

# Calcium loss from root canal dentin following EDTA and Tetracycline HCl Treatment with or without subsequent NaOCl irrigation and evaluation of microhardness of dentine

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## ABSTRACT

**Aim:** To assess Calcium loss from root canal dentin following EDTA and Tetracycline – HCL Treatment with or without subsequent NaOCl Irrigation and evaluation of microhardness of dentin. **Materials and methods:** Sixty freshly extracted single rooted premolars were selected. The teeth were sectioned longitudinally into two equal halves along the long axis using Diamond disc. The one half of the specimen is used for the Calcium loss evaluation by ICP-AES technique and the other one half with the acrylic block is used for the micro hardness study. The data was analysed using One – way ANOVA and Post Hoc Tuckey test.

**Results:** The maximum calcium loss was observed in Group V 17% EDTA +2.5% NaOCl ( $3.88 \pm 0.30$ ), followed by Group III 17% EDTA ( $3.46 \pm 0.43$  mg/L), Group VI 1% Tetracycline HCl + 2.5% NaOCl ( $1.71 \pm 0.28$ mg/L), Group IV Tetracycline HCl ( $1.46 \pm 0.29$ mg/L). Group I distilled water ( $0.17 \pm 0.25$ mg/L) and Group II 2.5% NaOCl ( $0.28 \pm 0.05$ mg/L) had the least calcium loss value with  $p < 0.001$ . Dentin Microhardness in Group I ( $67.6 \pm 1.70$  VHN), Group II ( $65.5 \pm 3.57$  VHN) Group IV and in Group VI ( $61.5 \pm 1.09$ ) showed significantly higher when compared to the mean values in Group III ( $59.7 \pm 4.3$ mg/L) and in Group V ( $55.2 \pm 2.53$ VHN) with  $p < 0.001$ . **Conclusion:** Group V 17% EDTA + 2.5% NaOCl resulted in maximum Calcium loss, but has reduced the microhardness. But Group VI 1% Tetracycline HCl + 2.5% NaOCl solution effectively removed Calcium without much altering the microhardness of the root dentine.

**Key words:** Calcium, spectrophotometry, irrigation, microhardness, root dentine

## 1 INTRODUCTION

An important objective in endodontic therapy is the removal of pulp tissue and dentinal debris from the root canal system. In addition to biomechanical preparation it is essential to use an irrigant or combination of irrigants. Since, it helps to clean the areas of the root canal system that could not be directly reached by root canal instrumentation [1].

Subsequent to biomechanical preparation, an amorphous irregular layer known as the "smear layer" is formed on the root canal walls. Smear layer contains inorganic and organic substances that include fragments of odontoblastic process, microorganisms and necrotic debris [2]. The smear layer consists of a superficial layer on the surface of the canal wall approximately 1 to 2  $\mu$ m in thickness and a deeper layer packed into the dentinal tubules to a depth of 40  $\mu$ m [3]. The presence of smear layer prevents the penetration of the intra canal medication into the irregularities of the complex root canal system and also prevents the complete adaptation of obturation material to the prepared root canal surface [4].

One of the most widely accepted irrigating solutions today is 2.5% Sodium hypochlorite (NaOCl). It has strong proteolytic effect and antimicrobial effect as long as free chlorine is available in the solution. It has been demonstrated that 5.25%

sodium hypochlorite dissolves vital tissue and was found to be significantly better than 2.5%, 1% or 0.5% as necrotic tissue solvent<sup>5</sup>. But such a high concentration is not advisable as a routine irrigant during endodontic procedures; therefore it is preferred NaOCl to be replenished frequently, when low concentrations are used.

Ethylene diamine tetra acetic acid (EDTA) is often suggested as an irrigating solution because it can chelate and remove the mineralized portion of smear layer. It also can decalcify upto a 50 $\mu$ m layer of the root canal wall if used liberally. 17% EDTA is normally used to remove smear layer in less than 1 min if the fluid is able to reach the surface of the root canal wall.

The irrigating solutions like NaOCl and EDTA helps in eradicating the microorganisms commonly found in the infected root canals. The most common organism, which is being isolated particularly in reinfected root canals, is Enterococcus faecalis.

The most common organism, which is being isolated particularly in reinfected root canals, is Enterococcus faecalis. Various investigators have demonstrated that tetracycline HCl has the ability to remove the smear layer [4], disinfect contaminated root canals, and found to be effective in eradicating

Enterococcus faecalis.

The use of the irrigating agents influence the dentin structure and change in the dentin properties. Calcium ions present in hydroxyapatite crystals are one of the main inorganic elements of dentin. Any change in the calcium ion ratio may change the original proportion of the organic and inorganic components. This in turn affects the micro hardness, permeability and solubility characteristics of dentin, sealing ability and adhesion of dental materials such as resin-based cements and root canal sealers to dentin [5],[6]. It has been further established that removal of the smear layer will enhance the adhesion of root canal sealers to the surface of dentin walls.

Therefore, the present study has been undertaken to evaluate the effectiveness of removal of Calcium loss and its effect on the micro-hardness of the root dentin after using different irrigants during endodontic procedure.

### Materials and Methods

Sixty single rooted freshly extracted premolars periodontic purpose were used for the study. It was stored in distilled water. The soft tissue covering the root surface was removed using gauze piece and a fine brush. Later, the teeth were decoronated at the cemento-enamel junction using a diamond disc and at apical end of the root to obtain 10mm length that was standardized for the entire specimen in different groups (Fig 1). Teeth were longitudinally split and pulp tissue was removed using a brush. Then teeth were then randomly divided into six groups with ten specimens in each group.

The treatment groups namely:

- Group1 - Distilled water
- Group 2 - 2.5% NaOCl
- Group 3 - 17% EDTA
- Group4 - 1% Tetracycline HCl
- Group 5 -17% EDTA + 2.5% NaOCl
- Group 6 - 1% Tetracycline HCl+ 2.5% NaOCl



Fig 1

### CALCIUM LOSS

In each group, the specimen was individually immersed in a magnetic stirrer bath containing 10ml of test solution for 2 minutes (Fig 2). Accordingly 1ml of solution was aspirated from bath after 2 minutes for Calcium Analysis. In-group IV-V, specimens were first immersed in the test solution respectively and then further immersed in 2.5% NaOCl solution. Same amount of solution was aspirated for each group. The

synergistic effect of both the treatment was then calculated by taking the sum of  $Ca^{2+}$  readings. The Ca level of each group was determined by ICP - AES technique i.e. inductively coupled plasma- atomic emission spectroscopy.



Fig 2

### MICROTOME SECTIONING

Teeth were mounted on an acrylic block and sectioned longitudinally into two equal halves along the long axis using a hard tissue microtome machine. The one half of the specimen is used for the SEM evaluation and the other one half with the acrylic block is used for the micro hardness study (Fig 3)



Fig. 3

### ROOT CANAL DENTIN MICROHARDNESS

The other half of the section with acrylic block was taken and 3 mm sectioning was done and later immersed in 10 ml of the irrigating solution for duration of 2 minutes as per the groups. Later the section was subjected to Micro Vicker's hardness testing (Fig.4). Hardness was measured under the condition of load 50 grams and a retentive duration of 15 seconds in five dentin areas on the middle third of the root canal of each specimen and the mean average of the five indentations was taken and the results were statistically evaluated.



Fig. 4

**Statistical Analysis**

Mean and standard deviation were estimated from the samples from each study group. Mean values were compared among different study groups by using One - way ANOVA followed by Post Hoc Tukey test.

**Descriptives**

Calcium Loss from Root canal Surface

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Distill w ater	10	.17170	.249035	.078752	-.00645	.34985	.050	.875
NaOCl	10	.28160	.047936	.015159	.24731	.31589	.238	.362
EDTA	10	3.46190	.434620	.137439	3.15099	3.77281	2.420	3.875
Tet	10	1.45590	.286189	.090501	1.25117	1.66063	1.079	1.851
EDTA + NaOCl	10	3.88300	.302270	.095586	3.66677	4.09923	3.328	4.235
Tet + NaOCl	10	1.70500	.281625	.089058	1.50354	1.90646	1.432	2.119
Total	60	1.82652	1.463460	.188932	1.44846	2.20457	.050	4.235

**Table 1a**

**Multiple Comparisons**

Dependent Variable: Calcium Loss from Root canal Surface

Tukey HSD

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Distill w ater	NaOCl	-.109900	.129845	.957	-.49353	.27373
	EDTA	-3.290200*	.129845	.000	-3.67383	-2.90657
	Tet	-1.284200*	.129845	.000	-1.66783	-.90057
	EDTA + NaOCl	-3.711300*	.129845	.000	-4.09493	-3.32767
	Tet + NaOCl	-1.533300*	.129845	.000	-1.91693	-1.14967
NaOCl	Distill w ater	.109900	.129845	.957	-.27373	.49353
	EDTA	-3.180300*	.129845	.000	-3.56393	-2.79667
	Tet	-1.174300*	.129845	.000	-1.55793	-.79067
	EDTA + NaOCl	-3.601400*	.129845	.000	-3.98503	-3.21777
	Tet + NaOCl	-1.423400*	.129845	.000	-1.80703	-1.03977
EDTA	Distill w ater	3.290200*	.129845	.000	2.90657	3.67383
	NaOCl	3.180300*	.129845	.000	2.79667	3.56393
	Tet	2.006000*	.129845	.000	1.62237	2.38963
	EDTA + NaOCl	-.421100*	.129845	.024	-.80473	-.03747
	Tet + NaOCl	1.756900*	.129845	.000	1.37327	2.14053
Tet	Distill w ater	1.284200*	.129845	.000	.90057	1.66783
	NaOCl	1.174300*	.129845	.000	.79067	1.55793
	EDTA	-2.006000*	.129845	.000	-2.38963	-1.62237
	EDTA + NaOCl	-2.427100*	.129845	.000	-2.81073	-2.04347
	Tet + NaOCl	-.249100	.129845	.402	-.63273	.13453
EDTA + NaOCl	Distill w ater	3.711300*	.129845	.000	3.32767	4.09493
	NaOCl	3.601400*	.129845	.000	3.21777	3.98503
	EDTA	.421100*	.129845	.024	.03747	.80473
	Tet	2.427100*	.129845	.000	2.04347	2.81073
	Tet + NaOCl	2.178000*	.129845	.000	1.79437	2.56163
Tet + NaOCl	Distill w ater	1.533300*	.129845	.000	1.14967	1.91693
	NaOCl	1.423400*	.129845	.000	1.03977	1.80703
	EDTA	-1.756900*	.129845	.000	-2.14053	-1.37327
	Tet	.249100	.129845	.402	-.13453	.63273
	EDTA + NaOCl	-2.178000*	.129845	.000	-2.56163	-1.79437

\*. The mean difference is significant at the .05 level.

**Table 1b**

**Descriptives**

Microhardness

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Distill w ater	10	67.6300	1.69774	.53687	66.4155	68.8445	64.50	70.30
NaOCl	10	65.4800	3.56832	1.12840	62.9274	68.0326	60.10	70.90
EDTA	10	57.9300	1.85715	.58728	56.6015	59.2585	54.80	60.30
Tet	10	63.4700	1.14412	.36180	62.6515	64.2885	61.70	65.30
EDTA + NaOCl	10	55.1600	2.52639	.79892	53.3527	56.9673	50.30	58.30
Tet + NaOCl	10	61.5200	1.08710	.34377	60.7423	62.2977	59.70	63.60
Total	60	61.8650	4.77606	.61659	60.6312	63.0988	50.30	70.90

**Table 2a**

**Multiple Comparisons**

Dependent Variable: Microhardness

Tukey HSD

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Distill w ater	NaOCl	2.15000	.96502	.242	-.7011	5.0011
	EDTA	9.70000*	.96502	.000	6.8489	12.5511
	Tet	4.16000*	.96502	.001	1.3089	7.0111
	EDTA + NaOCl	12.47000*	.96502	.000	9.6189	15.3211
	Tet + NaOCl	6.11000*	.96502	.000	3.2589	8.9611
NaOCl	Distill w ater	-2.15000	.96502	.242	-5.0011	.7011
	EDTA	7.55000*	.96502	.000	4.6989	10.4011
	Tet	2.01000	.96502	.312	-.8411	4.8611
	EDTA + NaOCl	10.32000*	.96502	.000	7.4689	13.1711
	Tet + NaOCl	3.96000*	.96502	.002	1.1089	6.8111
EDTA	Distill w ater	-9.70000*	.96502	.000	-12.5511	-6.8489
	NaOCl	-7.55000*	.96502	.000	-10.4011	-4.6989
	Tet	-5.54000*	.96502	.000	-8.3911	-2.6889
	EDTA + NaOCl	2.77000	.96502	.061	-.0811	5.6211
	Tet + NaOCl	-3.59000*	.96502	.006	-6.4411	-.7389
Tet	Distill w ater	-4.16000*	.96502	.001	-7.0111	-1.3089
	NaOCl	-2.01000	.96502	.312	-4.8611	.8411
	EDTA	5.54000*	.96502	.000	2.6889	8.3911
	EDTA + NaOCl	8.31000*	.96502	.000	5.4589	11.1611
	Tet + NaOCl	1.95000	.96502	.344	-.9011	4.8011
EDTA + NaOCl	Distill w ater	-12.47000*	.96502	.000	-15.3211	-9.6189
	NaOCl	-10.32000*	.96502	.000	-13.1711	-7.4689
	EDTA	-2.77000	.96502	.061	-5.6211	.0811
	Tet	-8.31000*	.96502	.000	-11.1611	-5.4589
	Tet + NaOCl	-6.36000*	.96502	.000	-9.2111	-3.5089
Tet + NaOCl	Distill w ater	-6.11000*	.96502	.000	-8.9611	-3.2589
	NaOCl	-3.96000*	.96502	.002	-6.8111	-1.1089
	EDTA	3.59000*	.96502	.006	.7389	6.4411
	Tet	-1.95000	.96502	.344	-4.8011	.9011
	EDTA + NaOCl	6.36000*	.96502	.000	3.5089	9.2111

\*. The mean difference is significant at the .05 level.

**Table 2b**

## Results

Mean calcium loss in Group I ( $0.17 \pm 0.25$  mg/L) and group II ( $0.28 \pm 0.05$  mg/L) are least and maximum in Group V ( $3.88 \pm 0.30$  mg/L). There is statistical significant difference in means of all six groups ( $F = 288.99$ ,  $df = 5,54$ ,  $p < 0.001$ ) (Table 1a & b)

Mean Dentin Microhardness in Group I ( $67.6 \pm 1.70$  VHN) and Group II ( $65.5 \pm 3.57$  VHN) are maximum and least in Group V ( $55.2 \pm 2.53$ ). There is statistical significant difference in means of all six groups ( $F = 47.007$ ,  $df = 5,54$ ,  $p < 0.001$ ) (Table 2a & b)

## Discussion

Dentin is mineralised tissue, composed of inorganic components of hard dental tissues, in which calcium and phosphorus are distributed in the form of hydroxyapatite crystals. The Ca/P ratio in hydroxyapatite is approx 1.67 and it depends on many factors like level of mineralisation, type of crystals, age of tissue and anatomical site [7].

Cleaning and shaping of the root canal system are considered the key requirements for success in root canal treatment. Irrigation of root canal system provides gross debridement, lubrication, destruction of microbes, dissolution of tissues and help in cleaning areas that are inaccessible for mechanical cleansing. During irrigation, radicular and coronal dentin is exposed to irrigating solution deposited in the pulp chamber, which may cause alteration on dentin and effect their interaction with material used for obturation [8].

During biomechanical preparation the remnants of organic matter form a smear layer, which might adhere, to canal walls, which consists of dentin particles, remnants of vital or necrotic pulp tissue and bacterial products [2]. Removal of the smear layer requires the use of irrigants that can dissolve both the organic and inorganic components [9].

The decalcifying efficacy of these acids and chelating agents depend on the root length, duration of application time, diffusion in to the dentin and the pH of the solution [10]. Currently, a final irrigation with a combination of ethylene diamine tetra acetic acid (EDTA) and sodium hypochlorite (NaOCl) is recommended to remove the inorganic as well as organic components of the smear layer [2],[9]. However, reports have indicated that the use of EDTA and NaOCl may lead to dentinal erosion of the root canal walls [11]. Baumgartner and Mader (1987) reported that EDTA and NaOCl solutions when alternately applied to a uninstrumented root canal wall, the dentin showed an eroded appearance of tubular orifice with enlarged diameter.

Further it has been reported that surface treatment of dentin with different agents may cause alterations in the chemical and structural composition of dentin, which in turn may change its permeability and solubility characteristics [12],[13].

Microhardness of dentin is sensitive to composition and surface changes of tooth structure, the effects of some chemicals such as guttapercha solvents, hydrogen peroxide, sodium perborate, EDTA and combination of hydrogen peroxide and sodium hypochlorite, EDTAC, CDTA and EGTA solution on the reduction of dentin hardness were evaluated by earlier studies [5],[12],[14].

The teeth were split longitudinally and divided into six groups with ten specimens in each for Calcium loss analysis using ICP- AES technique. The other half of teeth were mounted on an acrylic block and sectioned longitudinally into along the long axis using a hard tissue microtome machine and used for the micro hardness study.

Scelza et al, Studies have shown that the amount of extracted calcium increased with time when chelating agents are used. They observed that highest amount of Calcium ion extracted during the first 3 minutes of immersion in a 17% EDTA and also removes smear layer in about 1 minute. In the present study it has been observed that desired and optimum effect of irrigating solution of calcium loss has taken duration time of 2 minutes. This observation is more or less in agreement with other workers [13],[16].

ICP- AES (inductively coupled plasma - atomic emission spectrometry) technique is one of the most attractive detection systems for determination of trace elements in dentin<sup>17</sup>. The mineral content can be measured at ppb detectable level. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element.

In the present study the Vickers microhardness test was done to evaluate the surface changes of dental hard tissue specimens, treated with the chemical agents. The suitability and practicability of this study for evaluating the surface change of the dental tissues by Vickers hardness test has been also been endorsed by previous workers. Further Microhardness determination can provide indirect evidence of mineral loss or gain in the dental hard tissues [8]. To assess the Vickers hardness values indentation were made on different root canal regions and were done at 0.5mm level from the root canal space for standardization of the study and the methodology followed confirms with earlier workers [14].

The results were evaluated statistically using One-Way ANOVA and Post Hoc Tuckey test (Table 1 and Graph I). Among the Groups studied, Group III (17% EDTA), IV (1% Tetracycline HCl), V (17% EDTA+ 2.5% NaOCl) and VI (1% Tetracycline HCl+ 2.5% NaOCl) statistically significant calcium loss was seen. The maximum calcium loss from root canal dentin was observed in Group V, 17% EDTA + 2.5% NaOCl ( $3.88 \pm 0.30$ ), followed by Group III 17% EDTA ( $3.46 \pm 0.43$ ), Group VI 1% Tetracycline HCl + 2.5% NaOCl ( $1.71 \pm 0.28$ ), Group IV 1% Tetracycline HCl ( $1.46 \pm 0.29$ ). For the calcium loss the significance level was  $p < 0.001$ .

While in the second part of the study, Mean values of Dentin Microhardness (Table 2 and Graph II) Group I, Distilled water ( $67.6 \pm 1.70$ ), Group II 2.5% NaOCl ( $65.5 \pm 3.57$ ), Group IV, 1% Tetracycline HCl ( $63.5 \pm 1.14$ ) and Group VI, 1% Tetracycline HCl+ 2.5% NaOCl ( $61.5 \pm 1.09$ ) are significantly higher when compared to the mean values in Group III, 17% EDTA ( $57.9 \pm 1.86$ ) and in Group V, 17% EDTA + 2.5% NaOCl ( $55.2 \pm 2.53$ ).

Calcium loss is statistically non-significant when compared with Group I, (Distilled water). It removes organic component from dentin and also eliminates mineralization inhibitors and increases the porosity of residual dentin. Passage of Ca ions into irrigating solution would explain the some amount of decalcification results obtained with 2.5% NaOCl in present study.

EDTA, which is the most widely used chelating irrigant, was first used in root canal therapy by Nygaard - Ostby in 1957[18][19].

EDTA solution mainly contains disodium salts, which appear to give the best chelating action. Neutral ethylene diamine tertra acetic acid (EDTA) solution with 15% - 17 % concentration is effective in demineralizing the dentin and has been used to remove the smear layer. However, it does not dissolve organic matter.

Nygaard- Ostby explained the demineralisation of the dental hard tissue by the EDTA solution. The mineral component of dentin is mainly of calcium and phosphate, which are soluble in water. Chelators such as EDTA form a stable complex with calcium. When the disodium salts of EDTA is added to the equilibrium, calcium ions are removed from the solution. This leads to the dissolution of further ions from dentin so that the solubility product remains constant. Thus Chelators causes decalcification of dentin.

In Group V, a combination of 2.6% NaOCl and 17% EDTA for the final irrigation of the root canals is the positive control group in the present study. The association of EDTA and NaOCl solutions is effective in removing the smear layer formed during endodontic instrumentation. Studies have shown after the final irrigation, the dentin surface appears rough and EDTA and dissolution of the organic matrix by NaOCl irregularly enlarged dentinal tubule orifices due to decalcification of the inorganic component. EDTA however fails in its antibacterial action and therefore combined use of sodium hypochlorite is required.

The use of tetracycline is attributed to its antibacterial effect and concentrations used ranged from 0.5% to 200% and application period varied between 0.5 to 10 minutes. Local application of antibiotics within the root canal system is more effective mode for delivering the drug. Tetracyclines have been used to remove the smear layer from instrumented root canal walls and as irrigant and intracanal medicament [20].

Substantivity of tetracycline and their effect as endodontic irrigant to remove smear layer has been confirmed by Zahed Mohammadi [19]. Haznedaroglu and Ersev claimed that tetracycline HCl has demineralized peritubular dentin [20].

Sayin et al studied to determine extent of calcium removal on root canal dentin by treating with EDTA, EGTA, EDTAC and Tetracycline HCl with or without subsequent NaOCl irrigation. According to them Tetracycline removes less calcium as compared to other groups but in combination with NaOCl significant more calcium was removed irrespective of time

period used [13].

In the present study the results obtained, showed that calcium loss was more when tetracycline is combined with NaOCl than when it was used alone. This observation of our results agrees with the findings of earlier workers [13].

In our study in Group IV, 1% tetracycline HCl is used as the irrigant. Calcium loss was seen but it was less when compared to 17%EDTA + 2.5%NaOCl, 17%EDTA and 1% tetracycline HCl + 2.5% NaOCl, but microhardness of root canal dentin was more than these groups. Previous authors Tetracycline readily attaches to dentin and subsequently released without loosing their antibacterial activity. This property creates a reservoir of active antibacterial agents, which is released from dentinal surface slow and sustained manner [19]. The lower concentration of Tetracyclin HCl was significantly more effective in precence of NaOCl. No adverse effects of hypochlorite on doxycycline antibacterial activity were seen <sup>21</sup>

Use of the chelating agents and other root canal irrigants in the study have successfully removed the Calcium from the root dentin surface which might have cause demineralisation of the inter tubular and peri tubular dentin causing a significant change in the microhardness of root dentin.

A significant negative correlation between loss of calcium from root dentin and microhardness observed in all experimental and control groups. As the loss of calcium from root dentin increased, a reduction in microhardness of root dentin was observed. As mentioned previously, Panighi and G'Sell (1992) have reported that the hardness of dentin depends on the mineral content of dentin.

From the forgoing analysis and dicussion its evident that 2.5% NaOCl when used along with EDTA was found to be more effective in reducing the micro hardness of root dentin. Whereas, 2.5% NaOCl has showed minimal effect on reducing the dentin microhardness, but was not effective in removing the smear layer [20].

The present observations suggest that irrigation of root canal with 17% EDTA +2.5% NaOCl and the 17% EDTA effects the structural changes in root dentin as demonstrated by loss of calcium and reduction of microhardness . The combination of 2.5% NaOCl and 17% EDTA had the strongest effect while 1% tetracycline HCL +2.5% NaOCl and 1% tetracycline HCL showed the desired effect.

Taking into consideration, the dehydration and brittleness of the endodontically treated teeth, necessary precautions must be taken while using different irrigants, which may further reduce the strength of root canal dentin due to decalcification. Further studies are required to assess the extent to which these alterations affect the adhesion of sealers to the treated dentin surfaces, which may be useful for future clinical studies.

### Conclusion

From the present study it can be concluded that,the most erosive irrigating solution was 17%EDTA +2.5% NaOCl.

1% tetracycline+ 2.5% NaOCl solution effectively removed

the calcium from root canal dentin without much altering the microhardness of the root dentin.

17% EDTA also proved to be equally effective in removing the Calcium from root canal dentin but decreased the microhardness of the root dentin significantly.

Canal irrigation with these chemical solutions lead to structural changes, as evidenced by reduction of the microhardness of root dentin along with the removal of the calcium from root canal dentin.

From this study it is evident that 1% tetracycline HCl + 2.5% NaOCl can be considered an ideal irrigating solution in removing the Calcium ions as well as to preserve the microhardness of root dentin.

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