Original Article

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Mechanical and acid root treatment on periodontally affected human teeth - a scanning electronic microscopy

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Abstract

Aim: To evaluate the root topography of human teeth affected by periodontitis, after different root surface treatments. **Methods:** Forty-two periodontally affected single-rooted human teeth were selected and randomly divided into 7 groups (n=6): Cont- control group, which received no treatment; Sca- root surface scaling and root planning with curettes; ScaPh- Sca followed by 37% phosphoric acid gel etching for 15 s; ScaEdta- Sca followed by 24% EDTA gel pH 7 for 1 min; ScaCi- Sca followed by 30% citric acid pH 1.6 for 5 min; ScaTe- Sca followed by - mixture obtained by 500 mg tetracycline capsule dissolved in saline solution for 3 min; ScaTeg- Sca followed by 0.2 g/mL tetracycline gel pH 1.8 for 1 min. The specimens were analyzed by scanning electronic microscopy to verify the presence of calculus, demineralization level and residues of the product. **Results:** Calculus deposits were found in all control specimens. ScaEdta, ScaCi and ScaTeg removed completely calculus deposits and resulted in adequate demineralization without smear layer and smear plug on root surface. ScaTe produced great tetracycline residues with several demineralization areas on root dentin surface. **Conclusions:** ScaEdta, ScaCi and ScaTeg produced clean root surfaces associated with regular dentin demineralization.

Keywords: scanning electron microscopy; periodontitis; conditioning; dental scaling; root planning and demineralization

Introduction

Dental calculus is constituted by mineralized structure with numerous holes, which leading to the accumulation of a larger number of microorganisms[1]. 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[2-3]. To regenerate the periodontal structure affected by disease it is necessary to eliminate calculus, bacterial plaque and cytotoxic substances from the contaminated root surface[4]. In addition, exposed cementum removal by scaling and root planning has been recommended as part of periodontal therapy[5-6]. These procedures can also effectively to remove the bacterial deposits and endotoxin[2]. After root planning, the instrumented root surface is invariably covered by a smear layer, which contains remnants of dental calculus, contaminated root cementum, bacterial toxins, and subgingival plaque[7]. Therefore,

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the use of additional chemical protocols after scaling and root planning tends to be decisive to achieve successful in periodontal therapy. Acid agents produce a demineralization zone that improves periodontal regeneration, which is an important natural phenomenon in the healing process[8-9]. The use of demineralizing agents increases the degree of connective tissue attachment to denuded roots[10]. This procedure exposes dentin collagen fibrils and opens dentinal tubules[11], removes cementum-bound proteins[11] and eliminates the contaminated smear layer produced by root planning[8,12].

Several agents, such as phosphoric acid[7], ethylenediaminetetraacetic acid[7,13-14], citric acid[7-8,15] and tetracycline hydrochloride[6,8,11] have been used to chemically treat the periodontitis affected root surfaces. Phosphoric acid, with low pH 1.94[16], has been widely used in adhesive dentistry to remove the smear layer, smear plug and to demineralize the peritubular and intertubular dentin[16]. On the other hand, it is not frequently used in periodontal therapy. EDTA with neutral pH has recently been used not only to preserve periodontal cell vitality but also to demineralized and remove the smear layer of the dentin[14-15]. This product also has bactericidal effects[4] depending on the concentration[6]. Citric acid has been recommended for removing smear and exposing collagen in order to retard gingival epithelium down-growth[10,17-18]. Another product usually used on periodontal treatment is tetracycline hydrochloride because of its effective antibacterial action on periodontal pathogens[11,19]. This product also demineralizes the root surface, removes the smear layer[10], promotes fibrin clot stabilization[10-11], increases chemotaxis, adhesion, and growth of fibroblasts on the root surface[19-20] and inhibits matrix metaloproteinases[21-22]. Tetracycline has been used as root surface conditioning agent to enhance periodontal tissue regeneration[19]. 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.

All chemical products used after scaling and planning of the dentin should result in clean surface without calculus remaining and adequate demineralization. Scanning electronic microscopy (SEM) is an adequate tool used to characterized surface of dental structures[13]. This methodology can be employed to demonstrate the effect of the different chemical protocols used in association with scaling and root planning over root dentin.

The aim of this study was to analyze by SEM the effect of hand scaling and planning associated with different acid agents on the roots of periodontally affected teeth, regarding to 3 factors: presence of calculus, product residues after use, and presence of smear layer and smear plug on dentin surface.

Material and methods

Forty-two single root human teeth extracted due to severe periodontitis were used in this study after approval by the local Research Ethics Committee (protocol #051/05). The specimens were randomly divided into 7 groups (n=6): **Cont** (control)- no root surface treatment was performed; **Sca**- root surfaces were scaled with a Gracey curette (Hu-Friedy, Chicago, IL, USA) to remove calculus deposits and cementum, thus exposing visual clean dentin (this was the first step for all the others groups); **ScaPh**- after Sca the dentin was etched with 37% phosphoric acid gel (SDI, Victoria, Australia) for 15 s. **ScaEdta**- after Sca the dentin was etched with 24% EDTA gel pH 7.0 (Biopharma, Uberlândia, MG, Brazil) for 1 min; **ScaCi**- after Sca the dentin was etched with 30% citric acid pH 1.6 (Biopharma, Uberlândia, MG, Brazil) for 5 min; **ScaTe**- after Sca the dentin was etched for 3 min with a solution obtained by dissolving one 500 mg capsule of tetracycline (Tetraciclina, Medquimíca, São Paulo, SP, Brazil) in 2 mL of saline solution; **ScaTeg**- after Sca the dentin was etched 0.2 g/mL tetracycline gel pH 1.8 (Biopharma, Uberlândia, MG, Brazil) for 1 min. After chemical etching, all specimens were rinsed for 1 min with 10 mL of saline solution.

The specimen was mounted on aluminum stubs, sputter-coated with gold, and examined with a scanning electron microscope (LEO 435 VP, Carl Zeiss, Germany). Images of representative areas of each specimen were obtained at 500-4000x magnification. The SEM micrographs were analyzed by 3 calibrated examiners according to 3 parameters: *Presence of calculus*: 1 – presence of great amount on dentin; 2- absence of calculus; *Presence of smear layer*: 1- presence of great amount of smear layer and smear plug; 2- absence of smear layer and smear plug; Presence of the product residues: 1- presence of great amount of product residues; 2- no product residues left use. Data were described by distribution and percentages for 2 parameters on each level, and were Data were presented by score distribution frequency. Representative SEM images of each group were obtained.

Results

The parameter scores for all groups are shown in Table

Table 1. Parameter scores evaluated by scanning microscopy analysis for all groups

Groups	Presence of calculus		Presence of smear layer		Presence of product residues	
	1	2	1	2	1	2
1- Control	6	-	na	na	na	na
2- Scaling and root planing	-	6	6	-	na	na
3- Phosphoric acid etching	-	6	-	6	-	6
4- EDTA etching	-	6	-	6	-	6
5- Citric acid etching	-	6	-	6	-	6
6- Tetracycline capsule dissolved in saline	-	6	na	na	6	-
7- Tetracycline gel	-	6	-	6	-	6

na - not applied to this group.

1. The SEM analysis showed that all dentin specimens of Cont group were covered by calculus and debris (Figure 1). Sca resulted in considerable amount of debris, no residual calculus, irregular root surface and a smear layer and smear plug covering all dentin (Figure 2). The SEM of the ScaPh group showed absence of smear layer and smear plug in all specimens, and no remaining of phosphoric acid (Figure 3). In the ScaEdta group was found the complete removal of smear layer, more regularity of the dentin surface and no remaining of the EDTA (Figure 4). The ScaCi protocol resulted in complete smear layer removal and no remaining of citric acid (Figure 5). In the ScaTe group, irregular demineralized dentin with deeper depressions and the presence of a high amount of the residual tetracycline was found on the dentin surface. Over all specimens of this group the dentin tubules were completely closed by tetracycline residues (Figure 6). The ScaTeg protocol resulted in a uniform demineralization of the dentin tubules, absence of smear layer and smear plug, and no remaining of the tetracycline gel (Figure 7).

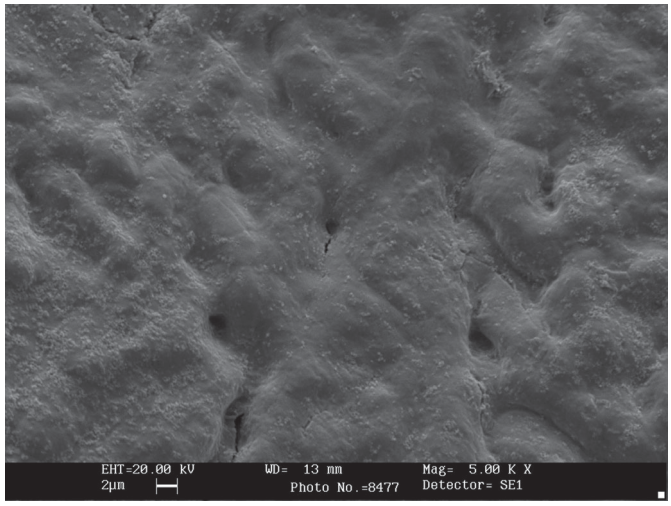

Fig. 3 - SEM image of the ScaPh group showing complete removal of smear layer and smear plug and no acid product residues.

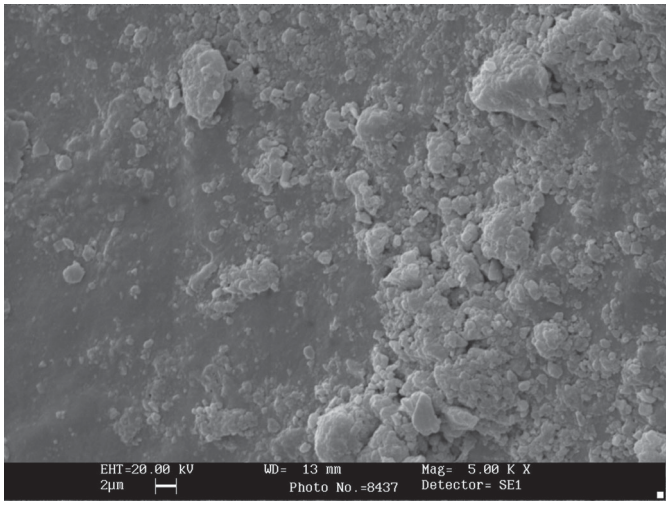

Fig. 1 - SEM image of the Cont group showing calculus and debris over root dentin, covering the entire root surface.

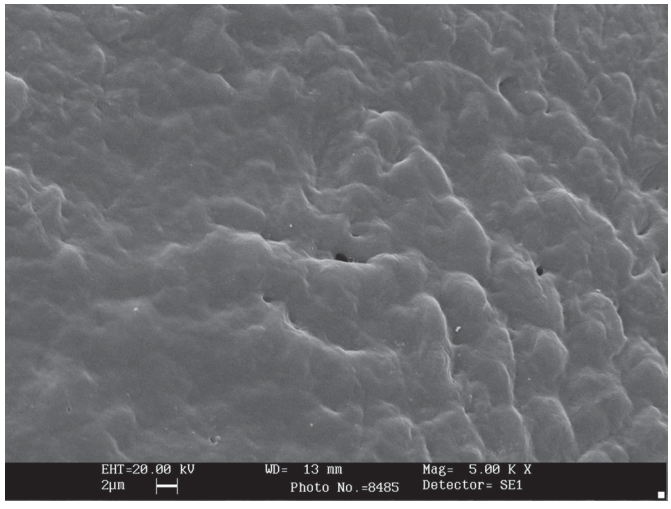

Fig. 4 - SEM image of the ScaEdta group showing complete removal of smear layer and no EDTA residues.

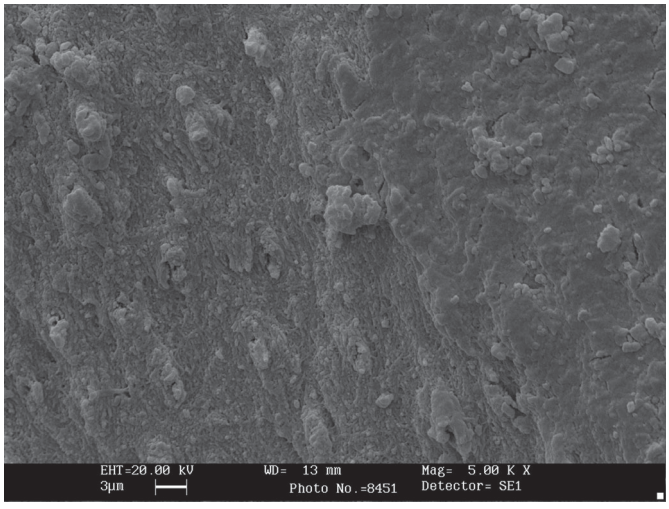

Fig. 2 - SEM image of the Sca group showing the presence of debris, no residual calculus, irregular root surface and smear layer and smear plug covering all root dentin.

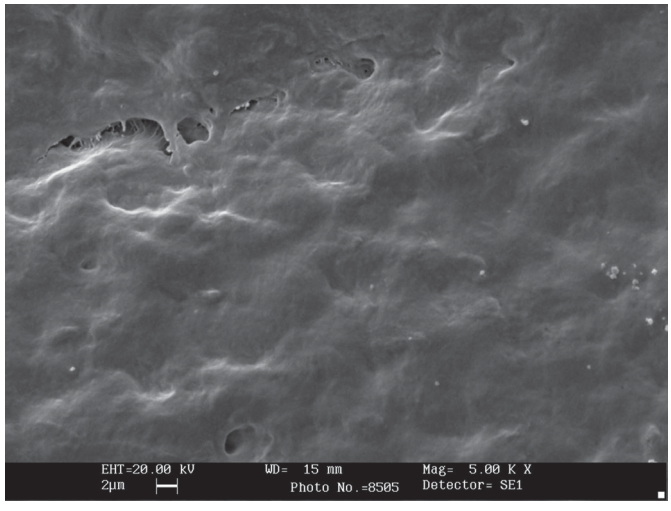

Fig. 5 - SEM image of the ScaCi group showing complete removal of smear layer and no citric acid residues.

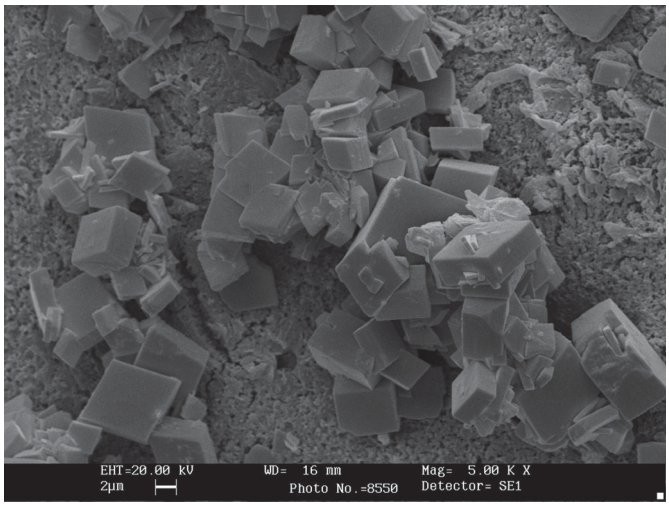

Fig. 6 - SEM image of the ScaTe demonstrating presence of residual tetracycline on dentin surface, and all dentin tubules closed.

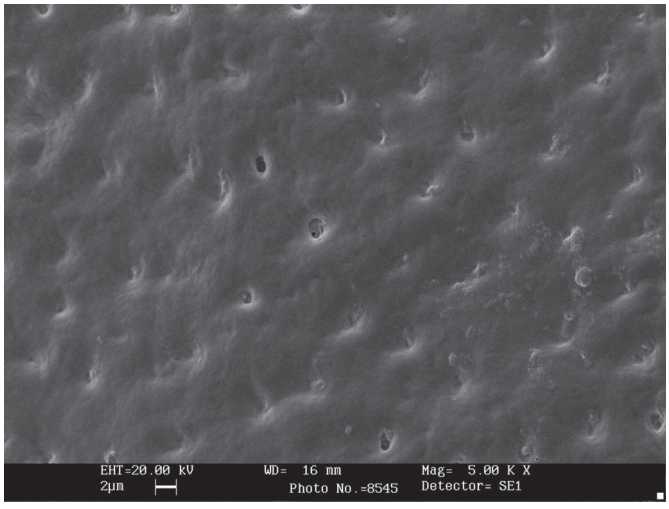

Fig. 7 - SEM image of the ScaTeg showing uniform demineralization of the dentin tubules, absence of smear layer and smear plug, and no tetracycline gel residues.

Discussion

One of the objectives of periodontal therapy is to convert the root surface affected by periodontitis into a surface biologically compatible with epithelial and connective tissue adherence and attachment[11]. The presence of dental calculus associated with plaque colonization is one of the main determinants of periodontal disease. This study demonstrated that all teeth (Table 1), extracted by the patients with periodontal disease, presented calculus with numerous holes (Figure 1).

The smear layer and mineralized debris formed after root surface instrumentation may serve as a physical barrier to the development of connective tissue attachment to the root surface[11]. In the present study, after scaling, the root dentin of all specimens (Table 1) was covered by smear layer smear plug (Figure 2). However, no calculus deposits were found after mechanical treatment (Table 1), demonstrating that this procedure should be the first protocol in all periodontal therapies. Since root scaling forms an irregular smear layer along the external root dentin, and it is invariably contaminated by toxins generate by previous disease present[5-6], additional chemical treatment is indicated in periodontal therapy.

The results of this study confirmed morphological modifications caused by the application of complementary substances. EDTA and citric acid were applied in the form of gel preparations, which are considered to increase the possibility of controlling the etching agent action[15]. The 24% EDTA used in this study was demonstrated by Blomlöf et al.[23] to be significantly more effective than the lower concentrations tested regarding to removal capacity of smear layer. The citric acid 30% used in this study is more effective to demineralize dentin than the lower concentrations ones[24]. Citric acid application causes superficial root surface demineralization[24], which is capable to eliminate bacterial endotoxinas[25]. This product is bactericidal[26] and capable to partially exposes dentin collagen[27]. This latter effect has been proven important to increase collagen splicing, improve fibrin linkage, and consequently inhibit epithelial down growth[28]. Although it was not assessed in this study, previous investigations have found that this effect stimulates the fibroblast attachment and migration[29], and facilitates new cementum formation[17,30]. In this study, the use of EDTA and citric acid after scaling and root planning resulted in effective smear layer and smear plug removal (Figures 4 and 5). However, it has recently been demonstrated that EDTA, which is used in a neutral pH (pH 7.0), is better to maintain the periodontal cell vitality adjacent to the etched surface than the citric acid[14] and phosphoric acid[14]. Hand scaled roots treated with phosphoric acid presented dentin demineralization with complete removal of the smear layer and smear plugs (Table 1 and Figure 3). However, this result should be carefully analyzed because this product has a high capacity of the demineralization. It could be a serious problem if some area was not covered by gingival after surgery, resulting in dentin hypersensitivity[31].

When the results of the groups that used tetracycline were compared, it could be observed that tetracycline gel removed the smear layer and no tetracycline residue was found. However, the tetracycline capsule dissolved in saline left tetracycline residues on the dentin root surface. The tetracycline from capsules for oral use bought at pharmacies and used to prepare an acid solution resulted in a significant amount of filler and other substances on dentin. This medication should be avoided until the effect of these materials on the root surface has been investigated[32]. The SEM results (Table 1) of this study showed a severe demineralization of root dentin substrate with the presence of a high amount of residues on the surface (Figure 6), occurred because tetracycline was not completely dissolved. The presence of residues of tetracycline particles during long time may result in continuous demineralization of the root dentin. Furthermore, this procedure caused an extremely irregular surface with many depressions after demineralization. For this reason, the application of tetracycline capsule dissolved in saline solution should be avoided. However, the same effect was not found with the tetracycline gel (Table

1 and Figure 7). The tetracycline gel tested in the present study presents additional benefits for use on dentin surfaces as the enhanced extracellular matrix glycoprotein fibronectin binding to dentin and stimulated fibroblast growth and attachment[19]. This substance has potent inhibitory effects on metalloproteinases[21-22], osteoclast function[21] and has antiinflammatory properties[19]. Furthermore, tetracycline is adsorbed to and subsequently released from dentin, maintaining its antimicrobial activity[33].

The results of this study suggest that tetracycline gel, EDTA or citric acids can be indicated to obtain an efficient and uniform demineralization of root dentin surface. Moreover, their pharmacological characteristics are important for periodontal disease management. However, other characteristics of these products should not be overlooked. Other researchers have suggested testing the viability of cellular fixation and the possibility of soft tissue alterations as a result of the use of these protocols.

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