

# Comparison of the efficacy of ethylenediaminetetraacetic acid and tetracycline hydrochloride as root conditioning agents: An *in vitro* study

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## ABSTRACT

**Objective:** Root surfaces of periodontitis-affected teeth are hypermineralized and contaminated with cytotoxic and other biologically active substances. Various root conditioning agents have been recommended as an adjunct to mechanical root surface debridement to expose dentin collagen and cementum bound proteins. The aim of the present *in vitro* study was to compare the efficacy of ethylenediaminetetraacetic acid (EDTA) and tetracycline hydrochloride (HCl) as root conditioning agents on planed root surfaces. **Materials and Methods:** A total of 20 human maxillary anterior teeth indicated for extraction due to chronic periodontitis were collected and root planed. The teeth were sectioned and specimens were divided into two groups – Group I and II. Group I dentin specimens were treated with EDTA and Group II specimens were treated with tetracycline HCl solution at a concentration of 10% by active burnishing technique for 3 min. The root surface samples were then examined by scanning electron microscope. **Results:** The results of this study showed that EDTA and tetracycline HCl were equally effective in removing the smear layer. It was observed that the total and patent dentinal tubules were more in number in teeth treated with tetracycline when compared to EDTA group. However, EDTA was found to be much more effective as root conditioning agent because it enlarged the diameter of dentinal tubules more than that of tetracycline HCl. **Conclusion:** The results of *in vitro* study showed that both the agents are good root conditioning agents if applied in addition to periodontal therapy. However, further studies are required to establish the *in vivo* importance of EDTA and tetracycline HCL as root conditioners.

**Key words:** Ethylenediaminetetraacetic acid, root conditioning, scanning electron microscope, tetracycline hydrochloride

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## INTRODUCTION

The primary etiological factor in periodontal disease is bacterial plaque. This results in an inflammatory lesion in the gingival tissues leading to progressive destruction of the supporting periodontal tissues. Periodontitis-affected root surfaces are hypermineralized and contaminated with cytotoxic and other biologically active substances.<sup>[1]</sup>

The main objective of periodontal therapy is the restoration of the lost periodontium and conversion of the periodontally

affected root surfaces into a substrate, which is biologically hospitable for epithelial and connective tissue cell adherence and attachment.<sup>[2]</sup> Methods to achieve this objective include scaling and root planning, as well as treatment of denuded root surfaces with various chemicals and antimicrobial agents.<sup>[3-5]</sup> It is not possible to decontaminate a periodontitis-affected root surface completely by mechanical means alone mainly because the bacterial toxins are not completely eliminated from the root surface and the instrumented surface will inevitably be covered by a smear layer, which contains remnants of dental calculus, contaminated cementum and subgingival plaque, which acts as a physical barrier between periodontal tissues and root surfaces and thus inhibiting the formation of new attachment.<sup>[1,3,6]</sup>

Various physical (laser) and chemical root conditioning agents (citric acid, phosphoric acid, tetracycline hydrochloride [HCl],

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doxycycline HCl, fibronectin, ethylenediaminetetraacetic acid [EDTA], minocycline HCl, hydrochloric acid, Cohn's factor, sodium deoxycholate etc.) have been tried following root instrumentation, to enhance new attachment.<sup>[7-13]</sup> These agents expose dentin collagen and cementum bound proteins and have been found to elute retained toxins from the altered root surfaces.<sup>[14]</sup> Such treatment enlarges dentinal tubules into which healing connective tissue can enter.<sup>[15]</sup> Thus, root conditioning has been recommended as an adjunct to mechanical root surface debridement.

EDTA has been extensively tried as a root conditioning agent. It appears to promote early cell tissue colonization by promoting a more biocompatible surface for cell and tissue attachment.<sup>[16]</sup> EDTA seems preferable to citric or phosphoric acid because EDTA will selectively remove hydroxyapatite, leaving most of the collagenous matrix intact, whereas cleaning with conventional etching agents such as citric or phosphoric acids will, in addition to dissolving the mineral, also dissolve the collagenous matrix.

The tetracyclines are broad spectrum antimicrobials that are used for root conditioning. When applied on root-planed dentin, tetracycline removed the smear layer, exposed the dentinal tubules and produced a fibrillar surface.<sup>[2,15,17,18]</sup> Tetracycline HCl demineralized dentin has been shown to demonstrate substantivity.<sup>[19]</sup> Dentin root surface demineralization by low pH tetracycline increases fibronectin, an extracellular matrix glycoprotein, binding. The absorbed or bound fibronectin enhances fibroblast attachment and growth while suppressing epithelial cell attachment and growth.

The aim of the present study was to compare the efficacy of EDTA and tetracycline HCl as root conditioning agents on planed root surfaces.

## MATERIALS AND METHODS

For the present study, 20 human maxillary anterior teeth indicated for extraction due to chronic periodontitis were collected from the patients visiting the Department of Periodontology and Oral Implantology, Guru Nanak Dev Dental College and Research Institute, Sunam.

### Criteria for the selection of teeth

- Teeth with hopeless prognosis due to periodontal disease.
- No history of periodontal treatment for the past 6 months.
- Teeth with vital pulps and no root surface caries or restorations.
- Absence of any endodontic treatment.
- Absence of internal or external root resorption.
- Minimal instrumentation during extraction.

### Root conditioning agents

The agents used were EDTA (10% with pH 4.7) and tetracycline HCl (10% with pH 2.2). Both solutions were made by mixing 1 g of EDTA/tetracycline HCl powder to 10 ml of distilled water and constantly stirring with a glass rod, till the powder got fully dissolved. The pH was tested using a pH meter.

### Preparation of the specimen

After extraction, the teeth were cleaned of blood and saliva with distilled water using a soft bristled brush. After rinsing, the root surfaces were thoroughly root planed with Gracey curette (No. 1/2) followed by finishing bur (No. 102R) in high-speed hand piece at a speed of approximately 400,000 rpm in an attempt to remove the cementum and achieve a smooth, hard glass like surface. Experimental surface was obtained by removing the crown of each tooth at the level of cemento-enamel junction (CEJ) and then a portion of root 5 mm from the CEJ was taken up. A longitudinal section of the obtained specimen along the pulp chamber was made in order to obtain two equal halves with the help of a double-sided diamond disc in a slow-speed hand piece under copious water irrigation. The pulpal side was flattened with a straight bur and a horizontal groove was made for identification purpose.

### Group formation

A total number of 40 specimens were prepared from extracted maxillary anterior teeth which were divided into two groups comprising of 20 specimens in each group.

Group I: Dentin specimens treated with EDTA solution for 3 min

Group II: Dentin specimens treated with Tetracycline HCl solution for 3 min.

### Application of the solutions

The dentin samples were rubbed with saturated cotton pellets of the test solutions by "Active Burnishing Technique" with the help of tweezers for 3 min, which were changed every 30 s so as to maintain a steady concentration. After acid treatment the area was rinsed for 2 min with distilled water.

The samples were viewed under scanning electron microscope (SEM) at  $\times 1500$  magnification. The micrographs were taken and were evaluated for:

#### 1. Efficacy of removal of smear layer.

Removal of smear layer was estimated by scale given by Madison and Hokett in 1997 in accordance with the following criteria:

- 0 = No removal or no apparent effect on the smear layer
- 1 = Greater than no effect, but less than one-half removal

- 2 = Approximately one-half removal of the smear layer
  - 3 = Greater than one-half but less than complete removal
  - 4 = Complete removal of the smear layer with clean and open dentinal tubules.
2. Total number of dentinal tubules present per specimen.
  3. Number of patent dentinal tubules from the total number of tubules present.
  4. Diameter of randomly selected ten patent dentinal tubules was measured using a digital vernier caliper with 0.03 mm accuracy.

## RESULTS

In both the experimental groups, the efficacy in removal of smear layer was near total except for few areas, which were covered by debris, the difference between the two groups being statistically insignificant [Table 1]. In Group I (EDTA), the range came out to be 2-4 with a mean of 2.95 and standard deviation of 0.394 [Table 2, Figure 1]. In Group II (tetracycline HCl), the range was found to be 2-4 with mean of 2.85 and standard deviation of 0.670 [Table 2, Figure 1].

It was observed that the total number of dentinal tubules in the experimental Group II were more when compared to the experimental Group I, the difference being statistically highly significant ( $P < 0.05$ ) [Table 3]. The total number of dentinal tubules in 20 specimens of Group I amounted to 1211 and in case of Group II, the total count was found to be 1997. In case of Group I, the range was 44-82 with a mean of 60.5 and standard deviation of 9.9 [Table 4, Figure 2]. In Group II, the range was 62-123 with a mean of 99.8 and standard deviation of 16.2 [Table 4, Figure 2].

It was observed that the number of patent dentinal tubules in the experimental Group II was more when compared to the experimental Group I, the difference being statistically

highly significant ( $P < 0.05$ ) [Table 5]. The number of patent dentinal tubules in 20 specimens of Group I was 760 and in case of Group II, the count was found to be 1010. In case

**Table 1: Comparison of mean of smear layer removal in experimental groups**

Treatment groups		Mean difference	Standard error	Significance
Group I (EDTA)	Group II (tetracycline HCl)	0.10	0.174	$P=0.569$ (NS)

NS = Non-significant; EDTA = Ethylenediaminetetraacetic acid; HCl = Hydrochloride

**Table 2: Range and mean of efficacy in removal of smear layer in experimental groups**

Treatment groups	Total no. of specimens	Range	Mean $\pm$ SD
Group I (EDTA)	20	2-4	$2.95 \pm 0.394$
Group II (tetracycline HCl)	20	2-4	$2.85 \pm 0.670$
Total	40	2-4	$2.90 \pm 0.545$

SD = Standard deviation; EDTA = Ethylenediaminetetraacetic acid; HCl = Hydrochloride

**Table 3: Comparison of mean of total number of dentinal tubules in experimental groups**

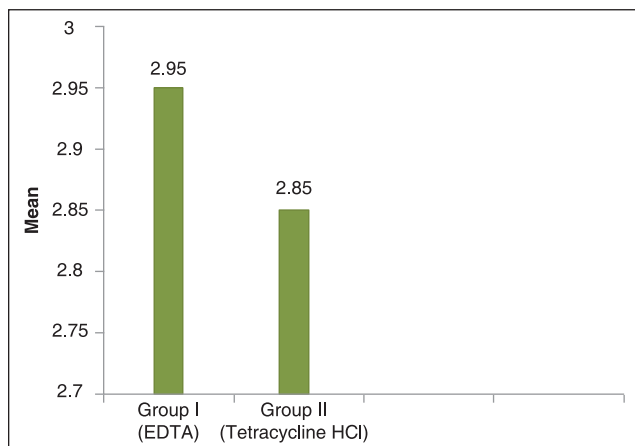
Treatment groups		Mean difference	Standard error	Significance
Group I (EDTA)	Group II (tetracycline HCl)	39.30*	4.256	$P=0.001$ (S)

S = Significant; EDTA = Ethylenediaminetetraacetic acid; HCl = Hydrochloride; \*The mean difference is significant at the 0.05 level

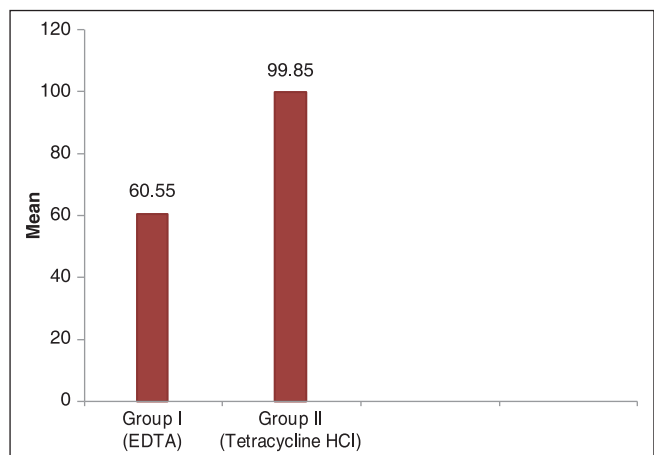
**Table 4: Range and mean of total number of dentinal tubules in experimental groups**

Treatment groups	Total no. of specimens	Range	Mean $\pm$ SD
Group I (EDTA)	20	44-82	$60.5 \pm 9.9$
Group II (tetracycline HCl)	20	62-123	$99.8 \pm 16.2$
Total	40	44-123	$80.2 \pm 23.93$

EDTA = Ethylenediaminetetraacetic acid; SD = Standard deviation; HCl = Hydrochloride



**Figure 1:** The bar diagram of the mean values of efficacy in removal of smear layer in two experimental groups



**Figure 2:** The bar diagram of the mean values of total number of dentinal tubules in two experimental groups

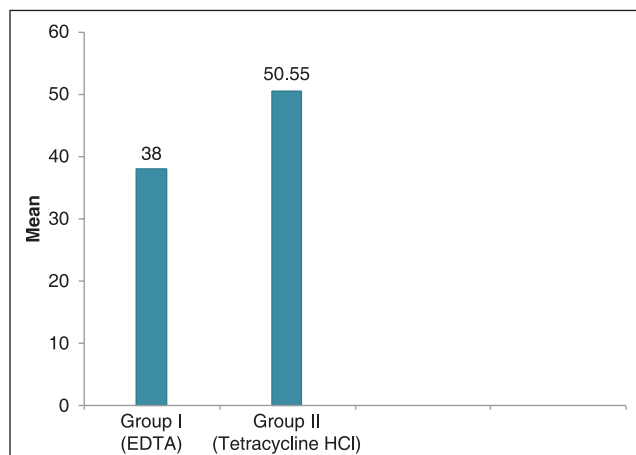
of Group I, the range was 20-60 with a mean of 38.0 and standard deviation of 8.0 [Table 6, Figure 3]. In Group II, the range was 30-67 with a mean of 50.5 and standard deviation of 10.6 [Table 6, Figure 3].

In the experimental Group I, the diameter of selected ten patent dentinal tubules was more as compared to the experimental Group II [Table 7]. In case of Group I, the range was 2.87-5.32 (mm) with a mean of 4.26 and standard deviation of 0.558 [Table 8, Figure 4]. In Group II, the range was 2.16-5.64 (mm) with a mean of 3.19 and standard deviation of 1.064 [Table 8, Figure 4]. The difference in the diameter of selected ten patent dentinal tubules came out to be statistically highly significant ( $P < 0.05$ ).

## DISCUSSION

Periodontitis causes pathological alterations of the periodontium, seen as loss of connective tissue attachment to tooth, loss of supporting alveolar bone and apical migration of the junctional epithelium along the root surface.<sup>[20]</sup> Periodontitis-affected cementum presents loss of collagen fiber insertion, it may harbor bacterial cells and may be contaminated by endotoxins which may suppress fibroblast migration and proliferation on cementum.<sup>[18]</sup>

Root surface conditioning by topical application of acidic solutions have been demonstrated to remove not only root instrumentation smear layer, but also any remaining root surface contaminants.<sup>[18]</sup> Demineralization of the root surface with root conditioning agents have been associated with uncovering and widening of the dentinal tubules with exposure of dentin collagen, thereby providing a matrix, which supports migration and proliferation of cells involved in periodontal wound healing resulting in enhanced connective tissue cell attachment to the root surfaces.<sup>[2]</sup>



**Figure 3:** The bar diagram of the mean values of patent number of dentinal tubules in two experimental groups

Considering the above findings, an effort was made in this study to compare the surface characteristics of diseased root surfaces after application of 10% EDTA and 10% tetracycline HCl as root conditioning agents using SEM.

**Table 5: Comparison of mean of patent number of dentinal tubules in experimental groups**

Treatment groups		Mean difference	Standard error	Significance
Group I (EDTA)	Group II (tetracycline HCl)	12.50*	2.979	P=0.001 (S)

S = Significant, EDTA = Ethylenediaminetetraacetic acid; \*The mean difference is significant at the 0.05 level

**Table 6: Range and mean of patent number of dentinal tubules in experimental groups**

Treatment groups	Total no. of specimens	Range	Mean $\pm$ SD
Group I (EDTA)	20	20-60	38.0 $\pm$ 8.0
Group II (tetracycline HCl)	20	30-67	50.5 $\pm$ 10.6
Total	40	20-67	44.25 $\pm$ 11.25

EDTA = Ethylenediaminetetraacetic acid; SD = Standard deviation; HCl = Hydrochloride

**Table 7: Comparison of mean of tubular diameter of patent dentinal tubules in experimental groups**

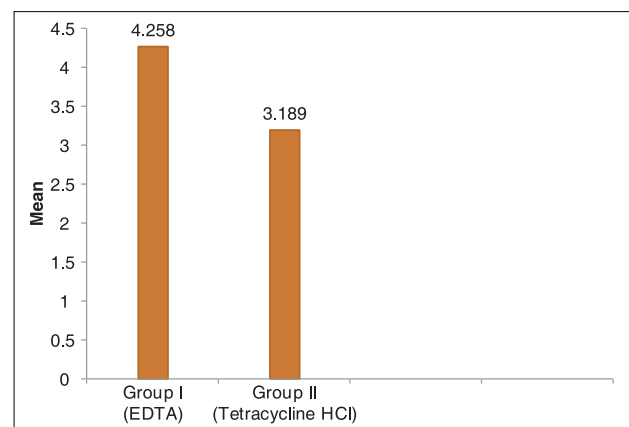
Treatment groups		Mean difference	Standard error	Significance
Group I (EDTA)	Group II (tetracycline HCl)	1.069*	0.269	P=0.001 (S)

EDTA = Ethylenediaminetetraacetic acid; S = Significant; HCl = Hydrochloride; \*The mean difference is significant at the 0.05 level

**Table 8: Range and mean diameter of patent dentinal tubule orifices in experimental groups**

Treatment groups	Total no. of specimens	Range	Mean $\pm$ SD
Group I (EDTA)	20	2.87-5.32	4.26 $\pm$ 0.558
Group II (tetracycline HCl)	20	2.16-5.64	3.19 $\pm$ 1.064
Total	40	2.16-5.64	3.72 $\pm$ 0.998

EDTA = Ethylenediaminetetraacetic acid; SD = Standard deviation; HCl = Hydrochloride



**Figure 4:** The bar diagram of the mean values of patent dentinal tubule orifices diameter (mm) in two experimental groups

The teeth used in this study were sectioned near the CEJ to obtain the experimental surface because the coronal part of the root contains less cementum as compared to its apical part so it is easy to remove the cementum and obtain a glass like dentin surface for root conditioning.<sup>[21]</sup> Instrumentation prior to application of root conditioning agents was done to remove the hypermineralized surface layer present on the periodontitis-affected roots and also to remove as much cementum as possible because root conditioning done on hypermineralized cemental surfaces did not induce collagen fiber attachment.<sup>[18,22]</sup>

In the present study, the concentration of both EDTA and tetracycline HCl was fixed as 100 mg/ml keeping into consideration the observation of various studies. Isik *et al.* used different tetracycline HCl concentrations of 0, 10, 25, 50, 75, 100, 125 and 150 mg/ml for root conditioning and found that concentration between 50 mg/ml and 150 mg/ml showed a statistically significant opening of dentinal tubules.<sup>[23]</sup> Hanes *et al.* used 0.5 mg/ml solution of tetracycline HCl and suggested that higher concentration of tetracycline HCl might be required to achieve complete demineralization.<sup>[2]</sup> Sterrett *et al.* found higher concentrations of tetracycline HCl demineralized dentin more effectively, which tend to leave more tetracycline on the root surface. They also demonstrated that tetracycline substantivity and desorption from the root surface was concentration dependent.<sup>[19]</sup> In a study by Frantz and Polson, greater number of attached cells were found at 100 mg/ml of tetracycline HCl concentrations as compared to untreated controls.<sup>[24]</sup> Terranova *et al.* also used the same concentration and found favorable results.<sup>[4]</sup>

In case of EDTA, the concentration mostly used was in the form of a gel having 24% EDTA. Babay used 8% EDTA and found similar results in removing smear layer and opening of dentinal tubules.<sup>[25]</sup> Lasho *et al.* used 15% EDTA and found similar results.<sup>[26]</sup> In the present study, to maintain the standardization, both solutions were used at 10% concentration and cotton pellets soaked in EDTA and tetracycline HCl were applied for 3 min at each experimental site. Isik *et al.*, Hanes *et al.*, Sterrett *et al.* and Register and Burdick also used the cotton pellets soaked in different root conditioning for 3 min at the experimental sites.<sup>[2,19,23,27]</sup>

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, in comparison to other application modes. Isik *et al.* applied tetracycline by different methods — immersion in tetracycline HCl solution, placement of saturated cotton pellets, burnishing with saturated cotton pellets vigorously rubbed using root planning pressure technique and camel hair brush application. They observed that burnishing technique produced maximum exposure of inter-tubular fibrils and tubular openings as compared to other methods of application.<sup>[7]</sup> Sterrett and Murphy and Trombelli *et al.* have also demonstrated similar findings.<sup>[18,28]</sup>

After conditioning with respective solutions, the specimens were then processed and examined under SEM at  $\times 1500$  magnification to evaluate: Efficacy in removal of smear layer, total number of dentinal tubules present per specimen, number of patent dentinal tubules and their diameter.

Efficacy in removal of smear layer in both the groups (10% EDTA and 10% tetracycline HCl) was near total and equivalent. Our results were consistent with the findings of Isik *et al.*, Mythili and Ahamed, Lafferty *et al.* who used similar concentrations and found similar results.<sup>[11,15,23]</sup> In the present study, debris were observed on some specimens. These debris may be (i) fragments of enamel, cementum, or dentin chipped off during instrumentation; (ii) foreign material that contaminated the surface during preparation of the specimen for SEM; (iii) precipitation artefacts resulting from interactions between buffer and fixative materials or between the specimen and these materials; or (iv) a combination of the above.<sup>[26]</sup>

Treatment with tetracycline HCl resulted in a higher number of dentinal tubules as compared to EDTA. This difference was probably due to lower pH of tetracycline HCl (pH = 2.2) compared to EDTA (pH = 4.7), so higher concentration of EDTA may be required to achieve the comparable results. Madison and Hokett compared the effects of different tetracyclines i.e., tetracycline HCl (pH 1.6), doxycycline (pH 2.2), minocycline (pH 3.8), sumycin (pH 4.4) on the dentin root surfaces of instrumented, periodontally involved human teeth. They observed that tetracycline HCl having lowest pH is the best form for root surface conditioning measured by its ability to affect both dentin smear layer removal and dentin tubule exposure.<sup>[29]</sup>

Treatment with tetracycline HCl resulted in a higher number of patent dentinal tubules as compared to EDTA. This again can be attributed to the lower pH of tetracycline HCl as compared to EDTA. Sterrett *et al.* compared tetracycline with citric acid having lower pH than tetracycline and found that citric acid solution was more effective than tetracycline in demineralizing dentin.<sup>[19]</sup> These findings were also supported



by Hanes *et al.* who used saturated solution of citric acid (pH = 1) and compared it with tetracycline HCl (pH = 3.2) and found citric acid being more effective in exposing dentinal tubules than tetracycline HCl.<sup>[2]</sup>

EDTA was more efficient in opening of dentinal tubules diameter as compared to tetracycline HCl. Although tetracycline HCl has lower pH as compared to EDTA but the results of EDTA came out to be better because etching at lower pH, in addition to dissolving the mineral also dissolves the collagenous matrix and also erodes the surface dentin<sup>[16,30]</sup> *In vivo* study by Blomlof reported that conditioning with EDTA solution with neutral pH did not evoke tissue necrosis, however, conditioning with acidic citric acid induced necrosis of flap along with adjacent periodontium even in 20 s of exposure.<sup>[30,31]</sup> EDTA acting at higher pH (pH 7.75) produced numerous patent dentinal tubules with a diameter of 2 to 3  $\mu$ .<sup>[26]</sup> Gamal and Mailhot observed that periodontal ligament fibroblasts adhere and differentiate on EDTA-conditioned root surfaces that were free from smear layer and presented exposed round to oval dentin tubule orifices.<sup>[32]</sup> Blomlöf *et al.* observed that EDTA (pH 7.0) has profoundly higher capacity to selectively expose collagen fibers which resulted in 10 to 15% more total histological attachment (long epithelial junction, connective tissue and reparative cementum), approximately 20% less long epithelial junction and approximately 20% more connective tissue in roots as compared to citric acid (pH 1.0).<sup>[16]</sup>

However, the findings of the present study are not in accordance with the findings of Babay who showed similar results with EDTA and tetracycline HCl as root conditioning agents on periodontally involved root surfaces. Both the agents removed the smear layer and exposed the dentinal tubules equally. The mode of application may have caused these observations since they used light pressure technique without burnishing and also they used 8% EDTA.<sup>[25]</sup> In the

present study, we used the active burnishing technique and 10% EDTA, giving different results.

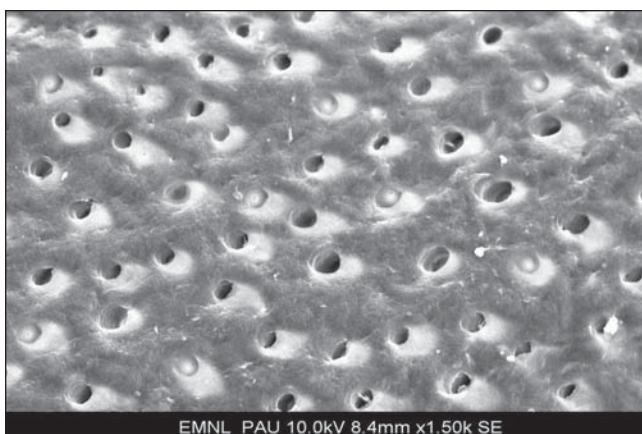
In the present study, it was found that root conditioning in both the experimental groups helped in the removal of smear layer, exposure of dentinal tubules and also widening of dentinal tubules. Hence, their application as root conditioner might have a significant role in periodontal wound healing and futuristic new attachment *in vivo*. The results of this study are limited to the physical findings of root surface changes and do not present *in vivo* differences that may result from the physiologic effects of EDTA or tetracycline HCl.

## SUMMARY AND CONCLUSION

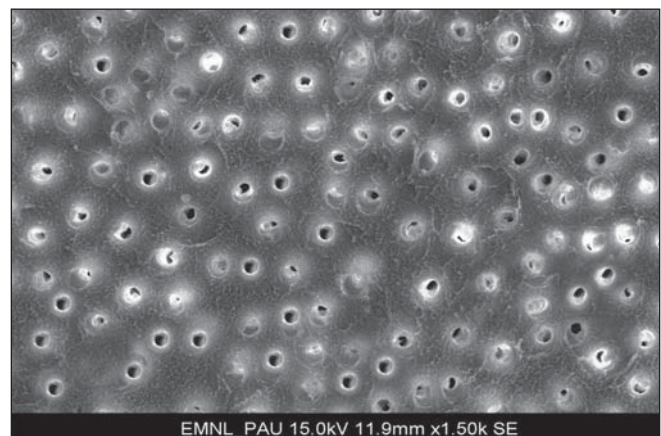
Both EDTA and tetracycline HCl were equally effective in removing the smear layer. There was no statistical significant difference ( $P > 0.05$ ) between the two groups. Both test solutions resulted in exposure of the dentinal tubules. The results of the tetracycline HCl [Figure 5] were highly significant ( $P < 0.05$ ) as compared to EDTA [Figure 6].

The number of patent dentinal tubules exposed by tetracycline HCl were statistically high ( $P < 0.05$ ) when compared to EDTA. Both the experimental groups resulted in widening of the patent dentinal tubules, but the results of EDTA were highly significant ( $P < 0.05$ ) for mean diameter of randomly selected 10 patent dentinal tubules as compared to tetracycline HCl. Although tetracycline HCl was more efficient in exposing the dentinal tubules, EDTA was much more effective as root conditioner because it enlarged the diameter of dentinal tubules more than that of tetracycline HCl.

In view of the present findings, further studies are necessary to establish the *in vivo* importance of EDTA and tetracycline HCl application as root conditioners as an additional step during periodontal therapy, especially in regenerative procedures.



**Figure 5:** Illustrates morphology of root surface treated with EDTA at  $\times 1500$  magnification



**Figure 6:** Illustrates morphology of root surface treated with tetracycline hydrochloride at  $\times 1500$  magnification

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