RESEARCH ARTICLE

ELECTRON MICROSCOPIC COMPARATIVE ANALYSIS OF SMEAR LAYER REMOVAL BY ULTRASONICALLY ACTIVATED AND DIODE LASER ACTIVATED - EDTA AND CHITOSAN: AN INVITRO STUDY

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ABSTRACT

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 novel chelating agents which are biocompatible, with minimal tissue toxicity and better antibacterial efficacy, in combination with newer irrigating systems which would aid in better debridement of the root canal would improve the clinical outcome of root canal treatment.

Methodology: This study was assessed 75 freshly extracted mandibular premolars with single canal in department of Conservative dentistry and Endodontics of Saraswati dental college and hospital, Lucknow. These were extracted due to poor periodontal prognosis and orthodontic reasons. X-ray was taken in both buccolingual and mesiodistal directions to confirm the presence of single canal. Completely formed teeth with intact apices, Teeth without anatomical variations, Teeth without caries and root canal fillings were included in inclusion criteria. Fractured roots, Teeth with multiple roots, Open apices, Calcifications in the canal, Root resorption and cracks on the surface were included in exclusion criteria.

Results: In the middle and apical third, ultrasonically activated EDTA had the highest efficacy. The dentinal tubule orifices were patent with clearly demarcated boundaries in both the groups. Normal saline had the least efficacy as compared to the other groups throughout the length of the specimen which showed occluded orifices on the dentin surface with florid debris.

Keywords: EDTA, Chitosan

INTRODUCTION:

Root canal treatment is an essential procedure carried out in various clinical situations which include teeth with deep caries and irreversible pulpitis, following trauma, attrition, resorption and in certain clinical conditions such as prosthetic rehabilitation of missing teeth, when the tooth/teeth need to be taken as an abutment. The success of an endodontic procedure majorly lies on 3 important factors that include – Creating a straight line access, proper cleaning and shaping of the canals and producing a 3 Dimensional obturation with a good seal. Shaping of the canal wall is carried out using endodontic hand files or rotary instruments by cutting the root dentin

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along the canal walls. This leads to the production of an irregular amorphous smear layer.[1]Cleaning the canal refers to the removal of smear layer, which can be done using chemical agents, ultrasonics and/or by the use of laser. Chemical agents such as sodium hypochlorite, EDTA and organic acids in combination with ultrasonics and laser agitation have also been used. Ethylenediaminetetraacetic acid (EDTA) is the most commonly used irrigant/chelating agent for removal of smear layer[2,3,4]. It promotes decalcification by chelating the calcium ions in dentine at approximate depths of 20–30 μm within 5 min . EDTA has harmful effect on periapical tissues and this has led researchers to seek more biocompatible material as an alternative. Weak acids, like apple cider vinegar and citric acid have also been studied [5,6,7]. Chitosan has gained attention in dental research because it is biocompatible, biodegradable possesses the property of bio adhesion and lack of toxicity[8,9] . Chitosan is obtained by the deacetylation of chitin, which is found in crab and shrimp shells[10.] Recently, the use of laser devices for agitating the irrigating solutions has gained popularity. Several studies have used Nd-YAG lasers for canal disinfection. The advantage of near-infrared diode laser is that the fibre is thin and flexible, which allows access into narrow and curved root canals, and it provides increased. Disinfection of the deep radicular dentin. The type of irrigating solution used and the laser wavelength determines the quantity of irrigant absorbed into the canal walls. One of the effectively used methods to determine the ability of smear layer removal is Scanning electron microscopy. Among the various scoring systems for quantifying the remaining smear layer, Gutmann's scoring criteria was followed in the present study. 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 novel chelating agents which are biocompatible, with minimal tissue toxicity and better antibacterial efficacy, in combination with newer irrigating systems which would aid in better debridement of the root canal would improve the clinical outcome of root canal treatment[1].

MATERIALS AND METHODS:

This study was assessed 75 freshly extracted mandibular premolars with single canal in department of Conservative dentistry and Endodontics of Saraswati dental college and hospital, Lucknow. These were extracted due to poor periodontal prognosis and orthodontic reasons. X-ray was taken in both buccolingual and mesiodistal directions to

confirm the presence of single canal. Completely formed teeth with intact apices, Teeth without anatomical variations, Teeth without caries and root canal fillings were included in inclusion criteria. Fractured roots, Teeth with multiple roots, Open apices, Calcifications in the canal, Root resorption and cracks on the surface were included in exclusion criteria.

Procedure:

1) **Removal of external residual tissues:** The residual tissues on the surface of the teeth were removed and were stored in 2.5% NaOCl solution for 10 minutes. Calculi were removed using hand scalers from the external surfaces and they were again stored in distilled water.

2) **Root canal therapy:** The collected samples were decoronated with a diamond disc to length of 14 ± 1 mm, measured with the help of a calliper. Access cavity preparation was done with copious water using high speed diamond burs on each teeth. A #10 K file was inserted in the root canal till it was visible at the apical end of the root. Working length determination was done by reducing 1 mm from this measurement. Pro Taper Universal rotary file was used for canal preparation. To simulate the clinical conditions the apices were sealed with sticky wax. A #20 K file was used for instrumentation of the canal after which it was instrumented up to size F3 ProTaper universal rotary files. 2ml of 3% NaOCl was used for irrigation after using each file. The irrigating solutions were delivered using a 27 gauge needle which was placed 1mm short of the measured working length. Finally, for the flushing out of debris 3ml of 3% NaOCl was used followed by a final rinse with distilled water.

3) **0.2% Chitosan preparation:** Electronic weighing device was used to measure 0.2g of low molecular weight Chitosan. The solution was prepared by dissolving 0.2g of Chitosan in 100 mL of 1% acetic acid . A heated magnetic stirrer was used to agitate this solution for 2 hours to obtain a homogenous clear solution.

Grouping Of Samples:

Group A (Control) – Normal Saline 1ml of Normal saline was used to flush the canals for 1 minute followed by flushing the canal with 3 ml of 3% NaOCl.

Group B1 – Ultrasonically activated EDTA 1ml of EDTA was used as a final flush to irrigate the canals and passive ultrasonic activation was done with #20 U file followed by flushing the canal with 3ml of 3% NaOCl.

Group B2 – Ultrasonically activated Chitosan :Final flush of 1ml of 0.2% Chitosan was used to irrigate the canals and then passive ultrasonic activation was done using #20 U file [Fig 8, 10] for 1 minute, followed by flushing the canal with 3ml of 3% NaOCl. In groups B1 and B2 the U file was placed into the canal so that it was 1mm short of the measured working length.

Group C1 – Diode laser activated EDTA : 0.8ml of 17% EDTA was used to irrigate the canal for 40 seconds and diode laser was used to activate the remaining 0.2ml for 20 seconds. The treatment was undertaken for four passes of each 5 seconds. Each pass was done at a fibre withdrawal rate of 1mm/second. A fiberoptic tip measuring 200-300µm, 970±15nm, with a power of 2W 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 seconds, followed by 3ml of 3% NaOCl.

Group C2 – Diode laser activated Chitosan 0.8ml of 0.2% Chitosan was used to irrigate the canal for 40 seconds and diode laser was used to activate the remaining 0.2ml for 20 seconds. The treatment was undertaken for four passes of each 5 seconds. Each pass was done at a fibre withdrawal rate of 1mm/second. A fiberoptic tip measuring 200-300µm, 970±15nm, with a power of 2W 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.Reintroduced to the apical region for an irradiation cycle of 20 seconds, followed by 3ml of 3% NaOCl. 5ml of distilled water was used as a final flush in all the samples to terminate the action of the other irrigants used. Scanning electron microscopic examination was carried out after the samples were dried and prepared.

RESULTS:

The mean values of the remaining smear layer scores were tabulated (Table 1). Analysis of the data was done using One-way analysis of variance (ANOVA), using the SPSS version 20. The values were considered statistically significant when P value < 0.05. There was statistically significant difference among all the tested groups except among Group B2 and C1 in the apical third, which had no significant difference (Table 2).

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

Area Recorded	Group A (Normal Saline)	Group B1 (Ultrasonics + EDTA)	GroupB2 (Ultrasonics + Chitosan)	Group C1 (Diode Laser + EDTA)	Group C2 (Diode Laser + Chitosan)
Coronal third	3.2	1.4	2.4	1	2.2
Middle third	3.33	1	2.4	1.2	2.8
Apical third	3.8	1	1.4	1.4	2
Total	10.33	3.4	6.2	3.6	7

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

GROUPS	CORONAL			MIDDLE			APICAL			OVERALL		
	Mean difference	P value	Sig	Mean difference	P value	Sig	Mean difference	P value	Sig	Mean Difference	P Value	Sig
A vs B1	1.800	0.000	S	2.333	0.000	S	2.800	0.000	S	6.933	0.000	S
A vs B2	0.800	0.000	S	0.9333	0.000	S	2.400	0.000	S	4.133	0.000	S
A vs C1	1.800	0.000	S	2.333	0.000	S	2.800	0.000	S	6.933	0.000	S
A vs C2	0.800	0.000	S	0.933	0.000	S	2.400	0.000	S	4.133	0.000	S
B1 vs B2	-1.000	0.000	S	-1.400	0.000	S	-0.400	0.400	S	-2.800	0.000	S
B1 vs C1	0.000	1.000	NS	0.000	1.000	NS	0.000	1.000	NS	0.000	1.000	NS
B1 vs C2	-1.000	0.000	S	-1.400	0.000	S	-0.400	0.004	S	-2.800	0.000	S
B2 vs C1	1.000	0.000	S	1.400	0.000	S	-0.400	0.400	S	2.800	0.000	S
B2 vs C2	0.000	1.000	NS	0.000	1.000	NS	0.000	1.000	NS	0.000	1.000	NS
C1 vs C2	-1.000	0.000	S	-1.400	0.000	S	-0.400	0.004	S	-2.800	0.000	S

Coronal third – C1 > B1 > C2 > B2 > A

Middle third – B1 > C1 > B2 > C2 > A

Apical third – B1 > B2 = C1 > C2 > A

Overall – B1 > C1 > B2 > C2 > A

Scanning Electron Microscopy analysis of the experimental specimens at 2000x and 5000x magnifications revealed that among the tested specimens, the efficacy of smear layer removal of Diode laser activated EDTA was highest at the coronal third. In the middle and apical third, ultrasonically activated EDTA had the highest efficacy. The dentinal tubule orifices were patent with clearly demarcated boundaries in both the groups. Normal saline had the least efficacy as compared to the other groups throughout the length of the specimen which showed occluded orifices on the dentin surface with florid debris.

DISCUSSION:

The disinfection of dentin walls using irrigants is adversely affected in the presence of smear layer by blocking them from entering dentinal tubules[2]. It also adversely affects sealer penetration and increases micro leakage following obturation leading to increased intra-canal microflora[11,12]. Hence, to enhance sealer penetration and a fluid tight seal it is necessary to remove the smear layer. There has been an increasing interest in developing new irrigating solutions due to the limitations of the presently available ones. Chitosan is a natural, cationic aminopolysaccharide copolymer of glucosamine and N-acetylglucosamine obtained by the alkaline, partial deacetylation of chitin. It is obtained from shells of crustaceans and shrimps[13] .According to Silva et al[14] the efficacy of 0.2% chitosan on the removal of smear layer was better than that of 1% acetic acid. This is important because in the present study 1% acetic acid was used in the preparation of chitosan solution.A.M.Darrag[15] studied the ability of ability of 17% EDTA, 10% CA, MTAD, and 0.2% chitosan solutions to remove the smear layer and concluded that 0.2% chitosan was better, but there is no significant difference among them. The effect of smear layer removal by Chitosan was compared to that of EDTA because EDTA is has been accepted as a gold standard for removal of smear layer[14.] A combination of NaOCl and EDTA has been successfully used in debridement and for enlarging narrow and obstructed canals. Fraser[16] in 1974 found that in the apical third the chelating ability of EDTA was minimal and it also caused root dentine erosion. EDTA also has limited antimicrobial activity compared to NaOCl. To minimise the harmful effects of EDTA the search continues for a newer material which is more biocompatible with enhanced antimicrobial effect.

In the present study, Chitosan solution preparation was done using 1% acetic acid. According to Silva et al[14], it was attributed

that the chelating ability of Chitosan was because of its own properties and not by 1% acetic acid. Thus, we could deduce that the chelating behaviour of Chitosan favoured its smear layer removal. In the current study, Ultrasonics and 970nm Diode laser were used as an adjuncts. This was because several studies showed that the addition of ultrasonics increased the smear layer removing by enhancing the penetration of irrigating solution into the narrow apical regions of the root canals[1,17,18,19]. According to Walmsley[20] et al, 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. This was in accordance with the current study in which EDTA and Chitosan which showed effective smear layer removal from coronal third. Comparing the overall efficacy of various combinations used in this study, Group B1 (EDTA+ Ultrasonics) produced better smear layer removal than Group B2 (EDTA+Diode Layer), which in turn was better than Group C1 and C2 i.e., a combination of Chitosan with Ultrasonics and diode laser respectively. Group A had the least efficacy in the removal of smear layer. In Group A (Normal Saline) there was thick smear layer all through the length of the root canal which is in accordance to a study conducted by Mensudar Rathakrishnan et al[21] .Arslan et al [22]evaluated the activation of 15% EDTA using 808-nm diode laser and concluded that on the removal of smear layer removal and concluded that agitation with diode laser was effective in the removal of smear layer. This was in accordance with the present study where EDTA activated with Diode laser had the greatest efficacy of smear layer removal .

In the middle and apical 3rd, Group B1 i.e., a combination of ultrasonics with EDTA was the most effective in the removal of smear layer. In the middle 3rd , following Group B1, Group C1 i.e., diode laser activated EDTA was better than Group B2 i.e., Ultrasonically activated Chitosan , which in turn was better than Group B2 and C2 i.e., Chitosan which was activated with Ultrasonics and Diode laser respectively. In the apical 3rd, Group B1 i.e., 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[23]. Following this, the efficacy of Group B2 i.e., ultrasonically activated Chitosan was better in the removal of smear layer than Group A i.e., Normal saline and Groups B2 and C1 i.e., Ultrasonically activated EDTA and Diode laser activated Chitosan produced similar results. These

results were not in accordance with the study conducted by A. M. Darrag et al[15] which showed 0.2% Chitosan to be more effective than 17% EDTA and 10% Citric acid.Comparing the overall efficacy of various combinations used in this study, Group B1 (EDTA+ Ultrasonics) produced better smear layer removal than Group B2 (EDTA+Diode Layer), which in turn was better than Group C1 and C2 i.e., a combination of Chitosan with Ultrasonics and diode laser respectively. Group A had the least efficacy in the removal of smear layer. According to a 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 [24,25,26] . EDTA potentiates the antibacterial activity of Chitosan and facilitates the entry of Chitosan into bacterial cell, this combination is known to restrain the growth of microorganisms by enzyme inhibition[25,26] . Hence, from the current study, it can be inferred that the combination of Diode laser with EDTA had the maximum efficacy in the coronal 3rd and a combination of Ultrasonics with EDTA had the maximum ability of smear layer removal in the middle and apical 3rd. Further studies i) using a combination of EDTA and Chitosan, ii) using higher concentration of Chitosan and iii) activation of irrigants using lasers, ultrasonics and newer adjuncts and more in vivo studies need to be carried out to support the results of the current study and to achieve clinical success.

CONCLUSION:

Under the limitations of the present study, 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. Normal saline had the least efficacy as compared to the other groups throughout the length of the specimen Hence from the results of the present study, it can be concluded that using Chitosan may be an alternative to EDTA, in the removal of smear layer considering the drawbacks of EDTA but further studies using higher concentrations of Chitosan and in vivo studies need to be carried out to support the results of the present study.

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FIGURES:

Fig 1: 75 Single rooted mandibular premolar

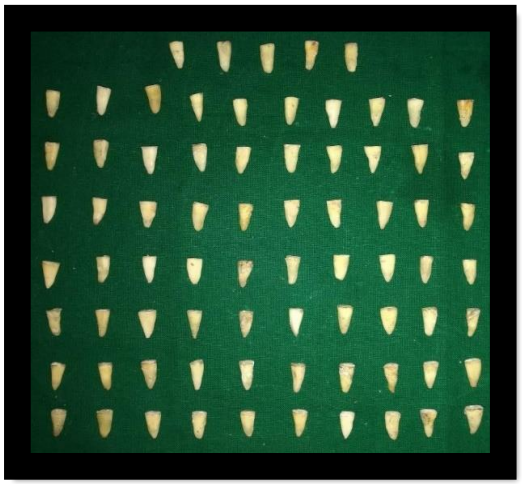

Fig 2: Decoronated samples

Fig 3: Armamentarium

Fig 4: Chitosan and Acetic acid

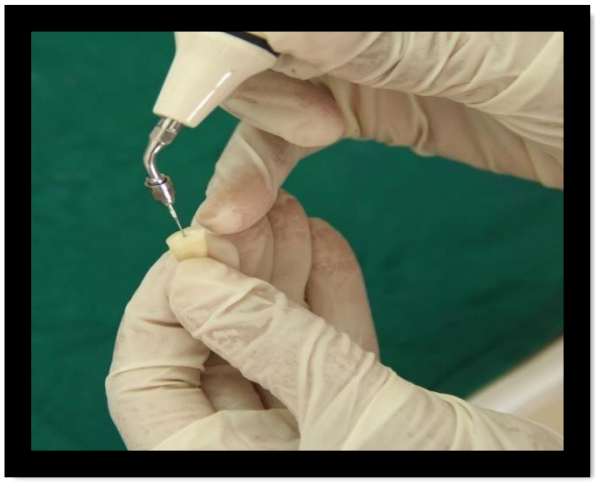

Fig 5: U File used for agitation of irrigant

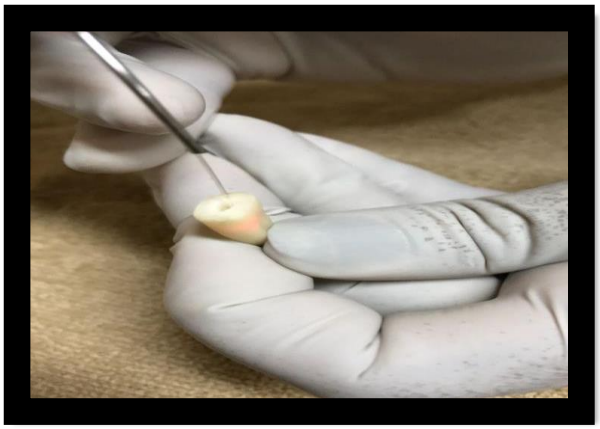

Fig 6: Diode Laser used for agitation of irrigant

Fig 7: Scanning Electron Microscope