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Various Root Conditioning Agents and Their Effectiveness in Peeiodontal Therapy- An *In Vitro* Scanning Electron Microscopic Study

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ABSTRACT

Introduction: Conditioning of root surfaces by the topical application of acid solutions has been introduced as a periodontal regenerative procedure to dissolve the smear layer and to aid in detoxification of any root surface contaminant remaining after root planing. Hence we aimed to investigate the scanning electron microscopic alterations produced on scaled and root planed dentin surfaces after application of citric acid, tetracycline hydrochloride and ethylenediaminetetraacetic acid (EDTA).

Materials and Methods: Eighty teeth were made to four groups. In group 1, control group specimens were treated with normal saline for 3 min, group 2 specimens were treated with citric acid, group 3 specimens were treated with tetracycline hydrochloride for 3 min and group 4 specimens were treated with 15% EDTA.

Results: Opening of dentinal tubules was seen in all specimens except for control group that was treated with normal saline. The total number of tubules seen was highest in citric acid group as compared to tetracycline hydrochloride and EDTA Group. The total number of patent tubule opening was highest in citric acid as compared to EDTA and tetracycline. The diameter of patent dentinal tubules in citric acid group was more than EDTA and tetracycline.

Conclusion: Thecitric acid group is more efficient than EDTA and tetracycline HCl group in removing the smear layer and in opening of number of patent dentinal tubules.

Key words: Citric acid, Ethylenediaminetetraacetic Acid, Root Conditioning Agents, TetracylineHcl

INTRODUCTION

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The periodontitis affected root surfaces are hypermineralized and contaminated with cytotoxic and other biologically active substances.[1‑ 5] It is not possible to decontaminate a periodontitis affected root surface completely by mechanical means alone.[6, 7] The instrumented surface will inevitably be covered by a smear layer following root planing.[8]Conditioning of root surfaces by the topical application of acid solutions has been introduced as a periodontal regenerative procedure to dissolve the smear layer and to aid in detoxification of any root surface contaminant remaining after root planing.[8] There are number of agents for conditioning of root surfaces that have been proposed for demineralization purposes. These include citric acid, phosphoric acid, tetracycline, doxycycline, minocydine, fibronectin, ethylenediaminetetraacetic acid (EDTA), sodium deoxycholate and Cohn's fraction IV.[9] Citric acid causes demineralization of root surfaces and removes bacterial endotoxins from the pathologically altered cementum surfaces.[8,10] EDTA etching also appeared to promote early cell colonization by providing a more biocompatible surface for cell tissue attachment.[11] Tetracyclines, which are well known as effective agents in control of periodontal pathogens, have been shown to produce a dentine surface that can enhance periodontal regeneration.[12]The investigations have shown that the application of these chemical agents results in the exposure of fibrils of the dentinal collagen matrix with inter‑ digitations of new and old collagen fibrils in soft tissues‑ root interface. Hence we aimed to investigate the scanning electron microscopic alterations produced on scaled and root planed dentin surfaces after application of citric acid, tetracycline hydrochloride and ethylenediaminetetraacetic acid (EDTA).

MATERIALS AND METHODS

Eighty human single‑ rooted teeth affected with severe periodontitis that were free from caries, cervical‑ restorations, erosion were extracted and used in the present study. They were stored in distilled water and stored in normal saline. After scaling and root planing, crown and healthy portion of root along with 2 or 3 mm of apical portion of the root was removed with a water cooled high‑ speed bur. The dentine specimens of dimension 5 mm × 5 mm were prepared for the study. The labial surface of each specimen was used for the study.

All 80 roots divided into 4 groups

- Group I: The root specimens were treated with normal saline for 3 min and used as control group
- Group II: The root specimens were treated with tetracycline HCl solution by placing the cotton pellets saturated solution that was changed every 20 s for the total time of 3 min
- Group III: The root specimens were treated with citric acid solution by placing the cotton pellets saturated solution when were changed every 20 s for total time of 3 min
- Group IV: The root specimens were treated with 15% EDTA solution by placing the cotton pellets saturated solution that was changed every 20 s for the total time of 3 min.

The solution was applied to specimen surfaces by light pressure burnishing.[13] After etching, the teeth were immediately immersed in approximately twenty ml of distilled water for 20 s and gently swirled to stop the chemical reaction. All specimens were dehydrated in a graded series of ethanol using l00% acetone as the final step. The teeth were dried for half an hour and were mounted on the stubs with an adhesive tape with the labial side of the toots facing the beam of the SEM and in such a way that the roots were placed in the center of the stub. The surface of the

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roots was scanned and observed on the computer screen fitted with the SEM at 3000×. The representative areas were photographed. All statistical analyses were carried out keeping $p<0.05$ significant.

RESULTS

The least number of tubules opening was seen in Tetracycline Hydrochloride group. On statistically comparing the difference between citric acid and tetracycline hydrochloride group of the total dentinal tubules the difference was significant. The difference was also significant between EDTA and tetracycline hydrochloride, citric acid and EDTA [Table 1].

The percentage of patent dentinal tubules was seen maximum in citric acid (452) followed by EDTA (252) and tetracycline HCl(168) [Table 2]. On comparing the mean of patent tubules in three experimental groups significant difference was found between the groups [Table 3].

Same observations were also observed for mean diameter of dentinal tubule orifice in the different groups. The mean diameter of patent dentinal tubules orifice was greater in citric acid (0.608 µm) as compared to EDTA group (0.571 µm) followed by tetracycline hydrochloride (0.468 µm) group which represented the minimum opening of the dentinal tubules [Table 4].

Table 1: Comparison of mean of total tubules in three experimental groups

	Number of specimens	Total number of dentinal tubules	Mean±SD	Groups compared		significance
I. Citric acid	20	601	30.05±5.00	I versus II	4.818	<0.001
II. EDTA	20	601	23.2±3.93	I versus III	9.738	<0.001
III. Tetracycline	20	354	17.7±2.68	II versus III	5.174	<0.001

Table 2: Comparison of percentage of patent tubules in three experimental groups

	Number of specimens	Total number of dentinal tubules	Mean±SD	Groups compared		significance
I. Citric acid	601	452	75.2	I versus II	8.629	<0.001
II. EDTA	601	452	54.3	I versus III	13.766	<0.001
III. Tetracycline	354	168	47.5	II versus III	2.287	0.026

Table 3: Comparison of mean of patent tubules in three experimental groups

	Number of specimens	Total number of dentinal tubules	Mean±SD	Groups compared		significance

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I Citric acid	20	452	22.6±3.52	I versus II	9.173	<0.001
II. EDTA	20	252	12.6±3.38	I versus III	16.437	<0.001
III. Tetracycline	20	168	8.4±1.60	II versus III	5.024	<0.001

Table 4: Comparison of mean diameter of dentinal tubule orifice (µm).

	Number of specimens	Total number of dentinal tubules	Mean±SD	Groups compared		significance
I. Citric acid	20	0.56-0.72	0.608±0.046	I versus II	2.064	0.046
II. EDTA	20	0.46-0.71	0.571±0.065	I versus III	8.389	<0.001
III. Tetracycline	20	0.36-0.55	0.468±0.058	II versus III	5.258	<0.001

DISCUSSION

In the present study, the light pressure burnishing technique was preferred over passive application. The solutions were applied for a total time of 3 min. The control group specimens were treated with normal saline. Counting of dentinal tubules orifice in saline group was not possible as smear layer covered the root surface. At × 3000 control group specimens were characterized by an irregular amorphous surface that served to correspond to smear layer. Depressions that seemed to correspond to dentinal tubules orifice were rare.These findings are in agreement with Lasho*et al*.[9] Citric acid conditioning of roots to produce new connective tissue attachment has been used since many years.[14] Some of these studies have reported reestablishment of connective tissue attachment on roots following citric acid conditioning. However due to its acid pH, it impairs the vitality of surrounding periodontal tissues Blomlof and Lindskog (1995).[15] EDTA exerts its action by chelating divalent cations at neutral PH. It has no deleterious effect on the surrounding periodontal tissues (Brannstrom*et al.* 1980,[16] Blomlof and Lindskog 1995).[15] EDTA etching has also been reported to promote early cell colonization and tissue colonization by providing more biocompatible surface for cell and tissue attachment. The tetracyclines have been shown to produce a dentine surface that can potentially further enhance periodontal regeneration. *In vitro* study of Terranova*et al.*[12] investigating the effect of tetracycline on dentine have suggested its potential usefulness. Tetracycline studies demonstrate multiple beneficial properties like enhanced attachment and growth of gingival fibroblast, good anti‑ collagenase activity, high substantivity and inhibition of parathyroid hormone that causes bone resorption. All the three experimental groups showed difference in mean number of dentinal tubules opening.Total number of dentinal tubules in tetracycline HCl group was 354, in EDTA group it was 464, and in the citric acid group it was 601. The number of tubules opened by the citric acid group was higher than the other 2 groups.[17] Mean number of tubule opening in the citric acid group was 30.05 greater than EDTA group which is 23.2 and tetracycline HCl

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17.7 The difference between tetracycline group and EDTA group for total number of dentinal tubules is statistically significant [Table 1]. This can be due to the substantivity of tetracycline hydrochloride on dentine surface as compared to EDTA. The proportion of patent tubules to the total number of dentinal tubules was 75.2% in citric acid group, compared to EDTA 54.3% and tetracycline hydrochloride group 47.5% [Table 2]. The lesser number of patent dentinal tubules in tetracycline HCl group may be attributed to the fact that tetracycline have a high substantivity on dentine surface. As the experimental agent was lightly burnished, the tetracycline powder remained on the test specimen and was seen, occluding the dentinal tubules. Partially occluded tubules were also counted as nonpatent or occluded. Same observations were also made by Madison and Hokett (1997)[18] with the use of tetracycline powder in their experimental study. The comparison of citric acid group showed higher number of patent dentinal tubules when compared to tetracycline HCl and EDTA group and results were statistically significant for comparison between tetracycline HCl group and citric acid group[17] and between EDTA and tetracycline HCl. The results of total number patent dentinal tubules in three experimental groups were statistically significant between tetracycline HCl, EDTA and citric acid group [Table 3].The comparison of mean diameter of the three experimental groups showed that the citric acid group had higher diameter (0.608 μm) as compared to the EDTA and tetracycline HCl group. EDTA group showed higher value of mean diameter opening when compared to tetracycline hydrochloride group. The mean diameter of EDTA is (0.571 μm). The mean diameter of tetracycline HCl is 0.468 μm [Table 4]. The tetracycline hydrochloride group showed the least diameter of dentinal tubule. It can be partially because of occupancy of tubules by substativity of tetracycline HCl and partially because of chelator effect of EDTA. In the present study, it was established that root conditioning in all the three experimental groups helped in the removal of smear layer and exposure of dentinal tubules. The agents not only remove debris but their demineralizing action also removes peritubular dentine, resulting in a wider orifice of the dentinal tubules (Goldburg and Ambranovich 1977).[18] Hence, their application as root conditioner may have a significant role in periodontal wound healing and future new attachment *in vivo*.

CONCLUSION

From the study, it was concluded that all the three agents namely citric acid, tetracycline hydrochloride, ethylenediaminetetraacetic acid were effective in removing smear layer. The total number of tubules seen was highest in citric acid group as compared to tetracycline hydrochloride and EDTA Group. The total number of patent tubule opening was highest in citric acid as compared to EDTA and tetracycline. The diameter of patent dentinal tubules in citric acid group was more than EDTA and tetracycline. Hence, citric acid group is more efficient than EDTA and tetracycline HCl group in removing the smear layer and in opening of number of patent dentinal tubules.

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