

Fibrin Clot Adhesion To Instrumented Conditioned Root Surfaces By MTAD And EDTA- A SEM Study

Dr Gaurav Didhra¹, Dr Jagjit Singh², Dr Parveen Dahiya³, Dr Sachin Upadhyay⁴, Dr Deepak Phor⁵, Dr Ankur Sharma⁶

¹ Dr Gaurav Didhra, M.D.S. (Periodontics and Implantology) Director at Dent-O-Care, Opp. UCO Bank Main bazar, Mehatpur, Una

² Dr Jagjit Singh, M.D.S (Periodontics and Implantology) Professor and HOD in Department of Periodontics and Implantology at B.R.S. Dental College Barwala, Haryana.

³ Dr Parveen Dahiya, M.D.S (Periodontics and Implantology) Professor in Department of Periodontics and Implantology at Himachal Institute of Dental Sciences, Paonta Sahib, University of Himachal Pradesh, Himachal Pradesh, India.

⁴ Dr Sachin Upadhyay, M.D.S (Orthodontics and Dentofacial Orthopaedics) Senior Lecturer in Department of Orthodontics and Dentofacial Orthopaedics at Himachal Institute of Dental Sciences, Paonta Sahib, University of Himachal Pradesh, Himachal Pradesh, India.

⁵ Dr Deepak Phor, M.D.S (Orthodontics and Dentofacial Orthopaedics) Senior Lecturer in Department of Orthodontics and Dentofacial Orthopaedics, Yamuna Dental College, Yamunanagar, Haryana.

⁶ Dr Ankur Sharma, M.D.S (Orthodontics and Dentofacial orthopedics) Resident in department of dentistry at Pt.J.L.N.G.M.C.&H. Chamba. (H.P)

Abstract:

Background: Smear layer removal and formation of stable bond between blood clot and root surface is desirable for periodontal healing, which can be achieved by using root biomodifiers. The objective of this in-vitro study was to evaluate the root surface changes in terms of fibrin clot adhesion subsequent to the application of MTAD and EDTA.

Material and methods: Thirty human single rooted teeth were collected and grouped in to Saline (control Group-I), 15% EDTA gel (Group-II), MTAD Biopure (Group- III) treatment groups containing 10 teeth in each group. After root conditioning, fresh human blood was applied to each root specimen and was allowed to clot, subsequently samples were dehydrated in graded series of ethanol and visualized under Scanning electron microscope.

Results: The highest blood clot attachment score was observed with roots treated with MTAD followed by EDTA and least results was observed with control group saline.

Conclusion: Specimens treated with MTAD best supported the fibrin clot attachment. EDTA appeared to be less effective in supporting the clot. Saline was least effective of all the samples, showing presence of smear layer.

Keywords : MTAD, Fibrin clot, Root biomodification, Periodontal regeneration

Date of Submission: 09-10-2018

Date of acceptance: 24-10-2018

I. Introduction

Regeneration of the periodontal ligament and bone destroyed by infectious processes is the ultimate goal of periodontal surgery. It has been demonstrated that the formation and maintenance of a stable bond between the blood clot and the root surface is critical for optimal periodontal healing, preventing the down growth of gingival epithelial cells and promoting new connective tissue attachment. But the root surfaces of periodontally involved teeth are heavily contaminated by bacteria and their endotoxins and this contamination may hinder the outcome of periodontal regenerative procedures by preventing new connective tissue attachment. Therefore to enhance the periodontal regenerative procedure root conditioning is the method to restore the biocompatibility of the root surface to promote the migration, attachment, proliferation, and synthesis of connective tissue macromolecules by connective tissue cells.^[1]

Root biomodifiers have shown to remove inorganic and organic materials and toxins from the root surfaces, furthermore, they have shown to expose dentin collagen and cementum bound proteins, resulting in a root surface which is conducive in promoting periodontal regeneration. Many chemical agents have been proposed for the purpose of root conditioning, includes citric acid, tetracycline hydrochloride group, ethylene

diamine tetra acetic acid. The other agents used are carbon dioxide laser, neodymium:yttrium, aluminum, garnet laser, and erbium: Yttrium, aluminum, garnet laser and many more.^[2]

The use of calcium chelators such as EDTA with neutral pH has been shown to hold considerable promise as root conditioning agent. EDTA selectively removes hydroxyapatite, leaving most of collagenous matrix intact, preserves the adjacent tissue vitality, promotes early cell and tissue colonization by providing a more biocompatible surface for cell and has been reported to give favourable results with respect to less flap failure and more connective tissue attachment.^[3]

Biopure MTAD™ (Dentsply Tulsa Dental, Tulsa, OK, USA) is a material originally developed for use during endodontic treatment as to remove the smear layer from the root canal walls. The use of MTAD has, so far, been confined to endodontics to aid bacterial removal from dentine tubules within the canal and enhancing better attachment of the sealant to the canal walls.^[4] Many of the unique properties of MTAD which contribute to its status as an endodontic irrigant may be transferable to the conditioning process in periodontal therapy.^[5]

In this study an effort has been made to compare the effect of EDTA and MTAD on adhesion of fibrin clot to periodontally diseased root surfaces.

II. Materials And Methods

Thirty human single rooted teeth were extracted due to severe periodontitis and were collected as study specimens from the department of Oral and Maxillofacial Surgery of Himachal Institute of Dental Sciences, Paonta Sahib. Inclusion criteria consisted of teeth with

grade III mobility or hopeless prognosis, bleeding on gentle probing, proximal bone loss on radiographic examination. Patients with history of systemic disease, oral prophylaxis in previous 6 months, carious teeth along with peri-apical infection and any history of acute pain and swelling necessitating tooth removal were not included in the study. Following extraction, the teeth were washed with distilled water and stored in normal saline 0.9% at room temperature till further study.

Preparation of samples

Two parallel grooves were made on the proximal surface of the roots of each tooth using diamond disk bur with micromotor handpiece under copious irrigation, first groove is prepared at cemento-enamel junction and another at 3 mm apical to it. The area between two grooves is debrided and planned with apico-cervical stroke movements till the surface becomes smooth and shiny, using Gracey curette no.5-6 (HU-Friedy, USA). After root planning the area between two grooves were sectioned to obtain dentinal blocks of adequate size. In such way 30 dentinal blocks are obtained and stored in normal saline until use.

Chemical treatment of the dentinal blocks – All 30 specimens are divided in to 3 groups

Group 1: Ten root specimens are treated with normal saline by cotton pellets saturated with it and changed after every 20 seconds for a period of 3 minutes.

Group 2: Ten root specimens conditioned with EDTA Gel by placing cotton pellets saturated with it and changed every 20 seconds for 3 minutes.

Group 3: Ten root specimens are conditioned with Biopure MTAD™ by placing cotton pellets saturated with it and changed every 20 seconds for 3 minutes.

Root conditioning was done by burnishing soaked cotton pellets with light pressure. Then the specimens were washed with 10 ml saline solution. Venous blood was collected from a healthy volunteer and a drop of blood was placed on each chemically treated root surface. The drop of blood was allowed to clot for 20 minutes at 37°C in a humidifier chamber. Then specimens were subjected to five minute rinses in phosphate-buffered saline thrice times, this was done to test the adhesion of clot.^[6] After that each block of specimen were fixed in 2.5% glutaraldehyde for 30 mins. Subsequently, the blocks were subjected thrice in phosphate buffered saline for five minutes, after that each block was subjected for dehydration in graded series of ethanol (10-90%) for 30 minutes each and final dehydration carried out in 100% acetone for 30 minutes more.^[7] The samples were dried overnight in a dehydration jar, mounted on metallic stubs with adhesive tape, and sputter-coated with gold. Finally the specimens were observed by using a SEM unit. The surfaces of the roots were scanned and representative photomicrographs were obtained on the computer screen at 5000X magnification.

Analysis of photomicrographs

Scoring of the samples was done according to the criteria given by Theodoro LH et al 2006^[8]

- Score 0: Absence of fibrin network and blood cells
- Score 1: Scarce fibrin network with no blood cells
- Score 2: Moderate fibrin network and moderate quantity of blood cells
- Score 3: Dense fibrin network and trapped blood cells

Statistical analysis

Comparison of median of different groups was done at 25th, 50th, and 75th percentile. Inter group analysis was done by applying Mann-Whitney U test. Significance is assessed at a 5% level of significance.

III. Results

The photomicrographs of the samples treated with saline showed the presence of heavy smear layer throughout the entire sample. There was no evidence of fibrin network and blood cells in all the samples. All the samples belonged to score 0. The median value of saline at maximum and minimum interval is 0 [Table 1,2 Figures 1,2]

Photomicrographs of the samples treated with EDTA showed maximum samples having moderate fibrin network and moderate quantity of blood cells i.e score 2. Observations in the present study indicate that the median values of EDTA at minimum and maximum interval is 0 and 3 respectively. [Table 1,2, Figure 1,3]

The photomicrographs of the samples treated with MTAD showed maximum number of samples with dense fibrin network and trapped cells i.e score 3. Median values of MTAD at minimum and maximum interval is 1 and 3 respectively. [Table 1,2 Figure 1,4]

Mean rank on intergroup comparison between group 1 and group 2 was 6.00 and 15.00 respectively and on comparison between group 1 and group 3 mean rank was 5.50 and 15.50 respectively. On applying Mann-Whitney U- test when both the experimental groups i.e group 2 and group 3 were compared with control group 1 the p value was found to be statistically significant ($p < 0.001$) showing that both the experiment groups i.e group 2 and group 3 are better than group 1 in terms of fibrin clot attachment. [Table 3,4]

When both the experimental groups was compared the mean rank between group 2 and group 3 was 7.60 and 13.40 respectively. On applying Mann-Whitney U-test between the experimental groups the p value was found to be significant at the level ($p < 0.020$). The above results show that EDTA is able to remove smear layer and open dentinal tubules and collagen matrix for fibrin clot attachment but is less efficacious than of MTAD BIOPURE™. [Table 5]

IV. Discussion

The prime objective of all the periodontal therapy is to convert a periodontitis affected root surface in to a substrate which is biologically acceptable for epithelial and connective tissue adherence.^[9] However the periodontitis affected root surfaces are hyper-mineralised and contaminated with cytotoxic and other biologically active substances, such surfaces are not biocompatible with the adjacent periodontal cells that plays an important role in periodontal wound healing^[10] and it is not possible to decontaminate the periodontitis affected root surface by mechanical mean alone.^[11]

Root biomodification is one 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 demineralization pretreatment procedure is thought to augment periodontal wound healing thereby enhancing periodontal regeneration. A variety of agents have been used in conjunction with root demineralization new attachment procedures such as hydrochloric acid, ethylenediamine tetra acetic acid, phosphoric acid, tetracycline, stannous fluoride and citric acid.^[12]

Considering the above facts an effort has been made in this study to determine the surface characteristics of diseased root surface by conditioning with EDTA 15% and MTAD BIOPURE™ under scanning electron microscope.

Biopure MTAD (Dentsply Tulsa Dental, USA) is a mixture of doxycycline (a tetracycline isomer), citric acid and polysorbate-80 (a detergent). It has been reported to remove the smear layer effectively, eliminate microbes that are resistant to conventional endodontic irrigants and dressings, and provide sustained antimicrobial activity.

In the present study 30 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 immediate past history of scaling and root planing were excluded as they may alter the root surface. Teeth with attrition, abrasion and erosion were not included in study as they have shown to produce secondary changes in tooth structure like alteration in mineral composition and formation of sclerotic dentine. Only proximal root surfaces was preferred because facial and lingual surfaces were marred by the extraction forceps during tooth extraction.^[11] The teeth in this study were root planed until the roots felt hard, velvety smooth, and glass-like to the touch of a 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.^[13] In the present study active burnishing of the samples with cotton pellet saturated with the respective conditioner is done and changed after every 20 seconds for a period of 3 minutes.

This active burnishing was found to increase the size of dentinal tubules and expose the large amount of collagen matrix.^[5] After root conditioning the samples were washed with 10 ml of saline solution to rid the specimens off any remaining/pooled conditioning agent on the root surface.^[14] On visualizing of samples under Scanning electron microscope the photomicrographs of the samples treated with saline showed the presence of heavy smear layer throughout the entire sample. All the samples belonged to score 0. When the instrumented conditioned root surface of samples treated with saline was rinsed in PBS following 20 minute incubation with blood, the blood clot washed away from all the samples along with SEM evaluation showing smear layer. This is in accordance with the study of Baker PJ et al 2000.^[15] Photomicrographs of the samples treated with EDTA showed maximum number of samples with moderate fibrin network and moderate quantity of blood cells i.e score 2 (seen in 5 samples). Studies have shown that the chelating agent EDTA working at neutral pH is the only one which exerts its demineralising effect through chelating divalent cation at neutral pH and has been found to be as effective as low pH root conditioners in respect to smear layer removal and superior in exposing root surface associated collagen.^[16] EDTA conditioning removed the smear layer exposing the dentinal tubules and collagenous matrix, it is likely to increase the wettability of dentin resulting in enhanced attachment of fibrin clot imposed on the root surface.^[6] EDTA inhibited blood element adsorption and adhesion to dentin surface, it may be attributed to a possible incomplete removal of the gel from the root surface. Also, EDTA is a calcium chelator; therefore, its residues may have inhibited or retarded coagulation events. This is in accordance with the study done by **Liete**

FRM et al 2005.^[17]

The photomicrographs of the samples treated with MTAD showed maximum number of samples with dense fibrin network and trapped cells i.e score 3 (seen in 6 samples). Doxycycline (a tetracycline isomer) is the primary ingredient of MTAD contributing to its antimicrobial activity. Citric acid has been shown to remove the smear layer in combination with doxycycline and allowed the penetration of doxycycline in to the root canal irregularities and dentinal tubules to show its antimicrobial activity. The third component i.e Tween-80 reduces the surface tension and enhances the penetrating ability and diffusion of acids in to the root canal wall and irregularities.^[18] Studies from the past had shown that individual components of MTAD have been used previously as periodontal conditioners. **Boyko GA et al.**, showed that the citric acid demineralisation of the root surfaces resulted in enhanced healing of periodontal defects by improving the adhesiveness of root surface as a substrate to which cells can adhere.^[19] **Polson AM et al.**, had shown that citric acid application to the root planned surface removed smear layer and exposed collagenous matrix that appeared to provide more hospitable environment for connective tissue attachment.^[20] **Baker DL et al.**, showed better efficiency of citric acid demineralisation of root surface to promote fibrin clot adhesion as compared to EDTA and protein constructs.^[6] **Weikesjo UME et al.**, showed tetracycline HCL removed smear layer and exposed dentinal tubules and can act as an improved substrate for connective tissue components vital to periodontal healing.^[21] **Minocha T et al.**, found increased attachment of fibrin clot when combination of citric acid and tetracycline is used as compared to when citric acid alone was used.^[13] On the basis of statistical analysis when both the experimental groups i.e group 2 and group 3 were compared with control group 1 the p value was found to be statistically significant ($p < 0.001$) showing that both the experiment groups i.e group 2 and group 3 are better than group 1 in terms of fibrin clot attachment. This is in accordance with the study done by **Zia A et al 2014**,^[14] **Gupta PK et al 2015**,^[22] **Houshmand b et al 2011**.^[5] When both the experimental groups was compared i.e. group 2 and group 3 the p value was found to be significant at the level ($p < 0.020$). The above results show that EDTA is able to remove smear layer and open dentinal tubules and collagen matrix for fibrin clot attachment but is less efficacious than of MTAD BIOPURE™. The better results for MTAD can be attributed due to its low pH (1.28) and presence of detergent (tween-80) enhancing its penetration and thus better removal of smear layer and subsequently better attachment of fibrin clot. This is in accordance with the study done by **Zia A et al 2014**,^[14] **Tandon C et al 2015**.^[2] Hence the overall statistics shows that the group III (MTAD) is better than group II(EDTA) and group I(Normal saline), indicating superior efficacy of MTAD as better root surface modifier as compared to EDTA and normal saline.

V. Conclusion

Within the limits of this study it was concluded that both EDTA and MTAD are effective in removing smear layer.

On inter-group comparison between EDTA and MTAD it was found that MTAD had shown better results in terms of collagen fibers exposure and creating a zone of demineralized matrix that is biologically acceptable for fibrin clot attachment to dentin surface.

References

- [1]. Rompen EH, Goffinet GH, Nusgens B. Human periodontal ligament fibroblast behaviour on chemically conditioned dentine: An in-vitro study. *J Periodontol* 1999;70:1144-52.
- [2]. Tandon C, Govila V, Pant VA, Meenawat A. Comparative in vitro SEM study of a novel root canal irrigant- MTAD and conventional root biomodifiers on periodontally involved human teeth. *J Indian Soc Periodontol* 2015;19:268-72.
- [3]. Batra P, Gupta H, Bharti V, Sharma A, Kaur R. Comparison Of Efficacy Of Citric Acid And Ethylene Diamine Tetra Acetic Acid (EDTA) As Root Conditioning Agents During Periodontal Flap Surgery-in Vivo Study. *Indian Journal of Dental Sciences* 2014;6:15-18.
- [4]. Ghandi M, Houshmand B, Nekoofar MH, Tabor RK, Yadeghari Z, Dummer PMH. The effect of MTAD, an endodontic irrigant, on fibroblast attachment to periodontally affected root surfaces: A SEM analysis. *J Indian Soc Periodontol* 2013;17:188-92.
- [5]. Houshmand B, Gandhi M, Nekoofar MH, Gholamii GA, Tabor RK, Dummer PMH. SEM Analysis of MTAD Efficacy for Smear Layer Removal from Periodontally Affected Root Surfaces. *Journal of Dentistry* 2011;8:157-64.
- [6]. Baker DL, Stanley Pavlow SA, Wikesjo UME. Fibrin clot adhesion to dentin conditioned with protein constructs: an in vitro proof-of-principle study. *J Clin Periodontol* 2005;32:561-66.
- [7]. Tandon C, Govilla V, Pant AV, Meenawat A. An in vitro scanning electron microscopic study comparing MTAD (intracanal irrigant) and various root biomodifiers on periodontally involved human teeth. *J Int Clin Dent Res Org* 2014;6:24-28.
- [8]. Theodoro LH, Sampaio JEC, Haypek P, Bachmann L, Zezell DM, Garcia VG. Effect of Er: YAG and diode lasers on the adhesion of blood components and on the morphology of irradiated root surfaces. *J Periodont Res* 2006;41:381-90.
- [9]. Hanes PJ, O'Brien NJ and Garnick JJ. A morphological comparison of radicular dentine following root planning and treatment with citric acid or tetracycline HCL. *J Clin Periodontol* 1991;18:660-68.
- [10]. Blomlof JPS, Blomlof LB, Lindskog SF. Smear removal and collagen exposure after non-surgical root planning followed by etching with an EDTA gel preparation. *J Periodontol* 1996;67:841-45.
- [11]. Jones WA, O'Leary TJ. The effectiveness of in-vivo root planning in removing bacterial endotoxin from the roots of periodontally involved teeth. *J Periodontol* 1978;49:337-42.
- [12]. Sterrett JD, Bankey T, Murphy HJ: Dentin demineralization. The effects of citric acid concentration and application time. *J Clin Periodontol* 1993;20:366-70.
- [13]. Minocha T, Rahul A. Comparison of fibrin clot adhesion to dentine conditioned with citric acid, tetracycline, and ethylene diamine tetra acetic acid: An in vitro scanning electron microscopic study. *J Indian Soc Periodontol* 2012;16:333-41.
- [14]. Zia A, Andrabi SM, Bey A, Kumar A, Fatima Z. Endodontic irrigant as a root conditioning agent: An in vitro scanning electronmicroscopic study evaluating the ability of MTAD to remove smear layer from periodontally affected root surfaces. *Singapore Dental Journal* 2014;35:47-52.
- [15]. Baker PJ, Rotch HA, Trombelli L, Wikesjo UME. An in-vitro screening model to evaluate root conditioning protocols for periodontal regenerative procedures. *J Periodontol* 2000;71:1139-43.
- [16]. Blomlof J, Blomlof L, Lindskog S. Effect of different concentrations of EDTA on smear removal and collagen exposure in periodontitis-affected root surfaces. *J Clin Periodontol* 1997;24:534-37.
- [17]. Leite FRM, Moreira CS, Theodoro LH, Sampaio JES. Blood ceel attachment to root surfaces treated with EDTA gel. *Braz Oral Res* 2005;19:88-92.
- [18]. Torabinejad M, Shabahang S, Aprecio RM, Kettering JD. The Antimicrobial Effect of MTAD: An In Vitro Investigation. *J Endod* 2003;29:400-3.
- [19]. Boyko GA, Brunette, Melcher AH. Cell attachment to demineralized root surfaces in-vitro. *J Periodont Res* 1980;15:297-3.
- [20]. Polson AM, Frederick GT, Ladenheim S, Hanes PJ. The production of a root surface smear layer by instrumentation and its removal by citric acid. *J Periodontol* 1984;55:443-46
- [21]. Wikesjo UME, Baker PJ, Christerrson LA et al. A biochemical approach to periodontal regeneration: Tetracycline treatment conditions dentin surfaces. *J Periodont Res* 1986;21:322-29.
- [22]. Gupta PK, Mahajan UP, Gupta K, Sheela NV. Comparative evaluation of a new endodontic irrigant- Mixture of tetracycline isomer, an acid, and a detergent to remove the intracanal smear layer: A scanning electron microscopic study. *J Int Oral Health* 2015;7:1-6.

FIGURES

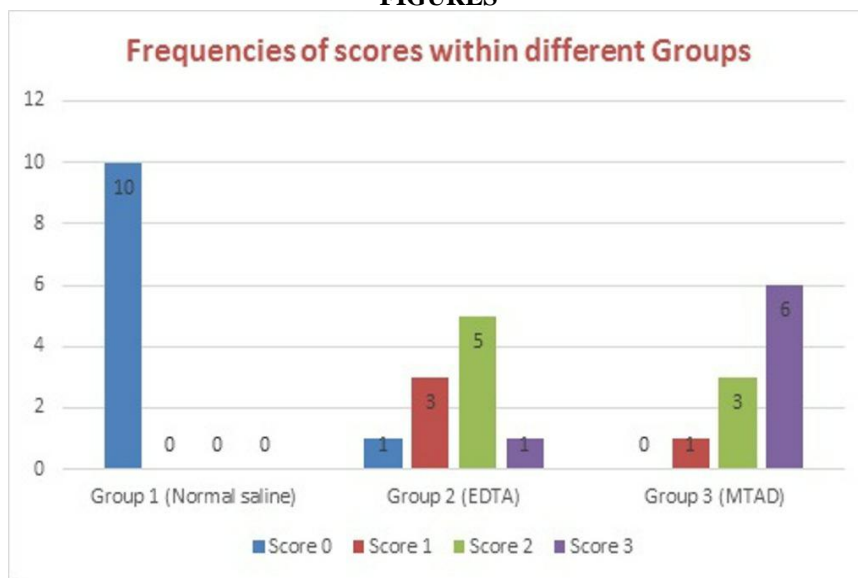


Figure 1: Frequencies of scores within different groups.

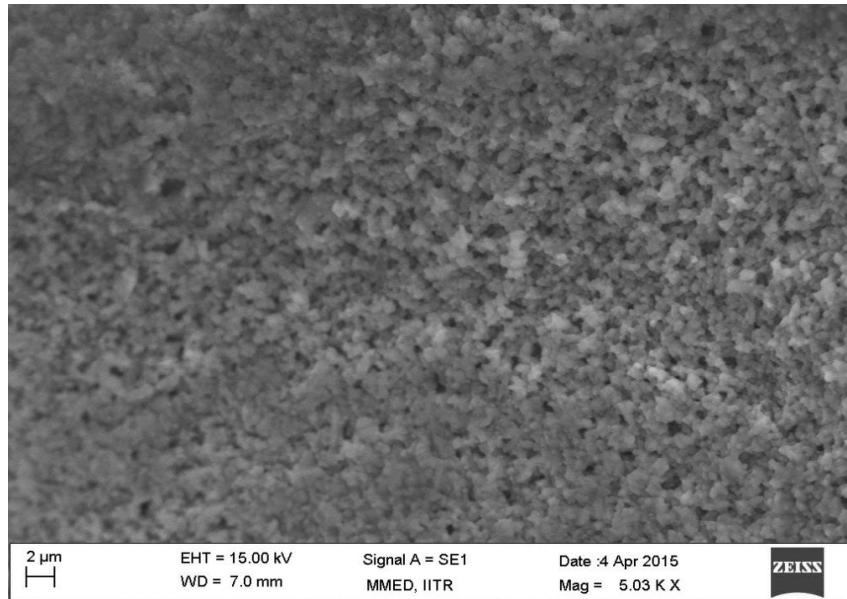


Figure 2: Photomicrograph of dentine samples treated with Saline (magnification 5000 X)

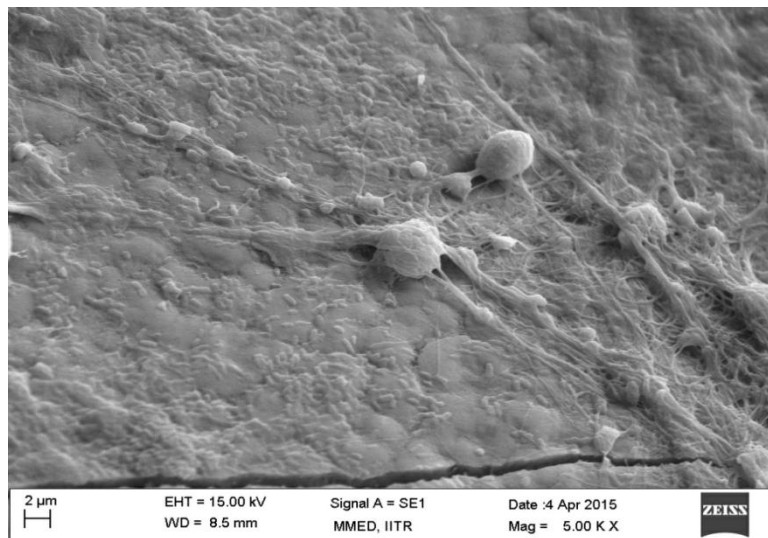


Figure 3: Photomicrograph of dentine samples treated with EDTA (magnification 5000 X)

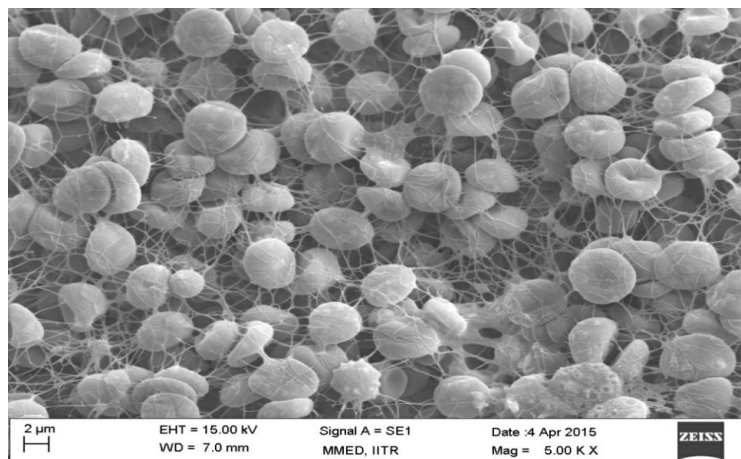


Figure 4: Photomicrograph of dentine samples treated with MTAD (magnification 5000 X)

TABLES:

GROUPS	SCORE 0	SCORE 1	SCORE 2	SCORE 3
GROUP: 1 (NORMAL SALINE)	10	0	0	0
GROUP: 2 (EDTA)	1	3	5	1
GROUP: 3 (MTAD BIOPURE®)	0	1	3	6

Table 1: Scoring of samples using criteria of Theodoro LH et al 2006

Groups	N	Minimum	Maximum	Percentiles			
				25th	50th (Median)	75th	
1	scores	10	0	0	0.00	0.00	0.00
2	scores	10	0	3	1.00	2.00	2.00
3	scores	10	1	3	2.00	3.00	3.00

Table 2: Comparison of median of different groups

SCORES	Groups	N	Mean Rank	Sum of Ranks	Mann-Whitney U	p- value
	1	10	6.00	60.00	5.00	0.001*
	2	10	15.00	150.00		

Table 3: Inter Group comparison between Normal Saline (group 1) and EDTA (group 2)

SCORES	Groups	N	Mean Rank	Sum of Ranks	Mann-Whitney U	p- value
	1	10	5.50	55.00	0.00	0.001*
	3	10	15.50	155.00		

Table 4: Inter Group comparison between Normal Saline (group 1) and MTAD (group 3)

SCORES	Groups	N	Mean Rank	Sum of Ranks	Mann-Whitney U	p- value
	2	10	7.60	76.00	21.00	0.020*
	3	10	13.40	134.00		

Table 5: Inter Group comparison between EDTA (group 2) and MTAD (group 3)

Dr Gaurav Didhra. "Fibrin Clot Adhesion To Instrumented Conditioned Root Surfaces By MTAD And EDTA- A SEM Study ." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 10, 2018, pp 91-97.