J Periodont Res 2006; 41; 381–390

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2005.00846.x

Effect of Er:YAG and Diode lasers on the adhesion of blood components and on the morphology of irradiated root surfaces

Letícia Helena Theodoro[1], José Eduardo C. Sampaio[1], Patrícia Haypek[2], Luciano Bachmann[3], Denise Maria Zezell[3], Valdir Gouveia Garcia[4]

[1]Department of Periodontology, Araraquara Dental School, State University of São Paulo, Araraquara, São Paulo, Brazil, [2]Experimental Laboratory Lasers in Dentistry – LELO, São Paulo Dental School, University of São Paulo, USP, São Paulo, Brazil, [3]Center of Laser Applications – CLA, Instituto de Pesquisas Energéticas,e Nucleares – IPEN, , São Paulo, Brazil and [4]Department of Periodontology, Araçatuba Dental School, State University of São Paulo, Araçatuba, São Paulo, Brazil

Theodoro LH, Sampaio JEC, Haypek P, Bachmann L, Zezell DM, Garcia VG: Effect of Er:YAG and Diode lasers on the adhesion of blood components and on the morphology of irradiated root surfaces. J Periodont Res 2006; 41: 381–390.

Objective: The aim of this study was to evaluate *in vitro*, by scanning electron microscopy (SEM), the adhesion of blood components on root surfaces irradiated with Er:YAG (2.94 μm) and GaAlAs Diode (808 nm) lasers and the effects on the morphology of irradiated root surfaces.

Methods: One hundred samples of human teeth were obtained. They were previously planed and scaled with manual instruments and divided into five groups of 20 samples each: G1 (control group) – absence of treatment; G2 – Er:YAG laser (7.6 J/cm^2); G3 – Er:YAG laser (12.9 J/cm^2); G4 – Diode laser (90 J/cm^2) and G5 – Diode laser (108 J/cm^2). After these treatments, 10 samples of each group received a blood tissue but the remaining 10 did not. After laboratory treatments, the samples were obtained by SEM, the photomicrographs were analysed by the score of adhesion of blood components and the results were statistically analysed (Kruskall–Wallis and Mann–Whitney test).

Results: In relation to the adhesion of blood components, the study showed no significant differences between the control group and the groups treated with Er:YAG laser ($p = 0.9633$ and 0.6229). Diode laser radiation was less effective than control group and Er:YAG laser radiation ($p < 0.01$).

Conclusions: None of the proposed treatments increased the adhesion of blood components in a significant way when compared to the control group. Although the Er:YAG laser did not interfere in the adhesion of blood components, it caused more changes on the root surface, whereas the Diode laser inhibited the adhesion.

Letícia Helena Theodoro, PhD, Departamento de Diagnóstico e Cirurgia, Disciplina de Periodontia, UNESP – Faculdade de Odontologia de Araraquara, Rua Humaitá, 1.680, 14801-903 Araraquara, São Paulo, Brazil
Phone/Fax: +55 16 3301 6369
E-mail: letheodoro@uol.com.br
perio@foar.unesp.br

Key words: blood; fibrin; lasers; root planing

Accepted for publication August 24, 2005

It has become increasingly apparent that the stability of the fragile attachment between the root surface and the gingival flap by the maturing fibrin clot is crucial for the outcome of reconstructive periodontal surgery (1). The fibrin of the clot forms an initial attachment to the root surface, preventing epithelial down-growth and forming a scaffold for the development of a cell and collagen fiber attachment mechanism (2). Connective tissue repair to the root surface seems critically dependent on an attachment between the fibrin clot and the root (1). Studies have shown that in the absence of stable fibrin attachment the defect may epithelialize (3–5). Root surface demineralization with acidic agents promotes the establishment of a new connective tissue attachment (3, 6). Conditioning of the root surface after scaling and root planing has been introduced as a promising procedure

for endotoxins and smear layer removal, and as a compensation for the limitations inherent to the mechanical root surface therapy (7–9).

Even though *in vitro* (7, 8) and *in vivo* (3, 10) studies have shown the effectiveness of chemical root conditioning agents, clinical studies in humans have not (11–13). On the other hand, conditioning may favor clot stabilization in the earliest stages of periodontal healing events by increasing blood cells and fibrin adhesion to the root surface (14).

Laser therapy has been studied in different applications in periodontics (15). Thermal and photodisruptive laser effects result in the elimination of periodontopathogenic bacteria (16–19) and might be beneficial to the treatment of periodontitis. Attention has been paid to the clinical applicability of the Er:YAG laser with 2.94 μm wavelength in the near infrared spectrum. This erbium laser light is well absorbed by water because its wavelength is resonant to the water molecule (20). The Er:YAG laser also enables the removal of calculus and a superficial layer of infected cementum without promoting undesirable thermal damage. Its effects on periodontally involved root surfaces have been examined *in vitro* (21–26) and *in vivo* (27–29). Nevertheless, the effect of the Diode laser with wavelength of 808–810 nm on root surfaces has not yet been thoroughly investigated (30, 31). Moreover, only a few studies have evaluated the effects of both laser irradiations on the root surface biocompatibility (31–33). The aim of this study is to evaluate *in vitro*, by scanning electron microscopy (SEM), the adhesion of blood components using an *in vitro* screening model proposed by Baker *et al.* (14) on root surfaces irradiated with Er:YAG (2.94 μm) and Diode (808 nm) lasers and the effects of these lasers on irradiated root surfaces.

Material and methods

This study was approved by the Ethical Committee of Araraquara Dental School, UNESP (CEP-FO/CAr.n°220–2002).

Sample preparation

Twenty-five extracted, single-rooted, periodontally diseased human teeth from non-smoking patients, with probing depths of at least 6 mm, 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.

Using a high speed cylindrical bur under copious irrigation, two parallel retention grooves were made on the proximal (mesio and distal) root surface of each tooth, one at the cementum/enamel junction and the other approximately 5 mm apically to the first groove. After this procedure, the areas between the grooves were treated with scaling and root planing with manual instruments (Gracey curets n°5/6, Hu-Friedy Co., Chicago, IL, USA), through 50 cervical-occlusal traction movements. The scaling and root planing was performed by the same experienced operator. The samples were prepared using a diamond disc. The roots were crosscut in the first groove separating them from the crown. The roots were cut lengthwise in the buccal–lingual orientation and then in the mesio–distal orientation until the second grove was reached apically. The samples were crosscut and separated in two samples of about 2 mm × 2 mm in the mesio and distal surfaces. Four samples of each tooth and a total of 10 samples divided into five groups of 20 samples each were obtained.

Treatment

Two samples from the same mesio or distal surfaces were kept in an identified bottle containing phosphate-buffered saline. The bottles were randomly divided into five groups of 10 bottles each, with a total of 20 samples in each group. The samples of each group received the following treatments:

G1 (control group) – absence of treatment;
G2 – irradiated with Er:YAG laser (7.6 J/cm^2 per pulse);
G3 – irradiated with Er:YAG laser (12.9 J/cm^2 per pulse);
G4 – irradiated with Diode laser (90 J/cm^2 per pulse);
G5 – irradiated with Diode laser (108 J/cm^2 per pulse).

The samples were randomly divided among the groups and the two from each bottle were deposited in a plastic container identified by the same number in order to distinguish them from each root surface and to allow the proposed treatment. The samples in G1 (control group) received no treatment apart from irrigation in 10 ml of distilled water.

Lasers

The samples of G2 and G3 were irradiated with a pulsed Er:YAG laser (KaVo Key Laser II, KaVo, Biberach, Germany) with a wavelength of 2.94 μm, 250–500 μs exposure duration, repetition rate of 10 Hz using a handpiece (2056, KaVo, Biberach, Germany) with a special application tip (1.1 mm × 0.5 mm), in the following parameters: energy at 60 mJ as indicated on the display, resulting in transmitted energy of 42 mJ at the tip of the handpiece (2056, transmission factor of 71%) and fluency of 7.6 J/cm^2 per pulse (G2); energy at 100 mJ as indicated on the display, resulting in transmitted energy of 71 mJ at the tip of the handpiece (2056, transmission factor of 71%) and fluency of 12.9 J/cm^2 per pulse (G3), focused and without contact, with water cooling.

The optical fiber was positioned perpendicularly to the surface of the sample. Ten samples were fixed in a specific equipment to receive the irradiation. Each sample was irradiated for 15 s with scanning movements. The irradiation was carried out manually to simulate clinical conditions.

The samples of G4 and G5 were irradiated with the GaAlAs Diode laser device (Soft Lase, Zap Laser Ltd, Pleasant Hill, CA, USA) with a wavelength of 808 nm ± 5 nm in the repeat wave mode (0.05 ms). Power outputs were measured with a power meter device. Laser light was delivered through a 400 μm contact optical fiber in the following parameters: power outputs of 0.9 W, fluency of 90 J/cm^2 per pulse (G4);

power outputs of 1.08 W, and fluency of 108 J/cm^2 per pulse (G5). The irradiation parameters used with the Diode laser were based on previous research that evaluated the pulpar thermal variation of teeth with Diode irradiated radicular surfaces in several parameters. It may be observed in this study that these two parameters did not promote undesirable pulpar temperature increase (34). The optical fiber was perpendicularly positioned to the surface of the specimens. Each sample was irradiated for 15 s, with scanning movements. The irradiation was carried out manually to simulate clinical conditions.

Preparation of human root blocks with blood tissue

Immediately after treatments, fresh human whole peripheral blood from a healthy female donor was applied to the external root surface of 10 blocks of each group. The blood was allowed to clot onto the root blocks for 20 min in a humidified chamber at 37°C. Blocks were then rinsed three times for 5 min in phosphate-buffered saline. Washes and rinses of the root blocks were carried out in small Petri dishes with gentle swirling motion using a rotating table-top shaker at low speed (14).

Immediately after rinsing, the blocks were fixed in 1% formaldehyde in phosphate-buffered saline for 15 min. After three 5-min phosphate-buffered saline rinses, the blocks were incubated for 10 min in 0.02 M glycine in phosphate-buffered saline and rinsed again, as previously. The samples were postfixed in 2.5% glutaraldeyde in phosphate-buffered saline for 30 min and rinsed again, as above. The samples were dehydrated through a graded ethanol series: 25%, 50%, 75%, 95% and three exchanges of 100%. The samples were subsequently dried in a CO_2 critical point drier (Baltec CPD 030, Chicago, IL, USA). The blocks were mounted on aluminum stubs with colloidal graphite, sputter-coated with gold palladium in a specific device (Baltec SCD 050), and stored and desiccated at room temperature for 3 days (14).

Preparation of human root blocks without blood tissue

The samples that did not receive blood tissue, 10 in each group, were postfixed in 2.5% glutaraldeyde in phosphate-buffered saline for 30 min and rinsed again, as above. The samples were dehydrated through a graded series of ethanol: 25%, 50%, 75%, 95% and three exchanges of 100%, all steps at room temperature. The samples were subsequently dried at room temperature. The blocks were mounted on aluminum stubs with colloidal graphite, sputter-coated with gold palladium in a specific device (Baltec SCD 050), and stored and desiccated at room temperature for 3 days.

Scanning electron microscopy observations

Three random photomicrographs were obtained from distinct areas of the samples treated with blood tissue (one central and two in distinct margin), increased by 2000 × ($n = 30$). From the samples that received no treatment with blood tissue, one photomicrograph from the central area of the samples was obtained, also increased by 2000 × ($n = 10$). All photomicrographs were obtained through a SEM analysis at 20 kV (Jeol JSM, Tokyo, Japan).

After the photomicrographs were obtained, they were identified and analyzed through scores in order to verify the adhesion of blood components and to analyze the morphological characteristics obtained in the treatment.

Using a single-blind method, the photomicrographs obtained from the samples that received blood tissue were examined three times by an operator who was previously trained and calibrated with two other operators (Kendall $p < 0.01$), in a score of 'blood components adhesion' (rating system). Each sample received the score that prevailed among the three readings.

Rating system

0 absence of fibrin network and blood cells.
1 scarce fibrin network and/or blood cells.
2 moderate fibrin network and moderate quantity of blood cells.
3 dense fibrin network and trapped blood cells.

The photomicrographs obtained from the samples that did not receive blood tissue were examined by a single operator, previously trained, who evaluated the morphological changes in root surfaces, comparing them to the control group photomicrographs.

Statistical analysis

Blood components adhesion scores were independently analyzed considering the groups (1–5). The non-parametric Kruskall–Wallis test ($p < 0.05$) was employed to compare the rank of the evaluated groups using Bioestat software (Bioestat 1998, Windows 95, Manaus, AM, Brazil). This procedure was followed by a non-parametric Mann–Whitney test when the Kruskal–Wallis test suggested a significant difference between the groups ($p < 0.05$). The comparison between the groups was performed with the Mann–Whitney test using Bioestat software (Bioestat 1998, Windows 95, Manaus, AM, Brazil).

Results

Scanning electron microscopy analysis

Group 1 — It was observed in Group 1 (control group) that most of the photomicrographs (15 samples) presented a blood components adhesion score of 3 (Fig. 1) characterized by the presence of a dense fibrin network and trapped blood cells. This was followed by a blood components adhesion score of 0 (eight samples) in which the photomicrographs did not present a fibrin network and trapped blood cells. Five samples presented a blood components adhesion score of 2, and the remaining two presented a scarce fibrin network and/or blood cells (score of 1), which demonstrates great variability of findings in the same experimental group (Table 1).

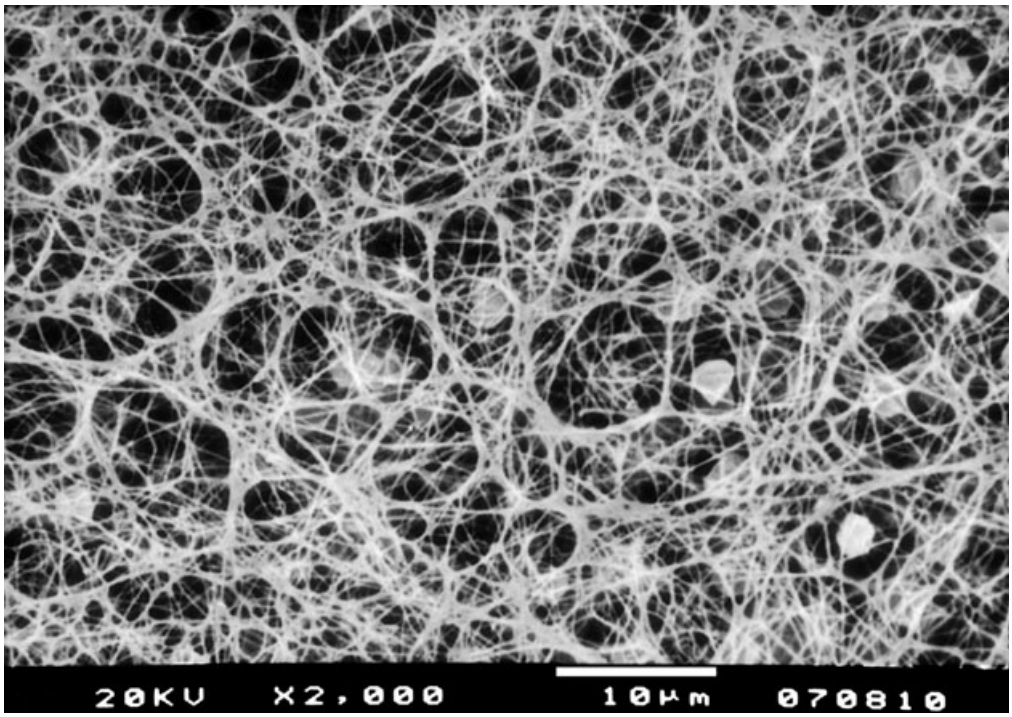

Fig. 1. Group 1 (control group/blood treatment). Root surface covered by dense fibrin network with trapped blood cells (score 3) (bar 10 µm; original magnification ×2000).

Table 1. Frequency and percentage (%) of blood components adhesion score BCA in groups G1, G2, G3, G4 and G5

	Groups Frequency (%)				
Scores	G1	G2	G3	G4	G5
0	8 (26.67)	2 (6.67)	–	17 (56.67)	16 (53.33)
1	2 (6.67)	8 (26.67)	10 (33.33)	5 (16.67)	3 (10.00)
2	5 (16.67)	10 (33.33)	10 (33.33)	1 (3.33)	3 (10.00)
3	15 (50.00)	10 (33.33)	10 (33.33)	7 (23.33)	8 (26.67)
Total (*n*)	30	30	30	30	30

Among the photomicrographs obtained from the samples that did not receive blood tissue, six presented a regular and smooth root surface without exposure of dentinal tubules, three presented an irregular root surface without exposure of dentinal tubules, and one presented a rough and irregular root surface, also without exposure of dentinal tubules.

The presence of grooves and smear layer in all samples caused by manual instrumentation was also evident.

Group 2 — The samples in Group 2 presented blood components adhesion scores of 2 (10 samples) and 3 (10 samples) in most of their photomicrographs (Fig. 2), demonstrating moderate or dense fibrin network and trapped blood cells. This was followed by a blood components adhesion score of 1 with eight photomicrographs, and only two photomicrographs did not show any evidence of fibrin network or blood cells adhered to the root surface (score of 0).

It was also possible to observe that the majority of the photomicrographs of Group 2 showed greater adhesion of blood cells in the irradiated root surfaces (Table 1). The samples that did not receive blood tissue presented an even surface on their photomicrographs, characterized by an irregular aspect with microroughness on the entire surface, similar to squamous and dentinal tubules obliteration, besides the absence of smear layer (Fig. 3).

Group 3 — The samples in Group 3 presented blood components adhesion scores of 2 (10 samples) and 3 (10 samples) on the photomicrographs, showing moderate or dense fibrin network and trapped blood cells (Fig. 4), and the other 10 samples presented scarce fibrin network and/or blood cells (score of 1) (Table 1).

Apart from this, none of the photomicrographs presented an absence of fibrin network. In the samples that did not receive blood tissue, the photomicrographs also presented an even surface (10), characterized by an irregular aspect with microroughness on the entire surface. This fact was more obvious than in the samples in G2, similar to squamous and also dentinal tubules obliteration, besides the absence of a smear layer on the root surface, also similar to G2.

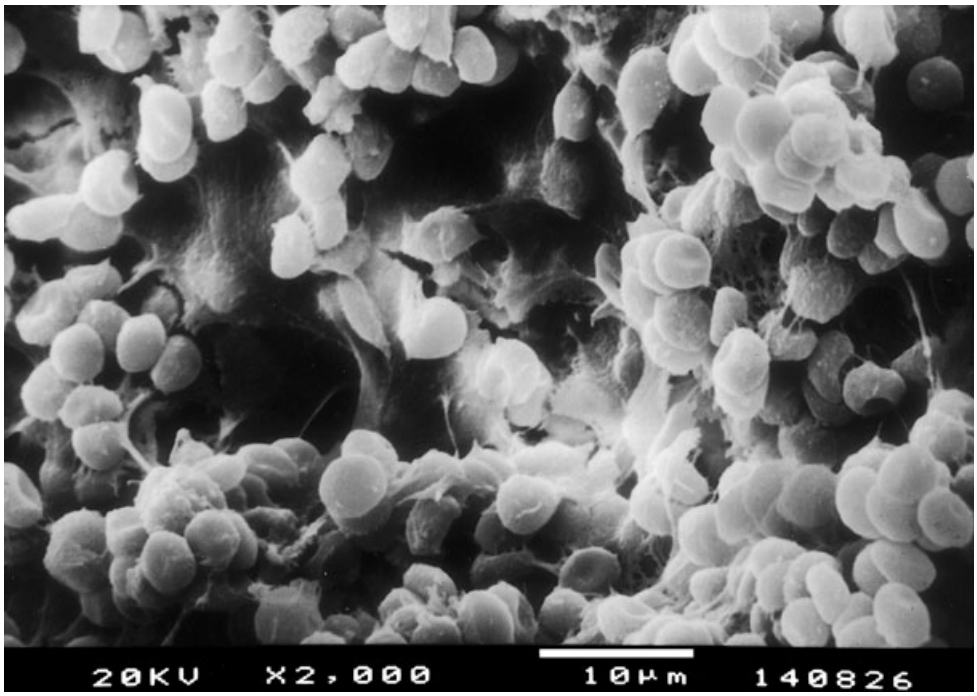

Fig. 2. Group 2 (Er:YAG 7.6 J/cm^2 per pulse, blood treatment). Root surface covered by dense layer of blood cells on the fibrin network (score 3) (bar 10 µm; original magnification ×2000).

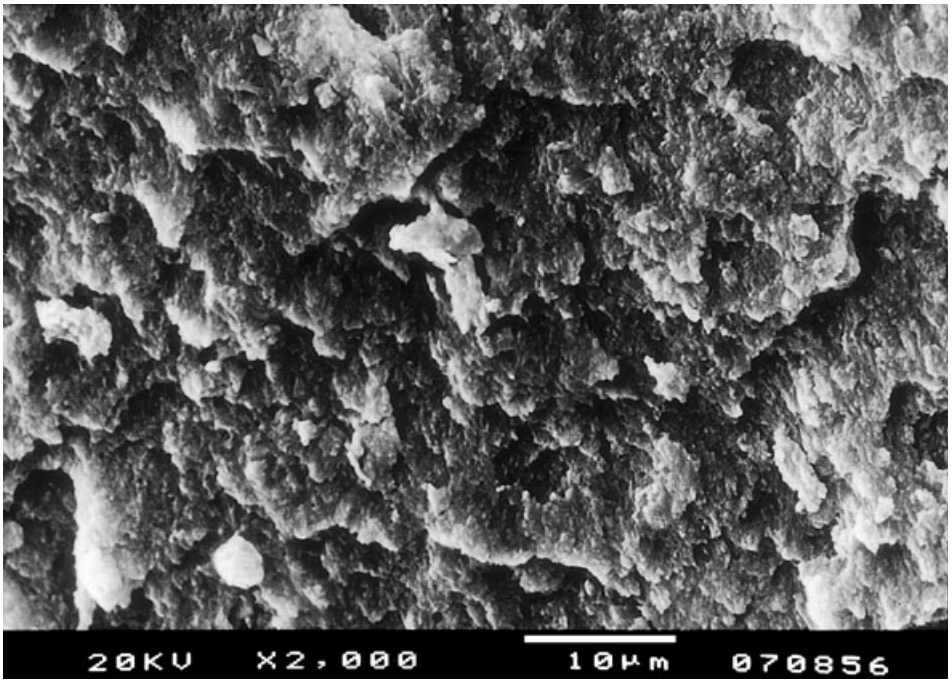

Fig. 3. Group 2 (Er:YAG 7.6 J/cm^2 per pulse, no blood treatment). Irregular root surfaces with microroughness, obliteration of dentinal tubules and absence of smear layer (bar 10 µm; original magnification ×2000).

Group 4 — The samples in Group 4 presented a blood components adhesion score of 0 in 17 of the photomicrographs, i.e. the photomicrographs did not present any fibrin network and blood cells (Fig. 5). Five samples presented scarce fibrin network and/or blood cells (score of 1), and eight photomicrographs showed moderate or dense fibrin network adhered to the surface (Table 1).

In relation to the photomicrographs from the samples that did not receive blood tissue, six presented an irregular

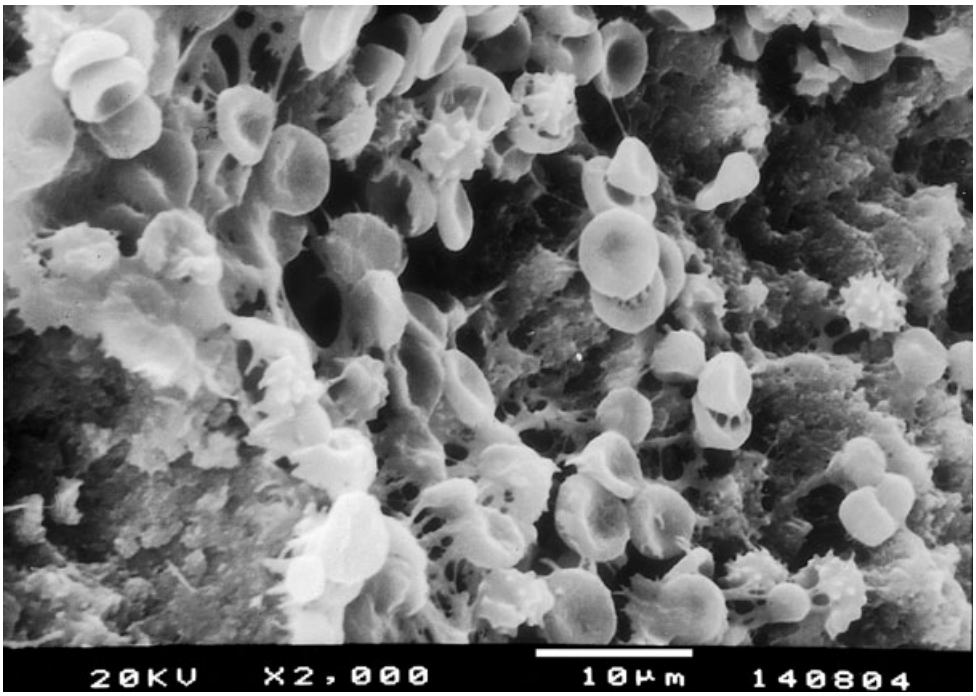

Fig. 4. Group 3 (Er:YAG 12.9 J/cm^2 per pulse, blood treatment). Root surface covered by dense layer of blood cells positioned over the fibrin network (score 3) (bar 10 µm; original magnification ×2000).

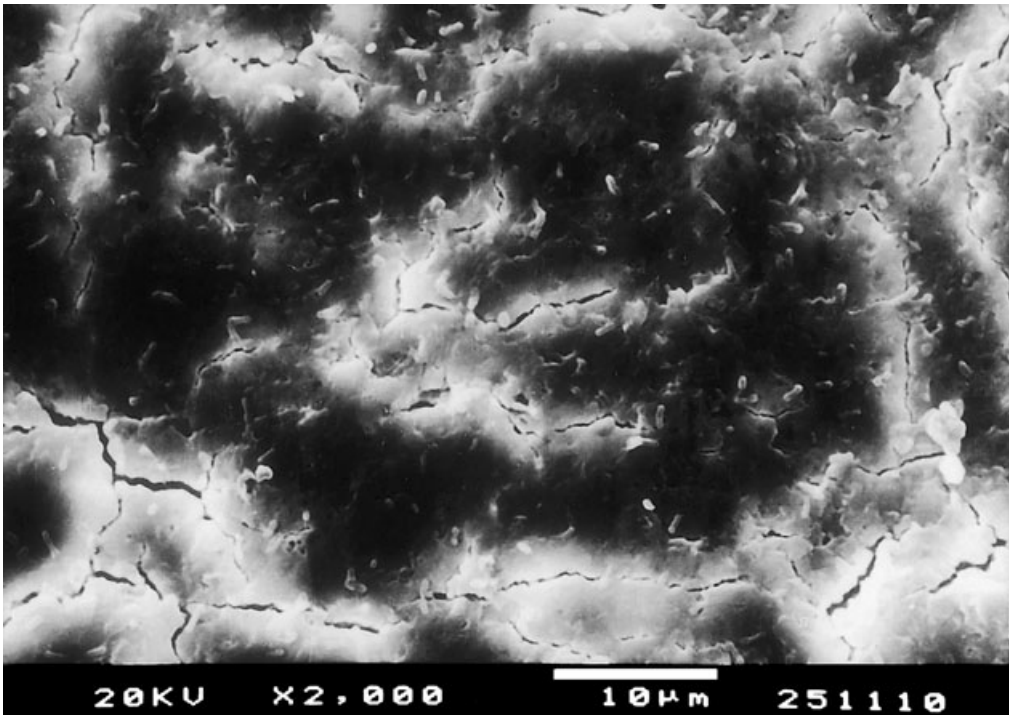

Fig. 5. Group 4 (Diode 90 J/cm^2 per pulse, blood treatment). Root surface showing absence of fibrin network and blood cells (score 0) (bar 10 µm; original magnification ×2000).

root surface and dentinal tubules obliteration, whereas four photomicrographs also presented an irregular root surface, although they were rough and had obliterated dentinal tubules.

Moreover, the presence of smear layer on the irradiated root surfaces without evidences of morphological changes on the root surfaces was clear.

Group 5 — The samples in Group 5 showed that a blood components adhesion score of 0 was predominant (16 samples), i.e. most of the photomicrographs did not show adhesion of

blood components to the root surface. Eight photomicrographs presented dense fibrin network and captured blood cells (score of 3), three photomicrographs had scarce fibrin network (score of 1) and other three had moderate fibrin network (score of 2).

All the photomicrographs of the samples that did not receive blood tissue presented an irregular root surface and dentinal tubules obliteration, besides the presence of a smear layer on the root surface, without evidence of morphological changes on the root surface.

Statistical results

There are limitations in the statistical analysis because this is a descriptive study comparing various treatments. Considering groups as an independent variable, the use of the non-parametric Kruskal–Wallis test showed a significant difference among the evaluated groups regarding blood components adhesion scores ($p = 0.0004$), resulting in a value of 20.6364.

The comparison between the rank of the groups (Mann–Whitney test) showed significant differences between Groups 1 and 4 ($p = 0.0041$), 1 and 5 ($p = 0.0164$), 2 and 4 ($p = 0.0036$), 2 and 5 ($p = 0.0144$), 3 and 4 ($p = 0.0008$) and 3 and 5 ($p = 0.0038$) (Table 2). Despite the fact that the photomicrographs of the groups irradiated with Er:YAG laser presented more samples with higher scores on blood components adhesion and also indicated a higher adhesion of blood components on the irradiated root surfaces than the control group, the statistical results showed that there were no significant differences between the control group (G1) and in the groups treated with Er:YAG laser (G2 and G3) in relation to the score of blood components adhesion ($p = 0.9633$ and $p = 0.6229$).

When comparing G4, in which the samples were irradiated with Diode laser (90 J/cm^2 per pulse), to the control group ($p = 0.0041$) and to the groups irradiated with Er:YAG laser (G2 and G3), it was observed that the blood components adhesion score was smaller in G4 than in the control group ($p = 0.0305$) or G2 ($p = 0.0036$) and G3 ($p = 0.0008$). In the same way, the group irradiated with Diode laser (108 J/cm^2 per pulse) (G5) was statistically different from the control group ($p = 0.0164$) and the Er:YAG laser groups ($p = 0.0144$ and $p = 0.0038$).

The Er:YAG laser (7.6 and 12.9 J/cm^2 per pulse) is more effective than the Diode laser in both of the parameters applied in this study (90 and 108 J/cm^2 per pulse) concerning the adhesion of blood components on the root surface.

Discussion

While analyzing the results obtained in the present study, as far as the adhesion of blood components is concerned, it could be observed that, when compared to the control group samples, which were irrigated with distilled water after scaling and root planing, no proposed treatment increased the adhesion of blood components. The effectiveness of scaling with hand instruments alone to promote a more compatible surface to the clot adhesion was demonstrated. Furthermore, the root surfaces irradiated with Diode laser (90 and 108 J/cm^2 per pulse) promoted an inhibition and small blood components adhesion when compared to the control group and to the groups irradiated with Er:YAG laser (7.6 and 12.9 J/cm^2 per pulse).

The different wavelengths and consequent tissue interaction can explain the differences obtained with the irradiation of both lasers used in the present study. The interaction mechanisms with the mineralized tissues have been described as photothermic in the case of the Diode laser, and photomechanical in the case of the Er:YAG laser. The absorbed energy depends on the absorption coefficient of each molecule in the tissue. The Diode laser is not well absorbed by water or hydroxyapatite; in this way, the absorbed energy is transformed into human heat, increasing the root surface temperature (20). This is the opposite to what happens to the Er:-YAG laser, which is highly absorbed by water.

Several studies have suggested that the initial healing processes, such as adsorption and adhesion of blood components along with a fibrin clot adhering to the formation of a blood clot on the root surface, are crucial determiners in the repairing process between the gingival flap and the root surface (1, 3–5).

Nowadays it is understood that the control of fibrin clot adhesion on the root surface in the protocol execution of reconstructive periodontal therapy is vital to the success of periodontal treatments (14).

The samples in the groups irradiated with Er:YAG laser (G2 and G3) indicated a higher adhesion of blood components when compared to the control group (G1), due to a higher frequency of samples that presented a higher score on blood components adhesion, although not presenting statistical differences from the control. On the other hand, this laser irradiation was significantly more effective than the Diode laser in the parameters performed in this study, in relation to the adhesion of blood components.

The fact that the Er:YAG laser presents a satisfactory adhesion of

Table 2. Rank and median values of the blood components adhesion score concerning groups G1, G2, G3, G4 and G5

Groups	Median	Rank**	p value – $p < 0.05$*
G1 ($n = 30$)	2.5	86.1167	1 and 4 $p = 0.0041$* 1 and 5 $p = 0.0164$*
G2 ($n = 30$	2.0	86.6333	2 and 5 $p = 0.0144$*
G3 ($n = 30$)	2.0	91.6333	3 and 4 $p = 0.0008$*
G4 ($n = 30$)	0	53.9333	2 and 4 $p = 0.0036$*
G5 ($n = 30$)	0	59.1833	3 and 5 $p = 0.0038$*

*Statistical differences between groups. Refers to the Mann–Whitney test ($p < 0.05$).
**Rank values – Kruskall–Wallis test ($p < 0.05$).

blood components in the parameters applied to G2 and especially to G3, although not presenting statistically significant differences from the control group (G1), is probably due to the fact that the laser with energy density of 7.6 and 12.9 J/cm^2 has greater power in reducing bacteria and endotoxins. According to Wikesjo *et al*. (1), the presence of endotoxins and bacteria could inhibit the adhesion of plasma proteins on the root surface. The Er:YAG laser irradiation promotes a removal of mineralized tissues producing holes and bumps and, consequently, promoting irregular root surfaces and a larger exposure area of collagen fiber. The exposure of collagen fiber could facilitate the adhesion and formation of primary homeostatic buffers through the adhesion of plaques to the exposed collagen (35). This fact should be further researched.

The Er:YAG laser parameters applied in this study may have caused bacteria reduction, as proposed by Ando *et al*., who demonstrated significant *in vitro* reductions of *Porphyromonas gingivalis* colonies (19). With relation to the morphology of root surfaces in the samples irradiated with Er:YAG laser (G2 and G3), it was observed that there was a significant difference when compared to the control group (G1), and to the Diode laser groups (G4 and G5). The irradiated root surfaces in G2 and G3 were found to be more irregular and rough, without smear layer. These results confirm the findings of several studies that have demonstrated the topography and the morphological features of root surfaces irradiated with Er:YAG laser (21, 23, 24, 26, 36–39).

These morphological features are probably due to this laser's high interaction with mineralized tissues once its wavelength is highly absorbed by the water present in the mineralized tissue expelled through micro explosions, a process known as explosive ablation.

It should still be considered that, in the present study, the samples were irradiated with the laser output positioned at a 90° angle perpendicularly to the root surface. This situation would promote great removal of mineralized tissue and consequently greater morphological changes on the root surface (37). This application angle (90°) was used to simulate the conditions of a periodontal surgery procedure to substitute for a chemical element on the root surface.

It is believed that the samples irradiated with Er:YAG laser could facilitate the adhesion of blood components also due to an increase in physical retention of the fibrin clot on the root surface or it could play a certain role in the cell retention, although this roughness would not be the crucial point in the adhesion of blood components.

Some studies have demonstrated that polymorphonuclear cells prefer to adhere to rough surfaces rather than smooth ones due to the functional differences of these cells (40, 41). This fact could justify the findings presented in the Er:YAG laser groups with higher adhesion of blood cells, probably erytrocytes and white cells.

Although some studies have not demonstrated significant effects on the presence of roughness on the root surface in the success of periodontitis treatment (42, 43), it could be considered that the roughness on the root surface caused by the Er:YAG laser can facilitate the retention of bacteria plaque. An increase in surface roughness could result in faster colonization and maturation of the dental plaque supragingivally, while the effect is less dramatic subgingivally, probably because this environment already offers more niches for bacterial adhesion and survival (44). Diode laser does not seem to be a promising treatment as a support to scaling and root planing when irradiated on the root surface, with relation to the adhesion of blood components, in spite of not promoting significant changes in structure on the irradiated root surfaces. This fact is probably due to the parameters applied in this study once they are not sufficient to promote a significant bacteria reduction that could increase the adsorption of plasma proteins. However, some studies demonstrated a significant effect on the microorganism reduction with the Diode laser when applied as support to periodontal therapy (18, 45).

On the other hand, the fact that Diode laser could also decrease the adhesion of blood components in a significant way can be associated with some damages in the collagen matrix or in the production of toxic substances in this laser interaction with the tissues, such as cyanate and cyanamid, which are produced in the presence of nitrogen (which can be produced in protein denaturation in high temperatures) (25). Other studies must be carried out in order to analyze the production of these substances on root surfaces irradiated with Diode laser.

In thermal analysis, the first chemical compound released from the tissue upon heating is water, while the organic matrix is changed between 100 and 400°C and a maximum loss around 320°C is identified (46). After laser irradiation with thermal action, as Er:YAG and Diode lasers in this work, the surface temperature increases at a maximum value that depends on irradiation parameters (46). The subsurface temperature of the irradiation site quickly decreases to values near the ambient temperature at deeper tissue layers. Therefore, the temperature rise at the dentine surface during laser irradiation changes mainly the water and organic matrix (47), while at the subsurface these compounds remain unchanged. Changes in the collagen structure may be reversible but only for temperatures below 175°C (47). The collagen degradation probably influences negatively the blood cell adhesion. For this work, the highest temperature values are expected to occur for the Diode laser and for this reason poor blood cells adhesion is expected to occur. It should also be considered that the Diode laser may promote undesirable thermal changes on the pulpal tissues (26) depending on the parameter applied.

Conversely, any significant differences on the root surfaces irradiated with this laser were observed due to the Diode laser weak interaction with mineralized tissues. However, G4 had the biggest number of samples with irregular surface when compared to the samples in the control group, although the samples treated with Diode laser did not present morphological changes

such as the presence of cracks, fractures, fusions or concavities.

While using Er:YAG laser and Diode laser, the operator must be aware of the possible risks involved, such as detrimental tissue effects or increase of pulpal temperature (26, 48). The power settings and the use of water as a coolant during Er:YAG laser irradiation to avoid harmful effects to the irradiated tissues must be controlled (48).

Due to the limitations of the methodology applied in the present study, it is not possible to evaluate the viability of blood cells adhered to the root surface and/or under the fibrin clot and it is not possible to distinguish which cells would be more predominant or even to evaluate the thickness of the fibrin clot adhered to each sample.

It is necessary to carry out *in vivo* studies to evaluate if the data obtained in this study are in agreement with the healing process of periodontal tissues, as this methodology allowed the evaluation of only the variable adhesion of fibrin clot and blood cells. It is known that the healing process is highly complex, because of the initial stages of clot formation, as well as the inflammatory process of granulation tissue formation and the maturing of this tissue with deposition of collagen fibers. Therefore, further studies are necessary to clarify the histological attachment of periodontal tissues to the irradiated root surfaces *in vivo*. Clinical studies must be carried out in order to analyze if the hypothesis of roughness on the root surface provoked by the Er:YAG laser could influence in the maintenance of a long-term periodontal treatment, as it increases the retention of bacteria plaque.

Based on the methodology applied and the limitations of this study, it is possible to assume that none of the proposed treatments was able to provide significant increase in the adhesion of blood cells on the root surfaces when compared to the control group. The Er:YAG laser appeared to be the most indicated laser for the irradiation of root surfaces when compared to the Diode laser in the parameters applied in the present study, although it promotes significant morphological changes on the root surfaces such as microroughness; the Diode laser did not promote significant changes on the root surfaces, but it can inhibit the adhesion of blood components.

Acknowledgements

This study was financially supported by Capes (Coordination for the Improvement of Higher Education Personnel). We would like to thank ZAP Laser Ltd, for supplying the equipment used, and the Experimental Laboratory Lasers in Dentistry of São Paulo University, USP, Center of Laser Application, Ipen and KaVo, Brazil. The authors gratefully acknowledge Dr Marie Oshiiwa for data management. We also thank Dr Solange Aranha for the English review.

References

1. Wikesjö UME, Nilvéus RE, Selvig KN. Significance of early healing events on periodontal repair: a review. *J Periodontol* 1992;**63:**158–165.
2. Caton JG, Greenstein G. Factors related to periodontal regeneration. *Periodontol 2000* 1993;**1:**9–15.
3. Polson AM, Proye MP. Fibrin linkage: a precursor for new attachment. *J Periodontol* 1983;**54:**141–147.
4. Wikesjö UME, Nilvéus R. Periodontal repair in dogs. effect of wound stabilization on healing. *J Periodontol* 1990;**61:**719–724.
5. Wikesjö UME, Claffey N, Egelberg J. Periodontal repair in dogs. Effect of heparin treatment of the root surface. *J Clin Periodontol* 1991;**18:**60–64.
6. Wikesjö UME, Claffey N, Nilvéus R, Egelberg J. Periodontal repair in dogs: effect of root surface treatment with stannous fluoride or citric acid on root reabsortion. *J Periodontol* 1991;**62:**180–184.
7. Blomlöf J, Blomlöf L, Lindskog S. Effect of different concentrations of EDTA on smear removal and collagen exposure in periodontits-affected root surfaces. *J Clin Periodontol* 1997;**24:**534–537.
8. Blomlöf J, Blomlöf L, Lindskog S. Smear layer formed by different root planing modalities and its removal by and ethylenediaminetetraacetic acid gel preparation. *Int J Periodontics Restorative Dent* 1997;**17:**243–249.
9. Blomlöf JPS, Blomlöf LB, Lindskog SF. Smear removal and collagen exposure after non-surgical root planing followed by etching with an EDTA gel preparation. *J Periodontol* 1996;**7:**841–845.
10. Lopez NJ. Connective tissue regeneration to periodontally diseased root planed and conditioned with citric acid implanted into the oral mucosa. *J Periodontol* 1984;**55:**381–390.
11. Caffesse RG, Nasjleti CE, Anderson GB, Lopatin DE, Smith BA, Morrison EC. Periodontal healing following guided tissue regeneration with citric acid and fibronectin application. *J Periodontol* 1991;**62:**21–29.
12. Blomlöf L, Bergman E, Forsgardh A *et al*. A clinical study of root surface conditioning with an EDTA gel. I. Non surgical periodontal treatment. *Int J Periodontics Restorative Dent* 2000;**20:**561–565.
13. Blomlöf L, Jonsson B, Blomlöf J, Lindskog S. A clinical study of root surface conditioning with an EDTA gel. II. Surgical periodontal treatment. *Int J Periodontics Restorative Dent* 2000;**20:**567–573.
14. Baker PJ, Rotch HA, Trombelli L, Wikesjö UME. An in vitro screening model to evaluate root conditioning protocols for periodontal regenerative procedures. *J Periodontol* 2000;**71:**1139–1143.
15. American Academy of Periodontology. Lasers in Periodontics. *J Periodontol* 2002;**73:**1231–1239.
16. Bem Hatit Y, Blum R, Severin C, Maquin M, Jabro MH. The effects of a pulsed Nd:YAG laser on subgingival bacterial flora and on cementum: An in vivo study. *J Clin Laser Surg Med* 1996;**14:**137–143.
17. Radvar M, Macfarlane TW, Mackenzie D, Whittres CJ, Payne AP, Kinane DF. An evaluation of the Nd:YAG laser in periodontal pocket therapy. *Br Dent J* 1996;**180:**57–62.
18. Moritz A, Schoop U, Goharkhay K *et al*. Treatment of periodontal pockets with a diode laser. *Lasers Surg Med* 1998;**22:**302–311.
19. Ando Y, Aoki A, Watanabe H, Ishikawa I. Bactericidal effect of erbium YAG laser on periodontopathic bacteria. *Lasers Surg Med* 1996;**19:**190–200.
20. Bachmann L, Diebolder R, Hibst R, Zezell DM. Infrared Absorption bands of enamel and dentin tissues from human and bovine teeth. *Appl Spectrosc Rev* 2003;**38:**1–14.
21. Aoki A, Ando Y, Watanabe H, Ishikawa I. In vitro studies on Laser scaling of subgingival calculus with an Erbium:YAG Laser. *J Periodontol* 1994;**65:**1097–1106.
22. Aoki A, Miura M, Akiyama F *et al*. In vitro evaluation of Er:YAG laser scaling of subgingival calculus in comparison with ultrasonic scaling. *J Periodont Res* 2000;**35:**266–277.
23. Folwaczny M, Mehl A, Haffner C, Benz C, Hickel R. Root substance removal with

Er:YAG laser radiation at different parameters using a new delivery system. *J Periodontol* 2000;**71:**147–155.
24. Israel M, Cobb CM, Rossmann JA, Spencer P. The effects of CO_2, Nd:YAG and Er:YAG lasers with and without surface coolant on tooth root surfaces. An in vitro study. . *J Clin Periodontol* 1997;**24:**595–602.
25. Sasaki KM, Aoki A, Masuno H, Ichinose S, Yamada S, Ishikawa I. Compositional analysis of root cementum and dentin after Er:YAG laser irradiation compared with CO_2 lased and intact roots using fourier transformed infrared spectroscopy. *J Periodontal Res* 2002;**37:**50–59.
26. Theodoro LH, Haypek P, Bachmann L *et al*. Effect of Er:YAG and diode laser irradiation on the root surface. Morphological and thermal analysis. *J Periodontol* 2003;**74:**838–843.
27. Schwarz F, Sculean A, Berakdar M, Georg T, Reich E, Becker J. Periodontal treatment with an Er:YAG laser or scaling and root planing. A 2-year follow-up split-mouth study. *J Periodontol* 2003;**74:**590–596.
28. Schwarz F, Sculean A, Georg T, Reich E. Periodontal treatment with an Er:YAG laser compared to scaling and root planing. A controlled clinical study. *J Periodontol* 2001;**72:**361–367.
29. Schwarz F, Putz N, Georg T, Reich E. Effect of an Er:YAG laser on periodontally involved root surfaces: an in vivo and in vitro SEM comparison. *Lasers Surg Med* 2001;**29:**328–335.
30. Kreisler M, Al Haj H, Daubländer M *et al*. Effect of diode laser irradiation on root surfaces in vitro. *J Clin Laser Med Surg* 2002;**20:**63–69.
31. Kreisler M, Meyer C, Stender E, Daubländer M, Willershausen-Zönnchen B, d'Hoedt B. Effect of diode laser irradiation on the attachment rate of periodontal ligament cells: an in vitro study. *J Periodontol* 2001;**72:**1312–1317.
32. Rossa C Jr., Silvério KG, Zanin ICJ, Brugnera A Jr, Sampaio JEC. Root instrumentation with an erbium:yttrium-aluminum-garnet laser: effect on the morphology of fibroblasts. *Quintessence Int* 2002;**33:**496–502.
33. Schoop U, Moritz A, Kluger WM *et al*. Changes in root surface morphology and fibroblast adherence after Er:YAG laser irradiation. *J Oral Laser Applications* 2001;**2:**83–93.
34. Haypek P, Theodoro LH, Bachmann L, Eduardo CP, Sampaio JEC, Zezell DM. Effects of the diode laser irradiation on root surfaces. Thermal analysis. *Braz Dent J* 2004;**15:**110.
35. Rapaport SI. *Mecanismos de hemostasia: Introdução à Hematologia*. São Paulo: Roca, 1990: 330–358.
36. Folwaczny M, Benner KU, Flasskamp B, Mehl A, Hickel R. Effects of 2.94 μm Er:YAG laser radiation on root surfaces treated in situ: a histological study. *J Periodontol* 2003;**74:**360–365.
37. Folwaczny M, Thiele L, Mehl A, Hickel R. The effect of working tip angulation on root substance removal using Er:YAG laser radiation: an in vitro study. *J Clin Periodontol* 2001;**28:**220–226.
38. Sasaki KM, Aoki A, Shikuzo I, Ishikawa I. Morphological analysis of cementum and root dentin after Er:YAG laser irradiation. *Lasers Surg Med* 2002;**31:**79–85.
39. Fujii T, Baehni PC, Kawai O, Kawakami T, Matsuda K, Kowashi Y. Scanning electron microscopic study of the effects of Er:YAG laser on root cementum. *J Periodontol* 1998;**69:**1283–1290.
40. Rich A, Harris AK. Anomalous preferences of cultured macrophages for hydrofhobic and roughened substrata. *J Cell Sci* 1981;**50:**1–7.
41. Eriksson C, Lausmaa J, Nygren H. Interactions between human whole blood and modified TiO_2-surfaces: Influence of surface topography and oxide thickness on leukocyte adhesion and activation. *Biomaterials* 2001;**22:**1987–1996.
42. Khatiblou FA, Ghodssi A. Root surface smoothness or roughness in periodontal treatment. A clinical study. *J Periodontol* 1983;**54:**365–367.
43. Oberholzer R, Rateitschak KH. Root cleaning or root smoothing. An in vivo study. *J Clin Periodontol* 1996;**23:**326–330.
44. Quirynen M, Bollen CML. The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. *J Clin Periodontol* 1995;**22:**1–14.
45. Moritz A, Doertbudäk O, Gutknecht N *et al*. Bacterial reduction in periodontal pockets through irradiation with a diode laser: a pilot study. *J Clin Laser Med Surg* 1997;**19:**33–37.
46. Bachmann L, Dielbolder R, Hibst R, Zezell DM. Changes in chemical composition and collagen structure of dentine tissue after erbium laser irradiation. *Spectrochim Acta A Mol Biomol Spectrosc* 2005;**61:**2634–2639.
47. Bachmann L, Gomes ASL, Zezell DM. Collagen absorption bands in heated and rehydrated dentine. *Spectrochim Acta A Mol Biomol Spectrosc* 2005;**62:**1045–1049.
48. Ishikawa I, Aoki A, Takasaki AA. Potential application of Erbium:YAG laser in periodontics. *J Periodontal Res* 2004;**39:**275–285.