Ministry of Higher Education and
Scientific Research
University of Al-Qadisiya
College of Veterinary Medicine

Effect of Low Level Laser Therapy on Second Degree Burns

A Research Project
Submitted to the council of Department of the Surgery and Obstetrics College of Veterinary Medicine/ University of Al-Qadisiyah in Partial Fulfillment of the Requirements for the Degree of Bachelor in Veterinary Medicine & Surgery

By

Wafaa Hussain Al-Fatlawiy

Lec.
Abbas Ali AL-lban

2017 1438

بسم الله الرحمن الرحيم

(هُوَ الَّذِي جَعَلَ الشَّمْسَ
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يُفَصِّلُ الآيَاتِ لِقَوْمٍ يَعْلَمُونَ)...

صدق الله العلي
العظيم

سورة يونس الآية ٥

Certificate of supervisor

I certify that ***Wafa Hussain*** has completed the fulfillment of her graduation project entitled ***the Effect of Low Level Laser Therapy on Second degree Burns*** for the year 2016/2017 under my construction.

Lecturer

ABBAS ALI AL-LBAN

2017

Certificate of Instructor

We certify that ***Wafaa Hussain*** has completed the fulfillment of her graduation project entitled ***the Effect of Low Level Laser Therapy on second degree of burns*** for the year 2016/2017 under our construction.

Instructor
Dr. MuthannaHadiHussain

Head of Department of Internal and Preventive Medicine
Dr. MuthannaHadiHussain
2017

Abstract

Burn is a severe injury that may result in loss of tissue fluids and is associated with infection, destruction, pain and even death.

Burn healing is a complex process including dynamic series of events involving clotting , inflammation, granulation tissue formation, epithelialization, collagen synthesis and tissue remodeling.

Healing of burns injury is still difficult to achieve although the evolution of antiseptic, medications and advanced operation procedures.

Death and disability are a significant incidences associated with Burn injuries.

The aim of treatment of burns is to prevent infections and achieve the best functional and aesthetic results is a shorter time with lower costs as well as avoidance of excessive scaring as soon as scar has formed, it is known to be difficult to treat because of its tendency to cause hypertrophy and contracture. The action of LLLT is based on absorption of tissues the laser light,which then generate modifications in cell metabolism.

Studies have revealed that laser therapy increased mitochondrial respiration and synthesis of ATP .

Laser irradiation affects calcium exchange through cell membrane, resulting in transient changes in the cytoplasmic calcium level. DNA ,RNA and cellocyte regulatory protein synthesis can be increased by these modifications and therefore stimulating cell proliferation and the latter is beneficial for the reestablishment of connective tissue during tissue repair and wound healing.

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1- Introduction

Since the first report on laser therapy many potential fields for its applications have been investigated .Among types of lasers certainly belong to the most significant advances of present century. So various kinds of laser light already become irreplaceable of modern medicine.

For approximately forty years, light in form of low level laser therapy (LLLT) has been used in treatment of a myriad of conditions. Low level laser therapy is used in photobiostimulation, leading to biological and physical effects have been reported which are the observed clinical symptoms of various cellular events. low level laser therapy has been found to modulate various biological processes (Karu, 2000), such as increasing mitochondrial respiration and ATP synthesis, facilitating wound healing (Conlan 2001) and promoting the process of regeneration (Yu W1997).The LLLT action depends on the radiation wavelength, power density and exposure time. The most effective irradiation is that in the red and near infrared range of the spectrum. The most commonly used sources are the helium-neon laser (He-Ne) (632.8 nm) and diode laser.

In this study, the effect of low level laser irradiation on the burn healing was investigated. In clinical practice LLLT could be considered for short-term relief of pain and treatment of many cases like wound injuries and burns, which was found to be highly affected with LLLT (Baxter 1994).

1-1 Aim of the study

The purpose of this study was evaluated enhancement effect of burn healing by using low level laser therapy.

2 - 1 The Skin:

The skin is the largest body's organ weighing about 6 pounds and it is the body's outer covering protecting layer. It protects the body against heat, light, injury, and infection. In fact, the skin is an essential part of our body's defense against infection from microbes in the environment. The skin also regulates body temperature, stores water, fat, and vitamin D. It is made up of two main layers; the outer layer is the epidermis, the inner layer is the dermis like in figure (Figure 1-1). The epidermis (outer layer of the skin) mostly made up of flat, scale-like cells called squamous cells. Under the squamous cells are round cells called basal cells. The deepest part of the epidermis also contains melanocytes. These cells produce melanin, which gives the skin its color. The dermis (inner layer of skin), contains blood and lymph vessels, hair follicles, sweat glands and sebaceous glands. Sweat glands produce sweats, which help regulating body temperature, and sebum secreted by sebaceous gland, is an oily substance that helps in keeping the skin from drying out. Sweat and sebum reaches the skin surface through tiny openings called pores (Rod, 1998).

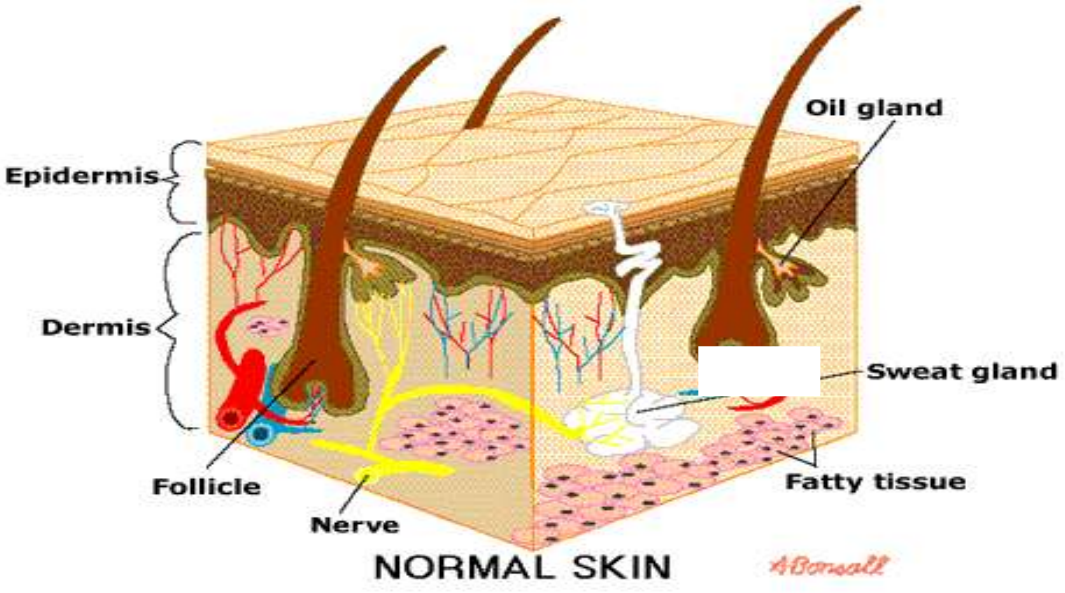

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Figure (1-1) skin structure (Wheater, 1987)

2 -1-1 Anatomy of the skin

The skin is divided into the epidermis and dermis. The epidermis varies in depth. It is thickest in the scalp and thinnest in the delicate skin of the eyelid. The dermis is composed of connective tissue and collagen. It contains nerves, blood vessels, muscles, lymphatics, sebaceous and sweat glands. The more superficial part of the dermis is termed the papillary dermis , which contains thin , randomly arranged collagenized fibers and the superficial vascular plexus .The deeper which is the thicker dermis, called the reticular dermis, contains bundles of collagen and the deep vascular plexus composed of larger vessels that surround skin appendages. (Rod 1998)

A- Hypodermis (subcutaneous tissue)

The hypodermis is loose connective tissue that contains collagen and elastic fibers located beneath the dermis.The hypodermis attaches the skin to underlying structures and it is a site of fat storage.

B- Skin Dermis

The dermis a dense, irregular connective tissue with few adipose cells is divided into two layers (reticular and papillary layers). The reticular layer which is the main fibrous layer and consists mostly of collagen. The papillary layer is well supplied with capillaries. (Rod 1998)

C- Skin Epidermis

The epidermis is a stratified squamous epithelium divided into:

1. The stratum basale consists of keratinocytes, which produce the cells of the more superficial stratum.
2. The stratum spinosum consists of several layers of cells held together by many desmosomes (tight connections between cells).
3. The stratum granulosum consists of cells filled with granules of keratohyalin. Cell death occurs in this layer.
4. The stratum Leucidum consists of cells or layer of dead transparent cells
5. The stratum corneum consists of many layers of dead squamous cells. The most superficial cells are desquamated.

• Keratinization is the transformation of the living cells of the stratum basal into the dead squamuos cells of the stratum corneum.Keratinized cells are filled with keratin and have a protein envelope. The cells are also held together by many desmosmes.The Intercellular spaces are filled with lipids that contribute to the impermeability of the epidermis to water.

• Soft keratin is found in the skin and the inside of the hair, whereas hard keratin occurs in nails and the outside of the hair. Hard keratin makes cells more durable, and these cells do not desquamate. (Rod 1998)

2-1-2 Thick and thin skin

Thick skin has all five epithelial strata. The dermis under thick skin produces finger-like projections. The thin skin contains fewer cell layers per stratum, and the stratum lucidum is usually absent. Hair is found only in thin skin.

2-2 Burn

Is a tissue injury from heat or cold applications or/ from the absorption of physical energy or chemical contact.

2-2-1 Common types of burns:

Burns are classified according to the causes into the following types:

- **Thermal burns** are caused by contact with intense heat, such as flames, steam, scalding liquids, hot metals and other sources of heat.
- **Radiation burns** are caused by contact with nuclear radiation (X-rays, etc.) or ultraviolet rays such as from sunlight.
- **Chemical burns** are caused by contact with caustic chemicals, such as acids, alkalis, detergents or solvents.
- **Electrical Burns** are caused by electric current passing through the body (Russfll 2000).

2-2-2 Severities of burns:

Burn depth in the thermal injury depends upon:

•The temperature of the burning agent.

•The mode of transmission of heat.

•The duration of the contact.

a- First degree burn:

Burn damages only the outer layer of skin, or epidermis. Sunburn is typically a first degree burn. The 1st degree superficial burn is usually red and turns white if you press on it. A first degree burn heals spontaneously in a week or so without scarring. These have the ability to heal themselves by epithelialisation alone. (Russfll 2000).

b- Second degree burn

Second degree burn involves the entire epidermis, and some portion of the dermis figure (1). Superficial second degree burn symptoms include redness ,pain,edema,and blisters .Healing takes approximately 2 weeks, and there is no scaring .If the burn goes deep into the dermis (deep second degree burn),however the wound appears red,tan,or white, may take several months to heal, and end with scar. In all second-degree burns the epidermis regenerates from epithelial tissue in hair follicles and sweat glands, as well as from the edges of the wound. A skin graft is usually recommended for deep second degree burns. (Russfll 2000)

C- Third degree burn:

Burn destroys the epidermis, dermis, and deeper tissue may be involved (figure 1). Third degree burns are surrounded by first-and second-degree burns. The region of second degree burn is painless because of the destruction of the sensory receptors. The third degree burns are dry, with a dark brown or leathery appearance. Most of third degree burns larger than 3 centimeters in diameter are best treated with removal of dead tissue and by immediate skin grafting. Deep partial-thickness and full-thickness burns take a long time to heal and form scar tissue with disfiguring and debilitating wound contraction. Skin grafts are performed to prevent these complications and to speed healing. (Russfll 2000)

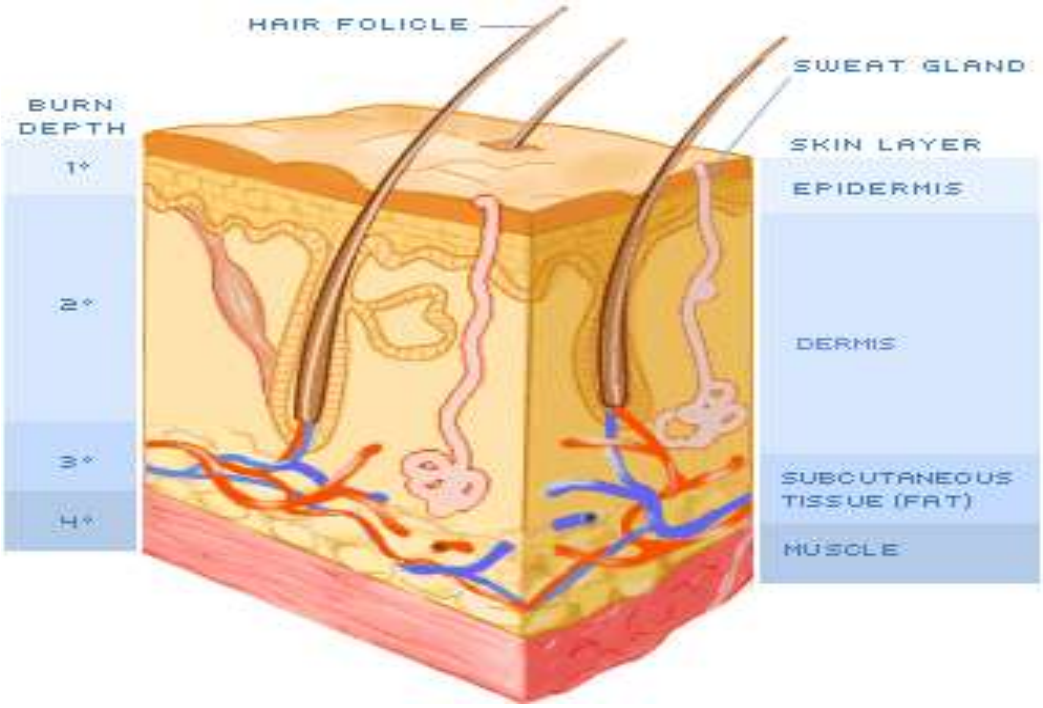


Figure (1) The depth of a burn injury (Russfll 2000)

2-2-3 Effects of burn injury:

The effects of the burn can be considered as:

A-Local effects:

1- Tissue damage.

Heating of the tissue results in direct cell rupture or cell necrosis, the periphery cells may be viable, but injured. In addition, the collagen is denatured and damaged.The capillaries are either thrombosed where the damage is severe or in less damaged areas there is an increased in capillary permeability such that the tissue becomes oedematous and there is external leakage of serous fluid. The essential difference between a partial-thickness and full-thickness skin loss is the depth of injury, but it is possible that the former may progress to the latter (Russfll 2000).

2-Inflammation

There is a marked, immediate inflammatory response. In the areas of least damage by burning, this is manifested simply as erythema. The

precise cause of this immediate vasodilatation may represent a neurovascular response. Mild area of erythema resolves within a few hours. More severely damaged tissue may develop a more prolonged inflammatory response. Macrophages produce inflammatory mediators or cytokines (e.g. transforming growth factor-B) and phagocytose necrotic cells. Neutrophils and later lymphocytes provide protection against infection. Damaged tissue separates by an active cellular process described as desloughing, generally completed by 3 weeks (Russfll, 2000)

3-infection

The damaged tissue represents a nidus for infection. Burn wounds will almost inevitably be colonized by microorganisms within 24-48 hours and this may remain as a local wound or regional infection. There may, in addition, be a bacteraemia or septicemia and infections may develop other sites. Bacteraemia is a common cause of fatality in a severe burn and may occur at any time from the first day until the point when all the wounds have entirely healed. Beta-haemolytic streptococci and pseudomonas produce protease enzymes that prevent skin graft adhesion (Russfll, 2000).

B-Systemic effects

When large areas of skin are severely burned, systemic effects are produced and can be life-threatening. Within minutes of a major burn injury, there is increased permeability in the capillaries, which are the small blood vessels in which fluid, gases, nutrients, and waste products are normally exchanged between the blood and tissues. As a result, fluid and electrolytes are lost from the burn wound and into tissue spaces. The loss of fluid decreases blood volume, which decreases the ability of the heart to pump blood. The resulting decrease in blood delivery to tissues can cause tissue damage, shock, and even death. Treatment consists of administering intravenous fluid at a faster rate than it leaks out of the capillaries. Although this can reverse the shock and prevent death, fluid continues to leak into tissue

spaces causing pronounced edema and a swelling of the tissues (Russfll, 2000).

C- Other effects of burns

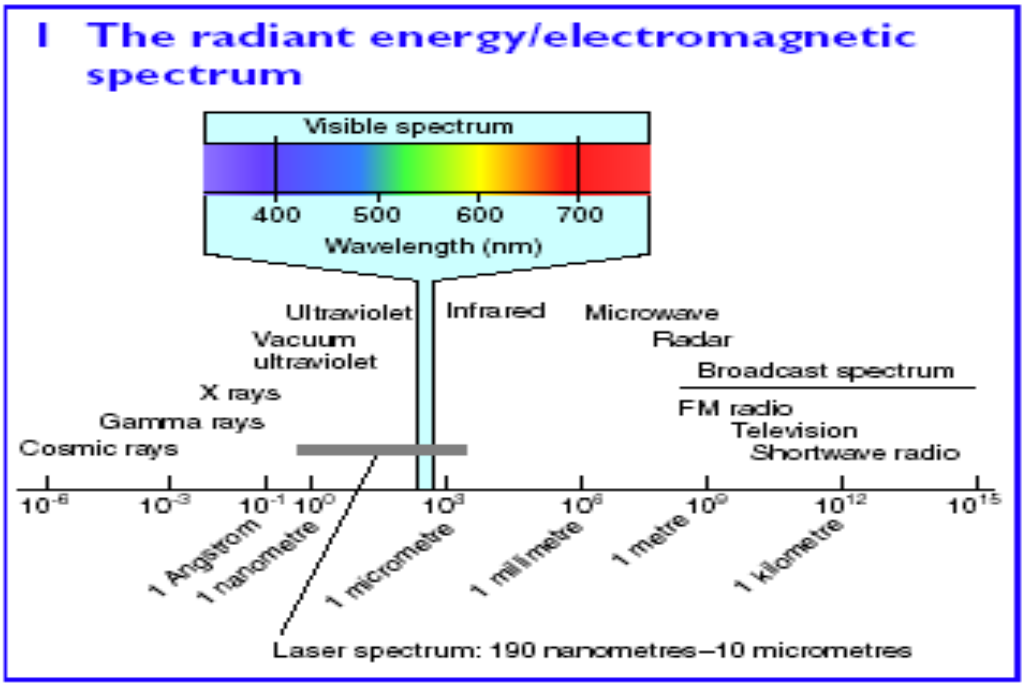
Severe burns, both physically and emotionally. Persons with severe burns may be left with permanent physical disabilities; loss of mobility, disfigurement, scarring, infection, muscle or tissue damage, nerve damage, respiratory system damage, loss of a limb, and permanent mental disabilities; nightmares or flashbacks from the traumatizing event and anguish from loss of a friend or family member. Severe burn injuries often necessitate long term medical care, nursing care, physical therapy, and psychological care.

2-3 Basics of lasers

The acronym laser shortly describes the process that generates laser light: **L**ight **A**mplification by the **S**timulated **E**mission of **R**adiation. In this process, photons are emitted and amplified by atoms and molecules within a special optical volume. Although the word "laser" was primarily created to describe the process of laser light generation, it has also come to name the device that creates the light (Baxter 1994).

2-3-1 The Electromagnetic (EM) spectrum

Generally , lasers function within the so-called optical spectrum. This is portion of the electromagnetic spectrum (Fig- 2) that includes the longer wavelength radiation,microwave and infrared segment, through the visible portion seen by the human eye and including the ultraviolet , gama rays and cosmic rays. Laser are torches of radiation from within the EM spectrum emiting beam of energy that possesses special properties that enable their use for



surgical and non-surgical applications (Guya, 1997).

Figure(2)the electromagnetic spectrum showing the range of wavelengths and categories of light waves, (Colonel 2001).

2-3-2 Nature of Light

Light is actually a form of energy that behaves like a wave and also as a stream of particles called photons. Photons behave differently from conventional particles. They have no mass and are not limited to a specific volume in space or time. Each photon gyrates and bounces at a unique frequency and exhibits electrical and magnetic properties. As a result, their waves are called electromagnetic (EM) waves. Not all photons are visible to the human eye. As shown in Figure (3), what we see as light is only a minute range of the spectrum of electromagnetic waves associated with photons. The entire spectrum includes radio waves, infrared radiation, visible light, ultraviolet rays, x-rays, gamma rays, and cosmic radiation. The photons of different regions of the electromagnetic spectrum vibrate differently and have different amounts of energy , Thus even though radio waves, infrared radiation, visible light, ultraviolet rays, x-rays, and gamma rays are photons, ie, light, they vibrate at different rates and differ in photon energy. Their waves have different wavelengths as well. A wavelength is the interval between two peaks of a wave (Figure 1-4), and relates to the color of visible light. For example, blue, green, red, and violet light have different wavelengths. This difference becomes clearer when one compares red and infrared light. Red light is visible; infrared is not visible, (Chukuka 2004).

2-3-3 Fundamentals of light wave :

The wave that radiates away from the impact point has amplitude, frequency, and wavelength. The peak of the wave or its vertical height is called the amplitude ,and this is related to the intensity of a light wave(figure 3) . The number of waves per second passing a specific static measuring point is the frequency expressed in hertz. Measuring the distance from one peak to the trough is called the wavelength. In light radiation, the wavelength and frequency determine the actual energy level of light and how it is distinguished or positioned within the EM spectrum. Longer wavelengths in

the EM spectrum, such as infrared, produce lower energy, and they include not only infrared,but microwaves, television waves, and radio waves. Shorter wavelength have higher energy and include ultravolet, x-ray, and gamma ratiation.

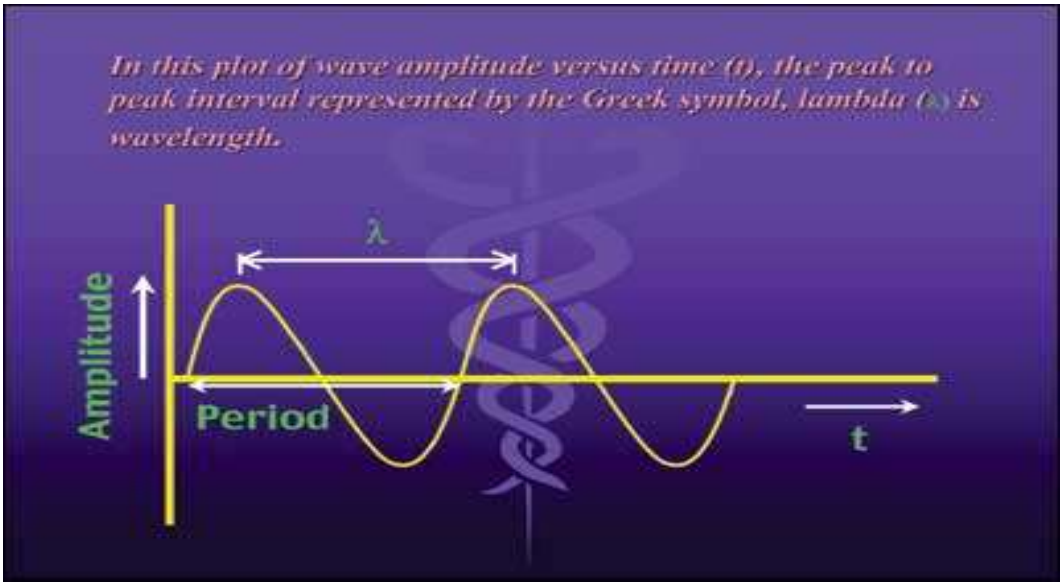


Figure (3) The wave nature of light. Light is transmitted as sinusoidal wave. A plot of the amplitude and time is shown (Chukuka 2004).

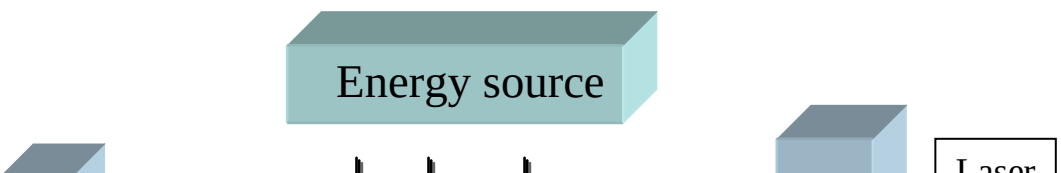
2-3-4 Elements and properties of Laser light:

All laser devices have three major components (figure 4):

1- Energy source

2- Active medium.

3- Laser cavity.



Mirror (R= 100%) Mirror (R< 100%)

Figure (4) Basic components of a laser system (Guya, 1997).

The laser light is generated within the active medium, which may be a solid, a liquid, a gas or a semiconductor. The energetic excitation or pumps of the active medium, which brings the great majority of the atoms or molecules within the active medium from a lower to a higher energy state in order to create a population inversion simultaneously, is done by an energy source. Different energy sources can be used in medical laser systems; most common is the use of flash lamps or electric current(Guya, 1997).

The light beam produced by stimulated emission during the multiple passage of light between the mirrors of the laser cavity has special properties, most of these photons escape from the active medium ,but these travel along the axis of the active medium a portion of the light that strikes the output coupler leaves the cavity as the output beam .pulsed pumping laser that supplies excitation energy in portions ,where as continuous pumping gives it up continuous which results from the fact that the characteristics of each stimulating photon are maintained in the emitted photons(Baxter 1994).

The laser beam has four distinctive characteristics.

- **Coherence**

All wave trains in a laser beam are exactly in-phase with each other, temporally (in time) and spatially (in space)

• **Collimation**

(Directionality) – all rays of the laser beam are relatively parallel over a long distance due to limited on small divergence .

• **Monochromaticity**

All individual wave trains have the same wavelength and frequency (extremely narrow spectral band of radiation). In medical systems and for most clinical applications, the bandwidth of the output beam is so small to be negligible, and these lasers can reasonably be referred to as operating at a single wavelength-as, monochromatic (Guya, 1997).

● **Brightness:**

Another property of laser light that distinguishes it from conventional light sources is brightness. This property arises form the parallelism or collimation of the laser light as it moves through space maintaining its concentration and, thus, the characteristic brightness.

Factor translates to high concentrations of energy when the laser is focused on small spot (Guya, 1997).

The laser beam can be focused on a very small cross-sectional area. These permit very high energy densities to be achieved and even solid tissue to be destroyed. Consequently the output of the laser process, a powerful light beam of very special characteristics is meanwhile widely applied in industry, science, defense, medicine and other fields.

2-3-5 Laser mode:

Depending upon nature of active medium, how the excitation energy is applied and the laser cavity configuration, the output beam of laser will be either real pulsed or continuous wave (cw). Cw lasers deliver constant power during a defined time or work, in chopped mode (it is cw which there are

defined breaks in power delivery).Real pulsed lasers deliver the whole energy collected in an active laser medium during the pulse time. The output of pulsed lasers can vary considerably in duration and energy of individual pulses as well as in their repetition frequencies. The pulses may be delivered individually in groups, or continuously over a broad range of frequencies (Baxter, 1994).

2-3-6 The parameters of laser radiation:

The parameters of laser radiation are wavelength, power, and exposure time, beam spot diameter, power density (irradiance) and energy density (fluence).

Wavelength		[nm]
Power p		[w]
Exposure time t		[s]
Energy	E=p· t	[w x s] = [J]
Spot area	D=π·r2	[cm2]
Power density	PD= P/D	[W/cm^2]
Energy density	ED=E/D=PD · t	[J/cm^2]

$r1 < r2$

$D1 = \pi \cdot r1^2 < D2 = \pi \cdot r2^2$

$PD1 > PD2$

$ED1 > ED2$

As the energy density can be considered as the product of power density and exposure time during treatment, the power density is the primary determinant of the rate of the tissue reaction. Increasing power density allows sufficiently rapid treatment to keep the exposure time to a minimum while still delivering the required total energy to treat the region concerned.

Besides the optical properties of tissue, the effects achieved by irradiation of the tissue with laser light are mainly determined by exposure time and effective power or energy density.

During the laser intact tissues processes the electromagnetic energy is transformed into heat after the absorption of laser light in tissue. Depending on the achieved temperature, the tissue is subjected to different structural changes. Up to 43˚C, all tissue changes are still reversible. For the temperature between 43˚C and 60˚C, denaturation of enzymes and loosing of membranes can be observed. Between 60˚C and 100˚C, coagulation (denaturation of proteins) occurs, followed by tissue necrosis. At 100˚C, tissue water vaporizes, the tissue dries out. At about 150˚C, charring of the tissue (carbonization) can be observed; and above 300˚C, solid tissue becomes vaporized and can thus be cut. However, it must be considered that tissue effects also depend on the time during which the heat affects the tissue (Markolf 1996).

2-4 low level laser therapy:

Low Level Laser Therapy (LLLT) is a form of phototherapy or light therapy. This involves the application of low power light to areas of the body in order to stimulate healing. It is also known as cold laser, soft laser or low intensity laser. Laser employing low-level radiant energy have been claimed to produce a positive effect on the biologic and biochemical processes of wound reconstitution (Baxter, 1994).

2-4-1 Mechanisms of low power laser light.

Low intensity laser photostimulation works through a photochemical response to laser light that induces biochemical alterations in cells, leading to physiological changes. Photons, which are particles of electromagnetic energy, are emitted from the low power laser. These particles enter the tissues and are absorbed in the mitochondria, which are tiny structures within the substance of each individual cell. The photonic energy is converted to chemical energy in the form of adenosine triphosphate (ATP) within the cell. Cell interaction with light at different wavelengths produces physiological changes, leading to alterations in cellular metabolism. Certain chromophores like rhodopsin and porphyrins absorb light energy and cause chemical reactions in the cell, resulting in changes in the metabolic activities of the cell. These photochemical reactions between cells and light form the fundamental mechanism of modern therapeutic low-intensity laser photostimulation (Kesava, 2003). The permeability of the cell membrane changes which in turn produces various physiological effects. These physiological changes affect a variety of cell types including macrophages, fibroblasts, endothelial cells and mast cells (Kesava, 2003) and (karu, 2001). Karu et.al (2001) results showed that cytochrome c oxidase becomes more oxidized (which means that the oxidative metabolism is increased) due to irradiation at all wavelengths used. Where Lutz, (1998) revealed that the mechanism of low-power laser therapy at the cellular level is based on the electronic excitation of chromophores in cytochrome c oxidase which modulates a redox status of the molecule and

enhances its functional activity. A cascade of reactions connected with alteration in cellular homeostasis parameters (pH, Ca^{+2}, cAMP, Eh, ATP and some others) is considered as a photosignal transduction and amplification chain in a cell (secondary mechanisms).

By stimulating the cells with this specific light source, their activity is hyper activated functionally ,speed up ,so that they can perform better, faster and more effective, The result is wound and injury healing that is fastly enhanced and that takes dramatically less time to complete. Also it stimulates the function of immune cells and the lymphatic and vascular systems. The current understanding of the cellular signaling cascade and amplification is that the receptors on the cell surface are the primary sites of action of low frequency electromagnetic fields. It is at this receptor that cellular responses are triggered by hormones, growth factors, neurotransmitters, antigens, or a single photon. Membrane signals closely associated with the receptors, such as adenylate cyclases and G proteins, are considered secondary messengers that couple a single molecular event at the cell surface to the influx of a huge number of calcium ions. Calcium ions entering the cell activate a variety of enzyme molecules and can produce a cascade of intracellular signals that initiate, accelerate, or inhibit biological processes. These enzymes, in turn, are catalysts and since catalysts are not consumed by reactions they can act again and again until calcium levels drop back to pre-stimulation levels (Valerie Hunt, 2002).

Karu (2003) revealed that the primary physical and/or chemical changes induced by light in photoacceptor molecules are followed by a cascade of biochemical reactions in the cell (photosignal transduction and amplification chains). These reactions are connected with changes in cellular homeostasis parameters. The crucial step here is thought to be an alteration of the cellular redox state: a shift towards oxidation is associated with stimulation of cellular vitality, and a shift towards reduction is linked to inhibition. It was shown that cells with a lower than normal intracellular pH, in which the redox state was shifted in the reduced direction, were more sensitive to parameters the

stimulative action of light as compared to cells in which the respective were optimal or near optimal. Light action on the redox state of a cell via the respiratory chain also explains the diversity as well as the versatility of low-power laser effects. The proposed redox-regulation mechanism may be a fundamental explanation for some clinical effects of irradiation, for example, the positive results achieved in treating indolent wounds, chronic inflammation, and ischemia, all characterized by acidosis and hypoxia.

2-4-2 Application of LLLT:

In the in vitro study was to evaluate a potential stimulatory effect of low-level laser irradiation on the proliferation of human periodontal ligament fibroblasts. With an 809-nm diode laser operated at a power output of 10 mW in the continuous wave (CW) mode at energy fluencies of 1.96-7.84 J/cm². The variable irradiation parameters were the time of exposure (75-300 S per well) and the number of irradiations (1-3) time. After laser treatment the irradiated cells revealed a considerably higher proliferation activity than the controls. The differences were significant up to 72 hour after irradiation **(**Kreisler, 2003).

Cultured fibroblasts were treated, during three consecutive days, either with an infrared LLLT or with a LED light source emitting several wavelengths (950 nm, 660 nm and 570 nm). : LED and LLL irradiation resulted in an increased fibroblast proliferation in vitro. Statistical analysis revealed a higher rate of proliferation ($p < 0.001$) in all irradiated cultures in comparison with the control **(**Vinck, 2003). In onther in vitro study focuses on the biostimulation of NIH-3T3 fibroblasts by a low-power Ga-As-pulsed laser. They studied cell growth and procollagen synthesis of cultured fibroblasts, with energy densities varying from 3 to 5 J/cm² over a period of 1-6 days. The light source was a 120 mW Ga-As diode laser (lambda = 904 nm). Irradiation of 3 and 4 J/cm² increased the cell numbers about three folds to six folds comparing with control cultures. Energy densities 5 J/cm² had no effect on cell growth (Pereira , 2002).

Laser irradiation was carried out with a gallium-aluminum-Arsenide (Ga-Al-As) diode laser (904 nm, 120 mW, energy density of 3 J/cm²). There were changes in the structure of cytoplasm organelles of treated cells. The procollagen was not altered by the laser irradiation; however, there were a significant reduction of the amount of protein in cells,
(Marques, 2004) .While Pogrel (1997) revealed that the gallium-aluminum-arsenide laser, when utilized at powers 5-100 milliwatts and times of between 10-120 seconds has no biostimulatory effects on fibroblasts or keratinocytes cultures as assessed by cell proliferation, adhesion, or migration.

A standardized low level laser (LLL) set-up was developed (812 nm, 4.5 ± 0.5 mW/cm²). LLL irradiation was performed for 0, 1, 3, 10, 32, 100, 316, or 1,000 seconds, respectively--corresponding to the radiant exposures 0, 4.5, 13.5, 45, 144, 450, 1,422, 4,500 mJ/cm2. Low level laser irradiation can induce increased DNA synthesis in cultured human oral fibroblasts following LLL exposure (Loevschall, 1994).

In the study which was done by Franek (2002) the ulcers were irradiated with laser light of wavelength 810 nm, so that a dose of 4 J/cm2 was applied in each procedure. At the end of the treatment a statistically significant reduction of the area and volume of the ulcers was found in all groups. No significant impact of laser light (lambda=810 nm, P=65 mW, p=4 J/cm²) on any of the stages of ulceration healing was observed. While in the study to evaluate the efficacy of low energy photon therapy (LEPT) in the treatment of venous leg ulcers. Treatment was given three times a week for 10 weeks, using two monochromatic optical sources. One source provided a wavelength (lambda) of 660 nm (red) while the second source delivered a wavelength of 880 nm (infrared). In this placebo-controlled, double-blind study LEPT was an effective modality for the treatment of venous leg ulcers (Gupta ,1998).Other used helium-neon laser at an energy density of 30 J/cm² three times weekly, complete epithelization could be induced by laser therapy (Schindl, 1999).While irradiation by He Ne lasers two of doses of 3.6 J/cm² produces an increase in new vessel formation, and a significant improvement in epithelialization. (Asencio, 1990)

In a study, irradiation of burns with a 250-mW/670-nm laser light produced no beneficial effects on wound-healing processes. One wound was irradiated with 670-nm laser light (2 J/cm²), whereas the other side remained untreated. Macroscopic evaluation of the wounds was performed daily; 10, 20, and 30 days after burning. Neither macroscopic nor histological examination of the irradiated wound showed acceleration of wound healing when compared with control wounds (Schlager, 2000).

karu, et al (2001) used He-Ne laser irradiation with dosages of 1.1 J/cm² during a 30-min exposure every third day, and 2.2 J/cm² during a 3-min exposure twice daily until wound closure. No significant differences in healing were observed between laser-treated wounds and untreated control wounds. Conversely, rat skin incisions exposed to 2.2 J/cm² for 3 min twice daily for 14 days demonstrated a 55% increase in breaking strength over control animals. Increasing the dosage to 4.5 J/cm² yielded a no significant 17% increase over the control rats 14 days postoperatively. While Wounds in rat were irradiated daily with Helium Neon laser at 4 j/cm² cm for 5 min. Wounds on right side were not exposed and served as controls. The time required for complete closure in irradiated test wounds took only 10.3 +/- 0.68 days (range 9-12 days) to heal. Granulation tissue was significantly ($P < 0.001$) more in test than in control wounds. Early epithelialisation with increased fibroblastic reaction, leukocytic infiltration and neovascularisation was seen in the laser irradiated wounds (Bisht, et al 1994). While Lowe (1998) recoded that the use of low intensity laser and monochromatic light diodes on mice were treated with (0.18, 0.54, and 1.45 J/cm², respectively) three times weekly using a GaAlAs 890 nm, at 0.18 J/cm² and 0.54 J/cm² had no effect upon the rate of wound closure. Petersen (1999) treated wounds that received a daily laser dosage of 2 J/cm². Low level gallinum aluminum arsenide (Ga Al As). There were no significant differences in wound contraction or epithelialisation between the laser treated and the control wounds. While In a study which was done by Yu W (1997) the laser biostimulation effects on wound healing in diabetic mice investigated. An argon dye laser at a wavelength of 630 nm and an output of 20 m W/cm2 was

used as the light source. Laser irradiation enhanced the percentage of wound closure over time as compared to the negative control group. Histological evaluation showed that laser irradiation improved wound epithelialization, cellular content, granulation tissue formation, and collagen deposition in laser-treated wounds as compared to the negative control group.

A study was done to measure tensile strength of wounds closed with and without tension in rats. Results suggested that tensile strength was not significantly different on day 5. However, wounds closed under tension showed significantly higher tensile strength on 7, 10, 14, and 21 following surgery (Pickett, 1996).Where Reddy, (2003) investigated wound treated with infrared radiation at 904 nm produced by a Ga-As laser at an energy density of 1.0 J/cm². The wounds were treated with a laser 5 days a week for 3 weeks. The results from the biomechanical analysis indicated that the Ga-As laser used in this study significantly increased wound tensile strain compared to the control wound. Stadler (2001) recorded the same results with laser irradiation (830 nm, 79 mW/cm², 5.0 J/cm). Daily low-level laser therapy (LLLT) occurred over 0-4 days, 3-7 days.

Cultured human lymphocytes were subjected to irradiation with a gallium-arsenide laser at energy fluence varying from 2.17 to 651 mJ/cm2.Cell proliferation were markedly inhibited by the laser irradiation at energy flounce as interfere with immune system in vitro. (Ohta et.al 1987)

Cultures of normal human keratinocytes were exposed to a single dose of 0 to 3.6 J/cm2 (0-180 sec) 780 nm low power diode laser irradiation lead to enhanced keratinocytes proliferation in vitro when exposed to 0.45-0.95 J/cm² was significantly enhanced. Exposure to other energy densities was considerably less effective in enhancing proliferation parameters. (Grossman et. al 1998)

Rezende (2001) analyzed the acceleration of the healing process of coetaneous lesions in mice. The laser intensity was 428 mW/cm², the total doses were 3 J/cm². All irradiated lesions presented acceleration of the healing

process with regard to the control group. The combination of the intensity value of 53 mW/cm2 and the dose of 1.3 J/cm2 led to optimal results, regarding the biometric and histological analysis, presenting faster lesion contraction, quicker information of epithelial and conjunctive tissue with more collagen fibers.

Oral ulceration, submitted to low intensity infrared laser 830nm, at 30 mw fluency per point 1.3 J/cm² at an exposure time ranging from 3s to 33s, depending on ulceration size single dose. Patients (those submitted to low-intensity infrared laser beam): showed that healing process was faster with reduction of sore area and immediate relief of pain following first irradiation. (Rodrigues, 2001)

In a study which was done to investigate the effects of low-power laser irradiation on exposed pulp tissue in dogs. GaAlAs laser (300 mW).Histopathological changes were observed at 1, 3, and 7 week after the operation. The results suggest that laser irradiation accelerates wound healing of the pulp and the expression of the lectins and collagens (Endod, 1998).while study of Efendiev et al (1992) on 218 white rats, with the infrared laser radiation ranging of from 1 to 150 mW (dosage of radiation 0.06-9.3 J/cm2) facilitated pronounced stimulation of the processes of collagen formation and significant increase in strength of a forming scar. In increasing the power of radiation, the reparative processes in a wound are slowed and disturbed, while Vegesna et al (1995) recoded that the radiation-enhanced wound tensile strength was greater and occurred earlier after higher radiation doses. Even though the effect of irradiation in enhancing wound tensile strength is transitory, it could be important in assisting early wound healing.

Karu (1988) showed that the mechanism of low-power laser therapy at the cellular level is based on the electronic excitation of chromophores in cytochrome c oxidase which modulates a redox status of the molecule and enhances its functional activity. Karu I(1999)drawn out both from in vitro cellular experiments as well as clinical "laser biostimulation" literature is that the cells which are in conditions of low oxygen concentration, acidic pH or

lack of necessary nutrients are much more sensitive (and susceptible) to irradiation than those in optimal or near optimal conditions. where Karu (2001)showed that the Redox absorbance changes in living cells (monolayer of HeLa cells) under laser irradiation at 633, 670, and 820 nm have been studied by the method of multichannel recording in spectral range 530-890 nm. It has been found that the irradiation causes changes in the absorption spectrum of the cells in two regions, near 754-795 nm (maxima at 757, 775, and 795 nm) and near 812-873 nm (maxima at 819, 837, 858, and 873 nm). Changes occur in band parameters (peak positions, width, and integral intensity). Virtually no changes occur in the red spectral region and a few changes are recorded in the green region near 556-565 nm. The results obtained evidence that cytochrome c oxidase becomes more oxidized (which means that the oxidative metabolism is increased) due to irradiation at all wavelengths used. L. Wilden **(**2000) revealed that with regard to radiation phenomena and its enhanced electron flow in the cellular energy transfer (respiratory chain) it is possible to explain the experimentally found increase of ATP-production by means of low level laser light on a cellular level.

Laser treatment 633 nm of 5 J/cm^2 was administered three times a week. Laser treatment in the diabetic burn model gave the best healing acceleration. It has been shown that light energy is absorbed by endogenous chromophores in the mitochondria and used to synthesize ATP. The resulting ATP is then used to power metabolic processes; synthesize DNA, RNA, proteins, enzymes, and other biological materials needed to repair or regenerate cell and tissue components; foster mitosis or cell proliferation; and restore homeostasis. The clinical benefits resulting from these demonstrated effects are pain control and tissue repair in the multitude of circumstances (Bill, 2004).

Laser irradiation was carried out with diode lasers with the following wavelengths: 670 nm, 780 nm, 692 nm, and 786 nm. The fluency was fixed in 2 J/cm^2. The Low level laser therapy (LLLT) acts by improving the in vitro fibroblast proliferation and a smaller laser exposure time results in higher proliferation (Almeida-Lopes, 2001), (Vinck et al 2003).In other study the

physical parameters of the output of the laser were as follow wavelength, 830 nm; average power output, 30 mW; spot size, 0.1 cm2; irradiance, 300 mW/cm2; continuous wave output. It would seem that LILT provides no advantages in the management of minor postoperative wounds over current practice. (Lagan et al 2001)

Wister male rats, which were irradiated with laser light (wavelength 590 nm and intensity of 50 mW/cm2 for 5 minutes after 70% hepatectomy.They conclude that the laser promotes a biostimulation effect on the early stages of liver regeneration without any detectable damage of the cells.(De Castro et al 2001)

Clinical studies were performed on 97 complicated operative wounds with partial dehiscence, involving the skin and the subcutaneous connective tissue. As a result of helium-neon laser therapy with power density 90 mW/cm2 and 70 mW/cm2 healing of these complicated operative wounds occurred in 5 to 18 days (after 5 to 18 irradiations). The studies showed that laser therapy dose (power density) of 90 mW/cm2 stimulated more effectively the tissue repair processes at exposure time of 1.5 min per field. (Burgudzhieva, 1989).

In a study was to evaluate a 980-nm gallium-aluminum-arsenide diode laser for wound healing. Using genetically diabetic and nondiabetic mice, laser treatment at different flunce and frequency of treatment: 5 W (18 J/cm^2) every 2 days, 5 W (18 J/cm^2) every 4 days, 10 W (36 J/cm^2) every 2 days, and 10 W (36 J/cm^2) every 4 days. For diabetic mice receiving 5 W every 2 days, the percentage of wounds healed after 19 days was 100% versus 40% in the control group. For nondiabetic mice, 100% of the wounds in the 5 W every 4 days and control subgroups were completely healed, whereas 90% of the wounds from the 5 W every 2 days and the 10 W every 4 days subgroups were completely healed, but the 10 W every 2 days subgroup had average closure of >90% (Jill S 2004) .

Under the influence of low-energy laser radiation was studied in albino rats by a complex of methods. High phagocytic activity of macrophages was observed as early as 6 h after trauma. It resulted in rapid debridement of the wound and prepared conditions for the proliferation phase. At later terms (5 and 10 days) the reaction to laser irradiation manifested itself as a powerful vascularization of the wound and increased amount of secreting fibroblasts (Petrova, 1992).

Wounds in experimental diabetes using a rat model. The wound of each animal was treated with a 632.8nm He:Ne laser at a dose of 1.0J/cm^2 for five days a week until the wounds closed (three weeks). Biochemical assays revealed that the amount of total collagen was significantly increased in laser treated wounds (Kesava et al 2001).

The wounds in the test group were irradiated twice a day with a combined HeNe (632nm, 5mW)-IR (904nm 68,8mW) laser. The results show a significant increase ($p<0.05$) of fibroblast proliferation. There is also a perfect match between number of fibroblasts and DNA activity. The adhesion of the scar with the underlying tissues disappeared after 10 days in the control group and after 4 days in the experimental group. The local edema disappeared in the test group after 8 days, while in the control group it lasted until 10 days. A considerable acceleration of the regeneration of both vein and lymph vessel was seen in the test group (Lievens et al 2000). LLLT is useful in treating swelling and edema in acute-phase injury and in accelerating healing of surgical wounds. (Yoshimi et al 2004)

Chukuka (2004) revealed that when healing is impaired, the tissues respond positively to appropriate doses of light, especially light wavelengths within the 600 to 1000 nm ranges. The exact fluency necessary to achieve optimal healing continues to be explored for each tissue, but there is a growing consensus that accelerated healing can be accomplished with doses ranging from 1 to 6 J/cm^2. The amount of light absorbed by each tissue differs, even when wavelength is kept constant. Therefore, the energy flounce needed to optimize healing will differ among tissues.

3- Conclusions

1. The diode laser is good therapy for enhancement of tissue regeneration and burn healing.
2. The best parameter of diode laser (790 nm) enhancing burn healing was Power density 0.255 W /cm² with exposure time 20 second and Power density 0.0589 W /cm² with exposure time 60 second.
3. Increase the Power density 0.888 W /cm² with exposure time 20 didn’t show any improving to the burn wound healing.

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