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Evaluation And Comparison of Effectiveness of Mixture of Tetracycline Isomer, Acid and Detergent (Mtad) and 17% Ethylenediaminetetraacetic Acid in Smear Layer Removal of Periodontally Affected Teeth - A Scanning Electron Microscopic Study.

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Abstract

Objectives: Periodontal diseases produce physical and chemical alterations in the root cementum. Various to pical applications as root conditioning agents have been recommended as an adjunct to mechanical root surface debridement to remove smear layer, endotoxins and to expose collagen fibers on dentin surface. The objectives of present study are to compare the effective ness of MTAD and 17% EDTA in smear layer removal of periodontally affected teeth. Method: Study sample consist of 66 extracted single rooted teeth with advanced Perio dontitis indicated for extraction, is assigned to three treatment groups of 22 each with inclusion and exclusion criteria.

1. Group I (control group): Received only saline rinse
2. Group II: Received MTAD (0.05% doxycycline, 10% citric acid ,10% tween80) for 5 m
3. Group III: Received 17% EDTA for 5 min.

The conditioning agents are applied with the cotton pallets which are replaced every 30 sec to ensure the

uniform contact of the root surface area, the border bet ween healthy and diseased root surfaces will be marked with a bur. The diseased root surfaces of all teeth are scaled with ultrasonic scaler and thoroughly planed with Gracey curettes crown and healthy portion of root along with 2 or 3 mm of apical portion of the root will be removed with a water-cooled high-speed bur. The specimens were then subjected to dehydration in a hot air oven and analyzed by SEM. Results and conclusion: Based on the results obtained, both the experimental groups removed smear layer and Group II showed statistically significant results when compared to Group III. And number of opened dentinal tubules and diameter of opened tubule is also higher in Group II compared to Group III.

Keyword: Smear layer, Scanning Electron Microscope, root biomodification, EDTA (Ethylenediaminetetraacetic acid), MTAD (Mixture of Tetracycline isomer, Acid and Detergent)

Introduction

Periodontitis is a chronic disease caused by periodontal pathogens and results in the destruction of periodontium. New connective tissue attachments to the root surfaces must first be made in order to restore periodontal tissue. The movement of fibroblasts and their attachment to the collagen fibrils on the root surface regulate this process.[1]

Root surfaces that have been damaged by periodontitis are too calcified and polluted with cytotoxic and other bio logically active agents. The proliferation of neigh Boering periodontal cells, which is essential for Perio dontal wound healing, is not bio compatible with such surfaces.[2]

Scaling and root planing can lessen the amount of Perio dontal infections and cytotoxic materials found in dental calculus and cementum on the root surfaces of Perio dontally com promised teeth. Never the less, these pro cedures invariably leave a smear layer that contains subgingival plaque, leftover dental calculus, and con taminated cementum that may affect periodontal tissue cells and prevent fresh attachment.[3]

The smear layer may range from 2 to 15um and serve as a physical barrier between the periodontal tissues and root surface inhibiting new connective tissue attachment to the root surface. Therefore, the root surfaces must be devoid of any smear layer in order to facilitate Perio dontal healing through regeneration or new attachment.[4]

Removal of the smear layer is of utmost relevance in reg en erative operations where it is necessary to recolonize vast portions of the root surface with Perio dontal connective tissue.[5]

As an adjunct to root surface instru mentation, etching agents such as mixture of tetracycline isomer, acid and detergent (MTAD), citric acid, ethylene dia mine tetra acetic acid (EDTA), phosphoric acid and tetracycline hydro chloride have been used to dissolve the smear layer.[6]

Ethy lenedia mine tetra acetic acid is a decalcifying agent operating at a neutral pH by chelating the divalent cations. It preserves the vitality of the remaining Perio dontal cells close to the root surface, and also has the advantage of biocompatibility (pH = 7.0) as compared to other root conditioning agents. EDTA etching appeared to promote early cell and tissue colonization by provid ing a more biocompatible surface for cell and tissue attach ment.[7]

MTAD (Dentsply, has been used as an antibacterial root canal irrigant. It stands for a mixture of TTC isomer (doxycycline), acid-CA, detergent

tween80®. It has the ability to remove the smear layer and also exert a potent antimicrobial activity. MTAD has been proved to be an effective root canal irrigant and also effective in smear layer removal.[7]

The purpose of this study is to evaluate and compare the effective ness of MTAD (Mixture of tetracycline isomer, Acid and Detergent) and 17% EDTA (Ethy lene dia mine tetra acetic acid) for smear layer removal.

Material and Methods

The study sample consists of 66 human periodontally involved single rooted teeth assigned to three treatment groups of 22 each with inclusion and exclusion criteria.

2.1 Inclusion criteria

Single rooted teeth with stage III and IV periodontitis according to 2017 world workshop on the Classification of periodontal and peri-implant disease and conditions.

2.2 Exclusion criteria

Teeth with dental caries, cervical restorations, Deciduous teeth, teeth with fracture, erosion and Teeth extracted for reasons other than periodontal disease.

Instructions were given not to instrument the root surface to be studied during extraction. Following extra ction, the teeth was washed with distilled water and stored in normal saline to avoid dehydration.

All 66 roots were divided into 3 groups:

Group I: (control group): Received only saline rinse

Group II: Received MTAD (0.05% doxycycline, 10% citric acid, 10% tween 80) For 5 min. Group III: Received 17% EDTA for 5 min.

The following parameter was selected for the study

Smear layer scoring given by Sampaio et al., 2005

SCORE 1- Root surface without smear layer, with the dentinal tubules completely Opened without evidence of smear layer in the dentinal tubules

SCORE 2- Root surface without smear layer, with the dentinal tubules completely opened, but with some evidence of smear layer in the dentinal tubules entrance.

SCORE 3- Root surface without smear layer with the dentinal tubules partially opened

SCORE 4- Root surface covered by a uniform smear layer, with evidence of dentinal tubules opening

SCORE 5- Root surface covered by a uniform smear layer without evidence of dentinal tubule opening

SCORE 6- Root surface covered by an irregular smear layer, with the presence of grooves and/or scattered debris.

Preparation

The indigenous solution was prepared with 0.05% doxycycline, 10% citric acid and 10% tween 80 at a ratio of 2:2:1. The ration has been chosen depending on the importance of the role played by each ingredient. 17% EDTA was obtained from a laboratory. Both the solutions were stored in an amber color bottle as they were sensitive to light.

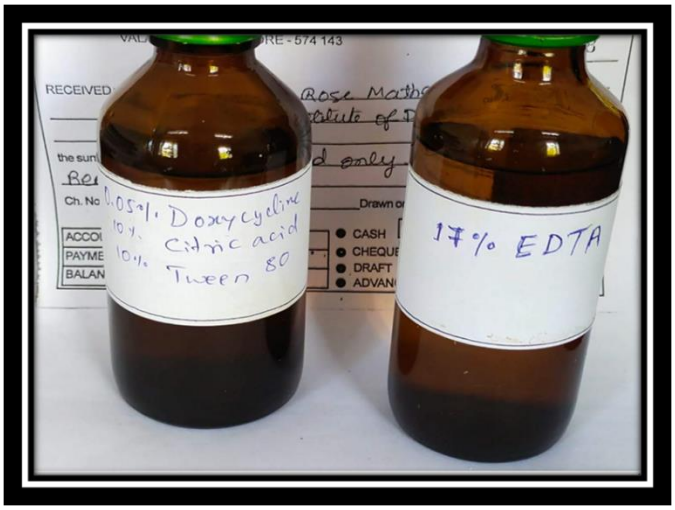


Figure 1: MTAD and 17% EDTA

Experimental Procedure

Sixty-six samples of teeth which were extracted due to periodontal disease were used. After extraction, the teeth were washed with water to remove blood and tissue tags. They were then stored in a container with saline solution to avoid dehydration of the specimens.

After extraction, the border between healthy and diseased root surfaces was marked with a bur. The diseased root surfaces of all teeth were scaled with ultra sonic scaler and thoroughly planed with Gracey

curettes (No. 1/2, 3/4, 5/6). After scaling and root plan ing, crown and healthy portion of root along with 2 or 3 mm of apical portion of the root was removed with a water cool highspeed bur.

The root conditioning of the specimen was done accord ing to a fixed protocol in all groups so that there is no difference in the method of conditioning and the time period for which the conditioning agent is in contact with the root surface of the specimen. The conditioning agents were applied with the cotton pallets which were replaced every 30 sec to ensure the uniform contact of the root surface area of each specimen with conditioning agent for 5 min (Figure 2). After this the samples were thoroughly rinsed with distilled water to remove any remaining conditioning agents on the root surface of the specimen. Washed samples were then dried and scheduled for scanning electron micro scopic evaluation.

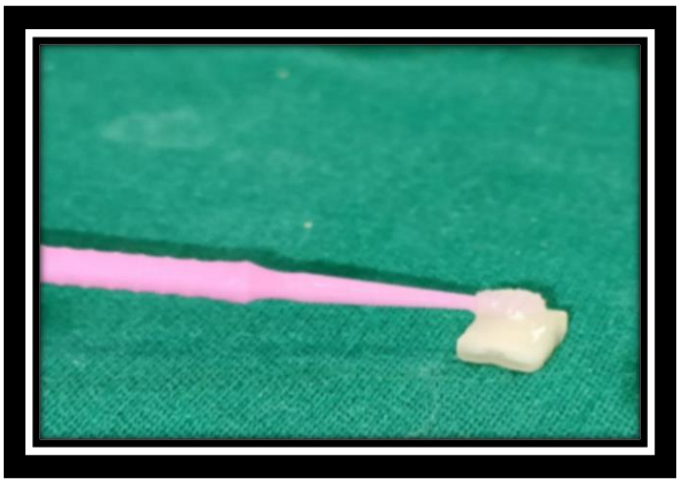


Figure 2: Solution being applied on to the tooth surface

Scanning Microscope Analysis

The microscope used for SEM analysis was ZEISS, Model- EVO LS 15 (Germany). All the specimens were dehyd rated with ascending concentration of ethyl alcohol (30 - 100%) and placed in a desiccator for at least 24hrs, mounted on metallic stubs, gold sputtered, gold sputtering was done for the specimens with the help of a gold sputtering unit (SPI-MODULE Sputter Coater) and viewed under scanning electron micro scope. Photo micro graphs were taken from each specimen surface examined at 1000x magnification under Scanning Electron Microscope. The roots were examined with respect to presence or absence of smear layer, total number of open dentinal tubules and diameter of open dentinal tubules.

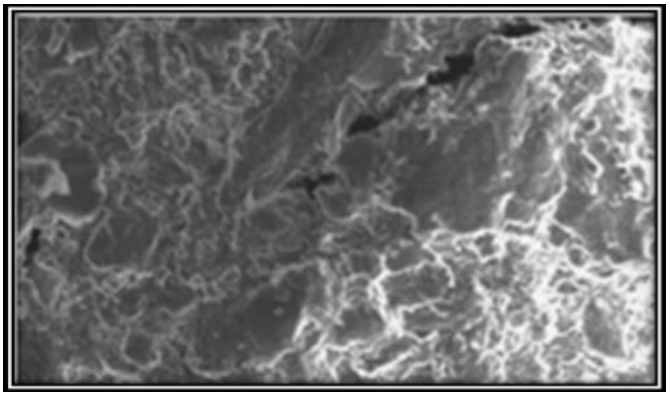


Figure 3: Sem Group I

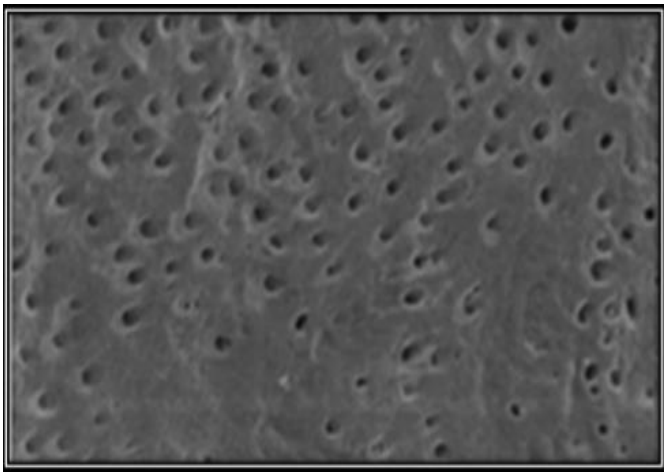


Figure 4: Sem Group II

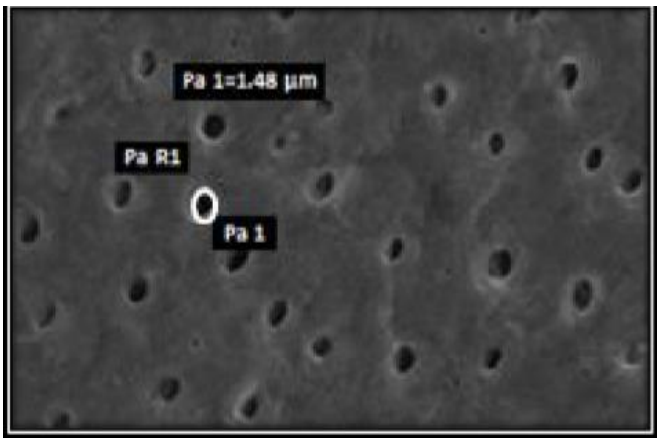


Figure 5: Diameter of tubule in group II

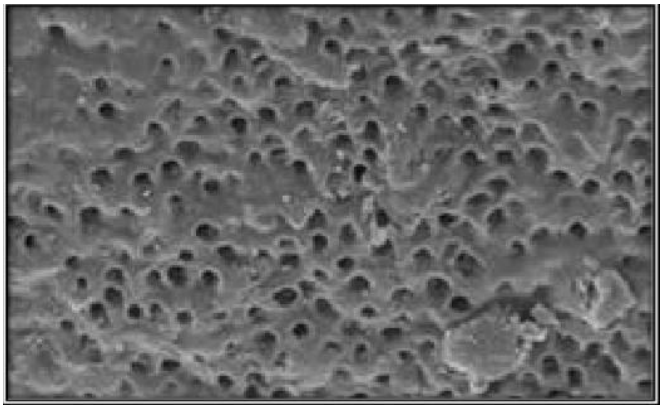


Figure 6: Sem Group III

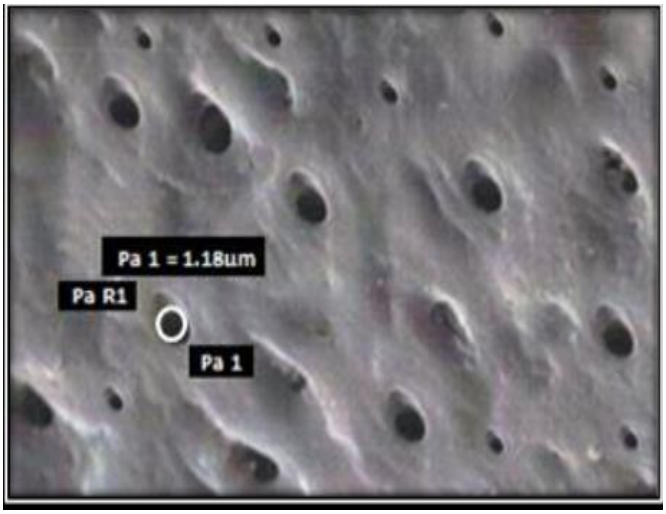


Figure 7: Diameter of tubule in group III

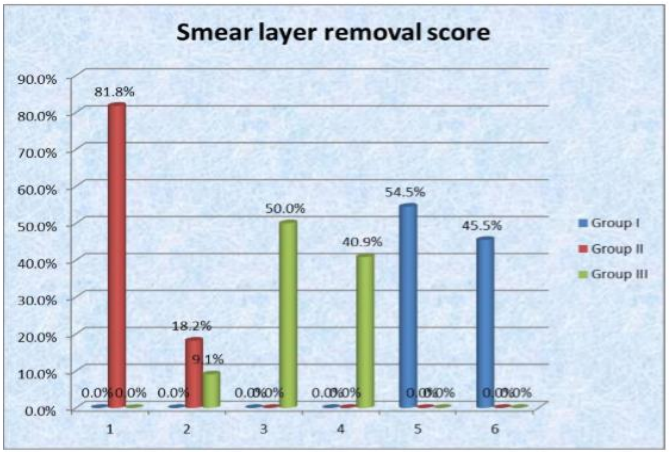
Results

Statistical analysis of the data was done by using the software SPSS23.0. Descriptive statistics were calculated and summarised which includes frequency, percentage, median, mean and standard deviation. Inferential statistics had been carried out in the present study. Com parison of smear layer removal score between the groups were done using chi square test. Comparison of number of tubules between the groups and diameter between the groups were done by Mann Whitney U test. Level of significance was set at 5%.

Table 1 shows in group III ,a majority of 11(50%) are with smear layer removal score 3,9(40.9%) with score 4 ,In group II majority of 18 (81.8%) are with smear layer removal score 1 and 4 (18.2%) with score 2.where as in group I majority 12(54.5%) are with smear layer removal score 5 and 10(45.5%) with smear layer removal score6.The chi square test used to find association between group and smear layer removal score shows chi square=124.0 with p value <0.05. This depicts there is significant association between smear layer removal score and the group. It is significantly higher in group I and lower in group II.

Smear layer removal score		Group			Total
		Group III	Group II	Group I	
	1.00	0	18	0	18
		0.0%	81.8%	0.0%	27.3%
	2.00	2	4	0	6
		9.1%	18.2%	0.0%	9.1%
	3.00	11	0	0	11
		50.0%	0.0%	0.0%	16.7%
	4.00	9	0	0	9
		40.9%	0.0%	0.0%	13.6%
	5.00	0	0	12	12
		0.0%	0.0%	54.5%	18.2%
	6.00	0	0	10	10
		0.0%	0.0%	45.5%	15.2%
Total		22	22	22	66
		100.0%	100.0%	100.0%	100.0%

Table 1: Showing cross tabulation of smear removal score

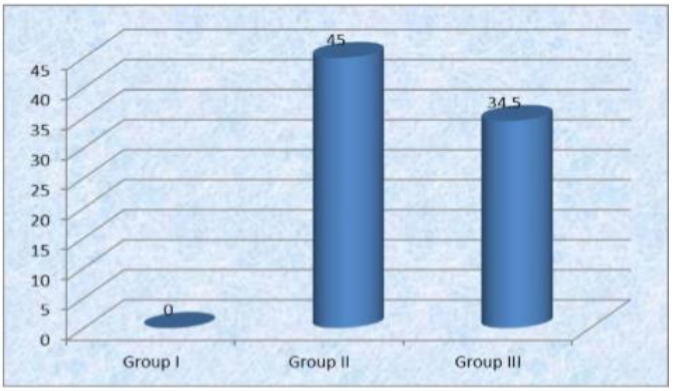


Graph 1: Showing smear layer removal score in group I, group II and group III

Table 2 shows in group III average number of tubules are 34.5 with range (29-39), in group II it is 45 with range (41-52) and in group number of tubules are nil. Comparison of number tubules between group II and group III shows U value=233.0 with p<0.05, shows significant difference between number of tubules of group II and group III. It is significantly higher in group II.

	Group III	Group II	Group I
Median	34.5	45	0
Range	(29-39)	(41-52)	
U value	233.0		
P value	P<0.05		

Table 2: Showing number of tubules group I, group II and group III

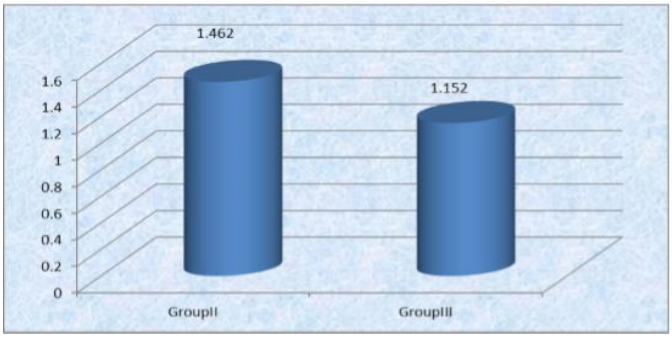


Graph 2: Showing number of tubules in group I, group II, group III

Table 3 shows in group III average diameter of tubules as 1.152±0.0234, in group II it is 1.462±0.024 and in group I it is nil. Comparison between group II and III shows U value=257.0 with p<0.05. It shows diameter of tubule is significantly higher in group II than group III

	N	Minimum	Maximum	Mean	Std. Deviation	U value	P value
Group III	22	1.12	1.19	1.152	.0235	257.0	P<0.05
Group II	22	1.39	1.49	1.462	.0240		
Group I	22			0.00	0.00		

Table 3: Showing diameter of tubules group I, group I, group III



Graph 3: Showing diameter of tubules group I, group II, group III

Discussion

The main aim of periodontal regeneration is to alter the root surface affected by periodontitis and make it a hospi table substrate to encourage and support migration, proliferation, attachment and proper phenotypic expression of periodontal connective tissue progenitor cells.[8]

However, the root surfaces affected by periodontitis are hyper-mineralized, contaminated with cytotoxic and other biologically active substances as such surfaces are not biocompatible with the adjacent periodontal cells, the proliferation of which is crucial for periodontal wound healing and it is not possible to decontaminate the root surface affected by periodontitis by mechanical mean alone.[9]With age systemic and local changes occur inside the oral cavity that may lead to alterations at micro scopic level causing hindrance in fibrin clot attach ment hence the teeth that were easy to remove and appeared clinically normal without any root surface alterations were selected.[10]

The surface which is instrumented will be covered by smear layer after root planing. This smear layer consists of remnants of dental calculus, contaminated root cemen tum and subgingival plaque and bacterial endotoxins. It serves as a physical barrier between the periodontal tissue and root surface and may inhibit new connective tissue attachment formation to the root surface.[11]

Root bio modification is a periodontal regenerative technique which has received

much attention. It has been shown to expose collagen fibrils and creates a zone of demineralized matrix of 3–20 µm thick. The tooth collagen exposed by this root 89 demineralization pre-treatment procedure is thought to augment periodontal wound healing, thereby enhancing periodontal regeneration. [7]

Demineralization of root surfaces during periodontal therapy has been performed to enhance regeneration of the lost periodontal attachment. Demineralizing agents have been shown to expose dentinal collagen, widening the orifices of dentinal tubules and cementum bound proteins.[13]

Furthermore, root conditioning after flap elevation, debridement, and root planing helps to remove the smear layer, a thin residual layer of organic and mineralized debris that results from any mechanical root preparation method, which also has been suggested to have a negative effect on regenerative outcomes.[14]

The smear layer is a layer composed of the collagen molecules and mineralized matrix that are present after mechanical tooth preparation. Open dentinal tubules (absence of smear layer) on the tooth surface are among the critical elements that determine the quality of fibro blast adhesion to the dental root surface. To ensure fibro blast proliferation on the radicular dentin, its surface should have open dentinal tubules without the presence of the smear layer. [15]

The clean surfaces with wider tubular openings appear to offer a more favourable environment for close adhesion of fibroblasts or a true organic attachment with new cementum formation .[16]

Application of appropriate root surface conditioning may, therefore, regulate the adsorption of plasma proteins, enhance adhesion of the blood clot and stimulate deposition of collagen against the root surface. Hence, their application as root conditioner will have a significant role in periodontal wound healing and future new attachment in vivo.[17]

A variety of agents have been used in conjunction with root demineralization new attachment procedures such as hydrochloric acid, ethylenediaminetetraacetic acid, phos phoric acid, tetracycline, stannous fluoride, and citric acid [18]The present study tested an endodontic irrigant MTAD (mixture of tetracycline, citric acid and detergent) for its root conditioning ability.

Considering the above facts an effort has been made in this study to determine the surface characteristics of diseased root surface by conditioning with MTAD, 17% EDTA and saline under scanning electron microscope.

In the present study, 66 specimens of single-rooted teeth affected by periodontitis with grade III mobility were extracted from the patients with no history of systemic disease. Teeth affected by caries were not included in this study, as it could have adversely affected the root surface topography. Minimal instrumentation during extraction was considered to avoid chipping of the root structure. Teeth with attrition, abrasion, and erosion were not included in the study, as they have shown to produce secondary changes in tooth structure like alteration in mineral composition and formation of sclerotic dentine. The teeth in this study were root planed until the roots felt hard, velvety smooth, and glass-like to the touch of an explorer and until no rough spots or deposits could be detected. After root planing, the samples are stored in saline to avoid dehydration of the specimens.

In the present study active burnishing of the samples with cotton pellet saturated with the respective conditio ner is done and changed after every 30 seconds for a period of 5 minutes. Change pellets after every 30 seconds to apply a constant concentration of drug over the application interval. This procedure enhances a mechanical/chemical action which chemically loosens surface debris and inorganic material, thereby exposing underlying dentin to fresh acid resulting in deminerali zation. After root conditioning, the samples were washed saline solution to rid the specimens off any remaining/ pooled conditioning agent on the root surface.

On visualizing the samples under the scanning electron microscope, the photomicrographs of the samples treated with saline showed the presence of a heavy smear layer throughout the entire sample, with smear layer removal score 5 and score 6.Samples treated with 17% EDTA (Group III) showed presences ,a majority are of with smear layer removal score 3 and others are with score 4 .Whereas in samples treated with MTAD (group II)majority are with smear layer removal score 1 and rest with score 2. The difference in the smear layer removal score is found to be statistically significant ($p<0.05$). Accor ding this study smear layer removal is signifi cantly higher in group treated with MTAD than group treated with 17% EDTA.

The mean number of tubules opening in MTAD group is 45 greater than 17% EDTA group which is 34.5, whereas it is nil in saline groups. The difference in the number of opened tubules found statistically significant($p<0.05$). The number of opened dentinal tubules in MTAD treated group is significantly higher compared to that of 17% EDTA treated group.

The mean diameter of tubules in MTAD group is 1.462 which is also greater when compared to 17% EDTA group which is 1.152. The difference in the tubule diameter found statistically significant ($p < 0.05$) The calculated mean surface area of the tubule's orifices in the MTAD treated group is significantly higher compared to that of 17% EDTA treated group.

An increase in diameter of the tubule leads to an increase in surface area which in turn maximizes the number of binding sites for factors and proteins which stimulate the regeneration processes.

EDTA,[19]has been found to be as effective as low pH etchants with respect to smear removal and superior in exposing root surface-associated collagen. Cell attachment and periodontal healing has been shown to be promoted by EDTA etching compared to etching with low pH agents like citric acid and phosphoric acid.

Various concentrations of EDTA have been used in the past. Lasho et al.[20] used 15% EDTA, Bergen Holtz and Babay 80 evaluated smear layer removal by 8% EDTA etching. Blomlöf et al[21]. compared 1.5%, 5%, 15% and 24% and suggested that the concentration of EDTA should be somewhere between 15 to 24% in order to obtain an acceptable smear removing and collagen exposing effect. MTAD (commercially available as Bio Pure ™ MTAD, Dentsply Tulsa Dental, Tulsa, OK, USA) was developed by Torabinejad et al[22]. as a final endodontic irrigant to disinfect the canal and remove the smear layer.[23]Shabahang et al. showed that Bio Pure MTAD was an effective disinfectant of the root canal system and a combination of 1.3% NaOCl and Bio Pure MTAD as a final treatment eliminated E. faecalis from human tooth cementum and dentin. They attributed the effectiveness of

Bio Pure MTAD to its ant collagenase activity, low pH, and ability to be released gradually over time. Bio Pure MTAD has been found to adsorb to hydroxyapatite with prolonged and gradual release at therapeutic levels]. In addition, presence of a detergent (Tween 80) in Bio Pure MTAD reduces its surface tension and thus improves its penetration into deep layers of dentin.

MTAD has been found to adsorb to hydroxyapatite with prolonged and gradual release at therapeutic levels[24]. In addition, presence of a detergent (Tween80) in Bio Pure MTAD reduces its surface tension and thus improves its penetration into deep layers of dentin.

In this study, we have utilized this novel agent as a root bio modifier for smear layer removal on periodontally involved human teeth. Doxycycline is the primary ingredient contributing to its antimicrobial activity. CA removes the inorganic materials and Tween-80 reduces

the surface tension and benefits the diffusion of acids into the root canal irregularities and dentinal tubules.

In a study [24] evaluating the antimicrobial substantivity of MTAD, chlorohexidine (CHX) and sodium hypochlorite, Bio Pure MTAD showed significantly higher anti micro bial sub stantivity than CHX and was retained in root canal dentin for at least 28 days. These properties of Bio pure MTAD can be useful in periodontal root con dition ing. Bio pure MTAD will remove the smear layer, show improved penetration into the root surface dentin and/ or cementum, expose the dentinal tubules, and provide antimicrobial activity.

Present study compared the root conditioning ability of 17%EDTA and MTAD (mixture of tetracycline, citric acid and detergent) with normal saline as control. Both the experimental solutions removed the smear layer successfully from the root surfaces and the experimental samples were significantly cleaner than the control group. The results demonstrated that the mean smear for samples treated with MTAD is lower than those treated with 17% EDTA, the results were statistically significant ($p < 0.05$).

The mean number of tubules opened and the mean diameter of tubules for samples treated with MTAD is higher than those treated with 17% EDTA, the results were statistically significant ($p < 0.05$). The cleaner root surfaces in the samples treated with MTAD can be attributed to its low Ph and presences of detergent (tween -80) enhancing its penetration and thus better removal of the smear layer and more effective opening of dentinal tubules.

Therefore, MTAD can be seen as a potential root conditioning agent with effective smear layer removal from the root surfaces. However, MTAD has anti micro bial activity, anti-collagenolytic activity and the presence of polysorbate-80 (TWEEN80) likely improves its penetration into the cementum and root dentine and hence make it more effective than 17% EDTA.

5.1 Limitation of the study:

Analysis by a scanning electron microscope is expensive and Limited sample size.

The result of present study is limited to physical findings of root surface changes and do not present in-vivo differences that may result from the physiological effect of these root conditioning agents.

Conclusion

The present study is carried out on dentin slabs prepared from periodontally diseased human teeth after applic ation of the three-root conditioning agent's namely saline as control, MTAD and 17% EDTA under SEM. From the study, it was concluded both the agents namely MTAD, 17% EDTA is effective in removing smear layer. Opening of dentinal tubules is seen in all the specimens except for control group that was treated with normal saline. The total number of tubules opened is highest in MTAD group as compared to 17% EDTA Group. The diameter of dentinal tubules in MTAD group was more than 17% EDTA. Hence, MTAD group is more efficient than17% EDTA group in removing the smear layer and exposing dentinal tubules.

Within the experimental protocol of the present study it can be concluded that the MTAD is an effective root conditioning agent showing significantly better smear layer removing ability and exposing dentinal tubules when compared to 17% EDTA.

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