Interaction Between High-power Diode Laser and Dental Root Surface. Thermal, Morphological and Biocompatibility Analysis

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Purpose: The aim of this study was to analyze the interaction between the high-power diode laser and the dental root surface.

Materials and Methods: Twenty-one single-rooted teeth were divided into 3 experimental groups. Group 1: Root surfaces were treated with scaling and root planing followed by high-power diode laser irradiation in the gated-pulsed mode (wavelength 808 nm, 400 μm optical fiber used parallel to the root surface, 1.5 W for 30 s, 10 Hz, pulse width of 50 μs). Group 2: Root surfaces were treated as in group 1, but irradiated in the continuous-wave mode. Group 3: Control – scaling and root planing using a Gracey curette. The temperature variation, root surface morphology, and proliferation of fibroblasts cultured on the root surfaces were analyzed.

Results: There was an increase in temperature within the biological safety limits; it was significantly higher for group 2. Irradiation modified the smear layer, which exhibited rough areas intermingled with smooth areas. Open dentinal tubules were not observed. The fibroblasts proliferated throughout the experimental time (0 to 3 days). The growth curves of all groups were similar.

Conclusion: Under the conditions of this study, we concluded that the use of high-power diode laser for root-surface conditioning is thermally safe and causes similar superficial morphological changes independent of the irradiation mode used. Additionally, these lased root surfaces are biocompatible, because cell adhesion and growth were not impaired.

Keywords: cell culture, diode laser, root surface.

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The main goal of periodontal treatment is to stop the progress of periodontitis and restore gingival health. Treatment is based on the removal of dental biofilm and calculus, as well as the contaminated cementum and dentin.[1] For this purpose, mechanical techniques are conventionally used, especially scaling and root planing. However, this treatment has limitations due to root anatomy and pocket depth, among other factors.[2] Thus, conventional periodontal treatment may require adjunct therapy in some cases.

Laser irradiation is an adjunct therapy able to improve many conventional periodontal treatments. Sev-

eral different lasers can be used in periodontics.[3] The high-power lasers (810, 980 nm) more commonly used in periodontics are CO_2, Nd:YAG, Er:YAG, Er,Cr:YSGG and, more recently, diode lasers. The thermal and photodisruptive laser effects are responsible for bacterial reduction, as well as inactivation of bacterial endotoxins in cementum.[4-11]

Besides decontamination, some in vitro studies have shown that lasers are able to change the root surface,[12-14] improving[15,16] (or not[17,18]) cell adhesion and proliferation of cultured cells to these lased root surfaces. These contrasting results probably occurred due to differences in the laser wavelengths, parameters, and irradiation procedures.

The diode laser has a bactericidal effect and helps to reduce inflammation in the periodontal pockets in addition to scaling.[19] Some studies observed that diode laser powers of 500 mW to 1.4 W could be safe to use on periodontal tissue.[20,21]

The aim of this study was to test whether the surface morphology obtained through diode laser irradiation of periodontally involved teeth previously scaled with curettes influences gingival fibroblast adhesion and proliferation. We searched for the most suitable irradiation mode to promote cell adhesion and proliferation, which are important steps in periodontal healing.

MATERIALS AND METHODS

Sample Collection

Twenty-one freshly extracted, periodontally involved human teeth were obtained at the Department of Surgery, School of Dentistry, University of São Paulo. The consent of the patients was obtained, and the Ethics Committee of the University approved the project. The teeth were selected using the following criteria: single-rooted, with visible calculus; proximal attachment loss ≥ 7 mm; no history of scaling, root planing, or antibiotic therapy in the previous 6 months. Immediately after extraction, the teeth were immersed in phosphate buffered saline (PBS) solution in glass containers and submitted to sterilization by autoclaving. They were then stored in these containers at 4°C.

The twenty-one teeth were used in the 3 steps of the experiments: analysis of the temperature variation (step A), observation of the root surface morphology (step B), and analysis of the adhesion and proliferation of cultured fibroblasts on the root surfaces (step C).

Temperature Variation Analysis (Step A)

Six teeth were used to analyze the temperature changes during the laser irradiation on the root surface. A rotating instrument was used to open the dental pulp chamber. The dental pulp was removed from the dental pulp chamber. Thermal changes were monitored by means of type K thermocouples (chromel-alumel; SR 510 lock-in amplifier, Standford Research System, Sunnyvale, CA, USA), with the 125-µm-diameter tip placed inside the pulp chamber. A thermal paste filled the dental pulp chamber in order to establish adequate contact between the thermocouple tip and the internal dentin walls. Data in triplicate were obtained during laser irradiation; the temperature rise represented the difference between the maximum and the initial temperature values. Data were presented as mean ± standard error of mean (sem). Comparisons between the groups were carried out and tested with the Student's t-test. The level of significance was 5%.

Experimental Groups

After calculus removal using a Gracey curette, the fragments were divided into three experimental groups, as follows:

Group 1: Root surfaces treated by scaling and root planing, followed by high-power diode laser irradiation in the gated-pulsed mode (n = 10);
Group 2: Root surfaces treated by scaling and root planing, followed by high-power diode laser irradiation in the continuous-wave mode (n = 10);
Group 3: Control (n = 10). Root surfaces treated by scaling and root planing using a Gracey curette (SG ¾, Hu-Friedy, Chicago, IL, USA).

The experimental conditions were established in a pilot study. The settings for irradiation energy and frequency were previously determined in step A of the experiment. The laser parameters used are presented in Table 1.

Specimen Preparation

In order to evaluate changes of the root surface morphology (step B) and adhesion and growth of fibroblasts on the root surfaces (step C), 30 fragments (5

Table 1 Laser parameter settings

Group	Fiber diameter (μm)	Power on the display (W)	Pulse length (ms)	Duty cycle (%)	Repetition rate (Hz)	Mean power (W)	Intensity (W/cm^2)	Irradiation time (s)
Gated-pulsed mode	400	1.5	50	50	10	0.75	298.5	30
Continuous wave mode	400	1.5				1.5	597.1	30

mm x 6 mm) were prepared from experimental root surfaces, 1 mm apical to the cementoenamel junction.

Laser Irradiation

The root surfaces of the fragments were irradiated using a GaAlAs high-power diode laser (Soft Lase, Zap Laser; Pleasant Hill, CA, USA) with a wavelength 808 nm. Power outputs were measured with a power meter. Laser light was delivered through a 400-μm contact optical fiber. Laser parameters on the display were set at 1.5 W power output in a gated-pulsed mode (Group 1): 10 Hz, pulse width of 50 μs, resulting in a the mean power of 0.75 W and power density of 298.5 W/cm^2 at the end of the fiber. Group 2: power output of 1.5 W in a continuous-wave mode and power density of 597.1 W/cm^2. The samples were irradiated for 30 s using a scanning motion. The irradiation was done manually with the optical fiber parallel to the root surface to simulate clinical conditions.

Root Surface Morphological Observation (Step B)

One specimen from each experimental group was prepared for surface topography visualization using scanning electron microscopy. These samples were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer solution (pH = 7.4) for 2 h at 4°C. The post-fixation was done in 1% osmium tetroxide in the same buffer solution. Samples were then dehydrated in ethanol and submitted to chemical drying in hexamethyl disilazane (HMDS, Electron Microscopy Sciences, Fort Washington, PA, USA). Specimens were then sputter coated with gold (Sputtering, SCD 020, Bal-Tec, Balzers, Liechtenstein) and examined in a scanning electron microscope (Leo 430, Leo Ltd, Cambridge, UK).

Cell Culture (Step C)

A cell line derived from human gingival cells (LMF cell line[22]) was used at the 5th passage. The cells were cultured in Dulbecco's modified Eagle medium supplemented by 10% fetal bovine serum and 1% antibiotic-antimycotic solution. The cells were maintained in an incubator at 37°C in a humidified 5% CO_2 atmosphere. Cultures were fed every other day. After sterilization, 27 root fragments (nine from each experimental group) were placed in 24-well petri dishes. Then, 10^3 cells were plated on the top of each root fragment. One, 2, and 3 days after seeding, three samples of each experimental group were fixed in 2% glutaraldehyde in a 0.1 M phosphate buffer solution (pH = 7.4) for 2 h at 4°C for scanning electron microscopy (SEM) analysis. The preparation of the specimens for this analysis was similar to that for root surface morphological observation, except that a different scanning electron microscope was used (Philips XL 30, Eindhoven, The Netherlands).

Cell Counting

To count the cells, the 27 root fragments were observed with a scanning electron microscope at the same working distance (20 mm) and same magnification (500X). For statistical analysis, the micrographs of five defined areas of each root fragment were taken and stored using the SEM software. On each image, the SEM software overlaid a grid of evenly spaced horizontal and vertical lines. This method previously determined by Feist et al[16] allowed for cell counting on the surface of each fragment.

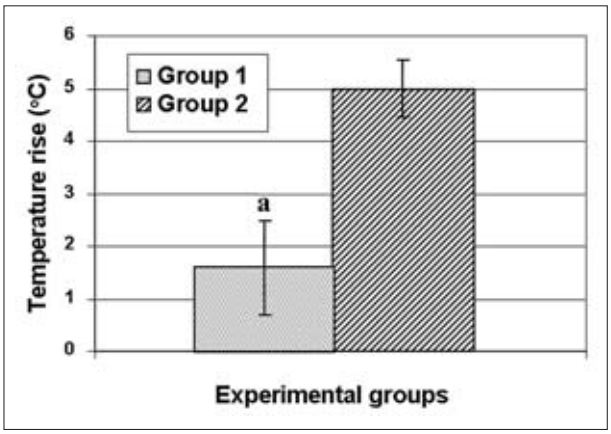

Fig 1 Graphic representation of the temperature rise (°C) inside the dental pulp chamber during the irradiation with the gated-pulsed (group 1) and continuous-wave modes (group 2). (a) Significantly smaller than group 2 ($p < 0.05$).

Proliferation Assay

For the proliferation assay, the number of cells adhered to the root fragments of each experimental group, in triplicate, was obtained 1, 2, and 3 days after seeding. This provided the data for constructin cell growth curves.

Statistical Analysis

Each data point corresponded to the mean ± standard error of the mean (sem) of the cell counts. The data obtained in triplicate were statistically compared using ANOVA, complemented by Tukey's test. The level of significance was 5%.

RESULTS

Temperature Variation Analysis (Step A)

The temperature measurements inside the pulpal chamber showed that with both irradiation parameters used, the temperature increased (Fig 1). This increase was significantly higher for group 2 compared to group 1 ($p < 0.05$). The mean temperature rise was 1.6°C ± 0.9 for group 1, and 5°C ± 0.5 for group 2. The extent of temperature rise depended on the tooth anatomy. In small teeth, such as the mandibular incisors, the temperature increase reached a maximum of 6.5°C.

Root Surface Morphological Observation (Step B)

The root surfaces of periodontally involved teeth after scaling and root planing followed by treatment with high-power diode laser irradiation in both irradiation modes are presented in Fig 2.

The root surfaces treated by the gated-pulsed mode (group 1) showed a modified smear layer, exhibiting smooth areas intermingled with rough areas (Figs 2A and 2B). These areas resembled parallel grooves in a panoramic view (Fig 2A). In detail (Fig 2B), the smooth areas exhibited fusion and resolidification of the root surface in a homogeneous aspect, whereas on the rough areas, these processes occurred in a more irregular fashion. Open dentinal tubules were not observed. On the root surfaces irradiated using the continuous-wave mode (group 2), the same aspects were observed in the scanning electron micrographs (Figs 2C and 2D). Fusion and resolidification of the root surface also occurred covering the dentinal tubules (Fig 2D).

Cell Adhesion and Proliferation (Step C)

The LMF cells became attached and increased in number throughout all root fragments, independent of the experimental groups (Fig 3). These gingival fibroblasts were either stellate or spindle shaped, and spread across the experimental root surfaces both in samples irradiated with the gated-pulsed mode (Figs 3a to 3C) and the continuous-wave mode (Figs 3d to 3f).

Cell numbers of all groups increased from the beginning to the end of the experimental period (1 to 3 days; Figs 4 and 5). Figure 4 shows the growth curve of the cells grown on the surfaces of samples of both lased groups (groups 1 and 2). The cultures grown on the root surfaces treated with the high-power diode laser in the gated-pulsed mode have higher cell numbers than those grown on the surfaces irradiated with the continuous-wave mode, however with no significant differences ($p > 0.05$). When the cell numbers of the control cultures were compared to the lased cultures, no differences were observed among the groups at the same experimental time (Fig 5).

DISCUSSION

Diode laser therapy in combination with scaling supports healing of the periodontal pockets by eliminating bacteria.[8,23,24] Additionally, the diode laser in combination with scaling produces moderate clinical improve-

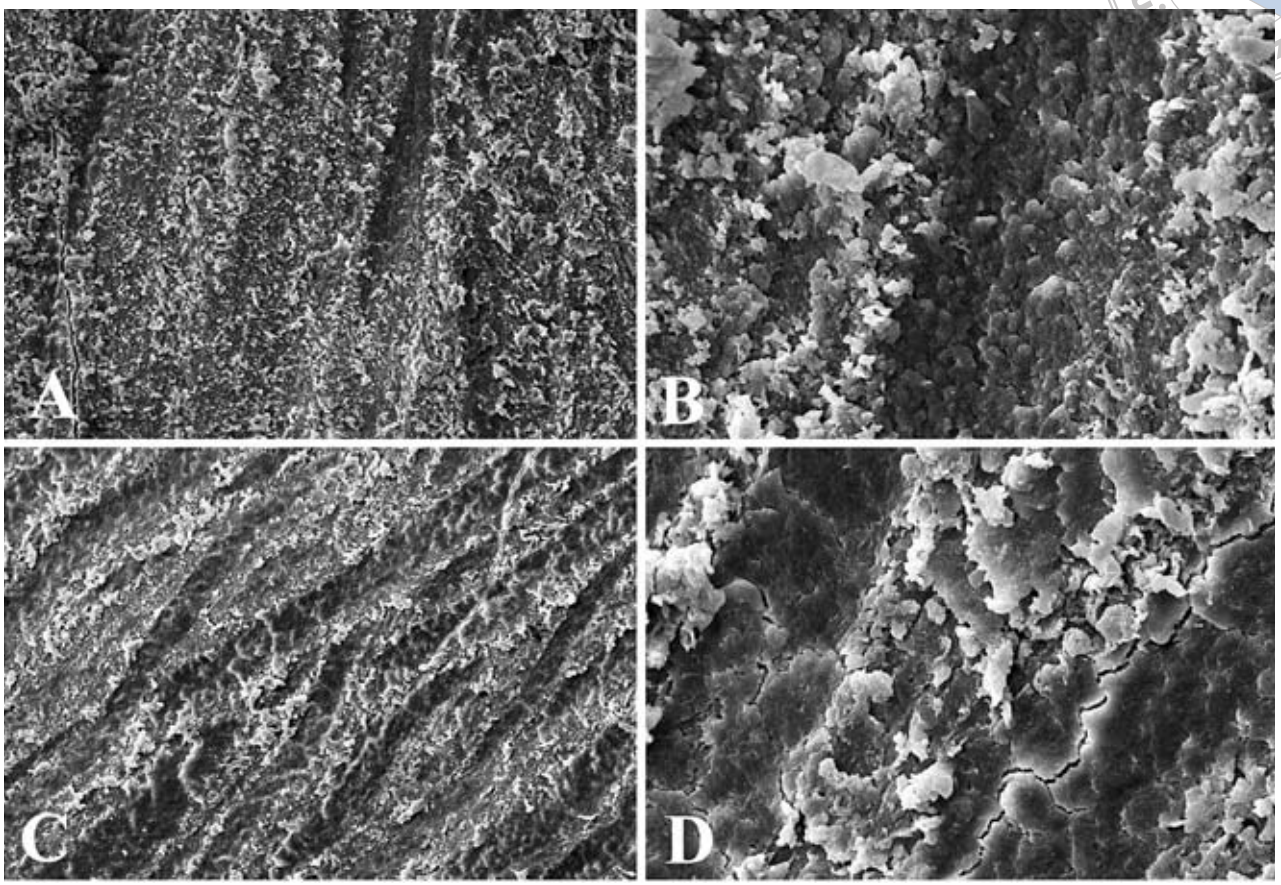

Fig 2 Scanning electron micrographs of the root fragments treated with the high-power diode laser with the gated-pulsed mode (A, B) and continuous-wave mode (C, D). Original magnification 200X (A, C) and 1000X (B, D).

ment over conventional treatment.[25] Although the FDA approved oral soft-tissue surgery in 1995 and sulcular debridement in 1998 by means of a diode laser,[3] the high-power diode laser irradiation of periodontally involved root surfaces needs more investigation.

The present study evaluated the effect of different irradiation modes (gated-pulsed and continuous-wave) of high-power diode laser (880 nm) on periodontally involved root surfaces. Initially, the temperature rose during irradiation using 1.5 W for 30 s with the optical fiber parallel and in contact with the root surfaces. Then, knowing that these parameters were thermally safe, the second step of the study was to evaluate the morphology of the lased root surfaces, and finally, the biocompatibility of such root surfaces was investigated through the adhesion and growth of cultured gingival fibroblasts. The main goal of the study was to find the best irradiation mode for the high-power diode laser in vitro before using this laser in vivo.

Monitoring the temperature during irradiation, it was demonstrated that although the continuous-wave irradiation mode caused a significantly higher temperature increase than that observed when the gated-pulsed irradiation mode was used, the overall temperature increases were within biologically safe limits.

The high-power lasers usually cause an increase in temperature in the target tissues during irradiation. For this reason, it is important to determine the appropriate parameters for each laser in each clinical application in dentistry. Laser light at 800 to 980 nm is poorly absorbed in water, but highly absorbed in hemoglobin and other pigments.[3] Thus, this laser basically does not interact with hard dental tissues; however, the temperature rise could be harmful to the dental pulp, which justified our concern about changes in temperature during diode laser irradiation of root surfaces. In fact, Kreisler et al[20] reported the risk of temperature elevation of the pulp during diode laser irradiation of the

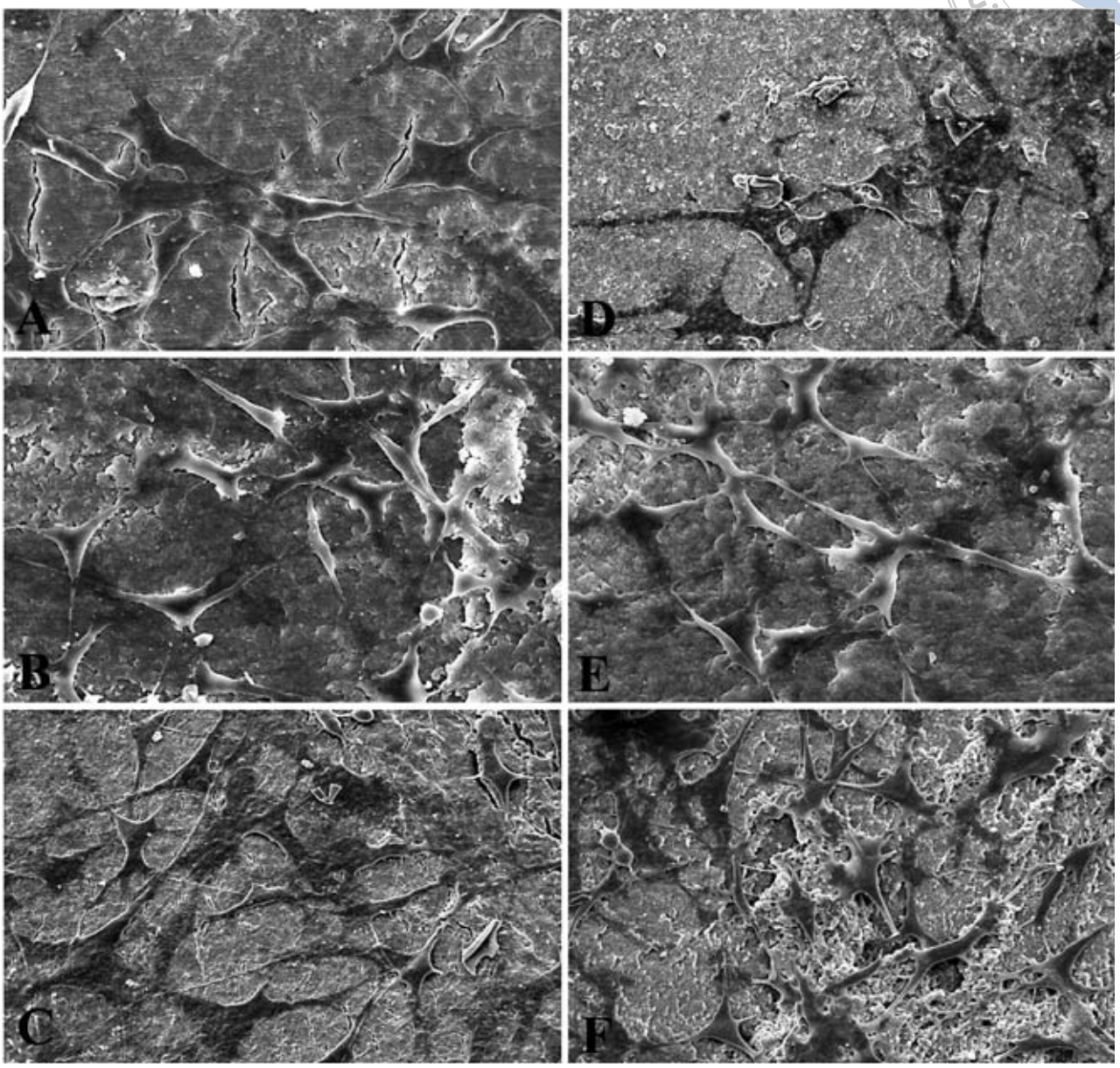

Fig 3 Scanning electron micrographs of the LMF cells attached to the root fragments treated with the high-power diode laser in gated-pulsed mode (A-C); in continuous-wave mode (D-E); at one (A, D), two (B, E) and three (C, F) days after seeding. Original magnification 500X.

root surface. They demonstrated that the temperature increase is energy and time dependent. This is the first study testing different irradiation modes; basic in vitro research on diode lasers applied to root surfaces have used only the continuous-wave mode with power outputs from 0.5 to 2.5 W and irradiation times from 10 to 120 s.[26-29]

The mean temperature variation was 1.6°C ± 0.9 when the gated-pulse mode was used, whereas this increases was 5°C ± 0.5 when the irradiation was done in the continuous mode. The extent of temperature rise depended not only on the irradiation mode used, but also on the tooth anatomy (see above). Although the classical work of Zach and Cohen[30] established 5.5°C as a biologically safe temperature increase limit

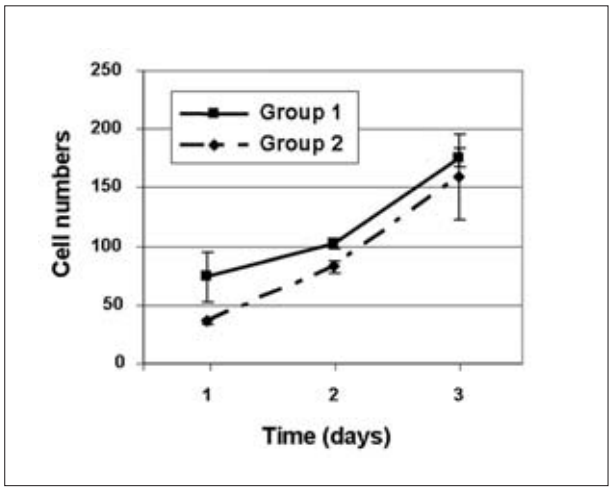

Fig 4 Growth curves of cells grown on lased root surfaces.

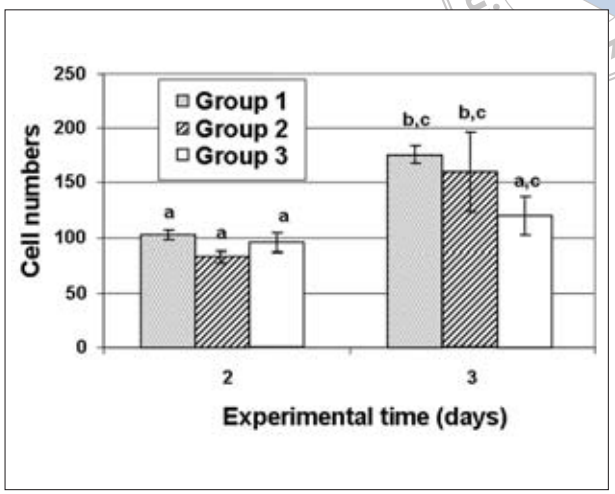

Fig 5 Graphic representation of the cell numbers on the root surfaces of all experimental groups, 2 and 3 days after seeding. Observe that there are no differences among the groups at the same experimental times. Different letters indicate significant differences ($p < 0.05$).

for preserving the dental pulp health, Baldissara et al,[31] using the same basic principle of research as Zach and Cohen[30] but in human teeth, showed no evidence of cellular injury for average temperature increases of 11.2°C. Thus, even in teeth with thin dentin walls, the temperature increases observed in this study were within safe biological limits.

Diode lasers at high energy levels, especially in a continuous mode, can cause root surface alterations in the presence of blood and elevated temperatures, depending on the power employed.[3] Kreisler et al[26] reported no detectable alterations on dry or saline-moistened root surfaces irradiated with diode laser at 1.5 W; detectable alterations were only noticed when a thin blood film covered the root surfaces. In contrast, in our study, even in root surfaces only moistened with saline, clear morphological alterations were observed. This discrepancy could be due to the analysis techniques used. Kreisler et al[26] used reflected light microscopy to look for blackening due to carbonization of the root surface, whereas in the present study, these surfaces were analyzed by scanning electron microscopy. In fact, in agreement with these authors, we also did not find carbonization, only fusion and resolidification. This result could also be due to the angle of irradiation, which was close to 0 degrees (parallel to the root surface). Kreisler et al[26] observed that the root surface damage is greater when angles close to 90 degrees were used.

Although morphological alterations were observed in our samples, these alterations caused no deleterious effect to those lased root surfaces; cell attachment and proliferation were not impaired. Similar results were observed by Kreisler et al,[27] who found similar periodontal ligament cell attachment on root surfaces submitted to scaling and root planing followed by air-powder abrasive treatment, or diode laser irradiation at 1 W in the continuous mode for 20 s, or the control group left unirradiated.

The root surfaces treated by either the gated-pulsed or continuous mode showed a modified smear layer, with smooth areas intermingled with rough areas resembling parallel grooves. The smooth areas exhibited fusion and melting in a homogeneous aspect, whereas on the rough areas, these processes occurred in a more irregular fashion. Open dentinal tubules were not observed. On both lased root surfaces, as well as on the control root surfaces, cell attachment and proliferation occurred in a similar fashion. This means that these alterations were not able to change the biocompatibility of the root surfaces. This is probably because no sharp scales or edges were present on these root surfaces. Feist et al[16] found that, when root surfaces were irradiated with Er:YAG laser at 100 mJ of energy, irregular roughness of the lased root surfaces impaired the cell attachment and proliferation.

Clinical studies have demonstrated that a diode laser facilitated bacterial elimination from periodontal pock-

ets, resulting in better healing. Moritz et al[8,24] concluded that diode laser therapy, in combination with scaling, supports healing of periodontal pockets by eliminating bacteria. Although the power outputs used in those studies were as high as 2.5 W in pulsed mode (50 Hz), there are other studies in which lower power outputs were used for periodontal pocket therapy, 2 W for curettage before mechanical debridement.[32,33] It would therefore be interesting to test the bactericidal effects of the parameters used in our study (1.5 W), since this power output – independent of irradiation mode – proved to be safe not only thermally, but also in terms of root surface alterations, which certainly occur but do not change the biocompatibility of the lased root surfaces. Thus, if these parameters were applied in periodontal pocket treatment under the conditions used in this study, improvement of periodontal healing could possibly be achieved.

CONCLUSION

High-power diode laser irradiation (wavelength 808 nm, 400-µm optical fiber used parallel to the root surface, 1.5 W for 30 s) applied in either continuous-wave or gated-pulsed mode (10 Hz) proved to be safe not only thermally, but also in terms of root surface alterations, which occur but do not change the biocompatibility of the lased root surfaces.

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