM ION DIFFUSION FROM CALCIUM HYDROXIDE AFTER ULTRASONIC ACTIVATION IN SIMULATED EXTERNAL ROOT RESORPTION-AN IN VITRO STUDY

Dental Science

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ABSTRACT

AIM: The aim of the present in vitro study was to analyze the hydroxyl ion as well as the calcium ion diffusion from Calcium Hydroxide pastes after ultrasonic activation, in simulated external root resorption

MATERIALS AND METHODOLOGY : For this in vitro study, single rooted sound human maxillary anteriors and premolars were used . To simulate external root resorption, cavities (1.4mmin diameter and 0.7 mm in depth) on the buccal surface of the roots were create The samples prepared were then divided into 3 groups according to the vehicle used .In GROUP 1 the vehicle was an aqueous vehicle-distilled water. In GROUP 2, the vehicle was a viscous vehicle – Propylene glycol. .In GROUP 3, vehicle was 2% chlorhexidene.. The root canals were filled with the paste using the Lentulo Spiral, at the standard working length of 14mm. The specimen were evaluated for PH and calcium ion after 24 hours, 7, 14, and 28 days .

RESULT: The results showed that all the experimental groups showed diffusion of hydroxyl and calcium ions. The pH of all these preparations was observed to be higher. The ion diffusion in all three groups was improved significantly when ultrasonic activation was used to agitate the medicament. The choice of vehicle seems to affect the efficacy of medicaments.

CONCLUSION : The present study concluded that the choice of vehicle seems to affect the efficacy of medicaments. The agitation of medicament with ultrasonic apparatus significantly improves the ion diffusion rendering it more effective.

KEYWORDS

calcium hydroxide root resorption ultrasonic activation

INTRODUCTION

Root resorption is described as a multifactorial process that results in the loss of the dentoalveolar complex. The causes may vary, depending on local or systemic factors. Root resorption types can be categorized mainly as internal and external; external inflammatory root resorption (EIRR) can be progressive unless treated.[1,2]

External resorption can exist as external surface resorption, external inflammatory resorption, external replacement resorption, invasive cervical resorption and transient apical breakdown

In endodontics, calcium hydroxide (CH) has been used in the treatment of root resorption as a component of root canal–filling cements and intracanal dressings.[5,6,9,11,14]When used as an intracanal dressing, CH has been combined with different vehicles in order to provide a paste-like consistency.[8,9,15-18]

The pH level of the paste can vary according to the vehicle used. The medicament vehicle plays a very important role in the overall disinfection process because it determines the velocity of ionic dissociation, causing the paste to be solubilized and resorbed at various rates by the periapical tissues and from within the root canal.[8]

There are three main types of vehicles used:.[8,9]

(1) Water soluble substances like water, saline, local anesthesia, ringers solution etc.
(2) Viscous vehicles like glycerin, polyethylene glycol, and propylene glycol (PG)
(3) Oil-based vehicles like olive oil, silicone oil, camphor, eugenol, metacresylacetate

It has been stated that calcium is important for the repair of external root resorption. Calcium can activate adenosine triphosphate, which is involved in cell migration, differentiation, and mineralization .Another author suggested that the calcium release reacts with carbon gas to form calcium carbonate, thus favouring mineralization. . Ultrasound is used in different endodontic procedures, ranging from coronal opening to endodontic surgery and it has been used for the removal of CH in the root canal . Ultrasound promotes a greater agitation of irrigating solutions, thus penetrating in an area of anatomic complexity and the dentinal tubules and resulting in a greater cleaning ability. Possibly, the agitation of Calcium Hydroxide paste can favour the penetration of particles inside the dentinal tubules, providing an increase in the pH level and calcium release in the exterior of the root.[12,13]

This in vitro study was conducted to evaluate the hydroxyl ion and calcium ion diffusion after ultrasonic agitation from Calcium Hydroxide pastes in simulated external root resorption.

MATERIALS AND METHODOLOGY

SAMPLE COLLECTION

30 single rooted sound human maxillary anteriors and premolars extracted for orthodontic or periodontal reasons, were used in this study.

Consent form along with subject information was given to patients explaining the entire procedure. **(Strict anonymization was observed)**

The teeth were cleaned of soft tissue and debris and stored in distilled water.

SPECIMEN PREPARATION

The teeth were decoronated by using a diamond disc mounted on a mandrel with micromotor and straight handpiece and root length of 14mm was maintained.

Endodontic access cavities were prepared by using air-rotar handpiece and sterile diamond burs and pulp tissue was extirpated by using #10 K –file.

The working length was confirmed by using #15 K-file, until it is seen at the apex and then subtracting 0.5 mm from its length. To simulate external root resorption, cavities on the buccal surface of the roots (1.4mm in diameter and 0.7 mm in depth) were created at 5 mm from the apex by using a 1.4-mm high-speed carbide round bur

The procedure was followed by instrumentation of the canals by using ProTaper Rotary system. Specimens were held in a table mounted vise and biomechanical preparation was carried out in the following sequence Sx S1 S2 F1. The canal irrigation was performed with 5ml of 5.25% sodium hypochlorite. On completion of the instrumentation, canals were irrigated again with 5 mL distilled water. The root canals were dried with paper points. The samples, thus obtained were externally made impermeable using a nail varnish , except for the

defected area, and divided into three main groups (n=20) on the basis of vehicles used , distilled water , propylene glycol and 2% chlorhexidine.

The groups were subdivided into one subgroup, subgroup A, (ultrasonic activation) The orifices of all samples were sealed and made impermeable with nail varnish and cavitG after filling the canals with the calcium hydroxide paste.

GROUP 1 (n=10)
(Distilled water and calcium hydroxide)

SUBGROUP 1A (N=10)
Calcium hydroxide measuring 1gm with 0.4ml of distilled water as a vehicle was mixed on a glass slab using a metal spatula in a paste like consistency. The canals of the specimens prepared were filled using a lentulospiral. The ultrasonic activation was done using an ultrasonic jet (Satelec p5 booster ultrasonic unit) for a total of 1 minute (3 cycles of 20 seconds each). The calcium hydroxide paste was placed after each cycle. The samples were individually immersed in airtight glass vials containing 20ml of distilled water(pH=6.8)

GROUP 2 (N=10)
(propylene glycol and calcium hydroxide)

SUBGROUP 2A (N=10)
In this group canals were filled with calcium hydroxide and propylene glycol as a vehicle, using a lentulospiral. After filling, the paste was activated using an ultrasonic jet. The procedure for ultrasonic activation was same as that in group 1.

GROUP 3 (N=10)
(Chlorhexidine and calcium hydroxide)

SUBGROUP 3A (N=10)
In this group, canals were filled with calcium hydroxide and 2% chlorhexidine as a vehicle, using a lentulospiral. After filling, the paste was activated using an ultrasonic jet. The procedure for ultrasonic activation was same as that in group 1 and group 2

SPECIMEN EVALUATION
The specimens were evaluated after 24 hours, 7, 14, and 28 days. The pH level of the leachate in the glass vial was measured by means of a pH meter and calcium ion release was measured by means of a UV spectrophotometer at 220nm.

PH MEASUREMENT:
The digital pH meter Lab man Pvt limited, India, was used to measure the pH.

The pH was determined by placing the electrode probe into each sample bottle and noting the pH reading. For each sample the pH value were recorded on 24 hours, day 7, day 14 and day 28.

CALCIUM ION DIFFUSION MEASUREMENT:
UV visible spectrophotometer (THERMOSCIENTIFIC GENESYS 10uv) was used to measure the calcium ion concentration at 220nm wavelength. The instrument was calibrated at zero absorbance using a blank solution. Absorbance values of a standard solution of known concentration were then obtained. Calcium release was then calculated using the line equation of the standard curve after absorbance of each sample was noted, on 24 hours, day 7, day 14 and day 28.

The calcium ion and hydroxyl ion readings were then subjected to statistical analysis.

STATISTICAL ANALYSIS
Descriptive and inferential analyses were carried out in the present study.

ANOVA test was used to compare the within the group and between group variances among the study groups.

Analysis of variance of these three study groups at a particular time interval revealed the differences among them.

ANOVA provided "f" value , where a higher "f" value depicted a higher intergroup difference.

Level of significance was fixed at p=0.001 and any value less than or equal to 0.001 was considered to be statistically significant.

RESULT
The results showed that:

- All the experimental groups showed diffusion of hydroxyl and calcium ions. The pH of all these preparations was observed to be higher.
- The ion diffusion in all three groups was improved significantly when ultrasonic activation was used to agitate the medicament.
- The choice of vehicle seems to affect the efficacy of medicaments. In this study Group 2 (propylene glycol vehicle) showed maximum ion diffusion followed by Group 3 (chlorhexidine vehicle) followed by Group 1 (distilled water vehicle).
- All three groups showed a gradually rising calcium ion concentration. There was a statistically significant difference in ion diffusion between all the groups from day1 to day 28. Group 1 showed maximum ion release at 24 hours however there was no significant increase in the release of calcium ions at 7, 14 and 28 day

TABLE 1: Mean pH releases at different intervals in samples with ultrasonic activation

Groups	N	24 hours/day1		Day 7		Day 14		Day 28	
		MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
GROUP 1	10	10.610	0.100	10.854	0.152	10.918	0.059	11.261	0.088
GROUP 2	10	9.662	0.155	10.304	0.083	10.791	0.131	11.069	0.067
GROUP 3	10	8.428	0.156	9.328	0.111	9.699	0.154	10.101	0.047
ANOVA (F)		616.92		422.35		301.67		808.69	
"P"		<0.001*		<0.001*		<0.001*		<0.001*	

mean ph release at 24 hours, day 7, day 14 and day 28 in study groups on the basis of ultrasonic activation ,using ANOVA test.p value*p<0.001 Statistically Significant, p>0.001 Non Significant. The p value in group 1(distilled water), group 2 (propyleneglycol) and group 3 (chlorhexidine) was less than 0.001 , which interprets a highly significant difference between groups with ultrasonic activation

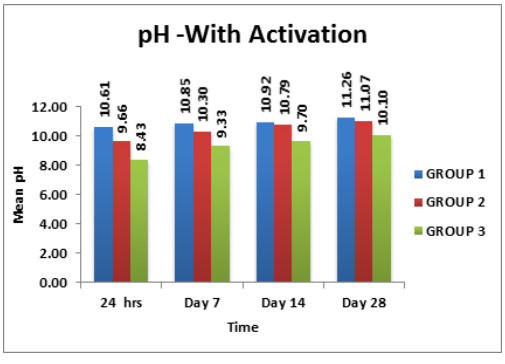

FIGURE.1:This bar diagram indicates the mean ph release in the study after ultrasonic activation at 24 hours day 7 day 14 and day 28.

TABLE 2 : Mean calcium releases at different intervals in samples with ultrasonic activation.

Groups	N	24 hours		Day 7		Day 14		Day 28	
		MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
GROUP 1	10	6.288	0.027	6.496	0.117	6.509	0.066	6.848	0.052
GROUP 2	10	6.037	0.012	7.039	0.029	8.721	0.051	9.044	0.048
GROUP 3	10	6.028	0.018	7.310	0.100	7.478	0.050	8.164	0.028
ANOVA (F)		538.22		208.8		3894.13		6290.45	
"P"		<0.001*		<0.001*		<0.001*		<0.001*	

This table shows the mean calcium ion release at 24 hours , day 7, day 14 and day 28 in group 1 group 2 and group 3 on the basis of ultrasonic activation ,using ANOVA test. p value*p<0.001 Statistically Significant, p>0.001 Non-Significant. The p value in group 1(distilled water , group 2(propyleneglycol) and group 3 (chlorhexidine) was less than 0.001 , which interprets a highly significant difference between groups with ultrasonic activation

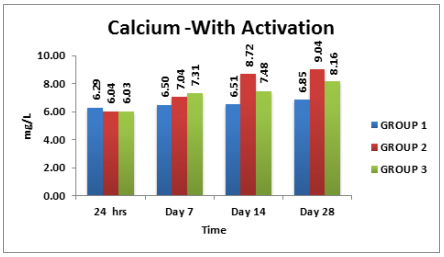

FIGURE 3:This bar diagram indicates the mean calcium ion release in the study groups after ultrasonic activation at 24 hours day 7 day 14 and day 28.

DISCUSSION

Root resorption is a physiological or pathological process that results in loss of hard tissue. The roots of the permanent teeth are not normally resorbed as they are protected by predentine and odontoblasts, on the pulpal side, and periodontal ligament, precementum, and cementoblasts on the root surface. If predentine or precementum is damaged or removed for some reason, resorption will result.[5,9]

External inflammatory root resorption can be efficiently managed by treating the underlying etiology. However, complete elimination of microorganisms from the root canal system is not possible simply with biomechanical preparation of the root canal.[2,6.]

Calcium hydroxide has emerged as a versatile and popular choice of intracanal medicament, since its introduction by Hermann in 1920, this material has come a long way in endodontics as the most suggested and investigated material because of its antibacterial, anti resorptive, tissue dissolving and osteogenic potential.[10.]

When used as an intracanal dressing, CH has been combined with different vehicles in order to provide a paste-like consistency. The vehicle plays an important role in the overall process.[15-18]It determines the velocity of ionic dissociation causing the paste to be solubilized and resorbed at various rates from the root canal as well as the periapical tissues. It has a direct relationship with the concentration and antibacterial activity. Thus an ideal vehicle should allow a gradual and slow release of calcium and hydroxyl ions.[8]

There are three main types of vehicles:[8]

- Water-soluble substances such as water, saline, anaesthetic solutions, carboxymethylcellulose,methylcellulose and Ringers solution.
- Viscous vehicles such as glycerine, polyethyleneglycol (PEG) and propylene glycol.
- Oil-based vehicles such as olive oil, silicone oil, camphor (the oil of camphorated parachlorophenol), some fatty acids (including oleic, linoleic, and isostearic acids), eugenol and metacresylacetate.
- The results of the present study revealed that, irrespective of the vehicle used in the calcium hydroxide paste, the ultrasonic activation groups showed maximum diffusion of hydroxyl and calcium ions. As far as the effect of vehicle is concerned, it was observed that the calcium hydroxide paste with propylene glycol as the vehicle showed maximum diffusion. It was also observed that the time for which the medicament was kept in the canal had influence on the diffusion of calcium and hydroxyl ions and diffusion was directly proportional to the time period for which medicament was in the canal.
- In all the experimental groups, there was a consistent release of ions during the given time period. However, Ultrasonic activation, regardless of the vehicle used, significantly ($P < .001$) favored a higher calcium and hydroxyl ion release from day 1 till day 28. In comparison of the groups on the basis of ultrasonic activation, a significantly ($P < .001$) higher pH level and calcium ion release was observed in the groups 1A, 2A, 3A (with ultrasonic activation) in all periods evaluated as observed in tables.
- The reason for the better diffusion in ultrasonic activation groups could be explained on the basis of mechanism of ultrasonic activation. The agitation of the calcium hydroxide paste using ultrasonic can favor the penetration of the particles inside the dentinal tubules providing an increase in the pH level and calcium release in the exterior of the root.
- The results showed that for all periods analyzed, the pH level was found to be higher when the CH paste was activated with ultrasound. Calcium release was significantly greater ($P < .05$) using ultrasonic activation after 7 and 30 days. They concluded that the ultrasonic activation of CH pastes favored a higher pH level and calcium release in simulated external root resorptions.[20]
- When the effect of vehicles was compared, it was observed that Group 2 with propylene glycol vehicle showed maximum diffusion of calcium ions and hydroxyl ions throughout the time intervals tested in the study. Group 3 with chlorhexidine vehicle showed less diffusion than propylene glycol group but more than that of Group 1 with distilled water for the tested time intervals. The distilled water vehicle group showed maximum diffusion of ions only for first 24 hours and even then diffusion continued to occur, it was not as sustained as propylene glycol group and chlorhexidine group.
- Propylene glycol has a hygroscopic nature,[8,17] which permits the absorption of water and ensures a good sustained release of calcium hydroxide for long periods. The high molecular weight of the propylene glycol minimizes the dispersion of calcium hydroxide into the surroundings and maintains the paste in desired area for longer intervals; this factor prolongs the action of the paste, and Calcium and hydroxyl ions will be given off at lower velocity.
- In the present study the diffusion of calcium and hydroxyl ions in propylene glycol group was best because of its hygroscopic nature and high molecular weight. Another explanation for better diffusion could be attributed to the lower surface tension of propylene glycol. The less surface tension of propylene glycol prevents itself from contacting the resorptive defect and hence the ions penetrate easily in the defect.
- In our study, Group 3 (with chlorhexidine) showed sustained release of calcium and hydroxyl ions from 24 hours to 28 days time interval. The diffusion of these ions was better than Group1 (with distilled water) but less than Group 2 (with propylene glycol).
- The reason for better diffusion with Chlorhexidine is because it is more effective at alkaline than acidic pH. In our study it is evident that the pH values for all the groups were above 6.8 indicating an alkaline environment throughout the study period. In this alkaline medium there could be an increase of the ionization capacity of the chlorhexidine molecule.[16.17]resulting in its better performance.The initial high pH in distilled water vehicle may be due its ability to promote a high degree of solubility of the Calcium hydroxide powder. Another reason for the initial rise of pH, and later drop may be due to the formation of insoluble calcium carbonate crust which blocks the dentinal tubules resulting in decrease in the pH and stabilization of ionic release.[16,18,19]
- From the results of present study, we can conclude that the choice of vehicle seems to affect the efficacy of medicaments. When an aqueous vehicle is used (distilled water) initial diffusion is rapid but the ion concentration does not increase. The viscous vehicles (propylene glycol) exhibit sustained release of ions and may remain in the canal for longer time periods. Chlorhexidine can be considered a good vehicle especially in refractory or failure cases because of its ability to maintain high concentration of calcium and hydroxyl ions in alkaline pH and proven antimicrobial properties. The agitation of medicament with ultrasonic apparatus significantly improves the ion diffusion rendering it more effective. We also suggest that the medicament be placed in the canal for approximately 14 to 28 days for desirable clinical outcome

CONCLUSION

Within the limitations of this in vitro study, following observations were made:

- All the experimental groups showed diffusion of hydroxyl and calcium ions. The pH of all these preparations was observed to be higher. The ion diffusion in all three groups was improved significantly when ultrasonic activation was used to agitate the medicament. All three groups showed a gradually rising calcium ion concentration. There was a statistically significant difference in ion diffusion between all the groups from day1 to day 28. Group

1 showed maximum ion release at 24 hours however there was no significant increase in the release of calcium ions at 7, 14 and 28 days Hence we conclude that the choice of vehicle seems to affect the efficacy of medicaments. When an aqueous vehicle is used (distilled water) initial diffusion is rapid but the ion concentration does not increase. The viscous vehicles (propylene glycol) exhibit sustained release of ions and may remain in the canal for longer time periods. The agitation of medicament with ultrasonic apparatus significantly improves the ion diffusion. The effectiveness of the medicament is better if it is kept in the canal for longer time period.

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