EFFECT OF DIFFERENT CHELATING AGENTS AS FINAL RINSE ON INTRACANAL SMEAR LAYER REMOVAL AT THE APICAL THIRD AREA OF SINGLE ROOTED TEETH USING APICAL NEGATIVE PRESSURE: AN IN VITRO SEM STUDY

Ashish Choudhary[1], Riyaz Farooq[2], Sachin Chadgal[3], Aamir Rashid Purra[4], Fayaz Ahmed Aangar[5]

[1]Resident, Department of Conservative Dentistry and Endodontics, Government Dental College, Srinagar, Jammu and Kashmir.
[2]Professor and Head, (Resident), Department of Conservative Dentistry and Endodontics, Government Dental College, Srinagar, Jammu and Kashmir.
[3]Resident, Department of Conservative Dentistry and Endodontics, Government Dental College, Srinagar, Jammu and Kashmir.
[4]Associate Professor, Department of Conservative Dentistry and Endodontics, Government Dental College, Srinagar, Jammu and Kashmir.
[5]Assistant Professor, Department of Conservative Dentistry and Endodontics, Government Dental College, Srinagar, Jammu and Kashmir.

ABSTRACT

BACKGROUND

Smear layer formed by mechanical action of endodontic instruments is the potential source of microbial infection. Various chelating actions have been used for managing the smear layer but none of them showed promising results at the apical third of root canal. The purpose of this study was to evaluate the intracanal smear layer removal efficacy of 10% citric acid, 7% maleic acid and 17% EDTA at the apical third area of single rooted teeth when irrigated with apical negative pressure system.

METHODS

Eighty single-rooted human premolars with straight canals and fully formed apex were selected. Samples were randomly divided into two groups- groups I and II of 40 patients each, depending on the method of irrigation. Root canals were then prepared with Pro Taper rotary files up to size F4. The samples were irrigated with 5% NaOCl solution during the preparation of root canals with a 30-gauge side vented, closed end needle and EndoVac in group I and II respectively. The samples were again divided into four different subgroups (n=10 each) in each group depending upon the chelating agent (distilled water, 17% EDTA, 10% critic acid, 7% maleic acid) used for smear layer removal in the final irrigation procedure. The apical third of the root canal was examined using scanning electron microscope at 1000X magnification as it was the area of concern in the present study. Analysis of variance (ANOVA) and Least Significant Difference (LSD) tests were employed for intra-group analysis of data. For inter group analysis, Student's independent t-test was used. A p-value of less than 0.05 was considered statistically significant.

RESULTS

10% citric acid and 7% maleic acid were able to remove the smear layer at the apical third, when irrigated with EndoVac, significantly better than all the groups tested. EndoVac did remove the smear layer significantly better than traditional needle irrigation but was not able to remove it completely at the apical third.

CONCLUSIONS

Use of EndoVac along with chelating agents, benefits smear layer removal from root canals.

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BACKGROUND

During the cleaning and shaping phase of endodontic therapy, the dentin debris, in association with organic tissue, microorganisms and auxiliary chemical substances form the so-called smear layer.[1] It has been demonstrated that the smear layer itself may be infected and may protect the bacteria within the dentinal tubules.[2] Many efforts have been made to remove smear layer and improve the adaptation of obturation materials to root canal wall. Decalcifying solutions such as phosphoric acid, citric acid, maleic acid and ethylene diamine tetra acetic acid (EDTA) have been reported as suitable for removing the smear layer.[3,4] However it has been reported that smear layer removal is less predictable in the apical region as compared to the coronal and middle third of the root canals.[5] This could be attributed to comparatively smaller apical dimensions of the root canals hindering the penetration of irrigants or the formation of apical vapor lock at the apical third area of root canals.

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Corresponding Author:
Dr. Sachin Chadgal,
#727, Puran Nagar,
Bhagwati Nagar,
Jammu- 180016, Jammu and Kashmir.
E-mail: sachinendo2@gmail.com
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Traditional needle irrigation is a positive pressure irrigation system which delivers solutions no further than 1mm past the tip of needle and is relatively ineffective in cleaning the apical third of the canal walls.[6] The EndoVac is a commercially available apical negative pressure irrigation (APN) system that combines a master delivery tip that delivers irrigant to the access cavity while drawing irrigant into the canal space by using macro and micro-cannulas to clean and disinfect the canal system.[7] The EndoVac has been shown to introduce a higher flow of irrigant and produce better debridement 1 mm from working length when compared with needle irrigation.[8] There is lack of studies evaluating the effect of citric acid or maleic acid on intracanal smear layer removal when used with APN system. Hence, the purpose of this study was to evaluate the intracanal smear layer removal efficacy of 10% citric acid, 7% maleic acid and 17% EDTA at the apical third area of single rooted teeth when irrigated with apical negative pressure system.

METHODS

A total of 80 non carious single rooted mandibular premolars with fully formed apices, extracted for periodontal or orthodontic reasons were taken for the study. The teeth were stored in 10% formalin solution until they were used for the study. The samples were decoronated with the help of a diamond disc under water irrigation to obtain a standardized root length of 14 mm measured with Dial calliper. After standardization of the root length, apical patency was confirmed with ISO Size 10 K file (DENTSPLY Maillefer, Switzerland). The working length of specimens were determined by deducting 1 mm from the length of the #15 K-file (DENTSPLY Maillefer, Switzerland) after it was passively placed in the canal until the tip of the instrument visibly penetrated the apical foramen. All canals were then subsequently instrumented with 15, 20, 25 ISO size K files (DENTSPLY Maillefer, Switzerland) to previously determined working length using the balanced force technique. All canals were then recapitulated with size #10 K-type files, approximately 1 mm beyond apices to maintain apical patency and loosen intracanal debris for subsequent irrigation and evacuation. The side vented closed end needle (Canal clean; Biodent Co. Ltd., Korea) of the Monoject syringe (12 ml) was taken to a point of apical binding or working length, choosing the shorter of the two, and retracting 1 mm. The syringe was then progressed and retracted in pumping motion over a length of approximately 5 mm careful not to extend beyond one mm short of working length. One ml of 5.0-percent sodium hypochlorite was expressed from syringe during this pumping motion. To simulate the clinical conditions, a closed system was made by embedding the samples in test tubes containing the poly vinyl siloxane impression material (Aquasil Ultra Monophase, Dentsply). All the samples were equally divided into two different groups depending on the method of delivery of irrigants (n=40 each): Group I (Needle) & Group II (EndoVac).

All canals were instrumented with the ProTaper (DENTSPLY Maillefer) nickel titanium rotary file system (S1-F4) according to manufacturer's recommendations. Each Pro Taper file was used five times before discarding. 2ml of 5.0-percent sodium hypochlorite (J.L. Morrison India Ltd.) for 30 seconds was introduced into the root canal system of all the samples between file transitions with the method of delivery varying between experimental groups. All specimens remained upright in a custom-made jig throughout all rotary instrumentation.

Irrigation Protocol During Rotary Instrumentation

Group I: Needle Group

40 randomly selected teeth were irrigated utilizing only a standard 12- ml Monoject syringe with a 30-gauge, side-vented, closed-end needle. The needle of the syringe was taken to a point of apical binding or working length, with the shorter of the two chosen, at which point 2 mm was retracted. The needle of the syringe was then progressed and retracted in a "pumping" motion over a length of approximately 5 mm, careful not to progress apical to 2 mm short of binding point/working length delivering 2ml of 5% NaOCl for 30 seconds after every instrument change.

Group II: EndoVac Group

Forty randomly selected teeth were irrigated with the EndoVac System. The stainless-steel cannula of the Master Delivery Tip (MDT) was placed "just inside the access opening of the tooth" expressing 2 ml of 5% sodium hypochlorite for 30 seconds after every instrument change. Excess irrigation solution delivered was simultaneously aspirated by the plastic hood of the MDT.

Final Irrigation Protocol

All the samples in both the groups were subsequently further divided into 4 subgroups depending on the chelating agent used as a final rinse:

Group IA: Needle + Distilled water DW (control)
Group IB: Needle + 17% Ethylene diamine tetra acetic acid
Group IC: Needle + 10% Citric acid
Group ID: Needle + 7% Maleic acid
Group IIA: EndoVac+ Distilled water (control)
Group IIB: EndoVac + 17% EDTA
Group IIC: EndoVac+ 10% Citric acid
Group IID: EndoVac + 7% Maleic acid

Group I (Needle)

3 ml of 5% sodium hypochlorite (NaOCl) was delivered via 30-gauge side vented, closed end needle constantly moving 2 mm from working length till orifice for 30 sec & waiting for 60 sec (simulating macro-cannula of EndoVac group). 3 ml of 5% NaOCl was delivered constantly moving the needle 2 mm from working length within 2 mm amplitude for 30 sec & waiting for 60 sec (simulating microcannula of EndoVac group). Then 5 ml of distilled water (DW) was delivered constantly moving the needle 2 mm from WL within 2 mm amplitude for 60 seconds & waiting for 60 seconds in group IA. Similarly, procedure was repeated with 17% EDTA (Prevest DenPro; Jammu), 10% CITRIC ACID (CA) & 7% MALEIC ACID (MA) IN GROUP IB, IC & ID respectively.

After the final rinse with chelating agent, irrigation needle was placed 2 mm from working length to aspirate the remaining fluid from the canal. Finally, 5 ml of distilled water was delivered to terminate any action of the irrigating solutions in the root canal & canals were later further dried with absorbent points. Canal orifices were sealed with cotton pellet & temporary filling material and test tubes were capped with their lids.

Group II (Endovac)

3 ml of 5% NaOCl was delivered via Master delivery tip over 30 sec as macrocannula was constantly moved from binding point/working length to orifice. After that macrocannula was removed followed by Master delivery tip (MDT) and irrigant was left in the canal undisturbed for 60 seconds.

1st cycle of micro irrigation: 3 ml of 5% NaOCl was delivered via Master delivery tip over 30 sec as microcannula was initially placed to working length & moved in an up/down motion of 2 mm amplitude every 6 seconds & irrigant was left in the canal for 60 seconds for soaking.

2nd cycle of micro irrigation:

5 ml of distilled water was delivered via Master delivery tip over 60 sec as microcannula was initially placed to working length & moved in an up/down motion of 2 mm amplitude every 12 seconds & irrigant was left in the canal for 60 seconds for soaking in group IIA. Similarly, procedure was repeated with 17% EDTA, 10% CITRIC ACID & 7% MALEIC ACID IN GROUP IIB, IIC & IID respectively.

After the end of 2nd micro irrigation cycle, the microcannula was left at working length without replenishment to suction the remaining fluid.

Finally, 5 ml of distilled water was delivered via MDT over 60 sec as microcannula was initially placed to working length & moved in an up/down motion of 2 mm amplitude every 12 seconds to terminate any action of the irrigating solutions in the root canal. Irrigant was then aspirated from the canal by using the microcannula placed till working length & canals were later further dried with absorbent points.

Scanning Electron Microscopy

The teeth were grooved along the buccal and lingual planes by using a diamond disc at low speed taking care not to perforate the root canal. The roots were then split longitudinally with a bi-bevelled chisel and a mallet, exposing the entire root canal. One half of each root was selected depicting the entire root canal length and prepared for scanning electron microscope examination. The selected samples were progressively dehydrated using graded concentrations of aqueous ethanol (70%, 80%, 90% and 100%) for 24 hrs at each concentration. After dehydration, the samples were placed in a vacuum chamber and sputter coated with a 30 nm gold layer. The samples were then analysed using scanning electron microscope S-3000 H (Hitachi, Japan). The dentinal wall of the root canals were examined at the apical third level (2-3 mm from the apex) at magnification of 1000 x for the presence or absence of smear layer and patency of dentinal tubules. Photomicrographs of the root canals were taken at apical level (2-3 mm from the apex) for scoring individually in a calibrated single blind manner according to the rating system developed by Hulsmann et al,[9] and modified by Caron et al.[10] This system measures the presence of the smear layer as follows: a score of (1) Indicates absence of smear layer with open dentinal tubules (2) Indicates small amount of scattered smear layer and dentinal tubules open (3) Indicates thin smear layer and dentinal tubules partially open (4) Indicates canal wall partially covered with thick smear layer (5) Indicates canal wall totally covered with thick smear layer. Representative SEM images of each group have been depicted in Figure 1. SEM images were assessed two times in random order by two-blinded observers at 1-week interval without knowing the previous result. Analysis of variance (ANOVA) and Least Significant Difference (LSD) test were employed for intra-group analysis of data. For inter group analysis, Student's independent t-test was used. A P-value of less than 0.05 was considered statistically significant. SPSS (Version 16.0) and Microsoft Excel software was used to carry out the statistical analysis of data.

RESULTS

The mean scores for smear layer removal regarding each auxiliary chemical substance and activation protocol were listed in Table 1. Intra-group and intergroup comparisons have been depicted in Table 2 and Table 3 respectively. With regards to the total remaining mean scores of different groups, Group IIC (EndoVac+CA) and Group IID (EndoVac+MA) had the least smear layer scores, with no significant difference between them (P=0.677). This was followed by Group IIB (EndoVac+EDTA), with significant difference in comparison to other groups of the study. This was followed by Group IC (Needle+CA), ID (Needle+MA) & IB (Needle+ EDTA) respectively, with no significant differences between them. The highest mean smear layer scores were for Group IA (Needle+DW) and Group IIA (EndoVac+DW) respectively, with no significant difference between them.

Group	Sub-Group	Mean± SD
Needle (Group I)	IA (DW)	4.7±0.48
	IB (EDTA)	3.6±0.52
	IC (CA)	3.3±0.48
	ID (MA)	3.4±0.52
EndoVac (Group II)	IIA (DW)	4.3±0.48
	IB (EDTA)	2.8±0.42
	IIC (CA)	2.1±0.57
	IID (MA)	2.2±0.63

Table 1. Mean ± Standard Deviation (SD) Smear Layer Score for Each Group and Sub Group

Intra Group Comparisons	Group I		Group II	
	Mean Difference	p Value	Mean Difference	Value
DW vs. EDTA	1.1*	<0.001	1.5*	<0.001
DW vs. CA	1.4*	<0.001	2.2*	<0.001
DW vs. MA	1.3*	<0.001	2.1*	<0.001
EDTA vs. CA	0.3	0.188	0.7*	0.006
Edta vs. MA	0.2	0.377	0.6*	0.016

MA vs. CA	0.1	0.657	0.1	0.677

Table 2. Intra Group Comparisons of Smear Layer Scores

*The mean difference is significant at the 0.05 level.

Multiple Comparison within Groups		Mean Difference	p-Value
Group II	**Group I**		
A (DW)	A (DW)	-0.4	0.081
	B (EDTA)	0.7*	0.006
	C (CA)	1.0*	<0.001
	D (MA)	0.9*	<0.001
B (EDTA)	A (DW)	-1.9*	<0.001
	B (EDTA)	-0.8*	0.001
	C (CA)	-0.5*	0.025
	D (MA)	-0.6*	0.011
C (CA)	A (DW)	-2.6*	<0.001
	B (EDTA)	-1.5*	<0.001
	C (CA)	-1.2*	<0.001
	D (MA)	-1.3*	<0.001
D (MA)	A (DW)	-2.5*	<0.001
	B (EDTA)	-1.4*	<0.001
	C (CA)	-1.1*	<0.001
	D (MA)	-1.2*	<0.001

Table 3. Inter-group Comparison of Smear Layer Scores

*The mean difference is significant at the 0.05 level.

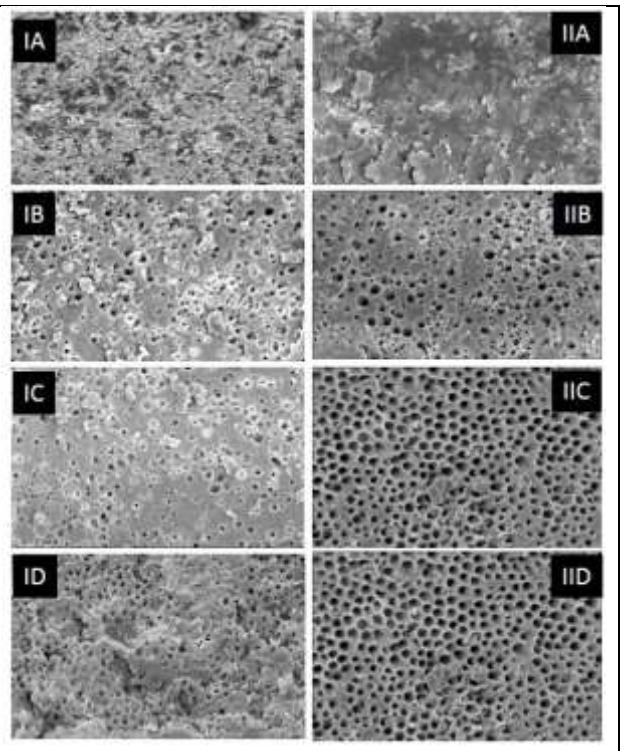

Figure 1. Representative SEM Images of Each Subgroup (IA, IB, IC, ID, IIA, IIB, IIC, IID)

DISCUSSION

Decalcifying solutions such as phosphoric acid, citric acid, maleic acid and EDTA have been reported as suitable for removing the smear layer.[3,4,11] This study compared the effectiveness of these chelating agents in removing the smear layer formed in the root canal system during chemo-mechanical preparation. In the current study, mean smear layer index of all the samples in group I were not significant to each other at the apical third except the control group. These results are in accordance with the previous studies proving that none of the chelating agents used in the study are effective in removing intracanal smear layer at the apical third of root, when delivered through traditional conventional needles.[12-14] According to Desai and Himel,[15] traditional needle irrigation delivers solutions no further than 1 mm past the tip of needle. To be fully effective, the chelating agent must reach the maximum length of the root canal as possible. The apical negative pressure is a type of hydrodynamic irrigation which pulls the irrigant down the canal walls towards the apex, creating a rapid turbulent current force towards the terminus of the microcannula which helps to overcome the vapor lock, thus enabling effective irrigation. Development of turbulent flow leads to more efficient replacement of irrigation solution which in turn also avoids saturation, precipitation of particles & favours the removal of debris in suspension inside the root canal.[16] In this study, the results showed that except for the control group all the chelating agents were effective in removing smear layer from the apical third of root canal system when agitated with EndoVac as compared to when delivered via conventional 30 gauge needles which was statistically significant and in accordance with previous studies.[8,17,18] But a statistically significant difference was found when mean smear layer index of 17% EDTA was compared to that of 10% Citric acid & 7% Maleic acid. This finding is in agreement with various other studies that have reported EDTA to be effective in smear layer removal only in coronal and middle thirds but not in the apical third.[5,19,20] EDTA may not have such a pronounced action in the apical third due to the presence of more sclerosed dentin in the area. Another explanation may be that the irrigation with 17% EDTA and 5% sodium hypochlorite caused deep erosions into the dentinal tubules and result in more organic and inorganic calcium matrix resolution.[21] This implies a negative impact on the dentin wall as it enhances dentin surface destruction which in turn might affect the consistency of the resulting smear layer.

In addition to this, it seems the application of higher volumes of citric acid over 1 minute improves its efficacy in removing the smear layer. Accordingly, Sterrett et al[22] showed that the effect of 10% citric acid on dentin demineralization was time dependent at 1, 2, and 3 minutes. Some investigators have reported that the application of 10% citric acid for more than 1 minute and in a volume more than 1 ml was more effective than 17% EDTA in terms of decalcifying ability.[21,23] These results are in contrary to the results of previous studies which showed no significant difference in terms of smear layer removal at the apical third by either chelating agent.[24,25] The difference in results may be due to the greater amount of chelating agent used in the current study with its continuous replenishment throughout the procedure via EndoVac. EDTA activity decreases over time due to its self-limiting activity due to lowered pH, while citric acid has no self-limiting activity and its activity increased as pH values decreased. Therefore, citric acid may penetrate deeper into the dentinal tubules.[26] 7% Maleic acid

as chelating agent was equally effective as 10% citric acid, in terms of smear layer removal which can be explained by the fact that both are organic agents with acidic pH. Maleic acid yielded significantly better results as compared to 17% EDTA which is in accordance with the study conducted earlier.[27] This might be attributed to the increased surface tension of 17% EDTA (0.0783 N/m) when compared with that of 7% maleic acid (0.06345 N/m). As EDTA is a chelating agent, it is not dependent on a high hydrogen ion concentration to accomplish decalcification and is effective at a neutral pH. The exchange of calcium from dentin by hydrogen results in a subsequent decrease in pH. Hence, the efficacy of EDTA decreases over time because of the decrease in ph.[28] Since maleic acid is highly acidic, it has a better demineralizing effect within a shorter period of time.

However, in spite of the turbulence created by EndoVac & inserting the microcannula till full working length, the apical negative pressure system was not able to completely remove the smear layer from the apical third as expected which is in accordance with previous study.[29] The most important problem regarding the EndoVac system is that a certain amount of irrigant is sectioned out of the canal before it reaches the apical region. Therefore, the amount of cleaning solution that comes into contact with the canal wall decreases gradually as it nears the apical region. This might explain the inability of EndoVac to completely remove the smear layer at the apical third.

CONCLUSION

All the groups irrigated with EndoVac, except distilled water were able to remove the smear layer at the apical third significantly better than conventional needle irrigation, but not completely. These findings point to the possibility that irrigation with EndoVac may be a promising adjuvant to improve the removal of smear layer from the apical third of root canals.

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