COMPARATIVE EVALUATION OF CHEMICAL ROOT SURFACE MODIFIERS POST ROOT PLANING OF PERIODONTALLY INVOLVED TEETH-AN SEM STUDY

***Dr. Bhagwan Dass Puri[1], Dr. Harveen Singh[2], Dr. Gaurav Kansal[3], Dr. Isha Sharma[4], Dr. Deep Gagan Gupta[5], Dr. Sayed Tahir Bukhari[6], Dr. Shridul Gupta[7], Dr. Ramanpreet Kaur[8]**

Article History: Received: 05.05.2023 **Revised:** 24.06.2023 **Accepted:** 20.07.2023

Abstract

Aim: The present study was carried out to evaluate the relative efficacy of topical application of root conditioning agents such as citric acid, minocycline HCl solutions and EDTA gel preparation on periodontally diseased root surfaces.
Material and methods: 60 specimens were obtained from the freshly extracted teeth and divided into4 groups, comprising of one control group and three experimental groups, each having 15 specimens. After scaling and root planing of teeth, these were resected first at level of cementoenamel junction and then longitudinally. Tooth was divided into 2 halves to obtain the dentin slabs of size 7x5 mm. These dentinal slabs were washed with and preserved in distilled water until the time of treatment. The particular solution or gel was passively applied to outer surface of dentin specimens with the help of cotton pellet saturated with that particular solution or gel preparation. These specimens were dehydrated in ascending order concentrations of aqueous alcohol solutions. Dried samples were mounted on SEM stubs. Specimens were then sputter coated with gold using sputtering device. The mounted specimens were evaluated using scanning electron microscope. The surface characteristics of root surfacewere evaluated descriptively, concerning the removal of smear layer, number of open dentinal tubules and the diameter of individual tubules, from the black and white camera prints. The data so obtained was compiled and subjected to statistical analysis.
Results: Out of all the three root conditioning agents, the results of citric acid were better than minocycline HCl (highly significant) and EDTA (Non-significant).
Conclusion: We concluded that root conditioning in all three experimental groups helped removal of smear layer, exposure of dentinal tubules and also the widening of dentinal tubules. Their application as root conditioner may have significant role in periodontal wound healing and future new attachment in-vivo.

Keywords: Citric acid; Minocycline HCl; EDTA; Smear Layer; Dentinal tubules.

[1]PG student, Department of Periodontology, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab.
[2]Professor and HOD, Department of Periodontology, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab.
[3]Professor, Department of Periodontology, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab.
[4]Sr. Lecturer, Department of Periodontology, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab.
[5]PG student, Department of Periodontology, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab.
[6]PG student, Department of Periodontology, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab.
[7]PG student, Department of Periodontology, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab.
[8]Sr. Lecturer, Department of Periodontology, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab.

***Corresponding Authors: Dr. Bhagwan Dass Puri**
G student, Department of Periodontology, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab.
Email:- puridentist@gmail.com

DOI: 10.31838/ecb/2023.12.s3.729

1. Introduction

The ultimate aim of periodontal treatment is regeneration of the periodontium in cases previously affected by periodontal disease.[1] For regeneration to occur, it is necessary to eliminate bacterial plaque, calculus and other cytotoxic substances on or within the diseased root surface.[2] Cementum surfaces exposed by periodontitis are pathologically altered. Such cementum surfaces have loss of collagen fiber insertion, alteration in mineral density and are contaminated by bacterial endotoxin. Cementum surface contaminants inhibit growth and viability of fibroblasts in vitro and may prevent new connective tissue attachment. It was suggested that with periodontal therapy one must either remove the toxic materials from the involved cementum or remove the cementum itself Disinfection and modification of the contaminated root surface in order to restore its biocompatibility and to favour the attachment of regenerated periodontal structures becomes the necessity. Scaling androot planing is effective in removing the bacterial deposits and accretions as well as in removing endotoxins from the exposed root surface.[3] However, mechanical instrumentation leaves a smearlayer, which is usually comprised of remnants of dental calculus and necrosed root cementum, microorganisms and their products. This smear layer acts as a barrier for connective tissue attachment to the root surface and can serve as a reservoir for microbial growth. Historically, the use of acids as a substitute for scaling and root planing was first reported in the New York Dental Record in 1846 and later by Younger (1893, 1897) and Stewart in 1899, who described an operation which included elevation of gingiva from the teeth, scrapping of tooth root surfaces to remove cementum and application of pure sulfuric or hydrochloric acid to decalcify the surface and reported considerable success. Minocyclines are broad spectrum antibiotics with activity against both gram +ve and gram -ve bacteria as well as mycoplasma, rickettsial and chlamydial infections. Tetracycline, doxycycline and minocycline are commonly used and all three have a similar spectrum of activity and along with their root conditioning property they also have additional benefits of (a) antibacterial activity (b) anticollagenase activity (c) substantivity. Minocycline Hcl can promote the attachment and proliferation of human periodontal ligament cells. can also stimulate the synthesis of dihydrotestosterone in the human gingival fibroblasts.[4] Thus, helping in periodontal regeneration. EDTA has been used in root conditioning as it has been seen to remove the smear layer, open dentinal tubules and also expose the collagen fibers when applied on periodontally affected root surfaces.[5] Thus the aim of the present study was to evaluate and compare the effects of topical application of citric acid, minocycline Hcl solutions and ethylene diamine tetraacetic acid (EDTA) gel on periodontal disease root surfaces, under scanning electron microscope (SEM).

2. Material and methods:

In this study, maxillary and mandibular single rooted human teeth indicated for extraction due to chronic periodontitis and having poor prognosis were collected amongst the patients visiting the Department of Periodontics, Genesis Institute of Dental Sciences and Research, Ferozepur.

3. Methodology

In this study, human 30 maxillary and mandibular anterior teeth indicated for extraction due to chronic periodontitis were collected from the patients visiting the Department of Periodontics, Genesis Institute of Dental Sciences and Research, Ferozepur. After instrumentation, a total of sixty specimens were obtained from the

roots of extracted teeth by sectioning them, first at cementoenamel junction and then longitudinally into two equal halves of dimension 7 mm x 5 mm. These 60 specimens were divided into four groups (one control and three experimental groups) comprising of 15 specimens in each group. **Group I:** Dentin specimens treated with saline for 3 minutes. **Group II:** Dentin specimens treated with citric acid (pH 1, 10%) for 3 minutes. **Group III:** Dentin specimens treated with minocycline HCl (pH 4.2, 10 %) for 3minutes. **Group IV:** Dentin specimens treated with EDTA gel (pH Neutral, 10 %) for 3 minutes. (Fig 1-4) After the dehydration process specimens were air dried. Dried samples were mounted on SEM stubs. Specimens were then sputter coated with gold in a smart coater (DII-29030SCTR) sputtering device (Figure 5 and Figure 6). The mounted specimens were evaluated using model JEOL JSM 7610 F Plus (Field Emission Scanning Electron Microscope) (Figure 7). The respective solutions or gel were passively applied to the experimental and control specimens. The specimens were then processed and scanned under scanning electron microscope at ×3000 magnification. Images were recorded on to black and white photographic film via camera linked to SEM. When observed under scanning electron microscope, the controlled specimens showed an irregular uneven surface which seemed to correspond to smear layer (figure 8). Counting the dentinal tubules orifices in saline (Control) group was not possible as the root surface was covered by smear layer. Hence, the comparison was made only between the three groups (citric acid, minocycline HCl and EDTA) of demineralizing agents used. So, total number of dentinal tubules present per specimen, number of patent dentinal tubules from the total number of tubules present and diameter of individual dentinal tubules were evaluated in three experimental groups.

Figure 1: Armamentarium

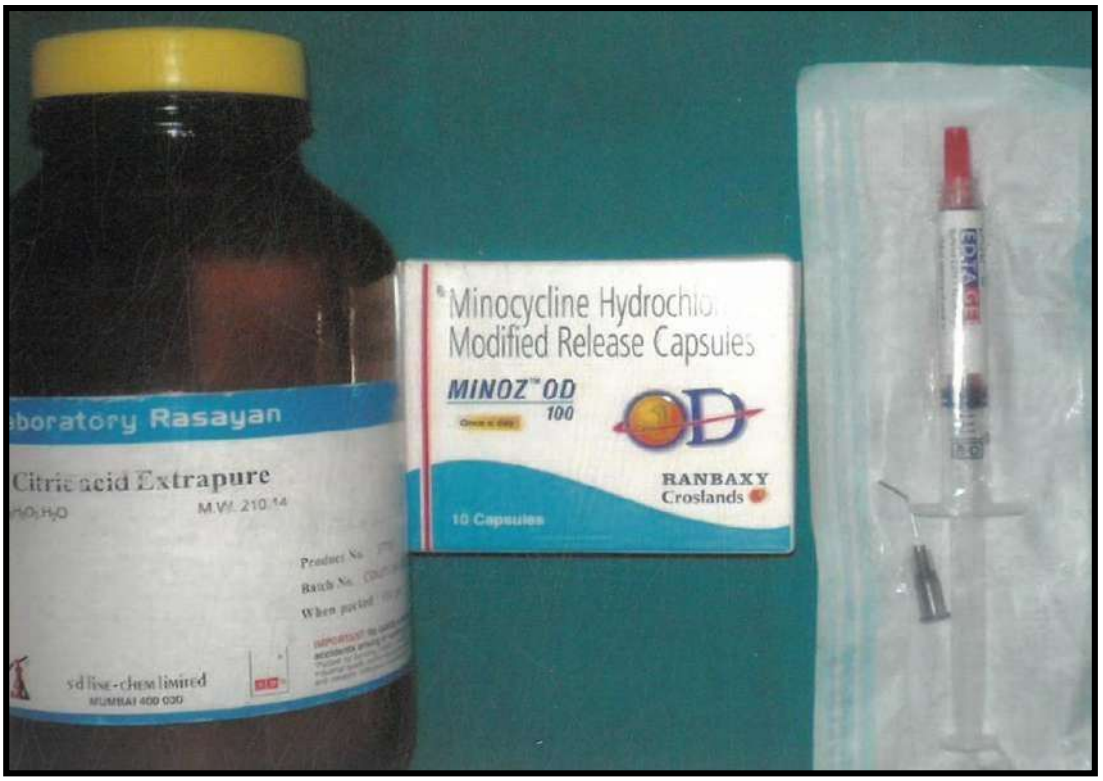

Figure 2: Showing root conditioning agents (citric acid, minocycline HCl and EDTA)

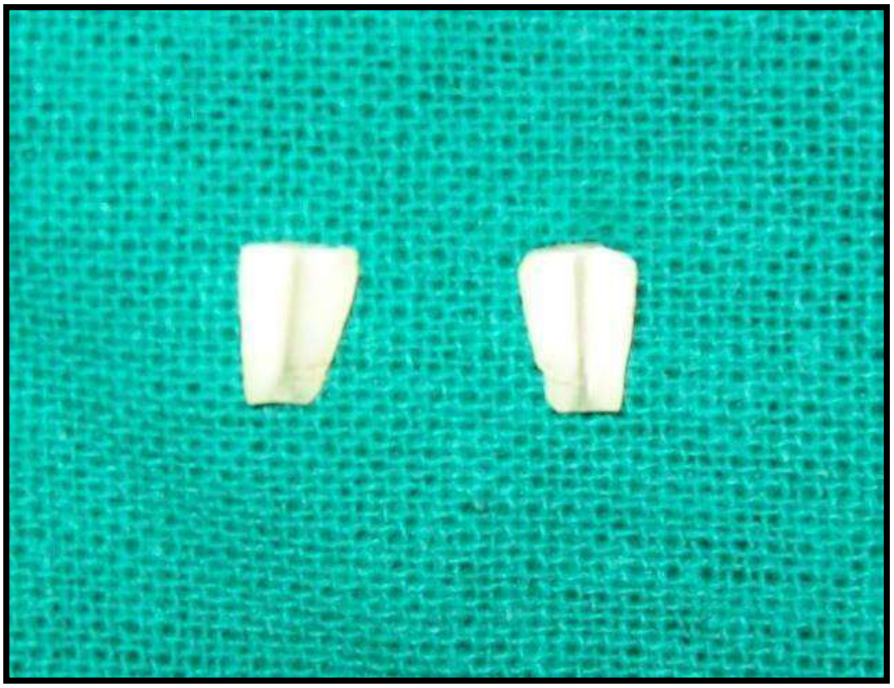

Figure 3: Sectioned dentin specimens being used for the study

Figure 4: Showing Digital Calliper

Figure 5: Sputter coating device being used for ion coating of the dentin specimens

Figure 6: Gold coated dentin specimens to be seen under SEM

Figure 7: SEM (Scanning Electron Microscope)

4. Results

The specimens in group II (citric acid) resulted in the removal of smear layer thus exposing the dentinal tubules in the range of 18-63 (Figure 9, Table 1) with the number of patent dentinal tubules as high as 58 and as low as 13 (Table 2). Tubular diameter of orifices of dentinal tubules range from 5.9µm to 6.5 µm (Table 3). The mean value for the total number of dentinal tubules was 36.60±13.64 (Table 4). While the mean value of total number of patent dentinal tubules was 27.93±11.50 (Table5).

The total mean tubular diameter was 6.23±0.23 (Table 6).

The specimens in group III (minocycline HCl) indicated that the total number of dentinal tubules exposed in the range of 5-29 (Figure 10, Table 1) with number of patent dentinal tubules highest at value 16 and lowest at 3 (Table 2). Tubular diameter of orifices of dentinal tubules range from 1.9µm to 3.9 µm (Table 3).The mean values for total number of dentinal tubules and for number of patent dentinal tubules were 14.31±8.33 (Table 4, and 7.47±3.76 (Table 5) respectively. Total mean tubular diameter was 2.53±0.55 (Table 6).

The specimens in group IV (EDTA) indicated that the total number of dentinal tubules exposed in the range of 16-62 (Figure 11, Table 1), with number of patent dentinal tubules highest at value 45 and lowest at 12 (Table 2,). Tubular diameter of orifices of dentinal tubules range from 4.9µm to 6.4 µm (Table 3). The mean values for total number of dentinal tubules and for number of patent dentinal tubules were 39.33±13.34 (Table 4 and 25.13±9.31 (Table 5) respectively. Total mean tubular diameter was 5.61±0.45 (Table 6).

On comparison between **group II (Citric acid) and group III (Minocycline),** itwas observed that results of group II were highly statistically significant than group III in the total number of dentinal tubules exposed, number of patent dentinal tubules and mean tubular diameter (Table 7,8 and 9).

On comparison between **group III (Minocycline HCl) and experimental group IV (EDTA),** it was observed that results of group III were highly statistically significant than group IV in total number of dentinal tubules, number of patent dentinal tubules and mean tubular diameter (Table 7,8 and 9).

Comparison between the **group II (Citric acid) and group IV (EDTA)** showedthat the number of patent dentinal tubules and mean tubular diameter was higher in group II than Group IV but result was statistically insignificant (Table 8 and 9) and total number of dentinal tubules were comparable in both the groups, also the result between these two groups was statistically insignificant(Table 7).

TABLE 1: TOTAL NUMBER OF DENTINAL TUBULES PER SPECIMEN IN THREE GROUPS

EXPERIMENTALGROUP II (CITRIC ACID)	EXPERIMENTAL GROUP III (MINOCYCLINEHCl)	EXPERIMENTAL GROUP IV (EDTA)
63	6	21
22	14	31
44	5	40
24	10	48
38	6	53
48	12	38

27	6	44
37	10	45
25	6	16
27	20	20
18	17	36
54	29	62
30	18	57
55	26	42
37	27	37

TABLE 2: TOTAL NUMBER OF PATENT DENTINAL TUBULES PER SPECIMEN IN THREE GROUPS

EXPERIMENTALGROUP II (CITRIC ACID)	EXPERIMENTALGROUP III (MINOCYCLINE HCl)	EXPERIMENTALGROUP IV (EDTA)
58	4	15
16	8	21
35	3	27
20	6	35
34	4	45
38	7	32
20	4	29
32	6	38
18	3	12
24	10	14

13	9	21
32	16	25
21	9	20
35	11	20
23	12	23

TABLE 3: TUBULAR DIAMETER OF ORIFICES (µm) PER SPECIMEN IN THREE GROUPS

EXPERIMENTALGROUP II (CITRIC ACID)	EXPERIMENTALGROUP III (MINOCYCLINE HCl)	EXPERIMENTALGROUP IV (EDTA)
5.9	2.0	6.0
6.5	2.4	6.0
6.1	2.2	6.3
6.5	2.6	6.4
6.5	2.2	5.7
6.4	2.2	5.5
6.1	2.1	5.7
6.4	1.9	5.2
6.2	2.2	5.9
6.4	3.0	5.6

6.2	3.3	5.5
5.9	2.6	4.9
6.4	2.4	5.3
6.1	3.9	5.1
5.9	2.9	5.1

TABLE 4: MEAN VALUE OF TOTAL NUMBER OF DENTINAL TUBULES

	Mean ± standard deviation
Group II (Citric Acid)	36.60±13.64
Group III (Minocycline HCl)	14.13±8.33
Group IV (EDTA)	39.33±13.34

TABLE 5: MEAN VALUE OF PATENT DENTINAL TUBULES

	Mean ± standard deviation
Group II (Citric Acid)	27.93±11.50
Group III (Minocycline HCl)	7.47±3.76
Group IV (EDTA)	25.13±9.31

TABLE 6: MEAN VALUE OF TUBULE ORIFICES (µM)

	Mean ± standard deviation
Group II (Citric Acid)	6.23±0.23
Group III (Minocycline HCl)	2.53±0.55

Group IV (EDTA)	5.61±0.45

TABLE – 7: COMPARISON OF MEANS OF TOTAL NUMBER OFDENTINAL TUBULES IN THREE GROUPS

		Mean Difference	p-value
Group II (Citric Acid)	Group III (Minocycline HCl)	22.47	0.001*
Group III (Minocycline HCl)	Group IV (EDTA)	25.20	0.001*
Group II (Citric acid)	Group IV (EDTA)	-2.73	1.000

TABLE 8: COMPARISON OF MEANS OF NUMBER OF PATENT DENTINAL TUBULES IN THREE GROUPS

		Mean Difference	p-value
Group II (Citric Acid)	Group III (Minocycline HCl)	20.47	0.001*
Group III (MinocyclineHCl)	Group IV (EDTA)	17.67	0.001*
Group II (Citric acid)	Group IV (EDTA)	2.80	1.000

TABLE 9: COMPARISON OF MEANS OF TUBULAR DIAMETER IN THREE GROUPS

		Mean Difference	p-value
Group II (Citric Acid)	Group III (Minocycline HCl)	3.71	0.001*
Group III (Minocycline HCl)	Group IV (EDTA)	3.09	0.001*
Group II (Citric acid)	Group IV (EDTA)	0.62	0.098

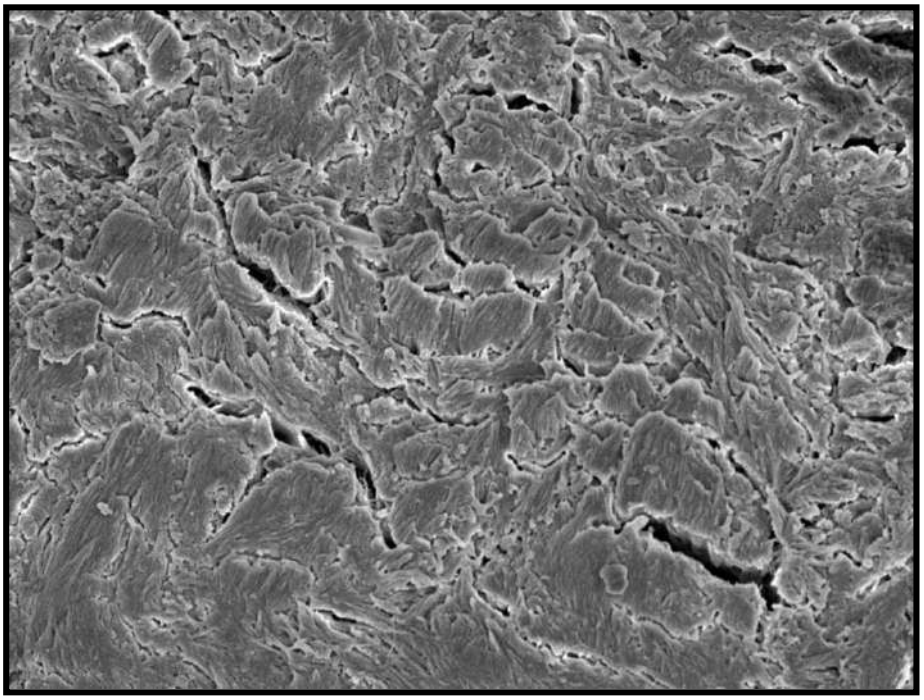

Figure 8: Showing Dentin Specimen treated with saline (Control Group I). The surface is uneven and irregular with considerable debris present (original magnification x3000)

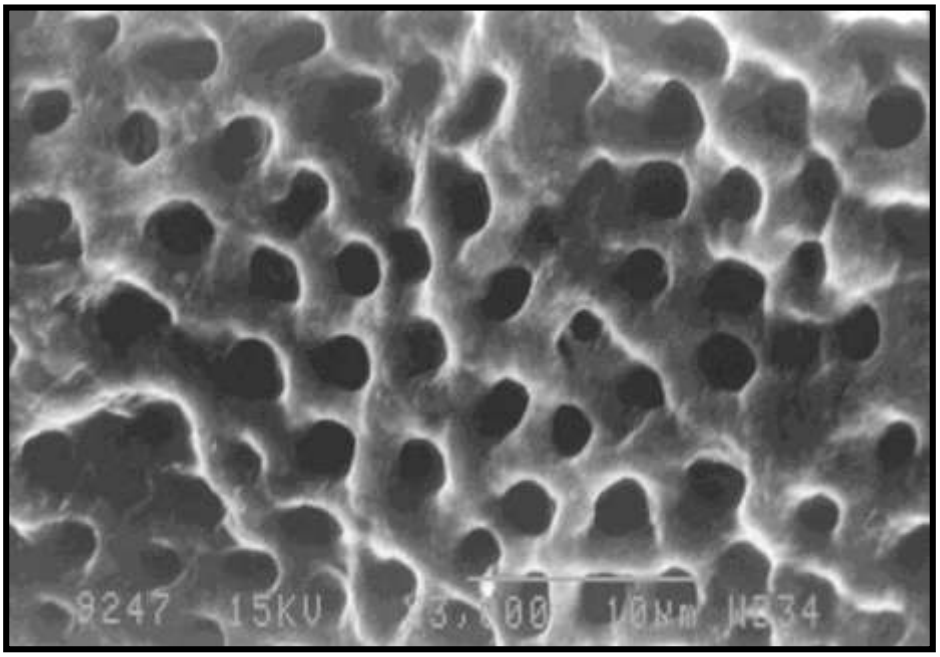

Figure 9: Showing dentin specimen treated with citric acid (Experimental Group II). The surface shows removal of smear layer thus exposing numerous patent dentinal tubules (original magnification x3000)

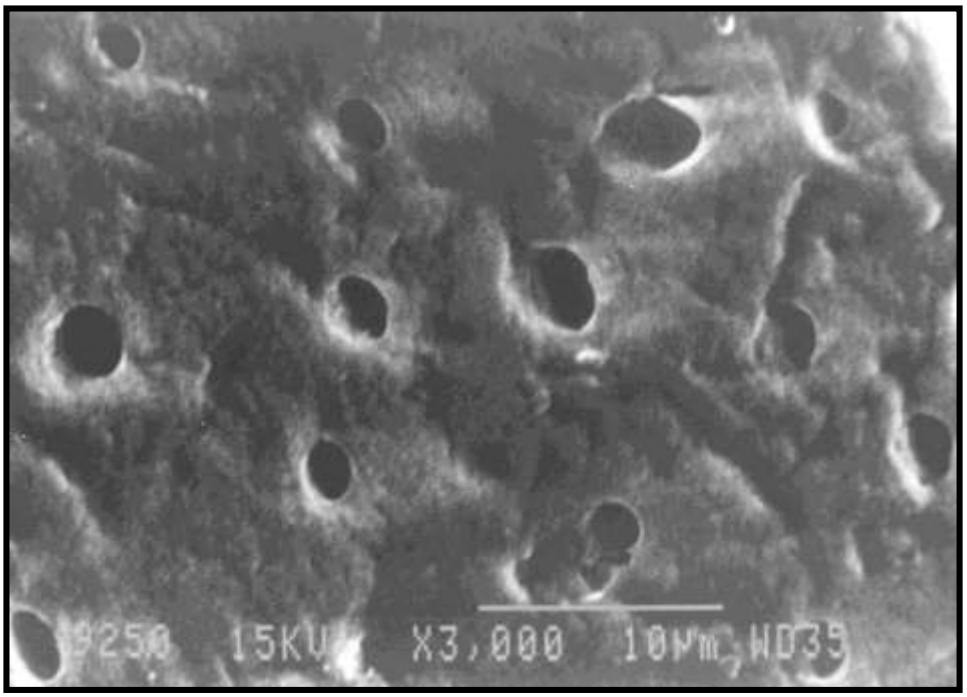

Figure 10: Showing dentin specimen treated with minocycline (experimental Group III). The surface shows few tubular openings, with some openings partially occluded (original magnification x3000)

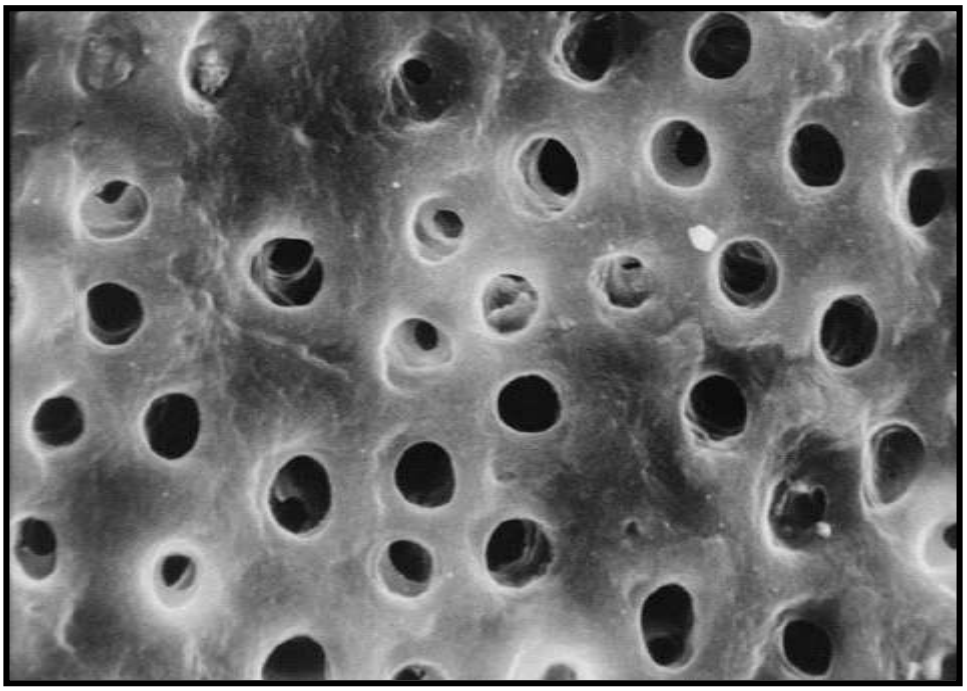

Figure 11: Showing dentin specimen treated with EDTA (experimental Group IV). The surface shows removal of smear layer thus exposing numerous patent dentinal tubules (original magnification x3000)

5. Discussion

In the present study, maxillary and mandibular anterior teeth indicated for extraction due to chronic periodontitis were used. Total of 60 specimen were obtained from the roots of extracted maxillary and mandibular anterior teeth, which were categorized into 4 groups **(**One control group - saline and three experimental groups citric acid, minocycline, EDTA**)** comprising of equally divided specimens in each group.

The teeth used in this study were sectioned near the cementoenamel junction to obtain the experimental surface because the coronal part of the root contains less cementum as compared to its apical part.[6] so it is easy to remove the cementum and obtain a glass-like surface for root conditioning. Instrumentation prior to application of root conditioning agents was done to remove the hypermineralized surface layer present on the periodontitis affected roots.[7]

The observations in the present study indicate that root conditioning with chemical agents as under In control group (saline), the specimens were characterized by an irregular uneven surface which correspond to smear layer. So the observation in this study indicate that mere instrumentation and rinsing with normal saline fail to remove the smear layer. This is in accordance with studies by **Lasho DJ et al (1983)** who reported that scaling/root planing and vigorous scrubing with distilled water and with tooth brush followed by ultrasonic cleaning failed to remove the smear layer.[8] **Polson AM et al (1984) and Wen CR et al (1992)** observed the presence of smear layer on instrumented root surface of periodontally diseased teeth.[9,10] **Garberoglio R et al (1994)** also found the presence of smear layer on the pulpal side of dentin after root canal instrumentation.[11]

Counting the dentinal tubules orifices in saline (control) group was not possible as the root surface was covered by smear layer. Hence, the comparison was made only between the three groups where demineralising agents were used. All three experimental groups showed some difference in the mean of total number of dentinal tubules exposed, number of patent dentinal tubules and in mean tubular diameter.

The observation in the present study indicate that group II (**citric acid**) resulted in the removal of smear layer thus exposing the dentinal tubules in the range of 18-63 with the number of patent dentinal tubules as high as 58 and as low as 13. The mean value for the total number of dentinal tubules was 36.60±13.64. While the mean for the number of patent dentinal tubules was 27.93±11.50. The total mean tubular diameter was 6.23±0.23.

These results are consistent with the findings of **Lasho DJ et al (1983)** according to whom citric acid application increased number of patent dentinal tubules with increased tubular diameter of the tubules orifices.[8]

Results of in vivo studies by **Register AA & Burdick FA (1976)** are also in favour of use of citric acid. They showed that the citric acid demineralization helps to increase clinical attachment level as well as promote cementogenesis by opening and widening of the dentinal tubules and exposing dentinal collagen matrix.[12]

Group III (Minocycline): The third root conditioning agent used was minocycline HCl with pH 4.2, 10%.

The results of group III (minocycline) indicated that the total number of dentinal tubules exposed in the range of 5-29 with number of patent dentinal tubules highest at value 16 and lowest at 3. The mean values for total number of dentinal tubules and for number of patent dentinal tubules were 14.31±8.33 and 7.47±3.76 respectively.

Total mean tubular diameter was 2.53±0.55µm.

Minocycline has been seen to (i) remove the surface inorganic smear layer created on the tooth surface during most dental treatments, (ii) to expose and widen the orifices dentinal tubules.[13] (iii) it also has good anticollagenase, anti-inflammatory activity and high substantivity.[14] **(**(iv) detoxifying effects. (v) enhanced attachment, proliferation of human periodontal ligament cells and can also stimulate the synthesis of dihydrotestosterone in human gingival fibroblasts, thus helping in periodontal regeneration.[4]

Group IV (EDTA): The second root conditioning agent used was EDTA.

Studies have shown that chelating agent (EDTA) working at neutral pH appears preferable with respect to preserving the integrity of exposed collagen fibers, early cell colonization and periodontal wound healing.[5]

The results of group IV (EDTA) indicated that the total number of dentinal tubules exposed in the range of 16-62, with number of patent dentinal tubules highest at value 45 and lowest at 12. The mean values for total number of dentinal tubules and for number of patent dentinal tubules were 39.33±13.34 and 25.13±9.31 respectively. Total mean tubular diameter was 5.61±0.45 µm.

These observations are consistent with the findings of **Lasho DJ et al 1983** according to whom the application of EDTA on instrumented periodontally diseased root surfaces produced numerous patent dentinal tubules with a diameter of 1-3 microns and also exposed collagenous matrix.[8] **Garberoglio R et al (1994)** also reported opened dentinal tubules with EDTA treatment in apical and middle part of the root canal.[11]

In contrast to these results, a study by **Pant V et al (2004)** had shown that EDTA caused a high level of surface cracking with several pits formation and very feeble removal of smear layer and poor opening dentinal tubules.[15]

6. Conclusion

We concluded that root conditioning in all three experimental groups helped removal of smear layer, exposure of dentinal tubules and also the widening of dentinal tubules. Their application as root conditioner may have significant role in periodontal wound healing and future new attachment in-vivo.

7. References

1. Stahl, SS & Froum, SJ 1977, 'Human clinical and histologic repair responses following the use of citric acid in periodontal therapy', *Journal of Periodontology*, 48, pp. 261-266.
2. Lafferty, TA, Gher, ME & Gray, JL 1993, 'Comparative SEM study on the effect of acid etching with tetracycline HCl or citric acid on instrumented Periodontolly involved human root surfaces', *Journal of Periodontology*, 64, pp. 689-693.
3. Jones, WA & O'Leary, TJ 1978, 'The effectiveness of in vivo root planing in removing bacterial endotoxin from the roots of Periodontolly involved teeth', *Journal of Periodontology*, 49, pp. 337-342.
4. Vanheusden, AJ, Goffinet, G, Zahedi, S, Nusgens, B & Rompen, EH1999, 'In vitro stimulation of gingival epithelial cell attachment to dentin by surface conditioning', *Journal of Periodontology*, 70, pp. 594-603.
5. Blomlof, L, Jonsson, B, Blomlof, J & Lindskog, S 2000, ' A clinical study of root surface conditioning with an EDTA gel II. Surgical Periodontol treatment', *International Journal of Periodontics and Restorative*

Dentistry,20, pp. 567-573.

6. Borghetti, A, Mattout, P & Mattout, C 1987, 'How much root planing is necessary to remove the cementum from the root surface', *International Journal of Periodontics and Restorative Dentistry*, 4, pp. 23-29
7. Trombelli, L, Scabbia, A, Zangari, F, Griselli, A & Calura, G 1995, 'Effect of Tetracycline HCl on Periodontally affected human root surfaces', *Journal of Periodontology*, 66, pp. 685-691.
8. Lasho, DJ, O'Leary, TJ & Kafrawy, AH 1983, 'A scanning electron microscope study of the effects of various agents on instrumented Periodontally involved root surfaces', *Journal of Periodontology*, 54, pp. 210-220.
9. Polson, AM, Frederick, GT, Ladenheim, S & Hanes, PJ 1984, 'The production of root surface smear layer by instrumentation and its removal by citric acid', *Journal of Periodontology*, 55, pp. 443-446.
10. Wen, CR, Caffesse, RG, Morrison, EC, Nasjleti, CE & Parikh UK 1992, 'In vitro effects of citric acid application techniques on dentin surfaces', *Journal of Periodontology*, 63, pp. 883-889
11. Garberoglio, R & Becce, C 1994, 'Smear layer removal by root canal irrigants- a scanning electron microscope study', *Oral Surgery, Oral Medicine, Oral Pathology* 78, pp. 359-367.
12. Register, AA & Burdick, FA 1976, 'Accelerated reattachment with cementogenesis to dentin, demineralized in situ II. Defect repair', *Journal of Periodontology*, 47, pp. 497-505.
13. Madison, JG & Hokett, SD 1997, 'The effects of different tetracyclines on the root surface of instrumented, Periodontally involved human teeth: A comparative scanning electron microscope study', *Journal of Periodontology*, 68, pp. 739-745.
14. Demirel, K, Baer, PN & McNamara, TF 1991, 'Topical application of doxycycline on Periodontally involved root surfaces in vitro: Comparative analysis of subtantivity on cementum and dentin', *Journal of Periodontology*, 62, pp. 312-316
15. Pant, V, Dixit, J, Agarwal, AK, Seth, PK & Pant, AB 2004, 'Behavior of Periodontol ligament cells on CO2 Laser irradiated dentinal root surfaces. An in vitro study', *Journal of Periodontal Research*, 39, pp. 373-379.