

Advances and Perspectives on Tissue Repair and Healing*

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General Aspects of Tissue Repair

WOUND HEALING is a complex process that involves both local and systemic responses, and these responses will widely reflect the etiology of the lesion, type of tissue, systemic condition of the subject, etc.

Despite being essentially the same for different wounds, the pattern of healing may change due to both intrinsic and/or extrinsic factors. These factors include age, gender, hormonal factors, nutritional deficiencies, the use of drugs, and some diseases. It is well known that some diseases or conditions such as uncontrolled diabetes, malnutrition, hypothyroidism, anemia, burns, etc., may cause delayed wound healing. The type of tissue has also to be considered as, despite showing similar phases, skin and mucosal wounds heal quickly than bone.

Several therapeutic approaches have been used to improve healing, and these include the use of stem cells,¹ growth factors,² drugs,³ hyperbaric oxygen,⁴ and phototherapies.^{5–8} Several light therapies have been used to improve both healing and repair. Laser, light emitting diodes (LEDs), and lamps have been used worldwide, both experimentally and clinically, to improve healing. It has been shown elsewhere that different phototherapies are able to stimulate the repair of skin, ligament, tendon, bone, and cartilage in laboratory animal wounds and human ulcers.

Phototherapy and Wound Healing

Soft tissues

Since the pioneer Mester's reports,^{9–11} the literature has shown that phototherapies, carried out with appropriated protocols, increases fibroblast proliferation, increases collagen synthesis, and influences both cellular and subcellular processes needed to increase the formation of both type I and type III pro-collagen pools of mRNA, ATP synthesis, and lymphocytic action. All of these would then initiate and promote the healing process, completing the cascading cycle of events.

Since the 1960s the volume of research into laser phototherapy has grown substantially and has focused on three

areas to assess the value of laser phototherapy (LPT) in wound repair: cellular function, animal studies, and human trials.^{7,8,12}

Despite early anecdotal reports on the use of light on biological systems, evidence of the effects of LPT on both soft and mineralized tissues have been widely reported for nearly half a century, especially regarding wounds and ulcerations, and have included the study of both local and systemic effects, including contralateral effects. The results of many of these reports are conflicting or controversial, and very often the positive effects are suggested to be related to general effects of the laser light as well as its ability to increase the rate of healing through mitochondrial ATP production and alteration in the cellular lipid bilayer. It has also been mentioned that the subsequent capacity of irradiated cells to alter their ion exchange rate, and thus influence the catalytic effects of specific enzymes and substrates, must also be considered.

There are several factors that are important for the outcome of treatment involving the use of light sources. Most of the proposed protocols used different parameters resulting in conflicting results. The choice of appropriate parameters is essential for the results of the treatment. These parameters include wavelength, power density, energy, duration, and frequency of application and others.^{7,8,12}

Over the last years, several reports on the benefits of the use of LEDs operating at several wavelengths have been published elsewhere both *in vitro* and *in vivo* on both normal and pathologic conditions^{13–16} as well as for facial rejuvenation.^{17,18} LED technology has been shown to be an effective alternative for the treatment of both cutaneous and mucosal wounds.^{13,19}

Our group has been studying the effects of different types of light on the healing of both soft and mineralized tissues using different models. We found that the use of laser and polarized light are effective on improving the healing of diabetic^{19,20} and malnourished animals.^{21,22} We also found that laser light is capable of improving the healing of drug-induced impairment²³ and on increasing the survival rate of musculocutaneous flaps on both diabetic and nondiabetic animals.²⁴ We have also studied and shown the influence of laser parameters on the healing of surgical wounds^{25,26} as

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well as on the postoperative period of both surgical and CO₂ laser wounds.^{27,28} Lately we verified the positive effect of LEDs on fibroblastic proliferation during healing.²⁹

Bone repair

Many techniques are used to improve the bone healing. Recently, laser light has been used for improving bone healing in several conditions, such as in dental implants^{30–33} and autologous bone graft,^{34,35} and several types of bone defects.^{36–44} Several studies have demonstrated that near infrared (NIR) LPT is the most suitable for bone repair due to its higher penetration depth in the bone tissue when compared to visible laser light.³⁶ Although the use of LPT on the bone healing has been growing steadily, and several studies have demonstrated positive results on the healing of bone tissue, there are few reports on the association of LPT and biomaterials.^{36–44} We have also used a fracture model to describe the effects of LPT.⁴⁵

It is possible that the effect of LPT on bone regeneration depends not only on the total dose of irradiation, but also on the irradiation time and the irradiation mode.^{36–45} Many studies indicated that irradiated bone, mostly with infrared (IR) wavelengths, shows increased osteoblastic proliferation, collagen deposition, and bone neoformation when compared to nonirradiated bone.^{36–45}

Closing Remarks

Tissue repair is a chemical, physical, and biological phenomenon that occurs after any kind of tissue lesion. These phenomena are of the utmost importance in the repair of tissues damaged by a wide range of pathologies. It is absolutely necessary to know what factors could prevent complete tissue repair. The repair process is influenced by a series of local and general conditions. Local conditions are good vascularization, absence of pathogenic microorganisms, absence of foreign bodies, and lack of trauma in area. Furthermore, systemic conditions also play a role on healing.

Several factors that are affected by LPT have been pointed out in the literature; these include: transforming growth factor- β , platelet-derived growth factor, Ca²⁺, enhancement of ATP production, prostaglandin reduction, improved cell wall permeability, and reactive oxygen species (H₂O₂, O₂²⁺). These factors are not only increased, but are modulated according to the oxidative state of the cell. For instance, cells in 2% fetal calf serum react best, those in 5% react well, and those in 10% hardly react at all. This is a key to understand the effect of light on living tissues. Some kind of deficiency has to be present. LPT is optimal when the function of the cell, that is the redox situation, is suboptimal. Healthy cells react little or do not react at all to light energy.

There are several specific effects of LPT that are widely reported on the literature; these include altered nerve conduction; altered blood flow and neoangiogenesis; increased metabolism of endorphins, acetylcholine, serotonin, and cortisol; enhanced ATP production; enhanced cell function (release of growth factors, cytokine reactions, replication); increased synthesis of DNA and proteins; and acceleration of healing. Secondary effects may be due to the fact that the laser-excited form of ATP behaves biochemically different than the nonexcited form. The former has an increased kinetics.

LPT is one of the areas of controversy in the use of lasers, and most of its applications are related to soft tissues. The true mechanism, which leads to a positive effect of laser light on different tissues, is not fully understood. Several hypotheses have been suggested. It has been suggested that the most probable hypothesis is that the laser energy stimulates porphyrins and cytochromes to increase cellular activity, increasing the concentration of ATP and the release of Ca. The magnitude of the biomodulative effect depends on the physiologic status of the cell at the irradiation time. This may explain why the biomodulative effect is not always detectable. The stimulant effect of the laser light occurs during the initial phase of proliferation and initial differentiation of undifferentiated cells. However, this does not occur during more advanced stages.

The dose is the most important parameter in LPT. At first glance, the dose seems very simple and obvious: simply give some joules per square centimeter of skin. A two-dimensional power distribution of the light on the tissue surface leads to a three-dimensional energy distribution in the tissue. This distribution depends on many factors.

Controversies regarding dose is still a hot topic among scientists, and previous reports have pointed out that the use of low doses result in positive effects on living organisms. On the other hand, higher doses have not been shown to produce positive effects in stimulating living tissues. Cellular proliferation is more intense 24 hours after the irradiation and decreases, in an energy-dependent way, up to 72 hours. If the same power output is kept and time is increased, the amount of energy within the tissue will increase in the same ratio, so a larger volume of tissue will receive a dose within the therapeutic window.

The efficacy of LPT depends on: amount of energy delivered to the tissue, exposure time, and delivery method. It is important to start the treatment using lower doses and intensity, particularly in elderly patients whose cellular response is relatively weak. Dose may be adjusted during treatment. Care has to be taken in order to avoid inhibitory threshold and overexposure during treatment. Controversies regarding dose continue, although previous reports have pointed out that low doses result in positive effects on living organisms.⁴⁶ On the other hand, higher doses have not been shown to have positive effects on stimulating living tissues.

Lower doses are recommended when irradiating mucous membrane or wounds due to the lack of skin (optical barrier). Dose and time interval will depend on the status of the process (chronic or acute), patient systemic status, and skin pigmentation. The recommended doses must be considered when using the contact method. When the noncontact technique is used, the loss of energy is difficult to calculate because of reflection. Usually the tissue reacts well to higher doses using HeNe and GaAlAs lasers; higher doses may cause inhibition or over-reaction.

Another question that is frequently asked regards the frequency of irradiation in clinical settings. So far there is no study that supports the theory that more frequent irradiation will further improve the final results. Dose has been found to be cumulative. Also, we will always get systemic effects. Our clinical experience shows that patients respond very well to treatment carried out every other day. There is no difference if the patient is treated at daily basis. Our clinical protocols always follow three sessions per week.

It is known that the nutritional status influences the healing process as well as the quality of the healed tissue and that the use of both visible and IR wavelengths result in a positive biomodulatory effect on undernourished subjects, and the effect is more evident with the use of 635 nm and SAEF of 20 J/cm² or 780 nm with spatial average energy fluence (SAEF) of 40 J/cm².^{21,22}

Most studies on soft tissues suggested that visible light produces more pronounced effects on cutaneous wounds than IR light. In our experience, in burns, lesions which deeply affects the tissues, IR wavelengths seems to be the better option since surface tissues are thermally damaged and less susceptible to be affected by light. Thus, the use of a wavelength with deeper penetration associated with pulsing, which has been shown to increase the penetration, may be accountable for the findings of this study. We have found that the use of polarized light emitting on both visible and invisible wavelengths positively affected the healing of burns.^{19,20}

On surgical flaps, we found the best responses in 790-nm irradiated subjects, with intense angiogenesis and fibroblastic proliferation. Angiogenesis is essential for wound healing and for the reestablishment of local blood flow on ischemic areas, such as on flaps where tissue necrosis is a common feature. In this case, we have reduction in tissue necrosis and less and precocious inflammatory reaction.²³ In cases in which some therapeutical approach may led to impairment of the healing, such as the use of some drugs, the use of LPT resulted in less inflammation and increased fibroblast proliferation and collagen deposition, favoring healing.²⁴

Our group has shown, using different models, that association of bone grafts, bone morphogenetic proteins, and guided tissue regeneration does improve the healing of bone tissue.³⁶⁻⁴⁵ Many studies indicated that irradiated bone, mostly with IR wavelengths, shows increased osteoblastic proliferation, collagen deposition, and bone neoformation when compared to nonirradiated bone.³⁶⁻⁴⁵

Several groups worldwide have been studying the effects of the use of different phototherapies on the healing and repair of both soft and mineralized tissue. From these reports and our own experience we have no doubt whatsoever that the use of phototherapies, carried out with appropriate parameters, promotes quicker tissue repair. Phototherapy is a highly effective form of treatment with increased supporting evidence from both experimental animal research and human studies. Controlled multicenter trials are necessary and mandatory for a worldwide acceptance of this therapeutic by the health authorities.

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