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Research Report

The difference of saline and sterile water for tetracycline hydrochloride solvents in cementum demineralization

Shinta Ferronika, Ahmad Syaify, and **Dahlia Herawati**
Department of Periodontics, Faculty of Dentistry, Universitas Gadjah Mada
Yogyakarta - Indonesia

ABSTRACT

Background: *The root cementum demineralization is an important step in regenerative periodontal therapy to smear layer removal on the root surface. Smear layer on the root surface becomes a barrier of the new attachment between periodontal tissues with the root surface. The use of tetracycline capsules as root surface demineralizing agent cannot be applied directly on the root surface and solvents such as saline or sterile water are needed.* ***Purpose:*** *The aim of this study was to determine differences between sterile water and saline solvent for tetracycline HCl (tetra HCl) as a cementum demineralization.* ***Method:*** *In this study the specimens were divided into three groups: a control, tetra HCl dissolved in saline, and tetra HCl dissolved in sterile water. Application using burnishing method for 3 minutes. Samples were dehydrated with ethanol series of 30% to 100%. Results of the root demineralization observed by scanning electron microscopy (SEM). Statistical analysis was performed using the Kruskal-Wallis followed by a Mann-Whitney nonparametric test.* ***Result:*** *Upon statistical analysis showed that the sterile water as a solvent of tetra HCl is more effective in smear layer removal and collagen structure exposure in the cementum.* ***Conclusion:*** *Tetra HCl dissolved in sterile water was found to be the best root cementum demineralization agent.*

Keywords: *root demineralization; cementum; tetracycline HCl; sterile water; saline*

Correspondence: Shinta Ferronika, Department of Periodontics, Faculty of Dentistry, Universitas Gadjah Mada. Jl. Denta Sekip Utara, Bulaksumur, Yogyakarta 55281, Indonesia. E-mail: drg.ferronika@gmail.com

INTRODUCTION

The root cementum demineralization is one important step in periodontal regenerative therapy. The aim of root surface debridement is to reduce the amount of bacteria and endotoxins on the root surface, treatment of the root surface with demineralizing agents such as acids, EDTA, and tetracycline primarily aims to expose collagen fibrils. To achieve this, the smear layer must be removed and the mineralized component of the supeficial layer of cementum needs to be decalcified.[1] Cementum is a mineralized tissue with primary function to insert the ligament fibers on the root surface.[2]

Biological concept of demineralization of the root surface is eliminating the smear layer and helps blood clot adhesion to collagen exposed, thus playing a supporting role in the formation of connective tissue new attachment.[3] The creation of a biologically acceptable root surface basically means that the root surface debridement should not hinder resolution of inflamation and without causing intentional removal of cementum.[4] The structure of root cementum are multiloculated for the insertion area of Sharpey fibers.[5]

Failure of connective tissue regeneration characterized by long junctional epithelium extending between the root surface and the gingival connective tissue.[6] Some research has been suggested that endotoxin present in the cementum could impair periodontal healing and should be removed to promote a more biologically acceptable surface than the one obtained only after scaling and root planing. Previous studies on tissue regeneration have used tetracycline hydrochloride (pH 1-2) to clean the root surface because it's bactericidal and demineralizing effect better than citric acids and EDTA. However, it is not clear the real performance of this protocol on the root dentin, mainly the use of tetracycline capsules, regarding product residues left after use and the smear layer removal capacity.[7]

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The use of tetracycline capsules cannot be applicated directly on the root surface. Tetracycline hydrochloride (Tetra HCl) is soluble in water, but Dibart and Karima explained that tetra HCl are dissolved in saline for root demineralization.[8,9] The tetra HCl capsule dissolved in saline showed a severe demineralization of root dentin substrate with the presence of a high amount of residues on the surface, but there is no residue appear when dissolved in sterile water with same concentration.[10,11] The solubility is dependent on the solvent intrinsic properties and solute-solvent interactions. It suggest that sodium chloride (NaCl) are soluble in water (H_2O), and less soluble in acid (HCl).[12]

The use of water in pharmaceutical industry is indispensable, especially in pharmaceutical liquid preparations. It serves many purposes such as an ingredient, solvent, excipients, for reconstitution of product, during synthesis, cleaning agents and other purposes in the production, processing and formulation of pharmaceutical products. Water as a universal solvent is able to dissolve, adsorb or suspend many different compounds.[13]

All that is fine for pure water, but in saline solution some other factors need to be considered especially with the ions in solution.[14] Saline solution containing sodium (Na) and chloride (Cl), which tetracycline HCl has the same chloride ions. It suggest that further study needed to observed the root demineralization outcome based on pharmacological characteristic of saline or sterile water and its use for tetra HCl application. The aim of this study was to determine differences between sterile water and saline solvent for Tetra HCl as a root cementum demineralization. Research about the difference between sterile water and saline for tetra HCl solvent as a root cementum demineralization need to be established for science information in dentistry, especially regenerative periodontal therapy.

MATERIALS AND METHODS

The design of this study was a laboratory experimental research and assessed by Scanning Electron Microscopy (SEM). Samples were obtained from fresh extracted tooth, with no caries or restoration on cemento enamel junction (CEJ), and no periodontal treatment for last 6 months. A total of 14 single-rooted human teeth were used in this study after approval by the Research Ethics Committee of Fakultas Kedokteran Gigi, Universitas Gadjah Mada, Yogyakarta, Indonesia (protocol #00243/KKEP/FKG-UGM/EC/2015).

Samples were scaled with ultrasonic scaler and root planed with Gracey currete for remove calculus and macro debris. Two parallel grooves with approximately 2 mm deep were made using a high speed cylindrical bur under copious irrigation. One groove was made at the CEJ and another one approximately 4 mm distant from the first, in the apical direction. The mesial and distal root surfaces of each tooth were used in sample preparation.

The specimens randomly divided into 3 groups (n=9/group): control - root surfaces were scaled with a ultrasonic scaler to remove calculus deposits, thus exposing visual clean dentin (this was the first step for all the others groups); saline + tetra HCl - after scaling the specimens was etched for 3 min with a solution obtained by dissolving 500 mg capsule of tetracycline HCl in 6.7 mL of saline solution; and sterile water + tetra HCl - after scaling the specimens was etched for 3 min with a solution obtained by dissolving 500 mg capsule of tetracycline HCl in 6.7 mL of sterile water solution. This method based on Ishi *et al.*[3] study that using concentration of tetra HCl 75mg/ml with stirring the capsule with solvents.

Application of the respective agents on the sample was done by burnishing method with cotton pellets saturated with the agent that were changed every 30 seconds for a total period of 3 minutes based on Vandana *et al.*[15] study. Following treatment, samples were rinsed with 10 ml sterile water and air dried. Samples were dehydrated in an increasingly graded series of ethanol: 30, 50, 70, 80, 95 and 100%. Then, the samples were dried overnight in a

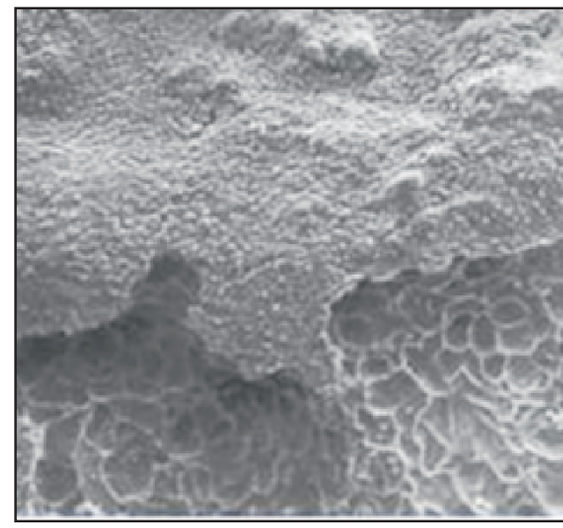

Figure 1. Cementum root surface with multilocular form and mineralized in the edge (magnif. 250x).

Table 1. Mean and SD smear layer percentage and collagen structure scoring on cementum after treatment

No.	Group	Mean and SD	
		Smear layer	Collagen structure
1.	Control	3.00 ± 0.00	1.00 ± 0.00
2.	Saline + tetra HCl	1.67 ± 0.50	2.00 ± 0.00
3.	Sterile water + tetra HCl	1.00 ± 0.00	2.67 ± 0.50

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dehydration jar, mounted on metallic stubs, sputter-coated with a thin titanium in a sputter coating machine (JEOL JEC-3000FC), and examined with a SEM (JEOL JSM-6510LA) at Laboratorium Penelitian dan Pengujian Terpadu, Universitas Gadjah Mada, Yogyakarta, Indonesia.

The surfaces of root specimens were scanned and observed on the computer screen fitted with the SEM at 100x and 1000x magnification. Each root surface was scanned in its entirety to obtain an overview of the general surface topography. Representative areas which were characteristic of the general surface topography were selected on each specimen and photographed.

The cemental SEM photographs were scored for smear layer based on Vandana *et al.*[15] using following scoring criteria: 0 = None; 1 = Smear layer involving random areas of surface that totals between 1-32% of total surface area; 2 = Smear layer involving random areas of surface that totals between 33-65% of total surface area; 3 = Smear layer involving > 66% of total surface area.[15] The SEM micrographs were scored according to ranking system by Houshmand *et al.*[21] for collagen structure exposure from cementum. Grade 3: Collagen structure could be seen and no debris present. Grade 2: Some collagen structure could be seen, although some debris is present. Grade 1: Significant debris may be seen and no collagen structure. The data were analyzed using the Kruskal Wallis statistical test.

RESULTS

Normality and homogeneity test were done for both groups with the results are non-homogen distribution and not normal data. Based on this results, data analyzed with Kruskal Wallis test. Kruskal Wallis test were done for the comparison of score percentage of smear layer per unit area and cementum collagen structure between the three study groups showed significance difference results ($p<0.05$). Mann-Whitney test were done for the mean difference of within groups showed that between saline + Tetra HCl and sterile water + Tetra HCl group are significantly difference ($p< 0.05$). Data was conducted using the SEM photomicrograph with the results as shown on Figure 2 and Figure 3.

DISCUSSION

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. 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.[16] Morphologic alterations and in vitro demineralization seem to be dependent on the nature

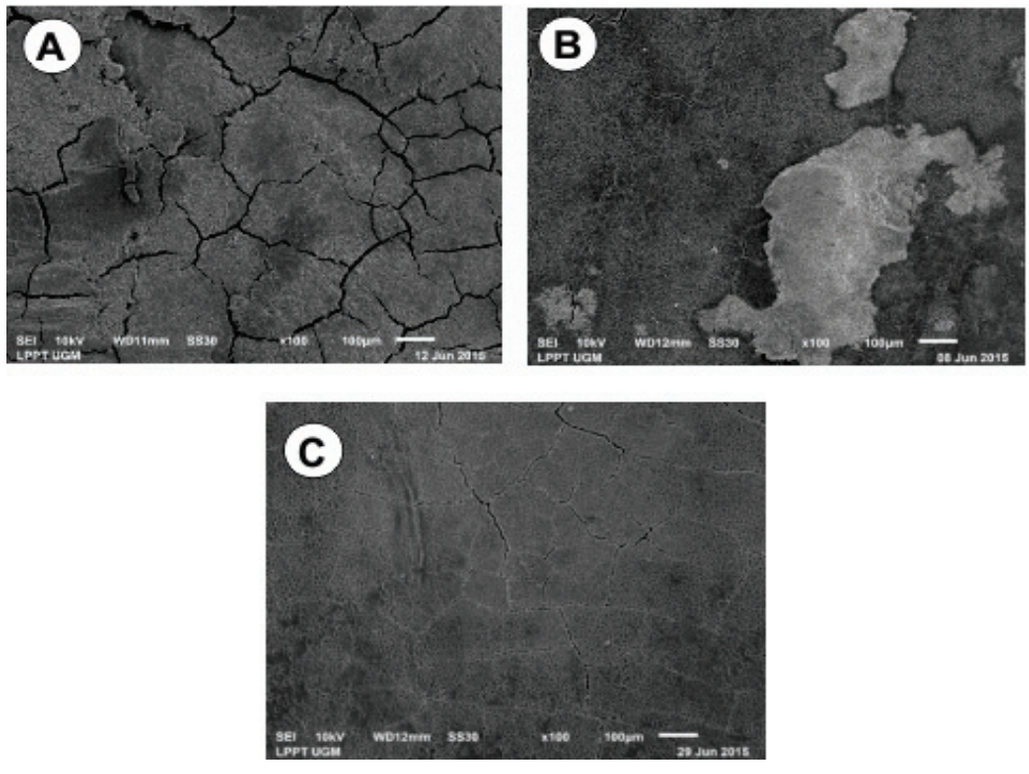

Figure 2. Surface morphology of smear layer cementum specimen (magnif. x100): (A) control; (B) saline + Tetra HCl; (C) sterile water + Tetra HCl.

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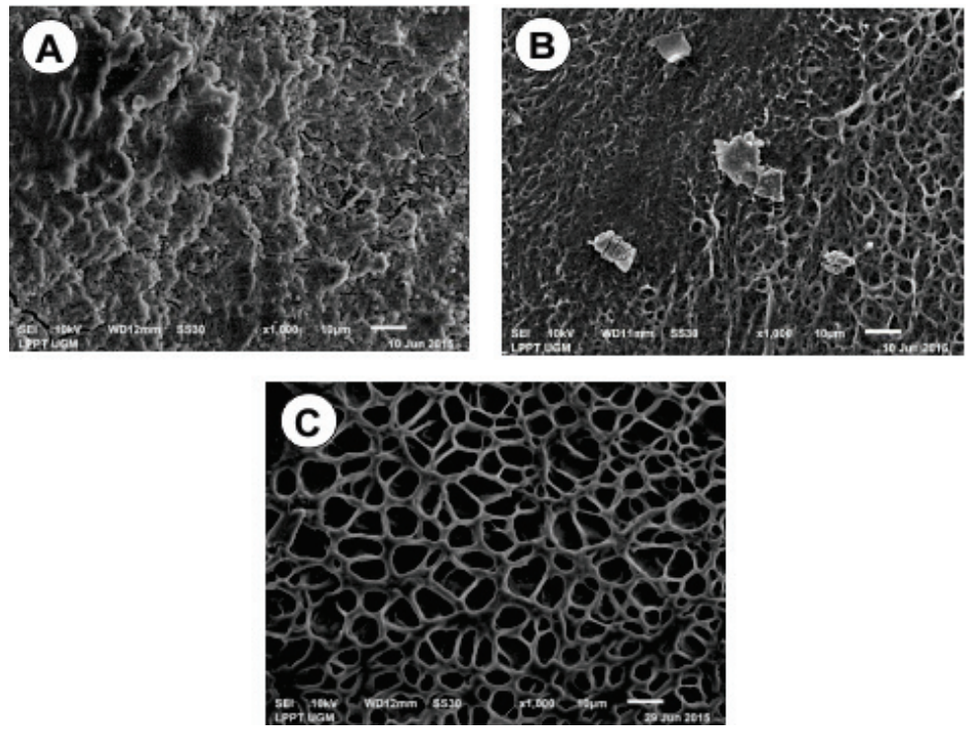

Figure 3. Surface morphology of cementum collagen structure (magnif. x1.000): (A) control; (B) saline + tetra HCl; (C) sterile water + Tetra HCl.

of the treated root surface, the method of application, the optimal concentration and application interval of tetracycline. These root surface characteristics which may support periodontal reconstructive therapy are important considerations.[17]

In the present study, the concentration of tetra HCl was fixed as 75 mg/ml in sterile water or saline solution keeping into consideration the observation of various studies. Previous study used different tetracycline HCl concentrations of 0, 10, 25, 50, 75, 100, 125 and 150 mg/ml for root demineralization and found that concentration between 50 mg/ml and 150 mg/ml showed a statistically significant opening of dentinal tubules. The solution was applied using "burnishing technique" in the present study. It has been observed by various studies that a burnishing technique resulted in a chemical/mechanical action that enhances the removal of chemically loosened inorganic material and surface debris, exposing the underlying root surface to the demineralization action of fresh acid solution. This may ultimately achieve an optimal degree of demineralization within a short period of time, in comparison to other application modes.[18]

Results of this study are discussed below on the percentage of smear layer and the collagen structure exposed cementum. Control group showed there is no apparent effect on the smear layer after scaling and water irrigation. This is in accordance with findings of Bhavikatti *et al*.[19] who found that water irrigation appeared to remove only the superficial portion of the smear layer, leaving root surface occluded with debris. The results of test groups showed the average percentage of smear layer on the root cementum with sterile water + Tetra HCl group was lowest and significantly different than saline + Tetra HCl group. This means that sterile water solvent at Tetra HCl was more effective in lowering the percentage of smear layer compared with saline solvent.

Tetra HCl well dissolved in sterile water (H_2O), while the solvent is saline (NaCl) in the Tetra HCl produce a crystal residue as seen on Figure 3B. The crystalline residue derived from excess chloride ion (Cl^-), which binds between hydrochloric acid and sodium chloride (NaCl), causing the precipitate in solution and create the effect of demineralization ineffective. Our results were consistent with the findings of Soares *et al.* who found similar results of crystalline residue from dissolving saline with Tetra HCl. Sodium chloride less soluble in hydrochloric acid as compared to water, due to the high concentration of chloride ions in a solution of hydrochloric acid. The solubility of the studied antibiotics in water is due to the presence of the hydrochloride group, which, in water, becomes Cl^-, which leads to the formation of ionic species and, thus, promotes an enhancement in the solubility.[20]

Results of this study showed that the means of amount of collagen structures exposed cementum on sterile water solvent tetra HCl group at most numerous and significant than saline solvent tetra HCl group. This means sterile water solvent at tetra HCl more exposed cementum collagen structure compared with saline solvent. The amount

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of collagen structure more exposed to the sterile water solvent at tetra HCl group due to the effect smear layer removal better than saline solvent, so that the collagen structure that was originally coated by the smear layer can be exposed. This showed that better smear layer removal, the cementum collagen structure will be more visible.[21] The result is supported by the action of chelating agents in tetra HCl as demineralization process in dissolving metals and other mineral.[22]

This study used same concentrations of tetra HCl (75mg/ml), application times (3 minutes), application method (burnishing technique), but with different solvents (saline or sterile water) for both test groups. The effect of demineralization with sterile water solvent for tetra HCl better than saline solvent, this means that sterile water as a solvent of tetra HCl is more effective for root cementum demineralization. This results was contrast with the findings of the Silva *et al.*[23] study where used saline as the solvent of tetra HCl, who found the residues showed after application on the root surface are probably related to the components present into the capsules, which were not completely dissolved.

This study presents some limitations such as the minimum samples were used. Further investigations needed for larger samples. Other relative limitation of this study is the difference on the time application for each substance. The difference time on the performance of the different products on the root dentin may impair on the results on this. However it is a relative limitation, because it is not possible to adjust the same time application for the different samples. Hence, additional studies of this variables are needed to validate the present findings.

Within the limits of this study, it can be concluded that tetra HCl dissolved in sterile water was better than in saline solution as a root cementum demineralization agent. In view of the present findings, further studies are necessary to establish the in-vivo importance of tetra HCl application as root demineralization agent as an additional step during periodontal therapy, especially in regenerative procedures.

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