LOW LEVEL LASER THERAPY

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1. Introduction
2. Mechanism of action
3. Cellular effects of LLLT during wound healing
4. LLLT and neural tissues
5. Clinical applications of LLLT
6. Frontiers of clinical practice for LLLT
7. LLLT technology
8. LLLT equipment
9. Conclusions
10. Acknowledgments

1. Introduction

Low level laser therapy (LLLT) is also known as "soft laser therapy" and bio-stimulation. The use of LLLT in health care has been documented in the literature for more than three decades. Numerous research studies have demonstrated that LLLT is effective for some specific applications in dentistry [1].

The LLLT literature is large, with more than 1000 papers published on this topic. A problem in dissecting this literature is the variation in methodology and dosimetry between different studies. Not only have a range of different wavelengths been examined, but exposure times and the frequency of treatments also vary. The inclusion of sham-irradiated controls in clinical studies is an important element, since placebo effects can be important, particularly in terms of the level of pain experienced and reported following treatment [1].

While broad band light can exert effects on cells [2-3], interest has been concentrated on using lasers as a light source because of their greater therapeutic effect. While much of the initial work with LLLT used the helium-neon gas laser (632.8 nm), nowadays most LLLT clinical procedures are undertaken using semi-conductor diode lasers, for example, gallium arsenide-based diode lasers operating at 830 nm or 635 nm wavelengths [4]. Since wavelength is the most important factor in any type of photo-therapy, the clinician must consider which wavelengths are capable of producing the desired effects within living tissues.

The typical power output for a low level laser device used for this therapy is in the order of 10-50 milliWatts, and total irradiances at any point are in the order of several Joules. Thermal effects of LLLT on dental tissues are not significant [5], and do not contribute to the

therapeutic effects seen. The wavelengths used for LLLT have poor absorption in water, and thus penetrate soft and hard tissues from 3 mm to up to 15 mm. The extensive penetration of red and near-infrared light into tissues has been documented by several investigators [6]. As the energy penetrates tissues, there is multiple scattering by both erythrocytes and microvessels. Because of this, both blood rheology and the distribution of microvessels in the tissue influence the final distribution pattern of laser energy [1].

2. Mechanism of action

The mechanisms of low level laser therapy are complex, but essentially rely upon the absorption of particular visible red and near infrared wave lengths in photoreceptors within sub-cellular components, particularly the electron transport (respiratory) chain within the membranes of mitochondria [2,7]. The absorption of light by the respiratory chain components causes a short-term activation of the respiratory chain, and oxidation of the NADH pool. This stimulation of oxidative phosphorylation leads to changes in the redox status of both the mitochondria and the cytoplasm of the cell. The electron transport chain is able to provide increased levels of promotive force to the cell, through increased supply of ATP, as well as an increase in the electrical potential of the mitochondria membrane, alkalization of the cytoplasm, and activation of nucleic acid synthesis [8]. Because ATP is the "energy currency" for a cell, LLLT has a potent action that results in stimulation of the normal functions of the cell. The specific actions of LLLT are summarized in Table 1.

Karu, who has studied the bio-stimulative effects of light on cell cultures in great detail, has demonstrated that cell cultures which are initially irradiated with laser light show a range of biological effects [7,9,10]. Of importance, if these cultures are then irradiated with non-monochromatic and incoherent light, the previous laser-produced biological effects are almost nullified. This suggests that there are more complex mechanisms at work than the simple excitation of polarization-sensitive chromophores in the cell.

It is crucial to recognize the optical distinction between irradiating human tissues, in which light will scatter very widely, and a thin transparent monolayer of cells in a laboratory. In this context, a key issue is polarization of the light, since polarized and non-polarized light can bring about different biological responses. In a thin layer of cells in culture, the polarization of laser light is maintained through the entire thickness of the cell layer. The work of Mester [11], which used leucocytes in the laboratory setting, indicates that both polarized laser light and polarized incoherent light can evoke bio-stimulation, whilst no such stimulation occurs with non-polarized incoherent light.

Considerable insight into the effect of wavelength on LLLT has been gained from the work of Karu, who over a period of years [7,9,10], has conducted extensive research using cell cultures of various types. Her work has provided an action spectrum for bio-stimulation of the rate of DNA synthesis in HeLa cells, and for the proliferation of bacteria and yeast colonies. These spectra show peaks in the blue (404 and 454 nm), red (620 nm), and near infrared (760 and 830 nm) wavelengths. Her findings also reveal that individual spectral bands may give antagonistic effects on the all-important electron transport chain, for example, blue versus red, and ultraviolet versus red, when these respective wavelengths are delivered in sequence [7]. In her own words: *"... it is possible to conclude that irradiation with monochromatic visible light in the blue, red and far red regions can enhance metabolic processes in the cell. The photobiological effects of stimulation depend on the wavelengths, dose and intensity of the light*".

By increasing the respiratory metabolism of the cell, LLLT can also affect the electro-physiological properties of the cell. This has relevance in terms of cells such as mast cells which are triggered to respond by ionic gradients.

3. Cellular effects of LLLT during wound healing

LLLT has also been shown to cause vaso-dilation, with increased local blood flow. This vaso-active effect is of relevance to the treatment of joint inflammation, such as may occur in the TMJ. LLLT causes the relaxation of smooth muscle associated with endothelium. This vaso-dilation brings in oxygen and also allows for greater traffic of immune cells into tissue. These two effects contribute to accelerated healing.

Furthermore, LLLT can exert vaso-active effects by its actions on mast cells. The effects of different types of light on mast cells are well recognized [12]. There is direct evidence [13] that 660, 820, and 940 nm light can trigger mast cell degranulation. Mast cells are distributed preferentially about the microvascular endothelium in skin, oral mucosa and dental pulp [14,15]. Mast cells in these locations contain the pro-inflammatory cytokine tumour necrosis factor-_ in their granules [16]. Release of this cytokine promotes leukocyte infiltration of tissues [17] by enhancing expression of endothelial-leukocyte adhesion molecules. In addition, mast cell proteases, such as chymase [18], alter basement membranes and facilitate entry of leukocytes into tissues. Because mast cells play a pivotal role in controlling leukocyte traffic, modulation of mast cell functions by LLLT can be of considerable importance in the treatment of sites of inflammation in the oral cavity.

Laboratory studies of low level laser effects have demonstrated a range of bio-stimulation effects (Table 2). For fibroblasts, increased proliferation, maturation and locomotion have been noted, as well as transformation to myo-fibroblasts, reduced production of pro-inflammatory prostaglandin E2, and increased production of basic fibroblast growth factor. These effects have been reported for fibroblasts from the skin, buccal mucosa and gingiva, all of which show increased proliferation at low doses (e.g. 2 J/cm^2). Of note, high dose LLLT suppresses both fibroblast proliferation and autocrine production of basic fibroblast growth factor [19].

LLLT effects on macrophages include increased ability to act as phagocytes, and greater secretion of basic fibroblast growth factor. Macrophages resorb fibrin as part of the demolition phase of wound healing more quickly with LLLT, because of their enhanced phagocytic activity during the initial phases of the repair response (for example, 6 hours after trauma). More rapid demolition of the wound establishes conditions necessary for the proliferative phase of the healing response to begin.

With LLLT, lymphocytes become activated and proliferate more quickly, while epithelial cells become more motile and are able to migrate across wound sites with accelerated closure of defects. Endothelium forms granulation tissue more quickly.

Early epithelialization, increased fibroblastic reactions, leucocytic infiltration, and neo-vascularization are seen in wounds irradiated using LLLT. Because of the overall impact of these influences, the time required for complete wound closure is reduced. Moreover, the mean breaking strength, as measured by the ability of the wound to resist rupture against force, is increased [20].

Wound healing consists of several distinct phases [21], all of which can be affected at the cellular level by LLLT. The initial, pro-inflammatory and vaso-active phases of inflammation include clotting of any cut blood vessels and deposition of a platelet plug, after which the site is infiltrated by neutrophils and macrophages. These infiltrating cells, together with resident tissue cells such as fibroblasts, release a variety of biologically active substances such as growth factors. Enhanced production of fibroblast growth factor, for example, can occur with LLLT from fibroblasts and macrophages.

The second phase of wound healing involves proliferation, with the formation of granulation tissue as a result of new blood vessel growth. This angio-genesis combined with the deposition of new connective tissue requires successful degradation of the wound matrix by macrophages. The final phase of wound healing, which is remodelling, can continue for months or years, and in this context accelerated formation of bone is of great clinical interest. Studies in the author's laboratory (in conjunction with Dr N. Doan and Prof. P.M. Bartold) which have measured DNA synthesis in cell culture have shown that bone derived cells and fibroblasts are stimulated to grow using low level laser therapy, at either 630, 670, or 830 nm. Of interest, the 830 nm wavelength exerts greater effects on bone cells than on fibroblasts. Similar findings for fibroblasts were obtained by Karu in her studies of the effect of the red light on DNA synthesis [7].

Direct evidence for enhanced collagen gene expression both in skin fibroblast cultures in vitro, as well as in animal models of wound healing in vivo, has been presented [22]. Biochemical assays of wounds have revealed that the amount of total collagen is significantly increased in laser treated sites, indicating accelerated collagen production. As well, there is a reduction in pepsin soluble collagen in laser treated wounds over control wounds, indicating higher resistance to proteolytic digestion. Together, these bio-mechanical and biochemical results suggest that laser photo-stimulation promotes the tissue repair process by accelerating collagen production and promoting overall connective tissue stability [23].

A final aspect of the effect of LLLT on cells relates to the effects of laser light on the cytoskeleton. Several studies have suggested that LLLT can modulate cell behaviour by causing re-arrangements of the cytoskeleton [24,25]. As shown by Medrado et al. [26], stimulation of connective-tissue cells toward a myoid phenotype can result in the differentiation of myofibroblasts. It is this cell type which mainly responsible for the contraction force during wound healing [27-29]. Myofibroblasts share morphologic features in common with fibroblasts and smooth muscle cells [30]. These cells are observed in normal tissue, granulation tissue, and some pathological conditions [31,32]. Because LLLT is an effective stimulator of differentiation to myofibroblasts, the process of wound healing should be accelerated. In the study of Medrado et al. [26], sequential semi-quantitative histological examination revealed that laser treatment shortened the exudation phase of wound healing in skin, and stimulated the reparative process. LLLT showed the greatest wound area reduction between 1 and 3 days after treatment, a finding which correlated with higher numbers of myofibroblasts. Their results confirm numerous earlier studies, such as the seminal investigation of Mester et al., [11] who used photos to document the faster wound contraction with LLLT.

Faster wound closure is of great importance in compromised patients, such as diabetics, and patients undergoing treatment for malignancies. Because LLLT can enhance the release of growth factors from fibroblasts, and can stimulate cell proliferation, it is able to improve

wound healing in such compromised patients. Histological studies have demonstrated that laser irradiation improves wound epithelialization, cellular content, granulation tissue formation, and collagen deposition in laser-treated wounds, compared to untreated sites [33, 8]. These findings have been confirmed in oral mucosal wound healing in clinical studies in humans [34].

4. LLLT and neural tissues

Following LLLT, neural tissues show reduced synthesis of inflammatory mediators, as well as more rapid maturation and regeneration, particularly axonal growth. LLLT has also been proven to reduce pain in patients suffering from post-herpetic neuralgia, from cervical dentinal hypersensitivity [5], or from periodontal pain during orthodontic tooth movement [35].

LLLT may also be of benefit in treating TMJ disorders. Clinical studies of LLLT used on patients with injuries to joints in other locations (ankle, knee, shoulder, and wrist) using either the AlGaAs 830 nm diode laser in continuous wave mode, or the He-Ne laser 632.8 nm combined with a diode laser 904-nm in pulsed mode, have shown clinical benefits in terms of a reduction in pain and swelling. Patients treated with LLLT obtain pain relief and recover function more rapidly compared to untreated patients [36,37]. Identical results have been obtained for LLLT of the TMJ. Active and passive maximum mouth opening, and lateral motion are significantly improved by LLLT, with similar results in myogenic and arthrogenic cases [38]. The number of tender trigger points was also reduced. Clearly, such effects may be mediated by a combination of both local and systemic effects.

Further evidence of the utility of LLLT was shown in a meta-analysis of 13 placebo-controlled clinical trials of LLLT, involving patients with rheumatoid arthritis affecting their hands. The duration of treatment ranged from 4 to 10 weeks. LLLT reduced pain (by 70%) relative to placebo. It also reduced morning stiffness, and increased flexibility, when applied over the joint or over the relevant nerves [39]. Consistent with this, positive reports of the benefit of LLLT used in the dental office to treat disorders including TMJ pain, trigeminal neuralgia, and muscular pain have been presented [40]. Once again, this suggests both a local and a systemic action of LLLT.

LLLT has proven to be very effective when applied to "trigger points" i.e., myofascial zones of particular sensibility and of highest projection of focal pain points, due to ischaemic conditions. Results obtained after clinical treatment of patients with pain of varying origin (headaches and facial pain, skeletomuscular ailments, myogenic neck pain, shoulder and arm pain, epicondylitis humery, tenosynovitis, low back and radicular pain, Achilles tendinitis) using LLLT have been particularly promising. In fact, in one study, the author commented that the results "*were better than we had ever expected*" [36].

An additional area of interest in this field is the use of LLLT to achieve an analgesic effect in the dental pulp prior to restorative procedures. First noted with the Nd:YAG laser in the early 1990's, the clinical use of "pre-emptive laser analgesia" is becoming more widespread now as a clinical technique with the Er:YAG and Er,Crt:YSGG laser. When operated at pulse rates between 15- and 20 Hz, at pulse energies below the ablation threshold of tooth structure, the erbium laser energy penetrates into the tooth, and is directed along hydroxyapatite crystals (which function like waveguides) towards the dental pulp. Here, the pulses of energy coincide with the natural bio-resonance frequency of Type C and other nerve fibers in the dental pulp.

The action of this type of LLLT is to cause a disruption in the action of the Na-K pump in the cell membrane, resulting in a loss of impulse conduction, and thus an analgesic effect. The duration of this effect is approximately 15 minutes. Direct examination of teeth lased to achieve this analgesic effect have not shown any evidence of adverse pulpal change at the histological level over the short or long term. There are parallels of the dental laser analgesic effect with several situations in medicine in which simultaneous non-destructive thermal and non-thermal bioactivation occur at the periphery of the target tissue. This phenomenon of "simultaneous LLLT" that may occur along with high level laser treatment has been explained in detail by Ohshiro and Calderhead [6,41].

In vivo studies of the analgesic effect of LLLT on nerves supplying the oral cavity have demonstrated that LLLT decreases the firing frequency of nociceptors, with a threshold effect seen in terms of the irradiance required to exert maximal suppression [42,43]. In vivo, LLLT selectively inhibits a range of nociceptive signals arising from peripheral nerves, including neuronal discharges elicited by pinch, cold, heat stimulation, and chemical irritation [44,45]. In contrast, neuronal discharges induced by brush stimulation are not affected by LLLT. There is some evidence that laser irradiation may selectively target fibers conducting at slow velocities, particularly afferent axons from nociceptors [46,47]. This explains why the LLLT effect of laser “analgesia” is not a complete “anaesthesia” of the lased tooth.

5. Clinical applications of LLLT

While there is extensive laboratory evidence on the effect of low level laser therapy on stimulating cells, the major interest in this technique clinically has been for accelerated wound healing or pain reduction. It is thought that the wound healing effects are due to local release of cytokines, chemokines and other biological response modifiers, while analgesic effects may result from both local and systemic effects. The latter may include release of endorphins.

Detailed and critical analysis of the LLLT literature reveals that the treatment exerts a range of effects, which in themselves are responsive to a range of experimental variables. In their classical work, Tuner and Hode [48] critically reviewed the parameter pitfalls found in many of the classic "negative" studies of LLLT. They assessed some 1,200 papers on LLLT, and examined carefully aspects of experimental design in 85 positive and 35 negative double-blind studies. The negative studies contained a variety of factors which in themselves could provide an explanation for a nil effect of the treatment.

Low level laser therapy has a range of dental, medical, physiotherapy, and veterinary applications. The latter group is of some interest, since when used in animals the possibility of any placebo effects of treatment (for example, on the perceptions of pain or discomfort) can be eliminated completely. LLLT benefits have been reported in both small and large animals [49,50].

Low level laser applications in dentistry include the promotion of wound healing in a range of sites, [1] including:

- surgical wounds to oral soft tissues,
- gingival incisions, [51]
- extraction sites (bone fill and soft tissue healing),
- lesions of recurrent aphthous stomatitis (canker sores), [52]
- the dental pulp, with secondary dentine formation after pulpotomy
- oral ulcerations (mucositis) induced by cancer chemotherapy, [42]

- TMJ injury or arthritic disease, and
- neuronal tissue which has been injured or transected, to accelerate regeneration.

A significant problem for the non-expert reader when examining the literature on LLLT is obtaining a fair comparison between studies. Several well-controlled experimental studies have indicated a beneficial effect upon wounds by laser biostimulation [53]. Despite similarities of dose, and a convergence in laser choice, significant methodological differences persist regarding experimental protocols [3,26]. Potential factors that may influence the effectiveness of LLLT are shown in Table 3, while clinical applications of LLLT are summarized in Table 4.

6. Frontiers of clinical practice for LLLT

There is increasing interest in the concept of pulsing the light source to achieve greater therapeutic benefit [3,6,54-56]. Diode lasers can be pulsed at high frequencies, and continuous wave lasers can be interrupted by means of a mechanical disk that "chops" the beam.

Another novel concept is using LLLT to enhance bone regeneration after extractions or implant placement. In cell culture studies, LLLT using a He-Ne laser stimulates the proliferation, differentiation, and calcification of cultured osteoblastic cells, but only when the cells are in a phase of active growth. If the in vivo parallel holds true, LLLT of healing sites within bone would be expected to increase bone deposition and promote bone regeneration.

In a study of wound healing after tooth extraction in rats, LLLT delivered on a daily basis for one week using an AlGaAs laser enhanced fibroblast proliferation and accelerated the formation of bone matrix [57]. Whether LLLT exerts positive results on bone regeneration following tooth extraction in humans remains uncertain, although there are reports that the formation of granulation tissue during post-extraction healing is accelerated [35].

A stimulatory LLLT effect has been observed on fracture healing in rats using the He-Ne laser, however studies of implant placement surgery have not found significant improvements in bone density or a positive effect of this laser on the process of osseointegration [58]. This lack of benefit may, of course, reflect a sub-optimal treatment protocol. It is important to bear in mind that the maximum benefit with LLLT occurs with repeated dosages, with the best effects being obtained when the treatment is applied daily. Less frequent treatment provides limited benefits [59]. Practically, for maximal bone stimulation, the patient may need to use a handheld LLLT device at home rather than relying on treatment administered at a dental clinic.

7. LLLT technology

A key issue with bio-stimulation using LLLT is the question of whether in fact coherent (laser) light is an absolute requirement. Because of greater destructive interference effects at tissue boundaries, one would expect that bio-stimulation effects with light emitting diodes (LEDs) would be less than with a laser when used at the same parameters [1,11]. As will be discussed further below, the available evidence suggests that this is so, both in clinical and laboratory settings. Because refraction, reflection and scatter can occur at boundaries between tissue types, the physical tissue volume which is irradiated during LLLT can be difficult to estimate [13, 60].

The light from a LED is neither monochromatic (singular in wavelength), nor coherent, and thus the interactions which occur as the light enters tissues and is transmitted, reflected, scattered, and absorbed can easily result in destructive interference. Nevertheless, several manufacturers of low level laser devices claim that treatment with lasers has the same effect as with LEDs [61], and they market units with LEDs for LLLT. Some so-called laser devices may be marketed with LEDs. High quality super-luminous LEDs have a spectral bandwidth of less than 15 nm, while less expensive units may have a spectral bandwidth of more than 50 nm. LEDs are much more difficult than diode lasers to focus into an optical fiber because of their wider divergence.

Several in vitro studies, with adequate blinding of the observers, have demonstrated that the effects of laser light are much greater than obtained with light from other sources, such as LEDs. Mester and colleagues [62] treated three groups of patients with long-standing crural ulcers with a He-Ne laser, a combination of He-Ne and GaAs lasers, and non-coherent unpolarized red light. The two laser groups demonstrated excellent healing, while only a small percentage healing response was seen in the normal red light group. In the study of Kubota and Ohshiro [55], an animal model was used in which no placebo effect is possible. In this study, LLLT with an 830 nm GaAlAs laser increased skin flap survival, with the irradiated sites showing better perfusion, and a greater number of large blood vessels. In contrast, there was no difference between non-irradiated animals, and animals treated with LEDs at 840 nm.

It is unclear whether monochromaticity and coherence are of equal importance in causing photochemical responses in living tissue which cannot be achieved by any other ways. The published literature contains a number of studies which cast doubt on the specificity of treatment effects with LLLT [7,63,64]. Their main argument is that a laser could be replaced by a non-coherent light with the same optical characteristics, since some loss of coherence may occur because of scattering within the tissue. This argument was effectively disproved by Tuner and Hode [48], who reasoned that the key property of coherence is not absolute, but varies across a range. In other words, a light source can be described as more coherent or less coherent than another.

8. LLLT equipment

Semiconductor diode lasers are compact and have a high conversion efficiency from electrical energy to laser energy. Unlike He-Ne lasers, semiconductor laser diodes do not require a high voltage supply, and so can be used in portable, battery-operated devices. It is also possible to pulse the light at various frequencies using simple external circuitry. Laser diodes have a typical life-expectancy of between 100,000 and 600,000 hours [65].

Semi-conductor diode lasers are generally variants of either Aluminium:Gallium:Arsenide (AlGaAs) which emit in the near infrared spectrum (wavelength 700-940 nm), or Indium:Gallium:Arsenide:Phosphorus (InGaAsP) devices which emit in the red portion of the visible spectrum range (wavelength 600-680 nm). Power outputs are typically in the order of 10-50 mW, when measured at the level of the diode laser itself. It is important to note that the final useable output (from the handpiece) will be less because of losses in the internal optical path or in the delivery system.

Since an increased temperature of a diode laser device during operation reduces the output power (and to a lesser extent also lengthens the wavelength), it is critical that the temperature

or output of the laser diode is monitored so that control circuitry can make the necessary adjustments to maintain a constant output. This is usually accomplished using an internal photo-transistor which is fitted within the package of the laser device. With an adequate heat sink and cooling system (with Peltier cooling for higher powered devices), the potential negative effect of temperature on laser output at the level of the treatment beam can virtually be eliminated.

The beam profile from a typical diode laser is rectangular, with a high divergence on the long axis (20 degrees from the centre axis), and a low divergence on the short axis (2 degrees). This gives a highly divergent oval or 'sweep' profile. Diode lasers may have integrated optics which produce collimated and focused light beams. To obtain a more useful beam, a series of lenses or a self-focusing graded index fibre can be used in front of the device to either deliver the treatment beam itself or to direct the laser output into a small diameter flexible optical fibre or a solid light guide (similar to the light tip on a curing light).

Whatever the delivery system used, it is important that the components which come into direct contact with patients are able to be protected adequately with a laser-transmissive disposable barrier, can be autoclaved, or are disposable. Similarly, it should be possible for the clinician to activate the laser into treatment mode without breaching asepsis. Some units employ footswitches or light-operated switches to allow hands-free operation.

Laser units used for LLLT are generally classified as Class III or Class IIIb in terms of the optical hazards which they pose to staff and patients. Because a low power treatment beam can be focused by the eye to give a high power density on the retina, the optical hazard is sufficiently great that laser safety standards mandate the wearing of appropriate protective glasses by patients and clinicians during treatment. Glasses are available which provide protection against common LLLT wavelengths in both the visible and near infrared spectrum.

It is not always possible to tell by looking quickly at a device whether it based on a laser diode or an array of LEDs, although the requirement of the manufacturer to place a laser label (e.g. Class IIIa Laser Device) because of international standards is helpful. With visible wavelengths, a simple test may help the prospective purchaser to determine the type of diode contained in the device. If the laser is visible, the beam can be pointed at a plain wall, and examined for speckle, a type of sparkling in which there are varying points of brilliance. Speckle only occurs with true laser light. The light from a LED does not speckle. For an invisible laser, the same property can be seen but with the aid of a domestic video camera, used in a darkened room to examine the impact of the beam on a wall. The charged coupled device (CCD) used in a video camera is sensitive to light in the near infrared region (extending to approximately 1100 nm), and this feature can be used to find laser beams from near infrared lasers, and to test the units for correct operation. An infrared phosphor board (that is capable of discriminating the wavelength of laser being tested) or a monochromator can be used in the laboratory setting to give a more detailed assessment of laser output. A monochromator will readily distinguish between a true laser diode and a LED.

9. Conclusions

Low level laser therapy has been found to accelerate wound healing and reduce pain, possibly by stimulating oxidative phosphorylation in mitochondria and modulating inflammatory responses. By influencing the biological function of a variety of cell types, it is able to exert a range of several beneficial effects upon inflammation and healing. LLLT exerts marked

effects upon cells in all phases on wound healing, but particularly so during the proliferative phase.

There is good evidence that the enhanced cell metabolic functions seen after LLLT are the result of activation of photo-receptors within the electron transport chain of mitochondria. The effect is specific for wavelength, and cannot be gained efficiently with normal, non-coherent, non-polarized light sources, such as LEDs.

Future trials of new LLLT applications in dentistry should make use of standardized, validated outcomes, and should explore how the effectiveness of the LLLT protocol used may be influenced by wavelength, treatment duration, dosage, and the site of application.

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Table 1. Effect of different wavelengths on biostimulation (modified from the work of Laakso, [3]).

Wavelength	**Energy Density**	**Effect**
540 nm, and 600 to 900 nm	0-56 J/cm^2	Dose and light intensity-dependent fibroblast proliferation
632.8 nm	2.4 J/cm^2	Vasodilation, mast cell exocytosis, interstitial oedema and opening of cell membrane pores
632.8 nm	2.4 J/ cm^2	Enhanced neutrophil phagocytosis
632.8 nm	2 J/ cm^2	Improved fibroblast metabolic rate
632.8 and 904 nm	0.25-4 J/ cm^2	Increased keratinocyte proliferation
660, 820, 870 and 880 nm	2.4 J/ cm^2	Stimulation of fibroblast proliferation by affecting macrophage responsiveness
660 nm	2.4-9.6 J/ cm^2	Enhanced macrophage responsiveness and proliferation
820 nm	2.4-7.2 J/ cm^2	Increased macrophage responsiveness and fibroblast proliferation
830 nm	10 J/ cm^2	Increased perfusion and angiogenesis in rat skin flaps
830 nm	10 J/ cm^2	Increased phagocytic activity of neutrophils
904 nm	76.4 J/ cm^2	Reduced oedema and improved rate of skin wound closure in rats

Table 2. Possible mechanisms involved in the acceleration of wound healing by LLLT [adapted from Reference 1]

Fibroblasts:
proliferation
maturation
locomotion
transformation into myofibroblasts
reduced secretion of PGE2 and IL-1
enhanced secretion of bFGF
Macrophages:
phagocytosis
secretion of fibroblast growth factors
fibrin resorption
Lymphocytes:
activation
enhanced proliferation
Epithelial cells:
motility
Endothelium
increased granulation tissue
relaxation of vascular smooth muscle
Neural tissue:
reduced synthesis of inflammatory mediators
maturation and regeneration
axonal growth

Table 3. Factors affecting the efficacy of LLLT

Patient selection factors
• standardized clinical presentation
• randomization
• blinding of the subjects and examiners
• sample size (number of patients and sites)
• statistical power of the study
• confounding factors, such as medications or other treatments
• use of anaesthesia
• inclusion of controls
• "sham" irradiation to identify the size of the placebo effect
• optimal "window" for the timing of treatment
• length of follow-up
Optical factors
• laser or LED light source
• wavelength
• spot size
• power density
• energy density
• mode of operation (continuous wave or pulsed)
• timing of treatments (single or multiple)

Table 4. Current LLLT applications in dentistry

Soft tissue modulation:
stimuation of wound healing
aphthous stomatitis
pulpotomy
mucositis
Neural modulation:
laser analgesia
neuronal regeneration
post-herpetic neuralgia
TMJ pain
? Post-surgical pain
? Bone regeneration

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