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Research Article

Evaluation of Root Biomodification by Citric Acid, Tetracycline and Doxycycline on Instrumented Periodontally Involved Root Surfaces Using a Scanning Electron Microscope - An *Invitro* Study

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Abstract

Background: A surface smear layer consisting of organic and inorganic material with minute particle sizes is found on root surface containing remnants of dental calculus, contaminated root cementum, and subgingival plaque after scaling and root planing. Various types of agents such as citric acid, conc. tetracycline HCl and doxycycline have been placed on root surface not only to remove the surface inorganic smear layer but also to enlarge dentinal tubules into which healing connective tissue can enter. They have also shown a long lasting substantivity on periodontally diseased root surfaces.

Aim and Objective: 1. To treat root planed surfaces of extracted teeth with citric acid, tetracycline HCl and doxycycline and observe the surface characteristics under Scanning Electron Microscope (SEM). 2. To compare changes in surface characteristics after treating with all the three agents. 3. To establish which of these three agents give most desirable root surface characteristics favourable for periodontal healing**.**

Materials and Methods: The study was done *in vitro* using scanning electron microscopy on a total of 5 single rooted anterior teeth with 15 samples indicated for extraction due to Stage III or IV with Grade B or C periodontitis. 5 samples were divided into three groups A, B, C of 5 each based on type of conditioning done, either with saturated citric acid (pH 1) or tetracycline HCl (pH 1.6) - 250 mg/ml or doxycycline (pH 2.2) - 100 mg/ml for 5 minutes. Scanning photomicrographs of root surfaces were taken at ×2000 and × 6000 magnification. The micrographs were taken and evaluated for presence or absence of smear layer, total number of dentinal tubules per unit area, number of patent tubules per unit area and diameter of randomly selected patent dentinal tubules were measured.

Results: All the three groups showed slight difference in mean number of total dentinal tubules and showed statistically significant difference. The proportion of patent dentinal tubules was (36 ± 1.51) in tetracycline HCl group compared to citric acid (31 ± 1.58) and doxycycline (28 ± 1.78) showing the differences statistically significant. Tetracycline group showed higher number of patent tubules and increase in tubule diameter when compared to citric acid and doxycycline and the difference was statistically significant.

Conclusion: Results of this study suggest that tetracycline is the best current tetracycline form for root surface conditioning as measured by its ability to affect both dentin smear layer removal, diameter and tubule exposure.

Keywords: *Root Biomodification; Root Conditioning; Citric Acid; Tetracycline; Doxycycline*

Introduction

Regeneration of supporting tissue to tooth surfaces affected by periodontitis has been an ideal step of periodontal therapy. Periodontitis affected root surfaces shows a number of changes that include hyper mineralized or demineralized surface because of presence of plaque and calculus, loss of collagen cross banding and surface contaminated with cytotoxic and other biologically active substances. Such surfaces are not biocompatible with adjacent periodontal cells in which the proliferation may play a pivotal role for periodontal wound healing [1]. It may also cause depression in the cell growth and viability of fibroblasts thus interfering with the new attachment.

A primary goal of periodontal therapy is the re-establishment of periodontium by removing the cytotoxic and endotoxin bacterial products which may terminate the progression of the disease process. Periodontal microbiota and bacterial endotoxins contaminate root surfaces in periodontal pocket and inhibiting the migration and attachment of fibroblasts cell. To accomplish this goal, To accomplish this goal, a complete removal of adhered plaque and calculus as well as infected cementum is necessary although complete removal of etiological factors is infrequent. In spite of visual appearances, scaling does not successfully contribute the root surfaces free of plaque and calculus even in the locations such as furcation areas are barely accessible to instrumentation [2].

Mechanical instrumentation leaves the root surfaces inevitable which is covered with smear layer that obliterates the orifices of dentinal tubules that may contain microbiota, bacterial endotoxins and residual contaminated root cementum. This could hamper the periodontal wound healing and regeneration of connective tissue attachment. Adequate removal of plaque, calculus, and cytotoxic substances from the diseased root surface appears to be essential for periodontal regeneration. In addition, the dentin smear layer produced by most forms of root manipulation could potentially affect fibroblast adaptation during the periodontal wound healing. Smear layer may have a composition close to that of intertubular dentin, however the smear layer present in deep dentin would reflect its lesser degree of mineralization. Smear layer is more permeable to bacterial toxins. It consists of an organic and inorganic material with a particle size of less than 0.5-1.5 microns.

An objective of periodontal treatment is the predictable regeneration of the periodontium in areas previously affected by periodontal disease. For regeneration to occur, it is necessary to eliminate calculus, bacterial plaque and other cytotoxic substances on or within the diseased root surface. Human root surfaces have been treated with many substances in an attempt to make the root physiologically acceptable for the regeneration of new connective tissue attachment. Conditioning of root surface by topical application of solutions has been introduced as a regenerative procedure to dissolve the smear layer produced by root instrumentation, to aid in detoxification of root surface, to expose embedded collagen fibres and helps in connective tissue attachment [3,4]. The use of demineralizing agents in periodontal therapy can be dated back to the turn of the century. For over 90 years, various types of agents and materials have been placed on root surfaces in attempts to modify the diseased tooth structure. Such treatment enlarges dentinal tubules into which healing connective tissue can enter [5].

The rationale for periodontal therapy is aimed at elimination of periodontal disease, restoration and maintainance of healthy functional state of periodontal tissue. The dental literature has clearly demonstrated that present modes of periodontal therapy are successful in achieving these goals; however, the ultimate goal of therapy is the regeneration of the attachment, which is lost during disease [6]. Traditional surgical and non-surgical periodontal therapies aim at arresting periodontal disease by removal of plaque- "invested" tissues from disease-affected roots. However complete removal appears not possible with only mechanical debridement. 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 fibres on the dentin surface [7].

A number of agents have been proposed for the demineralization procedure which include EDTA, citric acid, minocycline, tetracycline, doxycycline, fibronectin phosphoric acid, lactic acid, aromatic sulphuric acid, formic acid, Cohn's factor, sodium deoxycholate, fibronectin, PDGF-BB, IGF-1 and LASERS etc. These demineralizing agents when applied on the root surfaces remove the smear layer, eliminate the cytotoxic material like endotoxins, uncover and widen the orifices of dentinal tubules and expose the dentin collagen matrix. 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.

Tetracyclines are broad-spectrum antibiotics demonstrated to be effective in the control of periodontal pathogens. Concentrated tetracycline HCl is moderately acidic (pH 1 - 2) and removes the surface inorganic smear layer created on the tooth during most dental treatment. It exposes underlying dentin and its tubules. It acts as a calcium chelator and its application results in root surface demineralization. *Invitro* study on the effects of tetracycline HCl on dentin has revealed properties, which may be beneficial in periodontal regenerative therapy. Terranova., *et al*. (1986) stated that root surface demineralization with tetracycline HCl enhanced soft tissue attachment, increase in fibronectin, an extra cellular matrix glycoprotein binding and enhanced fibroblast attachment and growth, while suppressing epithelial cell attachment and growth [8]. Furthermore, topical tetracycline HCl is adsorbed to and released from the dentin surface maintaining an antimicrobial property for at least fourteen days post therapy.

Citric acid has been shown to alter the surface characteristics of treated root surface by removing the smear layer exposing the funnel shaped dentinal orifices, demineralizes the planed surfaces and evades the bacterial endotoxins from the pathologically altered cementum surfaces [9]. Furthermore, citric acid demineralization of underlying dentin may enhance new connective tissue attachment by either accelerating the cementogenesis or by its bactericidal properties.

Doxycycline is a semi-synthetic tetracycline invented and clinically developed in the early 1960s. Doxycycline has shown to inhibit collagenase activity *invitro*. It is a bacteriostatic drug that inhibits both 70S and 80S subunits. The mechanism of action is such that the drug reversibly binds to the 30S subunit at the A-site, which prevents the attachment of amino acyl t RNA, which causes the termination of the translation process. Doxycycline applied topically on root surfaces obtained from patients with periodontal disease *invitro* has shown high degree of substantivity and total exposure of dentin [10].

Scanning electron microscopy (SEM), which is also recognized as SEM analysis or SEM technique, has been used worldwide in many disciplines. It has been regarded as an effective method in analysis of organic and inorganic materials on a nanometre to micrometre (μm) scale. SEM works at a high magnification reaches to 300,000x and even 1000000 (in some modern models) in producing images very precisely of wide range of materials. Scanning Electron Microscopy (SEM) can be used *in-vitro* to evaluate the surface characteristics of the root surface morphology based on the number and diameter of dentinal tubules as well as presence or absence of smear layer following the application of root conditioning agents. In this study an attempt has been made to compare and evaluate the changes in root surface characteristics with application of citric acid, tetracycline and doxycycline on instrumented periodontally involved root surfaces *in vitro* using Scanning electron microscope.

Material and Methodology

Study population

A total of 5 single rooted anterior teeth were selected and washed with distilled water, ultrasonic scaling was done followed by root planning and samples were stored in distilled water and were randomly divided into three groups. This pilot study was done in the Department of Periodontology and Oral Implantology of National Dental College and Hospital, Derabassi, Punjab. An ethical approval for the study was obtained from the Institutional Ethical Board Committee.

Inclusion criteria: Freshly extracted single rooted anterior teeth indicated for extraction due to Stage III or IV with grade B or C periodontitis were selected that meet the following criteria:

1. No history of root planning or prophylaxis in past six months.
2. No history of acute pain or swelling necessitating their removal.

Exclusion criteria:

1. Presence of any restoration.
2. Proximal attachment loss of less than 5 mm.
3. Presence of dental caries.

Methodology

15 single rooted anterior teeth indicated for extraction due to Stage III or IV with grade B or C periodontitis were collected and stored in distilled water. The root surfaces of all the teeth were scaled with an ultra-sonic scaler and thoroughly planed with Gracey's Curette. Following extraction, the teeth were washed and cleaned using a soft bristle brush and was stored in distilled water. Samples were obtained from cervical 2/3rd of root by making two parallel grooves 0.5 mm depth with circular disc in a slow speed handpiece under copious water irrigation was used for sectioning. The first groove was positioned horizontally at CEJ and the second groove was sectioned off. Three longitudinal root sections were then be prepared from each tooth by cutting cervical 2/3rd of root into two halves first and then splitting one of the halves into two more halves by cutting perpendicular to the first cut. The three samples from each tooth were stored in an individual container containing distilled water.

After preparation of samples, these were randomly divided into experimental groups (group A, group B and group C) for root conditioning procedure:

I. Group A- Application of saturated citric acid (pH 1) for 5 min

II. Group B- Application of tetracycline HCl (pH 1.6) - 250 mg/ml for 5 min

III. Group C- Application of doxycycline (pH 2.2) - 100 mg/ ml for 5 min.

Preparation of citric acid solution

Saturated citric acid solution was prepared by slowly adding anhydrous citric acid powder to 50ml of distilled water using a magnetic stirrer until no more crystals dissolve in solution and pH of 1.0 was obtained which was checked using pH meter. A total of 35.25g of powder was needed to obtain desired pH.

Preparation of tetracycline HCl solution

Tetracycline HCl (250 mg/ml) was made by mixing 500 mg in 2 ml sterile water. The solution was thoroughly mixed till a slurry is produced. The ph. was checked using pH meter which should be 1.6.

Preparation of doxycycline solution

Doxycycline HCl solution (100 mg/ml) was made by mixing doxycycline HCl powder (100 mg) in sterile water (1 ml) so as to obtain a viscous slurry. The pH was tested using a pH meter. The pH of solution should be 2.2.

Application of solution

Application of respective agents on the samples were done passively with cotton pellets saturated with the agent that was changed every 30 seconds for a total of 5 min. Following treatment, samples were rinsed with distilled water for 20 seconds and air dried.

Preparation of dentin specimens for scanning electron microscopy

All the specimens were fixed in 2.5% glutaraldehyde at 4ºC. The buffer solution was changed after each washing. After draining the buffer, the specimens were placed in ascending concentration of aqueous alcohol solutions in following manner:

1. 30% ethanol for 15 minutes at 4ºC
2. 50% ethanol for 15 minutes at 4ºC

3. 70% ethanol for 15 minutes at 4ºC
4. 80% ethanol for 15 minutes at room temperature
5. 90% ethanol for 15 minutes at 4ºC
6. 95% ethanol for 15 minutes at room temperature
7. 100% ethanol three times consecutively for 15 minutes each at room temperature.

After dehydration process, specimens were placed in vacuum desiccator overnight. Specimens were inserted in Hitachi model (10/10) S sputter coating machine; the samples were sputter coated with 25 nm of gold for 10 minutes. All the specimens were mounted on SEM stubs and were examined in a Hitachi S-3400N at a magnification of 2000X and 6000X. Scanning photomicrographs of root surfaces were taken to evaluate the presence or absence of smear layer, total number of dentinal tubules per unit area, number of patent tubules per unit area and diameter of open dentinal tubules as shown in figure 1-6.

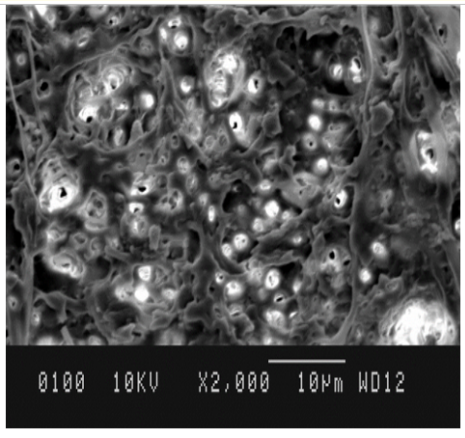


Figure-1: *Photomicrograph showing surface morphology of root specimen treated with citric acid at magnification of X 2000.*

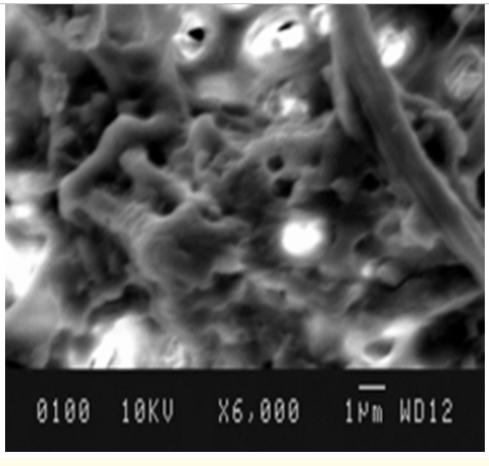


Figure-2*: Photomicrograph showing surface morphology of root specimen treated with citric acid at magnification of X 6000.*

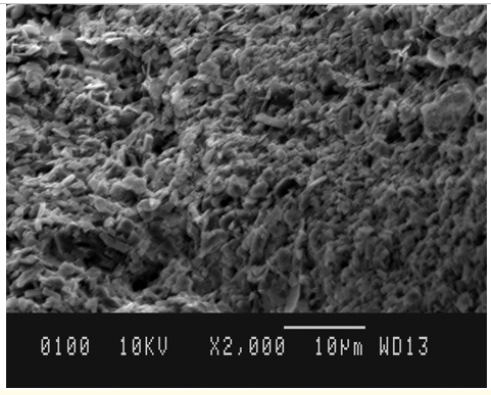


Figure-3: *Photomicrograph showing surface morphology of root specimen treated with tetracycline HCl at magnification of X 2000.*

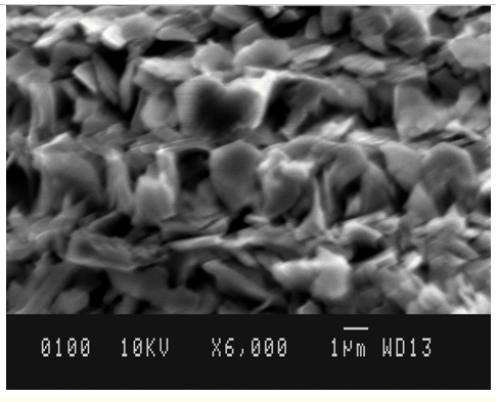


Figure-4: *Photomicrograph showing surface morphology of root specimen treated with tetracycline HCl at magnification of X 6000.*

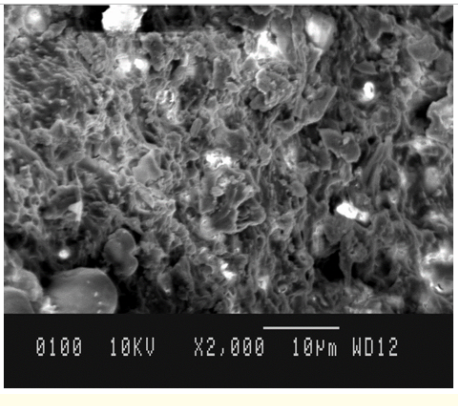


Figure-5: *Photomicrograph showing surface morphology of root specimen treated with doxycycline at magnification of X 2000.*

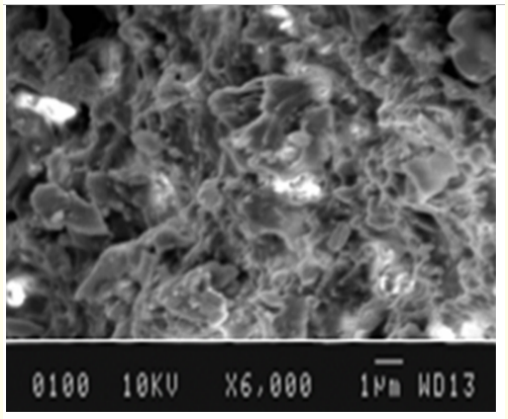


Figure-6: *Photomicrograph showing surface morphology of root specimen treated with doxycycline at magnification of X 6000.*

Results

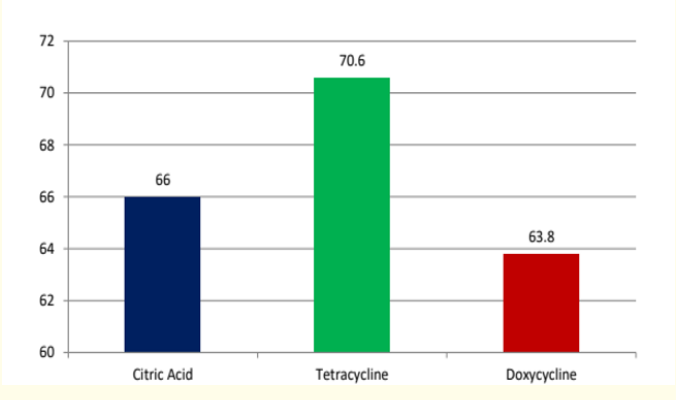
Statistical analysis

The data for present study was entered in the Microsoft Excel 2013 and analysed using SPSS statistical software 23.0 Version. The descriptive statistics analysis was expressed as mean and standard deviation for each group.

Experimental groups	Number of samples	Total no. of dentinal tubules	Mean ± SD	Std Error	P value
Citric acid (Group A)	5	330	66 ± 1.58	0.707	0.001**
Tetracycline (Group B)	5	353	70 ± 1.67	0.748	0.001**
Doxycycline (Group C)	5	319	63 ± 1.78	0.800	0.001**

Table 1: *Intragroup comparison of total number of dentinal tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

*P value < 0.05 indicates statistically significant**; P > 0.05 non significant*.*

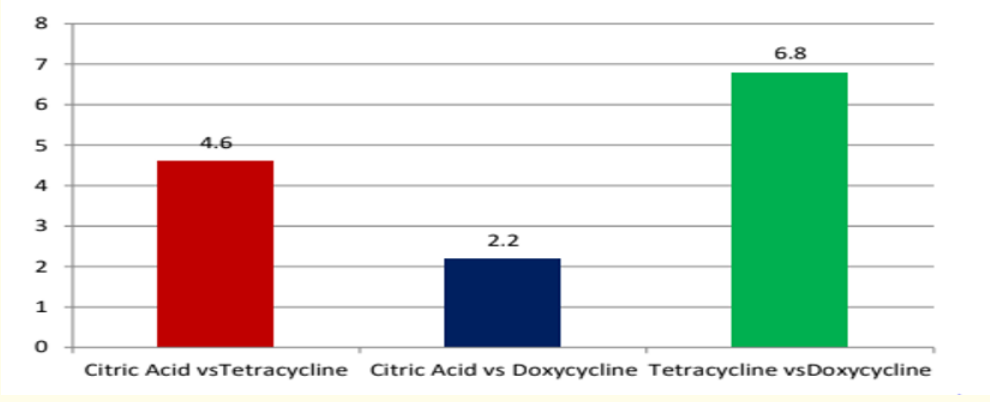


Graph 1: *Graphic representation of intragroup comparison of total number of dentinal tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

Experimental Groups (Post hoc tests)	Mean Difference	Std Error	P value
Citric acid vs tetracycline (Group A vs Group B)	4.60000	1.06458	0.006*
Citric acid vs doxycycline (Group A vs Group C)	2.20000	1.06458	0.041**
Tetracycline vs doxycycline (Group B vs Group C)	6.80000	1.06458	0.001**

Table 2: *Intergroup comparison of total number of dentinal tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

*P value < 0.05 indicates statistically significant**; P > 0.05 non significant*.*



Graph 2: *Graphic representation of intergroup comparison of total number of dentinal tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

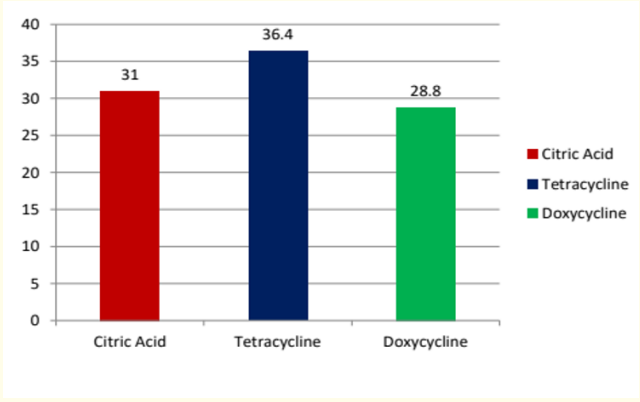
In table 1, graph 1, the mean number of total tubules per unit area in the tetracycline HCl-treated group (70 ± 1.67) was higher than in the citric acid group (66 ± 1.58) and the doxycycline group (63 ± 1.78). On intragroup comparison using One Way ANOVA, the difference between the groups was statistically significant (P = 0.001).

In table 2, graph 2, on intergroup comparison using One Way ANOVA and post hoc analysis, the mean difference in total number of tubules in specimens treated with tetracycline and doxycycline (Group B vs Group C) was significant. Similarly, the mean difference in total number of tubules in specimens treated with citric acid and doxycycline (Group A vs Group C) was significant, and the mean difference in total number of tubules in specimens treated with citric acid and tetracycline (Group A vs Group B) was not significant.

Experimental groups	Number of samples	Total no. of patent tubules	Mean ± SD	Std Error	P value
Citric acid (Group A)	5	155	31 ± 1.58	0.707	0.001**
Tetracycline (Group B)	5	182	36 ± 1.51	0.678	0.001**
Doxycycline (Group C)	5	144	28 ± 1.78	0.800	0.001**

Table 3: *Intragroup comparison of total number of patent tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

*P value < 0.05 indicates statistically significant**; P > 0.05 non significant*.*

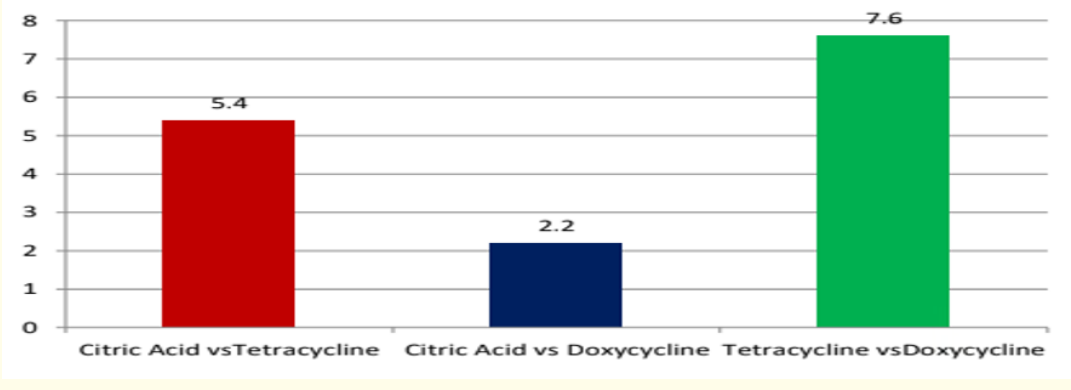


Graph 3: *Graphic representation of intragroup comparison of total number of patent tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

Experimental Groups	Mean Difference	Std Error	P value
Citric acid vs tetracycline (Group A vs Group B)	5.40000	1.03280	0.055**
Citric acid vs doxycycline (Group A vs Group C)	2.20000	1.03280	0.001**
Tetracycline vs doxycycline (Group B vs Group C)	7.60000	1.03280	0.001**

Table 4: *Intergroup comparison of total number of patent tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

*P value < 0.05 indicates statistically significant**; P > 0.05 non significant*.*



Graph 4: *Graphic representation of intragroup comparison of total number of patent tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

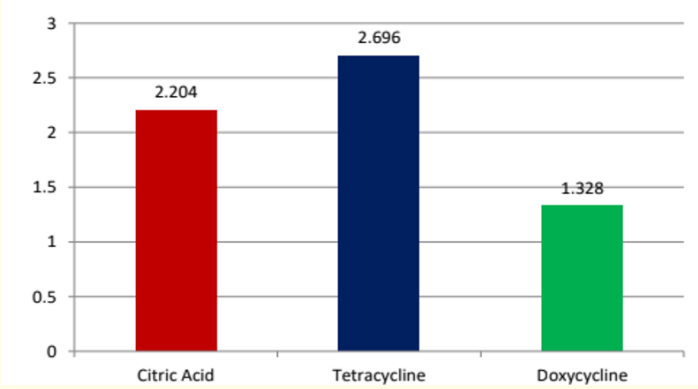
In table 3, graph 3, the mean number of patent tubules per unit area in the tetracycline HCl-treated group (36 ± 1.51) was higher than in the doxycycline group (28 ± 1.78) and the citric acid group (31 ± 1.58). On intragroup comparison using One Way ANOVA, the difference between the groups was statistically significant (P = 0.001).

In table 4, graph 4, on intergroup comparison using One Way ANOVA and post hoc analysis, the mean difference in patent tubules in specimens treated with tetracycline and doxycycline (Group B vs Group C) was significant. Similarly, the mean difference in patent tubules in specimens treated with citric acid and doxycycline (Group A vs Group C) was significant and the mean difference in patent tubules in specimens treated with citric acid and tetracycline (Group A vs Group B) was significant.

Experimental groups	Number of samples	Mean ± SD	Std Error	P value
Citric acid	5	2.2 ± 0.082	0.036	0.001**
Tetracycline	5	2.6 ± 0.084	0.037	0.001**
Doxycycline	5	1.3 ± 0.080	0.035	0.001**

Table 5: *Intragroup comparison of diameter of dentinal tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

*P < 0.05 (statistically significant) **; P > 0.05 (non significant)*.*

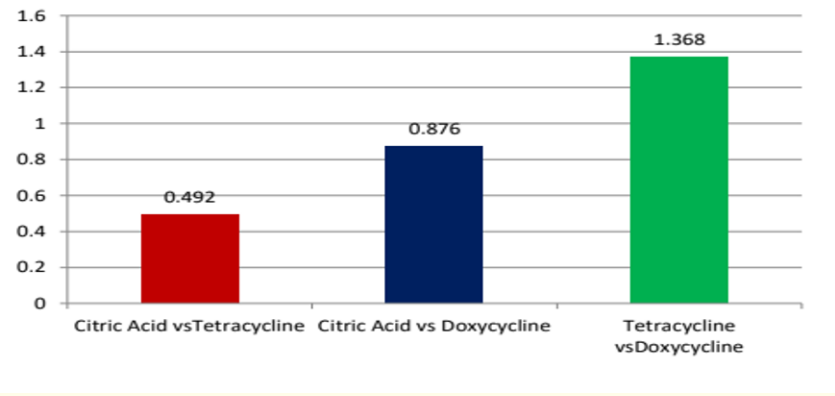


Graph 5: *Graphic representation of intragroup comparison of diameter of tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

Experimental Groups	Mean Difference	Std Error	P value
Citric acid vs tetracycline (Group A vs Group B)	0.49200	0.05200	0.001**
Citric acid vs doxycycline (Group A vs Group C)	0.87600	0.05200	0.001**
Tetracycline vs doxycycline (Group B vs Group C)	1.36800	0.05200	0.001**

Table 6: *Intergroup comparison of diameter of tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

*P < 0.05 (statistically significant) **; P > 0.05 (non significant)*.*



Graph 6: *Graphic representation of intergroup comparison of diameter of tubules in between citric acid (Group A), tetracycline (Group B) and doxycycline (Group C).*

In table 5, graph 5, the mean diameter of tubule in the tetracycline HCl-treated group was higher than in the doxycycline group and the citric acid group. On intra group comparison using One Way ANOVA, the difference between the groups was statistically significant.

In table 6, graph 6, on inter group comparison using One Way ANOVA and post hoc analysis, the mean difference in diameter of tubule was statistically significant.

Discussion

The nature of the periodontally exposed roots has been identified as one of the primary factors that may affect periodontal regeneration. There is a complexity in inflammatory, enzymatic and other biological factors associated with periodontal disease may give rise to physical as well as chemical alterations, particularly in the root cementum. The periodontitis affected root surface may harbour bacterial cells and may be contaminated by endotoxins, which may suppress the fibroblast migration and proliferation on the cementum during the wound healing after the periodontal therapy. The diseased root surfaces loose the collagen fibre insertion into it. Therefore, such surfaces may adsorb Ca, P, and F which develop to form a highly calcified layer [9]. Traditional treatment of pathologically altered root surfaces has relied on mechanical removal of etiological factors such as plaque, calculus, root bound toxins and contaminated cementum which is an important step for periodontal regeneration. However, it is not possible to completely decontaminate the root surface by mechanical therapy alone [10].

Demineralization of root surfaces during periodontal therapy has been performed to enhance regeneration of the lost periodontal attachment. Demineralizing agents have proven to expose dentin collagen, widening the orifices of dentin tubules and cementum associated proteins. Furthermore, demineralizing agents have been found to escape the retained toxins from the altered root surface. A number of agents have been proposed for the demineralization procedures such as phosphoric acid, EDTA, citric acid, PDGF-BB, IGF-1 and tetracycline [11]. Considering the above findings, an effort was made in this study to compare the surface characteristics of diseased tooth dentin after the application of tetracycline HCl, doxycycline and citric acid by scanning electron microscopy.

In the present study, single rooted anterior teeth indicated for extraction due to Stage III or IV with Grade B or C periodontitis were selected for application of tetracycline, citric acid and doxycycline as root conditioning agents. Teeth with no history of scaling or root planing in past 6 months and proximal attachment loss of 5 mm or more were included in the study [12]. Total of 15 specimens were

obtained from the roots of extracted teeth, which were categorized into 3 groups comprising of 5 specimens in each group. The various technique for the application of root conditioners have been tried for biomodification and conditioning of the root surface among the clinicians. One of the most common techniques is the placement of agent either passively (inactive method) by dropping or by burnishing (active method). Though both the techniques were found to be equally effective in removing the smear layer in a study done by Rosawen., *et al*. (1992) [12]. In the present study, each root conditioner was applied passively using cotton pellets that were completely soaked with the conditioning agent for a total period of 5 minutes with the cotton pellet being changed every 30 seconds. The passive application was preferred over burnishing technique as the latter may itself form smear layer which partially or completely obliterate the dentinal tubule openings. The cotton pellets were changed every 30 seconds to maintain a continuous stable dose of application of root conditioner. This procedure has been suggested to boost a mechanical/chemical act that would chemically loosen surface debris and inorganic material, thus exposing subsurface dentin to demineralizing role of fresh acid [13].

The primary difference between the optical microscope and the scanning electron microscope (SEM) is that with optimal microscope the light passing through a sufficiently thin specimen is magnified through glass lenses. On the other hand, in scanning electron microscope (SEM) observation, an electron beam that is finely focused by electron lenses is scanned over the specimen and the brightness on the cathode ray tube (CRT) is modulated by the signals obtained. SEM covers a wide range of magnification about x10 to x 1,00,000. Some of the most important features are easy magnification changing over, large depth of field (depth of focus) and stereographic image display. However, in the present SEM study, the characteristics of dentin surfaces were observed under magnification of x2000 and x6000.

Removal of smear layer in all the three experimental groups that is tetracycline HCl, doxycycline and citric acid was near total except for few areas, which were covered by debris. This observation was consistent with that of Parashis and Mitsis (1993) [14], Lafferty., *et al*. (1993) [5], Madison and Hokett (1997) [7], Mythili and Ahamed (2006) [15]. According to Wang., *et al*. the non-toxic dosage of tetracycline HCL has no smear layer removal ability and reports that declared this ability may have used higher dosage [16]. All three groups showed slight difference in mean number of total dentinal tubules. Citric acid, Tetracycline and doxycycline showed slight difference in the mean number of tubules. The intergroup comparison between Citric acid and tetracycline showed no significant difference however, intergroup comparison between the citric acid Vs doxycycline and tetracycline Vs Doxycycline showed statistically significant difference in the mean number of dentinal tubules. The results were in agreement with similar observation in the study conducted by Lafferty (1993) [5] and results were in contrast with Madison and Hokett (1997) [7].

The total number of patent dentinal tubules were (182) in tetracycline group compared to citric acid (155) and doxycycline (144) showing the differences which is statistically significant. However, on intergroup comparison the mean difference in patent tubules in specimens treated with tetracycline and doxycycline (Group B Vs Group C), Citric acid and doxycycline (Group A Vs Group C) and citric acid and tetracycline (Group A Vs Group B) were found to be statically significant. This may probably be attributed to the removal of smear layer to a lesser degree in doxycycline and citric acid as compared to tetracycline HCl owing to slightly lower pH of citric acid and doxycycline. Tetracycline group showed higher number of patent tubules when compared to citric acid, doxycycline and the difference was statistically significant. This difference was probably due to the lower concentration of citric acid (pH 1.0) as compared to less acidic Ph of doxycycline (pH 2.2) which causes exposure of comparable and substantial number of dentinal tubules compared to the other agents. Similar observations were made by Shetty., *et al*. (2008) [17] and Madison and Hokett (1997) [7]. The increase in the tubule diameter was significantly greater for tetracycline group than citric acid group in this study.

Our results showed that on intergroup comparison, tetracycline Vs doxycycline there was an increased the tubular diameter however, on intragroup comparison the mean diameter of tubule in the tetracycline group was higher than doxycycline and citric acid group. The application time used for each agent was 30 second for a total of 5 minutes. However, a previous study suggested that the application time should be limited to 2-3 minutes [4,18,19]. Since long etching time of 3 minutes and above could impair periodontal healing [9]. Doxycycline is able to enhance fibrin clot adhesion by exposing collagen matrix [18,19] and it enhances fibroblast chemotaxis and binding leading to a more stable initial clot formation [19]. Contrary to our study Chahal., *et al*. mentioned that removal of smear layer through tetracycline

and citric acid is more than doxycycline and the proportion of patent tubules and their diameter were less in the doxycycline group [20]. This finding may be due to the lower pH of tetracycline HCl (pH 1.8) and citric acid (pH 1) as compared to doxycycline (pH 2.2).

In the present study it was established that root conditioning in all three experimental groups helped in removal of smear layer and exposure of dentinal tubules. Hence, their application as root conditioner has a significant role in periodontal wound healing and future new attachment *in vivo*. The results of the present study are within the limitation of physical findings of root surface changes and do not present *in-vivo* variation that can result from the physiologic impact of the root conditioning agents. Difference between the results of our study with those of other studies in the literature may be related to the diseased dentin specimen utilized, the concentration, time and mode of application of demineralizing agent or a combination of these variables. Hence, additional studies of these variables are needed [21].

Conclusion

The use of root conditioning agents on the roots of human teeth may enhance the attachment through the connective tissue which results in enhancement of periodontal wound healing along with improvement in the reconstruction of periodontal treatment goal. In the present study all the three experimental groups showed effectively in removal of smear layer as well as dentinal tubule exposure. Results of this study suggest that tetracycline is more effective as a root surface conditioning agent and showed higher number of patent tubules and increase in tubule diameter when compared to citric acid and doxycycline and the difference was statistically significant.

Bibliography

1. Chandra RV., *et al*. "The Attachment of V79 and Human Periodontal Ligament Fibroblasts on Periodontally Involved Root Surfaces Following Treatment with EDTA, Citric Acid, or Tetracycline HCL: An SEM *in vitro* Study". *The Journal of Contemporary Dental Practice* 7.1 (2006): 044-059.

2. Theodoro Haypek., *et al*. "Effect of Er:YAG and diode laser irradiation on the root surface: Morphological and Thermal Analysis". *Journal of Periodontology* 74 (2003): 838-843.

3. Wen CR., *et al*. "*In vitro* effects of citric acid application techniques on dentin surfaces". *Journal of Periodontology* 63 (1992): 883-889.

4. Blomlof J and Lindskog S. "Periodontal tissue-vitality after different etching modalities". *Journal of Clinical Periodontology* 22 (1995): 464-468.

5. Lafferty TA., *et al*. "Comparative SEM study on the effect of acid etching with tetracycline HCL or citric acid on Instrumented periodontally involved human root surfaces". *Journal of Periodontology* 64 (1993): 689-693.

6. Smith BA., *et al*. "Effect of citric acid and various s concentrations of fibronectin on healing following periodontal flap surgery in dogs". *Journal of Periodontology* 58 (1987): 667-673.

7. Madison JS and Hokett SD. "The effect of different tetracyclines on the dentin root surface of instrumented, periodontally involved human teeth: A Comparative Scanning Electron Microscope study". *Journal of Periodontology* 68 (1997): 739-745.

8. Rosawen C., *et al*. "*In vitro* effects of citric and application techniques on dentin surfaces". *Journal of Periodontology* 63 (1992): 883-889.

9. Trombelli L and Okomoto H. "The effect of ultrasonic irrigation before and after citric acid treatment on collagen fibril exposure: An *invitro* SEM study". *Journal of Periodontology* 66 (1995): 887-891.

10. Labhan R., *et al*. "Root dentin morphology after different modes of citric acid and tetracycline hydrochloride conditioning". *Journal of Periodontology* 63 (1992): 303-309.

11. Minabae M., *et al*. "The effect of root conditioning with minocycline HCL in removing endotoxin from the roots of periodontally involved teeth". *Journal of Periodontology* 65 (1994): 387-392.

12. Rosawen C., *et al*. "*In vitro* effects of citric and application techniques on dentin surfaces". *Journal of Periodontology* 63 (1992): 883-889.

13. Nathalia GA., *et al*. "Comparison among four commonly used demineralizing agents for root conditioning. A scanning electron microscopy". *Journal of Applied Oral Science - SciELO* 19.5 (2011): 469-475.

14. Parashis AO and Mitsis FJ. "Clinical evaluation of the effect of tetracycline root preparation on guided tissue regeneration in the treatment of class II furcation defects". *Journal of Periodontology* 64 (1993): 133-136.

15. Mythili R and Ahamed BR. "Efficacy of tetracycline hydrochloride and doxycycline hydrochloride as a root conditioner-a scanning electron microscopic study". *Journal of Indian Society of Periodontology* 10 (2006): 227-232.

16. Wang Y., *et al*. "Tetracycline at subcytotoxic levels inhibits matrix metalloproteinase 2 and 9 but does not remove the smear layer". *Journal of Periodontology* 76 (2005): 1129-1139.

17. Shetty B., *et al*. "The comparative effects of tetracyclines and citric acid on dentin root surface of periodontally involved human teeth: a scanning electron microscope study". *Journal of Indian Society of Periodontology* 12.1 (2008): 8-15.

18. Penmatsa T., *et al*. "Effect of various concentrations of tetracycline hydrochloride demineralization on root dentin surface: A scanning electron microscopic study". *Journal of Pharmacy and Bioallied Sciences* 5 (2003): 48-53.

19. Preeja C., *et al*. "Fibrin clot adhesion to root surface treated with tetracycline hydrochloride and ethylenediaminetetraacetic acid: A scanning electron microscopic study". *Journal of Dental Research* 10 (2013): 382-388.

20. Chahal GS., *et al*. "Effect of citric acid, tetracycline, and doxycycline on instrumented periodontally involved root surfaces: A SEM study". *Journal of Indian Society of Periodontology* 18 (2014): 32-37.

21. Bhushan K., *et al*. "Root Biomodification in Periodontics - The Changing Concepts". *Journal of Dentistry and Oral Care Medicine* 2 (2016): 105-113.

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