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Effect of Final Irrigation Protocols on Fracture Resistance of Root Filled Teeth Using Universal Testing Machine- An In-Vitro Study

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Abstract

Background And Objectives: Application of irrigation solutions alters the physiochemical characteristics of dentin surfaces which in turn influences the fracture resistance of root-filled teeth. This study aimed to compare the effect of 17%Ethylenediaminetetraacetic acid (EDTA), 10%Citric acid (CA) with and without 2%Chlorhexidine (CHX) as final irrigants on fracture resistance of root-filled teeth by using Universal Testing Machine.

Materials And Methods: 100 extracted single-rooted premolar teeth were collected for this study. The root canals were instrumented with ProTaper Universal rotary instruments up to size F4. After instrumentation, the teeth were randomly divided into five groups (n=20) according to the final sequence of irrigation. All canals were irrigated with 5ml of irrigating solution for 1min.

- Group1- Distilled water(DW)
- Group 2- 17% EDTA and 3% NaOCl
- Group 3- 10% CA and 3% NaOCl
- Group 4- 17% EDTA and 3% NaOCl and DW and 2% CHX
- Group 5- 10% CA and 3% NaOCl and DW and 2% CHX

After irrigation procedures, all canals were obturated with ProTaper F4 gutta-percha following a single-cone technique using AH Plus sealer. All specimens were mounted in self-curing acrylic resin blocks. Specimens were then attached to the lower plate of a universal testing machine with a jig to measure the fracture resistance in newtons.

Results: The mean fracture resistance for distilled water group was 536.37 ± 89.55, for EDTA group was 648.80 ± 51.28, Citric Acid group was 741.56 ± 74.07, EDTA + CHX group was 809.98 ± 79.88 and Citric Acid + CHX group was 819.78 ± 61.94. The difference in the mean fracture resistance between 05 groups was statistically significant at P<0.001. In descending order, the fracture resistance of 05 groups was in the following order- Group 5>Group 4>Group 3>Group2>Group1

Conclusion: The results indicated that 10% of Citric acid provides more fracture resistance to root-filled teeth as compared to 17% EDTA. Irrigation with EDTA and CA followed by CHX in the final irrigation protocol increases the fracture resistance as compared to EDTA and Citric acid alone.

Keywords: Chlorhexidine; Citric acid; Ethylenediaminetetraacetic acid; Fracture resistance.

Introduction

Endodontic treatment involves procedures that intend to maintain the health of all or part of the dental pulp.[1] The objective of endodontic therapy is to remove the necrotic or inflamed pulp, the invasive bacteria, toxins, and inflammatory mediators, and then to prevent re-entry of bacteria. This is achieved through chemo-mechanical cleaning, debridement, and shaping of the root canals, obturation of the root canal system, followed by placement of a leak-resistant coronal restoration.[2]

Irrigation has a central role in endodontic treatment. Root canal irrigants are chemicals that are used during and after instrumentation, to facilitate removal of microorganisms, tissue remnants, and dentin chips from the root canal. Several studies using advanced techniques such as microcomputed tomography (CT) scanning have demonstrated that proportionally large areas of the main root-canal wall remain untouched by the instruments, emphasizing the importance of chemical means of cleaning and disinfecting all areas of the root canal.[3]

Various irrigants are used in endodontic treatment like Sodium hypochlorite, chelating agents like EDTA, acids like Citric acid and Phosphoric acid, antimicrobials like Chlorhexidine and many of herbal formulations like Neem, Morinda citrifolia, Turmeric, etc.

It has been seen that the application of irrigating solutions can alter the physiochemical characteristics of dentin surfaces.[4] Previous studies have reported that microhardness,[5] flexural strength,[6] elasticity,[7] erosion,[8] and fracture resistance of roots[9] might be negatively affected by the chemical properties and the concentration of irrigants and this chemically altered dentine may be associated with problems such as dentinal cracks and vertical root fracture.[10]

Chelating agents are used as irrigants in root canal therapy to remove the smear layer. Disodium salt of Ethylenediaminetetraacetic acid (EDTA) is generally accepted as the most effective chelating agent with prominent lubricant properties and is widely used in endodontic therapy. For effective removal of both the organic and inorganic components of the smear layer, EDTA followed by NaOCl (Sodium hypochlorite) are used.[11] However, there is no consensus on the duration of EDTA irrigation. When used in higher concentrations (5%, 10%, 15%)[8], and for longer durations[12], EDTA has been shown to cause dentinal erosion.

Another smear layer removal agent, Citric acid is a weak organic acid. R. Di Lenarda et al evaluated the cleansing and smear layer removal capability of canal irrigation with Citric acid and Sodium hypochlorite and concluded that irrigation with 1 mol L-1 Citric acid for 20secs was as effective in removing smear layer as 15% EDTA and was superior in specimens treated with rotary instruments.[13]

Once the smear layer has been eliminated, Chlorhexidine (CHX) seems to be a popular agent for the final irrigation of root canals.[14] Besides its wide range of antimicrobial activity and low cytotoxicity, CHX has substantivity: residual antimicrobial activity over time.[15] Further beneficial effects attributed to CHX have been reported such as increased wettability and decreased contact angle between root canal sealers and dentin surface.[16]

There is a clinical impression that endodontically treated teeth are more friable and fracture easily thus may have to be removed.[17] But the root canal instrumentation is an unavoidable step in endodontic therapy and any material that can compensate for this weakening effect could be useful. Thus, to reinforce the instrumented teeth against fracture, sealers are used in conjunction with a core filling material. The most commonly used sealers are AH Plus, Endosequence, MTA Fill Apex, Hybrid Root Sealer. AH Plus sealer is an epoxy resin-based sealer and among all the sealers has been reported to have highest bond strength to dentin and gutta-percha.[18]

It has been seen that the effect of irrigants on the dentin influences the interaction between the sealer and the dentin which in turn affects the fracture resistance of the tooth.[19] Moreover, it has to be considered that the use of different irrigants during root canal treatment will also result in chemical interactions between the irrigants as well as in cumulative effects on the root dentin.[20]

Hence, this study was undertaken to compare the effect of 17%Ethylenediaaminetetraacetic acid, 10%Citric acid with and without 2%Chlorhexidine as final irrigants on fracture resistance of root-filled teeth by using Universal Testing Machine.

Materials And Methods

Specimen Preparation: One hundred single-rooted premolar teeth that were indicated for extraction due to orthodontic reasons and periodontal problems were collected, cleaned carefully, and stored in 10% buffered formalin as per OSHA and CDC guidelines. They were then decoronated to standardize the tooth length to 12 mm. Only roots with a diameter of 4.5±0.5mm mesiodistally and 5.0±0.5mm buccolingually were used.

The canal lengths were visually established by placing a size 10 K file into each root canal until the tip of the file was visible at the tip of the apical foramen. The working length was established at 11 mm. The root canals were instrumented with hand K files up to size 15 K. ProTaper Universal rotary instruments were used afterward for instrumentation up to size F4. After each instrument, 1ml of 3% sodium hypochlorite was used for irrigation with a 27-gauge needle.

The specimens were randomly divided into five groups (n=20) according to the final sequence of irrigation: Group1- Distilled water; Group 2- 17% EDTA and 3% NaOCl; Group 3- 10% Citric acid and 3% NaOCl; Group 4- 17% EDTA and 3% NaOCl and distilled water and 2% CHX; Group 5- 10% Citric acid and 3% NaOCl and distilled water and 2% CHX. The irrigation protocol was performed using 5ml of each irrigant for 1min. After irrigation procedures, all canals were dried with paper points and then filled with ProTaper F4 gutta-percha following single-cone technique using AH Plus root canal sealer. The exposed area of obturating material was covered with a layer of Cavit. All specimens were stored in an incubator at 37°C in 100% humidity for 24hrs.

Preparation For The Fracture Resistance Test: Specimens were wrapped in one layer of light body silicone to mimic the periodontal ligament and were then embedded in self-curing acrylic resin covering the apical

9mm of each root. The fracture resistance test was conducted using Universal Testing Machine (Mecmesin). A needle with a tip diameter of 2mm was lowered vertically parallel to the long axis of the teeth and compressive loading was applied vertically at a speed of 1mm/min until a fracture occurs. The forces necessary to fracture each root were recorded in Newtons (N) on the monitor.

Statistical Analysis

The study data was analyzed using Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released in 2013. Armonk, NY: IBM Corp. The descriptive analysis included the expression of Fracture Resistance (in N) in terms of Mean & SD. One-way ANOVA test followed by Tukey's Post hoc analysis was used to compare the mean Fracture Resistance (in N) between 05 groups. The level of significance [P-Value] was set at P<0.05

Results

Table 1: Comparison Of Mean Fracture Resistance (In Newtons) Between Different Study Groups Using One-Way Anova Test

Groups	N	Mean	SD	Min	Max	P-Value
1-Distilled Water	20	536.37	89.55	314.6	671.7	<0.001*
2-17% EDTA+ 3% NaOCl	20	648.80	51.28	547.6	760.4	
3-10% Citric Acid + 3% NaOCl	20	741.56	74.07	605.8	867.7	
4- 17%EDTA+ 3%NaOCl + 2%CHX	20	809.98	79.88	639.9	985.5	
5- 10%Citric Acid + 3% NaOCl +2% CHX	20	819.78	61.94	713.8	915.4	

* - Statistically Significant

Table 01 demonstrates that the mean fracture resistance between different groups. The mean fracture resistance for distilled water group was 536.37 ± 89.55, for EDTA group was 648.80 ± 51.28, Citric Acid group was 741.56 ± 74.07, EDTA + CHX group was 809.98 ± 79.88 and Citric Acid + CHX group was 819.78 ± 61.94. This difference in the mean fracture resistance (in Newton) between 05 groups was statistically significant at P<0.001. Thus, in descending order, the fracture resistance of 05 groups was in the following order-

Group 5>Group 4>Group 3>Group2>Group1

Group 5 showed the highest mean fracture resistance while Group 1 showed the lowest mean fracture resistance.

Table 2: Multiple Comparisons Of Mean Difference In Fracture Resistance Between Different Groups Using Tukey's Post Hoc Analysis

(I) Group	(J) Group	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
Distilled Water	EDTA	-112.43	-176.28	-48.59	<0.001*
	Citric Acid	-205.19	-269.03	-141.35	<0.001*

	EDTA + NaOCl +CHX	-273.61	-337.45	-209.77	<0.001*
	Citric Acid +NaOCl + CHX	-283.42	-347.26	-219.57	<0.001*
EDTA	Citric Acid	-92.76	-156.60	-28.92	0.001*
	EDTA + NaOCl + CHX	-161.18	-225.02	-97.34	<0.001*
	Citric Acid + NaOCl +CHX	-170.98	-234.83	-107.14	<0.001*
Citric Acid	EDTA + NaOCl + CHX	-68.42	-132.26	-4.58	0.03*
	Citric Acid + NaOCl + CHX	-78.23	-142.07	-14.38	0.008*
EDTA + CHX	Citric Acid + NaOCl + CHX	-9.80	-73.65	54.04	0.99

* - Statistically Significant

In Table 02, Multiple comparisons of mean difference in the fracture resistance (in N) between groups revealed that Citric Acid + CHX group showed significantly highest mean fracture resistance as compared to Distilled water and EDTA groups [P<0.001] and Citric Acid alone [P=0.008]. This was followed by EDTA + CHX having significantly higher mean fracture resistance as compared to Distilled water & EDTA group at P<0.001 and Citric Acid at P=0.03. Later, Citric acid showed significantly higher mean fracture resistance as compared to the distilled water group at P<0.001 and EDTA group at P=0.001. However, there was no significant difference between EDTA + CHX and Citric Acid + CHX groups at P=0.99.

Discussion

Pulpal infection is considered as one of the major causes of periradicular lesions.[21] Hence the endodontic treatment is always directed towards the eradication of bacteria, their products, and substrate from the root canal system.[22] During endodontic treatment, bacterial reduction or elimination may be achieved by both chemomechanical preparation and intracanal dressings. In chemomechanical preparation, the removal of irritants from the root canal system is conducted through the mechanical action of instruments and the flow and backflow of the irrigating solution.[23]

Ideally, an irrigant should provide a mechanical flushing action, be microbiocidal, and dissolve remnants of organic tissues without damaging the periradicular tissues if extruded into the periodontium.[24] A large number of substances have been used as root canal irrigants, including acids (citric and phosphoric), chelating agent (ethylenediaminetetraacetic acid; EDTA), proteolytic enzymes, alkaline solutions (sodium hypochlorite) and antimicrobial agents (Chlorhexidine).[25] But there is no single irrigating solution that alone sufficiently covers all of the functions of an irrigant. Therefore, optimal irrigation is always based on the combined use of 2 or several irrigating solutions, in a specific sequence, to predictably obtain the goals of safe and effective irrigation.[26]

But it has been seen that the application of these irrigating solutions alters the physiochemical characteristics of dentin surfaces thus affecting the mechanical strength of the endodontically treated teeth.

In a study by Zhang et al.[6], it was observed that 1hr of 5.25%NaOCl exposure reduced the flexural strength of the dentin by more than one half of the value of untreated dentin. The reduction in flexural strength of dentin after NaOCl irrigation can be attributed to the generation of a brittle layer of apatite crystallites that are not supported by a structurally intact collagen matrix. This brittle layer increases the susceptibility of root-filled teeth to post-treatment crown or root fracture.

Similarly in a study by Ayrancı et al. it was seen that agitation of EDTA during irrigation, also reduced the fracture resistance of the teeth.[27] Even with an increase in time of irrigation, excessive peritubular and intertubular erosion has been seen.[28]

Thus, overzealous use of these irrgants might make root canal treated teeth more prone to fracture and it is significant to know that these vertical root fractures are one of the most common causes for failure of root canal treatment.[29]

Thus, the present study makes an attempt to find out which final irrigation protocol causes the least reduction in the fracture resistance of root canal treated teeth by comparing 17%EDTA and 10%Citric acid with and without 2%Chlorhexidine.

Results of the present study showed that the mean fracture resistance for distilled water group was 536.37 ± 89.55, for EDTA group was 648.80 ± 51.28, Citric Acid group was 741.56 ± 74.07, EDTA + CHX group was 809.98 ± 79.88 and Citric Acid + CHX group was 819.78 ± 61.94. This difference in the mean fracture resistance (in Newton) between 05 groups was statistically significant at $P<0.001$ except between Group 4 and Group 5, where the difference was statistically insignificant. The mean fracture resistance of the groups in decreasing order was- G5>G4>G3>G2>G1.

Group 1, the control group, which was irrigated by distilled water had the lowest mean fracture resistance value. This is because distilled water does not remove the smear layer and as the smear layer remains intact, there is no penetration and interaction of sealer with the root dentin.[30] This leads to poor bond strength between sealer and dentin and hence poor fracture resistance.

When comparing groups 2 and 3, group 2 had lower fracture resistance than group 3. The lower fracture resistance for EDTA than Citric acid can be explained by the erosive nature of EDTA on the root canal dentin and its inability to remove the smear layer from the apical third of the root canal, unlike Citric acid.

In a study done by Baumgartner and Mader, it was reported that the sequential use of EDTA and NaOCl caused a progressive dissolution of dentin at the expense of peritubular and intertubular areas.[31] EDTA has been seen to elute 62 ± 13 ppm of calcium from the root canal walls thus explaining the extent of its erosive nature.[32]

When comparing the erosive effects of EDTA and Citric acid, studies state that the erosive effect of Citric acid on the root canal walls is lesser than EDTA. This was proved by Yamaguchi et al. In his study, it was seen that the amount of calcium extracted with 0.1 M citric acid solution was less than that removed by 0.5 M EDTA solution.[33]

When it comes to smear layer removal, it was seen that EDTA has low efficiency in the apical third of the canal leading to less permeability of resin into dentinal tubules.[34]

The result of this study is in agreement with the study done by De-Deus et al. He reported in his study that 10%Citric acid for 1min was least effective in reducing dentine microhardness whilst 17%EDTA had the strongest effect.[35] EDTA's ability to erode the dentin can result in a decrease in microhardness and thus fracture resistance.

Group 4 and 5 in this study, showed higher fracture resistance values than other groups whereas in between groups 4 and 5, the difference in mean fracture resistance was not statistically significant. The fracture resistance of these groups can be explained by the various properties and functions of CHX.

CHX has been seen to increase the wettability and surface free energy of root canal dentin which improves the binding of sealer to the dentin thus increasing the bond strength. In a study by Prado et al. it was seen that 2%CHX increases the surface free energy, promoting high interactions between GP and sealer.[36] As it has been demonstrated that there is formation of covalent bonds between epoxide rings of AH Plus and naked amino groups in the collagen structure of dentine which determines the bond strength of AH Plus to dentin[37], increasing the wettability of the GP and dentin to sealer will, in turn, increase the bond strength and fracture resistance of the root canal treated teeth.

Moreover, Chlorhexidine possesses anti-collagenolytic property. It inhibits the activity of Matrix metalloproteinases (MMPs)[38] and cathepsins[39] thus preventing the hydrolysis of the hybrid layer and increasing the longevity of resin dentin bond, in-vivo

Hence, in the present study, it was seen that a final rinse with CHX increased the fracture resistance of root-filled teeth as compared to the teeth treated with only EDTA and Citric acid as final irrigants.

The results of the present study also corroborate the findings of Turk T et al. In his study, CHX rinse increased the fracture resistance of specimens treated with 5% and 17% EDTA.[40]

When comparing groups 4 and 5, final irrigation with Citric acid followed by CHX gave slightly higher fracture resistance than EDTA followed by CHX. Though the difference was statistically insignificant, this difference can be because of the Citric acid. It has been seen that Citric acid causes the least amount of para-chloroaniline (PCA) formation in the canal system when used as an intermittent irrigant.[41] PCA is a brown pigment that is precipitated when NaOCl and CHX are mixed[42] and has been seen to occlude the dentinal tubules, impairing cleaning and sealing of the root canal system.[43] The fact that EDTA had statistically more PCA present than saline and citric acid could be because of the combination of both NaOCl/CHX precipitate and EDTA/ CHX precipitate which may have made it more difficult to readily flush out the PCA from the canal and occluded dentinal tubules.[44]

One of the limitations of the present study was sectioning of the crowns of the teeth below the CEJ, in order to provide standardized experimental conditions. However, other factors like residual tooth structure, coronal restoration, occlusion, and parafunctional habits also affect the fracture resistance of endodontically treated teeth.[45] Better study designs are needed to evaluate the correlation between the irrigation regimes and other factors that may cause vertical root fractures in endodontically treated teeth. Further studies comparing the combination irrigation regimen using Citric acid and CHX as final root canal irrigants are required.

Conclusion

Under the limitations of this in-vitro study, it can be concluded that:

- Final irrigation protocols affect the fracture resistance of the root canal treated teeth.
- 10% of Citric acid provides more fracture resistance to root-filled teeth as compared to 17% EDTA.

- Irrigation with 17% EDTA and 10% Citric acid followed by 2% CHX in the final irrigation protocol increases the fracture resistance as compared to EDTA and Citric acid alone.

This study made an attempt to check only the fracture resistance of root canal filled teeth but other factors like sealer penetration and microleakage that affect the bond strength of root canal sealers to dentin should also be correlated in future studies.

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