Biostimulation effects of low-power laser in the repair process*

Efeitos bioestimulantes do laser de baixa potência no processo de reparo

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Abstract: The wound healing process has always been an excellent subject for researchers. The use of low-power laser on wounds during the postoperative phase has increased the speed of the healing process. It has been implied that low power radiation affects cellular metabolic processes and promotes beneficial biological effects (analgesic, anti-inflammatory, and healing). Laser biostimulation appears to influence the behavior of the repair process. This paper aims at reviewing the most interesting aspects of the use of low-power laser in the tissue-repair process.
Key words: lasers, low-level laser therapy, wound healing

Resumo: Os lasers de baixa potência promovem efeitos biológicos benéficos, de caráter analgésico, anti-inflamatório e cicatrizante, por meio de um fenômeno de bioestimulação. A radiação emitida pelo laser terapêutico afeta os processos metabólicos das células-alvo, produzindo efeitos bioestimulantes que resultam na ocorrência de eventos celulares e vasculares, os quais parecem interferir diretamente no processo de reparo. Este trabalho visa estudar o fenômeno da bioestimulação e destacar os principais efeitos bioestimulantes do laser de baixa potência na reparação tecidual.
Palavras-chave: Cicatrização de feridas; Lasers; Terapia a laser de baixa intensidade

INTRODUCTION

A laser is a device consisting of solid, liquid or gas substances which produce a light beam when excited by a source of energy. This device can be classified into two categories: high-power lasers or surgical lasers, featuring thermal effects with cutting, vaporization and hemostasis properties, and low power lasers or therapeutic lasers, with analgesic, anti-inflammatory and biostimulation properties (Silva et al, 2007; Barros et al., 2008). The latter category includes the Helium-Neon laser (He-Ne), which operates at a wavelength of 632.8 nm, that is, in the visible spectrum (red light); the Aluminium Gallium Arsenide (Al Ga As) or laser diode, which operates at a wavelength of approximately 780-830nm, outside the visible spectrum (infrared light); and the combined laser of Helium-Neon diode.[1-5]

The word laser is an acronym for "Light Amplification by Stimulated Emission of Radiation." Since a laser provides better inflammatory responses with edema reduction, pain reduction and cellular

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biostimulation, laser therapy constitutes an alternative to processes that present pain and inflammatory reaction and that require tissue regeneration.[6]

Since radiation emitted by low-power lasers has shown analgesic, anti-inflammatory and healing properties, they have been widely used in the process of tissue repair, for their wavelength and low densities of energy can penetrate tissues.[1-4]

It should be noted that therapeutic lasers do not have a direct healing effect. However, they act as an important pain-relieving agent providing the body with a better inflammatory response, as they help to reduce edema and minimize pain, in addition to promoting tissue repair of the injured region quite effectively through cellular biostimulation. In view of what has just been stated, this paper aims to review and discuss the phenomenon of biostimulation and the main biostimulation effects of low-power laser in the process of tissue repair.

LITERATURE REVIEW

Repair process

The repair process constitutes a dynamic tissue reaction, which comprises different phenomena such as inflammation, cell proliferation and synthesis of elements of the extracellular matrix, including collagen, elastic and reticular fibers.[7]

Inflammation is the reaction of vascular living tissues to a local injury. It serves to destroy, dilute or immobilize the injurious agent by triggering a series of biological processes that reconstruct the injured tissue as much as possible. It is intrinsically related to the repair process, which begins during the early stages of the influence of the injurious agent.[8]

Resolution of inflammation involves the removal of exudates and dead cells by enzyme dissolution and phagocytosis. These events are followed by the replacement of dead or damaged tissue with cells that derive from elements of the parenchyma or injured connective tissue. According to the authors, the repair process is an organic reaction that restores tissue destruction or loss. It may be accomplished by replacement of the original tissue with an identical one (regeneration), or by neoformation of connective tissue, which replaces lost or destroyed tissue, with alteration of tissue architecture (healing).

Neutrophil granulocytes are the first cells to appear in the injured tissue and they can be seen on the edges of wounds by means of electron microscopy three hours after injury. In the healing process, their main function is not phagocytosis, but the enzymatic destruction of fibrin. Soon after that monocytes appear, which will turn into macrophages on approximately the fifth day. These defense cells, which are major producers of proteolytic enzymes, are phagocytic par excellence. They also have functions such as those related to the formation and migration of fibroblasts in addition to stimulating formation of new blood vessels. Fibroblasts, in turn, are stimulated by macrophages in relation to formation and maturation of collagen.[10]

Synthesis of collagen fibers is a continuous event that lasts until the end of the repair process, as tissue remodeling occurs. As for elastic fibers, they allow the tissue to stretch during the healing process without being destructed. Long collagen fibrils are interspersed with elastic fibers in order to limit tissue expansion, preventing tearing of tissue during the repair process.[7]

Biostimulation effects of low-power laser in the repair process

The principle of biostimulation promoted by therapeutic lasers was introduced more than 20 years ago. It was first applied in dermatology, especially in the repair process of skin wounds. Soon after that, it was suggested that biostimulation could also be useful to accelerate the healing of wounds inside the mouth. Then it became largely used in cases of aphthous ulcer, herpes labialis, angular cheilitis, trismus, paresthesia, dentine hypersensitivity and in the postoperative phase.[4,5]

The therapeutic properties of lasers have been studied since their discovery, with their analgesic property being particularly observed in relation to the forms of chronic pain of several etiopathogeneses, from peripheral receptors to the stimulation in the central nervous system. [4]

According to Genovese's citations, [11] the biological effects caused by low-power lasers in tissues consist of light energy, which is deposited on the tissues and which become vital energy, thus producing primary effects (direct), secondary effects (indirect), and general therapeutic effects, which promote analgesic, anti-inflammatory, and healing reactions.

Laser therapy, when used in tissues and cells, is not based on heating, that is, the energy of the absorbed photons is not transformed into heat, but into photochemical, photophysical and/or photobiological effects.[4,12] Still in accordance with the authors, when laser light interacts with cells and tissues in an appropriate dosage, certain cell functions can be stimulated, among them are stimulation of lymphocytes, mast cell activation, increase of mitochondrial ATP production and proliferation of several types of cells, thus promoting anti-inflammatory effects.

In recent years, coherent light (laser) phototherapy has been used as a bio-stimulator for tissue repair, as it helps to improve local circulation, cell proliferation and collagen synthesis.[13-16]

In order to investigate the behavior of skin wounds caused in the dorsal region of Wistar rats, Rocha Junior, Andrade, Oliveira, Aarestrup and Farias[17] used a low-intensity laser with a dose of 3.8 J / cm^2 , power of 15mW and exposure time of 15 seconds and noticed increased neovascularization and fibroblast proliferation as well as reduction of inflammatory infiltrate in the surgical lesions submitted to laser therapy.

At the cellular level, low-power laser causes biochemical, bioelectric and bioenergetic changes, leading to increased metabolism, cell proliferation and maturation, increased quantity of granulation tissue and reduction of inflammatory mediators, inducing the healing process.[2,18] Molecular absorption of laser light allows for an increase in cellular metabolism characterized by stimulation of photoreceptors in the mitochondrial respiratory chain, changes in cellular ATP levels, release of growth factors, and collagen synthesis. [19.20]

The antiinflammatory and anti-edema effects exerted by laser occur through acceleration of microcirculation, resulting in changes in capillary hydrostatic pressure with edema reabsorption and disposal of the accumulation of intermediary metabolites.[5]

The monochromaticity and intensity of laser light cause selective excitation of atoms and molecules. Some research studies suggest that laser radiation can increase the percentage of molecular components produced during a chemical reaction. Studies show that laser therapy increases the levels of ascorbic acid in fibroblasts, thus increasing the formation of hydroxyproline and, consequently, the production of collagen, since ascorbic acid is a cofactor required for hydroxylation of proline during collagen synthesis.[21]

According to the authors mentioned above, biostimulation of cytoskeletal proteins promoted by low-power laser gives greater stability to the conformation of the lipoprotein layer of the cell membrane. Radiation emitted by therapeutic laser also affects cells as it modulates the production of growth factors.

In some studies, Catão [4] states that low-intensity laser therapy influences metabolic, energetic, and functional changes, since it promotes increased cellular resistance and vitality, rapidly promoting tissues to their normal state.

Some main biostimulation effects of low-power laser in the process of tissue repair include induction of mitotic activity of epithelial cells and fibroblasts, stimulation of collagen production by those cells, inhibition of secretion of some chemical mediators, change in capillary density and stimulation of local microcirculation.[2,6,21,22]

Low level-laser enhances the phagocytic and chemotactic activity of human leukocytes *in vitro.* In the process of wound repair, activation of lymphocytes by laser radiation can make them more responsive to stimulatory mediators present in injured tissues.[22] Still in accordance with the author's citations, therapeutic-laser treatment increases phagocytic activity of macrophages during the early stages of tissue repair, approximately 6 hours after trauma, facilitating cleaning of the wound and establishing the conditions needed for the subsequent proliferative phase.

At the vascular level, low-power laser stimulates proliferation of endothelial cells, resulting in formation of numerous blood vessels and increased production of granulation tissue. It also stimulates vascular smooth muscle relaxation, thus contributing to the analgesic effects of laser therapy.[22]

Low power laser possibly plays an important role in alveolar repair after tooth extraction, since it exerts pronounced effects on cultures of osteoblasts, influencing the processes of proliferation, differentiation and calcification.[22]

In some studies on new bone formation, it is suggested that the biostimulation effect of laser is not only due to its specific properties, but also to the creation of a series of local conditions that accelerate bone formation and resolution of edema.[6]

Several *in vitro* experiments show that therapeutic-laser treatment also stimulates proliferation and differentiation of fibroblasts as well as the synthesis of products of the extracellular matrix by those cells.[5,17,21-23]

There are several mechanisms through which low-power laser can induce mitotic activity of fibroblasts. This type of laser stimulates the production of basic fibroblast growth factor (bFGF), which is a multifunctional polypeptide secreted by fibroblasts. It is not only able to induce fibroblast proliferation but also differentiation and it affects the immune cells that secrete cytokines and other regulatory growth factors of fibroblasts. *In vitro* studies using macrophage lineage cells show that these cells release soluble factors that promote fibroblast proliferation when stimulated by low-power laser radiation. Maturation of fibroblasts and their movement through the matrix are also influenced by this laser.[22]

Several types of laser affect fibroblast proliferation and synthesis of procollagen and collagen *in vitro.* The laser which generates the most

positive results is the He-Ne low-power laser.[21] Other studies show that the tissue layer to be reached by the laser light depends on the type of laser, its power, wavelength, and irradiation time.[2] Depending on the wavelength of the laser device used, there will be varying effects on the production of procollagen, with increased synthesis of collagen when the He-Ne or Ga-As laser is used and dramatic reduction in its production when high-power Nd:YAG laser is used.[24]

According to the AAP[3], an experiment with cultured human-skin fibroblasts showed a reduction in DNA synthesis and in production of collagen when fibroblasts were exposed to radiation promoted by Nd: YAG laser.

In order to analyze the effect of low-power laser on the proliferation of gingival fibroblasts *in vitro,* Almeida-Lopes, Rigau, Zangaro, Guidugli-Neto, and Jaeger [23] applied a laser diode on cultured human gingival fibroblasts with a fluence of 2 J/cm^2 and the following wavelengths: L1= 670nm, L2 = 780nm, L3 = 692nm and L4 = 786nm. For the analysis of growth, non-irradiated cultures (control group) and those treated with the laser (experimental group) were placed on a 60mm-diameter Petri dish 12 hours before irradiation. In this experiment, the researchers concluded that low-level laser enhances proliferation of gingival fibroblasts regardless of the wavelength used and that the shorter the time of exposure to the laser, the higher the proliferation.

Kreisler, Christoffers, Al-Haj, and Willershausen [25] assessed the effect of Al-Ga-As laser diode with different power levels (0.5-2.5W) and duration (60-240 seconds per site) on cultures of human fibroblasts. The results showed that, depending on the different power calibrations, laser irradiation may cause a decrease in the number of cells. In addition, time of exposure is more important than power itself, since linear regression analysis showed no correlation between amount of energy and cell death when time of exposure was kept constant.

In another study[2] aimed to histologically evaluate the response of epithelial, bone, and connective tissues subjected to low-intensity laser therapy in an experimental model of dentoalveolar growth carried out with Wistar rats (*Rattus norvegicus albinus),* it was found that epithelial and connective tissues responded to laser-therapy stimulation with constant cell renewal, while there was an acceleration of new bone formation within normal limits in bone tissues. This study employed lasers with a wavelength of 660nm and 780nm and energy densities of 7.5 and 15J/ cm^2.

In their systematic review, the authors evaluated the results and methodologies of studies about the potential effects of low-level lasers on healing of periodontal tissues, suggesting the potential effect of this therapy on the healing process. This study considered the power of the applied laser, its action on pro-inflammatory mediators and fibroblasts as well as its effect on microcirculation and gengival bleeding.

CASE REPORT

Patient JFSS presented himself to the surgical clinic of the Universidade Estadual da Paraiba (State University of Paraiba) for extractions of teeth 36 (lower left first molar) and 47 (lower right second molar) due to extensive carious lesions. Laser therapy was applied only to tooth 47 on days 1 (Figure 1), 4, 8, 15 and 23 (Figure 2) after surgery. Tooth 36 was not treated with laser therapy, but was also evaluated on the days mentioned above (Figures 3 and 4).

DISCUSSION

In order for a low-power laser to exert a biological effect, the target tissue needs to absorb its light beam. [3] In his work, Walsh[22] reveals that proteins are the most important tissue components in the process of absorbing energy emitted by the red light and/or infrared beams of a therapeutic laser. However, the photoreceptors responsible for the biological effects of such a laser have not been identified yet. Several studies suggest participation of elements of the mitochondrial cytochrome system or endogenous porphyrins in the absorption of these light beams.

Increase in local circulation, cell proliferation and collagen synthesis are some of the effects observed during laser therapy on the process of tissue repair. [13-16] Furthermore, therapeutic laser radiation promotes analgesic, anti-inflammatory and wound healing effects.[11]

Low-power lasers show anti-edema and analgesic effects, stimulating the release of endorphins and thus inhibiting nociceptive signals and controlling pain mediators; anti-inflammatory effects, reducing tissue edema and vascular hyperemia; and wound healing effects, accelerating the healing of damaged tissues, stimulating bone repair and remodeling, restoring neural function after injury and modulating cells of the immune system to facilitate the repair process. [4-6,12,21,22]

Many investigations have attempted to determine the biological effects (analgesic, anti-inflammatory and healing) of low-intensity lasers on tissues, especially during the repair process. However, not all of them have produced satisfactory results. The mechanism of action of biostimulation promoted by this type of laser has not been clarified yet. Photoreception at the mitochondrial level may intensify the respiratory metabolism and

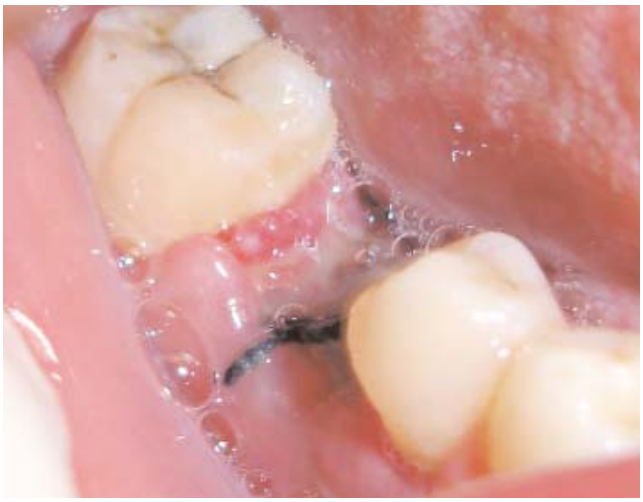

Figure 1: Area treated with laser therapy (element 47) 48 hours after surgery: pinkish edges, presence of after-surgery inflammation and edema, absence of extravasation from blood vessel walls, and sensory abnormalities (pain) at level 2 on the pain scale, as informed by the patient

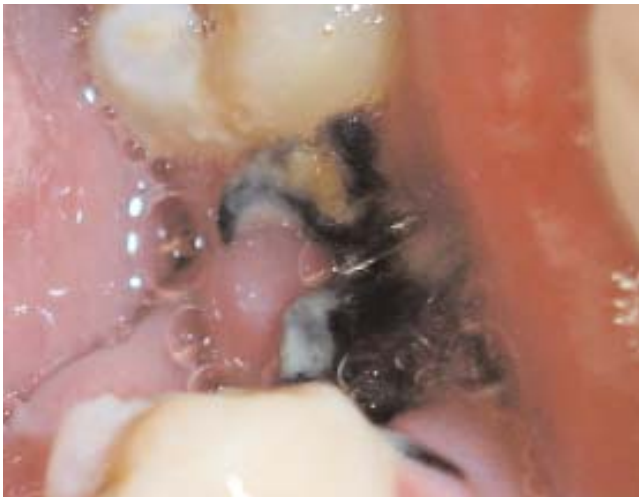

Figure 3: Area not treated with laser therapy (element 36) 48 hours after surgery: pinkish edges, presence of post-surgery inflammation, presence of extravasation from blood vessel walls, and sensory abnormalities (pain) at level 6 on the pain scale, as informed by the patient

electrophysiological properties of the membrane, thus promoting changes in cell physiology. Moreover, laser radiation increases the synthesis of ATP within mitochondria, thus accelerating the speed of cell mitosis. [11, 19-21]

In accordance with many authors cited in this study, [4,12,17,21,22] intracellular metabolic changes resulting from biostimulation promoted by low-power laser have resulted in: accelerated cell division, especially of fibroblasts and epithelial and endothelial cells; rapid production of extracellular matrix, particularly of collagen fibers; movement of leukocytes, fibroblasts and epithelial cells; and increased phagocytic activity of macrophages.

The phenomena of fibroblast and epithelial proliferation and high collagen synthesis, which constitute the main histological changes observed in wounds treated with laser therapy, have also been identified in *in vitro* studies using cultures of animal and human cells irradiated with low-power laser. However, the tissue layer to be reached by the laser light depends on the type of laser, its power, wavelength, and irradiation time. [2]

In their experiment, Almeida-Lopes et al. [23] found that, no matter the wavelength used, the laser diode increases the proliferation of gingival

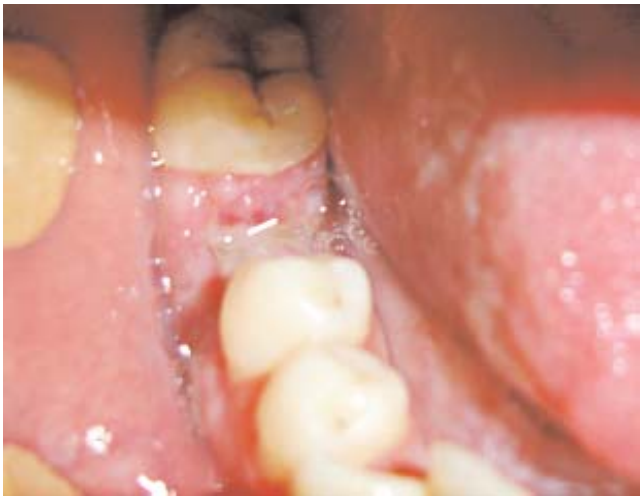

Figure 2: Area treated with laser therapy (element 47) 24 days after surgery: pinkish edges, absence of post-surgery inflammation and edema, absence of extravasation from blood vessel walls, and absence of pain, according to the patient

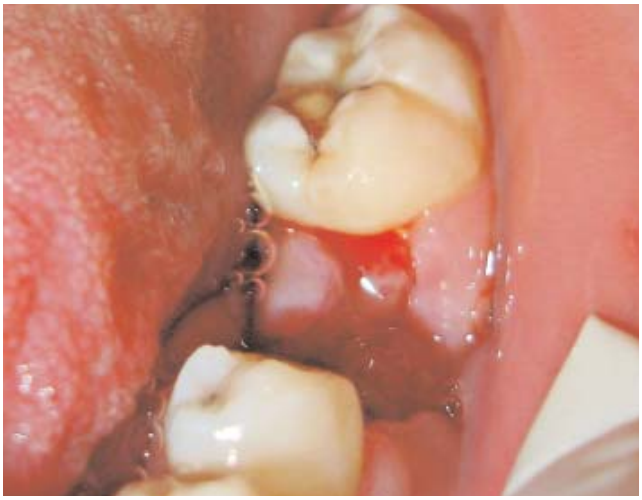

Figure 4: Area not treated with laser therapy (element 36) 24 days after surgery: pinkish edges, presence of post-surgery inflammation and edema, presence of high extravasation from blood vessel walls, and presence of pain, according to the patient

fibroblasts *in vitro,* thus suggesting that the wavelength of the laser device used does not interfere with the repair process. In addition, it can be concluded from their study that even though the wavelength of the Ga-As laser has no impact on fibroblast proliferation and, consequently, on the repair process, the time of exposure to laser radiation does. The authors found that the shorter the time of exposure to the laser, the greater the proliferation of fibroblasts. Likewise, Kreisler et al.[25] also consider the time of exposure to laser radiation important for the stimulation of fibroblast proliferation, which is even more relevant than the actual laser power.

In their systematic review, Barros et al.[1] state that further clinical studies must be conducted to evaluate the application of low-intensity laser, since different methodologies were found in the literature, with differences concerning wavelength, dosimetry, types of study and experimental designs. Although Silva et al.[2] have found positive results in relation to low-power laser application, their study used lasers with different wavelengths and energy densities, which makes further studies necessary to clarify the mechanisms of action of low-intensity lasers and the ideal parameters to be used in clinical practice.

Depending on the wavelength of the laser device used, there will be varying effects on the production of procollagen and, consequently, on collagen synthesis. [24] As stated by the authors, He-Ne and Ga-As lasers increase collagen production, while high-power Nd:YAG laser reduces collagen synthesis. Reports by the AAP[3] corroborate this information. In addition, Conlan, Rapley and Cobb[21] confirm that the He-Ne laser increases collagen production, thus accelerating the repair process. However, the Argon laser does not accelerate the healing process despite the fact that it also increases collagen synthesis.

FINAL CONSIDERATIONS

Although the effectiveness of biostimulation promoted by low-level lasers has not been proven yet, a review of the literature in this field and an analysis of the experiments carried out seem to clearly show a number of effects on biostimulation mediated by this kind of laser, including cellular events (fibroblast, endothelial and epithelial proliferation, high collagen synthesis, differentiation of fibroblasts into myofibroblasts, movement of leukocytes, fibroblasts and epithelial cells, and increased phagocytic activity of macrophages) and vascular events (vasodilation and angiogenesis), which play an important role in accelerating the repair process of injured tissues. ❑

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