Effect of Er,Cr:YSGG and Er:YAG laser irradiation on the adhesion of blood components on the root surface and on root morphology

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Abstract: The aim of this study was to conduct an *in vitro* evaluation, by scanning electron microscopy (SEM), of the adhesion of blood components on root surfaces irradiated with Er,Cr:YSGG (2.78 µm) or Er:YAG (2.94 µm) laser, and of the irradiation effects on root surface morphology. Sixty samples of human teeth were previously scaled with manual instruments and divided into three groups of 20 samples each: G1 (control group) - no treatment; G2 - Er,Cr:YSGG laser irradiation; G3 - Er:YAG laser irradiation. After performing these treatments, blood tissue was applied to 10 samples of each group, whereas 10 samples received no blood tissue application. After performing the laboratory treatments, the samples were observed under SEM, and the resulting photomicrographs were classified according to a blood component adhesion scoring system and root morphology. The results were analyzed statistically (Kruskall-Wallis and Mann Whitney tests, $\alpha = 5\%$). The root surfaces irradiated with Er:YAG and Er,Cr:YSGG lasers presented greater roughness than those in the control group. Regarding blood component adhesion, the results showed a lower degree of adhesion in G2 than in G1 and G3 (G1 × G2: $p = 0.002$; G3 × G2: $p = 0.017$). The Er:YAG and Er,Cr:YSGG laser treatments caused more extensive root surface changes. The Er:YAG laser treatment promoted a greater degree of blood component adhesion to root surfaces, compared to the Er,Cr:YSGG treatment.

Descriptors: Blood; Lasers; Root Planing; Microscopy, Electron, Scanning.

Introduction

Scaling and root planing performed with hand instruments is considered the gold standard in treating periodontal diseases.[1] However, this treatment has some limitations, such as the difficulty to access deep pockets and furcation areas, in addition to the long treatment time and patient discomfort.[1-3] Additionally, manual scaling produces a smear layer, which in turn may be related to a lower initial stability of the clot on the root surface, which marks the initial process of periodontal regeneration.[4]

Among the high-intensity lasers, Er:YAG (2.94 µm) and Er,Cr:YSGG (2.78 µm) lasers are the most suitable for laser irradiation on root surfaces. The wavelength of these lasers is well absorbed by water molecules; therefore, irradiation with these lasers promotes water heating in the hydroxyapatite crystals, causing a sudden evaporation of water and microbursts in the interpris-

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matic substance, removing hydroxyapatite crystals at a temperature below its melting point (~1,200°C).[5,6] Owing to this effect, these lasers abrade hard tissue without causing thermal damage. Some studies have shown that irradiation of root surfaces using Er:YAG lasers does not promote the formation of a smear layer.[4,7] Additionally, the Er:YAG and ErCr: YSGG lasers promote a selective removal of calculus with minimal damage to root surfaces,[8-10] and may be clinically effective for stabilizing the clinical parameters of periodontal disease.[2,3]

Although the Er:YAG[1] and Er,Cr:YSGG[3] lasers have been recommended for root instrumentation, to our knowledge there are no studies reporting a direct comparison between these tools. The objective of the present study was thus to conduct an *in vitro* evaluation, by scanning electron microscopy (SEM), of root surface morphology and of the adhesion of blood components on root surfaces irradiated with Er:YAG and Er,Cr:YSGG lasers.

Methodology

Sample preparation and groups

This study was approved by the Research Ethics Committee of the Araraquara Dental School, UNESP (CEP-FO/Car. n. 12/08). Fifteen extracted, single-rooted, periodontally diseased human teeth from non-smoking patients were used in this study. After extraction, the teeth were cleaned in distilled water and stored in phosphate-buffered saline, pH 7.4, at 37°C until the treatments were carried out.

A high-speed handpiece and a cylindrical bur under copious irrigation were used to make two parallel retention grooves on the proximal root surfaces of each tooth, one at the cementoenamel junction and the other approximately 5 mm apically to the first groove. After this procedure, the areas between the grooves were treated using scaling and root planing with manual instruments (Gracey curettes no. 5/6, Hu Friedy Co., Chicago, USA), by means of 50 apical-to-cervical traction movements. Scaling and root planing were performed by the same experienced operator. On each proximal surface (mesial and distal), the tooth was crosscut and separated in two samples of about 2 × 2 mm, to produce four samples per tooth. The total 60 samples were then divided into three groups of 20 samples each. The samples from each group (n = 20) received the following treatments:

- G1 (control group) - no treatment;
- G2 - the samples were irradiated with a pulsed Er,Cr:YSGG laser (Waterlase MD, Biolase, San Clemente, CA, EUA) at a wavelength of 2.78 µm and a repetition rate of 20 Hz, and using an optical fiber mounted on a handpiece (600 µm), set at the following parameters:
  - energy of 100 mJ and
  - fluency of 35.71 J/cm^2/pulse;
- G3 - the samples were irradiated with a pulsed Er:YAG laser (KaVo Key Laser II, KaVo, Biberach, Germany) at a wavelength of 2.94 µm and a repetition rate of 10 Hz, and using a handpiece (2056, KaVo, Biberach, Germany) with a special application tip (1.1 mm × 0.5 mm), set at the following parameters:
  - energy of 100 mJ and
  - fluency of 12.9 J/cm^2/pulse.

The optical fiber of the Er:YAG and Er,Cr:YSGG lasers was positioned perpendicularly to the surface of the sample, focused without contact, and the lasers were applied using water cooling. Ten samples were fixed onto a specific device to receive the irradiation. Each sample was irradiated for 15 s with scanning movements. The irradiations were carried out manually to simulate clinical conditions.

Preparation of human root blocks with blood tissue

Immediately after performing the treatments, fresh human whole peripheral blood from a healthy female donor was applied to the external root surface of 10 blocks from each group. The blood was allowed to clot on the root blocks for 20 minutes in a humidified chamber at 37°C. The blocks were then rinsed three times for 5 minutes in phosphate-buffered saline. Washes and rinses of the root blocks were carried out in small Petri dishes with gentle swirling motion using a table-top rotary shaker at low speed.

Immediately after rinsing, the blocks were fixed in 1% formaldehyde in phosphate-buffered saline for 15 minutes. After three 5-minute phosphate-buffered saline rinses, the blocks were incubated for 10 minutes in 0.02 M glycine in phosphate-buffered saline, and then rinsed again, as previously described. The samples were post-fixed in 2.5% glutaraldehyde in phosphate-

buffered saline for 30 minutes and rinsed once more, as described above. The samples were dehydrated through a graded ethanol series: 25%, 50%, 75%, 95% and three exchanges of 100%. The blocks were then mounted on aluminum stubs with colloidal graphite, sputter-coated with gold palladium in a specific device (Baltec SCD 050, Tokyo, Japan) and stored and desiccated at room temperature for 3 days.

Preparation of human root blocks without blood tissue

The samples that did not receive blood tissue, 10 in each group, were post-fixed, rinsed, dehydrated, mounted on aluminum stubs, sputter-coated, stored, and desiccated in the same manner as described above for the samples that did receive the blood tissue application.

Scanning electron microscopy observation

Three random photomicrographs were obtained from distinct areas of the samples treated with blood tissue (one in the center and two in distinct margins), at 2000× magnification (n = 30). One photomicrograph was obtained from the central area of the samples that received no blood tissue application at 2000× magnification (n = 10). All photomicrographs were obtained under SEM at 20 kV (Jeol JSM, Tokyo, Japan).

The micrographs were then identified and assessed using a scoring system to describe the degree of adhesion of blood components, and visually analyzed to describe the morphological characteristics observed after performing the treatments. Both the photomicrographs of the samples that received blood tissue and those that received no blood tissue application were examined three times by an operator (JECS) previously trained and calibrated with two other operators (Kendall test, $p < 0.05$), using a single-blind method. The photomicrographs were classified according to a blood component adhesion scoring system,[4] as follows:

- 0) absence of a fibrin network and blood cells;
- 1) scarce fibrin network and/or blood cells;
- 2) moderate fibrin network and a moderate number of blood cells;
- 3) dense fibrin network and trapped blood cells.

Statistical analysis

Blood component adhesion scores (BCA) were analyzed independently considering groups (G1 to G3). The non-parametric Kruskall-Wallis test ($p < 0.05$) was used to compare the ranking of the groups evaluated, using computer software (Bioestat 5.0, Windows 95, Belém, Brazil). This procedure was followed by applying the non-parametric Mann-Whitney test, which indicated a significant difference between groups ($p < 0.05$).

Results

Morphological root surface analysis

The photomicrographs taken of the G1 samples showed smooth and leveled root surfaces for the most part (9 samples); only one sample showed an irregular surface. A smear layer was formed and grooves with dentinal tubule exposure were observed on all samples (Figure 1A). However, the samples from group 2 (Figure 1B) presented greater dentinal tubule diameters and were less wrinkled than those from group 3 (Figure 1C). Additionally, the samples from group 3 showed a pattern of microroughness with a squamous appearance.

Blood component adhesion analysis

G1 (control) - In this group, most root surfaces (6 samples) were attributed a score of 3 for blood component adhesion, representing a dense fibrin network with extensive interlacing and trapped blood cells (Figure 1D), followed by scores of 1 and 2 (2 samples for each score), representing, respectively, the adhesion of a scarce fibrin network and/or blood cells, and a moderate amount of blood cells and a thinner fibrin network with little interlacing. No samples were attributed a score of 0 in this group (Table 1).

G2 (Er,Cr:YSGG laser irradiation) - The samples in this group received a score of 0 more frequently (8 samples), representing a situation in which the adhesion of blood elements was not observed (Figure 1E). The remaining samples received scores of 2 and 3 (one sample each), representing, respectively, the adhesion of a moderate amount of blood cells and thinner fibrin network with little interlacing, and the adhesion of a greater number of blood cells trapped in a dense fibrin network (Table 1).

G3 (Er:YAG laser irradiation) - The samples in this group were attributed a score of 3 more frequently (4 samples), representing extensive adhesion of blood cells trapped in a dense fibrin network (Figure 1F). The re-

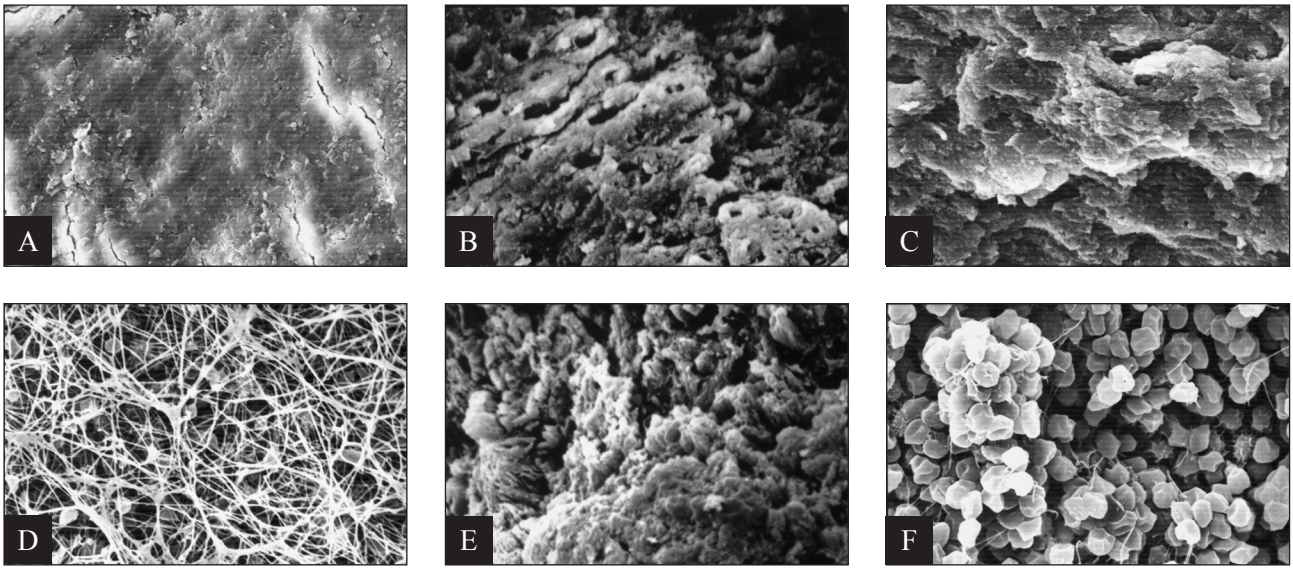

Figure 1 - Morphological Analysis. A: G1 (control). Flat and regular root surface, with occluded dentinal tubules and with the presence of a smear layer (Score 2); B: G2 (Er,Cr:YSGG laser irradiation). Irregular root surface with the presence of microroughness, with open dentinal tubules and no smear layer (Score 3); C: G3 (Er:YAG laser irradiation). Irregular root surface with the presence of craters, with open dentinal tubules and without a smear layer (Score 3). Blood component adhesion analysis. D: G1 (control). Root surface with the presence of a dense fibrin network with a great amount of adhered cells (Score 3); E: G2 (Er,Cr:YSGG laser irradiation). Root surface with no adhesion of fibrin network and blood cells (Score 0); F: G4 (Er:YAG laser irradiation). Root surface covered by a dense fibrin network with the presence of trapped blood cells (Score 3) (bar: 10 mm; original magnification: 2000×).

maining samples were attributed scores of 1 or 2 (3 samples each), representing, respectively, the adhesion of a scarce fibrin network and/or blood cells, and a moderate amount of blood cells and thinner fibrin network with little interlacing. No samples were attributed a score of 0 in this group (Table 1).

Statistical results

The nonparametric Kruskal-Wallis test revealed a statistically significant difference between groups ($p = 0.002$) regarding the adhesion of blood components. The Mann-Whitney test showed that the group irradiated with the Er,Cr:YSGG laser presented a lower adhesion of blood components compared to the other experimental groups (G1 × G2: $p = 0.002$; G3 × G2: $p = 0.017$). No statistically significant difference was observed for blood component adhesion between G1 and G3.

Table 1 - Frequency and percentage (%) of blood component adhesion.

Scores	G1	G2	G3
0	0 (0%)	8 (80%)	0 (0%)
1	2 (20%)	0 (10%)	3 (30%)
2	2 (20%)	1 (10%)	3 (30%)
3	6 (60%)	1 (10%)	4 (40%)
Total	10 (100%)	10 (100%)	10 (100%)

Discussion

After performing the treatments, we observed that the group instrumented with curettes showed a smoother surface characterized by occluded dentinal tubules and a smear layer. These characteristics were also demonstrated in other studies.[4,7] The surfaces irradiated with Er:YAG and Er,Cr:YSGG lasers showed similar morphological characteristics, including root surface roughness, no smear layer, open dentinal tubules and lack of thermal damage. These characteristics are consistent with those observed in other *in vitro* studies that evaluated the morphology of dentin irradiated with Er:YAG[4,8,11] and Er,Cr:YSGG lasers.[7,9,12,13]

The morphological characteristics of the surfaces irradiated with these lasers are explained by the laser tissue ablation mechanism, called photomechanical ab-

lation. This type of ablation occurs owing to the sudden evaporation of water from the amorphous interprismatic substance, which causes microbursts of the dental tissue and the elimination of hydroxyapatite crystals at temperatures below their melting point, without causing thermal damage to the dentin tissue.[5,6] The roughness observed in the samples may be explained by the heterogeneity of the dentin tissue. Dentin is formed by a mix of tubular, peritubular and intertubular tissue holding different concentrations of water, thus leading to an unequal ablation.[6]

We observed no differences between the degrees of adhesion of blood components on the surfaces irradiated with the Er:YAG laser and on those treated with curettes. This proves that irradiation with the Er:YAG laser does not lead to a root surface more biocompatible with blood cells and fibrin network than that obtained with the conventional manual scaling treatment.[4,14] However, although the Er,Cr:YSGG laser has a wavelength close to that of the Er:YAG laser and produces a root morphological pattern similar to that produced by Er:YAG, it afforded a lower degree of adhesion of blood elements on the irradiated root surfaces. One possible explanation for this is that surfaces irradiated by the Er,Cr:YSGG laser are relatively more thermally affected than those irradiated by the Er:YAG laser, insofar as the ablation produced by the Er:YAG laser is initiated at temperatures of approximately 300°C, whereas that produced by the Er,Cr:YSGG laser is initiated at approximately 800°C.[5]

On the other hand, studies that evaluated the adhesion of fibroblasts[10] and blood elements[13] on root surfaces irradiated with the Er,Cr:YSGG laser showed that irradiated surfaces present a biocompatibility greater than[10] or comparable[11,13] to that of surfaces scaled with hand tools. This may have occurred owing to the work angles used in these studies (~20-30° [10] and ~45° [13]), smaller than those used in our study.

In our study, the samples were irradiated at a working angle of 90°, and this angle could be used during surgical periodontal therapy. Studies that have evaluated the influence of the irradiation angle on root surface morphology have shown that greater angles of irradiation promote higher wear on root surfaces,[11,15] and this occurs because the removal of tissue is related to the energy density,[16] which, in turn, is directly proportional to the irradiation angle.[15]. The angles of irradiation used in non-surgical periodontal treatment are smaller than those used in the present study; reducing this irradiation angle should reduce thermal damage and improve the biocompatibility of the surfaces irradiated with the Er:YAG and Er,Cr:YSGG lasers.[11]

Another important consideration is that the adhesion of blood components represents only the first step in periodontal regeneration. A study that assessed the adhesion of fibroblasts on root surfaces irradiated with the Er:YAG laser revealed a lower degree of biocompatibility with the same parameters of irradiation angle and energy used in our study.[17]

Some characteristics of the samples irradiated with both lasers should be taken into consideration when applying them clinically. The roughness observed in the samples could serve as niches for bacterial colonization,[18] particularly if these rough surfaces are located supragingivally. In such cases, it is recommended that these surfaces be polished after irradiation.[19] When these rough surfaces are located in the subgingival environment, finishing the surfaces is not a requirement, since they facilitate the adhesion of cells that will mediate the healing process,[4,20] and supragingival plaque control will prevent bacterial colonization.[21] Another important feature is the presence of open dentinal tubules that may induce cervical dentin hypersensitivity in subjects where the root surface is exposed to the oral environment.[11] In both cases, one should take into account that after periodontal treatment, the inflamed gingival margin will retract, and surfaces that were once subgingivally located will eventually become supragengival.[13]

Conclusion

According to the results and the methodology used in this study, it can be concluded that the Er:YAG and Er,Cr:YSGG lasers produced rougher root surfaces than those instrumented with curettes, albeit with no smear layer. Moreover, for the irradiation parameters used in this study, the Er:YAG laser was more effective in promoting the adhesion of blood elements on root surfaces than was the Er,Cr:YSGG laser.

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