RESEARCH





# EVALUATION OF HYDROXYL ION DIFFUSION THROUGH DENTIN AFTER ENDODONTIC REINSTRUMENTATION AN IN VITRO STUDY

Research

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## ABSTRACT

**AIM:** This study was undertaken to evaluate the diffusion of hydroxyl ions through dentin after obturation with Gutta percha and various sealers and subsequent re-instrumentation.

**MATERIAL AND METHODOLOGY:**Sixty extracted single rooted maxillary incisors were used. The canals were cleaned and shaped till size 50 K-file. 3% Sodium Hypochlorite and RC Prep was used during cleaning and shaping. The samples were divided into 4 groups, stored in 10 ml of freshly prepared distilled water and pH of the distilled water was measured after 7 days-( E1).The canals were filled with calcium hydroxide and the pH was measured after 7, 14, 21, 28 days- pH 1. They were later obturated with Gutta percha and sealers, labelled as Group A- Endoflas, Group B- Endoseal MTA, Group C- Resino Seal and Group D- Apexit Plus. The pH was measured (F). The obturating material was removed and the pH was measured after 7 days-( E3). The samples were re-instrumented and the pH was measured ( E4). The empty canals were filled with calcium hydroxide and the pH was measured as (pH 2) at 7, 14, 21, 28 days. Statistical analysis was done using the Kruskal Wallis, Mann-Whitney U test and two way ANOVA.

**RESULT:**The results showed a statistically significant difference between the average values of pH 1 and pH 2 showing a rise in pH for pH 2 indicating in hydroxyl ion diffusion. The pH showed variations for different sealers showing difference in diffusion of hydroxyl ions with maximum values for Zinc Oxide Eugenol based sealers and least for resin based sealers.

**CONCLUSION:**Hence from this study it can be concluded that re-instrumentation increased the diffusion of hydroxyl ions from the dentin and the type of sealers obturating the canals did alter the diffusion.

**Keywords:**calcium hydroxide, re-instrumentation, hydroxyl ion diffusion.

## INTRODUCTION

The bacterial elimination is an important aspect for the successful outcome of treatment of primary and secondary infections before obturation has to be completed in the canals. However, persistence of microorganisms during the procedure of obturation, especially if they penetrate into the canals, increases the risk of treatment failure.[1] The bacteria associated with secondary infection is mostly Enterococcus faecalis. Calcium hydroxide has been long used as an intra- canal medicament for the elimination of bacteria.[2,3] The main reason for the antimicrobial property of Ca(OH)2 is the high pH in the range of 12.5-12.8. 4 This high pH of $Ca(OH)_2$ is attributed to the release of hydroxyl ions in the canals.1 Dentin permeability has a very important role to play in the diffusion of hydroxyl ions and hence the antibacterial effect. The diffusion of hydroxyl ions depends on the period of use and also on the diameter of the dentinal tubule.[4] Studies show that sealers can penetrate to different depths based on the properties of the sealer and dentine permeability.[5] There are very few studies which evaluate the diffusion of hydroxyl ions through dentin after retreatment. The purpose of this study was to evaluate the hydroxyl ion diffusion through dentin after endodontic re-instrumentation using various sealers.

## MATERIAL AND METHODOLOGY

Intact tooth extracted for orthodontic treatment or periodontal conditions were included in the study.

### Exclusion criteria:

Teeth with caries, calcified, extra or curved canals, external or internal resorption developmental anomalies were excluded in the study.

Sixty freshly extracted single rooted Maxillary Incisors were collected. The experimental teeth were then placed in 3% Sodium hypochlorite and stored in saline. Radiographs were taken to verify the presence of a single canal. The teeth were sterilized in an autoclave for 20 minutes at 120ºC following which the access cavity was made using a high speed air turbine handpiece and endo-access bur. The length of each tooth was measured using size 10 K file in the root canal until its tip was visible through the apical foramen. The canals were prepared using standardised technique using K- files, 1 mm short of the apex. The apical stop was created using 50 K-file at the apex. Finally, coronal enlargement was done with numbers 1, 2 and 3 Gates-Glidden burs. All this while, use of RC prep was advocated for cleaning of the canals. Intermittent irrigation was done with 2ml of 3% Sodium hypochlorite. Final rinse was done with 10 ml of distilled water to remove any traces of Sodium hypochlorite. Diamond burs and periodontal curettes were used for the removal of cementum layer. The samples were then stored in fresh distilled water for 24 hours. After 24 hours the canals were dried with paper points and external surface was dried with gauze. The coronal access was temporised with Cavit G. The apical third of the tooth was sealed by applying Dentin bonding agent and light cured.

### pH measurements before obturation

All the samples were randomly divided into 4 groups (n=15) and kept in plastic containers with freshly prepared 10 ml distilled water for 7 days.
Each of the containers were assigned an alphabet – A, B, C, and D; and the pH of distilled water was measured– Empty 1(E1) .
This was followed by removal of the temporary restoration, drying the outer surface with gauze and the inner with paper points. The apical third was again sealed with a dentin bonding agent externally and light cured. ApexCal (Calcium hydroxide with polypropylene glycol vehicle) was placed in the canal. The samples were restored with Cavit G and placed in respective cleaned containers with 10 ml of freshly prepared distilled water. The pH reading of distilled water was taken at 7, 14, 21, 28 days (pH1).
After 28 days the Ca(OH)2 was taken out with the help of files and irrigation was done with Sodium hypochlorite and distilled water. The samples were stored in distilled water for 24 hours for the neutralisation of Sodium hypochlorite. The canals were dried with paper points, sealed with Cavit G and the samples were placed in their respective containers with 10ml of freshly prepared distilled water for 7 days. The pH of the distilled water was measured -Empty 2 (E2).

### pH measurements after obturation

The samples were then obturated with different sealers for different groups –
Group A- (n=15) Endoflas and Gutta Percha
Group B- (n=15) Endoseal MTA and Gutta Percha

Group C- (n=15) Resinoseal and Gutta Percha
Group D- (n=15) Apexit Plus and Gutta Percha

The obturation was done using the Lateral Condensation technique. The sealers were placed according to the manufacturer's instructions. The teeth were then washed with distilled water and stored in their respective containers with 10ml of freshly prepared distilled water for 7 days. The pH of distilled water was measured again- filled (F).
After 7 days the obturating material was removed using H-files and an organic solvent, GP Cleanse. The canals were irrigated thoroughly, dried with paper points and the samples were temporised. The samples were placed in their respective containers and then the pH reading of the distilled water was taken 7 days later – Empty 3(E3).
The samples were re-instrumented till size 70 K-file at the apex. The canals were cleaned with RC Prep and irrigated with Sodium hypochlorite. The final irrigation was done with distilled water. The samples were stored in distilled water for 24 hours and then dried with paper points and sealed with Cavit G. The samples were kept in their respective containers with 10ml of freshly prepared distilled water and the pH of distilled water was measured after 7 days - Empty 4 (E4).
The canals were filled with ApexCal after the canals were dried and temporised. The samples were placed in their respective containers with 10 ml of freshly prepared distilled water and the pH was measured at 7, 14, 21, 28 days (pH 2).
The pH was measured with a pH meter which was pre-calibrated at pH 7. The measured pH values were subjected to statistical analysis. Three samples from each group at the end of the study were sectioned and evaluated under a microscope for the presence of cementum.

## RESULTS

SPSS for Windows version 20.0® was used for the statistical analysis of this study. The analysis was done using the Kruskal Wallis, Mann-Whitney U test, two way ANOVA.

### pH measurement before root canal obturation

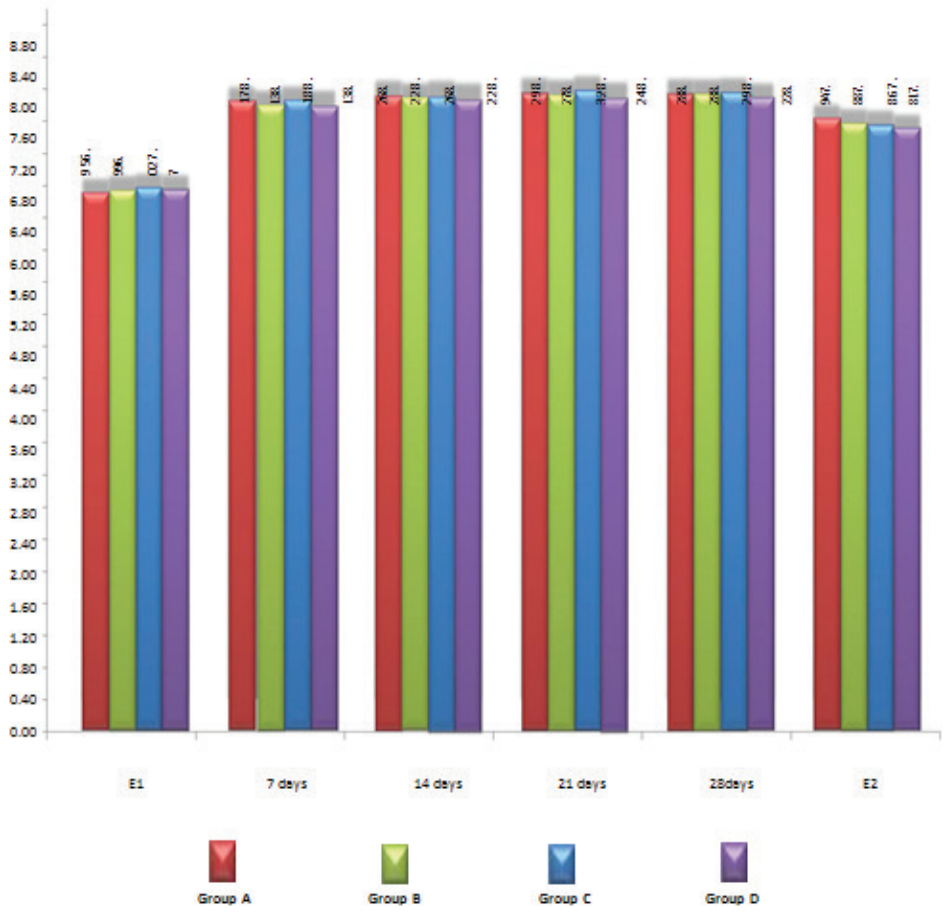
According to the statistical analysis it was seen that there was a rise of pH from E1 to pH 1, but the pH difference was the same in all the groups as seen in Graph 1. There was no statistically significant difference between any 4 groups in pH 1 as the p-value was more than 0.05 by Kruskal-Wallis Test .
A rise in pH was seen on the 7 day of calcium hydroxide application, with mild increase on day 14 and then remaining at the same level for day 21 and 28 for values of pH 1.The pH had a slight decrease from the pH 1 values for values of E2 (Graph 1). pH measurement after root canal obturation .The pH goes on decreasing for values of E3 and E4. There is tremendous increase seen in the values for pH 2 for all the groups as compared to E3 and E4 signifying an increase in the hydroxyl ion diffusion as seen on the Graph 2. A sharp increase is seen on pH measurements on day 7 and day 14 which later on maintains its level for day 21 and 28.

### Comparing values of pH for pH 1 and pH 2

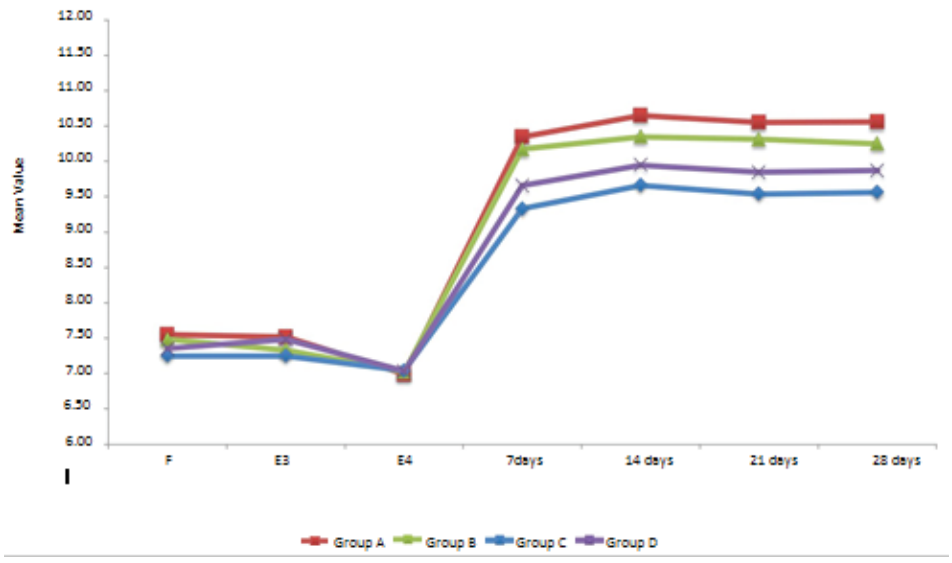
When comparing the values for before re-instrumentation (pH 1) and after reinstrumentation values (pH 2) there was a sharp increase in the pH (Graph 3). This sharp increase was attributed to the increase in hydroxyl ion diffusion from the samples after the teeth were re-instrumented. This was confirmed by the p-values of less than 0.001 for all the groups at different time periods for pH 2 .

### pH comparison of all 4 groups after obturation and calcium hydroxide placement

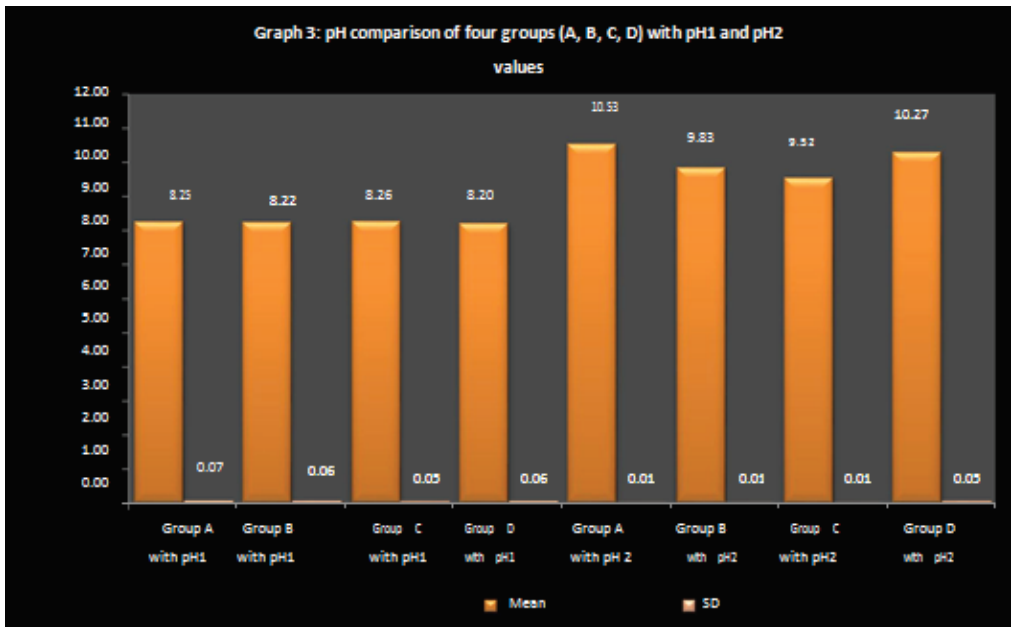
On evaluation of by Mann Whitney U test it was seen that the comparison between all the groups showed high significance between each other for values of pH 2 at different time periods. Graph 2 elaborated that the rise in pH was maximal for Group A (obturation with Gutta Percha and Endoflas) followed by Group B (Endoseal MTA), Group D (Apexit Plus) and lastly Group C (Resino Seal). This showed that the diffusion of hydroxyl ions was maximum for group obturated with Endoflas followed by Endoseal MTA,Apexit plus & least for resinoseal.




Graph 1: Comparison of pH values in four groups (A, B, C, D) at E1, pH 1- 7 days, 14days, 21 days, 28 days and E2




Graph 2 :Comparison of pH values of four groups (A, B, C, D) at F, E3, E4 and pH2 7 days,14 days,21 days,28 days




Graph 3: pH comparison of four groups (A, B, C, D) with pH1 and pH2 values

## DISCUSSION

Endodontic therapy is a sequence of treatment for the infected pulp of a tooth which results in the elimination of infection and the protection of the decontaminated tooth from future microbial invasion.[6] The need to remove bacteria lodged deep inside the dentinal tubules becomes necessary as its presence might lead to re-infection of the root canal system.[1] The use of Calcium hydroxide was potentiated for removal of such bacteria from the root canal system. The permeability of dentin is affected by the presence of cementum and smear layer.[7] The smear layer produced after instrumentation leads to decrease of permeability and after its removal with EDTA and Sodium hypochlorite enhanced the diffusion of hydroxyl ions.[8,9] To measure the diffusion of hydroxyl ions RC Prep and Sodium hypochlorite was used along with removal of cementum in this study as reported in previous studies.[4] In this study ApexCal is used which contains Calcium Hydroxide and propylene glycol as the vehicle.[10,11] Apexit Plus is a two component material , which sets by complex formation. Free Calcium ions react with the negatively charged oxygen atoms of phenolate and the carbonyl groups to form a chelate complex. In a disalicylate, $Ca(OH)^2$ does not react at an intramolecular but at an intermolecular level, hence the two salicylate groups provided by two different dimeric molecules will be linked by one calcium ion. As a result, an ionic polymer link is formed.[12,13] Endoflas,has the ability to disinfect dentinal tubules and hard-to-reach accessory canals that cannot be disinfected or cleansed mechanically.[14] Endoseal MTA, a finely pulverized pozzolan-based MTA has favourable mechanical characteristics such as fast setting time of around 4 min, higher washout resistance than other commercially available MTAs, and biologic effects including biocompatibility, mineralization potential, and odontogenic effect of the pozzolan cement had been previously reported.[15,16,17,18,19] The apical third of teeth have multiple apical foramen, lateral canals and ramifications; to prevent diffusion of hydroxyl ions through this way the apical third should be sealed.[20,21] Hence in the present study the apical third of the root was sealed with dentin bonding agent so that diffusion through the dentinal tubules can be measured. E. faecalis is one of the major organisms responsible for causing failure of endodontic therapy as it deeply penetrates into the dentinal tubules to a depth of 500 microns.[22] During retreatment procedure it is seen that the dentinal tubules might be obstructed by remaining Gutta Percha and sealer. Hence re-instrumentation seems necessary for the removal of inner dentinal wall layers so that the patency of these dentinal tubules is established.[23]

This study was conducted with an aim of determining the diffusion of hydroxyl ions through dentin after re-instrumentation by measuring the pH. A similar methodology was followed by dos Santos et al. Although, the teeth and canals were washed with distilled water, the possibility of Sodium hypochlorite solution or $Ca(OH)_2$ paste or root canal sealer might have increased the pH.[20] Comparing the values of E1 with the values of pH 1, an increase in the pH is seen after the placement of $Ca(OH)_2$ confirming the diffusion of OH ions through root dentin.[24,25] The mild variation in the pH of different groups shows that each tooth has a different permeability which in turn is affected by number, length and diameter of the dentinal tubules and even presence of lateral canals.[7] The pH peaked after 14 days of placement of $Ca(OH)_2$ which was in accordance with other studies 25 for readings of both pH 1 and pH 2. This can be explained by the buffering of hydroxyl ions by the dentin, which reinforces the concept that it takes time for the in-depth alkalization of the root dentin to occur.[26,27] Seven days after placement of $Ca(OH)_2$ a decrease in the pH was seen at E2, although no significant difference was observed. In this study, the choice of endodontic sealer was found to alter the pH of the dentin for each group in the F period, as supported by earlier studies.[28,29] Although sealers show alkalinity,[30,31,32] they were not able to show a significant increase in the values of pH for E3. When evaluating the values for pH 2 a statistically significant increase is seen as compared to values of pH 1 for all time periods in all groups, stating that the permeability was increased after the canals were re-instrumented. This is because of the reduction in the thickness of dentin after re-instrumentation as noted in previous studies,26,23 which leads to an increase in the diffusion of hydroxyl ions. For values of pH 2 for different groups Endoflas group has the highest diffusion of hydroxyl ions followed by Endoseal MTA, Apexit Plus and least for Resino Seal. This is because the chemical and physical properties of sealer influences their depth of penetration. Mamootil et al, 2007: noted that the resin based sealers showed deeper penetration than the zinc oxide eugenol based sealers.[34, 35] Also, Endoseal MTA with calcium silicate in its composition shows chemical bonding to dentin like that of MTA[31,36] while Apexit Plus shows formation of micromechanical tags into the dentinal tubules increasing the retention of the obturation material.[32] Therefore, it can be explained why re-instrumentation allowed a better removal of Endoflas than Endoseal MTA, Apexit Plus or Resino Seal allowing for greatest hydroxyl ion diffusion in the Endoflas group and least in the Resino Seal group.[37]

Both the null hypothesis were therefore rejected as a sharp increase was seen in the values of pH before and after obturation and re-instrumentation. Also, there was a difference in the hydroxyl ion diffusion shown by the difference in pH for the four different sealers with Endoflas showing maximum diffusion after removal followed by Endoseal MTA, Apexit Plus and Resino Seal in decreasing order. Even though each detail of the study was carefully reviewed, this study does not reproduce clinical conditions. The fluid surrounding the tooth is not distilled water which favors diffusion. Secondly, even though 3 samples from each group were sectioned and checked for the presence of cementum it is a possibility that some cementum may be left in few teeth leading to erratic readings and thereby, affecting the results. Also clinically cementum is present on the tooth except in cases of root resorption and hence, clinically, permeability is less leading to reduced diffusion of hydroxyl ions.[26,38] Thirdly, the apical foramen was blocked along with the apical third to prevent diffusion of hydroxyl ions but clinically, diffusion status through this passage could not be measured. This is in accordance with a previous study confirming that the diffusion of hydroxyl ions was more before and after obturation and retreatment and also confirming difference in the diffusion patterns after retreatment when different sealers were used.[20]

## CONCLUSION

Within the limitations of the present study, it is concluded

1) Hydroxyl ion diffusion was more through root dentin from Calcium Hydroxide after canal obturation and re-instrumentation.

2) Zinc oxide Eugenol Sealers least blocked the dentinal tubules allowing penetration of hydroxyl ions after obturation and re-instrumentation followed by MTA based, Calcium Hydroxide based and lastly with Resin based sealers.

These results are obtained within the experimental conditions and limitations of the present study.

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