Comparative Evaluation Of EDTA-S And EDTA-C As Root Conditioning Agents On Root Surfaces Of Extracted Human Teeth – An In Vitro Scanning Electron Microscope Study

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Abstract

Aim: This in vitro study aims to evaluate and compare efficacy of EDTA-S and EDTA-C in terms of remnant smear layer, number of patent dentinal tubules, and percentage patency of dentinal tubules following treatment on mechanically debrided periodontally involved root surfaces.

Materials and methods: Fifteen extracted periodontally compromised human teeth were subjected to scaling and root planing. The teeth roots were then sectioned yielding 30 samples and were allocated in group A : EDTA-S (n=15) and group B : EDTA-C (n=15). The freshly prepared EDTA-S and EDTA-C were applied to the respective root study samples for 3 minutes by active burnishing for 30 seconds. These samples were then sent for scanning electron microscope analysis.

Results: Statistically significant results were achieved in the EDTA-S group as compared to the EDTA-C group in the removal of remnant smear layer ($p = 0.000$), number of patent dentinal tubules ($p = 0.000$), and statistically nonsignificant results were achieved for the percentage patency of dentinal tubules ($p = 0.052$).

Conclusion: EDTA-S was found to be more effective in smear layer removal and establishing dentinal tubular patency and more no. of patent dentinal tubules than EDTA-C.

Keywords: Scaling and root planing, smear layer, dentinal tubules, root conditioning agent, EDTA, EDTA-S, cetrimide.

Introduction

Periodontitis can affect the root surface by creating altered cementum, debris, areas of demineralization and calculus involving calculo-cementum on the root surface.[1] To enhance the healing outcome scaling and root planing are performed. Instrumentation of the root surface, results in formation of a smear layer of organic and mineralized debris. This smear layer usually ranges from 2 to 15 μmin thickness and serves as a contaminated physical barrier between the periodontal tissues and the root surface inhibiting the proliferation of periodontal ligament cells and gingival fibroblasts, interfering in periodontal wound healing through connective tissue reattachment and regeneration.[2] In order to decontaminate the root surface and facilitate periodontal healing, the residual remnant smear layer and debris which remain after debridement is removed with the help of root conditioning comprised of chelating agents to make these surfaces more conducive to connective tissue reattachment and regeneration.[3-6]

EDTA is known to be an excellent chelating agent that leads to exposure of more intact collagen bundles, causes less periodontal tissue necrosis, enhances histological attachment by reducing the occurrence of long junctional epithelium formation, does not dissolve root collagen fibres, achieves a more biocompatible surface for cell and tissue attachment.[7-12] Results have shown that the concentration of EDTA should be 24% for effective smear layer removal.

Recently, EDTA is available in combination with certain surfactants that potentiate its action e.g. sodium lauryl sulfate, cetrimide, and tapoxon. They have been used as an alternative to recognized root conditioning agents like tetracycline and citric acid in periodontal root conditioning.

Therefore, this in vitro study aims to evaluate and compare remnant smear layer, no. of patent dentinal tubules, and percentage patency of dentinal tubules following treatment on mechanically debrided periodontally involved root

surfaces of extracted human teeth with either EDTA-S or EDTA-C.

The rationale of this in vitro SEM analysis study was to check whether the surfactant effect of cetrimide is comparable to that of sodium lauryl sulfate. If cetrimide shows comparable results then it can be used as a clinically applicable root conditioning agent as cetrimide has additional antiseptic properties. Literature comparing the effect of EDTA-S and EDTA-C in the removal of smear layer on mechanically debrided periodontally involved root surfaces is not available.

Materials And Methodology

The study was a double-blinded, mono-centric, prospective, parallel arm, quasi-experimental invitro scanning electron microscope study. The experiment was performed by one blinded operator and SEM analysis of samples was performed by one blinded assessor to avoid bias. The inclusion criteria of the study were single-rooted extracted teeth, anterior to the premolar, affected with periodontitis with a minimum of 5mm attachment loss with a hopeless prognosis indicated for extraction. The exclusion criteria of the study were teeth with root caries, non-carious cervical lesions, endodontic treatment, root restorations, acid etched or having bonded restorations, developmental anomalies, subjected to vital/non-vital bleaching, internal/external resorption, and subjects having undergone nonsurgical/ surgical periodontal therapy. Fifteen extracted periodontally compromised human teeth were collected using convenience sampling from the volunteer subjects meeting the inclusion criteria of the study and rendering informed consent.

Specimen preparation and sectioning procedure

All the samples were collected and stored in 1 % thymol solution for not more than 30 days. These samples were subjected to ultrasonic scaling and root planing using hand curettes. Each of the 15 teeth was mounted in plaster keeping a 5 mm distance from CEJ. The samples were sectioned at CEJ to remove the crown portion. The radicular root complex part was again sectioned longitudinally buccolingually yielding 30 samples. The pulpal surface of each root study sample was flattened with the help of a straight fissure bur and a groove was placed on the pulpal surface for identification purposes. (Figure 1)

The 30 samples were randomly allocated by onsite computer randomization method to 2 intervention groups of 15 samples each. (Figure 2)

Group A : Scaling and root planing + treatment with EDTA-S (n=15),

Group B: Scaling and root planing + treatment with EDTA-C (n=15).

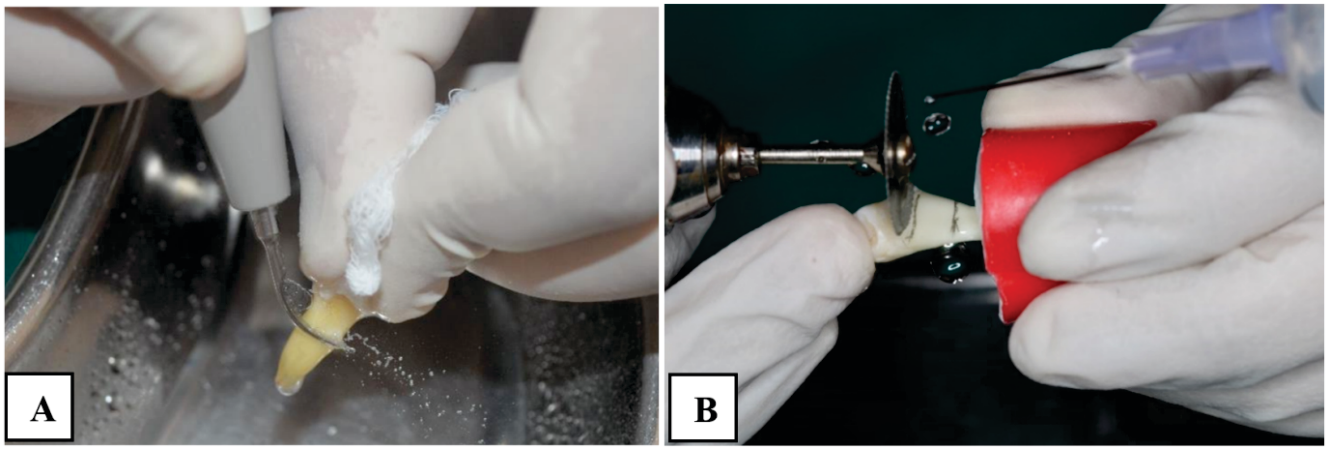

Figure 1 : (A) Scaling and Root planing (B) Tooth root sectioning

Preparation of root conditioning agents

All agents were freshly prepared i.e 24% EDTA-S [2.4 g EDTA + 10 mL distilled water + 2.4 g Sodium lauryl sulphate] and 24% EDTA-C [2.4 g EDTA + 10 mL distilled water + 2.4 g Cetrimide]. Each time the pH was maintained to 8 by using pH meter. Once the samples were obtained, they were stabilized on a separate wax block to avoid contamination of the adjacent sample and were treated with the respective agent.

Application of Root conditioning agents.

The agents were applied to the respective study sample for 3 mins by active burnishing which involves rubbing with the help of a cotton pellet. After every 30 seconds, cotton pellets were changed to ensure that the solution was applied consistently. After treatment with the respective agent, each sample was rinsed with distilled water for 30 seconds and sent for scanning electron microscope analysis at 3500x.

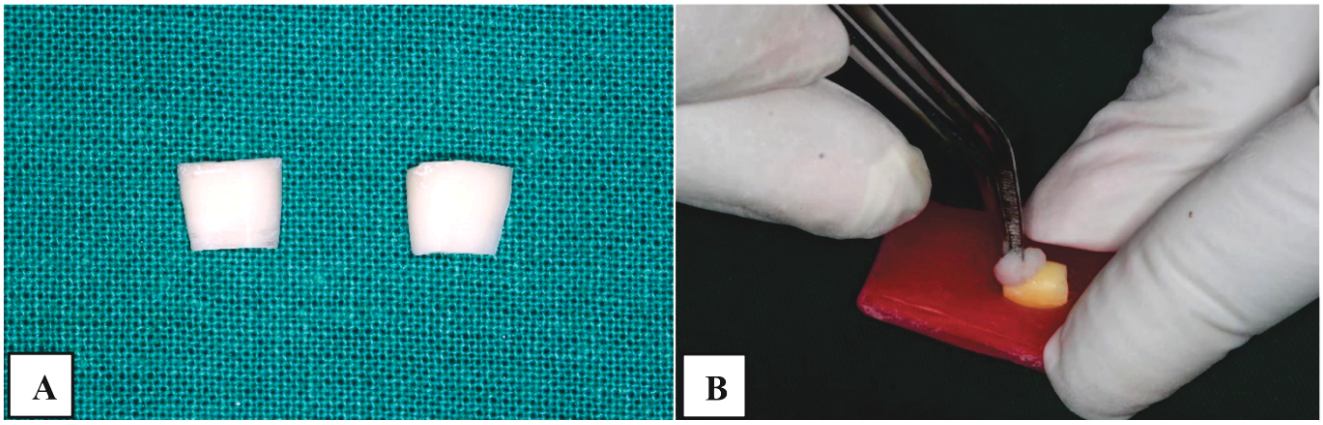

Figure 2 : (A) Toot study samples, (B) Active burnishing with root condiioning agents

Scanning electron microscopy

Each sample was dehydrated using a graded series of propranol at 50%, 60%, 70%, 80%, and 90% for 30 mins each. Each block was mounted on aluminum stubs and examined under 3500x magnification. (Figure 3 & 4)

Data analysis

The degree of smear layer removal and patency of dentinal tubules was evaluated according to Sampio's index.[13]

The percentage patency of dentinal tubules was calculated by using the formula: -

[(No. of patent dentinal tubules) x100] ÷ Total no. of dentinal tubules

Results

Statistical analysis

The data collected was compiled in a MS Office Excel worksheet and statistical analysis was carried out using SPSS software version 26. The descriptive data for Sampio's index score was depicted. Intergroup comparison was performed using arithmetic mean and standard deviation. Student's t-test was also used to compare the number of patent dentinal tubules and the percentage patency of dentinal tubules between both groups.

Data interpretation

Data interpretation was done by one blinded assessor. There was a statistically significant difference seen between EDTA-S group and EDTA-C group. Scanning electron microscope

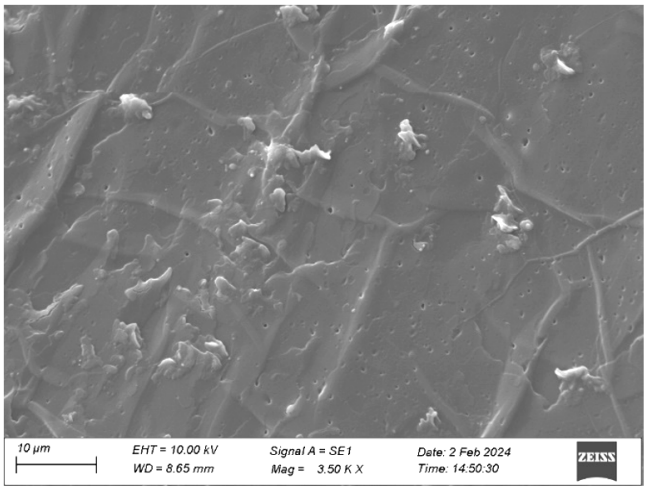

Figure 3 : EDTA-S group

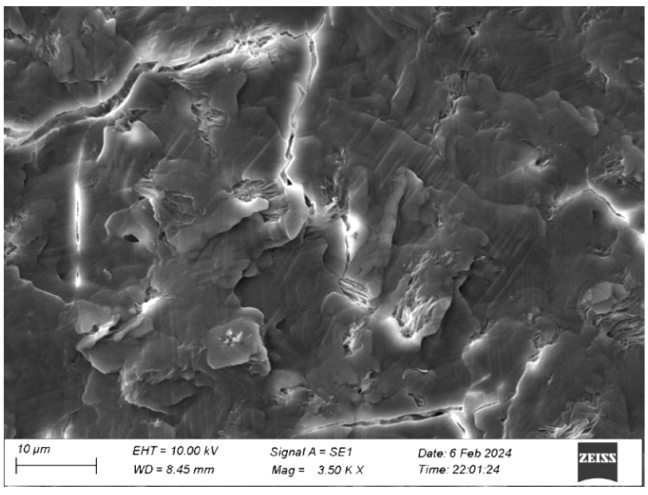

Figure 4 : EDTA-C group

of EDTA-S group shows that dentinal tubules were completely opened and there was no smear layer on the treated root surface. The dentinal tubule gap showed little or no evidence of a smear layer whereas the EDTA-C group showed the presence of a smear layer on treated root surfaces with the presence of grooves and scattered debris in uniform or irregular aspects. There was no evidence of dentinal tubule gaps (p=0.000) (Table 1).

More patent dentinal tubules were found in the EDTA-S group as compared to the EDTA-C group(p=0.000) (Table 2).

The percentage patency of dentinal tubules (Table 3) was found statistically nonsignificant (p=0.052).

Table 1: Descriptive statistics showing score distribution of remnant smear layer using sampio's index for Group A and Group B

Sampio's index score	Group		Total	Chi-Square value	P value of Chi-Square test
	A	B			
1	8	0	8	30.000	0.000**
2	3	0	3		
3	4	0	4		
4	0	5	5		
5	0	4	4		
6	0	6	6		
Total samples	15	15	30		

Table 2: Intergroup comparison of number of patent dentinal tubules

GROUP	N	Mean	Std. Deviation	Std. Error Mean	T value	p value of t test
A	15	50.27	14.200	3.666	8.481	.000**
B	15	17.47	4.764	1.230		

Table 3: Intergroup comparison of percentage patency of dentinal tubules

GROUP	N	Mean	Std. Deviation	Std. Error Mean	T value	p value of t test
A	15	89.0473	3.92396	1.01316	22.437	.052*
B	15	42.8847	6.93547	1.79073		

Discussion

This study aimed to evaluate and compare the remnant smear layer, no. of patent dentinal tubules, and percentage patency of dentinal tubules following treatment on mechanically debrided periodontally involved root surfaces of extracted human teeth with either EDTA-S or EDTA-C. At neutral pH, EDTA acts by chelating the divalent cations. It has been reported that EDTA does not have any deleterious effect on surrounding periodontal tissue.[14,15] EDTA provides a more biocompatible surface for cell attachment hence it promotes early cell colonization and tissue colonization.[16]

Other studies have reported that 24% EDTA has led to more fibroblast attachment on the treated root surfaces as compared to other concentrations of EDTA. EDTA leads to exposure of more collagenous structures which create a favorable root surface for cell reattachment by eliminating minerals selectively from the dentinal surface of treated root surfaces.[14-16]

Sodium lauryl sulfate is an excellent surfactant in terms of detergent activity.[13] Cetrimide is proven to be a known antiseptic and also has surfactant properties but is not yet proven to be a good root conditioning or root biomodification agent. Hence, in this study, we compared cetrimide and sodium lauryl sulfate as there is no collaborative literature that compares the efficacy of cetrimide alone or in combination with EDTA as a root conditioning agent.

In this experimental study, these agents were compared for remnant smear layer removal, no. Of patent dentinal tubules and percentage patency of dentinal tubules. The descriptive data demonstrated that EDTA-S has better smear layer removal efficacy as compared to EDTA-C which was measured using Sampio's index. This was in partial agreement with the study performed by Srirangarajan S. et al. in 2012. The intergroup comparison between EDTA-S and EDTA-C showed that EDTA-S has established greater tubular patency and more patent dentinal tubules as compared to EDTA-C. This indicates that the cetrimide does not have the same surfactant activity as that of sodium lauryl sulfate. To the best of our knowledge, there is no literature available to either agree or disagree upon the efficacy of EDTA-C in establishing dentinal tubular patency and no. of dentinal tubules.

More number of patent dentinal tubules were found with EDTA-S as compared to EDTA-C indicating that sodium lauryl sulfate is more effective as compared to cetrimide combined with EDTA. The percentage of tubular patency was found to be comparable in both groups.

The limitations of this study were: Assessment of antimicrobial properties of both combination agents was beyond the scope of this study.

Conclusion

EDTA-S was found to be more effective in smear layer removal, opening of dental tubules and resulted in establishing a better dentinal tubular patency compared to EDTA-C.

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