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Efficacy of Smear Layer Removal At Different Times By Irrigating Solutions :–An In Vitro Scanning Electro Microscopic Study

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Abstract

Introduction: Instrumentation produces smear layer which is removed by various irrigating solutions. **Objectives:** The aim of this study is to determine which irrigant effectively removes the smear layer from the apical third of the root canal at three different times.

Materials and Methods: Thirty six single-rooted human teeth were decoronated and biomechanical preparation was done through hand instrumentation up to size 50 k file with 3.5% NaOCl irrigation between each successive filing. The teeth were divided into Group I to IX containing 4 samples each and irrigated with 17% ethylenediamine tetraacetic acid (EDTA), 37% Phosphoric acid and normal saline respectively for 30 seconds, 1minute and 3 minutes respectively. The roots were then split with a chisel and mallet. One-half of each tooth at apical third was selected and then was analyzed using a scanning electron microscope. To observe the degree of smear layer removal, the scoring system described by Takeda et al. was used.Results: 37% Phosphoric acid is more effective for smear layer removal compare to 17% EDTA and normal saline from the apical third of canal. 37% Phosphoric acid and 17% EDTA is more effective at 3 minutes compare to 1 minute and 30 seconds. Conclusion: Within the limitations of the study, it can be concluded that both the solutions can remove the smear layer from apical third. However, 37% Phosphoric acid is the most efficacious irrigant in smear layer removal.

Keywords: Smear layer, Sodium hypochlorite, EDTA, Phosphoric acid, scanning electron microscope

Introduction

There are three basic phases in endodontic treatment[1]. First is the diagnostic phase, second is the preparatory phase and the third phase involves the filling or obliteration of the canal to gain a hermetic seal with an inert material as close as possible to the cementodentinal junction at the apex.

Microscopic examinations of root canals show that they are irregular and complex systems, with many cul-de-sacs, fins, and lateral canals[2]. The microorganisms present in the root canal not only invade the anatomic irregularities of the root canal system, but they also invade the dentinal tubules and can reinfect the root canals if they remain viable after inadequate root canal treatment[3]. However, an accurate canal preparation (cleaning and filing) with proper endodontic instruments is causing formation of a microlayer on root canal walls, known as smear layer[4].

From the chemical point, smear layer has two components, organic and inorganic. Organic part of

the smear layer contains dentine collagen fibres and glycosamineglycane, originating from extracellular matrix[5]. This part presents the base for the other, dominant inorganic.[6]

Different bacterial species (anaerobes) can be detected in smear layer existing on root canal walls[7]. Considering the complexity of root canals morphology and surfaces unreachable for endodontic instruments, significant numbers of microorganisms is left on hidden sites of root canal walls[8]. SEM analysis of human teeth with necrotic pulp, undertaken by Sen and co-workers[9], has shown that depth of bacterial penetration into dentinal tubules was up to 150 μm in apical two-thirds of the root.

The main argument of the greater number of scientists recommending removal of the smear layer is the fact that this layer obturates dentinal tubules in root canal and effects of canal medication are blocked, as well as the efficacy of disinfecting during endodontic treatment[10]. In addition, smear layer is containing significant amount of organic material (including bacteria and their products), which can act as a reservoir to irritation factors in canals' system and influence further disorders in periapical structures of the tooth[11]. Smear layer can be removed by chemical agents (EDTA, NaOCl, Phosphoric acid), by ultrasonic and laser techniques. Smear layer is consisted of organic and inorganic components that are highly acid soluble, which is the reason for acid use in smear layer removal[12]. Numerous studies[13] have confirmed that subsequent application of EDTA and NaOCl is a very efficient method for smear layer removal from root canal walls.

Phosphoric acid removed the smear layer and smear plugs, opened the dentinal tubules, and increased intertubular dentin surface porosities[14]. Ayad[15] (2001) obtained partial smear layer removal with a 10% concentration of this acid and total removal with a 32% concentration. Garberoglio & Becce[16] (1994) compared 17% EDTA, 3% EDTA and a combination of 24% phosphoric acid plus 10% citric acid for root canal cleaning and obtained similar results amongst the three solutions. A recent study[17] (Perez-Heredia et al. 2006) alternated aqueous solutions of 2.5% sodium hypochlorite with demineralizing solutions of 15% citric acid, 15% EDTA or 5% phosphoric acid, reporting the efficacy of these agents to remove the smear layer during root canal preparation. However, higher concentrations of phosphoric acid could cause reprecipitation of hydroxyapatite from the calcium phosphate solutions formed by the initial dissolution of root dentine[18]. The formation of new calcium phosphate complexes would reduce the extraction of calcium ions from exposed root dentine (Marshall et al. 1993).[19] Some studies have shown that 5% phosphoric acid in combination with 2.5% NaOCl have been effective in smear layer removal and have the ability to decalcify dentin [17].

The objective of this in vitro study is to evaluate and compare the efficacy of smear layer removal from the apical part of the canal by phosphoric acid with that of EDTA at three different times with the help of scanning electron microscope.

Materials And Method

Thirty six single rooted human teeth were used. The teeth were extracted due to periodontal or prosthetic reasons. The teeth with straight roots, mature root apex and similar anatomic characteristics were selected for this study. Samples thoroughly cleaned under running tap water and placed in sodium hypochlorite. Access cavity preparation done by round diamond abrasive (Dia-burs, Mani, Japan) mounted in airrotor hand piece (NSK, Japan). After preparing a conventional access preparation for each tooth, a #10 k file (Mani, Japan) was inserted into the canal until just visible at the apex to determine patency. One millimeter was subtracted from this measurement and this was the working length. Chemo-mechanical preparation was done with step back technique using K files (Mani, Japan). The apical portion was enlarged to no. 50 K file (Mani, Japan). During instrumentation of the root canals, all apices were covered with sticky wax (DPI Model cement, Batch no-12114, DPI, Mumbai) to allow the reflux of the substances, simulating clinical conditions. Between files the canals were irrigated with 1 ml of 3.5% sodium hypochlorite (Hypodent-D, Steri-chem, Calcutta). After instrumentation, the teeth were irrigated with 5 ml of distilled water (Purion. B.D.Pharmaceuticals works, Howrah). Then, the teeth were randomly divided into 9 groups of four teeth each according to the time and substances used.

The irrigation protocols and experimental time periods used in this study are described in table-

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Group	Irrigating solution	Time
G1	17% EDTA	30 seconds
G2	17% EDTA	1 minute
G3	17% EDTA	3 minutes
G4	37%Phosphoric Acid	30 seconds
G5	37%Phosphoric Acid	1 minute
G6	37%Phosphoric Acid	3 minutes
G7	Control-distilled water	30 seconds
G8	Control-distilled water	1 minute
G9	Control-distilled water	3 minutes

37% Phosphoric Acid Solution Preparation-

37% phosphoric acid solution was prepared from 85% phosphoric acid solution (Merck specialities private limited, Mumbai). In 85% phosphoric acid solution, 85 ml is present in 100 ml of solution. So 43.529 ml will be present in 37% phosphoric acid solution. 21.76 ml of 85% phosphoric acid was taken in a 50 ml volumetric flask and mixed with distilled water upto 50 ml mark, resulting 37% phosphoric acid was produced.

SEM evaluation-

To observe the degree of smear layer removal, the scoring system described by Takeda et al. was used but with modifications. Briefly,

Score 1= no smear layer, with all tubules cleaned and opened;

Score 2= few areas covered by smear layer, with most tubules cleaned and opened;

Score 3=smear layer covering almost all the surface, with few tubules opened; and

Score 4=smear layer covering all the surfaces.

Results And Observations:

Descriptive statistical analysis was performed to calculate the means with corresponding standard deviations (s.d.). Test of proportion was used to calculate the standard normal deviate (Z) to test the proportions. Also One Way Analysis of variance (ANOVA) followed by Tukey's Test was performed with the help of Critical Difference (CD) or Least Significant Difference (LSD) at 5% and 1% level of significance to compare the mean values. $p<0.05$ was taken to be statistically significant.

Table- 1: Distribution of smear layer score of apical region of root canal under different solutions.

Time and Score	Distilled water (Control)				17% EDTA solution				37% Phosphoric acid solution			
Smear layer score	1	2	3	4	1	2	3	4	1	2	3	4
30 sec	-	-	-	3	-	-	-	4	-	1	2	1
1 minute	-	-	-	3	-	-	2	2	-	4	-	-
3 minutes	-	-	-	3	-	3	1		3	1	-	-

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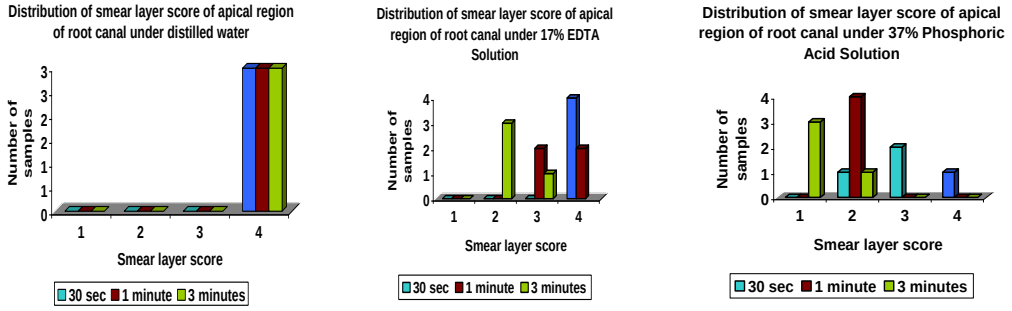
Table-2: Mean ± s.d. smear layer score of apical region of root canal under different solutions.

Time	Distilled water (Control)	17% EDTA solution	37% Phosphoric acid solution
30 sec	4±0	4±0	3±0.82
1 minute	4±0	3.5±0.58	2±0
3 minutes	4±0	2.25±0.50	1.25±0.50

Graph-1: Distribution of smear layer score of apical region of root canal under different solutions



Graph-2: Mean smear layer score of apical region of root canal under different solutions

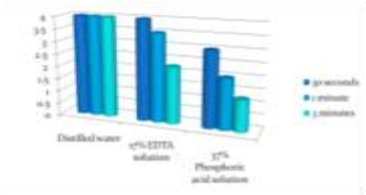


Table- 1: Distribution of smear layer score of apical region of root canal under different solutions. Test of proportion showed that smear score improved for significantly for 37% Phosphoric acid solution in comparison with other solutions (Z= 2.23;p<0.05) which was best at 3 minutes. Significant improvements were also observed for 17% EDTA solution compared to distilled water (p<0.05). **Graph-1: Distribution of smear layer score of apical region of root canal under different solutions.**

Table-2: Mean ± s.d. smear layer score of apical region of root canal under different solutions. t-test showed that there was no significant difference in mean smear layer score of apical region of root canal for distilled water for different times, for 17% EDTA solution the mean was significantly lower at 3 minute compared to at 1 minute (t_6=3.85;p<0.01) and at 30 sec (t_6= 7.82;p<0.01) and for 37% Phosphoric acid solution the mean was significantly lower at 3 minute compared to at 1 minute (t_6=2.64;;p<0.01) and at 30 sec (t_6= 6.67;p<0.01). ANOVA showed that there was no significant difference in mean smear layer score of apical region of root canal for distilled water, 17% EDTA solution and 37% Phosphoric acid solution at 30 sec ($F_{3,\ 13}$ =1.27;p>0.05). However, significant difference was observed at 1 minute ($F_{3,\ 13}$ =3.79;p<0.05) and 3 minutes ($F_{3,\ 13}$=4.13;p<0.05) and as per CD the mean for 37% Phosphoric acid solution was significantly lower than distilled water and EDTA solution (p<0.05). **Graph-2: Mean smear layer score of apical region of root canal under different solutions.**

Thus 37% Phosphoric acid solution showed better performance in reduction of smear layer score of apical region of root canal.

Discussion

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Lots of bacteria harbor inside dentinal tubules and these tubules need to be sterilized, as advocated by Shovelton (1962). Researchers have shown that adhesion and bonding strength of obturation material were highly improved after smear layer has been removed (Živkovic S, 1999)[20]. Because of its potential contamination and adverse effects on the outcome of root canal treatment, smear layer removal is recommended (Yamada et al. 1983)[21].

The most widely used irrigant for root canal treatment is sodium hypochlorite (NaOCl) at concentrations of 0.5 to 5.25 %. The tissue dissolving capacity and microbicidal activity of NaOCl make it an excellent irrigating solution (Pe´rez- Heredia et al. 2006)[17]. Higher concentrations of NaOCl may be toxic for the periapical tissue as they equally disolve necrotic and vital tissue[22]. In the present study, 3.5% NaOCl was used to evaluate the maximum effect of this solution. Smear layer removal requires a combination of NaOCL (an organic solvent) and acids such as, citric acid, tannic, polyacrilic, or phosphoric acid, or chelating agents such as EDTA or REDTA for the removal of the inorganic part.

Therefore in the present study EDTA and Phosphoric acid has been chosen to remove the smear layer from the root canal.

The other methods of smear layer removal such as laser technique, ultrasonic technique have played important role in endodontic therapy during the last decade. But they have various disadvantages.

Tewfik et al.[23] have recorded “destruction” of the smear layer but also recrystalisation of the dentine tissue lying beneath.

Baumgartner and Cuenin(1992)[24] have shown in their studies that NaOCl in ultrasonic technique was not completely efficient in removing smear layer from root canal walls.

So it has been noticed that to remove the smear layer from root canal, irrigating solution is the main requirement for any kind of technique.

In this project EDTA was used which is a well known chelating agents widely used to remove inorganic components of the smear layer. In the present study the results show that EDTA is less effective as smear layer removing agent from the apical third. This is supported by Mohammad Ali Mozayeni et. Al(2009)[25] who determined that when 17% EDTA was used as a final rinse, the smear layer was removed from the middle and coronal thirds of canal preparations, but it was less effective in the apical third of the canals. This is in agreement with Ciucchi et al(1989)[26] who stated that there was a definite decline in the efficiency of solution along the apical part of the canals. This can probably be explained to the fact that dentin in the apical third is much more sclerosed and the number of dentinal tubules present there is less.

Phosphoric acid shows better smear layer removal from the apical third of root canal than any other chemical used for that. Pashely(1985)[27] has reported that phosphoric acid in different concentration of 30% to 65% for 15sec has the ability to remove smear layer and was able to widen the dentinal tubules.

However, higher concentrations of phosphoric acid could cause reprecipitation of hydroxyapatite from the calcium phosphate solutions formed by the initial dissolution of root dentine[19]. Therefore, more predictable adhesion of sealer could be obtained directly with hydroxyapatite of partially demineralized dentin by removing the collagen.

The methodology employed in this study was similar to that undertaken by Teixeira et al.(2008)[28] During the final irrigation it was possible to introduce the needle tip up to 2 mm short of the working length and all solutions were used for different times (30 seconds, 1 minute and 3 minutes). Teixeira et al. showed that irrigation with EDTA and NaOCl for 3 minutes produced results as effective as those for 5 minutes and better than those for 1 minute, although there were no significant differences between the groups.

The lowest time period used here was 30 seconds, which is the ideal time for optimal action of phosphoric acid. However, EDTA resulted in lower performance comparable to the ones obtained with the control, which means that this solution was not able to remove the smear layer in 30 seconds. This finding is in accordance with other studies (Serper A, Calt S-2002)[29] assessing the use of EDTA for 1 minute, showing that it did not work well in this period of time. Gettleman et al.(*1991*) [30]showed that a contact time of 3 min with 17% EDTA was effective for smear layer removal. Calt and Serper

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demonstrated that 10-mL irrigation with 17% EDTA for 1 min was effective in removing the smear layer, but a 10-min application caused excessive peritubular and intertubular dentinal erosion. Therefore in this study three time periods (30 seconds, 1 minute, 3 minutes) has been taken to remove smear layer from the root canal without damaging.

In this in vitro study the apexes of the teeth were blocked using wax to simulate clinical situation with the tooth apex being surrounded by the bony socket [31]. This has been reported to cause entrapment of gas in the apical third, also referred to as vapor lock, thus hindering the complete removal of smear layer from this region [31]. This could explain the higher debris score for all specimens in the apical third.

In control group where distilled water was used as the only irrigant, dentinal walls were completely covered by smear layer. These results have already been found in other studies [32].

Summary And Conclusion

Based on the present findings, it can be concluded that:

None of the substances analyzed in this study was effective for removal of smear layer in 30 seconds.

At 3 minutes, all the substances worked well though Phosphoric acid solution exhibiting excellent results in the apical third.

Performing the same or similar procedure in clinical situation and subsequent SEM studies will help in revealing and establishing the usefulness of phosphoric acid and EDTA in smear layer removal individually as well as comparatively.

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