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EFFICACY OF NOVEL ROOT CANAL IRRTIGANT-MTAD AGAINST ROOT BIOMODIFIERS ON PERIODONTALLY INVOVLED TEETH IN PERIODONTAL REGENRATION- AN IN VITRO SCANNING ELECTRON MICROSCOPY STUDY.

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Abstract

B**ackground:** Smear layer removal and collagen fiber exposure may improve regeneration which can be accomplished by use of root biomodifiers. These enhance the degree of connective tissue attachment to denuded roots. The objective of this *in vitro* study was to evaluate and compare novel root canal irrigant and other root biomodifiers for smear layer removal on periodontally involved human teeth.

Materials and Methods: Forty human teeth were collected and stored in saline. After scaling and root planing, two samples were obtained from each tooth. A total of 100 dentin blocks were randomly divided into four groups: Mixture of tetracycline, acid and detergent (MTAD), tetracycline hydrochloride (TTC HCl), citric acid (CA), and ethylenediaminetetraacetic acid (EDTA). The agents were applied for 3 min by active burnishing. Immediately following treatment, the specimens were rinsed, dehydrated, fixed and prepared for scanning electron microscope and were examined at × 3500 magnification. Sampaio's index was evaluated by the previously trained blind examiner using photomicrographs. Groups were compared using analysis of variance followed by Tukey's *post-hoc* test.

Results: Mixture of tetracycline, acid, and detergent is most efficacious in removing the smear layer and showed statistically significant dentinal tubules opening, followed by EDTA, TTC HCl, and CA.

Conclusion: Mixture of tetracycline, acid and detergent and conventional root biomodifiers used in the study alters the dentin surface by smear layer removal and exposure of dentinal tubules. Hence, MTAD as a root biomodifier may have a significant role in periodontal regeneration.

Key words: Citric acid, ethylenediaminetetraacetic acid, MTAD, root biomodifier, scanning electron microscope study, tetracycline hydrochloride

Introduction

Regeneration is defined as reproduction or reconstitution of a lost or injured part, in contrast to repair, which describes healing of a wound by tissue that does not fully restore the architecture or the function of the part. Periodontal regeneration can be defined histologically as regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum over a previously diseased root surface.

There are various surgical and non-surgical periodontal therapies done at arresting periodontal disease by removal of plaque from disease-affected roots. Even though complete removal appears not possible with only mechanical debridment. So root conditioning has been recommended as an adjunct to mechanical root surface debridement to remove smear layer and root associated endotoxins and to expose collagen fibres on the dentin surface.

Smear layer is an amorphous, granulated, and irregular layer covering the root surface when observed under scanning electron microscope (SEM).It is constituted of inorganic (calcium, phosphate), and organic material (odontoblastic process, bacteria, and blood cells) and bacterial products (endo and exotoxins) resulting in hyper mineralization of root surface which may function as a physical barrier to the growth of a connective tissue attachment to the root surface.[1]

Root surface debridement (RSD) was carried out with the aim of facilitating reattachment of connective and gingival tissue to the periodontally affected root surface aims to remove soft bacterial deposits, calculus and endotoxins within the diseased cementum and is known to create a surface conducive to cell adherence and attachment.

The concept of acid demineralization in periodontal therapy was first introduced in the 1800s as a substitute for scaling and calculus removal. Citric acid alters the external characteristics of root surfaces, removes the smear layer after scaling and root planning, demineralizes the treated

surface leaving a "mat-like" collagen surface with exposed dentinal tubules, and eliminates bacterial endotoxins from pathologically altered cementum surfaces.

The main objective of this study includes scaling and root planning and use of demineralizing agents. scaling and root planing leads to the formation of smear layer which inhibits the growth of a connective tissue attachment to the root surface. Demineralizing agents have shown to expose the dentin collagen widening the orifices of dentinal tubules,remove cementum bound proteins and retained toxins from altered root surface.thus the root surface smear layer removal with chemicals was carried out during periodontal therapy to enhance regeneration of the lost periodontal attachment.

Numerous agents were used for conditioning of root surfaces that have been involved for demineralization purposes which include citric acid, phosphoric acid, tetracycline, doxycycline, minocycline, fibronectin, ethylenediaminetetraacetic acid (EDTA).

Tetracycline HCL is an effective antibiotic against periodontal pathogens which is absorbed into the root surface and slowly released in its active form (substantivity). As a root surface conditioning agent, TTC produces several actions with the potential of improving periodontal healing and regeneration including the Demineralization of the root surface and removal of the smear layer, Fibrin clot stabilization, increased chemotaxis, adhesion, and growth of fibroblasts on the root surface, inhibition of matrix metalloproteinases.[2]

Ethylene diamine tetra acetic acid(EDTA) is a decalcifying agent operating at a neutral pH by chelating the divalent cations. It preserves the vitality of the remaining periodontal cells close to the root surface, and also has the advantage of biocompatibility (pH = 7.0) as compared to other root conditioning agents.

Citric acid (CA) was been shown to alter the surface characteristics of treated root surfaces by removing smear layer. It causes demineralization of root surfaces and removes bacterial endotoxins from the pathologically altered cementum surfaces.

Bio PureMTAD (Dentsply, Tulsa Dental, and USA.) has been used as an antibacterial root canal irrigant. It stands for a mixture of TTC isomer (doxycycline), acid-CA, and detergent -tween80. It has the ability to remove the smear layer and also exert a potent antimicrobial activity. In the present study, mixture of tetracycline, acid, and detergent (MTAD) has been used as a novel approach for root bio modification.it has the ability to remove successfully smear layer from the root surface of periodontally affected teeth and whether this produced an environment conducive to periodontal cell attachment and growth.[3]

The solution was applied using "Active Burnishing Technique" in the present study. It has been observed by various studies that a burnishing technique resulted in a chemical/mechanical action that enhances the removal of chemically loosened inorganic material and surface debris, exposing the underlying root surface to the demineralization action of fresh acid solution. This may ultimately achieve an optimal degree of demineralization within a short period of time.

Taking into consideration by above that the present *in vitro* study is aimed to evaluate and compare novel root canal irrigant and other root biomodifiers for smear layer removal on periodontally involved human teeth which helps in periodontal regeneration.

Materials and Methods

The present in SEM study was conducted in the Department of Periodontology and Implantology, D.J. College of Dental College and Research, Modinagar (U.P.) in collaboration with the Indian Institute of Technology, Delhi . 100 freshly extracted single-rooted,

were collected from the Department of Oral and maxillofacial surgery, D.J. College of Dental Sciences and Research, Modinagar and stored in 10% formalin. The teeth with wasting diseases, tooth fracture, endodonticaly treated, and prosthodonticaly restored were excluded from the study. Written informed consent was taken prior to extraction from all the patients and ethical committee clearance from the institution was obtained.

Preparation of samples

Samples were obtained from the cervical third of the root by making two parallel grooves with a cylindrical bur under copious saline irrigation. The first groove was positioned horizontally at cementoenamel junction and second groove made parallel and 4 mm apical in relation to the first. The diseased root surfaces of all teeth were scaled with an ultrasonic scaler and thoroughly planed with #1–2, 3–4 Gracey curettes (Hu-Freidy) to remove all the diseased cementum. With the help of diamond disc under copious irrigation, the two samples are obtained first by transverse sectioning the root from the grooves and secondly by sectioning the sample longitudinally into two from the middle. The dentin samples of dimension 4 mm × 6 mm were prepared. The labial and lingual surface of each specimen was used for the study.

Total 80 samples were randomly divided into 4 groups:

- Group A: Biopure MTAD™
- Group B: Tetracycline HCl (50mg/ml)
- Group C: Citric acid (pH 1.0)
- Group D: Ethylene diaminetetraacetic acid (15%).

These agents were freshly prepared every time and were applied by "active burnishing" on the curetted root surfaces for 3 min. The cotton pellets are changed every 30 seconds to ensure consistent solution application. Following treatment, samples were rinsed thoroughly with distilled water.

Figure 1: MTAD

Preparation of samples for scanning electron microscope study Following the chemical treatment, all samples were dehydrated in a graded series of ethanol (10–90%) for 30 min each and finally in 100% acetone for 30 min more. The samples were dried under the lamp and then mounted on the aluminum stubs and inserted in SC7640 sputter coater machine for gold/palladium coating on specimens. All the specimens were examined in a Polaron-SEM (Leo-430) at a magnification of ×3500 and photomicrographs were evaluated to ascertain the extent of root biomodification by removal of smear layer, patent dentinal tubules in relation to the total number of dentinal tubules.

Analysis of photomicrographs

The photomicrographs were distributed to three calibrated, trained blind examiners to determine the degree of smear layer removal according to the root surface modification index (Sampaio's index).[4] The scores are as follows:

Score 1

Root surface without smear layer, with dentinal tubules completely opened; no evidence of smear layer in the dentinal tubule gaps.

Score 2

Root surface without smear layer, with dentinal tubules completely opened; evidence of smear layer in the dentinal tubule gaps**.**

Score 3

Root surface without smear layer, with the dentinal tubules partially opened.

Score 4

Root surface covered with smear layer, with uniform aspect; evidence of dentinal tubule gaps.

Score 5

Root surface covered with smear layer, with uniform aspect; no evidence of dentinal tubule gaps.

Score 6

Root surface covered with smear layer, with irregular aspect and presence of grooves and/or scattered debris.

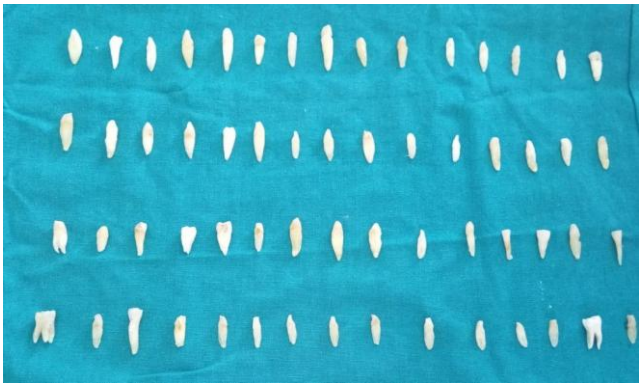


Figure 2: Specimens

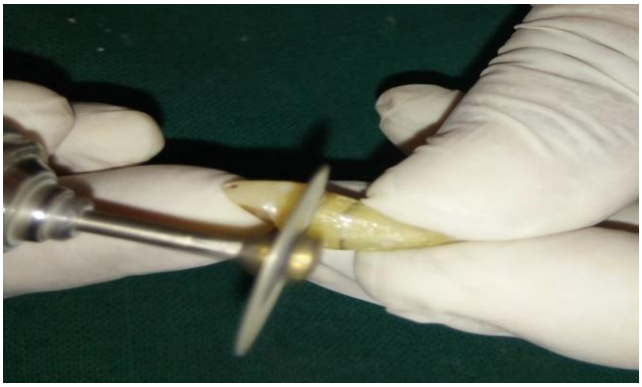


Figure 3: Tranverse section

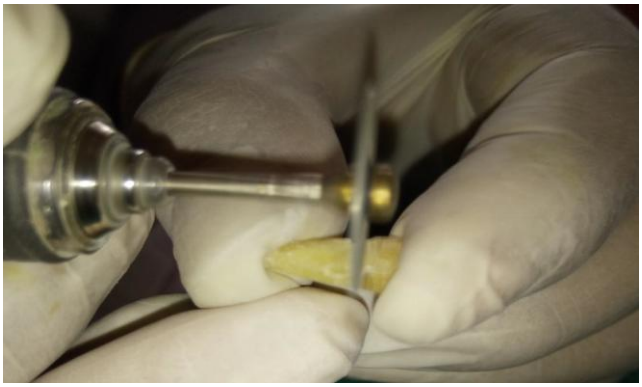


Figure 4: Transverse section

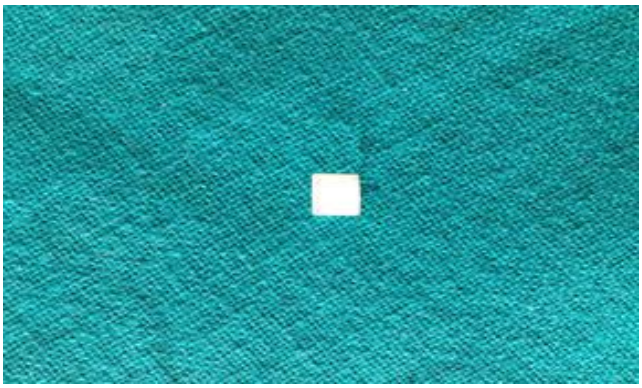


Figure 5: Tranverse cut block

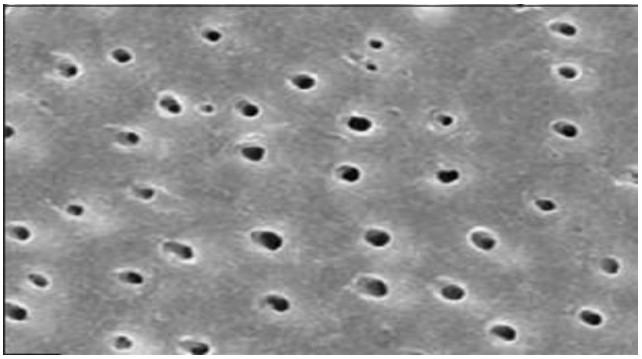


Figure 6: Photomicrograph 1
Opening of dentinal tubules patent for regeneration against MTAD

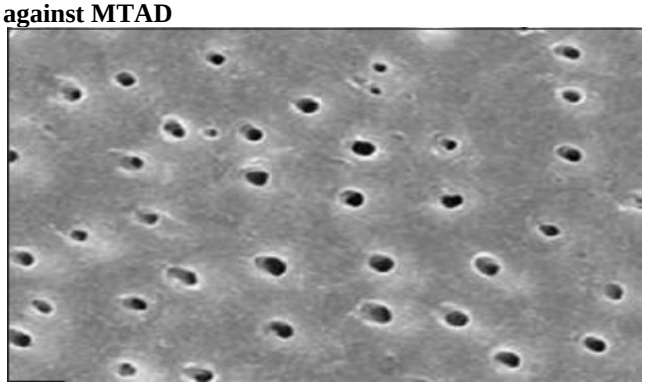


Figure 7: Photomicrograph 2
Opening of dentinal tubules patent for regeneration against EDTA

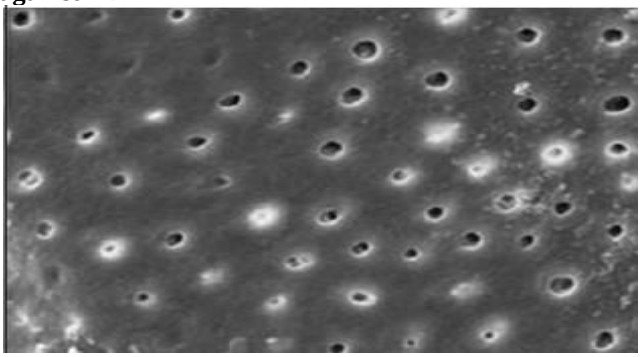


Figure 8: Photomicrograph 3
Opening of dentinal tubules patent for regeneration against TTC+HCL

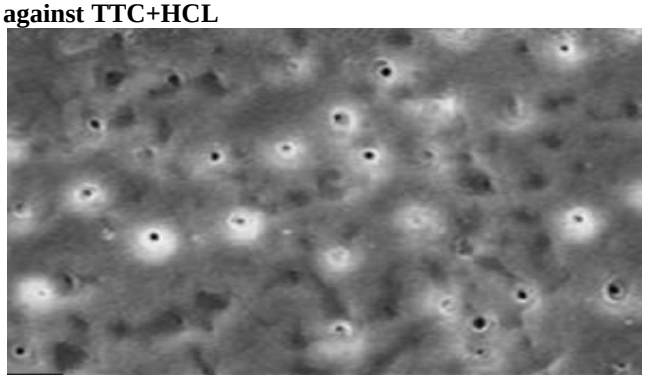


Figure 9: Photomicrograph 4
Opening of dentinal tubules patent for regeneration against citric acid

RESULTS

The Sampaio's index scores of Group A, B, C, D ranged from 1–2, 2–4, 4–5, and 1–3 respectively with mean ± SD 1.67 ± 0.49, 3.27 ± 0.59, 4.20 ± 0.41 and 2.33 ± 0.62, respectively. Comparing the mean scores of four groups, ANOVA revealed significantly different Sampaio's index scores (F = 64.09, P < 0.001), patent dentinal tubule scores (F = 140.42, P < 0.001) and mean percentage patency scores of four groups (F = 141.70, P < 0.001).Further, Tukey test revealed significantly different and lower mean Sampaio's index, mean patent dentinal tubule and percentage patency scores of MTAD group as compared to TTC HCl (P < 0.001), CA (P < 0.001) and EDTA (P = 0.006) groups. Similarly, the mean Sampaio's index, patent dentinal tubule and percentage patency scores of EDTA group also lowered significantly as compared to both TTC HCl (P < 0.001) and CA (P < 0.001). Further, the mean Sampaio's index scores of TTC HCL group was also found significantly different and lower as compared to CA (P < 0.001). However, like patent dentinal tubules (P = 0.974), the mean percentage patency scores also not differed between TTC HCL and CA (P = 0.427) that is, found to be statistically the same.

Intergroup comparison of sampio index scores between different groups

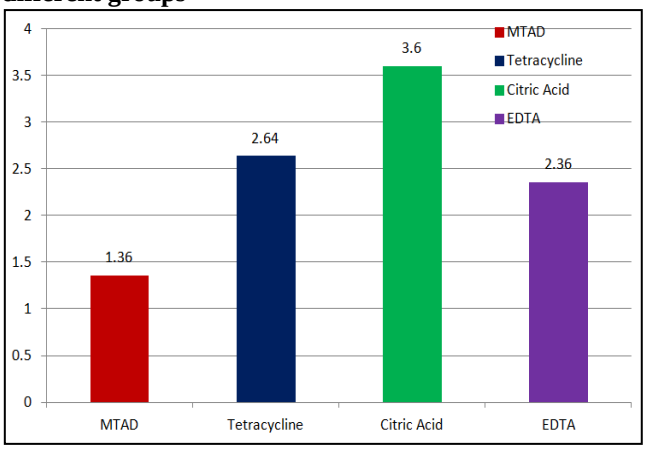
Table 1:

	N	Mean	Std. Deviation	Std. Error	P value
MTAD	25	1.36	0.489	0.097	0.001 (Significant)
Tetracycline	25	2.64	0.637	0.127	
Citric Acid	25	3.60	0.500	0.100	
EDTA	25	2.36	0.489	0.097	

Table-5 showed comparison of sampios index for removal of smear layer among 4 groups .

The mean of sampios index was significant difference among the groups, in which MTAD showed the highest score for smear layer removal followed by EDTA,TETRACYCLINE +HCL and CITRIC ACID group.

Intergroup comparison of sampio index scores between different groups



Intergroup comparison of patent opened dentinal tubules between different groups

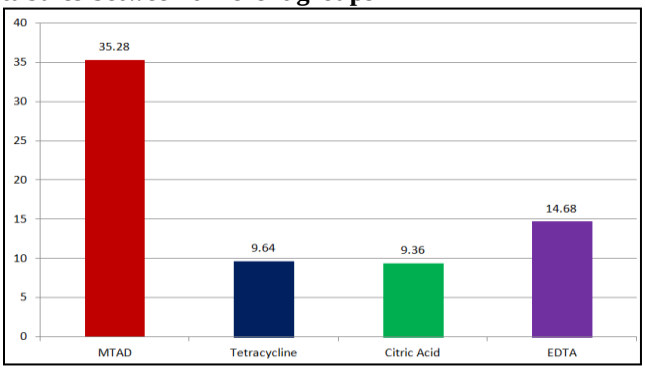
Table 2:

	N	Mean	Std. Deviation	Std. Error	P value
MTAD	25	35.28	1.307	0.261	0.001 (Significant)
Tetracycline	25	9.64	1.254	0.250	
Citric Acid	25	9.36	1.287	0.257	
EDTA	25	14.68	0.748	0.149	

Table 6- showed comparison of patency of dentinal tubules for regeneration among 4 groups.

The mean was significant difference among the groups, in which MTAD showed the highest patency dentinal tubules than other groups and after MTAD the better the agent for removal of smear layer is EDTA Hence MTAD was the best for removal of smear layer and helps in regeneration.

Intergroup comparison of patent opened dentinal tubules between different groups



Intergroup comparison of percentage patency between different groups

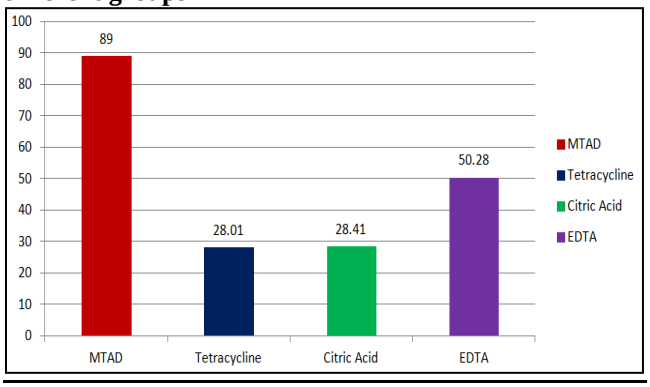
Table 3:

	N	Mean	Std. Deviation	Std. Error	P value
MTAD	25	89.00	3.741	0.748	0.001 (Significant)
Tetracycline	25	28.01	3.837	0.767	
Citric Acid	25	28.41	4.318	0.867	
EDTA	25	50.28	3.351	0.670	

Table-1 showed comparison of percentage of patency of dentinal tubules for regeneration among 4 groups .

The mean was significant difference among the groups, in which MTAD showed the highest percentage of patency dentinal tubules than other groups Hence MTAD was having the highest patent opened dentinal tubules which helps in regeneration.

Intergroup comparison of percentage patency between different groups



Descriptive data for smear layer removal by mtad

Table 4:

S.no	Sampios index	No. of dentinal tubules	Patency of Opened dentinal tubules	Percentage of opened dentinal tubules (%)
1	1	39	36	92
2	1	41	38	92
3	2	38	35	92
4	1	36	34	94
5	1	40	36	90
6	1	42	36	85
7	2	43	35	81
8	1	39	34	87
9	1	38	36	94
10	2	40	37	92.5
11	1	38	35	92
12	1	40	35	90
13	2	39	34	89
14	2	42	33	83
15	1	41	35	82
16	1	38	36	86
17	1	39	36	89
18	1	40	35	90
19	1	42	36	85
20	2	39	35	89
21	2	40	36	90
22	2	41	34	82
23	1	38	34	89
24	1	38	33	86
25	2	41	38	92

Descriptive data for smear layer removal by TTC+HCL

Table 5:

S.no	Sampios index	No. of dentinal tubules	Patency of opened dentinal tubules	Percentage of opened dentinal tubule(%)
1	3	35	10	28
2	2	34	12	28
3	3	36	9	30
4	3	32	8	25
5	2	33	11	25
6	3	36	10	33
7	2	35	10	27
8	3	34	9	28
9	2	36	8	26
10	3	33	9	22
11	2	31	10	27
12	2	35	8	32
13	2	36	8	22
14	2	32	10	22
15	4	35	11	31
16	2	36	12	31
17	3	34	10	33
18	3	36	8	29
19	4	35	9	22
20	2	35	8	25
21	2	36	11	22
22	3	33	9	30
23	3	34	10	27
24	3	35	10	29
25	3	35	11	28

Descriptive data for smear layer removal by citric acid
Table 6:

S.no	Sampios index	No. of dentinal tubules	Patency of opened dentinal tubules	Percentage of opened dentinal tubules (%)
1	4	34	8	23
2	3	33	9	27
3	4	34	8	23
4	4	32	9	28
5	3	35	11	31
6	4	33	12	36
7	4	33	11	33
8	4	31	10	32
9	3	33	8	24
10	3	35	9	25
11	4	31	12	38
12	4	32	10	31
13	4	33	8	24
14	3	35	8	22
15	3	35	9	25
16	4	34	8	23
17	3	32	8	25
18	4	35	9	25
19	3	32	9	28
20	3	33	10	30
21	4	34	11	32
22	4	32	10	31
23	4	31	10	32
24	3	31	8	25
25	4	33	9	27

Descriptive data for smear layer removal by EDTA
Table 7:

S.no	Sampios index	No. of dentinal tubules	Patency of opened dentinal tubules	Percentage of opened dentinal tubules (%)
1	2	29	14	48
2	3	30	16	53
3	2	31	15	48
4	2	28	16	57
5	3	29	16	55
6	2	30	14	46
7	2	28	15	53
8	3	29	14	48
9	2	29	14	48
10	3	28	15	53
11	2	30	16	53
12	2	29	15	51
13	3	28	14	50
14	3	30	14	46
15	2	29	15	51
16	2	30	14	46
17	3	28	14	50
18	3	29	15	51
19	2	30	14	46
20	2	28	14	50
21	2	29	15	51
22	2	32	14	43
23	3	31	14	45
24	2	28	15	53
25	2	29	15	51

Intergroup comparison of sampio index scores between different groups
Table 8:

	N	Mean	Std. Deviation	Std. Error	P value
MTAD	25	1.36	0.489	0.097	0.001 (Significant)
Tetracycline	25	2.64	0.637	0.127	
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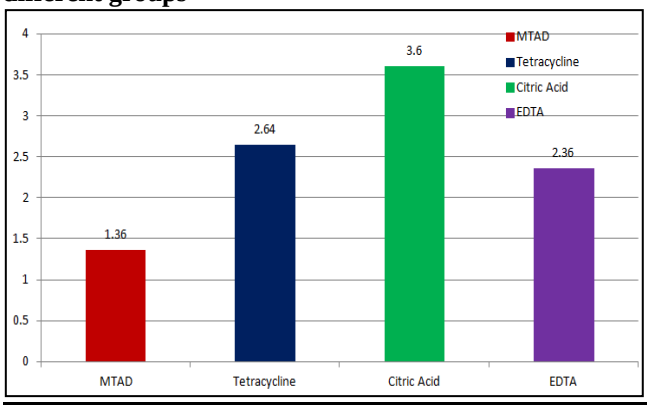
Intergroup comparison of sampio index scores between different groups
Post hoc analysis
Table 9:

(J) GP	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval		Significance
				Lower Bound	Upper Bound	
MTAD vs Tetracycline	-1.280	0.151	0.001	-1.579	-0.9807	Significance
MTAD vs Citric Acid	-2.240	0.151	0.001	-2.539	-1.9407	Significant
MTAD vs EDTA	-1.000	0.151	0.001	-1.299	-0.701	Significant
Tetracycline vs Citric Acid	-0.960	0.151	0.001	-1.259	-0.660	Significant
Tetracycline vs EDTA	0.280	0.151	0.066	-0.019	0.579	Non-Significant
Citric Acid vs EDTA	1.240	0.151	0.001	0.940	1.539	Significant

Intergroup comparison of sampio index scores between different groups



Intergroup comparison of patent opened dentinal tubules between different groups
Table 10:

	N	Mean	Std. Deviation	Std. Error	P value
MTAD	25	35.28	1.307	0.261	0.001 (Significant)
Tetracycline	25	9.64	1.254	0.250	
Citric Acid	25	9.36	1.287	0.257	
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Table 6- showed comparison of patency of dentinal tubules for regeneration among 4 groups.

The mean was significant difference among the groups, in which MTAD showed the highest patency dentinal tubules than other groups and after MTAD the better the agent for removal of smear layer is EDTA Hence MTAD was the best for removal of smear layer and helps in regeneration

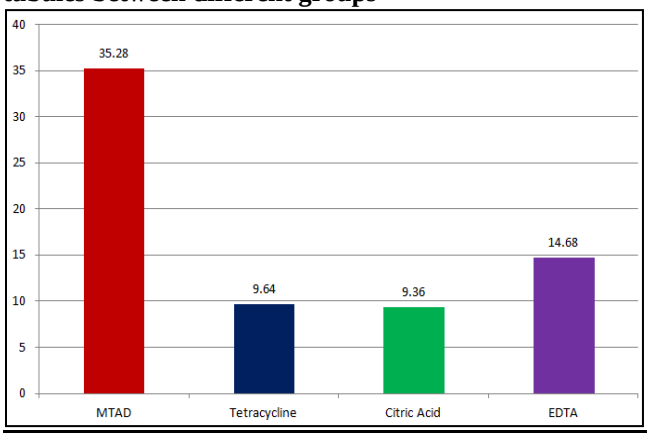
Intergroup comparison of patent opened dentinal tubules between different groups

Post Hoc Analysis

Table 11:

GP	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval		Significance
				Lower Bound	Upper Bound	
MTAD vs Tetracycline	25.64	0.331	0.001	24.98	26.29	Significant
MTAD vs Citric Acid	25.92*	0.331	0.001	25.26	26.57	Significant
MTAD vs EDTA	20.60*	0.331	0.001	19.94	21.25	Significant
Tetracycline vs Citric Acid	0.280	0.331	0.401	-.0.37	0.93	Non-Significant
Tetracycline vs EDTA	-5.040*	0.331	0.001	-5.69	-4.38	Significant
Ciytric Acid vs EDTA	-5.320*	0.331	0.001	-5.97	-4.66	Significant

Intergroup comparison of patent opened dentinal tubules between different groups



Intergroup comparison of percentage patency between different groups

Table 12:

	N	Mean	Std. Deviation	Std. Error	P value
MTAD	25	89.00	3.741	0.748	0.001 (Significant)
Tetracycline	25	28.01	3.837	0.767	
Citric Acid	25	28.41	4.318	0.867	
EDTA	25	50.28	3.351	0.670	

Table-1 showed comparison of percentage of patency of dentinal tubules for regeneration among 4 groups.

The mean was significant difference among the groups, in which MTAD showed the highest percentage of patency dentinal tubules than other groups Hence MTAD was having the highest patent opened dentinal tubules which helps in regeneration

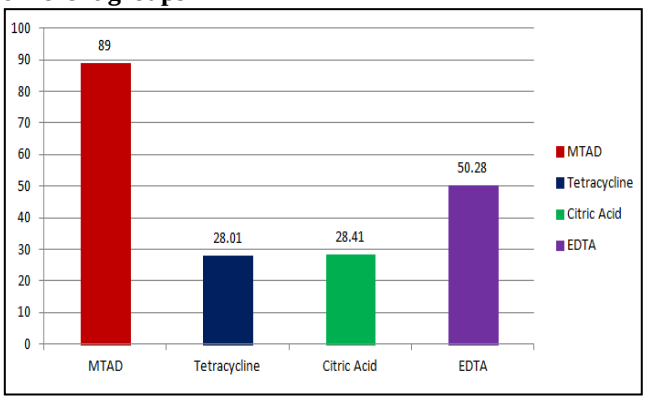
Intergroup comparison of percentage patency between different groups

Post Hoc Analysis

Table 13:

GP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		Significance
				Lower Bound	Upper Bound	
MTAD vs Tetracycline	60.993	1.082	0.001	58.8444	63.1425	Significant
MTAD vs Citric Acid	60.598*	1.082	0.001	58.4494	62.7475	Significant
MTAD vs EDTA	38.721*	1.082	0.001	36.5723	40.8705	Significant
Tetracycline vs Citric Acid	-0.394	1.082	0.716	-2.5440	1.7541	Non-Significant
Tetracycline vs EDTA	-22.272*	1.082	0.001	-24.4211	-20.1230	Significant
Ciytric Acid vs EDTA	-21.877*	1.082	0.001	-24.0261	-19.7280	Significant

Intergroup comparison of percentage patency between different groups



Discussion

One of the objectives of periodontal therapy is the restoration of the lost periodontium and conversion of the periodontitis–affected root surface into a substrate which is biocompatible for epithelial and connective tissue cell adherence and attachment. Methods to achieve this objective include scaling and root planing, and use of demineralizing agents. Scaling and root planing leads to the formation of smear layer which inhibits the growth of a connective tissue attachment to the root surface.[4,5] Demineralizing agents have shown to expose the dentinal collagen, widening the orifices of dentinal tubules, remove cementum bound proteins and retained toxins from altered root surface. Thus, the root surface smear layer removal with chemicals was carried out during periodontal therapy to enhance regeneration of the lost periodontal attachment apparatus.[6] Many chemical agents have been proposed as root conditioning agents, some of them are CA, TTC HCl group, EDTA.[4] The other agents used are carbon dioxide laser,[7] neodymium:yttrium, aluminum, garnet laser,[8] and erbium: Yttrium, aluminum, garnet laser[9] and many more.

As previously established in a systematic review by *Mariotti* (2003)[10,11] and American Academy of Pediatrics, position paper (2005), TTC HCl, CA, and EDTA remove the smear layer but do not provide clinical benefit to the patient during regenerative procedures. As BioPure

MTAD® (Dentsply Ltd., USA.) is used as an efficacious root canal irrigant for smear layer removal, so in the present study, we have utilized this novel agent as a root biomodifier 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. *Torabinejad et al.* (2003)[6] demonstrated that MTAD is an effective solution for the removal of the smear layer; it also did not significantly change the structure of the dentinal tubules. TTC HCl, 50 mg/ml concentration was used according to previous studies in which *Ishi et al.* (2008), *Isik et al.* (2000)[2,4]inferred that TTC HCl concentrations between 50 and 125 mg/ml might alter dentin surfaces by removing smear layer and also maximize tubule openings in a short period of time, if repeated applications were performed. Another agent used was CA pH 1 was also applied for 3 min as recommended by earlier studies.[12-14] EDTA of 15% concentration, pH of 4.35 was used as given by *Lasho et al.* (1983)[15] and Sampaio *et al.* (2003).[16]

The present study photomicrographs were evaluated using the root surface modification index (Sampaio's index) for smear layer removal. The lowest score was achieved in Group A/MTAD which indicates complete smear layer removal and complete opening of dentinal tubules, which was in accordance with the study by *Mozayeni et al.* (2009)[17] who stated that MTAD revealed the presence of more abundant and larger dentinal tubule opening when compared with other agents.The result of MTAD was statistically significant with TTC HCl, CA, and EDTA.[5,10,11] However, to date, no *in vitro* or *in vivo* studies have been conducted utilizing MTAD as a root biomodifier.

The patent dentinal tubules and percentage patency intergroup comparison by *Z*-test showed highly statistically significant difference among the groups. MTAD had higher statistically significant number of patent and percentage patency of dentinal tubules when compared with TTC HCl ($P = 0.0001$), CA ($P < 0.0001$) and EDTA ($P = 0.0455$).

This was in agreement with the study done by *Torabinejad et al.* (2003), and *Mozayeni et al.* (2009) who inferred that MTAD was more efficacious in removing smear layer and opening dentinal tubules of instrumented root canals because of being a combination agent of doxycycline, CA and a detergent tween-80 which reduces the surface tension and helps in better penetration of these acids into smear layer. *Zhang et al.* (2003)[18] demonstrated in his study that MTAD is less cytotoxic to the surrounding cells and tissues than EDTA.

Ethylenediaminetetraacetic acid was statistically significant than TTC HCl and CA, because EDTA is better in smear layer removal and exposure of dentinal tubules by making the root surfaces more biocompatible according to *Silverio et al.* (2007), and *Soares et al.* (2010)[19] *Blomlöf and Lindskog* (1995)[20] also stated that EDTA in a neutral pH was more effective to demineralize dentin than the acidic agents like CA, and selectively remove mineral from a dentin surface, exposing a collagenous matrix. TTC HCl when compared with CA ($P = 1.0000$) did not had any statistically significant difference which was in accordance with the studies done by Lafferty *et al.* (1993) who demonstrated comparable results of CA and TTC because of their similar acidic pH (TTC HCl 1.75 pH, CA pH 1).[21]

Conclusion

In summary, our results confirm that the root condition agents/root biomodifiers are effective in removing the smear layer and exposing dentinal tubules. MTAD being most efficacious followed by EDTA, TTC HCl, and CA. Despite the limitations of the study, further longitudinal *in vitro* and *in vivo* studies to establish MTAD as a root conditioning agent are warranted.

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