Morphology and adhesion on blood components in root surfaces treated by a piezoelectric ultrasonic: an in vitro study

Análise da morfologia e da adesão de elementos sanguíneos em superfícies radiculares instrumentadas com ultrassom piezoelétrico: estudo in vitro

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ABSTRACT

Objective
The aim of this study was to evaluate the morphology and adhesion of blood components on root surfaces instrumented with piezoelectric ultrasonic Piezon Master Surgery.

Methods
10 teeth were used in this study. The teeth had their proximal divided into four areas that received different treatments: Group 1: untreated control Group 2: scaling with manual instrument; Group 3: scaling with ultrasound; Group 4: Scaling with manual instruments and ultrasound. We obtained 20 samples, 10 of which were used to analyze the morphology and the other 10 were used for analysis of adhesion of blood components. The specimens were analyzed by scanning electron microscopy. Photomicrographs were analyzed by the scores of adhesion of blood components and the index of root morphology. The results were statistically by the Kruskall-Wallis and Mann-Whitney with a significance level of 95%.

Results
The morphological analysis showed that the Group 1 had a surface unchanged in relation to other groups (Group 1 X Group 2 = 0.0025; Group 1 X Group 3 = 0.0003; Group 1 X Group 4 = 0.0003) and Group 2 presented a smoother surface compared to Group 1 and groups instrumented with ultrasound (Group 2 X Group 3 = 0.0025; Group 2 X Group 4 = 0.0025) there were no statistical differences between the Groups 3 and 4. analysis of adhesion of blood components showed that the Groups 2, 3 and 4 had no statistically significant differences between themselves, but more biocompatible surfaces promoted the surface untreated control (Group 1 X Group 2 = 0.02; Group 1 X Group 3 = 0.04; Group 1 X Group 4 = 0.005).

Conclusion
The instrumentation with piezoelectric ultrasonic promoted an irregular root surface, but did not negatively affect the adhesion of blood components.

***Indexing terms**: Dental scaling. Regeneration. Ultrasonic.*

RESUMO

Objetivo
Avaliar a morfologia e a adesão de células sanguíneas em superfícies radiculares instrumentadas com o ultrassom piezoelétrico Piezon Master Surgery.

Métodos
Foram utilizados nesse estudo 10 dentes que tiveram suas proximais divididas em 4 áreas que receberam diferentes tratamentos: Grupo 1: controle sem tratamento; Grupo 2: raspagem com instrumento manual; Grupo 3: raspagem com ultrassom; Grupo 4: associação instrumento manual e ultrassom. Foram obtidas 20 amostras, sendo que 10 foram utilizadas para análise da morfologia e as outras 10 foram utilizadas para a análise de adesão de elementos sanguíneos. Os espécimes foram analisados em microscópio eletrônico de varredura. As fotomicrografias foram analisadas através dos scores de adesão de elementos sanguíneos e pelo índice de morfologia radicular e os resultados foram analisados estatisticamente através dos testes de Kruskall-Wallis e de Mann-Whitney com nível de significância de 95%.

Resultados
A Análise morfológica demonstrou que o Grupo 1 apresentou uma superfície inalterada em relação aos outros grupos (Grupo 1 X Grupo 2 = 0.0025; Grupo 1 X Grupo 3 = 0.0003; Grupo 1 X Grupo 4 = 0.0003), e o Grupo 2 apresentou uma superfície mais lisa em relação ao Grupo 1 e aos grupos instrumentados com ultrassom (Grupo 2 X Grupo 3 = 0.0025; Grupo 2 X Grupo 4 = 0.0025). Não houve diferenças estatísticas entre os Grupos 3 e Grupo 4. A análise de adesão de elementos sanguíneos demonstrou que o Grupo 2, Grupo 3 e Grupo 4 não apresentaram diferenças estatisticamente significantes entre si, porém promoveram superfícies mais biocompatíveis que a superfície controle sem tratamento (Grupo 1 X Grupo 2 = 0.02; Grupo 1 X Grupo 3 = 0.04;Grupo 1 X Grupo 4 = 0.005).

Conclusão
A instrumentação com ultrassom promoveu uma superfície radicular irregular, porém não afetou negativamente a adesão de elementos sanguíneos.

Termos de indexação: Raspagem dentária. Regeneração. Ultrassom.

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INTRODUCTION

Periodontitis is a set of chronic destructive inflammatory diseases of the supporting structures of the tooth, which is mainly caused by periodontal pathogens with their endotoxins and other antigens found in biofilm, which induces an inflammatory response in the host[1-2]. The main objective of periodontal treatment is biofilm removal with consequent reduction in the inflammatory process, causing the stagnation of disease progression[3-4].

Scaling and root planing (SRP) with manual curettes is considered the gold standard treatment of periodontitis, due the efficacy of removing the calculus and biofilm attached to the root surface, and the clinical outcomes promoted by this tool in the treatment of periodontitis[2,5]. However, some studies have reported limitations of SRP when treating regions with difficult access,such asfurcations, deep pockets and high dependence on the manual skill and experience of the clinician in obtaining good outcomes[2,4,6]. Additionally, SRP promotes irregularities on the root surface and a high degree of tooth wear[4-5].

Therefore, other tools have been proposed for the treatment of periodontal diseases with the aim of facilitating access to the root surfaces, such as ultrasonic scalers[7-8], lasers[9], air-polishing devices[10], and sonic scalers[3]. Ultrasonic scalers have increasingly been applied in periodontics because they have several advantages such being easy to use, efficient during scaling procedures, availability of a variety of tips for access to different anatomical regions[3,11], causing reduced operator fatigue, and reducing treatment time[4,8].

The Piezon Master Surgery ultrasonic scaler (Piezon Master - Electro Medical Systems) was introduced on the market with an indication for endodontic and surgical procedures, and thevibratory movements provided by this instrument are described as delicate, allowing its use in procedures such as augmentation of the maxillary sinus floor[12]. This device has specific tips for periodontal treatment, but no research has evaluated the characteristics of root surfaces instrumented with this ultrasonic scaler. The aim of this study was to evaluate, by scanning electron microscopy (SEM), the morphology and adhesion of blood components to root surfaces instrumented with a piezoelectric ultrasonic scaler (Piezon Master - Electro Medical Systems).

METHODS

Ten single-rooted or multirootedteeth, extracted due to several factors were used in this study. The teeth were obtained from the Tooth Bank of the Araraquara Dental School (FoAr-UNESP), and this project was accepted by the Ethics Committee on Studies Involving Human Beings (CEP-12/09), respecting the ethical principles contained in the Declaration of Helsinki (2000).

Sample preparation and treatment

The proximal surfaces of the roots were delimited with grooves made with the aid of a multilaminar carbide bur (7664FKG Sorensen, Barueri, Brazil). The coronal groove was placed at the cemento-enamel junction, and the apical groove 4 mm apically to the coronal groove. The anterior and posterior grooves were placed on the boundary region of the dihedral angle between the proximal surfaces, with the buccal and lingual / palatal surfaces, respectively. The enclosed area was divided into four regions with similar area (2x2mm). The regions were treated with different protocols as follows (Figure 1): Group 1 (G1): the control group without treatment; Group 2 (G2): the samples were treated by SRP, which was performed in 50 traction movements in the cervical-occlusal direction,using a manual curette (Gracey curette, no. 5-6; Hu-Friedy, Chicago, IL, USA); Group 3 (G3): the samples were treated using the piezoelectric ultrasonic scaler (Piezon Master Surgery; Electro Medical Systems, Nyon, Geneva, Switzerland), with tip RS3, which was used in standard mode with 30 kHz power and a water rate of 30 ml/min for 30 s; Group 4 (G4): the samples were treated by SRP associated with the ultrasonic scaler used with the same parameters as those used in Groups 2 and 3. All the treatments were performed by only one trained operator.

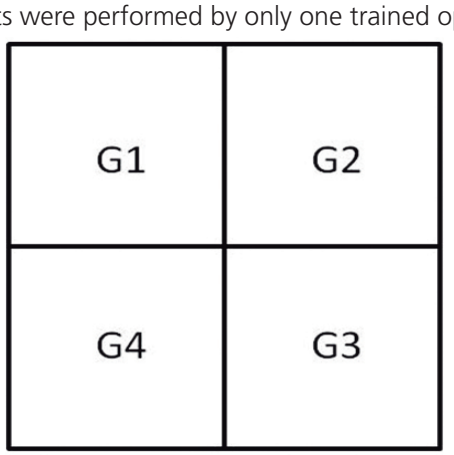

Figure 1. Model of the sample used in the study.

After this,using a diamond bur (KG Sorensen, Barueri, SP, Brazil) under slow rotation, the teeth were cross-sectioned in the following manner: The roots were crosscut at the first groove, thereby separating them from the crown; the second cut was performed in the bucco-lingual direction in middle third of the root and finally the third cut were made at the second groove located 4 mm apically of the first groove. Next, a markup was made in the region of Group 1 in order to facilitate orientation of the samples during the SEM analysis. Thus, one sample was obtained for the mesial surface and one sample for the distal surface, measuring about 1mm in thickness, totaling 2 samples per tooth and therefore 20 samples. Ten of these samples were used for the morphology analysis, and the other 10 samples were used for the analysis of blood component adhesion.

SEM analysis of blood component adhesion and morphology

After the treatment of the samples, 10 ml of blood was obtained from the peripheral blood vessels of an adult non-smoker without systemic impairment, via puncture with a syringe and disposable needle. This was performed at the School of Pharmaceutical Sciences in Araraquara, after the patient had signed the Term of Free and Informed Consent document.

In all of the experimental groups, a drop of blood (1 ml) was deposited on the samples with the aid of a syringe and needle and kept in a humidifying chamber for 20 min. The samples were then immediately washed three times for 5 min with a phosphate buffer solution (PBS, pH 7.0) by means of a shaker. Immediately afterwards, the samples were identified and fixed in formaldehyde in a phosphate buffer solution (1%) for 15 min. After three 5-min washes with phosphate buffer solution, the samples were incubated for 10 min in 0.02 M of glycine in phosphate buffer solution and washed again. They were then fixed in glutaraldehyde in phosphate buffer solution (2.5%) for 30 minutes and washed again. Subsequently, the samples were dehydrated in a graded series of ethanol (25%, 50%, 75%, and 95%) for 10 min in each solution, and washed three times for 10 min in absolute ethanol.

For the morphological analysis of the root surfaces, the samples were subjected to dehydration in a graded series of ethanol (25%, 50%, 75%, 95%, and 100%) for 1 h in each solution. After this procedure, the samples were placed on acrylic slides and hexamethyldisilazane (HMDS) was applied. First, 0.8 ml of HMDS + 0.8 ml of absolute alcohol, measured by means of an automatic pipette (Boeco, Hamburg, Germany) was placed in each well, and the samples remained in the HMDS/alcohol mixture for 30 min. The solution was then removed and the wells were filled with 1 ml of pure HMDS, in which the specimens remained for 10 min.

All the samples subjected to these processing procedures were dried in ambient air for 20 min. After subsequent drying with a carbon dioxide critical point apparatus, the specimens were fixed on metal stubs and placed in a vacuum desiccator for 48 h. After this period, the samples were sputter-coated using a Balt-Tec SCD-050 device for 120 s. The samples were analyzed by 20 kV SEM (Jeol-JSM, Tokyo, Japan) and photomicrographs were obtained at 1,000 and 2,000X magnifications. Next, the photos were analyzed by an experienced, calibrated examiner, who was blind to the identity of the samples, and made the description of blood component adhesion on the root surfaces according to the index of blood component adhesion[6]: 0) the absence of a fibrin network and blood cells; 1) a scarce fibrin network and/or blood cells; 2) a moderate fibrin network and a moderate number of blood cells; 3) a dense fibrin network and trapped blood cells.

The same examiner evaluated the root morphology according to an index of root surface morphology: 0) an intact cementum; 1) a smooth root surface; 2) an irregular root surface with the presence of intact dentin and cementum; 3) an irregular root surface without the presence of intact dentin and cementum.

Statistical analysis

The Bioestat 5.0 (Belém, Brazil) software program was used for statistical analysis. To evaluate the significant differences between the groups with regard to blood component adhesion and root surface morphology, the non-parametric Kruskal-Wallis test was used. With the occurrence of significant differences between the groups, the non-parametric Mann-Whitney U test was used to identify the groups that exhibited significant differences.All the tests used in this study were applied using a significance level of 95%.

RESULTS

Root surface morphology

Group 1 (without treatment) - the majority of the samples of this groups presented an intact layer of cementum (80%), representative of score 0(Figure 2A). The other samples presented a smooth pattern,

with the presence of smear layer and cementum (20%), representative of score 2.

Group 2 (SRP)-All the samples of this group (100%) presented a smooth aspect, with the presence of smear layer and occluded dentinal tubules, and these features are representative of score 2 (Figure 2B).

Group 3 (Ultrasonic Scaler)- An irregular root surface, with the presence of grooves promoted by the ultrasonic tip; presence of smear layer and occluded dentinal tubules were the main features observed after the ultrasonic scaler treatment (80%), representative of score 3 (Figure 3 C). In 20 % of the samples, a smooth surface was observed, with the presence of smear layer, and occluded dentinal tubules, representative of score 2.

Group 4 (SRP + Ultrasonic Scaler)- The pattern observed in this group was similar to that observed in Group 3, in which the majority of the samples presented score 3 of the index of root surface morphology, which is characterized by an irregular root surface, with the presence of grooves, smear layer, and occluded dentinal tubules (80%)(Figure 2D). The other samples (20%) presented a smooth surface, with the presence of smear layer, and occluded dentinal tubules, representative of score 2.

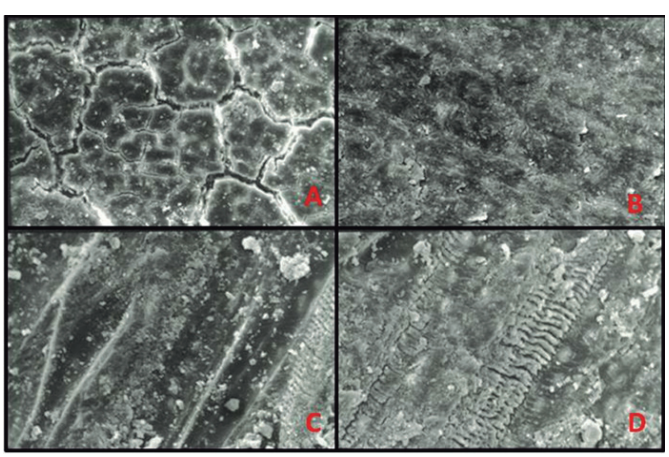

Figure 2. Morphological analysis- A: Group 1 (Control). Root surface with intact cementum (score 1); B: Group 2 (SRP). Smooth and regular root surface, with occluded dentinal tubules, and presence of smear layer (score 2); C: Group 3 (Ultrasonic scaler). Irregular root surface with presence of grooves, occluded dentinal tubules, and smear layer (score 3); D: Group 4 (Ultrasonic scaler+SRP). Irregular root surface with presence of grooves, occluded dentinal tubules, and smear layer (score 3) (bar:10µm; original magnification: X 1000).

The Kruskal-Wallis test showed significant differences among the groups (p≤0.0001). The Mann-Whitney U test showed that the control group presented a layer of intact cementummore frequently than the others groups (G1XG2=0.0025; G1XG3=0.0003; G1XG4=0.0003), and the SRP group presented a smoother surface than the groups treated with the ultrasonic scaler (G2XG3=0.0025; G2XG4=0.0025). There were no statistical differences between groups treated with the piezoelectric ultrasonic scaleralone, or in association with the manual curettes.

Blood component adhesion

Group 1 (without treatment): the majority of the samples of this group presented score 0 for blood component adhesion (60%), which is characterized by an absence of blood component adhesion on the root surfaces (Figure 3A). The other samples presented a scarce (20%) or a moderate (20%) adhesion of blood cells interwoven in a thin fibrin network, which is represented by scores 1 and 2, respectively.

Group 2 (SRP): in this group the majority of the samples (40%) presented score 3 for blood component adhesion, which represented a dense fibrin network with large entanglement and trapped blood (Figure 3B), followed by score 2 (30%), which represented a moderate number of adherent blood cells within a thin fibrin network;and 20 % of the samples presented score 0, due to the absence of blood component adhesion. Score 1 was observed in 10 % of the samples, in which scarce adhesion of blood components occurred.

Group 3 (Ultrasonic Scaler): 50 % of the samples of this group presented score 3, due to the extensive adhesion of blood components trapped in a dense fibrin network (Figure 3C). In 40 % of the samples score 0 was shown, due to lack of blood component adhesion. In 10% of the samples score 1 was observed, due to the scarce adhesion of blood cells trapped in a thin fibrin network.

Group 4 (SRP+ Ultrasonic Scaler): the majority of the samples of this group presented score 2 (40%), due to moderate adhesion of blood cells trapped in a thin fibrin network (Figure 3 D). The other samples of this group were equally distributed between scores 3 and 1 (30% each), which represented extensive and scarce adhesion of blood components on the root surfaces, respectively.

The Kruskal-Wallis test showed that there were significant differences among the groups (p=0.006). The Mann-Whitney U test showed that the SRP, Ultrasonic scaler, and the SRP+Ultrasonicscaler groups presented no statistical differences among them, however, these groups showed a higher blood component adhesion than the control group without treatment (G1XG2=0.02;G1XG3= 0.04;G1XG4=0.005).

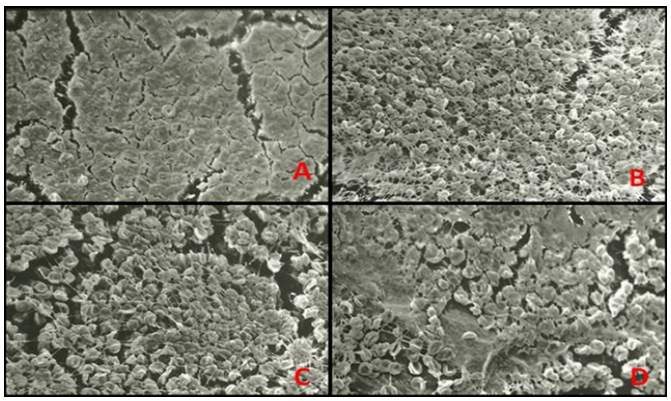

Figure 3. Blood Component Adhesion Analysis- A: Group 1 (Control). Absence of blood components adhered to the root surfaces (score 0); B: Group 2 (SRP). Root surface with the presence of dense fibrin network with a huge number of adhered cells (score 3); C: Group 3 (Ultrasonic scaler). Root surface with huge number of adhered blood cells intertwined in a dense fibrin network (score 3); D: Group 4 (Ultrasonic scaler+SRP). Root surface with a huge number of blood cells trapped in a dense fibrin network (score 3) (bar: 10µm; original magnification: X 1000).

DISCUSSION

Knowledge of the root surface characteristics after periodontal treatment is of fundamental importance, because it provides information about the ability of this surface to facilitate biofilm adhesion, or whether it is compatible with the regenerative processes. Therefore, the aim of this study was to evaluate, by SEM analysis, the morphology and blood component adhesion on root surfaces treated with the piezoelectric ultrasonic scaler (Piezon Master Surgery) which was recently proposed as a tool for periodontal treatment.

Morphological analysis of the root surfaces showed that 80% of the samples of the control group presented a intact cementum, and this pattern was expected because the surfaces did not undergo any periodontal treatment during the study. In this group, 20% of the samples presented a smooth pattern, with the presence of cementum and smear layer, and it is likely that these areas had undergone some form of periodontal treatment prior to extraction. The SRP group presented 100% of the samples with smooth surface, with the presence of smear layer, and occluded dentinal tubules, and this pattern was similar to that found in other studies[4,6]. The groups treated using the piezoelectric ultrasonic scaler alone or associated with the manual curettes (Group 3 and Group 4), presented 80% of the samples with an irregular surface, presence of grooves, smear layer, and occluded dentinal tubules and this morphological features is predominant after the treatment with ultrasonic scalers[4]. The irregularities promoted by the ultrasonic scaler are caused by the vibrational motion of the tip and the cavitation activity of the jet of water[13-14]; and due to the higher roughness and irregularities[15], a higher degree of biofilm adherence may occur on the root surface.

This research showed that the groups treated with the piezoelectric ultrasonicscaler alone or associated with SRP using manual curettes presented the same level of blood component adhesion as SRP with manual curettes alone. The results of this study are in agreement with the researches of Kishida et al.[16] and Crespi et al.[17], who evaluated the adhesion of fibroblasts on root surfaces after the ultrasonic instrumentation, which confirms the good biocompatibility of the root surfaces after ultrasonic instrumentation. As in this study, other treatments have not demonstrated superiority to SRP with manual curettes as regards the adhesion of blood components, such as treatment with lasers[6], and substances used for the biomodification of root surfaces[18]. These results reaffirm the efficiency of SRP with manual curettes as the gold standard treatment of periodontal disease. The untreated control group did not show a good adhesion of blood components, with a significantly lower biocompatibility when compared with the treated groups. The reason for this lower biocompatibility is that the root surfaces used in this study were obtained from patients with periodontal disease. Endotoxins present on the root surface have been associated with the poor outcomes in fibroblast adhesion, and it is likely that this factor also hindered the adhesion of blood components in this study[19].

A factor to be considered is the presence of irregularities and grooves observed on root surfaces after the use of piezoelectric ultrasonic scalers. These irregularities are associated with a higher adhesion of biofilm, which can hinder the regeneration of periodontal tissues[20]. However, this effect can be observed only supragengivally, because the rough surface would be exposed to the oral environment and facilitate biofilm retention, and this effect is not observed in the middle subgingival environment[21]. There fore supragingival root planing and polishing would be indicated on the surfaces treated with the piezoelectric ultrasonic scaler[22].

It should be pointed out that this topic was not the objective of this study, asthere were no calculus adhering on the root surfaces, which demonstrates the effectiveness of all protocols used in this study for the periodontal treatment. This may explain the good result of blood component adhesion, and the good clinical outcomes found in this study, when SRP was performed with manual curettes associated with the ultrasonic scalers[2,23].

The use of the piezoelectric ultrasonic scaler alone or in association with the manual curettes is an alternative approach to SRP with manual curettes alone. It may be advantageous to apply the piezoelectric ultrasonic scaler in areas of difficult access to instrumentation, such as in deep pockets and in furcation defects. The piezoelectric ultrasonic scalers have been shown to promote less root wear and are also good alternatives as tools for instrumentation during the maintenance phase of periodontal treatment[7,23-24], however, further in vitro and in vivo studies are necessary to confirm these properties, also with the use of the piezoelectric ultrasonic scaler Piezon Master Surgery.

CONCLUSION

Based on the results obtained with the methodology applied, it can be concluded that the root surfaces instrumented with the piezoelectric ultrasonic scaler presented a higher degree of irregularities than the surface profile of roots treated with manual curettes. Furthermore, the root surfaces treated by SRP with manual curettes and with the piezoelectric ultrasonic scaler alone or in association with manual curettes had significantly higher adhesion of blood components than the untreated control.

Collaborators

JN TSURUMAKI and BHM SOUTO implemented the methodology and participated in the writing of the article. GJPL OLIVEIRA interpreted the data and participated in writing the article. JEC SAMPAIO analyzed the data and participated in writing the article. RAC MARCANTONIO supervised the research and participated in study design, data interpretation and writing of the article.

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