ISSN 0976-4003



Volume 14 • Issue 2 • April-June 2022

INDIAN JOURNAL OF DENTAL SCIENCES

An Official Publication of Himachal Pradesh University

www.ijds.in



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Original Article

Scanning Electron Microscopic Comparative Analysis of Smear Layer Removal using Ethylenediaminetetraacetic Acid and Chitosan Activated by Ultrasonics and Diode Laser: An *In vitro* Study

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Abstract

Context: The inorganic component of the smear layer is traditionally removed using ethylenediaminetetraacetic acid (EDTA). **Aims:** This study was done to compare the ability of smear layer removal by ultrasonic activation and diode laser activation of EDTA with Chitosan – which is a novel nanoparticle-based chelating agent. **Settings and Design:** Prospective *in vitro* experimental study conducted in a tertiary care Dental college and hospital for 6 months. **Subjects and Methods:** Seventy-five mandibular premolars with single canal were decoronated to standardize the root length to 14 mm. Canals were prepared up to Protaper F3 and the specimens were randomly divided into three groups and two subgroups based on the irrigation protocol. Group A (Normal saline), Group B1 and B2 (ultrasonically activated–EDTA and Chitosan, respectively), Group C1 and C2 (Diode laser-activated– EDTA and Chitosan, respectively). Samples were processed for scanning electron microscopic (SEM) analysis. Photographs were taken in the apical, middle, and coronal thirds. Data were collected using Gutmann's scoring criteria. **Statistical Analysis Used:** Statistical analysis was performed using one-way analysis of variance and Tukey's *post hoc* test ($P < 0.05$). **Results:** Diode laser-activated EDTA had the highest efficacy of smear layer removal at the coronal third. In the middle and apical third, ultrasonically activated EDTA had the highest efficacy. **Conclusions:** Chitosan may be considered as an alternative to EDTA, in the removal of smear layer considering the drawbacks of EDTA.

Keywords: Chelating agent, Chitosan, diode laser, ethylenediaminetetraacetic acid, nanoparticle, scanning electron microscope, smear layer, ultrasonics

Submitted: 04-May-2021; **Revised:** 07-Jun-2021; **Accepted:** 03-Aug-2021; **Published:** 26-Apr-2022

Introduction

Cleaning and shaping of root canal lead to the formation of smear layer which contains inorganic debris and organic material.[1,2] Literature currently available supports the removal of the smear layer for improved disinfection.[3] The organic portion of the smear layer is routinely removed using sodium hypochlorite, whereas the gold standard for the removal of inorganic components is ethylenediaminetetraacetic acid (EDTA).[4]

Although EDTA is an effective chelating agent, it has several disadvantages such as dentinal erosion, harmful effect on periapical tissues, inactivation of sodium hypochlorite, and lack of antimicrobial properties.[5] This has led researchers to seek more a biocompatible material as an alternative.

Access this article online

Quick Response Code:

Website: www.ijds.in

DOI: 10.4103/IJDS.IJDS_56_21

Chitosan, a recently developed biopolymer, is reported to be effective in the removal of smear layer. It has an additional antibacterial property and is highly biocompatible.[6]

Several studies have shown that ultrasonic activation improved the efficacy of the chelating agents in the removal of the smear layer.[7,8] Recently, the use of laser devices for agitating irrigating solutions has gained popularity because the fiber

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How to cite this article: Vineetha CS, Ravi V, Sampathkumar SJ, Prasad SA, Soundappan SP, Chittrarasu M. Scanning electron microscopic comparative analysis of smear layer removal using ethylenediaminetetraacetic acid and chitosan activated by ultrasonics and diode laser: An *In vitro* study. Indian J Dent Sci 2022;14:74-8.

is thin and flexible, allowing access into narrow and curved root canals.[9]

In this study, the efficacy of Chitosan and EDTA in combination with Ultrasonic and Diode lasers for agitation was compared and the ability of smear layer removal was evaluated.

Hence, the use of a novel chelating agent which is biocompatible with better antibacterial properties, in combination with newer irrigating systems would aid in better debridement of the root canal, thus improving the clinical outcome.

Subjects and Methods

A total of 75 freshly extracted mandibular single-rooted premolars (calculated with a confidence interval of 95%, power of 5%, and considering 20% attrition) were taken for the study. Teeth extracted for periodontal or orthodontic reasons were included in the study. Radiographs were taken in the buccolingual and mesiodistal directions of all the samples to confirm the canal patency. Teeth were stored in 10% formalin solution until they were used for the study.[10,11] The root surfaces were cleaned and then decoronated using a diamond disc under constant water irrigation to obtain a standard root length of 14 mm. Instrumentation of root canal was initiated with ISO hand files up to #20 followed by ProTaper rotary files up to size F3, employing the crown down technique. In between every change of instrument, the canal was irrigated with 2 mL of 3% NaOCl. The irrigants were delivered with a disposable syringe with 30G side vented needle placed 1 mm short of the working length. Finally, 3 mL of 3% NaOCl was used to flush out the debris from the root canals followed by a final rinse with 3 mL of distilled water to terminate any action of the solvents remaining in the canal.[7,12] The electronic weighing device was used to measure 0.2 g of low molecular weight chitosan. The solution was prepared by dissolving 0.2 g of chitosan in 100 mL of 1% acetic acid. A heated magnetic stirrer was used to agitate this solution for 2 h to obtain a homogenous clear solution.[13]

Specimens were randomly divided into 3 groups and 2 subgroups (15 samples each) based on the irrigation protocol.

Group A (Control) – Normal saline

One milliliter of normal saline was used to flush the canals for 1 min followed by flushing the canal with 3 ml of 3% NaOCl.

Group B1 – Ultrasonically activated ethylenediaminetetraacetic acid

One milliliter of 17% EDTA was used to flush with passive ultrasonic activation (using #20 U file) for 1 min, followed by flushing the canal with 3 ml of 3% NaOCl.

Group B2 – Ultrasonically activated Chitosan

One milliliter of 0.2% Chitosan was used to flush with passive ultrasonic activation (using #20 U file) for 1 min, followed by flushing the canal with 3 ml of 3% NaOCl. In groups B1 and B2, the U file was placed into the canal such that it was 1 mm short of the measured working length.

Group C1 – Diode laser activated ethylenediaminetetraacetic acid

0.8 ml of 17% EDTA was used to irrigate the canal for 40 s and diode laser was used to activate the remaining 0.2 ml for 20 s. A fiberoptic tip measuring 200–300 µm, 970 ± 15 nm, with a max power of 7W was used for laser activation of the canal up to the working length. In a helicoid movement, the tip was withdrawn to the coronal region and reintroduced to the apical region for an irradiation cycle of 20 s, followed by 3 ml of 3% NaOCl.

Group C2 – Diode laser-activated Chitosan

0.8 ml of 0.2% Chitosan was used to irrigate the canal for 40 s and diode laser was used to activate the remaining 0.2 ml for 20 s. A fiberoptic tip measuring 200–300 µm, 970 ± 15 nm, with a maximum power of 7W was used for laser activation of the canal up to the working length. In a helicoid movement, the tip was withdrawn to the coronal region and reintroduced to the apical region for an irradiation cycle of 20 s, followed by 3 ml of 3% NaOCl. 5 ml of distilled water was used as a final flush in all the samples to terminate the action of the other irrigants used.[7]

The teeth were grooved along the buccal and lingual planes by using a diamond disc at low speed under constant water coolant. The roots were then split longitudinally with a bi-beveled chisel and a mallet. One-half of each root was selected depicting the entire root canal length and prepared for SEM examination. The selected samples were progressively dehydrated using graded concentrations of aqueous ethanol (70%, 80%, 90%, and 100%) for 24 h at each concentration. The dehydrated samples were sputter-coated with 30 nm gold layer in a vacuum chamber. The root canal wall was examined at coronal, middle, and apical thirds at a magnification of ×2000 [Figure 1] and ×5000 [Figure 2] for the presence or absence of smear layer and patency of dentinal tubules.

Photographs of the root canals were taken at the coronal, middle, and apical level [Figure 1] for scoring individually in a calibrated single-blind manner according to the rating system developed by Gutmann *et al*.[14]

- Score 1 - Little or no smear layer; covering 25% of the specimen most tubules were visible and patent or almost complete laser melting
- Score 2 - Little to moderate or patchy mounts of smear layer, covering 25%–50% of the specimen; many tubules visible and patent, or laser melting
- Score 3-Moderate amounts of scattered or aggregated smear layer; covering 50%–75% of the specimen; minimal to no tubule visibility or patency, or scattered laser melting
- Score 4 - Heavy smear layer covering >75% of the specimen; no tubule orifices were visible or patent; or no visible laser melting.

Statistical analysis of data was performed using a one-way analysis of variance using SPSS version 20 (IBM Corp, Armonk, NY, USA) and Tukey's *post hoc* tests ($P < 0.05$). Analysis of the data was performed using one-way analysis of variance, using the SPSS version 20 SPSS version 20 (IBM Corp, Armonk, NY, USA). The values were considered statistically significant when $P < 0.05$.

Results

The mean values of the remaining smear layer scores were tabulated [Table 1 and Graph 1]. Scanning electron microscopy analysis of the experimental specimens at ×2000 and ×5000 revealed that the efficacy of smear layer removal of Diode laser-activated EDTA was highest at the coronal third. Ultrasonically activated EDTA had the highest ability to remove the smear layer in both the apical and middle third of the root canal [Table 2].

Discussion

There is increasing interest in developing new irrigating solutions and activation systems due to the limitations of the currently available ones.

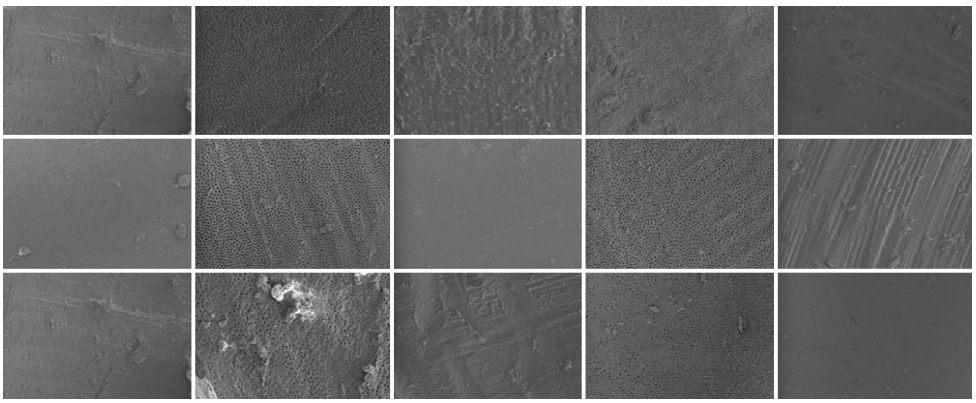


Figure 1: Scanning electron microscope photomicros of specimens of all the five groups with configurations (on the top left) 1,2 and 3 representing coronal, middle and apical third portion of root canal, respectively, whereas A, B1, B2, C1 and C2 represent their respective groups-image obtained at ×2000 magnification

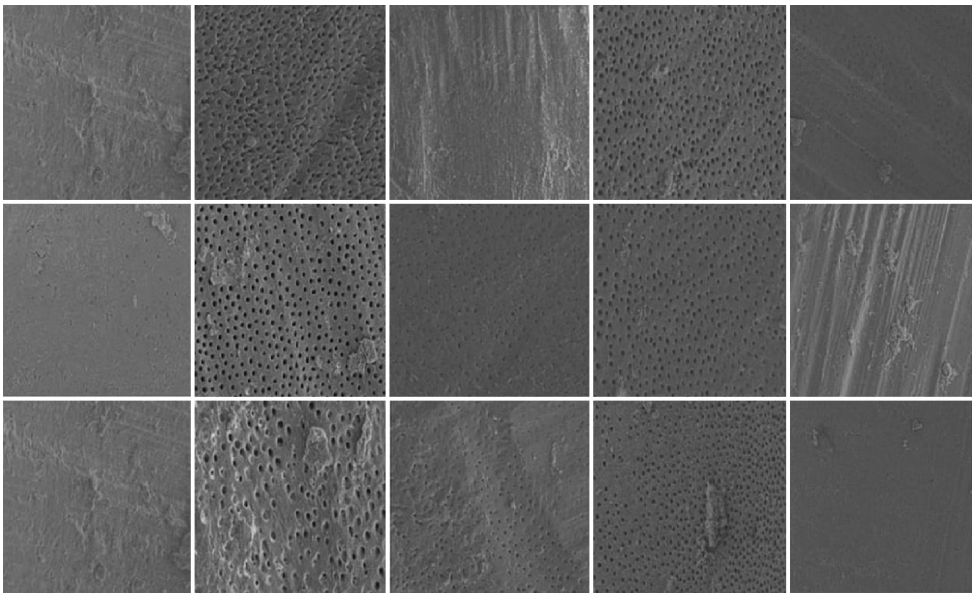
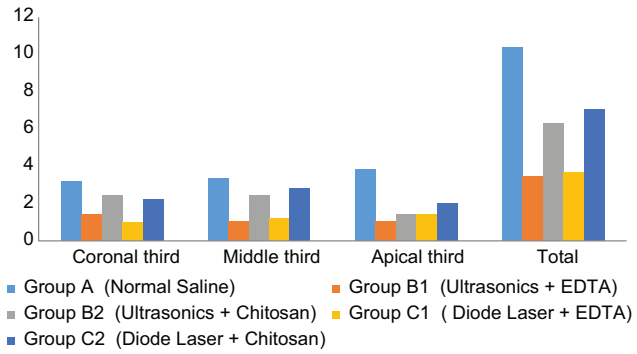


Figure 2: Scanning electron microscope photomicrographs of specimens of all the five groups with configurations (on the top left) 1, 2 and 3 representing coronal, middle and apical third portion of root canal, respectively, whereas A, B1, B2, C1 and C2 represent their respective groups-image obtained at ×5000 magnification

Table 1: Scores of mean remaining smear layer among various groups

Area recorded	Group A (normal saline)	Group B1 (ultrasonics + EDTA)	Group B2 (ultrasonics + chitosan)	Group C1 (diode laser + EDTA)	Group C2 (diode laser + chitosan)
Coronal third	3.21	1.41	2.42	1.01	2.21
Middle third	3.33	1.02	2.45	1.20	2.81
Apical third	3.84	1.03	1.44	1.44	2.02
Total	10.38	3.46	6.31	3.65	7.04

EDTA: Ethylenediaminetetraacetic acid



Graph 1: Scores of mean remaining smear layer among various groups

In this study, we have compared ultrasonics and 970 nm-diode lasers as an adjunct to irrigation because several studies showed that they increase the efficacy of smear layer removal. Ultrasonic activation enhances the penetration of irrigating solution into the narrow apical regions of the root canals.[15] The U-file oscillations are primarily responsible for the production of acoustic streaming causing cavitation.[16] Diode laser activation has also shown to increase the efficacy of the irrigating solution by the absorption of laser energy. The near-infrared wavelength of laser causes the formation of vapor bubbles followed by the collapse of these bubbles, which finally leads to cavitation, which enhances smear layer removal.[17,18]

In our study, EDTA was compared to Chitosan, which is a natural, cationic amino polysaccharide having the ability to chelate metal ions such as Fe^{2+}, CO^{2+}, Mg^{2+}, Zn^{2+} and Cu^{2+} in acid conditions.[19] According to several recent studies, a combination of Chitosan-EDTA (1:1) can perform as a root canal disinfectant and can also be used in the removal of smear layer.[20] EDTA potentiates the antibacterial activity of Chitosan and facilitates the entry of Chitosan into the bacterial cell, this combination is known to restrain the growth of microorganisms by enzyme inhibition.[21] The NH^{3+} moieties present in the chitosan were reported to react with COO– moieties of EDTA to form ionic EDTA– CHT complex.[22]

According to Walmsley the smear layer removal at the apical third was found to be the least because of the constriction in the root canal, which restricted the oscillation of the ultrasonic tip. The apical part is the most affected due to attenuation of oscillation because the amplitude is greatest at the tip of the instrument.[23] This was in accordance with the current study in which EDTA and Chitosan which showed effective smear layer removal from the coronal third due to the greater diameter of the canal.

Group A had the least efficacy in the removal of the smear layer, which is in accordance to a study conducted by Rathakrishnan *et al*. in which there was thick smear layer all through the length of the root canal.[24]

In the coronal third, a combination of EDTA with diode laser had the least remaining smear layer score. This was similar to the study Neelakantan *et al*., in which diode laser was found to be better than ultrasonics in the disinfection of dentinal tubules.[17] and a study carried out by Arslan *et al*. which

Table 2: Intra group comparison of remaining smear layer scores at various levels

Groups	Coronal			Middle			Apical			Overall		
	Mean difference	*P*	Significance	Mean difference	*P*	Significance	Mean difference	*P*	Significance	Mean difference	*P*	Significance
A versus B1	1.80	0.00	S	2.31	0.00	S	2.81	0.00	S	6.92	0.00	S
A versus B2	0.80	0.00	S	0.90	0.00	S	2.40	0.00	S	4.10	0.00	S
A versus C1	1.80	0.00	S	2.13	0.00	S	2.40	0.00	S	6.33	0.00	S
A versus C2	1.00	0.00	S	0.52	0.00	S	1.82	0.00	S	3.34	0.00	S
B1 versus B2	–1.01	0.00	S	–1.43	0.00	S	–0.41	0.40	NS	–2.85	0.00	S
B1 versus C1	0.40	1.00	NS	–0.20	1.00	NS	–0.41	1.00	NS	–0.21	1.00	NS
B1 versus C2	–0.80	0.01	S	–1.80	0.00	S	–1.00	0.01	S	–3.60	0.00	S
B2 versus C1	1.41	0.00	S	1.25	0.00	S	–0.00	0.40	NS	2.70	0.00	S
B2 versus C2	0.20	1.00	NS	0.00	1.00	NS	–0.60	1.00	NS	–0.40	1.00	NS
C1 versus C2	–1.00	0.00	S	–1.60	0.00	S	–0.60	0.004	S	–3.20	0.00	S

S: Significance, NS: Not significance

showed that the activation of 15% EDTA using 808-nm diode laser was more effective in the removal of smear layer.[18]

In the apical 3rd, Group B1 (a combination of ultrasonics with EDTA) had the maximum efficacy of smear layer removal. This was similar to a study conducted by Amin *et al*.[7] Following this, the efficacy of Group B2 (ultrasonically activated Chitosan) was better than Group A (Normal saline) and Groups B2 and C1 (ultrasonically activated EDTA and Diode laser-activated Chitosan respectively). These results were not in accordance with the study conducted by Darrag which showed 0.2% Chitosan to be more effective than 17% EDTA and 10% Citric acid. This can be due to the use of ultrasonics as an adjunct in the present study, which shows better results when used in combination with EDTA.[25]

Hence, from the current study, it can be inferred that the type of chelating agent used and the method of activation has varying effect at different levels of the root canal. Further *in vivo* studies need to be carried out to support the results of the current study.

Conclusions

From the results of this study, it can be concluded that Chitosan also possesses chelating ability. Hence it can be considered as an alternative to EDTA in the removal of the smear layer because of its additional antibacterial property.

Limitations

- Being an *in vitro* study, the results cannot be directly correlated to the clinical situations
- In the present study, properties such as biocompatibility and antibacterial efficacy were not assessed
- In this study, only 0.2% chitosan was used, further studies using higher concentrations of Chitosan are needed to support the results of the present study.

Acknowledgment

We sincerely thank Dr. Sashidharan Nair, HOD of Department of Applied Sciences, PSG Institute of Technology for the help he has provided with the usage of the magnetic stirrer in our material preparation ans we are also thankful to Dr.Anil Mathew M.D., Department of Community Medicine, PSG Institute of Medical Sciences for his guidance in the statistical works of this study.

Ethical clearance

Ethical Clearance was obtained by the Institution's Ethics Committee of Vivekanandha Dental College for Women.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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