Journal of
Periodontology
&
Implant Dentistry

Research Article

A Comparative Evaluation of the Efficacy of Citric Acid, Ethylene Diamine Tetra Acetic Acid (EDTA) and Tetracycline Hydrochloride as Root Biomodification Agents: An *In Vitro* SEM Study

Harpreet Singh Grover[1] • Anil Yadav[2] • Prashant Nanda[3]*

[1]Professor & Head, Department of Periodontics & Oral Implantology, SGT Dental College, Hospital & Research Institute, Gurgaon, India
[2]Assistant Professor, Department of Periodontics & Oral Implantology, SGT Dental College, Hospital & Research Institute, Gurgaon, India
[3]Post-Graduate Resident, Department of Periodontics & Oral Implantology, SGT Dental College, Hospital & Research Institute, Gurgaon, India
*Corresponding Author; E-mail: drprashantnanda@gmail.com

Received: 2 May 2011; Accepted: 23 September 2011
J Periodontol Implant Dent 2011;3(2):73–78
This article is available from: http://dentistry.tbzmed.ac.ir/jpid

Abstract

Background and aims. Root conditioning is recommended as an adjunct to mechanical root surface debridement to remove smear layer and root associated endotoxins. The aim of this study was to compare the efficacy of citric acid, ethylenediaminetetraacetic acid (EDTA), and tetracycline hydrochloride as root biomodification agents.

Materials and methods. Fifteen freshly extracted teeth were root planed and specimens obtained from the cervical two-thirds of the root. Each tooth root provided four specimens to be treated by saline (used as control, citric acid, EDTA and tetracycline hydrochloride for a total of three minutes using the passive burnishing technique. The specimens were then observed under a scanning electron microscope (SEM). The specimens were evaluated for presence or absence of smear layer, total number of tubules visible, number of patent tubules and diameter of patent tubules. Statistical analysis was performed using paired -test.

Results. All three test groups effectively removed the smear layer in comparison to the control. The number of patent tubules present in the citric acid and EDTA test groups was significantly higher than those in the tetracycline hydrochloride test group. However, the average diameter of the patent tubules was greater in the tetracycline hydrochloride group compared with citric acid and EDTA groups.

Conclusion. All three agents are equally effective root biomodification agents. In clinical practice, EDTA might be more useful owing to its neutral pH.

Key words: **Citric acid, EDTA, root conditioning, SEM, tetracycline hydrochloride.**

Introduction

The primary etiologic factor in periodontal disease is dental plaque. Root surfaces affected by periodontitis are hypermineralized and contaminated by bacteria and their endotoxins,[1] and as a result, they are not biocompatible with the adjacent periodontal cells, the proliferation of which is pivotal for periodontal regeneration.[1] Therefore, the outcome of most regenerative periodontal therapies depends on the modification and disinfection of the contaminated root surface, to restore its biocompatibility and to allow for re-attachment of the periodontal tissues.[2]

Scaling and root planing are effective in removing the bacterial deposits and accretions as well as endotoxins from the exposed root surface. However, it is not possible to completely decontaminate a periodontitis-affected root surface by mechanical means alone.[3] The instrumented surface will inevitably be covered by a smear layer which is produced by most root manipulation techniques and could potentially affect fibroblast adaptation in the healing of the periodontal wound.[1] This smear layer contains remnants of dental calculus, contaminated root cementum and subgingival plaque, which act as a physical barrier between periodontal tissues and root surface, thus inhibiting the formation of new attachment.[4] Furthermore, the smear layer is resistant to saline rinsing.[5,6]

Thus, root conditioning has been recommended as an adjunct to mechanical root surface debridement to remove smear layer and root associated endotoxins and to expose collagen fibers on the dentin surface.[7]

Ethylenediaminetetraacetic acid (EDTA) appears to promote early cell tissue colonization by promoting a more biocompatible surface for cell and tissue attachment.[8] EDTA is not dependent on a low pH, nor does it present any toxicological side effects. This eliminates the necrotizing effects on surrounding periodontal tissues of an etching agent operating at a low pH.[9]

Citric acid has been shown to alter the surface characteristics of treated root surface by removing the smear layer, demineralizing the planed surfaces and eluting bacterial endotoxins from the pathologically altered cementum surfaces.[10] Furthermore, citric acid demineralization of underlying dentin may enhance new connective tissue attachment by either accelerating cementogenesis or by its bactericidal properties.[11,12]

The tetracyclines are broad spectrum antimicrobials which are used for root conditioning as well. When applied on root-planed dentin, tetracycline removes the smear layer, exposes the dentinal tubules and produces a fibrillar surface.[13] Tetracycline hydrochloride demineralized dentin has been shown to be (i) bacteriostatic, a feature which increases with increase in concentration (ii) retains more antimicrobial properties than penicillin treated root surfaces and (iii) demonstrates substantivity.[14] Tetracycline's anticollagenase activity appears to produce favorable clinical results.[15]

The aim of the present study was to compare the efficacy of citric acid, ethylenediaminetetraacetic acid (EDTA), and tetracycline hydrochloride as root biomodification agents.

Materials and Methods

The study sample included fifteen freshly extracted teeth procured from the Department of Oral and Maxillofacial Surgery at the SGT Dental College, Hospital and Research Institute as per following criteria:

Inclusion Criteria

1. Single rooted periodontally compromised teeth demonstrating an attachment loss of 5 mm or more at the time of extraction
2. Grade II or III mobility present at the time of extraction
3. No history of periodontal treatment i.e. scaling or root planing in the last 6 months

Exclusion Criteria

1. Root surface caries and/or restorations
2. Root surface abnormalities
3. Cervical abrasion and/or erosion

Preparation of the Specimen

Following extraction, the teeth were thoroughly washed with distilled water and transported in the same.

Meticulous root planing was then done with a Gracey curette to obtain a smooth, shiny and hard surface.

Specimens were obtained from the cervical 2/3rd of the roots by making two parallel grooves with a fine diamond tapered bur. The first groove was positioned horizontally at the CEJ and the second groove was made parallel and apical in relation to it, 2 or 3mm above the apex of the root. The portion of the tooth above and below these groves was then sectioned off respectively.

Four longitudinal root sections were then prepared from each tooth by cutting the cervical 2/3rd of the root into 2 halves first then splitting (cutting perpen-

dicular to the first cut) the halves into two more halves making the total number of specimens from each tooth equal to four. A shallow identification groove was then made on the pulpal side.

Each of these specimens was then treated with the various chemical agents and saline respectively.

Root Conditioning Procedure

1. One specimen from each sample was randomly allocated to one of the following:
   a. Control Group (CG) – Rinse with normal saline (control agent)
   b. Test Group 1 (TG1) – Application of citric acid (pH 1)
   c. Test Group 2 (TG2) – Application of EDTA (24%, pH 7.4)
   d. Test Group 3 (TG3) – Application of tetracycline hydrochloride (50 mg/ml, pH 1.8)
2. Application of the respective chemical agents on the specimens was done by passively burnishing with a solution soaked cotton pellet for 3 minutes. During this time the conditioning solution was renewed every 30 seconds. Rinsing of the control group was done with the help of a needle and syringe.
3. The specimens were then flushed with distilled water for 30 seconds to stop the reaction.

Preparation for SEM Evaluation

All specimens were then fixed in 2.5% gluteraldehyde in cacodylate solution for at least 24 hours before SEM analysis.

The specimens were then dehydrated in an ascending concentration of aqueous alcohol solutions. After the dehydration process specimens were air dried. Dried specimens were mounted on SEM stubs. Specimens were then sputter coated with gold in a gold sputtering unit. The mounted specimens were evaluated using a scanning electron microscope. Photomicrographs were taken from approximately the center of the specimen at a magnification value of ×3000.

The photomicrographs were evaluated, concerning the presence or absence of smear layer, total number of dentinal tubules present, number of patent dentinal tubules and the diameter of the patent dentinal tubules.

The obtained data were then statistically analyzed using the paired *t*-test.

Results

On evaluation, specimens treated by the test group solutions revealed wide open tubules except for a few areas which were covered with debris (Figures 1a,b,c). The control group on the other hand revealed a homogenous smear layer with no patent tubules visible (Figure 1d).

The mean total number of tubules visible in each sample was calculated for the test groups. It was seen that the mean total number of tubules in TG1 was 13.66 ± 1.291, in TG2 it was 13.86 ± 1.457, and in TG3 it was 1.42 ± 1.0998. When these results were compared it was seen that the p-value for all pairs was more than 0.05, therefore the difference in total number of tubules seen in the different test groups was not significant. (Table 1, Figure 2).

The mean number of patent tubules in each test group was then calculated and found to be 8.00 with a standard deviation of ± 0.655 for TG1, 7.67 with a standard deviation of ± 0.724 for TG2, and 5.87 with a standard deviation of ± 0.743 in TG3. When these results were compared it was observed that the p-values for TG1 vs TG3 and TG2 vs TG3 were less than 0.05 (<0.001), but p-value for TG1 vs TG2 was more than 0.05 (0.096). Which meant there was a statistically significant mean difference while comparing TG1 with TG3 and TG2 with TG3, but the mean difference of TG2 with TG3 was not significant (Table 2, Figure 2).

Mean diameter of patent dentinal tubules was then calculated for each specimen in each test group, following which the mean diameter of the patent tubules was calculated to be 2.285 ± 0.039 in TG1, 2.293 ± 0.046 in TG2 and 2.717 ± 0.038 in TG3. On comparing the same it was seen that the p-value for TG1 vs TG3 and TG2 vs TG3 was less than 0.05, but p-value for TG1 vs TG2 was more than 0.05, which meant that there was a significant difference in the mean values while comparing TG1 with TG3 and TG2 with TG3, while this difference between the mean values of TG1 with TG2 was not significant (Table 3, Figure 2).

Table 1. Mean total number of tubules present

	Mean	N	Std. Deviation	t-value	p-value
Pair 1					
TG 1	13.6667	15	1.291	0.676	0.51
TG 2	13.8667	15	1.4573		
Pair 2					
TG 1	13.6667	15	1.291	1.964	0.07
TG 3	14.2667	15	1.0998		
Pair 3					
TG 2	13.8667	15	1.4573	1.871	0.082
TG 3	14.2667	15	1.0998		

TG1: Test Group 1– application of citric acid (pH 1); TG2: Test Group 2 – application of EDTA (24%, pH 7.4); TG3: Test Group 3 – application of tetracycline hydrochloride (50 mg/ml, pH 1.8).

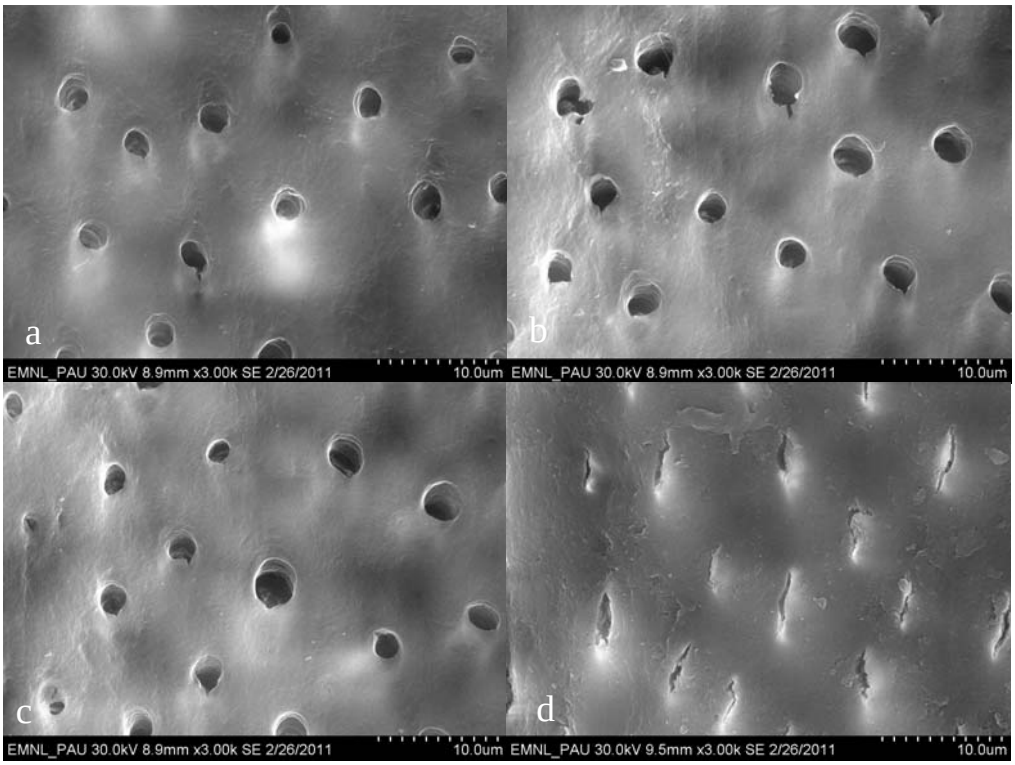

Figure 1. Citric acid specimen (a), EDTA specimen (b), tetracycline hydrochloride specimen (c), and control (normal saline) specimen (d).

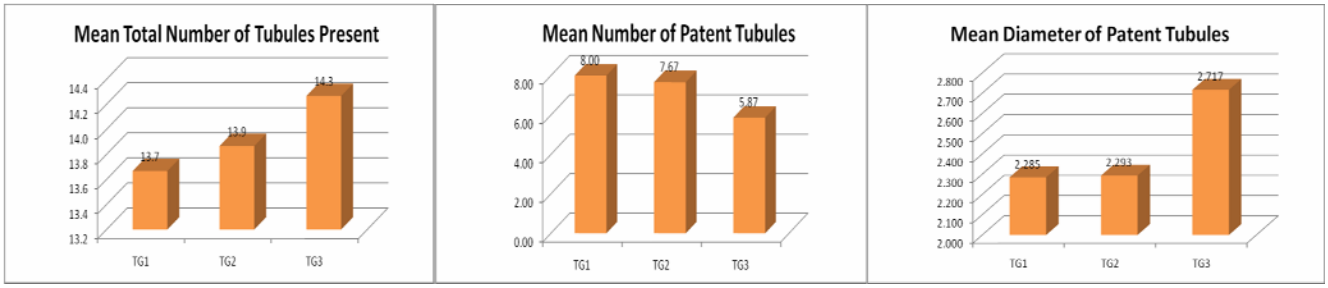

Figure2. Mean total number of tubules, and mean number and diameter (mm) of patent tubules. TG1: Test Group 1– application of citric acid (pH 1); TG2: Test Group 2 – application of EDTA (24%, pH 7.4); TG3: Test Group 3 – application of tetracycline hydrochloride (50 mg/ml, pH 1.8).

Discussion

Periodontitis causes pathological alterations of the periodontium, seen as loss of connective tissue attachment to the tooth, loss of supporting alveolar bone and apical migration of the junctional epithelium along the root surface.[7] Periodontitis-affected root surfaces, harbor bacterial cells, and may be con-

Table 2. Mean number of patent tubules

	Mean	N	Std. Deviation	t-value	p-value
Pair 1					
TG1	8.00	15	0.655	1.784	0.096
TG2	7.67	15	0.724		
Pair 2					
TG1	8.00	15	0.655	11.117	<0.001**
TG3	5.87	15	0.743		
Pair 3					
TG2	7.67	15	0.724	6.874	<0.001**
TG3	5.87	15	0.743		

TG1: Test Group 1– application of citric acid (pH 1); TG2: Test Group 2 – application of EDTA (24%, pH 7.4); TG3: Test Group 3 – application of tetracycline hydrochloride (50 mg/ml, pH 1.8).

Table 3. Mean diameter of the patent tubules

	Mean	N	Std. Deviation	t-value	p-value
Pair 1					
TG1	2.285	15	0.039	1.504	0.155
TG2	2.293	15	0.046		
Pair 2					
TG1	2.285	15	0.039	20.863	<0.001**
TG3	2.717	15	0.060		
Pair 3					
TG2	2.293	15	0.046	19.103	<0.001**
TG3	2.717	15	0.060		

TG1: Test Group 1– application of citric acid (pH 1); TG2: Test Group 2 – application of EDTA (24%, pH 7.4); TG3: Test Group 3 – application of tetracycline hydrochloride (50 mg/ml, pH 1.8).

taminated by endotoxins which suppress fibroblast migration and proliferation on cementum.[13] Root surfaces exposed to periodontitis have been shown to have higher mineral content than healthy root surfaces, having a higher content of calcium, phosphorus and fluoride.[16]

The traditional treatment of pathologically altered root surfaces has relied on mechanical removal of plaque and calculus and contaminated cementum. 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, and subgingival plaque.[1] 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.[2]

Root surface conditioning by topical application of acidic solutions has been demonstrated to remove not only root instrumentation smear layer but also any remaining root surface contaminants. Demineralization of the root surface with such agents has 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,[13] resulting in enhanced connective tissue cell attachment to the root surfaces.[2]

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, EDTA and tetracycline hydrochloride as root conditioning agents under a scanning electron microscope.

Specimens were obtained from the cervical 2/3rd of the roots because it contains less cementum as compared to the apical third so it is easy to remove the cementum and obtain a glass like dentin surface for root conditioning.[17] Also the test and control treatment was done in the same tooth root specimen to reduce variability.

Application of the respective chemical agents on the specimens was done with a cotton pellet for 3 minutes. During this time the conditioning solution was renewed every 30 seconds.[18,19]

The method of applying the root conditioning agents have varied among clinicians while some prefer active burnishing, others prefer the passive burnishing technique. Both methods, however, produce similar results. In the present study, we prefer the passive burnishing technique as the latter may itself form a smear layer and obliterate the dentinal tubule openings.[20]

The control group showed a uniform homogenous smear layer with no patent dentinal tubules. All three test groups showed almost complete removal of the smear layer except for a few areas. These observations were consistent with those of Lafferty et al,[21] Babay,[18] and Lasho et al,[6] who found similar results. Debris was found in some area of the test groups which could be attributed to (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 artifacts resulting from interactions between buffer and fixative materials or between the specimen and these materials; or (iv) a combination of the above.[6]

There was no statistically significant difference in the number of total tubules visible in the different test groups. These observations were consistent with those of who found comparable surface morphology after treating with the same solutions.[18,21]

Citric acid and EDTA samples had statistically greater number of patent tubules when compared with tetracycline hydrochloride treated samples. However, there was no significant difference in the number of patent tubules seen in the citric acid and EDTA samples. These observations are similar to those of Ahn et al[22] who obtained similar results.

On comparing the average diameters of the patent dentinal tubules it was seen that the diameters of the patent tubules were significantly larger in the tetracycline hydrochloride samples compared with citric acid and EDTA samples. Also, there was no statistically significant difference in the average diameters between the citric acid and EDTA groups. These observations are also similar to those reported by Ahn et al.[22]

In the present study, it was found that root conditioning in all three test groups helped in the removal of smear layer, exposure of dentinal tubules and also widening of dentinal tubules. The use of EDTA in clinical practice may be considered beneficial given its neutral pH and high efficacy in removing the smear layer and other toxins from the root surface.

The results of this study, however, 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 citric acid, EDTA or tetracycline hydrochloride.

References

1. Blomlof JPS, Blomlof LB, Lindskog SF. Smear removal and collagen exposure after non-surgical root planing followed by

etching with an EDTA gel preparation. *J Periodontol* 1996;67:841-5.
2. Hanes PJ, O'Brien NJ, Garnick JJ. A morphological comparison of radicular dentin following root planing and treatment with citric acid or tetracycline HCl. *J Clin Periodontol* 1991;18:660-8.
3. Jones WA, O'Leary TJ. The effectiveness of in vivo root planing in removing bacterial endotoxin from the roots of periodontally involved teeth. *J Periodontol* 1978;49:337-42.
4. Hanes PJ, Polson AM, Frederick GT. Initial wound healing attachments to demineralized dentin. *J Periodontol* 1988;59:176-183.
5. Blomlof J. Root cementum appearance in healthy monkeys and periodontitis-prone patients after different etching modalities. *J Clin Periodontol* 1996;23:12-18.
6. Lasho DJ, O'Leary TJ, Kafrawy AH. A scanning electron microscope study of the effects of various agents on instrumented periodontally involved root surfaces. *J Periodontol* 1983;54:210-20.
7. Lowenguth RA, Blieden TM. Periodontal regeneration: Root surface Demineralization. *Periodontology 2000* 1993;1:54-68.
8. Blomlof J, Jansson L, Blomlof L, Lindskog S. Root surface etching at neutral pH promotes periodontal healing. *J Clin Periodontol* 1996;23:50-5.
9. Blomlof L, Jonsson B, Blomlof J and Lindskog S. A clinical study of root surface conditioning with an EDTA gel II. Surgical periodontal treatment. *Int J Periodontics Restorative Dent* 2000;20:566-573.
10. Labahn R, Fahrenbach WH, Clark SM, Lie T, Adams DF. Root dentin morphology after different modes of citric acid and tetracycline hydrochloride conditioning. *J Periodontol* 1992;63:303-9.
11. Register AA, Burdick FA. Accelerated reattachment with cementogenesis to dentin, demineralized in situ I. Optimum range. *J Periodontol* 1975;46:646-55.
12. Register AA, Burdick FA. Accelerated reattachment with cementogenesis to dentin, demineralized in situ II. Defect repair. *J Periodontol* 1976;47:497-505.
13. Trombelli L, Scabbia A, Zangari F, Griselli A, Wikesjo UME, Calura G. Effect of Tetracycline HCl on periodontally affected human root surfaces. *J Periodontol* 1995;66:685-91.
14. Sterrett JD, Simmons J, Whitford G, Russell CM. Tetracycline demineralization of dentin: the effects of concentration and application time. *J Clin Periodontol* 1997;24:457-63.
15. Madison JG, Hokett SD. The effects of different tetracyclines on the root surface of instrumented, periodontally involved human teeth: A comparative scanning electron microscope study. *J Periodontol* 1997;68:739-45.
16. Polson AM, Hanes PJ. Cell and fiber responses to cementum from periodontitis-affected root surfaces after citric acid treatment. *J Clin Periodontol* 1989;16:489-97.
17. Borghetti A, Mattout P, Mattout C. How much root planing is necessary to remove the cementum from the root surface? *Int J Periodontics Restorative Dent* 1987;4:23-9.
18. Babay N. Comparative SEM study on the effect of root conditioning with EDTA or Tetracycline Hydrochloride on periodontally involved root surfaces. *Indian J Dent Res* 2000;11:53-7.
19. Babay N. Smear layer removal from root dentin using tetracycline hydrochloride concentrations: An SEM study. *Saudi Dent J* 2003;15:22-7.
20. Shetty B, Dinesh A, Seshan H. Comparative effects of tetracyclines and citric acid on dentin root surface of periodontally involved human teeth: A scanning electron microscope study. *J Indian Soc Periodontol* 2008;12:8-15.
21. Lafferty TA, Gher ME, Gray JL. Comparative SEM study on the effect of acid etching with tetracycline HCl or citric acid on instrumented periodontally involved human root surfaces. *J Periodontol* 1993;64:689-93.
22. Ahn SH, Chai JK, Kim CK, Cho KS. The effect of EDTA, tetracycline-HCl, and citric acid on diseased root surfaces: The SEM study. *J Korean Acad Periodontol* 1999;29:561-76.