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Research Article

In-Vitro Evaluation of Smear Layer Removal Using 2.5% Sodium Hypochlorite, 0.2% Chitosan and 17% Edta Solution with the three Different Irrigating Solutions Either Applied Alternatively or Mixed Together After Using K-Flexo File System, an Sem Study

Rohit Chadha*

Conservative and Endodontics, Rajiv Gandhi University of Health Sciences, India

***Corresponding Author:** Rohit Chadha, Conservative and Endodontics, Rajiv Gandhi University of Health Sciences, India.

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Abstract

Aim and Objectives: This study was undertaken to compare and evaluate, the cleaning efficacy of a 2.5% Sodium hypochlorite, 0.2% Chitosan and 17% EDTA solution with the three solutions either applied alternatively or mixed together for smear layer removal after the use of K-Flexo file system in different root thirds under Scanning Electron Microscope.

Methods: 30 single-rooted human maxillary premolars were used which were decoronated to obtain uniform working length of 17mm using a diamond disc and divided into three groups. Manual instrumentation was performed with K-Flexofiles with the crown-down technique and step back technique (hybrid technique) and divided into:-

Group 1: Irrigation was performed with 2.5% NaOCl mixed with 17% EDTA and 0.2% Chitosan in the root canal.

Group 2: Irrigation was performed alternately with 2.5% NaOCl, 17% EDTA and 0.2% Chitosan.

Group 3: Irrigation only with 2.5% NaOCl was used during all instrumentation and EDTA plus Chitosan for 3 minutes as the final irritant.

The mean scores for the smear layer removal after the use of K-Flexo file system was calculated and analyzed by SEM.

Results: The result was statistically analyzed using one way ANOVA and Post Hoc Bonferroni tests. p-value less than 0.05 was considered significant.

Conclusions: Irrigation with only 2.5% NaOCl during all instrumentation and combination of EDTA and Chitosan for 3 minutes as the final irrigant showed the best smear layer removal from the canal walls, when compared with all the irrigants i.e. 2.5% NaOCl, 17%EDTA and 0.2% Chitosan mixed together and irrigants 2.5% NaOCl, 17% EDTA and 0.2% Chitosan used alternately.

Keywords: Chitosan; K-Flexo; Sodium Hypochlorite; Bonferroni

Introduction

Success in root canal treatment is dependent on elimination of infection and on permanent obliteration of the root canal by a nontoxic material [1]. During instrumentation of the canal system, a superficial smear layer containing organic and inorganic particles; namely, pulpal remnants, dentinal debris, odontoblastic processes and bacteria are left behind on dentinal walls [2]. The elimination of microorganisms from the root canal is an important step in the success of endodontic therapy [3]. Bacteria play a major role in the development and progression of pulpal and periapical disease [4].

Smear layer is an amorphous, irregular entity formed during cleaning and shaping of the root canal space. It contains dentin debris and organic material such as vital or necrotic pulp tissue remnants, bacteria, and their metabolic by-products [5].

Endodontic files produce dentine debris and a smear layer as a consequence of their action on root canal walls. The smear layer is reported to prevent the penetration of irrigating solutions, medications and filling materials into dentinal tubules and many researchers believe that it is detrimental [6].

This layer of debris is estimated to be 1 - 2µm thick and may become packed into the dentinal tubules up to a depth of 110µm creating a smear plug [7,8].

Smear plugs may entrap bacteria within the tubules and potentially prevent adequate cleaning of the canal system [9].

The literature is inconclusive to whether the smear layer should be removed prior to obturation. Some studies suggest smear layer removal is advantageous because it eliminates trapped bacteria,

allows for a higher quality seal, and decreases bacterial leakage. Other studies do not recommend smear layer removal because it increases dentin permeability, creates an additional avenue for bacterial leakage or disrupts the apical seal [2,7,9,10,11].

Studies advocating leaving the smear layer intact have theorized its presence may prevent the initial penetration of bacteria into dentinal tubules [12].

These conflicting studies may explain a 2001 survey that revealed that more than three-fourths of the dental students and nearly two-thirds of the endodontic residents are not being taught for routine smear layer removal [13].

The smear layer is tenaciously attached to the dentin wall and cannot be removed by rinsing with saline alone [14]. Suchithra MS., et al. showed that the use of saline as the only irritant left a typical amorphous smear layer on the root canal walls [15].

A chemo mechanical instrumentation regimen that incorporates the chelating agent Ethylene diamine tetra acetic acid (EDTA) has shown to effectively remove the smear layer and expose dentin tubules [2,14]. The literature supports using 1ml of 17% EDTA over a 1-minute exposure followed by 3ml of full strength sodium hypochlorite (NaOCl) as a final irrigation protocol prior to obturation [16]. This combination effectively removes the smear layer while minimizing erosion of the dentinal walls [17]. Other irrigants and techniques reported to remove the smear layer include hydrogen peroxide, citric and other weak acids, Bio Pure® MTAD®, Qmix® and activated irrigation using ultrasonic and lasers. However, these methods have been found to be less effective than the combination of EDTA and NaOCl [2,14,18-21].

Sodium hypochlorite is used as an irritant because it combines important properties such as tissue dissolving capability and microbicidal activity. The organic tissue-dissolving activity of NaOCl is well known and increases with rising temperatures [22].

Chitosan is a natural polysaccharide, which has attracted attention in dental research because of its biocompatibility, biodegradability, bio adhesion and lack of toxicity. Chitosan is obtained by the deacetylation of chitin, which is found in crab and shrimp shells and has become ecologically interesting for various applications because of its abundance in nature and low production costs [23].

Hence the purpose of the study was to evaluate the "In-Vitro evaluation of smear layer removal using 2.5% sodium hypochlorite, 0.2% chitosan and 17% EDTA solution with the three different irrigating solutions either applied alternatively or mixed together after using K-Flexo file system, A SEM study".

SEM has been the most widely used methods to evaluate the removal of the smear layer. However, one of the main drawbacks related to this methodology is that the SEM allows the evaluation of only reduced areas, without considering the entire area of the root canal [24].

Scanning electron microscope studies of cavity preparations was demonstrated by Brannstrom and Johnson (1974). The first researchers to describe the smear layer on the surface of instrumented root canals were Mc Comb and Smith (1975) [25].

Materials and Methods

Source of data

30 single rooted human maxillary premolars.

2.5% NaOCl (VIP, Vensons, India)

0.2% Chitosan (Everest biotech, India) and

17% EDTA (Avue prep, India)

Gates-Glidden (GG) #3 and #2 (Dentsply/Maillefer, Ballaigues, Switzerland)

#30 gauge Prorinse (Dentsply Sirona, USA)

0.30-mm-thick diamond disc (KG Sorensen, São Paulo, SP, Brazil)

Stainless chisel (GDC, India)

Analysis of three irrigants on their smear layer removal efficacy was done by Scanning Electron Microscope at Ruska Labs, Hyderabad.

Methods of collection of data (including sampling procedure, if any)

Methodology

Sample preparation

A total of 30 freshly extracted permanent single rooted premolars, with a complete root formation, extracted for orthodontic or periodontal reasons, were used. The samples were randomly divided equally into three groups.

Root canal instrumentation

After coronal flaring, the working length was established with the introduction of a #10 K-file (MANI Inc. Japan) in the root canal, which was visualised in the foramen; this measurement was reduced by 1 mm to obtain the working length of each sample. Thus, a cervical preparation of the samples with Gates-Glidden (GG) #3 and #2 (Dentsply/Maillefer, Ballaigues, Switzerland) was performed. The apical preparation was extended up to a #40 K-file (MANI Inc. Japan) following the crown-down technique with step back technique (hybrid technique), and irrigation using an up-and-down motion was performed at every change of file. The irrigation was performed with the regimen established for each group.

Irrigation procedures

The auxiliary chemicals used in this study were a 2.5% Sodium Hypochlorite (VIP, Vensons India) and a 17% Ethylenediamine

tetra-acetic acid (Avue prep, India), 0.2% Chitosan (Everest biotech, India). The irrigation was performed with a plastic syringe and needles of # 30 gauge Prorinse (Dentsply Sirona, USA) inserted to the proximities of the working length. The solutions were combined for the following proposed irrigation schemes.

Group 1: NaOCl with EDTA and Chitosan simultaneously (at same time) in the canal. The irrigation cycles were inundated with equal parts of solutions used in accordance with the permitted volume to each canal, instrumentation for 2 minutes, and this process was repeated for each instrument.

Group 2: NaOCl alternated with EDTA and Chitosan. The irrigation cycles consisted of irrigation with 1 mL NaOCl, instrumentation for 2 minutes, and irrigation with approximately 1 mL of 17% EDTA, instrumentation for 2 minutes, and final irrigation with 1 mL of 0.2% chitosan. This procedure was repeated for each file until the use of the largest file.

Group 3: Irrigation with NaOCl solution at first and final irrigation with EDTA and Chitosan. The irrigation cycles consisted of irrigation with 1 mL NaOCl, instrumentation for 2 minutes, and final irrigation with 1 mL of EDTA and 1ml of Chitosan mixed together. This procedure was repeated for each file until the use of the master apical file.

In all groups, the samples conducted to SEM were irrigated with an additional final irrigation of 2 mL of saline solution to eliminate the waste from the irrigating substances. The materials to be used in the study were tabulated as follows.

Groups	Samples	Irrigants Used Alternatively Or Mixed
Group 1	10	2.5% NaOCl mixed with 17%EDTA and 0.2% Chitosan.
Group 2	10	Alternately with 2.5% NaOCl, 17% EDTA and 0.2% Chitosan.
Group 3	10	Only 2.5% NaOCl during all instrumentation EDTA plus Chitosan for 3 minute at the final.

Table

SEM observation

To examine the removal of the smear layer during chemo mechanical instrumentation of the root canal, samples were used from each group and the results were compared.

Crowns were sectioned at the cemento-enamel junction with a diamond bur, and longitudinal grooves were made using a 0.30-mm-thick diamond disc (KG Sorensen, São Paulo, SP, Brazil) on the root surface. The root was then split with a stainless steel chisel into two corresponding halves. The most suitable hemi-section of each tooth sample was selected for SEM examination. The specimens were dried and mounted on a single stub, sputter-coated with gold in a high-vacuum evaporator, and analyzed under a scanning electron microscope (HITACHI S-3700N, TOKYO, JAPAN) with 2000× magnification.

Results

The result was statistically analysed using one way ANOVA and Post Hoc Bonferroni tests. p-value less than 0.05 were considered significant.

(Table 1) shows the distribution of groups based on smear score. Group 1 showed 7(70%) moderate presence of smear layer at apical region, 5(50%) at middle region and 4(40%) at cervical region. Group 2 showed 5(50%) smear layer completely covering the canal wall at apical region, 6(60%) at middle region and 7(70%) at cervical region. Group 3 showed 5(50%) canal wall with absence of smear layer at apical region, 6(60%) at middle region and 7(70%) at cervical region.

		Apical-N (%)	Middle N (%)	Cervical N (%)
Group 1	Canal wall with absence of smear layer	2 (20)	2 (20)	4 (40)
	Moderate presence of smear layer	7 (70)	5 (50)	4 (40)
	Canal wall completely covered by smear layer	1 (10)	3 (30)	2 (20)
Group 2	Canal wall with absence of smear layer	1 (10)	1(10)	1 (10)
	Moderate presence of smear layer	4 (40)	3 (30)	2 (20)
	Canal wall completely covered by smear layer	5 (50)	6 (60)	7 (70)
Group 3	Canal wall with absence of smear layer	5 (50)	6 (60)	7 (70)
	Moderate presence of smear layer	5 (50)	3 (30)	3 (30)
	Canal wall completely covered by smear layer	0	1 (10)	0

Table 1: Distribution of The Groups Based On Smear Score.

One-way Anova	F Value	p value
Apical	27.64	0.00*
Middle	16.75	0.00*
Cervical	32.65	0.00*

Table 2: Comparision Of the Groups Based on The Regions Using Anova.

*significant

Table 2 ANOVA showed statistical significant difference among the groups at apical region (F=27.64; p=0.00); middle region (F=16.75; p=0.00); cervical region (F=32.65; p=0.00*).

Table 2A Post-hoc Bonferroni showed significant difference between group 1 and group 2 at apical region (p = 0.00); group 2 and group 3(p = 0.00). Similarly significant difference was seen between group 1 and group 2(p = 0.003) and group 2 and group 3(p = 0.00) at middle region and cervical region- group 1 and group 2(p = 0.00); group 2 and group 3 (p = 0.00).

Groups	Region	Regions	Mean Difference	p value
Apical	Group 1	Group 2	-1.50	.000*
		Group 3	.400	.448
	Group 2	Group 3	1.90	.000*
Middle	Group 1	Group 2	-1.30	.003*
		Group 3	.700	.168
	Group 2	Group 3	2.00	.000*
Cervical	Group 1	Group 2	-1.80	.000*
		Group 3	.500	.319
	Group 2	Group 3	2.30	.000*

Table 2A: Post-Hoc Bonferroni.

*significant

Graph 1: Smear layer removal efficacy of group 1 in cervical, middle and apical region.

Graph 2: Smear layer removal efficacy of group 2 in cervical, middle and apical region.

Graph 3: Smear layer removal efficacy of group 3 in cervical, middle and apical region.

Graph 4: Distribution of the groups based on smear score.

Master chart

The root canal walls were evaluated for smear layer under SEM, and the scoring criteria were based on the rating system developed by Rome., *et al.* [1] as follows:

1. No smear layer, dentinal tubules open and free of debris;
2. Moderate smear layer,outline of dentinal tubules observable or partially filled with debris; and
3. Heavy smear layer, cannot distinguish outlines of tubules.

Figure 1

Discussion

The elimination of microorganisms from the root canal is an important step in the success of endodontic therapy. The colonization of dentinal walls with biofilm, along with the anatomical complexity of the root canal and the possibility of invasion of dentinal tubules, can compromise the success of endodontic therapy [1].

The root canal wall, when submitted to the action of each instrument (manual or rotary), becomes coated with a layer predominantly composed of grinding debris, and reported as the smear layer. Because it is of dentinal origin, it is composed of organic and inorganic matter [1,5]. The morphology of the smear layer is composed of two layers. The superficial layer is firmly adhered to the dentin surface, and the deep layer is formed by smaller particles that are compacted into the dentinal tubules, making the deep layer difficult to remove [8].This compaction causes the reduction of dentin permeability by 25 - 49%, which would protect the bacteria previously installed inside the dentinal tubules [5,6].

Numerous techniques and irritant delivery devices have been proposed to improve the distribution of irrigating solution within the root canal system. But regardless of the techniques used, effectiveness of irrigating solutions remains limited in the apical third of a prepared canal. When canal curvatures are present, effective irrigant delivery becomes even more difficult [5].

The smeared layer associated with endodontic instrumentation has received considerable attention since the report by McComb and Smith in 1975 [7].

Hand and/or Rotary instrumentation with needle irrigation does not productively clean the entire root canal. Furthermore, the intricacies in the apical third of the root canal system make thorough debridement a clinical challenge [36].

An *In-Vitro* study conducted by Dr. Suchithra MS., *et al.* (2017) concluded that the use of EGTA effectively removed smear layer from the root canals without inducing erosion of the tubules, the most effective irrigation regime was the use of EDTA in combination with NaOCl and H_2O_2, as it completely removed the smear layer from both the middle and the apical thirds [15].

According to Russell S. Yamada., *et al.* the question is still open to debate to the significance of removing the smeared layer. For instance, it may interfere with the adaptation of filling materials to the canal wall by imposing an additional interface; packed into the openings of dentinal tubules, it could block the antimicrobial effects of intracanal medicaments into the tubules; it could contain potentially deleterious necrotic tissue and bacterial remnants within its structure; and opening all of the tubules can perhaps provide a better seal by allowing sealer or filling material to penetrate. These speculations remain to be investigated [14].

The smeared layer has been shown to be a protective diffusion barrier and capable of preventing bacterial penetration into the dentinal tubules [7,9,16,26].

A study conducted by Julio Cesar Emboava Spano., *et al.* proposed that the use of 15% EDTA resulted in the removal of greatest concentration of calcium ions causing a treated tooth prone to facture [18].

The smeared layer may be deleterious as it is a layer of material which covers prepared areas and prevents medicaments and filling materials from penetrating the dentinal tubules, or even contacting the canal wall [7,26].

Dorothy Mc Comb., *et al.* concluded that the most effective cleaning procedure was the use of REDTA sealed in the canal for 24 hours. Canals treated in this way were free of a smeared layer and superficial debris. The cement sealers adhered to the canal wall much more desirably and are a further reason for the production of a smooth clean surface [2].

Sung –Eun Yang., *et al.* in year 2002 conflicted in their own study and concluded that a significantly greater number of bacteria were found to adhere to those teeth in which a smear layer was present. Smear layer produced during root canal preparation promoted adhesion and colonization of P. nigrescens to the dentin matrix; it might also increase the likelihood of canal reinfection [9].

A recent 2012 survey reported 77% of endodontists routinely removed the smear layer prior to obturation [27], because the smear layer may be a liability to the success of root canal treatment because of the possibility that it harbors bacteria [10].

Many irrigating solutions have been studied extensively to determine which best exhibit these ideal properties, but the ideal irritant has not yet been realized [1].

In this study, a commercially available and routinely used irritants i.e., 17% EDTA and 2.5% NaOCl is compared with a Nano particle 0.2% Chitosan which is either mixed, used alternatively or as a final irrigant, as there is no single solution that has the ability to dissolve organic tissues and to demineralize the smear layer completely.

EDTA is widely used as a chelator in endodontic therapy. A chelator reacts with calcium ions in hydroxyapatite crystals, removing calcium ions from the dentin. EDTA is used at various concentrations and combinations in root canal treatment[17]. The effects of EDTA within the canal are known to be self-limiting. Seidberg and Schiller determined that EDTA will react with 73%

of the available inorganic dentin component, forming equilibrium within 7 hrs [16,29]. However a 10 -min application of EDTA caused excessive peritubular and intratubular dentinal erosion. Therefore a study conducted by Semra Çalt, suggests that this procedure should not be prolonged >1 min during endodontic treatment [17,19]. The overuse of this compound has increased considerably its concentration in rivers and lakes. In addition, EDTA is not originally found in nature and is therefore considered to be a pollutant (Spano., *et al.* 2009) [23].

Sodium hypochlorite (NaOCl) is the most commonly used irrigant in root canal treatment, and has proven to be an excellent irrigating solution, due to its tissue dissolving capability and microbicidal activity. According to Moorer and Wesselink (1982), the active principle of NaOCl solutions is the amount of undissociated HOCl molecules, which are consumed in the interaction with organic matter. However, its action does not affect inorganic material [22].

The organic tissue-dissolving activity of NaOCl is well known and increases with rising temperatures. However, the capacity to remove the smear layer from the instrumented root canal walls has been found to be insufficient. Cytotoxic and genotoxic effects on human peripheral lymphocytes have been observed with the usage of NaOCl [25]. Surprisingly, this toxic irritant is still widely used for disinfecting root canals during endodontic therapy in most parts of the world. It is usually employed at 0.5 - 6.0% concentrations. Extrusion of NaOCl into periapical tissues can cause severe injury to the patient [35].

The search for more biocompatible solutions than EDTA and NaOCl, aiming at minimizing its harmful effect on periapical tissues continues. Environmental concerns have also led researchers to seek alternatives.

Chitosan is a natural polysaccharide, which has attracted attention in dental research because of its biocompatibility, biodegradability, bio adhesion and lack of toxicity (Senel., *et al.* Akncbay., *et al.* 2007). It has a high chelating ability for various metal ions in acidic conditions and has been applied widely for the removal or recovery of metal ions in different industrial areas (Kurita 1998). Chitosan is obtained by the deacetylation of chitin, which is found in crab and shrimp shells (Kurita 1998) and has become ecologically interesting for various applications because of its abundance in nature and low production costs (Peter 1995) [23].

Applications for this substance are being seen mainly in the areas of dentistry, medicine and pharmaceuticals (antibacterial and antitumor agent, drug carrier, wound healing accelerator), biotechnology (enzyme and cell carrier, chromatography resin), environment (water treatment), agriculture (seed preparation), cosmetics and food (iron and calcium absorption accelerator, fiber source) (Jeon., *et al.* 2000). In dentistry, the antifungal effect of a 2% chitosan gel containing 0.1% chlorhexidine against Candida albicans has been demonstrated (Senel., *et al.* 2000), and its addition to calcium hydroxide paste as an intracanal medication has been shown to promote prolonged calcium ion release (Ballal., *et al.* 2010) [35,38].

In this study root canal shaping was performed in extracted human teeth, intentionally creating a 'Smear Layer'.

Scanning electron microscopy has been used to determine the effectiveness of various irrigants to remove the smear layer. Scanning electron microscopy allows an examination of morphologic details of the surfaces of prepared root canal [24].

Luiz Fernando Machado Silveiraa., *et al.* (2013) in their literature stated that the alternate or mixed use of EDTA during instrumentation with 2.5% sodium hypochlorite was the most effective form of irrigation for the removal of smear layer on the cervical and middle thirds. No form of irrigation was sufficiently effective to remove the smear layer in the apical third [1].

In the present study when NaOCl was used during all instrumentation and EDTA plus Chitosan (combination) for 3 minutes as a final irrigant i.e. Group 3, the dentin surface was free of smear layer and showed open dentinal tubules in middle, cervical and as well as in apical third of which the result is depicted in Table 1 i.e. 5(50%) canal wall with absence of smear layer at apical region, 6(60%) at middle region and 7(70%) at cervical region.

A study conducted by Kiran S., *et al.* concluded that alternate irrigation with NaOCl and EDTA is effective in the removal of debris and smear layer in the coronal and middle level, but the effectiveness in the apical third was less [28], which was found to be similar in our study Table 1 in Group 2 i.e., alternative irrigation with 2.5% NaOCl, 17% EDTA and 0.2% Chitosan showed 5(50%) smear layer completely covering the canal wall at apical region, 6(60%) at middle region and 7(70%) at cervical region.

Shabnam Hosseini., *et al.* (2016) in her research work on a new Nano-Chitosan irritant concluded that Nano-chitosan (Nano-CS) appears to be a relatively more effective penetrating root canal irritant than EDTA, NaOCl and regular Chlorhexidine [35].

In our study when NaOCl was mixed with EDTA and Chitosan, the dentinal tubules were moderately devoid of smear layer i.e. Group 1 showed 7(70%) moderate presence of smear layer at apical region, 5(50%) at middle region and 4(40%) at cervical region.

Based on the results of this investigation, photomicrographs of group 3 and 1 showed that after biomechanical preparation and final irrigation there was less accumulation of smear layer on the walls of the canal. In the case of irrigation system of group 2, the tubules were obliterated by deposits created by NaOCl which reduced dentine permeability to EDTA and Chitosan; this fact showed that

reduce effectiveness of EDTA and Chitosan when sodium hypochlorite was used in the early stages of preparation which was stated by Luiz Fernando Machado Silveira(2013) [1].

Irrigation with only 2.5% NaOCl during all instrumentation and combination of EDTA and Chitosan for 3 minutes as the final irrigant showed better removal of smear layer from the canal walls, when compared with all the irrigants i.e. 2.5% NaOCl, 17%EDTA and 0.2% Chitosan mixed together and irrigants 2.5% NaOCl, 17% EDTA and 0.2% Chitosan used alternately. However, Chitosan needs more clinical trials on its mode of action and potential to remove the smear layer from the canal walls in future studies.

Conclusion

Within the limitations of this study, it can be concluded that, smear layer removal efficacy was highest when the root canal was irrigated using only 2.5% NaOCl during all instrumentation and 17% EDTA plus 0.2% Chitosan (combination) for 3 minutes as a final irritant.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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