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Comparative Evaluation of Chemical Root Surface Modifiers Post Root Planning of Periodontally Involved Teeth-An Sem Study

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Abstract

Introduction

The ultimate aim of periodontal treatment is the regeneration of the periodontium in cases previously affected by periodontal disease. For regeneration to occur, it is necessary to eliminate bacterial plaque, calculus and other cytotoxic substances on or within the diseased root surface. To achieve this, various topical root conditioning agents have been used for both detoxification and enhancement of new attachment. Although meticulous root planing by hand instrumentation or by ultrasonic scalers have been advocated for elimination of all toxic substances from the periodontally affected root surfaces, the results have not been consistent and satisfactory at all times. Presently, to enhance the effectiveness of root planing, various physical and chemical root conditioning agents have been tried following root instrumentation, to enhance new attachment. These include laser, citric acid, tetracycline, fibronectin, growth factors, ethylene diamine tetra acetic acid ***(****EDTA****)****, minocycline HCl, phosphoric acid, sodium deoxycholate etc.*

Aim and Objectives

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.

Method

*60 specimens were obtained from the freshly extracted teeth and divided into 4 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 7*5 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 surface were 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

Within the limits of the study, it can be 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.*

Introduction

The ultimate aim of periodontal treatment is regeneration of the periodontium in cases previously affected by periodontal disease **(**Stahl SS, Froum SJ 1977**)**. For regeneration to occur, it is necessary to eliminate bacterial plaque, calculus and other cytotoxic substances on or with in the diseased root surface **(**Lafferty TA et al 1993**)**. 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 **(**Hanes PJ et al 1991**)**. It was suggested that with periodontal therapy one must either remove the toxic materials from the involved cementum or remove the cementum itself **(**Jones WA, O'Leary TJ 1978**)**. 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 (Pant V et al 2004). Scaling and root planing is effective in removing the bacterial deposits and accretions as well as in removing endotoxins from the exposed root surface (Jones WA, O'Leary TJ 1978). However, mechanical instrumentation leaves a smear layer, which is usually comprised of remnants of dental calculus and necrosed root cementum, microorganisms and their products (Pashley DH 1984). This smear layer acts as a barrier for connective tissue attachment to the root surface and can serve as a reservoir for microbial growth (Hanes PJ, Polson AM, Frederick GT 1988).

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 (Lowenguth RA, Blieden TM 2000).

Root conditioning has been recommended as an adjunct to mechanical root surface debridement to remove smear layer (Lasho DJ, O'Leary TJ, Kafraway AH 1983) and root associated endotoxin (Daly CG 1982) and to expose collagen fibers (Garrett JS, Crigger M, Egelberg J 1978) on the dentin surface. The root conditioning agents causes an increase in tubule diameter which may increase the surface area and the amount of exposed collagen available for new attachment (Register AA & Burdick FA 1976).

Presently to enhance the effectiveness of root planing, various physical (laser) (Pant V et al 2004) and chemical root conditioning agents (citric acid, phosphoric acid, tetracycline hydrochloride, doxycycline hydrochloride, fibronectin, ethylene diamine tetraacetic acid (EDTA), minocycline hydrochloride, sodium deoxycholate etc.) have been tried following root instrumentation, to enhance new attachment.

It has been suggested that following the removal of periodontally involved cementum by root planing and then citric acid demineralization of underlying dentin may enhance new connective tissue attachment by either accelerating cementogenesis (Register AA & Burdick FA 1976) exposing collagen fibrils in the dentin matrix (Garrett JS, Crigger M & Egelberg J 1978) or providing an optimal substrate for cell attachment (Boyko GA, Brunett DM & Melcher AH 1980).

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 **(**Thomas BH et al 1999**)**. Minocycline Hcl can promote the attachment and proliferation of human periodontal ligament cells **(**Rompen EH, Goffinet GH & Nusgens B 1999**)** and can also stimulate the synthesis of dihydrotestosterone in the human gingival fibroblasts **(**Vanheusden AJ et al 1999**)**, 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 **(**Blomlof J et 2000**)**

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 diseased root surfaces, under scanning electron microscope **(**SEM**)**.

Aim

The aim of present study is 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.

Objectives

1. To evaluate number of dentinal tubules exposed per 100 µm2 using different root conditioning agents.
2. To evaluate and compare the diameter of dentinal tubules after using different root conditioning agents.

Materials & Methodology

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.

Criteria For Selection of Teeth

1. Absence of attrition, abrasion or erosion.
2. Absence of root caries or root fracture and teeth without any restoration on any aspect.
3. Absence of internal and external root resorption.
4. Absence of endodontic treatment.
5. Minimal instrumentation during extraction.

Armamentarium (Figure 1**)**

- Dentin block
- Mouth Mask
- Gloves
- Micromotor hand piece
- Air rotor
- Finishing bur
- Straight bur
- Protective eye wear
- Gracey curette **(**No. 1/2**)**
- Double sided diamond disc
- Tweezer
- Dappen dish
- Cotton pellets
- Digital caliper
- pH meter
- Syringe
- Gold sputtering unit
- Scanning Electron Microscope

Solutions used for preparation of specimens

- Alcohol **(**40%, 70%, 90%, 100% alcohol**)**
- Hydrochloric acid
- Distilled water

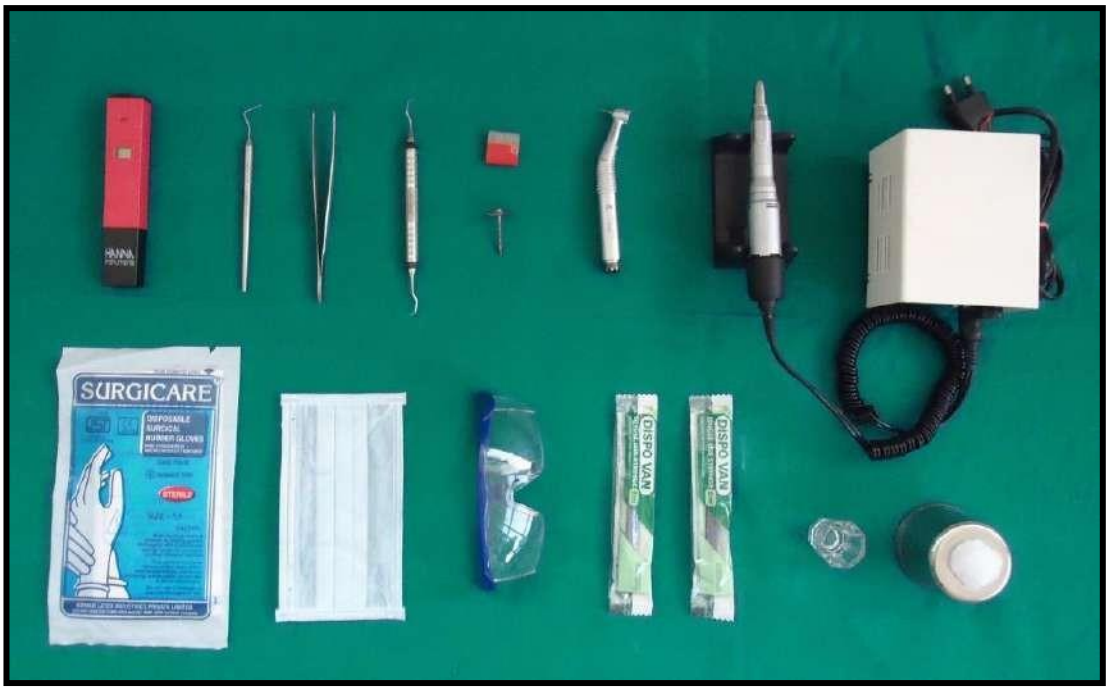


Figure 1: Armamentarium

Root conditioning agents (Figure 2**)**

Solutions of

- Citric acid
- Minocycline Hcl
- EDTA **(**Gel**)**

Preparation of citric acid solution: 10 gms of citric acid was dissolved in 100 ml of distilled water and hydrochloric acid was added drop by drop to adjust the pH at 1 which was checked using the pH meter.

EDTA Gel – Commerically available EDTA gel preparation **(**Neutral pH**)** was used.

Preparation of minocycline solution: 2 gms of Minocycline HCl was dissolved in 20 ml of distilled water to obtain minocycline HCl solution at pH 4.2 and was checked using pH meter.

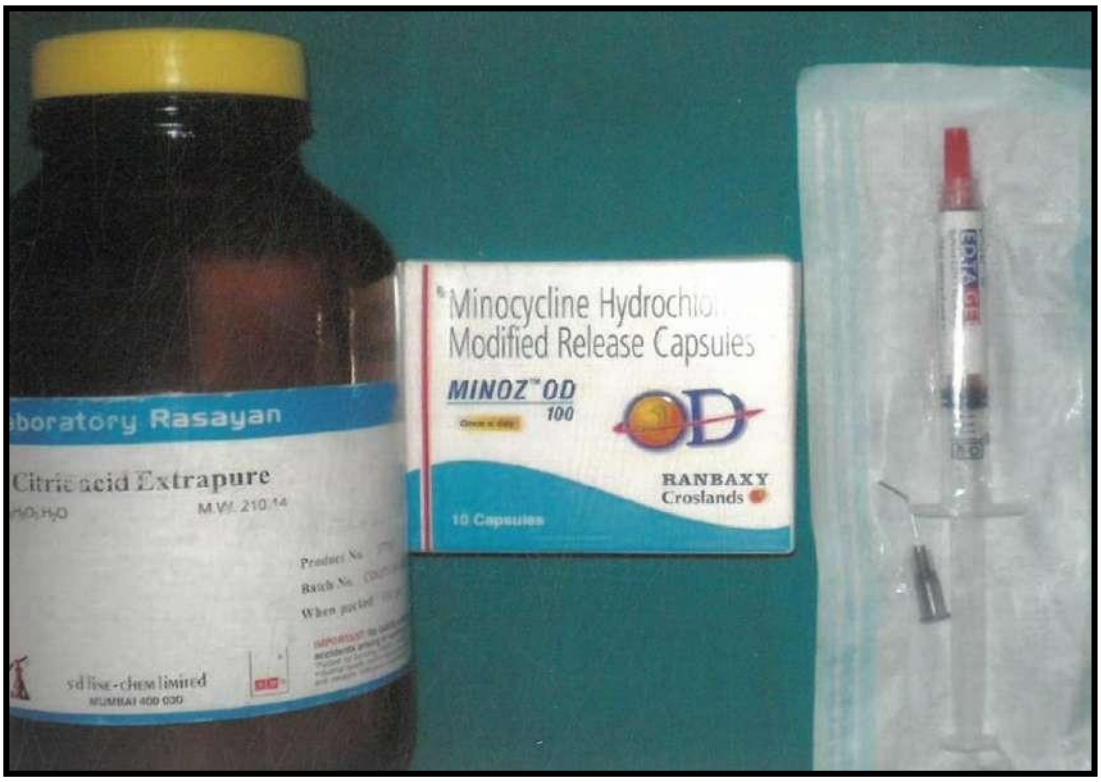


Figure 2: Showing root conditioning agents **(**citric acid, minocycline HCl and EDTA**)**

Method

Preparation of specimen **(**Figure 3**)**

Sectioning- A total of 60 samples were prepared. All tooth cuts were made with a double sided diamond disk in a slow speed hand piece under copious water irrigation. To obtain an experimental surface, the crown of each tooth at the level of cemento-enamel junction **(**CEJ**)** and apical third of each root was removed and the remaining root was sectioned longitudinally through the root canal to produce a 7×5mm tooth section using digital caliper **(**Figure 4**)**. The dentin root surface was then instrumented by using a sharp Gracey 1-2 periodontal curette to achieve a smooth glass like surface. These dentin specimens were then washed with and stored in distilled water until the time of treatment.

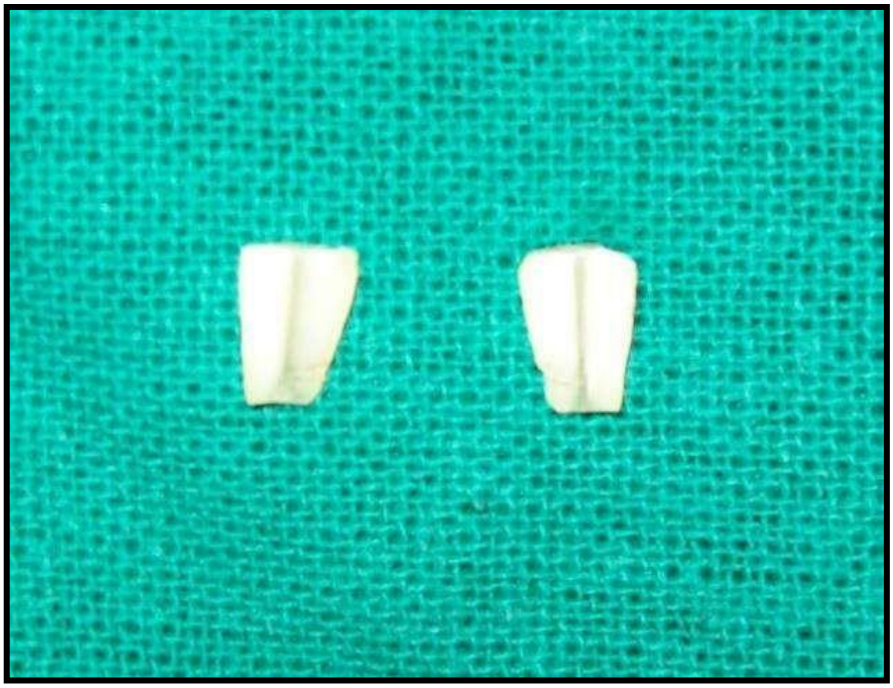


Figure 3: Sectioned dentin specimens being used for the study

Figure 4: Showing Digital Calliper

A total number of 60 specimens were prepared from extracted teeth which were randomly equally 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 3 minutes.

Group IV: Dentin specimens treated with EDTA gel **(**pH Neutral, 10 %**)** for 3 minutes.

Application of the Solutions and Gel

For all the three experimental groups and one control group mentioned aforehand, the method of application of their respective solutions or gel was same as given below:

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. To ensure a steady concentration of particular solution or gel, the saturated cotton pellets were changed every 20 seconds for a total period of 3 minutes. After 3 minutes the treated specimens were immediately rinsed with distilled water for 1 minute and again preserved in distilled water.

Preparation for Sem

All the specimens were fixed in 10 % Formalin for 48 hours and then these specimens were dehydrated in an ascending concentration of aqueous alcohol solutions in the following manner.

- 40% Alcohol for 5 Minutes
- 70% Alcohol for 5 Minutes
- 90% Alcohol for 5 Minutes
- Absolute Alcohol for 15 Minutes

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 surface characteristics of root surfaces were evaluated descriptively, concerning the removal of smear layer, number of patent tubules out of total number and their diameter. Scanning

photomicrographs were taken from the center of test area at x3000 magnification. Images were recorded on to black and white photographic film via camera linked to SEM.The micrographs were at appropriate magnification and were evaluated for:

1. Number of dentinal tubules. From the total number of tubules counted, number of patent dentinal tubules exposed per 100 µm2 were counted using different root conditioning agents.
2. The diameter of dentinal tubules was measured using digital caliper with 0.03 mm accuracy.

The data so obtained was compiled and subjected to statistical analysis. Intergroup comparison of treatment was made with the help of one way Anova test.

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**)**

Results

The present scanning electron microscopic (SEM) study was designed to compare the efficacy of topical application of citric acid, minocycline and ethylenediaminetetraacetic acid (EDTA) on periodontally diseased root surfaces as root conditioning agents.

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. 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.

The photomicrographs taken at appropriate magnification and were evaluated for:

I. Total number of dentinal tubules present per test area.
II. Number of patent dentinal tubules from the total number of tubules present.
III. Diameter of dentinal tubules.

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.

Statistical 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, Graph 1) with the number of patent dentinal tubules as high as 58 and as low as 13 (Table 2, Graph 2). Tubular diameter of orifices of dentinal tubules range from 5.9µm to 6.5 µm (Table 3, Graph 3). The mean value for the total number of dentinal

tubules was 36.60±13.64 **(**Table 4, Graph 4**)**. While the mean value of total number of patent dentinal tubules was 27.93±11.50 **(**Table 5, Graph 5**)**. The total mean tubular diameter was 6.23±0.23 **(**Table 6, Graph 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, Graph 1**)** with number of patent dentinal tubules highest at value 16 and lowest at 3 **(**Table 2, Graph 2**)**. Tubular diameter of orifices of dentinal tubules range from 1.9µm to 3.9 µm **(**Table 3, Graph 3**)**.The mean values for total number of dentinal tubules and for number of patent dentinal tubules were 14.31±8.33 **(**Table 4, Graph 4**)** and 7.47±3.76 **(**Table 5, Graph 5**)** respectively. Total mean tubular diameter was 2.53±0.55 **(**Table 6, Graph 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, Graph 1**)**, with number of patent dentinal tubules highest at value 45 and lowest at 12 **(**Table 2, Graph 2**)**. Tubular diameter of orifices of dentinal tubules range from 4.9µm to 6.4 µm **(**Table 3, Graph 3**)**.The mean values for total number of dentinal tubules and for number of patent dentinal tubules were 39.33±13.34 **(**Table 4, Graph 4**)** and 25.13±9.31 **(**Table 5, Graph 5**)** respectively. Total mean tubular diameter was 5.61±0.45 **(**Table 6, Graph 6**)**.

On comparison between group II **(**Citric acid**)** and group III **(**Minocycline**)**, it was 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**)** showed that 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**)**.

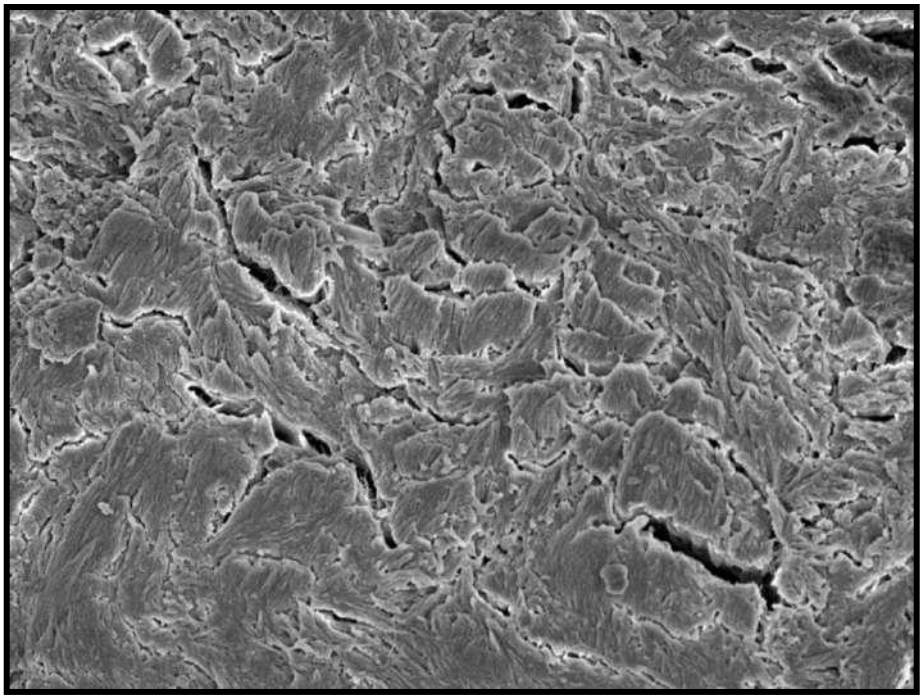


Figure 8: Showing Dentin Specimen treated with saline **(**Control Group I**).** The surface is uneven and irregular with considerable debris present **(**original magnification x3000**)**

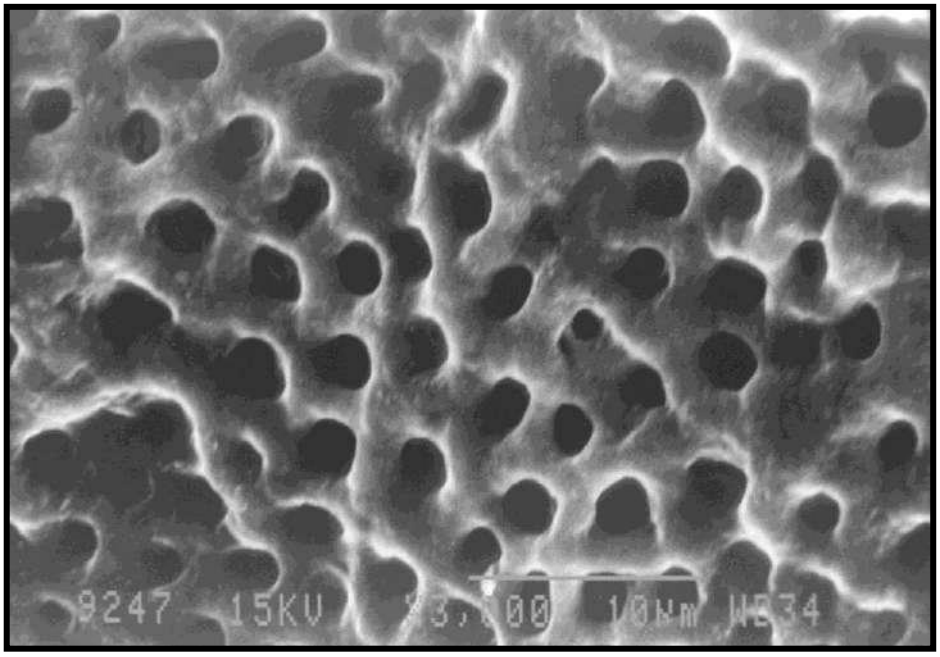


Figure 9: Showing dentin specimen treated with citric acid **(**Experimental Group II**).** The surface shows removal of smear layer thus exposing numerous patent dentinal tubules **(**original magnification x3000**)**

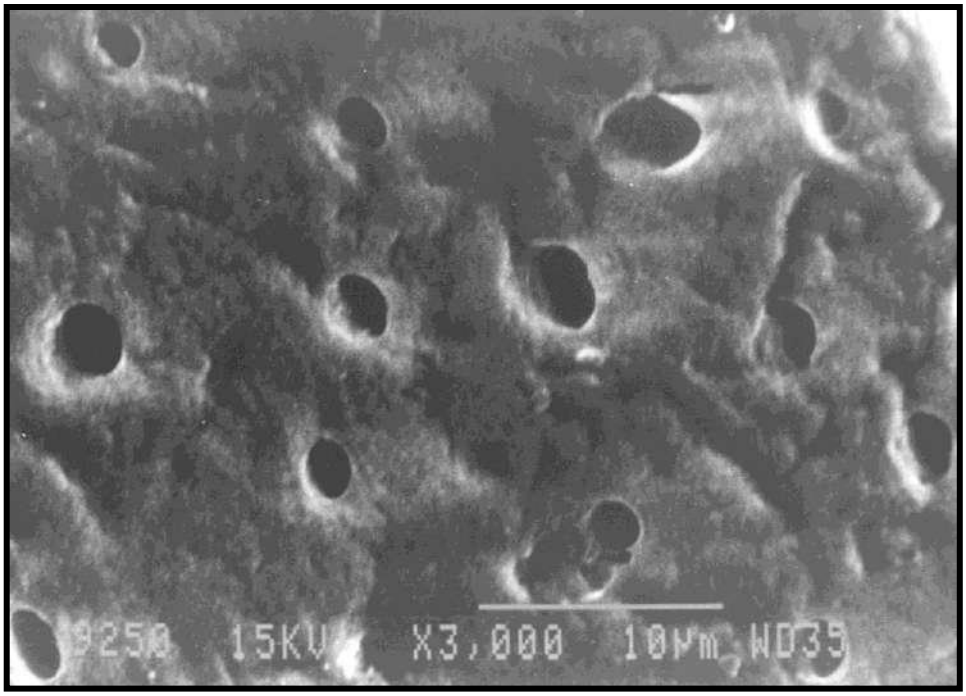


Figure 10: Showing dentin specimen treated with minocycline **(**experimental Group III**)**. The surface shows few tubular openings, with some openings partially occluded **(**original magnification x3000**)**

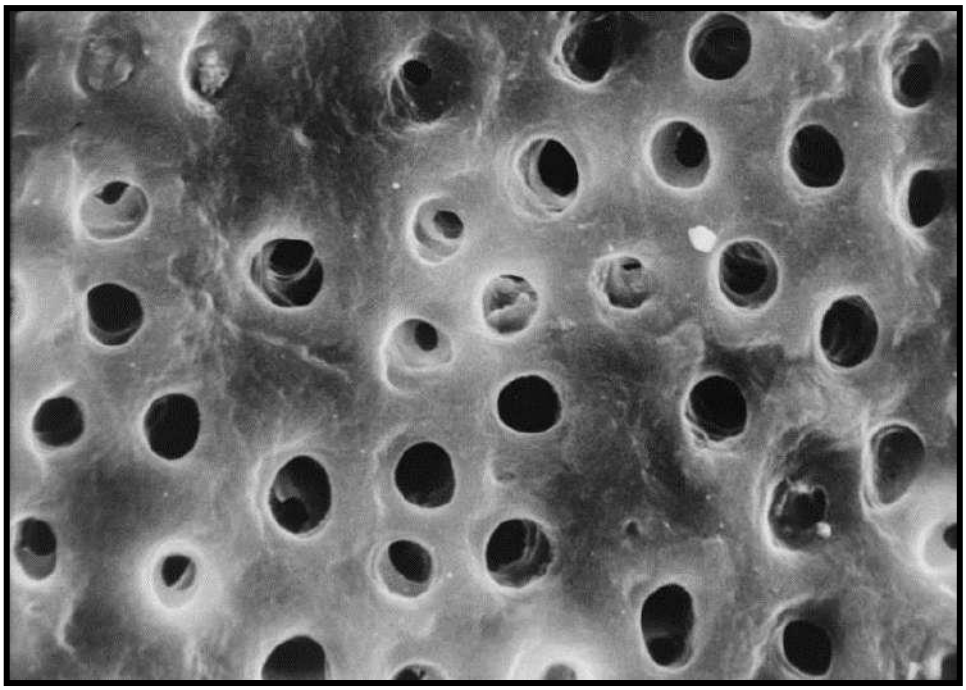
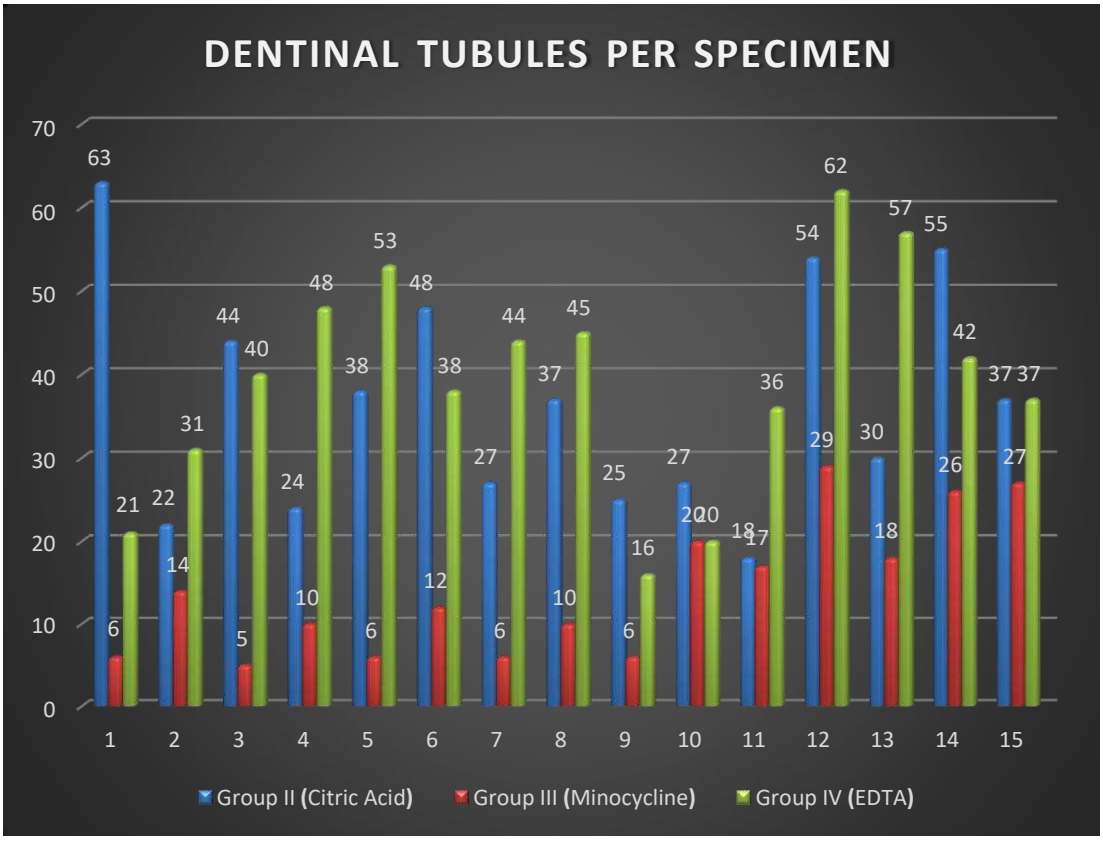


Figure 11: Showing dentin specimen treated with EDTA **(**experimental Group IV**)**. The surface shows removal of smear layer thus exposing numerous patent dentinal tubules **(**original magnification x3000**)**

S. NO	Experimental Group II (CITRIC ACID)	Experimental Group III (Minocycline HCl)	Experimental Group IV (EDTA)
1.	63	6	21
2.	22	14	31
3.	44	5	40
4.	24	10	48
5.	38	6	53
6.	48	12	38
7.	27	6	44
8.	37	10	45
9.	25	6	16
10.	27	20	20
11.	18	17	36
12.	54	29	62
13.	30	18	57
14.	55	26	42
15.	37	27	37

Table 1: Total Number of Dentinal Tubules Per Specimen in Three Groups

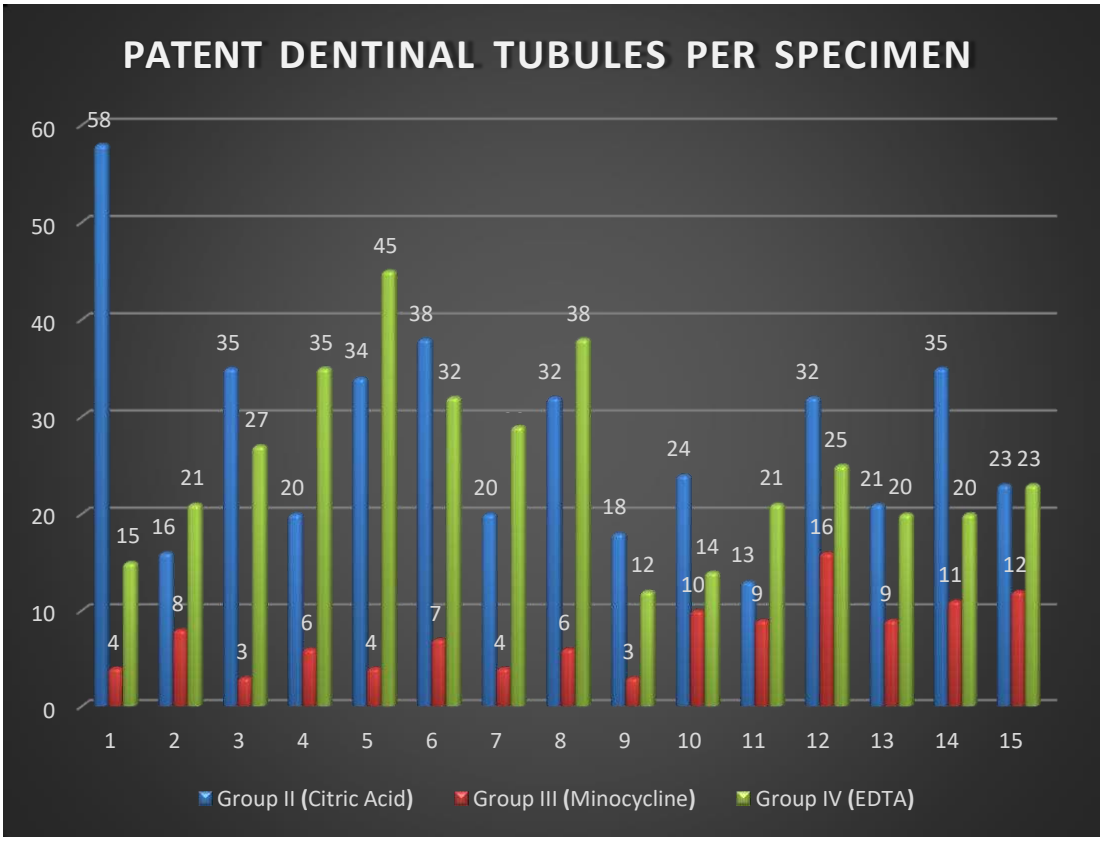


Graph 1: Total Number of Dentinal Tubules Per Specimen In Three Groups

S. NO	EXPERIMENTAL GROUP II (CITRIC ACID)	EXPERIMENTAL GROUP III (MINOCYCLINEHCl)	EXPERIMENTAL GROUP IV (EDTA)
1	58	4	15
2	16	8	21
3	35	3	27
4	20	6	35
5	34	4	45
6	38	7	32
7	20	4	29
8	32	6	38

9	18	3	12
10	24	10	14
11	13	9	21
12	32	16	25
13	21	9	20
14	35	11	20
15	23	12	23

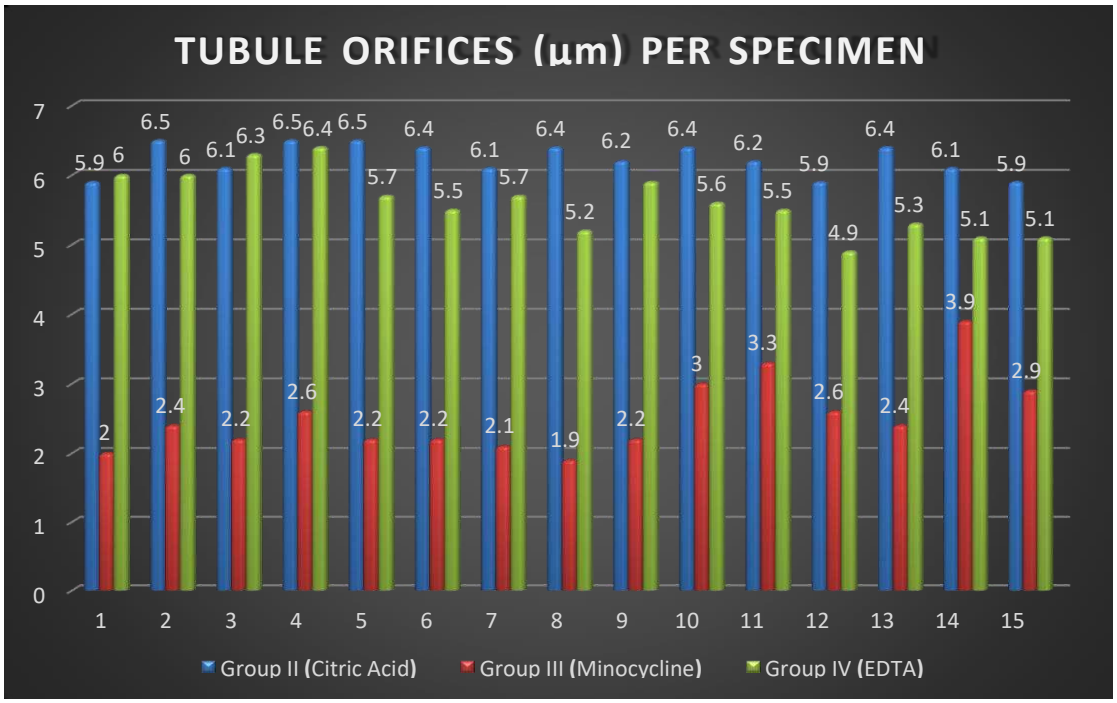
Table 2: Total Number of Patent Dentinal Tubules Per Specimen in Three Groups



Graph 2: Total Number of Patent Dentinal Tubules Per Specimen in Three Groups

S. NO	EXPERIMENTAL GROUP II (CITRIC ACID)	EXPERIMENTAL GROUP III (MINOCYCLINE)	EXPERIMENTAL GROUP IV (EDTA)
1	5.9	2.0	6.0
2	6.5	2.4	6.0
3	6.1	2.2	6.3
4	6.5	2.6	6.4
5	6.5	2.2	5.7
6	6.4	2.2	5.5
7	6.1	2.1	5.7
8	6.4	1.9	5.2
9	6.2	2.2	5.9
10	6.4	3.0	5.6
11	6.2	3.3	5.5
12	5.9	2.6	4.9
13	6.4	2.4	5.3
14	6.1	3.9	5.1
15	5.9	2.9	5.1

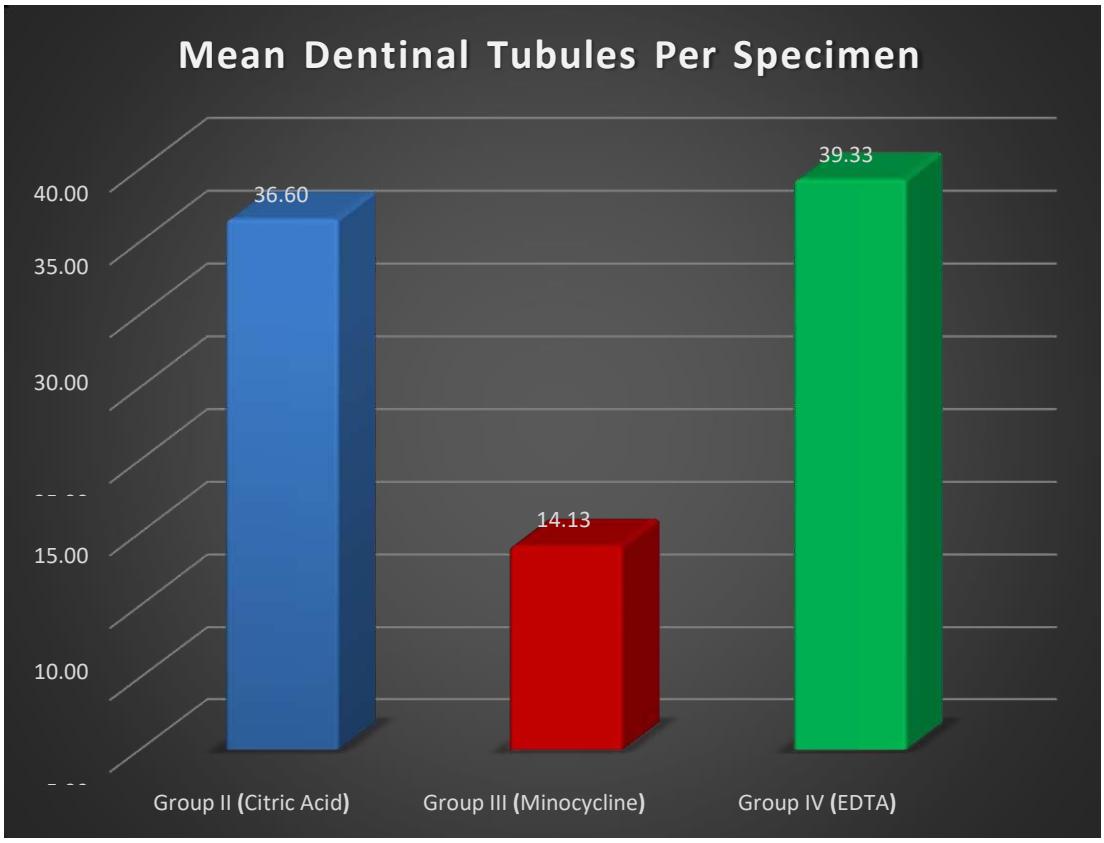
Table 3: Tubular Diameter OF Orifices **(**µM**)** Per Specimen in Three Groups



Graph 3: Tubular Diameter of Orifices **(**µm**)** per Specimen in Three Groups

	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

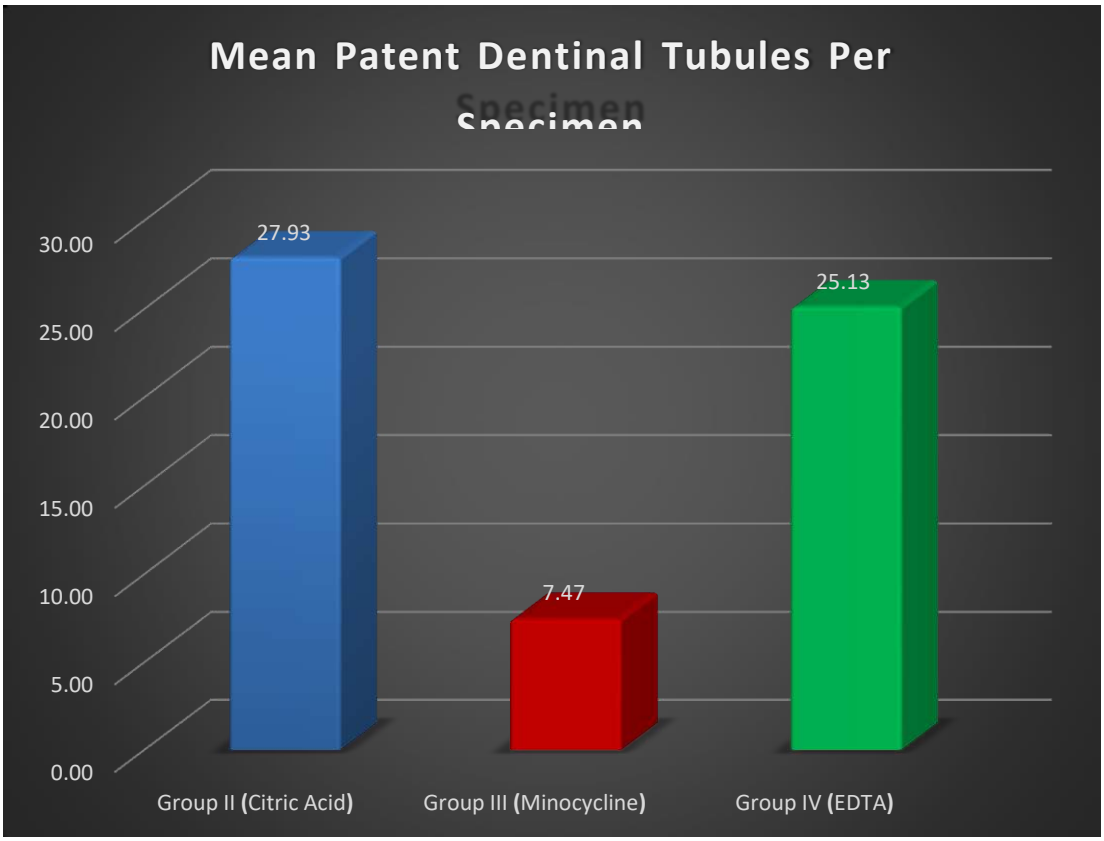
Table 4: Mean Value of Total Number of Dentinal Tubules



Graph 4: Mean value of total number of 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

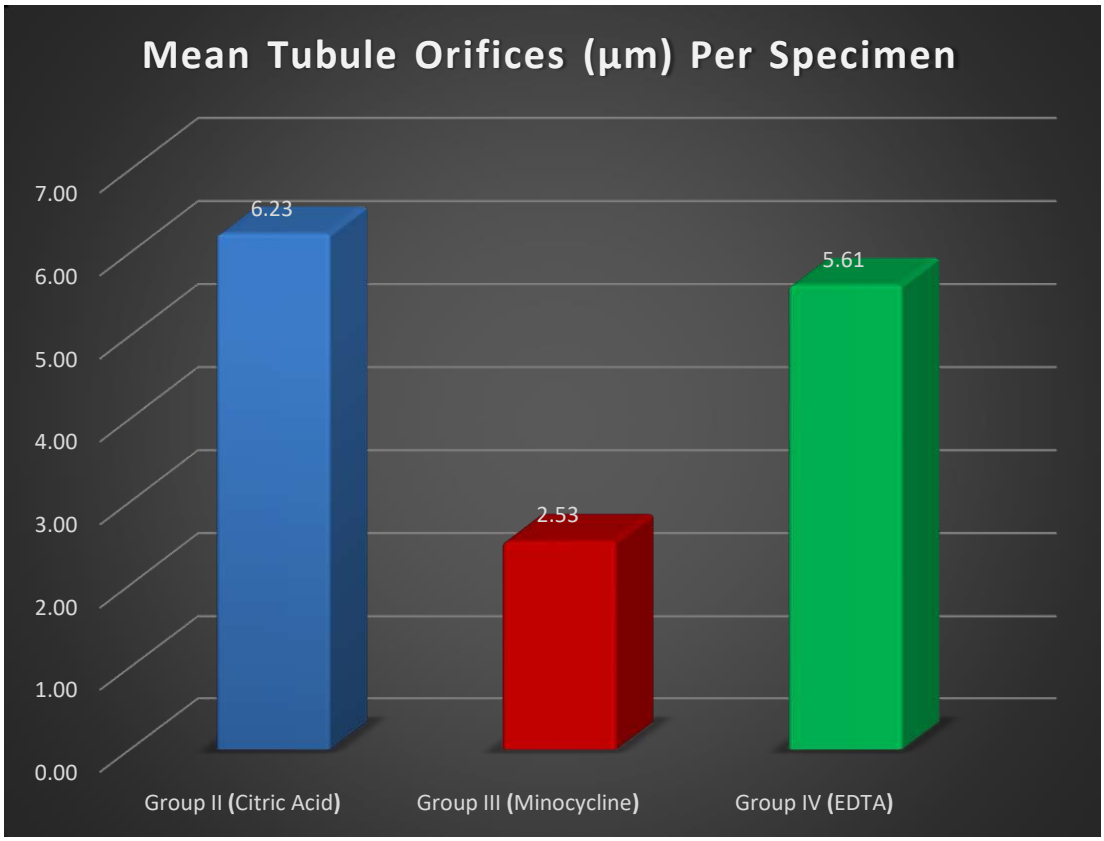
Table 5: Mean Value of Patent Dentinal Tubules



Graph 5: Mean Value of Patent Dentinal Tubules

	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 6: Mean Value of Tubule Orifices (µM)



Graph 6: Mean Value of Tubule Orifices (µm)

		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 – 7: Comparison of Means of Total Number of Dentinal Tubules in Three Groups

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

**** Statistically significant***

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)	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

**** Statistically significant***

Table - 9: Comparison of Means of Tubular Diameter in Three Groups

Discussion

The ultimate aim of periodontal therapy is regeneration of periodontium in cases affected by periodontal disease. Cementum surfaces exposed by periodontitis are pathologically altered, contaminated by bacterial endotoxins and have been shown to be higher in mineral content than normal root surfaces and having a higher content of calcium, phosphorus and fluoride **(**Hanes PJ et al 1991**)**. Cementum surface contaminants inhibit growth and viability of fibroblasts in vitro and may prevent new connective tissue attachment **(**Polson AM et al 1984, Hanes PJ et al 1988, 1991**)**.

The traditional treatment of pathologically altered root surfaces has relied on mechanical removal of plaque and calculus, root-bound toxins, and "contaminated"/diseased cementum. Curettes and ultrasonic scalers have been the primary instruments used to accomplish these goals **(**Labahn R et al 1992**)**, but it is not possible to decontaminate a periodontitis-affected root surface completely by mechanical means alone. The instrumented surface will inevitably be covered by a smear layer following root planing. This smear layer contains remnants of dental calculus, contaminated root cementum, micro-organisms, saliva, water and subgingival plaque **(**Blomlof JPS et al 1996**)**. It is thought to serve as a physical barrier between the periodontal tissues and the root surface and may inhibit the formation of new connective tissue attachment to the root surface **(**Hanes PJ et al 1991**)**.

Various chemical treatments of root surface have been suggested as method to detoxify the root surface or to demineralize the root surface. Demineralization of the root surface removes the smear layer, uncovers and widens the orifices of dentinal tubules **(** Lasho DJ et al in 1983, Polson AM et al in 1984, Wen CR et al 1992**)** and exposes the dentinal collagen matrix **(**Garrett S et al in 1978**)**. This collagen matrix is thought to provide a substrate which supports the chemotaxis, migration and attachment of those cells involved in wound healing and formation of new connective tissue attachment **(**Polson AM et al in 1984, Hanes PJ et al 1991**)**.

Considering the above findings, an effort was made in this study to compare the surface characteristics of diseased root surfaces after application of citric acid, ethylenediaminetetraacetic acid **(**EDTA**)** and minocycline as root conditioning agents using scanning electron microscopy.

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 (Borghetti A et al 1987) 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 (Trombelli L et al 1995).

Passive application was preferred over burnishing technique as the latter may itself form smear layer which partially or completely obliterate the dentinal tubule opening (Wen CR et al 1992).

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. 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. Garberoglio R et al (1994) also found the presence of smear layer on the pulpal side of dentin after root canal instrumentation.

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 demineralizing 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.

Group II (Citric acid) with pH 1 has been extensively used as root conditioning agent. It has been shown to (I) remove the smear layer, uncover and widen the orifices of the dentinal tubules (Lasho DJ et al 1983, Wen CR et al 1992), (II) Induce Cementogenesis (Register AA & Burdick FA 1975)

(III) promote collagen splicing (Garrett S et al 197831), (IV) Augment fibronectin-fibrin-collagen binding thereby inhibiting the epithelial apical migration (Polson AM & Proye MP 1982), (V) Enhance fibroblast chemotaxis, migration and attachment (Boyko GA et al 1980) (VI) Has antibacterial property (Daly CG 1982)

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), Labahn R et al (1992), Sterret JD et al (1993) and Wen CR et al (1992) according to whom citric acid application increased number of patent dentinal tubules with increased tubular diameter of the tubules orifices.

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.

However, the results of in vivo study by Nyman S et al (1981) failed to prove any beneficial effects of citric acid on periodontal healing. Speculative explanations for these inconsistent findings have included variation in animal models (Polson AM & Proye MP 1982), inconsistent flap adaptation (Polson AM & Proye MP 1982), inadequate demineralization of periodontitis affected root surfaces (Garrett S et al 1978, Hanes PJ et al 1989) and repopulation of the root surface with inappropriate cell types (Melcher A 1976, Nyman S et al 1981).

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 (Madison JG et al 1997) (iii) it also has good anticollagenase, anti-inflammatory activity and high substantivity (Demirel K et al 1991) (iv) detoxifying effects (Minabe M et al 1994) (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 (Rompen EH et al 1999, Vanheusden AJ et al 1999).

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 (Blomlof J et al 1995, 1996, 2000)

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, Blomlof J et al 1996 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. Garberoglio R et al (1994) also reported opened dentinal tubules with EDTA treatment in apical and middle part of the root canal. Blomlof J et al (1996) also observed that EDTA had profoundly higher capacity to selectively expose collagen fibers.

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.

On comparison between group II (Citric acid) and group III (Minocycline), it was observed that results of group II were highly statistically significant than group III in the total number of dentinal tubules exposed ($p<0.001$), number of patent dentinal tubules ($p<0.001$) and mean tubular diameter (<0.001). This difference was probably due to the lower pH of citric acid (pH=1) as compared to minocycline (pH=4.2) so a higher concentration of minocycline may be required to achieve the comparable results. These findings are in accordance with studies of Madison JG et al (1997). However studies by Hanes et al (1991) and Madison et al (1997) have shown that using a higher concentration may lead to crystals precipitation out of the solution.

On Comparison between the group II (Citric acid) and group IV (EDTA) showed that the number of patent dentinal tubules and mean tubular diameter were higher in group II but the result was statistically nonsignificant ($p=1.000$). Total number of dentinal tubules was comparable between two groups and result was statistically insignificant. Although citric acid is acting at a lower pH as compared to EDTA but the results between the two are comparable because EDTA is a chelating agent and forms the stable complex with calcium, and dentin demineralization by EDTA is best at neutral or alkaline pH. These results are consistent with findings of Lasho DJ et al (1983). They reported that citric acid as well as

EDTA treated specimens resulted in removal of smear layer leading to numerous patent dentinal tubules with an orifice diameter of approximately 2-3 µm.

On comparison between group III **(**Minocycline HCl**)** and group IV **(**EDTA**)**, it was observed that results of group IV were highly statistically significant than group III both in total number of dentinal tubules, number of patent dentinal tubules and mean tubular diameter **(**$p<0.001$**)**. Although minocycline is acting at lower pH as compared to EDTA but results of EDTA are significantly better than minocycline because EDTA acts best at neutral pH and a higher concentration of minocycline may be required to achieve the comparable results.

In the present study, it was observed 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. However, the results of present are 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.

Difference between the results of the present study and those of other studies may be related to the disease status of the dentin specimen utilized, concentration, time and mode of application of the demineralizing agent or a combination of these variables. Hence, additional studies both in-vitro and in- vivo of these variables with better standardization are needed.

Summary and Conclusion

The present in vitro study was undertaken to assess the effects of various conditioning agents on surface topography of the instrumented diseased root surfaces of periodontally involved teeth.

In this study maxillary and mandibular teeth indicated for extraction were selected and total of 60 specimens were obtained from the roots of extracted teeth. These 60 specimens were equally divided into four groups.

After scaling and root planing, sectioning of teeth was done first at cementoenamel junction and then longitudinally to size 7mm×5mm. The respective solutions were applied to the experimental specimens. These specimens were then processed and evaluated under scanning electron microscope at ×3000 magnification. Chemical solutions employed for experimental groups were citric acid, minocycline HCl and EDTA whereas normal saline was applied for control specimens. Presence or

lack of smear layer, total number of dentinal tubules, number of patent dentinal tubules, diameter of exposed tubule orifices were evaluated and following conclusions were drawn.

In control group **(**saline**)** an irregular uneven surface was seen which corresponds to smear layer. So can be concluded that hand instrumentation alone followed by normal saline treatment is incapable of removing the smear layer or opening of dentinal tubules.

Root conditioning agents **(**citric acid, minocycline HCl and EDTA**)** in all the three experimental groups were effective in removing the smear layer, exposing and widening the dentinal tubules orifices.

On comparison between experimental group II **(**Citric acid**)** and experimental group III **(**Minocycline**)**, it was 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.

Comparison between the experimental group II **(**Citric acid**)** and experimental group IV **(**EDTA**)** showed that the number of patent dentinal tubules and mean tubular diameter were higher in group II than Group IV but the result were statistically insignificant. Total number of tubules were comparable between two groups though the results between these two groups were statistically non-significant.

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

Out of all the three root conditioning agents, the results of citric acid were better than minocycline HCl **(**highly significant**)** and EDTA **(**Non-significant**)**

Within the limits of the study, it can be 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.

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