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Original Research Article

Effect of different irrigation porotocols on root dentine micro-hardness-An invitro study

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ABSTRACT

Introduction: Irrigation solutions used and time of use has a definite effect on the micro hardness & other physical properties of dentin which in turn have direct consequence on the longevity functional performance of root canal treated teeth.

Aim: To evaluate the effect of different irrigation solutions on micro hardness of root dentin.

Materials and Methods: Forty extracted single rooted lower premolars were used. After instrumentation all the root halves were randomly assigned into 4groups (n=10) and brought in contact with one of the following irrigants for 5 minutes.

Group I: 10 ml of 5% Sodium Hypochlorite (NaOCl).

Group II: 10 ml of 17% ethylene diamine tetra-acetic acid (EDTA) followed by 10 ml of 5% NaOCl.

Group III: 10 ml of 5% NaOCl followed by 10 ml of 2% chlorhexidine digluconate (CHX).

Group IV: 10 ml of 5% NaOCL followed by flush of 10 ml distilled water then by 10ml of 2% CHX.

Dentin micro hardness was measured at baseline and after treatment to determine the change in micro hardness, using Vickers tester.

Results: Data was analyzed using following parametric tests t-test, ANOVA test and Post Hoc test. Group II ie final irrigation with EDTA showed the highest percentage decrease in micro hardness values, followed by group III, then group IV and the lowest was group I. All groups showed a significant difference between each other ($P < 0.05$), except group III and IV. The coronal third showed the highest percentage decrease with significant difference between apical and middle thirds ($P < 0.05$).

Conclusions: EDTA with NaOCl causes greatest changes in dentine micro hardness, an intermediate flush with normal saline should be given for prevention of precipitation with NaOCl & CHX.

1. Introduction

With the advent of latest methods for instrumentation and chemo mechanical debridement of canals using various irrigant solutions the success rate of the root canal therapy has progressed greatly over a short period of time.[1] For an ideal irrigant in addition to adequate removal of organic, inorganic debris and antimicrobial properties the irrigant used should have least effect on the physiochemical properties of the residual root canal dentine.

Sodium hypochlorite (NaOCl), is the only irrigating solution which has organic tissue-dissolving properties and at has same time has antimicrobial action and has been long set as the gold standard irrigant used in endodontics. A balance should be aimed between used of NaOCl in high concentration which is cytotoxic to periapical tissues[2] and the effect of NaOCl on physicochemical and adhesive properties of root dentin structure.[3]

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Ethylene diamine tetra acetic acid (EDTA) is a Calcium chelating agent. EDTA acts on the organic debris in the canal and can open up the dentinal tubules, & increases the number of lateral canals to be filled.[4] The smear layer is a combination of both organic and inorganic debris and for a proper canal debridement a combination of sodium hypochlorite (2.5-5%) and EDTA (10-17%) is most effective. The only disadvantage with use of higher concentration of EDTA is its erosive action on dentine.[5]

Chlorhexidine gluconate(CHX) is a bigauide and has both bacteriostatic and bactericidal effects. It can effectively eliminate both gram positive and gram negative bacteria.[6] Since it possess broad-spectrum antimicrobial action, has property of Substantivity, does not alter the physiochemical properties of root dentine and is non toxic. Its use as a irrigating solution has been advocated. CHX does not have a tissue solvent action so its use as an irrigant is limited but it can be used a complementary irrigant after NaOCl use. The combined use of NaOCl and CHX results in a dense, orange-brown precipitate.[7] So a intermediate flush with saline is advocated.

The loss or gain of minerals results in changes in composition and physiochemical properties such as adhesion and sealing ability of dental materials to the dentine.[8] Therefore, this study evaluated the effect of irrigating solutions on the microhardness of the root dentine.

2. Materials and Methods

2.1. Sample preparation

Forty (N=40) human premolars teeth with single canal and mature apices were selected. Exclusion criteria included root caries, cracks, curved canals, prior endodontic treatment, internal resorption or calcification. Radio-graph was taken to confirm the presence of a single canal. After decontamination the samples were stored in sterile saline solution throughout the duration of study. The crowns of the samples were decoronated at the Cemento-enamel junction. The root canals were prepared up-to master apical file 40k file using step back technique and recapitulated using 10 k file. Canals in all groups were irrigated with a standardized volume of 2ml of distilled water using a universal 27-gauge needle between each change of instruments. Each root sample was sectioned into buccal and lingual segments. Dentin surface of each specimen was exposed as half of specimen was embedded into acrylic resin.

2.2. Micro-hardness measurement

Micro hardness changes was measured using Vickers Micro hardness Tester using a 25gm load for 10 seconds before and after application of different irrigating solution protocols. The indentation was made on the dentin surface approximately 0.5 mm from the root canal space. Two points were selected respectively in coronal middle and apical thirds, the first point of measurement represents the baseline value and the second point represents the post application value after irrigant used.

2.3. Micro hardness for the tested irrigants

40 samples were randomly divided into four groups (n=10) according to the irrigation protocol used. Each sample was immersed in each tested irrigant solutions for 5 minutes.

- Group I: 10ml of 5% NaOCl.
- Group II: 10ml 17% EDTA followed by 10ml of 5% NaOCl.
- Group III: 10ml of 5% NaOCl; followed by 10ml of 2% CHX.
- Group IV: 10ml of 5% NaOCl then 10ml normal saline.
  Followed by 10ml of 2% CHX

2.4. Statistical analysis

Statistical analysis was performed using [SPSS] 22.0 Armonk, NY: IBM Corp. The qualitative data is normally distributed and described using parameters such as mean, standard deviation, minimum and maximum. Parametric tests such as Student t-test was used for Comparison between two independent variables while comparisons between more than two groups were analyzed using the F-test (ANOVA) and Post Hoc tests. The level of significance was set at $P<0.05$

3. Results

The pre-treatment mean values for Vickers's hardness number (VHN) in all the groups are not statistically significant. After 5 minute contact with the respective irrigating solution all irrigating solutions significantly decreased the microhardness of the canal dentine.

Group II (EDTA+NaOCl) showed the highest percentage decrease in microhardness values and was equal to 31.97±5.70 VHN, followed by Group III (NaOCl + CHX) where the percentage decrease was equal to 18.12±3.42 VHN, then group IV (NaOCl+ saline+CHX) where the percentage decrease was equal to 13.07±2.02VHN.

The lowest was Group I (NaOCl) and the percentage decrease was equal to 9.70±1.42VHN. All the tested groups showed statistically significant difference ($P < 0.05$), except group III and IV, as shown in (Table 1). The coronal third showed the highest percentage decrease in microhardness values with significant difference when compared with apical and middle thirds ($P < 0.05$), there was no significant difference between apical and middle thirds (Table 2)

4. Discussion

The number diameter and density of tubules play an important role in the effectiveness of irrigants.

Table 1: Overalldecrease value of the mean of the three sections.

Coronal, middle & apical thirds	G I	G II	G III	GIV
Range	8.19 -11.78	18.97-39.08	14.92 -22.95	12.29 -15.63
Mean ± S.D	9.70±1.42	31.97±5.70	18.12±3.42	14.07±2.02
F		24.1		
P		0.0001*		
A1	0.0002*	0.003*		0.032*
A2		0.001*		0.001*
A3				0.077

A1 Group I and all other Groups.
A2 Group II and Group III, IV
A3 Group III and IV
F-test (ANOVA) * P < 0.05 (significant)

Table 2: Decrease in microhardness in the a different root thirds.

Range Mean±S.D	Group I		Group II		Group III		Group IV	
Apical	7.61-11.38	8.36±1.16	9.62-38.41	29.56±8.01	14.55-22.10	17.18±2.35	11.11-14.45	13.23±1.01
Middle	7.67-11.38	8.92±1.08	10.34-38.69	30.37±8.02	14.67-22.34	17.68±2.52	12.08-15.55	13.82±1.10
Coronal	8.70-12.59	10.02±1.15	20.17-40.15	32.98±6.06	15.53-24.42	19.15±3.09	13.19-16.88	15.15±1.25
A1	0.314		0.086		0.452		0.236	
A2	0.011*		0.043*		0.036*		0.047*	
A3	0.021*		0.032*		0.042*		0.036*	

A1 Apical and middle thirds
A2 Apical and coronal thirds
A3 Middle and coronal thirds
F-test (ANOVA)

Microhardness in-turn depends on physiochemical properties of the dentine and irrigating solution used.

During root canal treatment the different irrigants used for debridement come in contact first with the most superficial layer of dentine in the lumen. So in concordance with previous studies done by Cruz-Filho et al[9] samples were spilt longitudinally for more accurate representations of clinical situations.

As reported by Pashley et al,[10] dentine microhardness shows inverse relation with tubular density. Increased diameter of dentinal tubules near the pulp leads to decreased amount of calcified matrix between tubules decreases and thus leads to decreased dentinal microhardness. A decrease in microhardness reduces the strength of the root structure as well as has negative effect on adhesion of resin based dental materials, as a result an increase incidence of fracture is seen in root canal-treated teeth.[11]

Mechanism of action of an irrigant are dependent on time of contact with dentine and concentration of irrigant used.[12] Longer duration of action by EDTA and NaOCl have deleterious effects on root dentine. In this study, the irrigating solutions were used for a contact time of 5 minutes. This is in concordance with studies done by Ulusoy & Görgül[13] and Sayin et al.[14] A 5 minute use of the root canal irrigant in the micro-hardness tests, is more practical and can be replicated in clinical practice. The concentration of the irrigation solution has marked effect on the post-treatment micro-hardness values.[15] Higher concentration of NaOCl increases, its antimicrobial and smear layer removal efficacy.[16,17] 5% NaOCl application for 5 minutes is capable of inhibiting 100% of the Enterococcus faecalis. As 5% NaOCl has greater effectiveness than 0.5% and 1% concentrations so 5% concentration was used.[14]

The results showed that all irrigation solutions significantly decreased the microhardness value of dentine. The changes in microhardness values seen in group I are due to the fact that 5% NaOCl reduced significantly dentin microhardness when compared to control value. Inter tubular dentine is a mesh -work of minerals deposited in collagen matrix. NaOCL is an efficient organic tissue solvent that results in dissolution of collagen by the breakdown of the bonds between carbon atoms and disorganization of the protein's primary structure and change in magnesium and phosphate ions.

The highest decrease in microhardness value is seen in Group II, the effect is mainly from EDTA not from NaOCL,

as the EDTA solution has chelating potential so it causes an adverse softening potential on the calcified components of dentin and resultant reduction in the microhardness values is seen.[18]

Chlorhexidine (CHX) shows no marked effect on the dentin microstructure and its physical properties as its does not have an inherent proteolytic or chelating potential when compared to EDTA or NaOCL,[19] An orange brown precipitate of 4-chloroaniline forms in due to the combination of NaOCL with CHX without an intermediate flush in between. The dentin microhardness of group III was less than that of group I and group VI. This precipitate formed can result in decrease in microhardness values of the dentine as see from the results.

No significant difference is seen between groups III & IV but change in microhardness value in group IV greater than in group I. This can be due to the fact that the precipitate formed is flushed out by intermediary use of distilled water.

The coronal aspect of the samples had significant changes in dentin microhardness in comparison with the middle and the apical segments in all tested groups. As this portion of root canal is wider than the other section, it comes in contact with greater volume of irrigating solution and is better instrumented to remove smear layer. Another contributing factor can be the histological pattern of the root canal dentin. Carrigan et al[20] reported that dentinal tubule density decreased from cervical to apical region in a canal & there is an inverse relation between dentine microhardness and tubular density.

Apical section shows age related and functional changes such a secondary dentine, cementum-like tissue deposited over time marked variations in structure, dentinal sclerosis, accessory root canals low content of non-collagenous proteins (NCPs). EDTA may not have such a pronounced action in apical third because it acts on smear layer and non collagenous proteins (NCPs) and difficulty in reaching apical thirds due to narrow canal size and various dynamic phenomenons like vapor lock during clinical situations. The irrigating solutions are not able to reach the apical thirds due to its inability to reduce surface tension and little amount of irrigating solution in contact with root canal walls at apical third.[14]

Further studies that better simulate anatomic and biological considerations of natural human teeth and surrounding peri oral structures are needed to come to a substantial conclusion. various other factors such as designing a study at body temperature and use of rotary instruments that reduce smear layer and help better penetration of irrigants. Can reduce the limitation of the study. Remineralizing agent can be used before bonding procedures for enhancing remineralization and improving the microhardness of eroded dentin.

5. Conclusions

Within the limitations of this study, it could be concluded that:

1. All irrigating solution interact with the mineral components of the dentine and leading to a decreased dentin microhardness.
2. 17% EDTA followed by 5% NaOCl showed the highest percentage decrease in microhardness value
3. The precipitate formed as a result of the interaction between 2.5% NaOCl and 2% CHX has significant effect on dentin microhardness values.

6. Conflict of Interest

The author declares no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

7. Source of Funding

None.

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