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An In-Vitro Comparison of Laser and Chelating Agents in Removal of Smear Layer from Root Canal: A Scanning Electron Microscopic Study

Dental Science

KEYWORDS : Dentinal Tubules, Smear layer, Chelating agents, MTAD, EDTA, Laser.

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ABSTRACT

***Purpose**: The aim of this in vitro study was to compare the efficacy of chelating agents & Laser in removing the smear layer from root canal walls.*

***Methodology**: Sixty freshly extracted human premolar teeth with single root were selected for the study. The teeth were decoronated in such a way that the remaining root of every sample was 16 mm long. Vertical grooves were prepared on mesial and distal sides of each root. A standard conventional access preparation & instrumented using a protaper rotary files to the working length was done. the canal irrigated with 1ml of 5.25% NaOCl between files. All the samples thus were randomly divided into 4 groups and received 4 different treatment protocols. After the treatment procedures each root was split into two halves. And then examined under Scanning Electron Microscope for the presence or absence of smear layer. **Results:** The Scanning Electron Microscopic examination revealed most samples from EDTA group showed no smear layer and open dentinal tubules and maximum samples of LASER group showed closed dentinal tubules. Statistically significant difference was observed in EDTA group with all other groups.*

INTRODUCTION:

The main objectives of root canal therapy are cleaning and shaping and then obturating the root canal system in three dimensions to prevent reinfection.[1] Mechanical instrumentation alone will not completely eliminate bacteria from a root canal system. In order to predictably eliminate bacteria from the root canal system, it is necessary to use the supporting action of disinfecting agents such as irrigants.[2]

McComb and Smith first reported the presence of an amorphous layer on dentinal

walls created by instrumentation, calling it a "smear layer".[3] The smear layer has been defined as a layer of debris on the surface of dental tissues created by cutting a tooth. It varies in thickness, roughness, density and degree of attachment to the underlying tooth structure according to the surface preparation.[4]

An ideal intracanal irrigant or medication should be able to disinfect the dentin and its tubules in one visit. In addition, it should have sustained antimicrobial effect after use. Furthermore, it must be biocompatible with live host tissues.[5]

Sodium hypochlorite is commonly used in concentrations ranging from 0.5% to 5.25%. It can be removed by the chelating agent ethylene diamine tetra-acetic acid (EDTA) and solutions containing EDTA, which have been recommended for irrigation. More recently a new intra-canal irrigant – MTDA was proposed as a final attempt to remove the smear layer. Laser techniques have been used to remove smear layer on root canal walls. So the purpose of present in vitro Scanning Electron Microscopic study was to compare efficacy of various irrigating solutions and Laser to remove of smear layer from instrumented root canals.

MATERIALS AND METHODS:

In this in-vitro study sixty freshly extracted human premolar teeth with single root had been extracted for orthodontic reasons were selected. Teeth having fused roots, external root resorption, fracture lines, severe curvature, incompletely formed apex and root canal treated were excluded from the study.

The teeth were decoronated in such a way that the remaining root of every sample was 16 mm long. Vertical grooves were prepared on mesial and distal sides of each root. The depth of the grooves was kept in dentin, not to perforate the canal space. A standard conventional access preparation was done and each canal was then instrumented using a protaper rotary files to the working length. The apical preparation of each root was completed with a size 30 file, while the canal irrigated with 1ml of 5.25% NaOCl between files.

All the samples thus obtained were distributed randomly into 4 groups (n=15). The roots in each group received the following treatment protocol:

Group A: 5.25% NaOCl as a final flush, for 5 min.

Group B: 17% liquid EDTA as a final flush, for 5 min.

Group C: BioPure MTAD will be used as a final flush according to manufacture's instruction.

Group D: Specimens from this group will be irradiated with Laser. The laser tip was introduced into the canal, reaching to working length and parallel to the root canal wall. The tip was placed in contact with the wall and the laser device was activated for 5 seconds at the apex. Three additional laser exposures each of 5 seconds duration were applied to the canal, the tip being withdrawn from the apex to the orifice along the root canal wall for a total exposure of 20 seconds.

After the treatment procedures for all the samples were completed, each root was split into two halves using chisel and mallet.

SEM evaluation was done for middle third of each specimen.

The specimens were dehydrated using a series of graded ethanol solutions (70, 80, 90, 100%). The samples were kept in all concentrations of ethanol solution for half an hour. The micrographic images were obtained using SEM at 2000X magnification. For evaluation of presence of smear layer only middle third of each sample was examined.

The presence or absence of smear layer was scored as follows

1 - No smear layer. (No smear layer on the surface of the root canals; all tubules clean and open).
2 - Moderate smear layer. (No smear layer on the surface of root canal, but tubules containing debris).
3 - Heavy smear layer. (Smear layer covering the root canal surface and the tubules).

The scoring was performed in a blind manner based on a three-grade scale by a technician who was unaware of the treatment protocol. The scores thus obtained were subjected to for statistical analysis, using ANOVA" and "KRUSKALL WALLIS TEST".

RESULTS:

Descriptive statistics were expressed as numbers and percentages for each group. The efficacy of different techniques for smear layer removal was assessed by comparison of groups using Kruskal-Wallis ANOVA and Mann- Whitney U test Statistical analysis reveals that in group A (NaOCl) the percentage of samples loaded with heavy smear layer was 33.3, moderate smear layer samples were 46.7% while no smear layer samples were only 20%. In group B (EDTA) the percentage of samples loaded with heavy smear layer was zero, moderate smear layer samples were 46.7% while no smear layer samples were 53.3%. In group C (MTAD) the percentage of samples loaded with heavy smear layer was 26.7, moderate smear layer samples were 33.3% while no smear layer samples were 40%. In group D (Laser) the percentage of samples loaded with heavy smear layer is 60%, moderate smear layer samples were 26.7% while no smear layer samples were only 13%.

Table no.1: Efficacy of 5.25% NaOCl for removal of smear layer

	Score, n (%)		
	No smear layer (1)	Moderate smear layer (2)	Heavy smear layer (3)
NaOCl	3 (20)	7 (46.7)	5 (33.3)

Figure no.1: Efficacy of 5.25% NaOCl for removal of smear layer

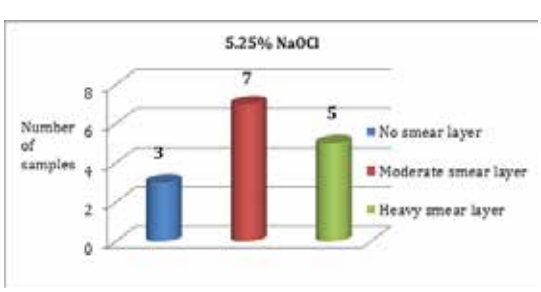


Table no.2: Efficacy of EDTA for removal of smear layer

	Score, n (%)		
	No smear layer (1)	Moderate smear layer (2)	Heavy smear layer (3)
EDTA	8 (53.3)	7 (46.7)	0 (0)

Figure no.2: Efficacy of EDTA for removal of smear layer

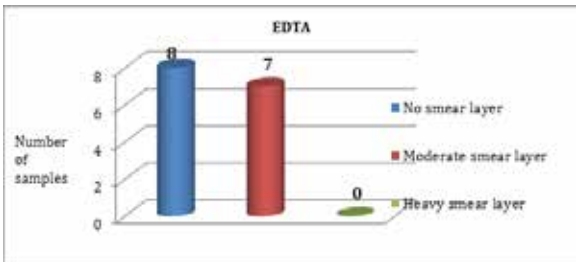


Table no.3: Efficacy of MTAD for removal of smear layer

	Score, n (%)		
	No smear layer (1)	Moderate smear layer (2)	Heavy smear layer (3)
MTAD	6 (40)	5 (33.3)	4 (26.7)

Figure no.3: Efficacy of MTAD for removal of smear layer

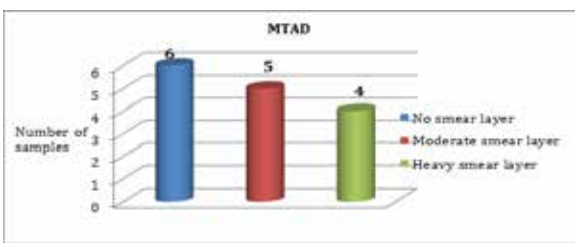
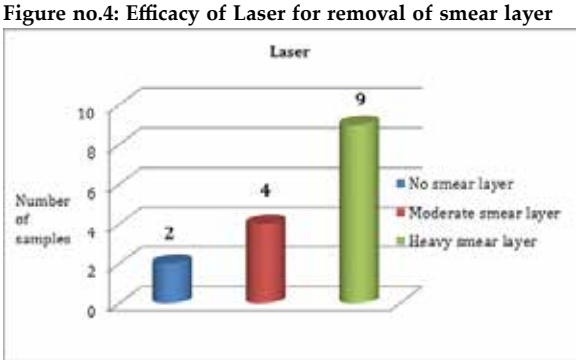


Table no.4: Efficacy of Laser for removal of smear layer

	Score, n (%)		
	No smear layer (1)	Moderate smear layer (2)	Heavy smear layer (3)
Laser	2 (13.3)	4 (26.7)	9 (60)

Figure no.4: Efficacy of Laser for removal of smear layer



DISCUSSION:

The success of endodontic treatment depends on the eradication of microbes from the root canal system and prevention of reinfection. As root canal morphology is complex and contains numerous ramification and anatomical irregularities, the microorganisms in the root canal not only invade the anatomic irregularities of the root canal system but also present in the dentinal tubules. Current techniques of root canal debridement may leave areas of the root canal system completely untouched by the instrument. It has also been shown that mechanical instrumentation without irrigation reduces but does not eliminate bacteria in the canal.[6]

The ideal irrigant or combination of irrigants kills bacteria, dissolves necrotic tissue, lubricates the canal, removes the smear layer and does not irritate healthy tissues. Many ir-

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rigating solutions and varying concentration of materials to achieve these goals are described in the literature. Some of these solutions used are sterile saline, NaOCL, MTAD, EDTA, citric acid.[7]

Identification of the smear layer was made possible using the electron microprobe with scanning electron microscope (SEM) attachment, and first reported by Eick et al. (1970). These workers showed that the smear layer was made of particles ranging in size from less than 0.5–15 μm.[8]

Brannstrom & Johnson (1974) and **Mader et al.** (1984) concluded that the tubular packing phenomenon was due to the action of burs and instruments. Components of the smear layer can be forced into the dentinal tubules to varying distances (Moodnik et al.1976, Brannstrom et al. 1980, Cengiz et al. 1990) to form smear plugs. However, Cengiz et al. (1990) proposed that the penetration of smear material into dentinal tubules could also be caused by capillary action as a result of adhesive forces between the dentinal tubules and the material.[8]

Some authors suggest that maintaining the smear layer may block the dentinal tubules and limit bacterial or toxin penetration by altering dentinal permeability (Michelich et al. 1980, Pashley et al. 1981, Safavi et al. 1990). Others believe that the smear layer, being a loosely adherent structure, should be completely removed from the surface of the root canal wall because it can harbour bacteria and provide an avenue for leakage (Mader et al. 1984, Cameron 1987a, Meryon & Brook 1990). It may also limit the effective disinfection of dentinal tubules by preventing sodium hypochlorite, calcium hydroxide and other intracanal medicaments from penetrating the dentinal tubules.[8]

Various irrigants like NaOCl (sodium hypochlorite) and acids are used to remove the smear layer. The components of the smear layer are very small particles with a large surface-mass ratio, which makes them very soluble in acids. Because of this characteristic, acids have been used to remove the smear layer.[9]

Sodium hypochlorite is the most commonly used irrigant, which has unique capacity to dissolve necrotic tissue and organic components of smear layer, however it cannot dissolve inorganic dentin particles and hence is unable to prevent the formation/removal of smear layer during instrumentation. Therefore demineralizing agents, which can dissolve inorganic dentin particles, have been recommended as adjuvant in root canal therapy. One of the most commonly used demineralizing agent is ethylenediamine tetraacetic acid (EDTA).[10]

EDTA was first introduced in endodontics by **Nygaardθstby** in 1957; it contains disodium salt of EDTA, Aqua dest and 5M sodium hydroxide. EDTA in its pure form already has a lower surface tension than 1% or 5% NaOCL, saline solution or distilled water. EDTA proves to have a greater antimicrobial effect than saline (Yoshida et al 1995) furthermore, the combined use of EDTA and 5% NaOCl has a greater antimicrobial effect than NaOCL alone (**Bystrom and Sundqvist** 1985)106 and also has an effect on removal of smear layer.[11]

Recently, MTAD (mixture of tetracycline isomer, acids and detergent) and Tetraclean, two new irrigants based on a mixture of antibiotic, citric acid, and detergent have been developed. MTAD is the first irrigating solution created which is capable of both removing the smear layer and disinfecting the root canal system.[12] MTAD is a mixture of 3% doxycycline hyclate, 4.25% citric acid and 0.5% polysorbate-80 (Tween 80) detergent. Tween 80 has been added to decreases the surface tension of MTAD solution.

In most studies, its effect on the smear layer is attributed to the existence of doxycycline and citric acid. These two components in the solution have been separately reported as being effective in the removal of the smear layer.[13]

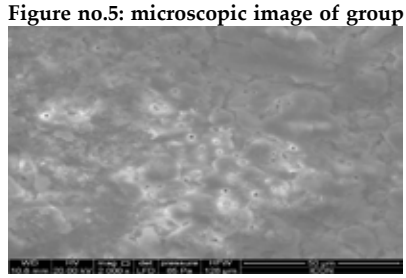
Recently the use of laser has certainly shown great promise in root canal therapy and its main application is to remove the smear layer remaining on the instrumented root canal walls. The effects of laser irradiation in endodontics have been investigated previously. An argon laser had efficient cleaning activity on the instrumented root canal surfaces (Harashima et al. 1997a). The Nd:YAG laser was used to irradiate root canal walls and caused melted, recrystallized, and glazed surfaces (Dederich et al. 1984). The Nd:YAG laser was able to produce clean root canals when combined with hand filing and showed a general absence of smear layer and tissue remnants on the root canal wall (Goodis et al. 1992). The CO2 laser system has been used to remove organic tissue from the root canal, to fuse hydroxyapatite, and to open dentinal tubules (Onal et al.1993).[14]

Hence the purpose of present in vitro Scanning Electron Microscopic study was to compare efficacy of various irrigating solutions and Laser to remove of smear layer from instrumented root canals.

We evaluated sodium hypochlorite (Group A), EDTA (Group B), 'BioPure' MTAD (Group C), Laser (Group D) for their efficacy to remove the smear layer from the instrumented samples.

The Group A of our study was treated with NaOCl; as per the scoring criteria 3 samples had no smear layer, 7 samples had moderate and 5 had heavy smear layer. The photomicroscopic images of most of the samples had dentinal tubules loaded with debris.

Figure no.5: microscopic image of group 1 (NaOCl group)



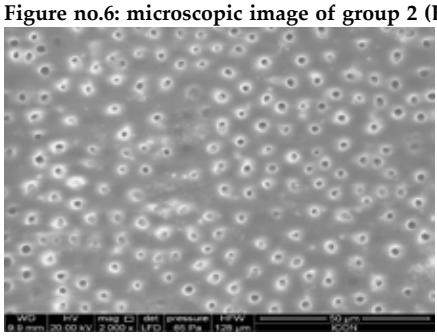
Luiz FM Silveira, CF **Silveira et al** (2013) conducted a study to compare, cleaning efficacy of a 2.5% sodium hypochlorite (NaOCl) and a 17%ethylenediaminetetra-acetic acid (EDTA) solution with the two solutions either applied alternately or mixed together for smear layer removal after the use of each endodontic file in different root thirds, by scanning electron microscopy analysis. They concluded 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.[15]

In the results of our study we found that photomicroscopic images of 12 samples (7 samples with moderate smear layer, 5 samples with heavy smear layer) of NaOCl group were loaded with smear layer. This could be because of the fact that NaOCl cannot dissolve inorganic dentin particles,

which are the components of smear layer composition. It has been proved that NaOCl can dissolve necrotic tissue, and organic components of smear layer but cannot dissolve inorganic dentin particles, hence is unable to prevent the smear layer formation/removal during instrumentation.[10]

The samples from group B were irrigated with EDTA as final flush. This group showed best results as far as smear layer removal is concerned. As per the scoring criteria we observed that 8 samples showed no smear layer, 7 had moderate smear layer and no samples with heavy smear layer. The photographic images of maximum samples showed open dentinal tubules.

Figure no.6: microscopic image of group 2 (EDTA group)



Semra Çalt **and Ahmet Serper** (2002) studied effects of EDTA on smear layer removal and on the structure of dentin after 1 and 10 min of applications. The results showed that 1 min EDTA irrigation is effective in removing the smear layer. However a 10-min application of EDTA caused excessive peritubular and intertubular dentinal erosion.[16]

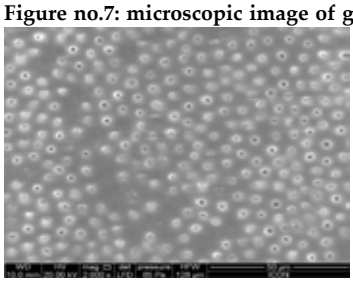
This group (EDTA Group) of our study also gave the best results in removing the

smear layer. 8 samples from this group had no smear layer. This could be attributed to the chelating effect conferred by EDTA. EDTA facilitates chelation of the inorganic portion of dentin. This action of EDTA must have helped in clearing the smear layer from the canal

walls of samples in this group.

In our study, Group C received the treatment with 'Bio-Pure' MTAD. As per the scoring criteria 6 samples showed no smear layer, 5 samples had moderate smear layer and 4 samples were with heavy smear layer. Photomicrographs of maximum samples (6 samples) showed dentinal tubules covered with moderate smear layer.

Figure no.7: microscopic image of group 3 (MTAD group)



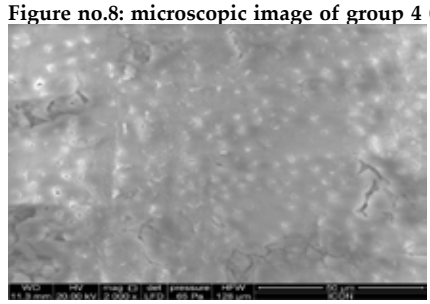
M Torabinejad, AA Khademi, et al (2003) conducted a study to investigate the effect of MTAD. Forty-eight extracted maxillary and mandibular single-rooted human teeth were prepared, Sterile distilled water or 5.25% sodium hypochlorite was used as intracanal irrigant. The canals were then treated with 5 ml of one of the following solutions as a final rinse: sterile distilled water, 5.25% sodium hypochlorite, 17% EDTA, or a new solution, MTAD. The presence or absence of smear layer and the amount of erosion on the surface of the root canal walls at the coronal, middle, and apical portion of each canal were examined under a scanning electron microscope. They concluded that MTAD is an effective solution for the removal of the smear layer and does not significantly change the structure of the dentinal tubules when canals are irrigated with sodium hypochlorite and followed with a final rinse of MTAD.[17]

In our study, there was no statistically significant difference observed between EDTA and MTAD also. Based on our results and previous investigations it seems that MTAD has the ability to remove most of the smear layer. The smear layer removing ability of MTAD could be related to its composition. MTAD contains citric acid as one of its components. Being acidic in nature, citric acid might have helped to remove smear layer from root canal walls of the instrumented samples of MTAD group.

The samples from group D of our study were irradiated with Laser. This group gave very poor results as compared with other groups in the study. Though the statistical analysis showed no significant difference between laser and NaOCl groups and laser and MTAD groups, the EDTA and laser group showed statistically significant difference in the percentage values of samples showing smear layer removal.

As per the scoring criteria for laser group, we observed that 2 samples showed no smear layer, 4 had moderate smear layer and 9 samples had heavy smear layer. The photomicrographic images showed that maximum samples (13 samples) of this group did not show open dentinal tubules.

Figure no.8: microscopic image of group 4 (LASER group)



FH Takeda, T Harashima et al (1998) evaluated efficacy of Er:YAG laser irradiation at two power settings (1 W and 2 W) in removing intracanal debris and smear layer. Control specimens showed an amount of debris and heavy smear layer obscuring the dentinal tubules at all levels in the canals. The root canal walls irradiated by Er:YAG laser were free of debris, with an evaporated smear layer and open dentinal tubules.[18]

These finding are contrary to our results. Photomicrographic images in our study showed blocked dentinal tubules in the laser group samples.

In SEM evaluation of the canal wall dentin following variants of Nd:YAG laser irradiation, Dederich et al and Tewfik et al reported a range of findings from no change or disruption of the smear layer to actual melting and recrystallization of the dentin35. Our findings are somewhat similar to this investigation. The photomicrographs of the laser group showed blocked dentinal tubules. Knowing the potential of laser to melt and vaporize dentin-like structure, laser might be effective in removing smear layer as well. The dentinal tubules of laser group in our study could be

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blocked with porous layers of melted minerals of vaporized dentine formed by microexplosion from laser irradiation.

So from the observations of this in vitro study we can conclude that EDTA is most effective in removing smear layer from instrumented root canal walls. However, there is enough literature available that suggests that the method of choice to remove the smearlayer seems to be the alternate use of EDTA and NaOCl solutions. As several new sealers and core materials have recently been introduced, further investigations are required to determine the role of smear layer in the outcome of endodontic treatment.

CONCLUSION

Within the limitation of this in vitro study, we conclude that:

1. The samples treated with EDTA gave best results in removing smear layer and photomicrographic images of maximum samples showed open dentinal tubules.

2. The MTAD showed better results than NaOCl irrigation group.

3. In the laser irradiation group photomicrographic images of maximum samples did not show the open dentinal tubules. We are not sure whether the tubules were covered with smear layer

or the porous layers of melted minerals of vaporized dentine formed by microexplosion from laser irradiation.

Hence we can conclude that EDTA is capable in removing the smear layer and cleaning dentinal tubules efficiently. Thus it contributes to the success of endodontic treatment.

REFERENCES

1. A New Solution for the Removal of the Smear Layer
2. Smear layer: a brief review of general concepts. Part II. The most common agents to remove endodontic smear layer
3. Efficacy of various concentrations of citric acid at different pH values for smear layer removal
4. The influence of the dentin smear layer on adhesion: a selfetching primer vs. a total-etch system
5. The Antimicrobial Effect of MTAD: An In Vitro Investigation
6. Markus Haapasalo et al, irrigation in endodontic. DCNA 2009 291-312.
7. Cohen's pathways of pulp tenth edition.
8. The smear layer in Endodontics : a review D.R.Violich and N.P> chandler. International endodontic journal, 43, 2-15, 2010.
9. Clinical implications of the smear layer in endodontics: A review.
10. M Zehnder, root canal irrigation. Journal of Endodontics 2006 389-398.
11. M. Hulsmann, et al chealating agents in root canal treatment: mode of action and indication for their use. International Endodontic Journal Vol 36, Issue 12, 810-830 December 2003.
12. Cohen's pathways of pulp Eighth edition.
13. Dr. Torabinajad A new Solution for the removal of the smear layer; Journal of Endodontics 29, 2003; 170-175(A126).
14. A comparative study of the removal of smear layer by three endodontic irrigants and two types of laser International Endodontic Journal, 32, 32±39, 1999.
15. Evaluation of the different irrigation regimens with sodium hypochlorite and EDTA in removing the smear layer during root canal preparation Journal of Microscopy and Ultrastructure 2013 51–56
16. Time-Dependent Effects of EDTA on Dentin Structures JOURNALOF ENDODONTICS VOL. 28, NO. 1, JANUARY 2002.
17. A new solution for removal of smear layer JOURNAL OF ENDODONTICS VOL. 29, NO. 3, MARCH 2003.
18. Efficacy of Er:YAG Laser Irradiation in Removing Debris and Smear Layer on Root Canal Walls JOURNAL OF ENDODONTICS VOL. 24, NO. 8, AUGUST 1998.