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# Debris and smear layer removal efficacy and changes in morphology of dentinal tubules after using citric acid, tetracycline-hydrochloride and mixture of tetracycline and acid and detergent

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## ABSTRACT

**Background:** In periodontal diseases, contamination of roots with bacteria and their active biologic agents and smear layer formation following periodontal treatments, prevents reattachment of periodontal cells. As a matter of fact biomodification of root surface and removal of smear layer and debris is critical for regeneration of periodontal structures. The aim of this study was to compare the efficacy of smear layer removal by citric acid, tetracycline-hydrochloride (TTC-HCL) and mixture of tetracycline and acid and detergent (MTAD).

**Materials and Methods:** In this *in vitro* and experimental study, 12 impacted third molars were sectioned from cervical enamel junction (CEJ) to mid-root area into four dentinal specimens ($n = 48$). Three groups were conditioned by citric acid 3%, TTC-HCL 50 mg/mL and MTAD by means of cotton pellets, which were changed every 30 s for 3 min. The control group was conditioned by distilled water. Images from scanning electron microscope (SEM) were examined by two different analyzers. Data was analyzed by Kroskal Wallis and Mann–Whitney statistical analysis.

**Results:** The mean of dentinal tubules diameter was the most in MTAD group and the least in TTC-HCL and this difference was statistically significant ($P = 0.05$).

**Conclusion:** According to the present study, TTC-HCL could remove debris and smear layer but citric acid and MTAD had better debris and smear layer removal efficacy and demineralization effect in comparison with TTC-HCL.

**Key Words:** Citric acid, mixture of tetracycline and acid and detergent, smear layer, tetracycline hydrochloride

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## INTRODUCTION

Regeneration of destructed periodontal ligament and alveolar bone is the most important goal in periodontal surgical treatments.[1] The key factor to meet this goal is optimum removal of plaque, calculus and cytotoxic materials from diseased root surface. Whereas pathologic changes of root surface may change the outcome of reconstructive treatment, it is recommended to remove the diseased denuded cementum as a part of periodontal treatment to complete root planning.[2] The root surface of periodontally diseased teeth has much more mineralized substance, because they resorb more calcium, phosphorous, and fluoride.[3] Bacteria, their endotoxins and other biologically active factors, contaminate the root surface and the periodontally diseased cementum may lose its collagen fibers.[1,4,5] After root planning with or without flap procedure, root surfaces will be covered by smear layer, which prevents the new attachment of periodontal fibers and inhibits the migration and proliferation of fibroblasts.[1,3,6]

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In order to increase the chance of new attachment in regenerative periodontal treatments, use of biochemical modifying agents on root surface has been considered for a long-time. Bogle, *et al*. in 1980 showed new attachment after surgical therapy and modification of root surface with citric acid in old dogs with periodontal disease.[4] Rompen, *et al*. 1999 also observed more protein and collagen synthesis and proliferation of fibroblasts in periodontal ligament of root surfaces treated with citric acid and tetracycline (TTC) in comparison with untreated roots.[1]

Citric acid is reported as the most widely used material but not the golden standard, which is useful for biomodification of root surface, especially in root coverage with free gingival grafts.[7] Its low-price, availability and efficacy has made it one of the most used materials. In this study, 3% solution was used for matching with other available reports.[1,7-10]

TTC has been reported as an effective anti-microbial acidic material suitable for smear layer removal and inhibiting collagenase and proteinase and bone resorption.[5,7] In the present study, pure TTC-hydrochloride (TTC-HCL) was used as a demineralizing agent because oral TTC capsules have other filling materials and agents with unknown effect on root surface. In the present study, TTC-HCL 50% was used like some other available reports suggestions.[1,3,10,11]

Mixture of tetracycline and acid and detergent (MTAD) is a new material in endodontics and loads of researches were performed on it but, it is still new in periodontics. It contains citric acid and doxycycline, the two materials with the most usage among other materials in biomodification of root surface.[5] Doxycycline has the most inhibiting effect on collagenase among TTC family.[1]

Most of the regenerative treatments require modification and decontamination of diseased root surface in order to regain its biocompatibility, which is needed for new attachment of new periodontal structures.[12] However, some other reports declared that there is no difference among clinical indices when root planning is the only treatment and no smear layer removal is performed.[13] Because there were adverse outcomes in some of the reports and bias among reporters, this study was designed to compare the morphologic changes of dentinal tubules (diameter of dentinal tubule) and debris and smear layer removal efficacy of MTAD, TTC-HCL, and citric acid.

## MATERIALS AND METHODS

In this study, 12 freshly extracted third molars were used. Teeth were rinsed by normal saline exactly after extraction and the remnant tissues were polished by brushing and prophylactic paste and were stored in distilled water and kept in refrigerator. The crowns were removed from their cervical enamel junction (CEJ) and their root trunks (the part between CEJ and furcation) were sectioned into four dentinal samples with dimension of $4 \times 3 \times 1$ mm, each of them were accidentally entered in one of three experimental groups or control group ($n = 12$). A narrow groove was ditched on external surface of each sample for later identification of the used surface.

Group 1 was treated with solution of 3% citric acid, provided in Department of Pharmacology in Isfahan University of Medicine. Each sample was robbed with solution by means of a cotton roll for 30 s (burnishing method). A new cotton roll was used after 30 s. Six cotton rolls were used for each sample and the robbing process for each sample was 3 min. Each sample was washed with distilled water for 30 s. Group 2 and 3 were treated as mentioned with fresh TTC-HCL 50 mg/mL provided in Department of Pharmacology in Isfahan University of Medicine and Bio-Pure MTAD (Dentsply – Tulsa-USA). Group 4 was treated with distilled water and served as control.

Finally, all of the samples were dehydrated in ascending alcohols, coated with gold and directly examined using a scanning electron microscope (PHILIPS XL-30, Eindhoven, Netherlands). They were observed for changes of dentinal tubule diameter and existence of smear layer with ×1500 magnification and an image was taken for each sample. Each image was given a code, so the investigation could be double-blinded. Each sample was observed by two trained examiners and in cases of versatility, a third trained examiner observed the image.

Dentinal tubules diameter reflects their amount of mineralization. To find out this diameter the widest diameters in one image were measured by means of a caliper and the mean of these numbers were calculated. The actual dentinal tubule was analyzed according to the image scale[9] [Figures 1-5].

A score of 1-8 was used for the evaluation of photomicrographs:[14]

1. Score 1: The surface is devoid of any debris and smear layer

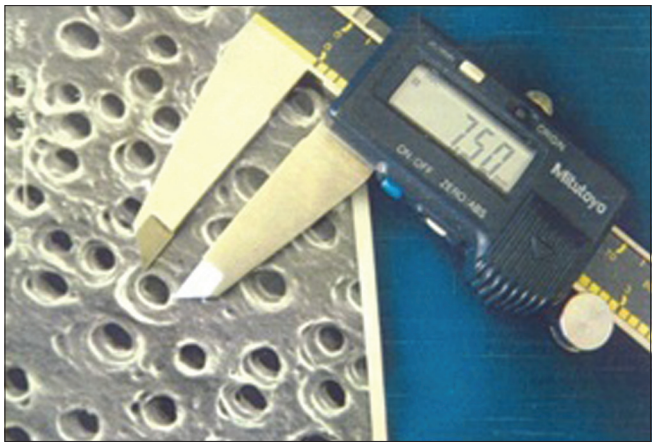

**Figure 1:** A caliper was used to measure the dentinal tubules diameter

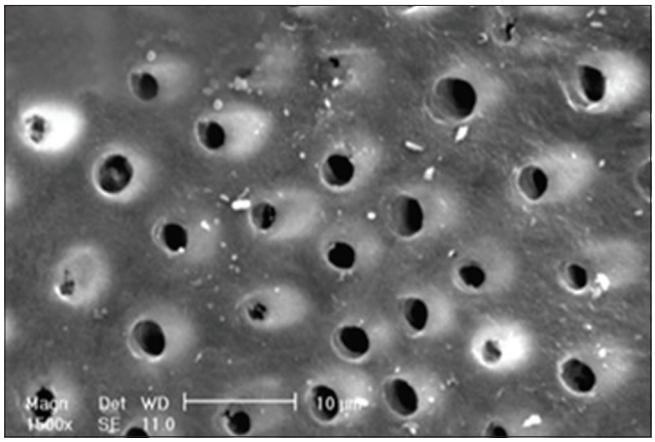


**Figure 3:** Group 1, citric acid

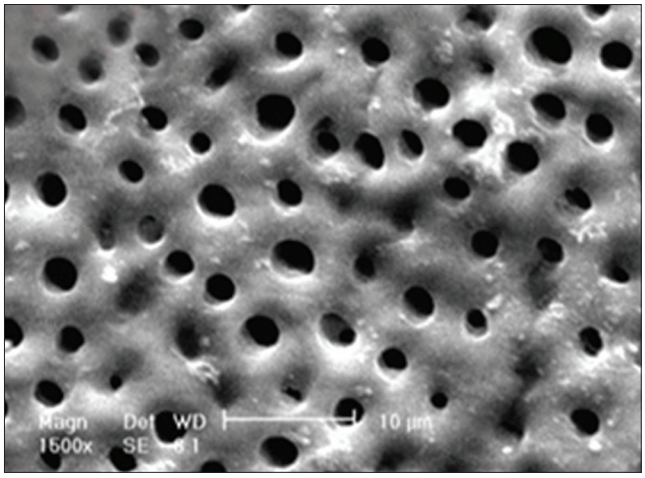


**Figure 5:** Group 3, mixture of tetracycline and acid and detergent

2. Score 2: The surface is devoid of smear layer, but little debris is obvious
3. Score 3: The surface has been cleaned, but both smear layer and debris are dispersedly observed

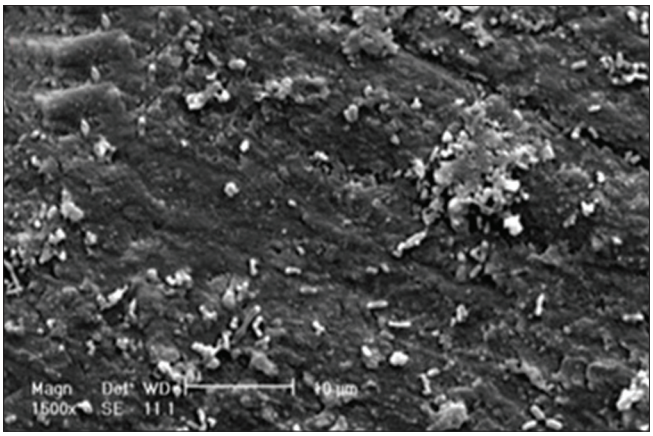


**Figure 2:** Control group

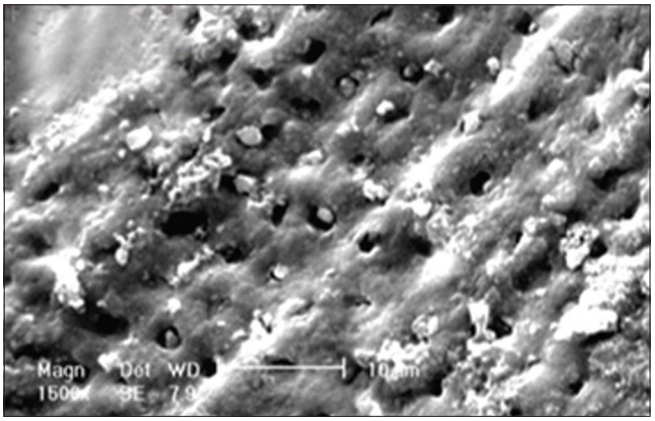


**Figure 4:** Group 2, tetracycline-hydrochloride

4. Score 4: The surface has been cleaned, but level of smear layer and debris is also noticeable
5. Score 5: The clean surface is bit greater than unclean surface
6. Score 6: Almost half of the smear layer and debris have been removed
7. Score 7: The greater part of smear layer and debris are left
8. Score 8: The surface is completely covered with smear layer and debris

The data was analyzed by Kruskal Wallis and Mann–Whitney statistical analysis.

## RESULTS

According to Kruskal Wallis analysis, the mean of dentinal tubules diameter was the most in MTAD group and the least in TTC-HCL and this difference of dentinal tubule diameter among all of the groups was statistically significant ($P = 0.05$).

The results showed significant difference between MTAD and citric acid with TTC-HCL in smear

layer removal amount, but the difference between MTAD and citric acid was not significant. TTC-HCL had the least efficacy in comparison with others. The difference of smear layer removal scoring was statistically significant among all groups except between citric acid and MTAD [Figure 6].

## DISCUSSION

Smear layer is defined as very tiny particles of mineralized matrix of collagen. Different studies have reported smear layer to act as a physical barrier between root surface and surrounding connective tissue and inhibit the adhesion between them.[3]

It is reported that biomodification of root surface has three different advantages:

1. Biomodification of root surface can prepare a suitable surface for new attachment of new connective tissue.
2. Increase in bond strength of root surface and fibrin layer by better adhesion of blood clot.
3. Interference in apical migration of epithelium.[8]

Biomodification and conditioning of root surface is used with two different ways of dropping (inactive method) and burnishing (active method). It seems that burnishing technique is more effective in smear layer removal because this technique can remove debris and loose organic materials with a chemical-mechanical method, and it is reported that dropping technique can not remove debris and smear layer effectively.[3,4,14,15] Hence, the present study used burnishing technique for 3 min. The 3 min time was suggested in a number of reports and hence it was used for matching the technique.[3,7-9,14,16-18]

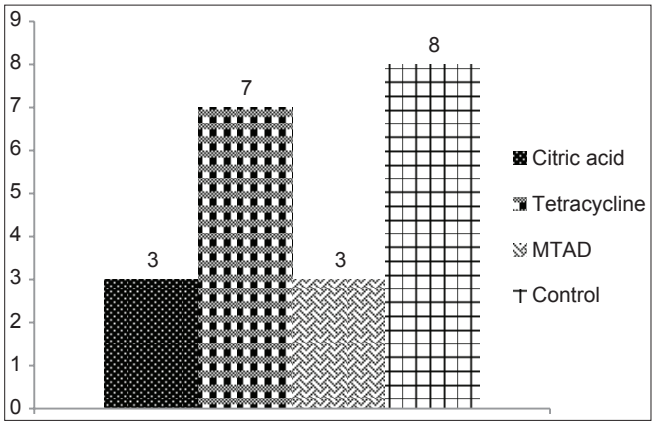


**Figure 6:** Mean of diameter of dentinal tubule in studied groups

The findings of present research about the ability of smear layer removal by citric acid, MTAD, and TTC-HCL, are supported by earlier reports[2,5,7,16,19-24] except for the reports that declare the power of smear layer removal by TTC-HCL is the same as citric acid.[1] According to Wang, *et al*.[16] the non-toxic dosage of TTC-HCL has no smear layer removal ability and the reports that declared this ability may have used higher dosage.

The present study showed that MTAD has the most and TTC-HCL has the least demineralization power. The ability of demineralization has also been found in citric acid. These results are confirmed by previous reports.[1,2,12,18,24]

Although TTC-HCL has the ability of smear layer removal and demineralization ability, former reports found citric acid more effective.[2,5,25,26] Chandra, *et al*.[15] reported citric acid and ethylene diamine tetraacetic acid (EDTA) more effective than TTC-HCL in fibroblast adhesion to root surfaces. In other hands, Wikesjo, *et al*.[23] declared the effect of TTC-HCL and citric acid on dentinal tubules surface had no difference. This observation might be found because they did not use citric acid in their experiment and they just compared their SEM images with other experiments.

Vanhuesden, *et al*.[8] reported the same effect for citric acid and TTC-HCL in their histologic experiment. This finding may be different from the present study because of their method of study and scoring.

Torabinejad, *et al*.[27] and Tay, *et al*.[28] found MTAD more effective in smear layer removal, if sodium hypochlorite was used before it. However, Tay declared that the use of sodium hypochlorite with MTAD may weaken its anti-bacterial effect. So it is recommended not to use sodium hypochlorite in periodontal surgeries because of its danger for the surrounding tissues and weakening of anti-bacterial effect.[28] Recently in 2010, different researches showed efficacy of MTAD with least side effects. Yasuda, *et al*.[29] showed that MTAD has the least toxicity among other detergents, even in comparison with chlorhexdine. On the other hands, Shokouhinejad, *et al*.[30] found MTAD the most efficacious in smear layer removal even in comparison with EDTA and Shabahang, *et al*.[31] and Tay, *et al*.[32] declared that anti-bacterial efficacy of MTAD is statistically significantly more than 2% chlorhexidine gel.

The differences between present results and other studies may be because of interfering factors such as differences between primary dentinal tubules diameter, demineralization efficacy, nature and dosage of biomodification materials and the method and time of application of them on root surfaces.[14]

## CONCLUSION

According to present study, TTC-HCL could remove debris and smear layer but citric acid and MTAD had better debris and smear layer removal efficacy and demineralization effect in comparison with TTC-HCL.

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