ISSN 1054-660X, Laser Physics, 2011, Vol. 21, No. 5, pp. 958–964.

Evaluation of Bone Repair after Radiotherapy by Photobiomodulation—An Animal Experimental Study[1]

M. R. S. Freire[a], *, D. Almeida[b], J. N. Santos[a], and V. A. Sarmento[a]

[a] *Federal University of Bahia, Rua Araújo Pinho, n. 62, Salvador-Bahia, Brazil CEP: 40110-150*
[b] *Federal University of Reconcavo of Bahia, Rua Rui Barbosa, s/n, Cruz das Almas-Bahia, Brazil CEP: 44380-000*
*e-mail: rosariofreire@ufba.br

Received June 30, 2010; in final form, November 4, 2010

Abstract—This research evaluated the effect of GaAlAs lasertherapy at the healing of surgical wounds produced in Wistar rat femurs a few days before the beginning of the radiotherapy. An orifice was artificially produced in the femur bone of the rats and they were submitted to an external radiotherapy with a radioactive source of cobalt in the dosage of 3000 cGys. The experimental group received additionally seven sessions of 780 nm, 40 mW, 100 or 5 J/cm^2 in four points around the surgical wound, at each 48 h, initiated at the day of surgery. These animals were then sacrificed at three and five weeks. The results were based on the clinical and histological analyses. Clinically, even though the rats had gained body mass within the time of the experiment ($p < 0.05$), those who has been submitted to the lasertherapy presented cutaneous inflammatory reactions. Regarding the histological findings, the number of osteocites ($p < 0.0001$) and Harvers channels ($p < 0.0001$) was significantly larger in the groups that had been radiated with laser during the experiment.

DOI: 10.1134/S1054660X11070085

1. INTRODUCTION

Radiotherapy is one of most useful methods to treat head and neck cancers, with radioactive sources that not only kill the tumor cells but also damage healthy surrounding tissue. Most of the time several side effects appear including mucositis, pain, xerostomy, loss of taste, trismus, radiation decay, candidiasis, HSV infections, gingival bleeding and osteoradionecrosis, that can appear from the first month until years later. Diagnosis delays can occur at the level of patients, primary care, systems and secondary care [1].

Prevention should be the best way to treat these patients, with dental care and hygiene education before the start of treatment and re-evaluation during and after radiotherapy treatment. The patient needs to know exactly the problems that can occur, alternatives and ways to treat them.

After head and neck radiotherapy, exodontias or others invasive procedures, can be associated with insufficient blood irrigation and determine the beginning of the infection. Clinicians treat with antibiotics, debridment procedures but the results, at times, aren't good enough and osteoradionecrosis probably can be initiated; most of times the only treatment is bone resection, with functional and esthetic problems.

Osteoradionecrosis is a permanent risk in the individual undergoing radiotherapy in the jaw and, technically, is a wound with hypoxia due to sclerosis of the small vessels. This compromises the ability and defensive properties of irradiated tissue repair, which predisposes the mandible to infection, bone sequestration, functional limitation and facial deformity. The incidence varies from 4 to 15% in irradiated cases at different treatment centers and is related to the organic condition of the patient, the method of splitting the total dose, the total dose, the radiotherapy technique used, anatomical characteristics of the irradiated fields, trauma and/or infection [2]. In an extensive lesion located in floor of the mouth and irradiated with doses above 6500 cGy the occurrence of osteoradionecrosis may reach 25% [3].

At this time, phototherapy may represent an effective tool to prevent and alleviate most of the symptoms. The biomodulation of low level laser is well known and should be applied more often in oncological patient. The only place that cannot be irradiated with the laser is the base of the tumor, and researchers are continuing to look for protocols of light delivery. Based on studies, the target is to find the best fluence, output power, spotside and wavelength observing the responses of the body and using the phototherapy to give a better quality of life to patients and less cost for Public Health. The aim of this research is to evaluate the effect of the GaAlAs photobiomodulation in the healing of surgical wounds produced in Wistar rat femurs, a few days before the beginning of the radiotherapy.

2. METHODS

2.1. Population and Sample

Sixteen male *Rattus norvegicus albinus, Rodentia mammalia, Wistar*, young adults, with an average

---

[1] The article is published in the original.

Fig. 1. Animals prepared to receive radiotherapy.

weight of three hundred grams, obtained from the Adventist School of Physiotherapy—Cachoeira–BA. The animals were housed individually in standard cages, measuring 22.5 × 15.0 × 14.5 cm^3, in a room with controlled temperature (21 + 1°C) and light (6 to 18 h); fed with Nuvilab CR1®, in the form of pellets, and hydrated ad libitum in the animal house of UNIME—Metropolitan Union of Education and Culture, Lauro de Freitas—BA.

The animals received care in compliance with Ethical Principles of Animal Experimentation formulated by Brazilian College for Animal Experimentation according to the guidelines approved by the Council of the American Psychological Society for the use of animals in experiments.

2.2. Surgical Procedures

The surgical procedure was performed on day zero. After weighing, the rats were anesthetized by intraperitoneal injection of sodium thiopental at a dose of 0.2 ml/100 g of body weight. Trichotomy was performed in the hip joint of the hind leg; half of the animals used the left leg and the other half, the right.

With the skin exposed, the disinfection was done with iodine alcohol 0.5%. The incision was 2 cm above the knee, using Bard Parker scalpel (no. 15), following the long axis of the femur. After the incision, a dissection of the underlying tissues with a hemostatic instrument to the periosteum. Then, the periosteum was also incised and detached using the small end of a spatula (no. 7).

The exposure and visualization of the femur was measured with a ruler the distance of 1 cm from the knee and at that point a monocortical hole was made with a drill (no. 1), using a low-speed Dremel Minimite® (10000 rpm) with constant saline solution irrigation. Finally, suturing was performed in layers, using an absorbable suture internally (no. 3.0) and mononylon externally (no. 4.0).

2.3. Laser Photobiomodulation

After surgery the animals were divided into four groups, namely:

—Group I (*BLUE*)—Consisting of five rats that immediately after surgery, underwent seven sessions of Laser photobiomodulation, λ 780 nm, 40 mW, ∅4 mm, 100 J/cm^2 (TWIN LASER®, MMOptics, São Carlos-SP, Brazil), 100 s, totaling four points around the surgical area, every 48 h. These animals were sacrificed after three weeks.

—Group II (*RED*)—Consisting of five rats that immediately after surgery, underwent to seven sessions of Laser photobiomodulation, λ 780 nm, 40 mW, ∅4 mm, 100 J/cm^2 (TWIN LASER®, MMOptics, São Carlos-SP, Brazil), 100 s, totaling four points around the surgical area, every 48 h. These animals were sacrificed after five weeks.

—Group III (*BLACK*)—Consisting of three rats that were submitted only to surgery and radiotherapy, and sacrificed after three weeks.

—Group IV (*GREEN*)—Consisting of three rats that were submitted only to surgery and radiation therapy, and sacrificed after five weeks.

2.4. Radiotherapy

After surgical wounding of the animals, they were submitted to radiotherapy 3000 cGy, four days after surgery. The rats were first anesthetized with sodium thiopental 0.2 ml/100 g and fixed with tapes in an acrylic holder, built especially for this procedure (Fig. 1) and based on another experiment [4]. This support allowed the irradiation of eight animals at the same time.

The apparatus used for irradiation was the THERATRON 780® (CO^{60}). The focus-surface distance was 80 cm. The size of the field, measured and estimated for each rat, was 2 × 2 cm^2, obtained by interposing pieces of lead. There was one session of 3000 cGy, in the femoral area.

2.5. Samples Sacrificies

To obtain the samples, the animals were sacrificed at three and five weeks after radiotherapy by administering a lethal dose (3–4 ml) of chloral hydrate10% intraperitoneally.

The femur was disarticulated and removed with a no. 3 scalpel. After were removed the posterior leg. The samples were then fixed in 10% formaldehyde until the time of histological preparation. Each container was identified with the number of the animal and the color of the group to which it belonged.

2.6. Data Analysis

The normal distribution of data was evaluated by the Kolmogorov and Smirnov test. The data analysis applied was the *t* Student test for paired and unpaired samples, Analysis of Variance (ANOVA), Mann Whitney and Chi-square for an error probability of 5%.

3. RESULTS AND DISCUSSION

In this study the bone chosen to be studied was the femur. Despite the fact that the femur does not have the same vascularization as the jaw or the same type of ossification (the jaw is formed mostly by intramembranous ossification and the femur by endochondral ossification), the femur bone is easily accessible. This facilitates the surgical procedure and immobilization of the animal, both during surgery and postoperatively, for irradiation with laser. In addition, the femur was the bone of choice in many previous studies [5–7]. In the Bastos [8] study, the photobiomodulation was applied on lesioned Achilles tendon of the rats and they compared lasers (λ 685 and 830 nm) and LEDs (*light emitting diode*) at λ 630 and 880 nm, and concluded that applications of infrared laser at 830 nm and LED 880 nm were more efficient.

The bone defect was done here to add the inflammation factor to the healing process. Among other causes, the risk of bone necrosis increases with bone trauma [9]. It is known that before radiotherapy, patients have to extract one or more infected teeth without other therapeutic options, to prevent a possible osteoradionecrosis after irradiation. However, if radiotherapy is initiated before the resolution of this process, the area may not heal.

The radiotherapy dose of 3000 cGy was chosen because the average doses used in previous research, which was used in the femur of rats of the same lineage in a single irradiation [10]. Other research used a dose of 3000 cGy of X-irradiation in rats in order to induce osteomyelitis [11]. To examine the effects of fractionated radiotherapy on wound healing, another study divided the animals into two groups, with doses of 4000 and 4800 cGy [12].

Several studies have developed techniques of lasertherapy in doses from 1.8 to 5.4 J/cm^2 in surgical wounds [6, 7, 13–15] and discussing how to apply it in four points around 1 cm injured bone at a dose of 5 J/cm^2 [16].

In this study, on day zero, there was no statistical difference in body mass of the rats in the four groups ($p = 0.5478$; Analysis of Variance). In the third week of the experiment when the animals in the black and blue groups were sacrificed also no significant difference between the four groups ($p = 0.4474$) was found. Comparing groups two by two, the weight of the rats between groups black and blue, no statistical difference was observed ($p = 0.7041$; *t* Student test), also between red and green groups ($p = 0.0578$; *t* Student test). This might indicate that the application of different therapies (radiotherapy and lasertherapy) did not affect weight gain in rats. Also in the period between day zero and the third week of the experiment, all rats gained body mass.

Table 1. Number of rats that had clinically inflammatory reaction in the wound, the day of sacrifice, depending on the group evaluated

Groups	Normal healing	Inflamatory reaction	Total
Black	3(100%)	0	3
Green	3(100%)	0	3
Blue	3(60%)	2(40%)	5
Red	4(80%)	1(20%)	5
Total	13	3	16

In the fifth week of the experiment there were only the animals from the green and red groups with no weight difference between them ($p = 0.4354$; *t* Student test). There was observed a gain in body mass of animals in the red group, higher than the green group, after five weeks. In the red group, the rats were submitted to lasertherapy, which may explain the increase in activity, appetite and body weight in these animals.

These results differ from others found in the literature, which show a decrease in weight over time in animals subjected to radiation at two weeks [10]. The weight gain in this work should be related to the phase in which the animals were—between fifty to sixty days of life at the stage of puberty [17]. From the beginning of the experiment the animals were older than thirty days, they were in a growth phase to reach 350 g, which is the average weight for an adult [15].

Prior to sacrifice, the entire sample was analyzed clinically, in order to determine whether there was a correct healing of the surgical wound in soft tissue, or the occurrence of inflammatory reactions with the formation of abscesses and fistulas. It was observed in black and green groups (controls) that the wounds healed uneventfully, but those groups of blue and red (lasertherapy), the majority had abscess formation and fistulas. The data is shown in Table 1. This suggests that the laser enhances inflammatory reactions, since the low-intensity laser is able to increase the production of prostaglandins and other chemical mediators of inflammation [14, 16, 17].

It is proven that low intensity laser, in interaction with the tissue causes changes in the cell membrane, alters its permeability, increases ATP synthesis and other metabolic activities, promoting a series of physiological changes. Different wavelengths appear to be absorbed by different receptors. Most authors agree that the improvement in wound healing is due to the increase in cell proliferation. The changes promoted by the photochemical action of light, both in the cell membrane and in the nucleus of the cell, are increas-

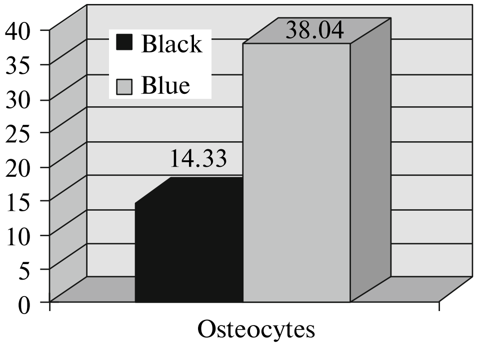

Fig. 2. Average number of osteocytes (ten fields) in rats in the black and blue groups.

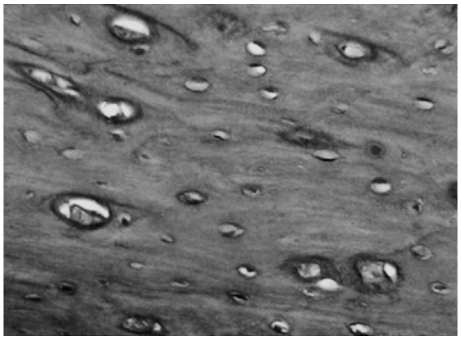

Fig. 3. Histological findings at group I (blue) showing the large quantity of osteocytes (HE, 40X).

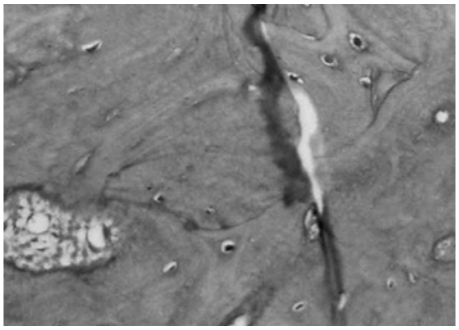

Fig. 4. Histological findings at group III (black) showing the small quantity of osteocytes (HE, 40X).

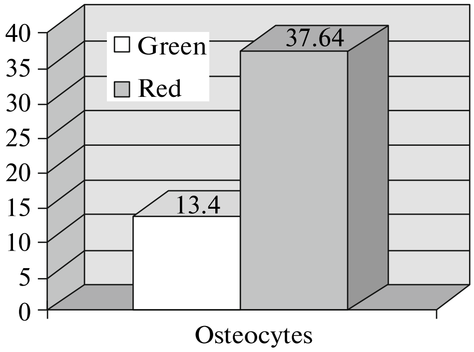

Fig. 5. Average number of osteocytes (ten fields) in rats in green and red groups.

ing local vasodilatation, angiogenesis, production of fibroblasts, collagen synthesis, production of T and B lymphocytes, release of endorphins and local changes in local prostaglandins (physiological changes as a result of laser light in the soft tissues). This type of therapy is used in the stimulation of healing of soft tissues such as inflammation, pain relief and to stimulate the immune system to mitigate the effects of infection [16–20]. In this study the laser photobiomodulation appears to have increased inflammatory reaction in soft tissues, especially after three weeks of the experimental procedures in the green group. In the red group, also submitted to lasertherapy, there was an inflammatory reaction in soft tissues but in smaller intensity, which suggests the reverse of the state, during the time.

3.1. Count of Osteocytes

In histological analysis, after the femur treated with radiotherapy and lasertherapy or not, being dissected, they were demineralized, sectioned and stained by the methods of HE and Picrosirius; the slides originally were analyzed using light microscopy, with increases of twenty, forty and a hundred times. In addition to the subjective description of the cuts, some parameters were quantified. The results of the count of osteocytes, osteoblasts and Harversian systems in ten fields of each rat in different groups are presented below.

Comparing the rats in the black and blue groups that were sacrificed after three weeks, it was observed that there was a statistical difference ($p < 0.0001$; t Student test), at the average number of osteocytes, higher in the blue sample irradiated by laser photobiomodulation (Fig. 2). This seems to indicate that the cellular activity was more intense after the technique, in agreement with clinical findings, which showed an inflammatory reaction in this group (Figs. 3 and 4).

Also in green and red groups, with sacrifice after five weeks of the experiment, there was a statistical difference ($p < 0.0001$; t Student test), at the average number of osteocytes in the fields assessed. It was observed that the group that underwent photobiomodulation (red) there was a higher number of osteocytes (Fig. 5), demonstrating a more intense cellular activity.

The comparision of the black and green groups to each other, neither subjected to photobiomodulation, there was no statistical difference in the average number of osteocytes in the fields evaluated ($p = 0.4139$;

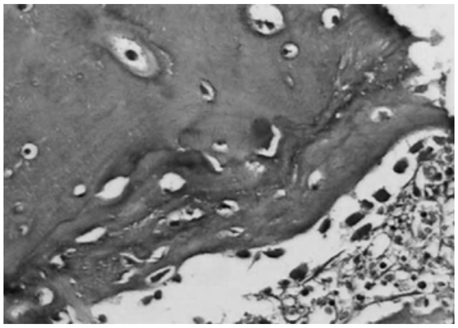

Fig. 6. Histological findings at group I (blue) showing the presence of osteoblasts at the bone cortical periphery (HE, 40X).

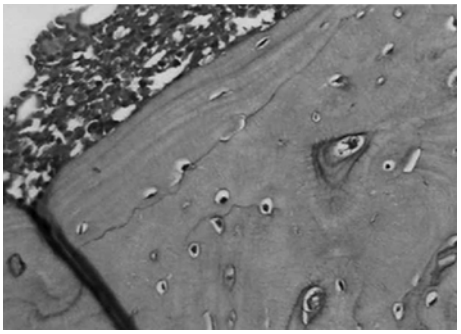

Fig. 7. Histological findings at group III (black) showing the small quantity of osteoblasts at the bone cortical periphery (HE, 40X).

t Student test). Similarly, between the blue and red groups, both submitted to lasertherapy after radiotherapy, no statistical difference between the average number of osteocytes was observed ($p = 0.8304$; *t* Student test), although in both cases the number of osteocytes decreased with time.

This shows that the lasertherapy was responsible for the increased number of osteocytes in blue and red groups and radiotherapy determined a decrease in the number of osteocytes, which has been confirmed in another study [10]. In conclusion it is observed that the number of cells of both cortical bone and marrow decreases over time and the activity of bone reabsorption increases with time, according to another study [15].

The results also showed that the number of osteocytes per field is higher in the laser irradiated groups, confirming the results of research where the lasertherapy was applied in bone tissue, although different in the wavelengths in 830 [5–7] and 660 nm [16–18] but in all of them the laser photobiomodulation increased the number of bone cells by promoting new bone tissue, and as found in this study, with a wavelength of 780 nm.

3.2. Presence of Osteoblasts in the Periphery of Cortical Bone

Some works [14, 15, 20–23] analyzed the effects of laser radiation in the rate of cell proliferation of osteoblasts, fibroblasts and odontoblasts. The results observed in these studies show that lasertherapy increased the number of these cells both in vitro and in vivo. When were compared two different wavelengths, Pretel's research [21] found a better response to accelerate dentin barrier formation in 785 nm. Another study [24] evaluated in vitro the effects of LLLT (low-level lasertherapy on cell metabolism (MTT assay), alkaline phosphatase (ALP) expression and total protein synthesis; they concluded under the tested conditions and the LLLT parameters used, that it did not influence cell metabolism, but reduced slightly the expression of some specific proteins.

In this analysis there was observed an association ($p < 0.05$; chi-square test) between the presence or absence of osteoblasts at the cortical bone periphery and study groups (Figs. 6 and 7). In the groups irradiated with laser, the presence of these cells occurred more frequently than in the groups that received no treatment (Table 2).

3.3. Count Harvers Channels

Based on the results of descriptive histological analysis of the groups, it can be noted significant difference only between the animals that were irradiated with laser and the presence of some Harversian formations. Counting the average number of Harvers channels in ten fields of each histological section obtained from the studied groups, it was observed that there was a significant difference ($p < 0.0001$; Mann–Whitney test), in the amount of Harvers channels between the black and blue groups and between the green and red groups, being higher in the groups submitted to lasertherapy (blue and red groups). This means that the laser light increased the amount of Harvers channels, due to the intensification of the inflammatory process that occurred in these rats. The data can be viewed in Table 3.

Comparing the black and green groups, it was noted that there was statistical difference ($p < 0.02$; Mann–Whitney test), being the average number of Harvers channels slightly higher in the green group

Table 2. Association between the presence of osteoblasts in the periphery of cortical bone and the group evaluated

Groups	p (Chi-square test)
Black × blue	$X_c^2 = 14.86$ ($p < 0.05$)
Green × red	$X_c^2 = 18.76$ ($p < 0.05$)

Table 3. Average number of channels Harvers between the groups evaluated

Groups	Average	Mann–Whitney test
Black × blue	1.3 × 1.97	$p < 0.0001$
Green × red	4.02 × 4.04	$p < 0.0001$

Table 4. Average number of channels Harvers between groups evaluated

Groups	Average	
Black × green	1.3 × 4.02	Mann–Whitney test $p < 0.02$
Blue × red	1.97 × 4.04	t Student test $p = 0.95$

(sacrificed at five weeks). This indicates that over time there is a tendency to form a greater number of Harvers channels, which probably has been diminished under the effect of radiotherapy. Between the blue and red groups, it was observed that there was no statistical difference ($p = 0.95$; t Student test) in the amount of Harvers channels, although in the red group (sacrificed at five weeks) the average was higher than in rats of the blue (sacrificed three weeks). This data can be viewed in Table 4.

The presence of Harversian formations and bone cells increased in irradiated groups with lasertherapy is very important since bone tissue submitted to radiotherapy suffered loss of bone cells, vasoconstriction and decrease the ability of bone to regenerate [25, 26], which are some of the reasons that facilitate the initiation of osteoradionecrosis after a bone infection.

From the foregoing, it can be concluded that the use of laser photobiomodulation stimulates the process of repair of surgical wounds before the radiotherapy started in rats under the protocols studied here; but it also triggered inflammatory skin reactions of considerable magnitude. With the presence of infected areas, methods such as PDT (Photodynamic Therapy) can also be applied as a healing procedure, and the combined techniques are tested recently and successfully, in an investigation with photoditazin as the photosensitizer [27]. Pain is another complaint of the patient undergoing cancer treatment, where the laser photobiomodulation is also indicated, as used at Clavijo's clinical study [28]. This determines a high expectation of the use of lasertherapy in humans who undergo radiation treatment and have teeth extracted or indication of needing surgery before the start of radiotherapy sessions.

4. FUTURE DIRECTIONS

Admittedly, however, that further investigations must be carried out to assess the safety of the method. If the laser photobiomodulation was indicated in such situations, it could stimulate tissue repair and thus prevent the initiation of an infection and osteoradionecrosis after radiotherapy. Proteomics and biomarkers should be the next stage to solve this side effect.

ACKNOWLEDGMENTS

To UNIME represented by the Prof. Dra. Ana Verena Madeira and Prof. Dr. Marcos Borges Ribeiro. To Santa Izabel Hospital represented by Dra. Maria Eulina Ramos Tavares, at the Radiotherapy Center, and also the physic Jailton Caeano de Souza. Thanks to my collegue Dra. Darcy Almeida, my orientator Dra. Viviane Almeida Sarmento and to Dr. Robert Prehatney, who worked with the translation of this paper and all the people that contributed to this research.

REFERENCES

1. R. D. Neal, Brit. J. Cancer **101**, S9 (2009).
2. J. F. Góis, E. E. Fukuyama, P. J. Valentim, and A. A. Feitosa, Rev. Soc. Bras. Cancer **12**, 4 (2000).
3. D. W. Klotch, T. Ganey, and H. Greenburg, Head Neck Surg. **114**, 620 (1996).
4. F. V. Mello-Filho, M. Auader, E. Cano, R. L. Carrau, E. N. Myers, and C. E. Miles, Am. J. Otolaryngol. **24**, 231 (2003).
5. A. L. B. Pinheiro, M. G. Oliveira, P. P. M. Martins, L. M. P. Ramalho, M. A. M. Oliveira, A. N. Júnior, and R. A. Nicolau, Laser Ther. **13**, 73 (2001).
6. C. B. Lopes, A. L. B. Pinheiro, S. Sathaiah, N. S. Silva, and M. A. C. Salgado, Photomed. Laser Surg. **25**, 96 (2007).
7. M. E. M. Gerbi, A. M. C. Marques, L. M. P. Ramalho, E. A. C. Ponzi, C. M. Carvalho, R. C. Santos, P. C. Oliveira, M. Noia, and A. L. B. Pinheiro, Photomed. Laser Surg. **26**, 55 (2008).
8. J. L. N. Bastos, R. F. Z. Lizarelli, and N. A. Parizotto, Laser Phys. **19**, 1925 (2009).
9. Y. Maegawa, T. Itoh, T. Hosokawa, K. Yaegashi, and M. Nishi, Lasers Surg. Med. **27**, 427 (2000).
10. S. Kiyohara, T. Sakurai, and I. Kashima, Dentomaxillofac. Radiol. **32**, 30 (2003).
11. N. Ozbek, E. Guneren, L. Yildiz, D. Meydan, S. Cakir, and M. Coskun, Oral Maxillofac. Surg. **34**, 185 (2005).
12. I. G. F. Freitas, V. Baranauskas, and M. A. Cruz-Höfling, Appl. Surf. Sci. **154**, 548 (2000).
13. O. Dörtbudak, R. Haas, and G. Mailath-Pokorny, Clin. Oral Implants Res. **11**, 540 (2000).
14. A. L. B. Pinheiro, E. T. Cavalcanti, T. N. R. Pinheiro, M. J. P. C. Alves, and C. T. A. Manzi, J. Clin. Laser Med. Surg. **15**, 181 (1997).
15. S. S. Cunha, V. Sarmento, L. M. P. Ramalho, D. Almeida, E. B. Veeck, N. P. Costa, A. Mattos, A. M. Marques, M. Gerbi, and A. C. Freitas, Photomed. Laser Surg. **25**, 197 (2007).
16. J. E. Harkness and J. E. Wagner, *Biologia e Clínica de Coelhos e Roedores* (Roca, Rio de Janeiro, 1999).

17. R. A. Nicolau, V. Jorgetti, J. Rigau, M. T. T. Pacheco, L. M. Reis, and R. A. Zangaro, Lasers Med. Sci. **18**, 89 (2003).
18. Y. Franks, J. Commun. Nursing **13**, 14 (1999).
19. T. I. Karu, IEEE J. Quantum Electron. **23**, 1703 (1987).
20. P. Torricelli, G. Giavaresi, M. Fini, G. A. Guzzardella, G. Morrone, A. Carpi, and R. Giardino, Biomed. Pharmacother. **55**, 117 (2001).
21. H. Pretel, J. A. Oliveira, R. F. Z. Lizarelli, and L. T. O. Ramalho, Laser Phys. Lett. **6**, 149 (2009).
22. M. Gordjestani, L. Dermaut, and H. Thierens, Int. J. Oral Maxillofacial Surg. **23**, 54 (1994).
23. L. Almeida-Lopes, J. Rigau, R. A. Zangaro, J. Guidugli-Neto, and M. M. M. Jaeger, Lasers Surg. Med. **29**, 179 (2001).
24. C. F. Oliveira, J. Hebling, P. P. C. Souza, N. T. Sacono, F. R. Lessa, R. F. Z. Lizarelli, and C. A. S. Costa, Laser Phys. Lett. **5**, 680 (2008).
25. E. M. N. Caccelli and A. Rapoport, Rev Bras. Cir. Cabeça Pescoço **37**, 198 (2008).
26. A. A. S. Lima, M. A. Z. Figueiredo, S. Krapf, and F. Souza, Rev. Bras. Cancerolog. **50**, 287 (2004).
27. A. B. Soiovieva, P. I. Tolstih, N. S. Melik-Nubarov, T. M. Zhientaev, I. G. Kuleshov, N. N. Glagolev, A. V. Ivanov, G. I. Karahanov, M. P. Tolstih, and P. S. Timashev, Laser Phys. **20**, 1068 (2010).
28. E. M. A. Clavijo, V. R. G. Clavijo, M. C. Bandéca, M. R. Nadalin, M. F. Andrade, J. R. C. Saad, and A. A. Pelegrine, Laser Phys. **19**, 2041 (2009).