

Effectiveness of EDTA and EDTA-T brushing on the removal of root surface smear layer

Efeito do EDTA e EDTA-T aplicado de forma ativa na remoção de "smear layer" de superfícies radiculares

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ABSTRACT: The purpose of this study was to compare the removal of root surface smear layer following active application of EDTA gel and EDTA-T (texapon) gel in different concentrations (5%, 10%, 15%, 20% and 24%), using scanning electron microscopy. A total of 220 dentin blocks obtained from the root surfaces of extracted teeth were divided into 3 groups: Group I - (control) application of saline solution (n = 20); Group II - EDTA gel (pH 7.0) was applied in the following concentrations: 5%, 10%, 15%, 20% and 24% (n = 100); Group III - EDTA-T gel (pH 7.0) applied in the same concentrations described above (n = 100). The photomicrographs were evaluated by one calibrated examiner using a smear layer removal index and following statistical analysis (Kruskal-Wallis test). The results demonstrated that the specimens treated with EDTA and EDTA-T gel presented a better smear layer removal than the control group (p < 0.01); no statistically significant differences were observed between the EDTA and EDTA-T groups and between the concentrations tested (Mann-Whitney, p > 0.05). Within the limits of this study, it can be concluded that all treatment modalities effectively removed the smear layer from the root surface. The addition of texapon into the EDTA gel formulation did not increase its effectiveness.

DESCRIPTORS: Dental scaling; Periodontics; Smear layer; EDTA.

RESUMO: O objetivo do presente estudo foi comparar, através de microscopia eletrônica de varredura, o efeito da aplicação de gel de EDTA e de EDTA-T (texapon) em diferentes concentrações (5%, 10%, 15%, 20% e 24%) na remoção de "smear layer" de superfícies radiculares previamente raspadas com instrumentos manuais. Duzentos e vinte espécimes de superfícies radiculares submetidas à raspagem foram divididos em 3 grupos. Grupo I - (controle) solução salina (n = 20); Grupo II - gel de EDTA (pH 7,0) nas concentrações de 5%, 10%, 15%, 20% e 24% (n = 100); Grupo III - gel de EDTA-T (pH 7,0) nas concentrações acima descritas (n = 100). As fotomicrografias obtidas foram avaliadas por um examinador calibrado através da aplicação de um índice de remoção de "smear layer", e os dados foram analisados através de análise estatística (teste de Kruskal-Wallis). Os resultados demonstraram que todos os tratamentos com EDTA e EDTA-T foram mais efetivos na remoção de "smear layer" quando comparados com o grupo controle (p < 0,01), e que não houve diferenças estatisticamente significantes entre os grupos tratados com EDTA e EDTA-T nas diversas concentrações (Mann-Whitney, p > 0,05). Dentro dos limites deste estudo, pode-se concluir que todas as modalidades de tratamento foram efetivas na remoção de "smear layer" e a adição de texapon ao gel de EDTA não aumentou a efetividade do tratamento.

DESCRITORES: Raspagem dentária; Periodontia; Camada de esfregaço; EDTA.

INTRODUCTION

One of the goals of periodontal therapy is the predictable regeneration of the periodontium in areas previously affected by periodontal disease^{14,24}. The key role of the diseased root surface in this process has been described²¹, and acid condition-

ing of the root surface after scaling and root planing has been introduced as a promising procedure for endotoxins and smear layer removal^{17,20,22}.

In several *in vitro* studies different agents such as citric acid¹⁶, ethylenediaminetetraacetic acid⁴ (EDTA) and tetracycline hydrochloride²⁵ have been employed. However, clinical studies do not demon-

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strate significant clinical differences regarding smear layer removal^{5,9}. On the other hand, dentinal tubules exposure may favor clot stabilization in the earliest stages of periodontal healing by increasing blood cells and fibrin adhesion to the root surface³, or even by improving the retention and contact of some substance, as the enamel matrix, to the root surface. To this extent, the smear layer removal would act as a growth factor in the periodontal healing processes¹³.

Regarding cervical dentinal hypersensitivity therapy, smear layer removal could lead to a higher permeability of the desensitizing chemical agents through the dentinal tubules, since it has not been possible to demonstrate their diffusion through the dentinal tubules in the presence of the smear layer^{15,19}.

The use of decalcifying agents operating at a neutral pH, such as EDTA, has recently demonstrated that it not only preserves the vitality of the remaining periodontal cells close to the root surface, but also removes calcium ions from the collagenous dentin matrix more selectively than low-pH etching agents⁵. The gel preparations may provide a better control of the etching agent⁵. However, in periodontal therapy, limited attention has been given to EDTA in gel preparations.

Thus, the aim of the present study was to compare, by scanning electron microscopy (SEM), the removal of smear layer following topical application, by brushing on the root surface, of EDTA gel and EDTA gel with the texapon detergent, added to EDTA in order to decrease the surface tension and to facilitate the spreading over the root surface. It also aims to evaluate the influence of the concentration and application time of these substances on smear layer removal from mechanically treated root surfaces.

MATERIAL AND METHODS

Teeth preparation

The ethical committee of the Araraquara Dental School, São Paulo State University (UNESP), approved this study. On hundred and three premolars and third molars indicated for extraction due to orthodontic reasons were used. After extraction the teeth were stored in a recipient with saline solution to avoid dehydration of the specimens.

Using a high-speed cylindrical bur under copious irrigation, two parallel retention grooves were made on the root surface of each tooth: one at the cementum/enamel junction and the other approx-

imately 4 mm apically to the first groove. After the two grooves were made, cementum was removed with the same bur between the two grooves on the buccal and lingual surfaces. After this procedure, scaling and root planning procedures were carried out using a Gracey 5-6 curette (Hu-Friedy®, Chicago, IL, USA) to remove all the remaining cementum layer, leading to dentin and dentinal tubules exposure, and creating a smear layer to be removed with the EDTA and EDTA with texapon (EDTA-T) gels.

The samples were prepared using a diamond disc (KG Sorensen, Barueri, SP, Brazil) where the roots were crosscut in the first groove, separating them from their crowns. Then the roots were cut lengthwise in the mesiodistal orientation until the second groove was reached apically where the sample was crosscut and separated in two samples of about 2 mm wide by 3 mm long, similarly to the samples removed from the premolars. The next step was the storage of these samples in a recipient with saline solution to avoid specimen dehydration.

Treatment groups

The 220 specimens obtained were randomly distributed according to the following treatment groups:

- Group I (control): Application of saline solution (n = 20).
- Group II: EDTA gel (pH 7.0) was applied in the following concentrations: 5% (n = 20), 10% (n = 20), 15% (n = 20), 20% (n = 20) and 24% (n = 20).
- Group III: EDTA-T gel (pH 7.0) was applied in the following concentrations: 5% (n = 20), 10% (n = 20), 15% (n = 20), 20% (n = 20) and 24% (n = 20).

In groups II and III, EDTA and EDTA-T gels were applied with a cotton pellet, which was replaced every 30 seconds. For each concentration the gels were applied for 1 (A), 2 (B) or 3 minutes (C), followed by a copious irrigation with saline solution (10 ml), or they received a 1-minute-application for 3 times, with a 10 ml saline solution irrigation between each 1-minute gel application (D).

For each subgroup (A, B, C and D) five samples were used in a total of 20 samples for each concentration of EDTA and EDTA-T. Thus, one hundred samples were used in groups II and III, and twenty samples in group I (control).

Sample preparation for SEM

After treatment of the root surfaces, samples were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.3) for 24 hours and washed three times each in phosphate buffer. The specimens were then dehydrated in a graded series of aqueous-ethanol solutions (50%, 70%, 85%, 95% and 100% ethanol) for 10 minutes each. Then the samples were dried overnight in a dehydration jar, mounted on SEM stubs, and sputter-coated with gold.

SEM examination

Photomicrographs were obtained with a 3,500 X magnification from a random area for each specimen, using a scanning electron microscope. One previously calibrated and trained examiner evaluated the photomicrographs, in order to determine smear layer removal from the root surface using the following scores²³:

- Score 1: Root surface without smear layer with the dentinal tubules completely opened without evidence of smear layer in the dentinal tubules.
- Score 2: Root surface without smear layer with the dentinal tubules completely opened, but with some evidence of smear layer in the dentinal tubules entrance.
- Score 3: Root surface without smear layer with the dentinal tubules partially opened.
- Score 4: Root surface covered by a uniform smear layer, with evidence of dentinal tubules opening.
- Score 5: Root surface covered by a uniform smear layer without evidence of dentinal tubules opening.
- Score 6: Root surface covered by an irregular smear layer, with the presence of grooves and/or scattered debris.

Statistical analysis

Smear layer removal scores were independently analyzed considering group (control, EDTA and EDTA with texapon), concentration and application time as independent variables. The non-parametric Kruskal-Wallis test was used to compare the average rank for each group, concentration, as well as for each application time tested. If $p \leq 0.05$, Dunn's Multiple Comparison test was applied to detect statistically significant differences between groups, concentrations and application times tested. The non-parametric Mann-Whitney test was used to compare smear layer removal scores

between groups II (EDTA) and III (EDTA with texapon) for each concentration tested ($p \leq 0.05$). The statistical analyses were performed using a computer software (Graph Pad InStat Software 3.05, San Diego, CA, USA).

RESULTS

Group I (control)

All specimens in this group had smear layer. In ten specimens the root surface was covered by a uniform smear layer, with evidence of dentinal tubules opening (score 4), and eight specimens were covered by an irregular smear layer, with presence of grooves and/or scattered debris without evidence of dentinal tubules (score 6) (Figure 1). The other two specimens were covered by a uniform smear layer without evidence of dentinal tubules opening (score 5).

Group II (EDTA)

All specimens demonstrated dentin exposure. In the 5% EDTA group, nineteen specimens had no smear layer (scores 1, 2 and 3) and seven of them presented the dentinal tubules completely opened (score 1) (Figure 2). In the 10% EDTA group all specimens had no smear layer covering the root surface and eleven specimens demonstrated dentinal tubules completely opened, but with some evidence of smear layer in the dentinal tubules entrance (Figure 3). All specimens in the 15% EDTA group had no smear layer (scores 1 and 2) and in twelve specimens the dentinal tubules were completely opened without evidence of smear

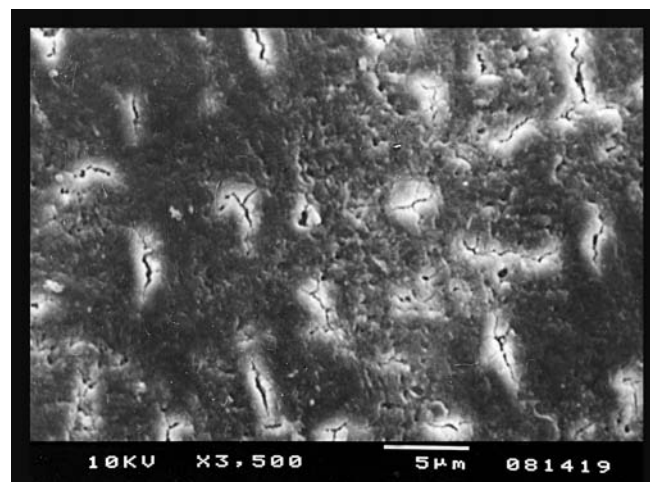


FIGURE 1 - SEM of a specimen from the control group showing a uniform smear layer, without evidence of dentinal tubules (score 6). (3,500 X; bar = 5 μ m).

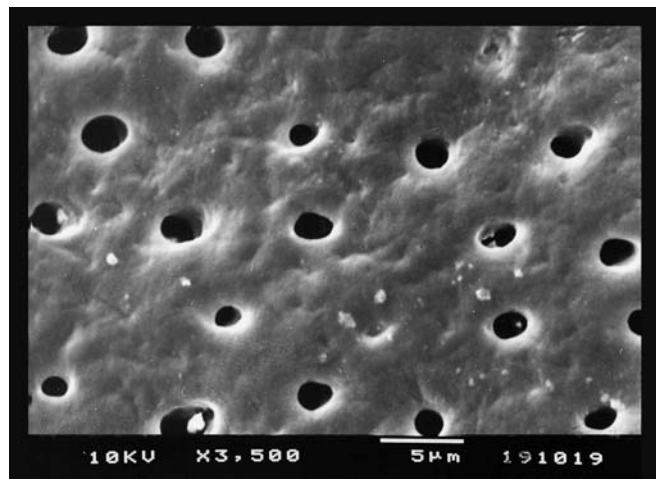


FIGURE 2 - SEM of a specimen from the 5% EDTA group showing a root surface without smear layer, with the dentinal tubules completely opened (score 1). (3,500 X; bar = 5 µm).

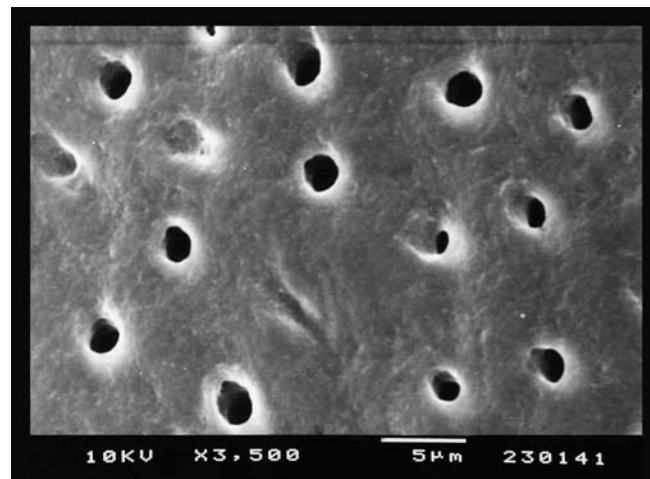


FIGURE 4 - SEM of a specimen from the 20% EDTA group showing a root surface without smear layer, with the dentinal tubules completely opened (score 1). (3,500 X; bar = 5 µm).

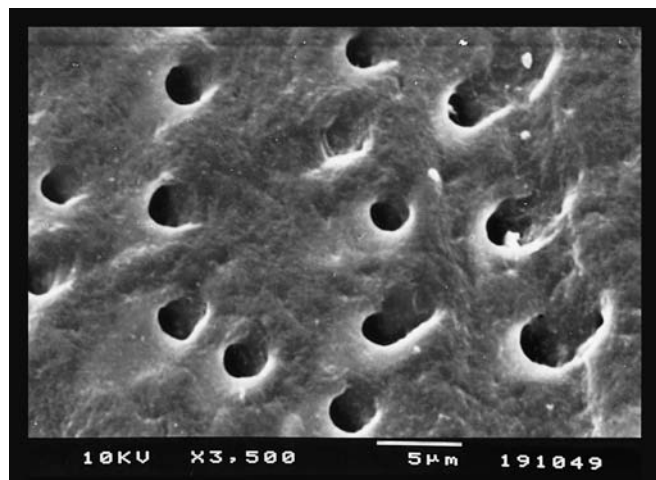
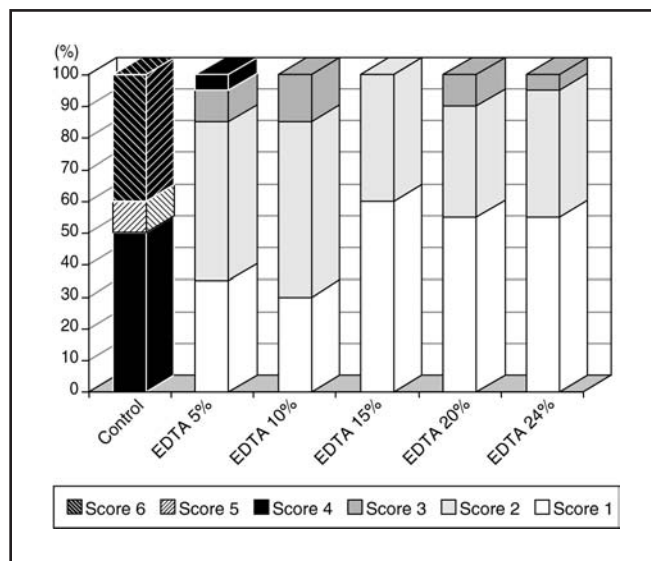


FIGURE 3 - SEM of a specimen from the 10% EDTA group showing a root surface without smear layer with the dentinal tubules completely opened, but with some evidence of smear layer in the dentinal tubules entrance (score 2). (3,500 X; bar = 5 µm).



GRAPH 1 - Distribution of smear layer scores for each concentration tested in the EDTA group.

layer in dentinal tubules. In the 20% EDTA group all specimens had no smear layer on the root surface (scores 1, 2 and 3) and in eleven specimens the dentinal tubules were completely opened without evidence of smear layer in dentinal tubules (Figure 4). In the 24% EDTA group, all specimens had no smear layer on the root surface (scores 1, 2 and 3) and in most of the specimens (11) the dentinal tubules were completely opened without evidence of smear layer in the dentinal tubules (score 1) (Graph 1).

Group III (EDTA-T)

As in group II, all specimens had their dentin exposed. In the 5% EDTA-T group nineteen specimens had no smear layer (scores 1, 2 and 3) and twelve of them had the dentinal tubules completely opened without evidence of smear layer in the dentinal tubules (Figure 5). In the 10% EDTA-T group all specimens had no smear layer (scores 1, 2 and 3), and twelve of them showed completely opened dentinal tubules, but with some evidence of smear layer in the dentinal tubules entrance

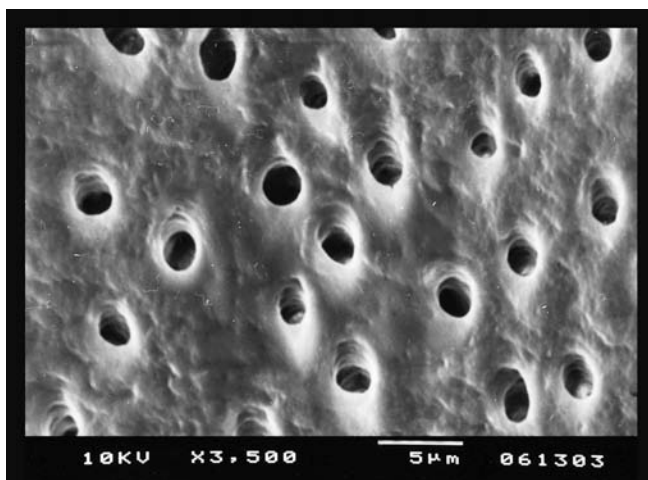


FIGURE 5 - SEM of a specimen from the 5% EDTA-T group showing a root surface without smear layer, with the dentinal tubules completely opened (score 1). (3,500 X; bar = 5 µm).

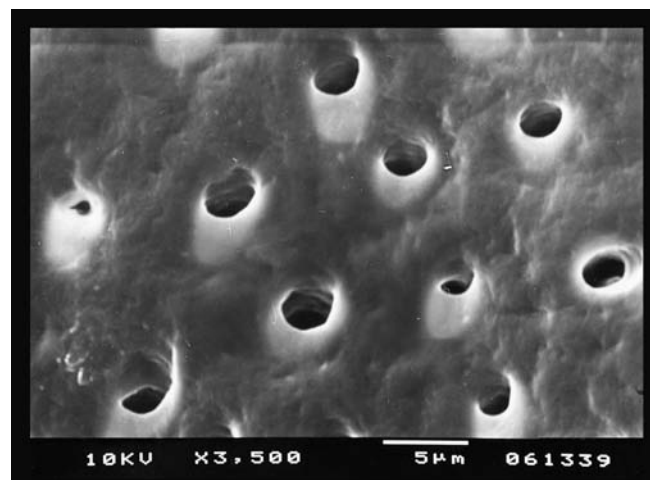


FIGURE 6 - SEM of a specimen from the 15% EDTA-T group showing a root surface without smear layer, with the dentinal tubules opened (score 1). (3,500 X; bar = 5 µm).

(score 2). In the 15% EDTA-T group all specimens had no smear layer on the root surface (scores 1, 2 and 3) (Figure 6), but three specimens had partially opened dentinal tubules (score 3). In the 20% EDTA-T and 24% EDTA-T groups the specimens did not show smear layer on the root surface, as in the other EDTA-T groups (scores 1, 2 and 3) (Figure 7) (Graph 2).

Data analysis

Considering concentration as an independent variable, the non-parametric Kruskal-Wallis test showed that there was a statistically significant difference between the concentrations tested regarding smear layer scores ($p < 0.0001$). Dunn's Multiple Comparison test demonstrated that all the EDTA gels exhibited statistically significant lower smear layer scores, compared to those of the control group ($p < 0.01$), but there was no difference between the different concentrations of EDTA gel tested ($p > 0.05$).

When the EDTA-T gels were analyzed, the non-parametric Kruskal-Wallis test also showed that there was a statistically significant difference between the gels tested regarding smear layer scores ($p < 0.0001$). All EDTA-T gels exhibited a lower smear layer score compared to that of the control group ($p < 0.001$), but no difference could be found between the different concentrations of EDTA-T gels tested ($p > 0.05$). The non-parametric Mann-Whitney test demonstrated that, when the smear layer removal scores between groups II (EDTA) and III (EDTA with texapon) were tested for

each concentration, there was no difference between the groups for any of the concentrations tested.

Regarding the application times tested, the 20% EDTA gel application for 3 minutes resulted in an improved smear layer removal when compared to that of the groups where the gel was applied for 1 and 2 minutes. For the 5% EDTA gel a better smear layer removal was also noted when the gel was applied for 2 and 3 minutes, compared to that of the 1-minute group.

DISCUSSION

In this study, the specimens treated by scaling and root planing followed by saline solution application (Group I) demonstrated an irregular smear layer formation along the root surface, in accordance with several previous studies^{1,2,11,12}. Smear layer is formed by residual calculus, microorganisms and their endotoxins, cementum and dentin fragments which should be removed by the use of root surface conditioning agents in order to favor a new connective attachment with new cementum formation after regenerative procedures¹⁷, although some clinical trials have failed to demonstrate significant clinical differences between conditioned root surfaces and controls, in both surgical and non-surgical periodontal therapies^{5,9}.

The use of EDTA gel with or without a detergent (texapon) after scaling and root planing procedures resulted in an effective smear layer removal with dentinal tubules exposure in many speci-

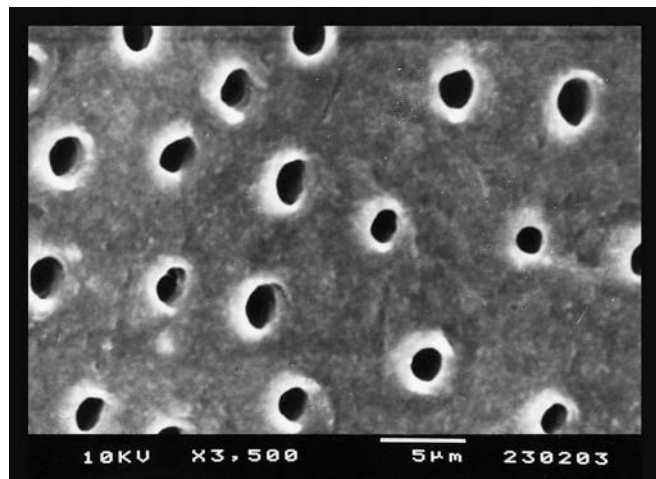


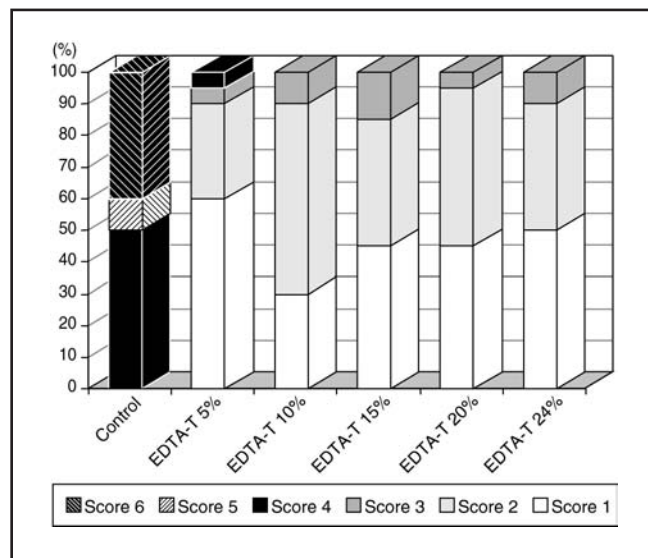
FIGURE 7 - SEM of a specimen from the 24% EDTA-T group showing a root surface without smear layer, with the dentinal tubules opened (score 1). (3,500 X; bar = 5 µm).

mens in SEM photomicrographs. These findings are corroborated by several studies where the use of EDTA led to a complete smear layer removal from the root surface^{6,7,8}.

Root surface conditioning, by exposing collagen fibers from the dentin extracellular matrix^{1,2}, may also favor fibrin deposition and consequently clot stabilization in the earliest phase of periodontal healing³, increasing the retention and contact of substances actually used during regenerative procedures, such as enamel matrix derivative proteins, which could act as a growth factor during the periodontal healing process¹³.

Many substances have been proposed for root surface conditioning after scaling and root planing. EDTA, which operates in a neutral pH, has recently been demonstrated to maintain periodontal cell vitality adjacent to the etched surface, in comparison to agents which operate in an acid pH, such as citric acid. In the present study, a detergent named texapon was added to the EDTA gel formulation (EDTA-T) to examine its effects on smear layer removal and also on dentinal tubules exposure. Although detergents used alone seem to remove bacteria and toxins from the root surface, they do not lead to a selective removal of hydroxyapatite and collagen exposure from the cementum or dentin extracellular matrix¹⁸.

According to the methods used in this study, the data analysis demonstrated that there was no statistically significant difference in smear layer scores between the groups treated by EDTA or EDTA-T application. Thus, the addition of a deter-



GRAPH 2 - Distribution of smear layer scores for each concentration tested in the EDTA-T group.

gent such as texapon did not lead to additional benefits considering smear layer removal compared to the use of EDTA alone in a gel formulation.

Considering EDTA gel concentration, no statistically significant difference could be found between the different groups concerning smear layer removal. This may be explained by the fact that the EDTA gel formulation exhibited a higher viscosity for the higher concentrations, hindering EDTA gel spreading over the mechanically treated root surface. However, an effective contact of the EDTA gel molecules to the root surface probably occurred, both for the lower and the higher concentrations, due to the act of brushing the gel on the root surface. Therefore, the active brushing of the gel on the root surface overcame the undesirable higher viscosity of the 24% EDTA gel, for example.

In this study the application time influenced the effectiveness of the smear layer removal and dentinal tubules opening by the EDTA gel formulations. These findings are corroborated by several studies that have analyzed the influence of the application time of this root surface conditioning agent^{4,5}.

CONCLUSION

Within the limits of this study, it was possible to conclude that the EDTA gel formulation effectively removed smear layer from the instrumented root surface, and that the addition of a detergent (texapon) in its formulation did not improve the re-

sults usually found with the EDTA gel. There was no difference between the different EDTA gel concentrations tested, and the application time could influence the effectiveness of smear layer removal. Further studies are needed to establish the real importance of EDTA gel application as an additional step during periodontal therapy, especially in regenerative procedures, in order to provide a

biologically acceptable environment that could favor connective tissue cell colonization of the diseased root surfaces.

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