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The Effect of Low-Level Laser Treatment (LLLT) on Peri-Implant Hard and Soft Tissue Healing: A Review of Experimental Studies

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Abstract: Osseointegration of titanium dental implants is the most essential factor, which is predictive of the stable function of structures that are based on implants. Additionally, the adequate regeneration of peri-implant soft tissues, provides protection against the bacterial invasion and consequently averts the development of peri-implantitis. Nowadays, several types of laser are widely used to accelerate healing of epithelium, connective tissue and bone. Especially, the application of low-power laser, seems to have a stimulatory action on peri-implant tissues repair. The aim of this study is to show the results of several studies, mainly based on animal experimental models, that investigate the beneficial action of low-power laser, when it is applied to enhance the regeneration of alveolar bone, of soft tissues and to increase the integration of several types of graft materials.

Key words: Low level laser treatment, titanium implants, oral surgery, bone healing, hard tissue, soft tissue

INTRODUCTION

Nowadays, lasers have become widely used in dentistry. The lasers used in dental applications have included high-level lasers such as the CO_2, the Nd: Yttrium Aluminum Garnet (Nd: YAG) laser, the argon laser, the Er: YAG laser and the excimer laser and low level lasers such as the He-Ne laser and the Gallium-Aluminum-Arsenide (GaAlAs) laser. During the past decade, there has been an explosion of research in the application of Low Level Laser Treatment (LLLT) to general dental practice. LLLT has been used for >30 years and no adverse effects have been reported (Goldman *et al*., 1987; Qadri *et al*., 2005). It is supposed to reduce pain, accelerate would healing and reduce inflammatory processes. Furthermore, it enhances bone remodeling, attenuates pain and modulates the immune system (Walsh, 2003; Goldman *et al*., 1987).

Particularly, in hard tissues, low-power laser irradiation was shown to speed up vasculation and to increase the number of trabeculae in fractured mouse tibiae (Trelles and Mayayo, 1987). In another study in rats, alkaline phosphatase was significantly increased 6 days after irradiation of standardized bone defects, which reflected an enhanced osteoblastic activity (Barushka *et al*., 1995). In an *in vitro* experiment of rat osteoblasts, three low-power laser irradiations significantly increased the osteoblast count after an interval of 8 days (Dortbudak *et al*., 2000). Stimulation with LLLT creates a number of environmental conditions that appear to accelerate the healing of bone in *in vivo* and *in vitro* investigations (Pinheiro and Gerbi, 2006). LLLT-related effects include stimulation of blood flow, recruitment and activation of osteoblasts, osteosynthesis, a decrease in osteoclastic activity and anti-inflammatory action (Nicolau *et al*., 2003).

In soft tissues, researchers evidenced faster healing of wounds after laser therapy, with an increase in mitotic activity, fibroblast number and the synthesis of collagen and neovascularization (Gomez-Villamandos *et al*., 1995). Yu *et al*. (1994) observed that the production of FGF by fibroblasts in culture medium increased considerably after irradiation. Besides that Bisht *et al*. (1994) observed a development of granulation tissue and faster re-epithelization on the wounds of rats treated with He-Ne laser.

In addition to the above mentioned, LLLT functions in the milliwatt range, leading to a cascade of photobiological events, which can stimulate periodontal healing (Yamamoto *et al*., 1996; Kreisler *et al*., 2003). Part of the laser penetrates during irradiation into periodontal pockets, resulting in reduction of inflammatory response and cell proliferation, improving periodontal attachment and bone reconstruction (Nomura *et al*., 2001; Almeida-Lopes *et al*., 2001; Shimotoyodome *et al*., 2001; Aoki *et al*., 2004).

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It is generally believed that quick hard and soft tissue healing without adverse effects, is the most vital factor for successful results in the field of oral surgery. Especially in dental implantology, the absolute osseointegration of implants is needed. Cellular reactions at the interface between host and biomaterial are the major determinants of clinical success of osseointegrated implants. In the search for the optimal implant-tissue interaction, the effect of laser therapy on these cells is an important field of investigation.

The aim of this study is to provide the results of recent studies on LLLT and to show its influence on bone repair and on soft tissue healing. Nevertheless, an overview of the available literature on the interaction between the LLLT and the osseointegration of implants and of bone grafts is analyzed.

MATERIALS AND METHODS

Biostimulation provided by LLLT is an area of controversy. Many investigations have indicated a positive effect by the use of low-power lasers on bone reconstruction either *in vivo* (Silva *et al*., 2002; Gerbi *et al*., 2005) or *in vitro* (Pinheiro *et al*., 2001; Stein *et al*., 2005). Contrarily, other researchers did not find any effect of LLLT on the healing of soft and hard tissues (Luger *et al*., 1998; Anneroth *et al*., 1988). However, the amount of studies that concluded the positive effect of LLLT action is considerably bigger.

There are few studies that inquire the LLLT effect on soft tissues around dental implants. It is widely accepted that long term stable function of dental implants depends not only on the integrity of osseointegration, but also on the health of the epithelium and the quality of attachment of the connective tissue to the titanium surface (Listgarten *et al*., 1991; Buser *et al*., 1992; Berglundh, 1993). As in the normal epithelium, the healthy mucosa has an essential role in protecting the peri-implant sulcus from bacterial invasion. The peri-implant mucosa and the free marginal gingival have a lot of features in common (Berglundh *et al*., 1991; Lindhe *et al*., 1992). However, the peri-implant soft tissue is less stable than the natural periodontal soft tissue (Ericsson *et al*., 1992; Lindhe *et al*., 1992). Lack of attachment around implants may lead to peri-implantitis and consequently to failure of the treatment (Lang *et al*., 1994). Regeneration of mucosa and creation of an intact functional barrier at the transmucosal passage of the implant abutment is one of the main benefits claimed for therapy with low-power laser. In a recent study, Khadra *et al*. (2005) investigated the effect of gallium-aluminum-arsenide diode laser on proliferation and attachment of Human Gingival Fibroblasts (HGF) cultured on titanium implant material. This laser system operates at a continuous wavelength of 830 nm and a power output of 84 mW. The distance from the probe to the cell layer was 9 cm. The HGF exposed at dosages of 1.5 or 3 J cm^{-2} and cell profile areas were measured after 1.3 and 24 h. The main result was that the percentage of attachment of laser-exposed fibroblasts was significantly higher than the non-exposed cells. Furthermore, the irradiated cells showed significantly higher proliferation rates. However, the study showed no considerable differences between the 2 laser doses, indicating that increasing the dose from 1.5-3 J cm^{-2} does not further promote the initial HGF reaction. Additionally, LLLT stimulated greater spread of HGF over the titanium surface. Another study by Pugliese *et al*. (2003) indicated that the application of low-power laser punctually, in contact with the wound, which had been performed on the back of 72 rats, induced a biomodulation of the collagen and elastic fibers, evidenced through an increase in the deposit of these fibrilar elements. The laser had been applied in a single dose, immediately after the operation. Webb *et al*. (1998) and Almeida-Lopes *et al*. (2001), also concluded that the 4 J cm^{-2} energy density provided more significant results than the 8 J cm^{-2}. Other experiments, have reported better tissue healing at HeNe laser exposure levels between 1 and 4 J cm^{-2}. Skinner *et al*. (1996) used a pulsatile Ga-Al-As laser applying different energy densities in cultures of fibroblasts of human embryos and observed that there was a significant increase in the levels of collagen in the irradiated cells. Additionally, still within the sphere of biochemical studies (Loevschall and Bindeslev, 1994; Passarela *et al*., 1984) demonstrated an increase in DNA and ATP synthesis *in vitro*. Laser enhanced biostimulation has been reported to induce intracellular metabolic changes, resulting in faster cell division, rapid matrix production (increased collagen, myofibroblasts, etc.) and cell migration. Karu *et al*. (1996) evaluated the adhesion of HeLa cells to glass after irradiation with monochromatic low-intensity light or laser irradiation. They showed increased cell-cell and cell-glass adhesion following laser irradiation. It has been suggested that the intensity of ion fluxes through the cell membrane depends on the intensity of the signal received from the photoacceptor, which in turn depends on the primary reactions occurring in/with photoreceptor during irradiation with light at different wavelengths. However, higher doses are reported to inhibit proliferation (Karu, 1989, 1990). Furthermore, according to the study of Khadra *et al*. (2005), the increase in initial fibroblast attachment supports the hypothesis that the main healing effect is associated primarily with the early, most sensitive stages

of the healing process. Kreisler *et al.* (2003) showed considerably higher proliferation activity 24 h after irradiation, decreasing in an energy-dependent manner over 48 and 72 h, indicating that repeated laser applications are necessary to achieve a positive effect on cell proliferation. These results are in accordance with Khadra's conclusions, who supported the growth of HGF after laser irradiation for 3 consecutive days. Other studies have supported that LLLT affects on the release of specific growth factors from fibroblasts (Andres *et al.*, 2002). Yu *et al.* (1994) demonstrated that fibroblast production of basic Fibroblast Growth Factor (bFGF) can be enhanced by laser irradiation with 2, 16 J cm^{-2} at a wavelength of 660 nm and concluded that stimulation of fibroblast proliferation may be associated with the autocrine secretion of bFGF from fibroblasts. It is obvious that the results from all these studies are very encouraging and may show a path to the generation of intact and stable peri-implant soft tissues.

Many researchers believe that LLLT creates a number of environmental conditions that accelerate the healing of peri-implant bone defects. Dortbudak *et al.* (2002) examined the effect of low-energy laser irradiation on osteocytes and bone resorption at bony implant sites. They irradiated the bone defects of 5 male baboons with a 100 mW energy laser (690 nm) for 1 min (6 Joule) immediately after drilling and then inserted the implants to the sites. They showed that the osteocyte viability was significantly higher in the samples that were subjected to laser irradiation. The bone resorption rate was not affected by laser application. Their results suggest that more vital bone tissue is present in the irradiated area than the non-irradiated and that peri-implant bone healing can be expected to be accelerated. In another investigation, Jakse *et al.* (2007) performed a bilateral sinus floor elevation to 12 sheeps, with cancellous bone from the iliac crest. Implant insertion followed 4 and 12 weeks later. Unilaterally, the grafted sinus and during the 2nd-stage surgery, the implant sites were irradiated intraoperatively, 3 times during the 1st postoperative week with a diode laser (75 mW, 680 nm) with an energy density of 3-4 J cm^{-2} per irradiation. The osseointegration measurements resulted in a considerably higher bone/implant contact but, conclusively, the researchers did not confirm any positive LLLT effect on bone regeneration. Guzzardella *et al.* (2003) inserted ceramic implants in distal femurs of 12 rabbits. They used a Ga-Al-As laser with a wavelength of 780 nm and the overall applied energy on the test site was 300 J cm^{-2}. The histomorphometric analysis showed a significantly higher bone microhardness due to osteocyte viability in the LLLT group compared with the control group. They also concluded that postoperative LLLT enhances the bone-implant interface. Khadra *et al.* (2004a, b) inserted coin-shaped titanium implants into the cortical bone of proximal tibiae in rabbits. A Ga-Al-As diode laser with a wavelength of 830 nm and an output power of 150 mW was used, immediately after surgery. Histomorphometric analysis showed more bone-implant contact than the controls. The results were concluded as a favourable effect of LLLT on healing and attachment of titanium implants. Nicolau *et al.* (2003) perforated the femurs of 48 rats (2 groups) and the irradiated group was treated with a Ga-Al-As laser (660 nm, 10 J cm^{-2}) of radiant exposure on the 2nd, 4th, 6th and 8th day postsurgically. They concluded that LLLT increases the activity in bone cells (resorption and formation) around the site of the repair without changing the bone structure. LLLT especially augmented osteoclast activity. They assumed that 2 possible mechanisms were involved. First, this laser wavelength directly stimulated osteoclasts. Osteoclasts are multinuclear vells, with many mitochondria of high activity. According to Karu (1999), the mitochondrial cytochromes absorb the photon energy in the visible part of the electromagnetic spectrum and this absorption increases ATP synthesis and improves the potential activity of the cells. Second, osteoclast activity may influence posterior osteoblast activity (MacDonald and Gowen, 1993) and virse versa. Nevertheless, Nicolau *et al.* (2003) found that LLLT, used in the period when inflammation is beginning, early after the operation, stimulates the enhanced activity of both osteoclasts and osteoblasts. In addition, it is mentioned by Ozawa *et al.* (1998), Barushka *et al.* (1995) and Yaakobi *et al.* (1996) that LLLT is probably a modulator responsible for the increased alkaline phosphatase levels into the mature bone. All these investigators found augmented amounts of alkaline phosphatase after low-power laser application. Specifically, Yaakobi *et al.* (1996) indicated that irradiation of animals provided 50% higher rate of calcium accumulation comparatively to the non-irradiated group. This finding seems to be essential because of the importance of the adequate and rapid bone maturation around titanium implants. This kind of bone formation is one of the vital factors that provide increased stability to implants. Sathaiah *et al.* (1999) using a He-Ne laser, found that 15 days after the operation the concentration of alkaline phosphatase increased and they also suggested an enhanced osteoblast activity and therefore, better formation of the organic bone matrix and mineralization. Pretel *et al.* (2007) treating 30 Holtzman rats with a Ga-Al-As laser (p = 35 mW, λ = 780 nm, D = 178 J cm^{-2}), concluded that a single application directly to the bone defects, stimulated the bone healing

by modulating the initial inflammatory response with earlier resolution to the normal conditions. They also found the presence of organizing connective tissue, numerous blood capillaries and fibroblasts at 15 days. However, the difference in collagen fiber maturation between the 2 groups at 45 days was not significant and finally, there were no difference at 60 days of the experiment. Nevertheless, bone formation was higher in the irradiated group at 45 and 60 days.

Regarding to bone grafts osseointegration, low-power laser application had been rarely used to enhance bone formation. Gerbi *et al.* (2005) indicated that the combinational use of lyophilized bovine bone and low-power laser had a positive biostimulating effect on the repair of surgical defects created in the rat femur after 15 and 30 days, but they attribute this effect mainly to LLLT. Pinheiro *et al.* (2003a) found that the application of LLLT (4 J cm^{-2} at each of 4 spots around the bone lesion grafted with inorganic bovine material) increased bone formation and the amount of collagen fibers in the area around the graft, as early as the 15th day postoperatively. The same positive results about increased bone formation were suggested by the investigation of Lopes *et al.* (2007), who treated bone defects placing implants, calcium hydroxyapatite graft and irradiating with low-power laser (λ = 830 nm, 7 sessions at 48 h intervals, 21, 5 J cm^{-2} per point, 10 mW, 86 J $session^{-1}$). They found that infrared LLLT stimulated bone healing 15, 30 and 45 days after operation. Rochkind *et al.* (2004) applied low-power laser, alone and combined with Bio-Oss, for 14 consecutive days and indicated that bone healing was enhanced and mineralization was higher. Likewise, in another investigation, it was concluded that bone healing was significantly higher, after filling the defects with autologous bone graft and then irradiating with LLLT (λ = 830 nm, 50 mW, 10 J cm^{-2}). The dose was 10 J cm^{-2} per session, applied on the bone graft (Weber *et al.*, 2006). The investigators found a positive modulative effect for the 1st, 15-30 postsurgical days. However, Ninomiya *et al.* (2003) and Takeda *et al.* (1988), who indicate that LLLT has no effect on bone regeneration after the first 15 days postsurgically and that LLLT affection is time-dependent because the modulatory effect takes place only at the beginning of the immature precursors differentation. They did not confirm any effect during later stages. Coombe *et al.* (2001) affirm this theory, showing that LLLT with a single dose applied daily for up to 10 days, was mostly effective in the first 2 postoperative days. Single dose laser application had no effect on the new mineralized bone, indicating that the LLLT action is time-and dose-dependent and that LLLT affects bone formation by stimulating the immature cells only.

Except from all the above mentioned, the idea that LLLT enhances bone formation is contradicted by many researchers. Goldjestani and Thierens (1994) found no difference in bone metabolism, after irradiating experimental circular defects with gallium laser, with a power of 33.3 mW and fluence 20 J cm^{-2} and applied daily for 28 days. Pogrel *et al.* (1997) concluded that there were no significant increase in cell proliferation, adhesion or migration in either fibroblasts or keratinocytes threated with Ga-Al-As laser. In another study, David *et al.* (1996) suggested that HeNe LLLT had no effect on bone healing acceleration. In that experiment, osteotomies performed at 62 rats were irradiated with HeNe laser with 0, 2 or 4 J every other day for 2-6 weeks. According to a more recent study by Jakse *et al.* (2007), who investigated the effect of LLLT on bone healing and osseointegration of titanium implants in a sinus graft model, it could not be confirmed that irradiation with low-power laser stimulates bone regeneration. Nevertheless, the researchers indicated that treatment with low-power laser probably could be profitable for the enhancement of implant osseointegration.

One the other hand, there are some researchers who provide a suggestion that the stimulative action of LLLT on the 1st week postoperatively, can change to an inhibitory effect after this period of time. Barushka *et al.* (1995) and Yaakobi *et al.* (1996) found that the volume fraction of trabecular bone and the concentration of alkaline phosphatase and of calcium in the regenerated experimental bone defects decreased more quickly in the irradiated with He-Ne laser group than the non-irradiated group. The results from Garavello-Freitas *et al.* (2003), showing also that the application of low-power laser for longer than 5 min (i.e. for 15 min) did not improve the reaction of the bone regarding the bone healing process. Garavello-Freitas *et al.* (2003), suggest that more future experiments need to be performed in order to find if stopping irradiation after the first 7 days would be more effective for bone regeneration.

RESULTS AND DISCUSSION

Nowadays, dental implantology is developing aggressively. Various theories and techniques have been proposed in order to achieve the enhancement of implant osseointegration. It is widely known that osseointegration is a direct structural and functional contact between a loaded implant and bone at the light microscopic level (Carlsson *et al.*, 1986). The establishment of adequate osseointegration depends on the stability of bone around implant and of soft tissues around the abutment. The use of LLLT has been proposed for the enhancement of soft

tissue healing and bone regeneration. The regulatory mechanism of low-power laser is photochemical in nature, with the energy probably being absorbed by intracellular chromophores and converted to metabolic energy (Belkin *et al*., 1988; Karu, 1989). The irradiation of bone defects leads to absorption by cellular structures activating the biochemical processes of the osteoblast nucleus and DNA-RNA-protein synthesis, thus accelerating normalization of the structure of the organic and inorganic bone mineral collagen, resulting in enhanced bone healing (Rochkind *et al*., 2004). Some studies showed a greater concentration of collagen fibres after irradiation of bone defects with low-power laser (Gerbi *et al*., 2005; Pinheiro *et al*., 2003b). The increase in production of collagen protein can occur through a higher proliferation of fibroblasts or through an increase in the anabolic cellular activity per se. Therefore, laser would act through one of these mechanisms, inducing proliferation of fibroblasts or the process of protein synthesis and secretion, also existing the possibility of both mechanisms occurring simultaneously. As collagen is a vital component of the extra-cellular bone matrix, large amounts of collagen fibers would represent increased new bone formation after mineralization of the matrix. Furthermore, Lewandrowski *et al*. (1996) and Romanos *et al*. (2000), indicate that LLLT improves osteoblast adhesion and vessel migration towards the surface and prepare an adequate implant site to reduce tissue damage. Irradiation with low-power laser at dosages of 1, 5 or 3 J cm^{-2} has been reported to stimulate fibroblast numbers *in vitro* (Khadra *et al*., 2005). Pugliese *et al*. (2003) indicated that 4 J cm^{-2} energy density provided more significant results than 8 J cm^{-2}. In other studies, it is supported that multiple doses, rather than the intensity of irradiation, are more effective for bone formation and for implant tissue interaction (Coombe *et al*., 2001; Ninomiya *et al*., 2003), while Pretel *et al*. (2007) concluded that single dose was effective in accelerating bone healing. In addition, it has been referred that other researchers affirmed that duration of the positive effect of LLLT is not longer than 1 week postoperatively (Barushka *et al*., 1995; Yaakobi *et al*., 1996; Garavello-Freitas *et al*., 2003).

It is obvious that conflicting results have been reported about the stimulatory action of LLLT. Some discrepancies may be attributable to differences in the species of cells, the wavelength, the energy density, the exposure time and the surgical procedure (Khadra *et al*., 2005). These parameters show that standardization of the methodology used must be established for accurate comparison of the results from several studies (Pugliese *et al*., 2003). Furthermore, laser irradiation has a wide range of effects on tissues and overexposure or underexposure can significantly change clinical or experimental results (Dew *et al*., 1993; Dortbudak *et al*., 2000). Thus, it is of great importance to adopt the proper stimulation method (Guzzardella *et al*., 2003). Additionally, the animal experimental model seems to be a useful technique for investigating the tissue reactions to the bioactive material, but the results reported from such experimental models cannot safely be put into practice to humans (Khadra *et al*., 2005). Unfortunately, only a few studies based on humans exist to support the positive effect of LLLT on alveolar bone regeneration (Nagasawa *et al*., 1991; Abe, 1990). This indicates the need for more human studies to help researchers securely support the positive or the negative action of LLLT.

It can be supported that LLLT has the potential of beneficial effects on peri-implant hard and soft tissues regeneration. Under stable and no hurtful surgical conditions, irradiation with low-power laser could reduce healing time and accelerate implants osseointegration (Dortbudak *et al*., 2002). Nevertheless, further investigations will be necessary to define stable protocols about low-power laser use, such as type of laser, treatment duration, energy dose, optimum wavelength and distance from the irradiated tissues, in order to accomplish the best stimulatory action of LLLT.

CONCLUSION

Irradiation with low-power laser could be a beneficial adjuvant therapy used by surgeons, when negative general and local parameters, predictive of poor hard and soft tissues regeneration exist. LLLT's stimulatory effect on bone healing could accelerate the osseointegration of titanium implants. Nevertheless, standardized experimental methodology and especially more studies based on humans are required.

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