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Comparing the efficacy of Minocycline Citric Acid and EDTA as a root biomodification agents: An in-vitro SEM study

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Abstract

Context: Periodontal disease alters the root cementum. Complete removal of smear layer and endotoxins is not possible with mechanical debridement. Therefore, root biomodification is an adjunct to root planning to alter the root cementum.

Aims: The aim of the present study was to compare the efficacy of minocycline (MN), citric acid (CA) and ethylenediaminetetraacetic acid (EDTA) as root biomodification agents.

Methods and Material: Forty-five periodontally involved freshly extracted teeth were scaled and root planed and then treated with minocycline (pH 3.8), citric acid (pH 1) and EDTA (pH 7.3). Thereafter, they were fixed and viewed under scanning electron microscope (SEM) to evaluate the presence or absence of smear layer, total number of dentinal tubules and number of patent dentinal tubules.

Statistical analysis used: The data obtained was then statistically analyzed using the ANOVA and Post-Hoc tests alongwith non-parametric Kruskal-Wallis Test and Mann-Whitney Test.

Results: All the three agents effectively removed the smear layer. When compared minocycline and EDTA showed no significant difference and minocycline and citric acid showed no significant difference. However, EDTA and citric acid showed statistically significant differences, EDTA being most effective and citric acid being least.

Conclusions: Root biomodifiers provide a biologically acceptable environment that could favor connective tissue attachment to previously diseased root surfaces.

Keywords: Smear layer, Root Biomodification, SEM, Endotoxins, Cementum.

Introduction

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. Periodontal therapy is directed at arresting the

progression of these events, with the goal of stabilizing the long-term prognosis of the periodontium. Although these therapies have been proven to be effective, they do not restore the normal anatomy of the periodontium. Instead, these methods result in the repair of periodontal wounds in which the healed tissue does not replicate the original architecture and function. More recently, the desired objective of periodontal treatment is the predictable regeneration of the periodontium. In periodontics, regeneration implies the formation of new cementum, periodontal ligament and alveolar bone adjacent to a previously pathologically exposed root surface.[1] This type of healing would result in the complete renewal of original architecture and function. Thus, removal of bacterial deposits, calculus, and endotoxins from the cementum is generally considered essential for the formation of new connective tissue attachment.[2] However, studies suggested that demineralization of root surface, exposing the collagen of dentin, would facilitate the deposition of cementum by inducing the mesenchymal cells in the adjacent tissue to differentiate into cementoblasts.[3] It is based on the concept that exposure of collagen fibres of the dentin matrix may facilitate adhesion of blood clot to the root surface thereby favoring migration of fibroblasts. Traditional surgical and nonsurgical periodontal therapies aim at arresting the degeneration of periodontal tissue. Complete removal of smear layer and endotoxins 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 dentin surface.[4] A number of agents have been proposed for the root biomodification procedure which includes ethylenediaminetetraacetic acid (EDTA), citric acid, minocycline, tetracycline, doxycycline, fibronectin, phosphoric acid, Cohnns factor, sodium deoxycholate etc. Thus, root biomodification agents when applied onto root surfaces remove smear layer, eliminate 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 attachment. The aim of this study was to compare the efficacy of citric acid, EDTA and minocycline hydrochloride as a root biomodification agents.

Materials & Method

Forty-Five, periodontally involved teeth and indicated for extraction, were freshly extracted and taken from the Department of Oral and Maxillofacial Surgery, Swami Devi Dyal Hospital and Dental College, Panchkula.

Preparation of specimen

After extraction, the teeth were washed under running tap water and then were cleaned of blood, saliva and other debris with a soft bristled toothbrush and distilled water. The teeth were scaled and root planed. The strokes were directed apico-coronally. The tooth was frequently flushed with water to avoid dryness.

Preparation of acidic solution

Minocycline hydrochloride: Minocycline Hydrochloride solution was prepared by adding pure minocycline hydrochloride in distilled water and pH was adjusted to 3.8 by adding 1N of HCl.

Citric Acid: 65 grams of anhydrous citric acid crystals was dissolved in 100 ml of distilled water to obtain citric acid at pH 1.

EDTA: 8% EDTA solution was prepared by combining 8 gm of EDTA in 88 ml of distilled water and pH was adjusted to 7.3 by adding sodium hydroxide.

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The chemical agents were applied with cotton pellets using passive burnishing method for 4 minutes. After biomodification the specimens were washed in distilled water for 2 minutes.

Sectioning procedure

The instrumented tooth root surface was then re-examined to ensure complete removal of calculus. Then the crown portion was sectioned at CEJ with the high speed rotary instrument with diamond disk under continuous supply of water. The left over root was marked dividing into 3 parts the cervical, middle and apical. The root was horizontally sectioned at marked points dividing into cervical1/3rd, middle 1/3rd, and apical 1/3rd. Then the middle 1/3rd portion of the root was sectioned longitudinally along the pulp canal and a specimen was obtained to be viewed under scanning electron microscope.

Experimental design

The 45 specimens were randomly assigned equally (i.e. 15 specimens) to one of the 3 groups:-

Group 1 – Biomodification with saturated minocycline hydrochloride (pH 3.8) for 4 minutes

Group 2 – Biomodification with citric acid (pH1) for 4 minutes

Group 3 – Biomodification with 8% ethylenediaminetetraacetic acid (pH 7.3) for 4 minutes.

Preparation of specimen for SEM analysis

After treatment of root surfaces, samples were fixed in 2.5% glutaraldhyde in phosphate buffer (pH 7.3) for 24 hours. The specimens were then dehydrated in graded series of aqueous solutions (70%, 85%, 95%, 100%) for 10 minutes each and then were dried overnight. They were then mounted on SEM stubs and sputter coated with gold. The microphotographs of representative areas were obtained at required magnification.

The photomicrographs were evaluated concerning –

1) Presence or absence of smear layer
2) Total number of dentinal tubules present
3) Number of patent dentinal tubules

The presence or absence of smear layer was evaluated using the index proposed by Sampiao.[5]

Results

The data obtained was then statistically analyzed using the ANOVA and Post-Hoc tests alongwith non-parametric Kruskal-Wallis Test and Mann-Whitney Test.

The total number of dentinal tubules in citric acid, EDTA and minocycline were found to be 298 with mean of 19.87 ± 3.68, 378 with mean of 25.20 ± 6.05 and 334 with mean of 22.27 ± 3.47 respectively. The total number of patent dentinal tubules in citric acid, EDTA and minocycline were found to be 239 with mean of 15.93 ± 3.26, 350 with mean of 23.33 ± 7.06 and 295 with mean of 19.67 ± 4.1 respectively. [Fig 1, Fig 2, Fig 3, Fig 4, Fig 5]

The mean score of smear layer in citric acid, EDTA and minocycline came out to be 1.33±0.488, 1.60±0.507 and 1.33±0.488. [Fig 1, Fig 2]

The mean difference of total number of dentinal tubules and patent dentinal tubules between citric acid and EDTA was statistically significant ($p<0.05$). The mean difference of smear layer between the two groups was however non-significant ($p>0.05$). [Fig 1, Fig 2]

The mean difference of total number of dentinal tubules and patent dentinal tubules between citric acid and minocycline hydrochloride was statistically non significant ($p>0.05$). The mean difference of smear layer between the two groups was however non-significant ($p>0.05$). [Fig 1, Fig 2]

The mean difference of total number of dentinal tubules and patent dentinal tubules between EDTA and minocycline hydrochloride was statistically non significant ($p>0.05$). The mean difference of smear layer between the two groups was however non-significant ($p>0.05$). [Fig 1, Fig 2]

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Discussion

Dental calculus is constituted by mineralized structure with numerous holes, which leading to the accumulation of a larger number of microorganisms.[6] Associated with plaque colonization it is one of the main determinants of periodontal disease. Diseased root surfaces are unfavorable to cell attachment probably due to endotoxin adsorption.[7] To regenerate the periodontal structure affected by disease it is necessary to eliminate calculus, bacterial plaque and cytotoxic substances from the contaminated root surface.[8] Complex inflammatory, enzymatic and other biologic influences, which accompany periodontal diseases, produce physical or chemical alterations, which are particularly apparent in the root cementum. Periodontitis affected root surfaces, harbor bacterial cells, and may be contaminated by endotoxins which suppress fibroblast migration and proliferation on cementum.[9] Root surfaces exposed to periodontitis have been shown to have higher mineral content than healthy root surfaces, having a higher content of calcium, phosphorous and fluoride.[10]

The traditional treatment of pathologically altered root surfaces has relied on mechanical removal of plaque and calculus, root- bound toxins, and contaminated cementum. Curettes and ultrasonic scalers have been the primary instruments to accomplish these goals,[11] 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. The smear layer contains remnants of dental calculus, contaminated root cementum, and subgingival plaque.[12] Its 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.[13]

Root surface conditioning by topical application of acidic solution has been demonstrated to remove not only root instrumentation smear layer but also any remaining root surface contaminants. Demineralization of 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[9] resulting in enhanced connective tissue cell attachment to the root surfaces.[13] Presently to enhance the effectiveness of root planing, various physical (lasers) and chemical root conditioning agents (citric acid, phosphoric acid, Tetracycline hydrochloride, doxycycline hydrochloride, EDTA, minocycline hydrochloride etc) have been tried following root instrumentation, to enhance new attachment.

In the present study we used supersaturated solution of citric acid (pH1)[14,15,16], EDTA 8% (pH 7.3)[17] and minocycline hydrochloride (pH 3.8).[18]

All the three test groups showed almost complete removal of smear layer except for few areas and therefore the results were non-significant (p-value > 0.05) between all the three test groups. These observations were consistent with those of Babay N 2000[17], Lasho et al 1983[8], Minabe et al 1994[19], Thomas BS et al 1999.[20] Debris was found in some area of 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.[8]

On comparing the mean of total number of dentinal tubules, the p-value was greater than 0.05 among the citric acid versus minocycline hydrochloride and minocycline

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hydrochloride versus EDTA groups. However, the p-value was less than 0.05 among the citric acid and the EDTA groups. These results were consistent with those of study done by Lafferty et al 1993[21] and Babay N 2000[17] who found comparable surface morphology after treating with tetracycline hydrochloride and citric acid. Though the agent used in these studies was tetracycline hydrochloride but it is of the same group as minocycline hydrochloride.

Similarly on comparing the mean of number of patent number of dentinal tubules it was observed that p-value was greater than 0.05 among the citric acid versus minocycline hydrochloride and the minocycline hydrochloride versus EDTA. However, the p-value was less than 0.05 among the citric acid versus EDTA group. These results were consistent with those of study done by Shetty B et al 2008[4] who found that greater number of patent dentinal tubules using tetracycline hydrochloride when compared with citric acid. Though the agent used in the study was tetracycline hydrochloride but it is of the same group as minocycline hydrochloride. Babay N 2000[17] found similar morphological characteristics when compared EDTA and tetracycline hydrochloride. However, the results were contradictory to the study done by Thomas BS et al 1999[20] who found that minocycline hydrochloride is not as effective as citric acid in removal of smear layer and exposure of dentinal tubules.

In the present study it was found that root biomodification in all the three test groups helped in removal of smear layer, exposure of dentinal tubules and also widening of dentinal tubules. Hence, their application as root biomodification agents might have a significant role in periodontal wound healing new attachment in vivo. However, similar studies with greater sample sizes should be conducted to further evaluate the role of root biomodifiers in periodontal regeneration.

Conclusion

In view of above findings, further studies are necessary to establish in-vivo significance of application of Citric Acid, EDTA and Minocycline Hydrochloride as root biomodification agents during periodontal therapy, to provide a biologically acceptable environment that could favor connective tissue attachment to previously diseased root surfaces.

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Legends Figure and Table

Fig.1: Table 1. Mean and comparison of number of total and patent tubules in three experimental groups

Groups	No. of specimen	Total number of dentinal tubules	Mean ± S.D	Total number of patent dentinal tubules	Mean ± S.D	Difference between groups	
						Groups compared	
1. Citric Acid	15	298	19.87±3.68	239	15.93±3.26	1 &2	p<0.05
2. EDTA	15	378	25.20±6.05	350	23.33±7.06	1&3	p>0.05
3. Minocycline Hydro-chloride	15	334	22.27±3.47	295	19.67±4.10	2&3	p>0.05

p>0.05 is non-significant , p<0.05 is significant

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Fig. 2: Graph 1: Depicting number of total, patent and closed dentinal tubules in three groups

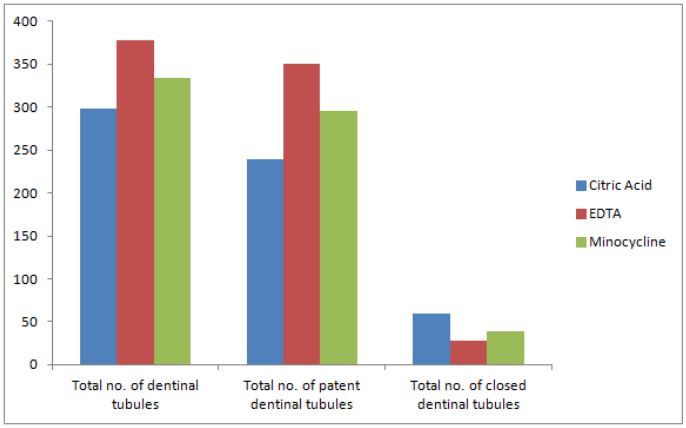


Fig. 3: SEM photograph of citric acid specimen

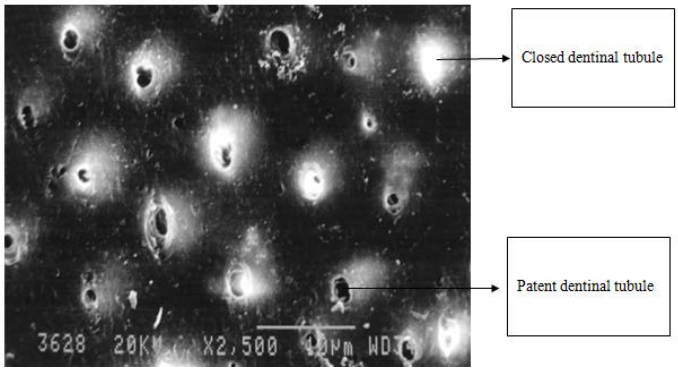


Fig.4 :SEM photograph of EDTA specimen

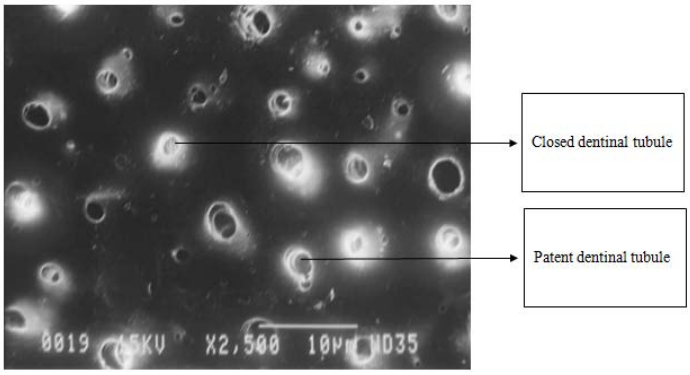
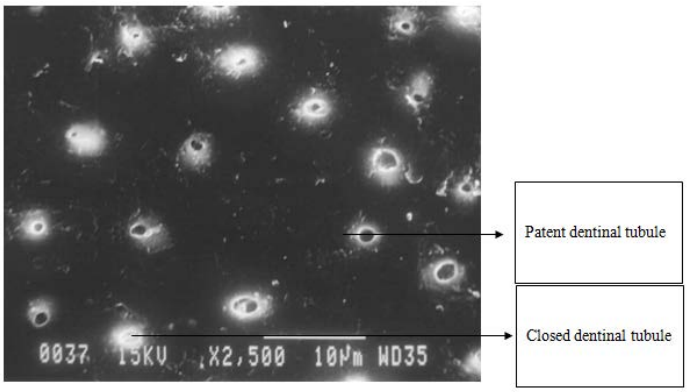


Fig. 5: SEM photograph of minocycline hydrochloride specimen



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