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Possible Role of Low level laser therapy on sciatic nerve regeneration in white male Albino rat ; experimental model ; Review article

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Abstract: Background: the use of low-power lasers (as opposed to high-power lasers that can destroy tissue by a photothermal effect) has steadily increased in diverse areas of medical practice that require healing, prevention of tissue death, pain relief, reduction of inflammation, and regenerative medicine. Nevertheless, this modality, which is variously known as LLLT or photobiomodulation, remains controversial. The reasons for this lack of general acceptance among both the medical community and the general public at large are 2-fold. First, widespread uncertainty and confusion exists about the mechanisms of action of LLLT at the molecular, cellular, and tissue levels. Many of the most compelling applications of LLLT are in the field of neurology (both central and peripheral). Many serious brain diseases and injuries can be successfully treated with noninvasive transcranial laser therapy. Furthermore, in the peripheral nervous system, LLLT can be used effectively for nerve regeneration and pain relief. Experimental studies that were conducted during World War II by Gutmann and Young indicated that the rate of outgrowth and axon numbers were not affected by delayed nerve repair. These findings, with evidence of extreme atrophy of the denervated muscles, led to the erroneous conclusion that poor functional recovery after nerve injury was due to irreversible denervation atrophy of muscle and its inability to accept innervation, especially after long periods of time. Recent studies showed that low-level laser therapy (LLLT) accelerates the regeneration process of injured peripheral nerve tissue.

Keywords: *Low level laser , nerve regeneration*

Introduction

The use of low-power lasers (as opposed to high-power lasers that can destroy tissue by a photothermal effect) has steadily increased in diverse areas of medical practice that require healing, prevention of tissue death, pain relief, reduction of inflammation, and regenerative medicine **(1).**

Nevertheless, this modality, which is variously known as LLLT or photobiomodulation, remains controversial. The reasons for this lack of general acceptance among both the medical community and the general public at large are 2-fold. First, widespread uncertainty and confusion exists about the mechanisms of action of LLLT at the molecular, cellular, and tissue levels **(2).**

Second, a large number of parameters (e.g., wavelength, fluence, irradiance, treatment timing and repetition, pulsing, and polarization) can be chosen in designing LLLT protocols. Furthermore, a biphasic dose response exists in laser therapy, which describes the observation that increasing the overall “dose” of the laser either by

increasing the power density or by increasing the illumination time may have a counter-productive effect compared with the benefit obtained with lower doses **(3).**

Taken together, these considerations may explain why a number of negative studies have been published; however, this should not be taken to imply that LLLT in general does not work but rather that the laser parameters used in those particular studies were ineffective **(4).**

In recent years, the development of light-emitting diodes (LEDs) as alternative light sources for LLLT has added to the confusion. These devices produce light with wavelengths similar to those of lasers, but they have broader output peaks (i.e., they are less monochromatic) and lack the coherence that is a particular feature of laser light **(5).**

LEDs have the advantage of being significantly less expensive than laser diodes (by a factor of approximately 100 on a milliwatt basis), and the LLLT community is engaged in a vigorous ongoing debate about their respective benefits **(6).**

Many of the most compelling applications of LLLT are in the field of neurology (both central and peripheral). Many serious brain diseases and injuries can be successfully treated with noninvasive transcranial laser therapy. Furthermore, in the peripheral nervous system, LLLT can be used effectively for nerve regeneration and pain relief **(7).**

Mechanism of action of LLLT:

LLLT is a stream of the same photons as in other light sources but has only one energy (or wavelength). Therefore, the effect of laser light on living patterns seems to obey the laws of classical photobiology, however, laser-induced bioeffects are fundamentally different from the known responses **(1).**

Photobiological processes can be schematically represented in the following sequence: acceptors, absorption spectrum of which coincides with incident light wavelength, absorbed photons then activate and trigger biochemical or physiological responses that are typical (specific) for these absorbing elements **(8).**

Considering laser-induced bioeffects, it seems that there are no specific acceptors and responses of biological system (cell, organ, organism), interaction is entirely non-specific. To demonstrate existing differences in processes (responses) that occur as a result of absorption of laser (monochromatic) and ordinary light let us consider as an example a special case of photobiology – photosynthesis, in which activation of photosynthetic pigments by light starts the process of formation of organic substances from carbon dioxide and water **(9).**

All absorbing acceptors, providing photosynthesis, "work" only in visible part of the spectrum that is 400-700 nm. This is so-called action spectrum, i.e. wavelength range in which the effect is observed. For other spectral ranges, light energy absorption by acceptors is excluded. Will photosynthesis occur when plants are irradiated by infra-red (IR) lamp? No **(3).**

The second response is continuously growing with saturation, result depending on magnitude of absorbed energy, - the more sunlight there is, the more active photosynthesis is and the more biomass there is (if there are nutrients and water). If the intensity of light is reduced at 104-106 times, i.e. it is almost dark, will photosynthesis occur? Of course not! However, everything is different when LILI interacts with biological systems **(7).**

The first thing that surprises is lack of action spectrum in case of laser modulation. This statement requires explanation. Of course, depending on the chosen model of study, e.g. cell type or tissue, investigated effect, localization effects (in vitro or in vivo), "action spectrum" is observed, this is a fairly well-known fact **(2).**

There are a lot of similar studies, but they do not clarify the situation, but even confuse, because action spectra vary significantly depending on experimental conditions, which does not allow marking out only one specific acting factor (acceptor). If we consider the issue in detail, we can find out the following facts **(6).**

First, responses "wavelength – effect" are found only for some parameters of one experiment model. If we consider an integral response of a biological system at laser impact, it becomes clear that absorption by a whole cell is important but not a selective absorption of any component of a living cell, which everyone is

unsuccessfully searching for many years. It is not so important, what kind of intracellular structure absorbs, but definitely the fact that absorption is required **(4).**

Well-known works show significantly different action spectra for some of intermediate stages of culture HeLa cell cycle: DNA and RNA synthesis, adhesion, but their united spectra characterizing the whole division process, corresponds to the absorption of a cell as a whole **(9).**

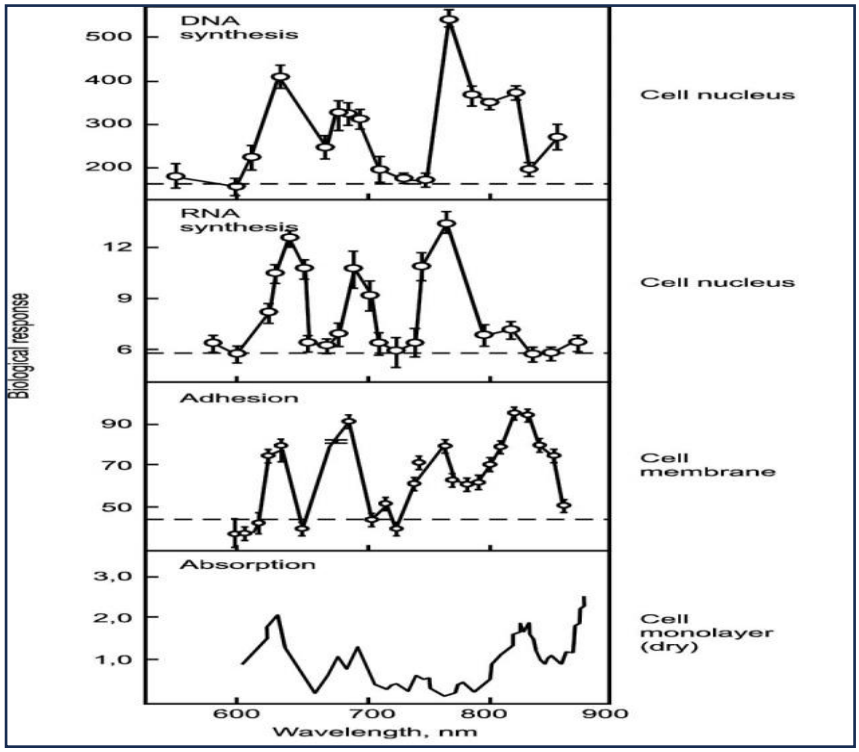

Figure (1): Action Spectra for Different Biological Effects, Hela Cell, Optimal Energy Density – 0.01 J/cm^2 (3).

However, it is impossible to conclude on the basis of the received spectra (614-624, 668-684, 751-772 and 813-846 nm) that there is a universal cellular mechanism of biomodelling action of LILI. Moreover, these spectra cannot be recommended for clinical practice because clinical effectiveness of LLLT with such parameters is not confirmed **(8).**

Secondly, analysis of literature has shown that in experimental and clinical studies, successful (to a variable degree) results of biomodulatory LLLT effect are obtained at more than 100 wavelengths in the range of 248 to 10600 nm. If optimal space and energy LILI parameters are ensured, the effect can be achieved to some degree in any experiment with a laser source in all spectral ranges. Besides, the more complex a biological system is, the easier it is to get a positive response to laser irradiation **(5).**

It is absolutely impossible that action spectrum is absent in photobiology, e.g. photosynthesis occurs only in visible spectrum (400-700 nm). But laser light successfully provokes similar effects, such as increase of

biomass or increase of cell proliferation for different cells and in a very wide wavelength range that matches the absorption spectrum of cells or tissues **(9).**

Since molecules and molecular complexes have quite a narrow absorption band, each of them cannot act as the only absorbing element (acceptor), but rather absorb all in aggregate, also water, as we know, has no transmission windows **(7).**

This is what lack of a specific action spectrum is and we can explain this fact only by the thermodynamic nature of LILI interaction with a living cell, when effecting on absorbing centers, temperature gradient causes a trigger launch of the physiological regulation system, which are, as we assume, intracellular calcium stores that can release Ca2+ under the influence of many external factors **(1).**

Energy responses are even more surprising than spectral regularities. Let us review some basic concepts and fundamentals, laser therapy "axioms." For an effective impact of LILI, it is necessary to provide optimum power and PD, i.e. distribution of light energy over the area of cells in vitro and area and/or volume of tissues in animal experiments and clinical settings **(8).**

Exposure time to one area is important, which needs to be strictly in the range of 100-300 s (1.5-5 minutes), except for methods like acupuncture (20-40 seconds) and intravenous laser irradiation of blood (up to 20 minutes). As a result, we receive PD per time unit or energy density (ED). For pulsed lasers (pulse duration of 100-150 ns), when frequency increases, the average power increases proportionally, that is ED effect **(3).**

The most well-known energy response of biomodulating LILI action is the presence of optimum dependencies "ED-effect," sometimes called "biphasic." Similar effect is certainly not observed in photobiology. The most important fact is that increase of Ca2+ concentration occurs only from intracellular stores (where it is again deposited after physiological cycle in 5-6 minutes), and not due to ions entering from outside, as many scientists consider **(9).**

First, there is no correlation between ATP level in cells and outside transport of Ca2+ into a cell, mitochondrial activation is performed only by increasing Ca2+concentration from intracellular stores. Secondly, removal of calcium ions from serum does not delay increase of Ca2+ concentration in cell cycle anaphase, i.e. activation of cell proliferation by LILI action is absolutely disconnected with extracellular calcium, membranes, specifically dependent pumps and etc. **(2).**

All these responses can be easily explained if we represent mechanisms of laser BMA in the following sequence: as a result of LILI a temperature gradient occur inside a cell and there is a short-time increase in concentration of calcium ions (Ca2+) released from intracellular stores, with development of cascade of organism responses to an external effect: work of immune and vascular systems normalizes, metabolic and proliferative processes are activated, there is analgesic effect and etc. **(6).**

All biological effects are Ca2+-dependent, nonlinear response "ED–effect" and "exposure–effect" can be explained by peculiarities of intracellular calcium stores work, lack of spectrum can be explained by its nonspecific inclusion **(4).**

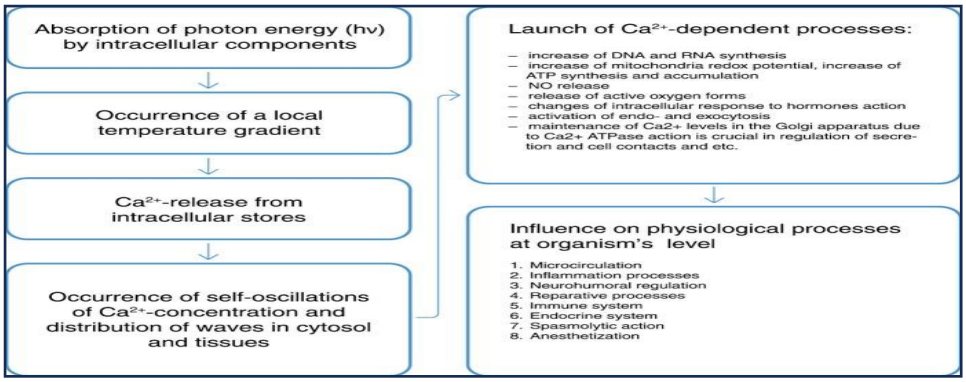

Figure (2): Mechanisms of Biological and Therapeutic Effect of LLLT (3).

Cellular and molecular effects of LLLT:

LLLT uses low-powered laser light in the range of 1-1000 mW, at wavelengths from 632-1064 nm, to stimulate a biological response. These lasers emit no heat, sound, or vibration. Instead of generating a thermal effect, LLLT acts by inducing a photochemical reaction in the cell, a process referred to as biostimulation or photobiomodulation **(5).**

The best outcome of neurotization can be expected in short denervation times and in the presence of proximal healthy roots **(5).**

Photo- biology works on the principle that, when light hits certain molecules called chromophores, the photon energy causes electrons to be excited and jump from low-energy orbits to higher-energy orbits. In nature, this stored energy can be used by the system to perform various cellular tasks, such as photosynthesis and photomorphogenesis **(7).**

Numerous examples of chromophores exist in nature, such as chlorophyll in plants, bacteriochlorophyll in blue-green algae, flavoproteins, and hemoglobin found in red blood cells. The respective colors of chromophores are determined by the part of the spectrum of light they absorb chlorophyll is green, flavoprotein is yellow, and hemoglobin is red **(8).**

Mitochondria are considered the power generators of the eukaryotic cell, converting oxygen and nutrients through the oxidative phosphorylation process and electron transport chain into adenosine triphosphate (ATP) **(6).**

The basic idea behind cellular respiration is that high-energy electrons are passed from electron carriers, such as reduced nicotinamide adenine dinucleotide (NADH) and the reduced form of flavin adenine dinucleotide (FADH2), through a series of transmembrane complexes (including cytochrome c oxidase [CCO]) to the final electron acceptor, generating a proton gradient **(3).**

The gradient is used by FOF1 ATP synthase to produce ATP. Various in vitro experiments, such as those that use rat liver isolates, found that cellular respiration was upregulated when mitochondria were exposed to an HeNe laser or other forms of illumination **(9).**

Laser irradiation caused an increase in mitochondrial products (such as ATP, NADH, protein, ribonucleic acid [RNA]) and a reciprocal augmentation in oxygen consumption. A similar effect is produced when tissue that contains mitochondria is exposed to low-level radiation. Visible and near- infrared (NIR) light is absorbed by the organelle, and an upregulation of cellular respiration is observed **(9).**

Once it was observed that LLLT's mechanism of action is at the level of the mitochondria, it remained to be determined what specific structure within the mitochondria acted as the chromophore. Four membrane-bound complexes have been identified in mitochondria, each constituting an extremely complex transmembrane structure embedded in the inner membrane **(1).**

Complex IV, also known as CCO, is a large transmembrane protein complex found in mitochondria, which is a component of the respiratory electron transport chain. CCO appears to absorb the same spectrum of light as that observed for the action spectra for the biological response to light in the NIR range **(9).**

Thus, it is reasonable to assume that CCO acts as an important chromophore in LLLT. CCO consists of 2 copper centers and 2 heme-iron centers that are capable of absorbing light over a wide range, including NIR. The next reasonable question to consider is: What action does CCO modulate once it absorbs the energy from light? On the cellular level, LLLT may cause photodissociation of nitric oxide (NO) from CCO **(2).**

In a stressed cell, NO produced by mitochondrial NO synthase displaces oxygen from CCO, which results in a downregulation of cellular respiration and a subsequent decrease in the production of energy-storing compounds, such as ATP. By dissociating NO from CCO, LLLT prevents the displacement of oxygen from CCO and thereby promotes unhindered cellular respiration **(5).**

Increased CCO enzyme activity can be measured; increased ATP production and increased electron transport also have been reported. The basic idea behind cellular respiration is that high-energy electrons are passed from electron carriers, such as NADH and FADH2, through a series of transmembrane complexes (including CCO) to the final electron acceptor **(4).**

Increased cellular ATP produced by LLLT may contribute to the positive effects, both by raising cellular energy levels and by upregulating the cyclic AMP molecule (biochemically formed from ATP) that is involved in many signaling pathways **(7).**

Oxygen acts as the final electron acceptor and is, in the process, converted to water. Part of the oxygen that is metabolized produces reactive oxygen species (ROS) as a natural by-product. ROS (e.g., superoxide and hydrogen peroxide) are chemically active molecules that play an important role in cell signaling, regulation of cell cycle progression, enzyme activation, and nucleic acid and protein synthesis **(5).**

Because LLLT promotes the metabolism of oxygen, it also acts to increase ROS production. In turn, ROS activates certain redox-sensitive transcription factors such as nuclear factor-nB [NF-nB] and activator protein 1, which leads to the upregulation of various stimulatory and protective genes. The ultimate effect of LLLT is likely to be produced by transcription factor activation, which modulates the host's downstream cellular and tissue responses **(6).**

Almost certainly, other mechanisms through which LLLT produces its effects are at play in addition to the one just described. For example, NO is a potent vasodilator via its effect on cyclic guanine monophosphate production. Cyclic guanine monophosphate is also involved in many other signaling pathways **(8).**

LLLT may cause the photodissociation of NO from intracellular stores (i.e., nitrosylated forms of both hemoglobin and myoglobin, in addition to CCO). LLLT promotes the synthesis of deoxyribonucleic acid (DNA) and RNA and increases the production of proteins **(9).**

LLLT also modulates enzymatic activity, affects intracellular and extracellular pH, and accelerates cell metabolism. The expression of multiple genes related to cellular proliferation, migration, and the production of cytokines and growth factors also have been shown to be stimulated by low-level light **(2).**

Light is a powerful force and has a myriad of effects. The specific mechanisms of action may vary among various clinical applications of LLL and will be discussed in the respective sections below. Furthermore, in spite of a great number of studies that explored how LLLT works, the exact mechanism of action remains to be fully elucidated **(9).**

LLLT for peripheral nerves regeneration:

The use of new therapeutic instruments such as electric stimulation, ultrasound, and LLLT for peripheral nervous system regeneration is currently being investigated in an attempt to achieve early functional recovery. LLLT has been used in several clinical and experimental research studies on peripheral nerves injuries **(3).**

Several authors showed that postoperative 780-nm laser phototherapy enhances the regenerative process of the peripheral nerve after reconnection of the nerve defect by using a PGA neurotube. Morphologically, the laser-treated subjects showed an increased total number of myelinated axons **(8).**

These researchers also reported that, in patients with long-term peripheral nerve injury, 780-nm laser therapy (250 mW) can progressively improve nerve motor function, which leads to significant functional recovery. In addition, pulsed (905 nm) continuous (808 nm) combined laser biostimulation can achieve the best effectiveness in promoting peripheral nerve regeneration **(1).**

Requirements for protocols of laser therapy methods:

These protocol requirements are obligatory, as it is proved that all parameters of listed below methods must be set. If even one of the parameters is incorrect, predictable and appropriate response to laser light and desired therapeutic effect cannot be achieved **(7).**

Moreover, in most cases minimum LILI energy is required for successful laser therapy techniques, and increase in power and exposure (energy) leads to inhibitory effect, this is a well-known fact **(4).**

All laser therapy techniques must contain the following information:

1. Wavelength of laser light is measured in nanometers [nm]. It is not allowed to shine simultaneously laser and/or non-coherent light sources with different wavelength on one area due to inhibitory interaction. The most common wavelengths in laser therapy are:
   - 365-405 nm – ultraviolet (UV) spectrum,
   - 440-445 nm – blue spectrum,
   - 520-525 nm – green spectrum,
   - 635 nm – red spectrum,
   - 780-785 nm – infrared (IR) spectrum,
   - 890-904 nm – IR spectrum.
2. Mode of laser operation: continuous, modulated, pulsed.
3. Radiation power of LILI.

Average power of continuous lasers operating in continuous or modulated modes is measured in milliwatts (mW), impulse (peak) power of pulsed lasers is measured in watts (W).

4. Modulation frequency or pulse repetition frequency for a pulsed mode - number of oscillations (pulses) per time unit (second) is measured in hertz (Hz, 1/s).
5. An important parameter of pulsed lasers is duration of light pulse, which is a constant (usually only 100-150 ns). Average power of pulsed lasers (Pav) is directly proportional to pulsed power (Pp), pulse duration (τp) and frequency (Fp): $Pav = Pp \times \tau p \times Fp$.
6. Illumination area is measured in square centimeters (cm^2).

Required area is almost always ensured by a technique without unnecessary measurements, e.g. in a contact-mirror technique area is supposed to be 1 cm^2. Laser diodes in matrix emitters must be positioned so that impact area is multiplied by PD **(6).**

For example, 8 (most often) pulsed laser diodes, 10 W each are placed on a surface area of 8 cm^2 and skin contact PD is 10 W/cm^2 accordingly. During laser acupuncture or intravenous laser irradiation of blood (ILIB) area is not specified, because impact area is too small, and diffusion and absorption of laser light in volume of biological tissues is primary **(9).**

7. PD is measured in watts or milliwatts per square centimeter (W/cm^2 or mW/cm^2).
8. Exposure (exposure time) on one area (zone) and total time of a treatment is measured in seconds (s) or minutes (min). Total time of laser therapy treatment (consistent effect on all areas) should not exceed 20 minutes, for one area – 5 minutes (except for intravenous laser irradiation of blood). This is a very important parameter that almost never can be changed.
9. Localization of impact (technique).
10. Number of procedures for the course and its periodicity **(2).**

Energy calculations, measured in joules (J or W×s) and ED (J/cm2or $W \times s/cm^2$) are not carried out, because this information is not necessary for effective laser therapy. One of the general effect methods (laser acupuncture or ILIB) can be included into a laser therapy scheme as well as direct impact method on the affected area (local, transdermal or abdominal techniques, and also combined method – laser phoresis) **(5).**

Local LILI exposure is carried out directly on the affected area, close to the body surface, or contact through a mirror head, or distantly, at a small distance from the surface (1-2 cm), stable. Sometimes associated

physiotherapy method - magnetic laser therapy (MLT) is used, effecting through an opening of a permanent magnet with induction of 35-50 mT **(9).**

These types of LILI are most often used for local laser exposure:

- continuous LILI of red spectrum (635 nm), PD – 10-15 mW/cm^2,
- pulsed LILI of red spectrum (635 nm), PD – 4-5 W/cm^2, pulse duration of 100-150 ns, frequency 80-10000 Hz
- pulsed infrared LILI (890-904 nm), PD – 8-10 W/cm^2, pulse duration of 100-150 ns, frequency 80-10000 Hz **(3).**

Frequency for pulsed laser varies depending on desired effect: for regeneration and anti-inflammatory effect it is 80-150 Hz, analgesia - 3000-10000 Hz. There are up to 2-3 local zones for one area, exposure to each is 2-5 minutes. A more than 5-minute exposure is not allowed **(9).**

Local LILI exposure on skin in projection of affected organ differs from surface illumination, because only pulsed infrared lasers are used, preferably matrix, providing a therapeutic effect to a depth of 15 cm: wavelength – 890-904 nm, PD – 8-10 W/cm^2, pulse duration – 100-150 ns, frequency – 80-10000 Hz **(7).**

When the frequency of pulsed lasers is increased, the average irradiation power increases proportionately, that allows to effect on deeper areas. There are up to 2-3 local zones for one area, exposure to each is 2-5 minutes. A more than 5-minute exposure is not allowed **(4).**

Laser acupuncture is performed by a special acupuncture head designed to concentrate laser beam energy to an area 1-2 mm in diameter. Wavelength is 635 nm (red spectrum), modes are continuous or modulated, output power is 2-3 mW, exposure to one corporal acupuncture point is 20-40 seconds, on auricular – 5-10 seconds **(6).**

Laser irradiation of blood has two techniques as options: intravenous or non-invasive (over venous, external, transdermal, transcutaneous). These are accordingly ILIB and non-invasive laser irradiation of blood (NLIB). A continuous mode is always used for ILIB, exposure is carried out intravenously through special disposable sterile light guides with paracentetic needle **(1).**

Different techniques using laser light of different spectrum that are applied for ILIB are:

- ILIB-635 (wavelength – 635 nm, red spectrum, power – 1.5-2 mW, exposure – 10-20 minutes) has a universal effect, a positive effect on immune system and trophic provision of tissue.
- ILIB-525 (wavelength – 525 nm, green spectrum, power – 1.5-2 mW, exposure – 7-8 minutes) is recommended for maximum enhancement of trophic provision of tissue.
- UV laser irradiation of blood (ULIB, wavelength – 365 nm (or 405 nm), power – 1.5-2 mW, exposure – 3-5 min) is preferable for correction of immune disorders as a result of illness or injury **(9).**

NLIB is carried out on large blood vessels (arteries or veins), close to lesion focus. Pulsed lasers are mostly used, preferably of red (635 nm) or infrared (890-904 nm) spectrum and matrix (8 laser diode) emitters, or, as an option, with a single laser and mirror head:

- pulsed LILI of red spectrum (635 nm), PD – 4-5 W/cm2, pulse duration – 100-150 ns, frequency – 80 Hz.
- pulsed infrared LILI (890-904 nm), PD – 8-10 W/cm2, pulse duration – 100-150 ns, frequency – 80 Hz **(3).**

Frequency is fixed. Impact on symmetric zones is possible, exposure to each is 2-5 minutes. A more than 5-minute exposure is not allowed. Laser phoresis is one of modern physico-pharmacological methods of combined effects of percutaneous LILI and medicine **(5).**

As a result of LILI on the area where a biologically active substance (gel or aqueous solution) was preliminary applied, activation of its penetration through skin (pores and hair follicles) occurs. Such transcutaneous non-injectional way of bringing the substance is only possible for low molecular (500 kDa) and hydrophilic compounds **(7).**

All presented LILI techniques are widely used worldwide for treatment of musculoskeletal, nervous and cardiovascular systems diseases, diseases of ear, nose and throat, after injuries and surgery, in dermatology and cosmetology, in obstetrics and gynecology, urology and andrology, pulmonology and phthisiology, i.e. in almost all areas of modern clinical medicine **(2).**

Associated and combined laser therapy methods are being actively developed: magnetic laser therapy, laser phoresis, laser-vacuum massage, etc. The concepts of combining LILI with taking medicine of different groups were worked out, that helps to reduce medicamental doses and risk of antibiotic resistance, to increase the effectiveness of the treatment **(1).**

Nerve injuries trigger complex cell-molecular interactions that are essential for axonal regeneration and, subsequently, for meaningful functional recovery for patients. These cellular and molecular responses of the nonneuronal cells of the peripheral nervous system (PNS), namely Schwann cells (SCs), create a growth-permissive environment for injured axons contrary to the central nervous system (CNS) response to injuries ***(10).***

However, there is a dichotomy between the capacity of injured PNS neurons to regenerate their axons after injury and suboptimal return of function after nerve injuries. Significant advancements have been made in the technique of microsurgery of injured nerves that sometimes results in improved outcome for patients **(11).**

Unfortunately, functional recovery is frequently still poor after peripheral nerve injuries, especially when nerve trunks close to the spinal cord and far from the target organs are injured. These injuries include most avulsion-type injuries, nerve lacerations, and nerve contusions. After these types of nerve injuries, injured neurons are required to regenerate their axons over long distances at the very slow rate of 1 mm/d **(12).**

At this sluggish rate of regeneration, reestablishment of a functional motor unit or sense organ reinnervation may take months or even years, a condition referred to as chronic axotomy. Likewise, SCs in the distal nerve stumps and the target organs remain denervated for long periods, conditions known as chronic SC denervation and chronic muscle denervation, respectively **(13).**

Regenerative response after nerve injury and its role in functional outcome:

- Initial phase of axonal regeneration:

After nerve injury, the proximal and distal stumps of the injured nerve undergo structural and molecular changes in preparation for the process of axonal regeneration. The proximal stump undergoes die back degeneration up to the first node of Ranvier and then each injured axon elaborates multiple daughter axons **(14).**

Many of the daughter axons are pruned; those that remain begin the process of elongation through the distal nerve stumps and constitute regenerating units. This initial stage of axonal regeneration is sustained by both the availability of locally produced cytoskeletal materials and the neuronally produced and anterogradely transported cytoskeletal proteins such as actin and tubulin **(13).**

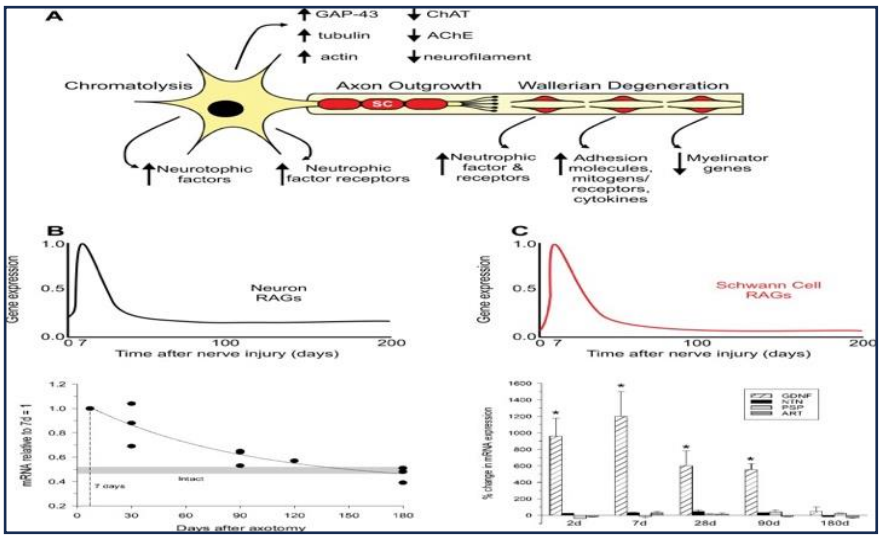

Figure (3): After nerve injury, regeneration-associated genes (RAGs) are upregulated transiently in the neurons while genes associated with normal synaptic transmission are downregulated (A and B). Schwann cells in the denervated nerve stumps undergo proliferation during Wallerian degeneration and express many RAGs while myelin-associated genes are downregulated (A). The gene profiles support the outgrowth of axons, but the expression is very short lived such that over time (while regenerating axons grow at a slow rate of 1 mm/d) the expression of RAGs is downregulated and the capacity of injured neurons to regenerate their axons and Schwann cells to support regeneration is diminished (C). Examples of progressive decline in mRNA levels are plotted for tubulin in neurons and GDNF in Schwann cells in the graphs **(15).**

Axon regeneration proceeds at a rate of 1-3 mm/d, the rate corresponding with the slow rate of transport of the cytoskeletal materials. Further elongation and regeneration through the distal nerve stump are dependent on the growth-supportive milieu provided by the SCs of the distal nerve stumps. Lack of Schwann cell–laden endoneurial channels (bands of Büngner) results in misdirected regeneration and formation of neuromas **(11).**

Role of schwann cells in axonal regeneration:

The distal nerve stumps of severed nerves undergo a degenerative process named after Augustus Waller, Wallerian degeneration. Wallerian degeneration is an essential preparatory stage of the process of axonal regeneration via which molecules that could be inhibitory to regeneration are eliminated **(12).**

Recent reviewes break the events into three morphologically discernible stages: acute axonal degeneration, a latency period, and an abrupt granular degenerative stage. Within an hour of injury, die back in both the proximal and distal stumps is mediated by channel-mediated calcium influx and activation of calpains that cleave neurofilament and microtubular-associated components such as spectrin and tubulin ***(10).***

In the proximal stump, this die back is to the first node of Ranvier. The next stage of approximately 24 hours, during which time axon potentials continue to be conducted, is followed by a longer period of disassembly of cytoskeletal proteins by calcium-activated calpains and the ubiquitin-proteasome system **(12).**

SCs play a major role in this process by way of phagocytosis of the axonal and myelin debris. They also secrete chemoattractive factors such as interleukin-1 and monocyte chemoattractant protein-1 that recruit macrophages into the denervated distal nerve stumps which contribute significantly to the phagocytosis of axon and myelin debris **(16).**

The axon debris releases mitogens that promote mitotic SC division and initiates a network of cytokines and transcription factors that stimulates myelin breakdown and macrophage invasion and targets the myelin for phagocytosis by the SCs and macrophages **(11).**

- Role of regeneration-associated genes:

Immediately after a nerve is injured, loss of axonal contact triggers the SCs to proliferate and switch their phenotype from a myelinating to a nonmyelinating growth-supportive phenotype. The messenger RNA (mRNA) expression of myelin-associated proteins such as P0 and myelin-associated glycoprotein are downregulated **(14).**

Neurotrophins (e.g., nerve growth factor, brain-derived neurotrophic factor, and glial-derived neurotrophic factor), their receptors (e.g., p75, GFRA-1, GFRA-2), and adhesion molecules (e.g., neural-cell adhesion molecule) are upregulated in preparation for the process of axonal regeneration **(13).**

These upregulated genes are collectively called regeneration-associated genes (RAGs). The change in the gene expression and the myelin and axonal degeneration and clearance are key features of the process of Wallerian degeneration **(11).**

Likewise, neurons whose nerves have been injured downregulate mRNAs of proteins required for neurotransmission and upregulate those for rebuilding their peripheral processes. Hence, actin, tubulin, and GAP-43 are upregulated immediately after injury **(12).**

However, the upregulation of RAGs is not sustained in either the injured neurons or the SCs. By 6 months in experimental animals, most of the upregulated mRNAs are downregulated, thereby losing the growth-supportive environment for regenerating axons **(17).**

The implication of the time-limited upregulation of RAGs is demonstrated in the progressive decline in the capacities of injured neurons to regenerate their axons and of SCs to support regenerating axons as the duration of nerve repair is prolonged ***(10).***

Assessment of axonal regeneration after nerve injury and microsurgical repair:

Experimental studies that were conducted during World War II by Gutmann and Young indicated that the rate of outgrowth and axon numbers were not affected by delayed nerve repair. These findings, with evidence of extreme atrophy of the denervated muscles, led to the erroneous conclusion that poor functional recovery after nerve injury was due to irreversible denervation atrophy of muscle and its inability to accept innervation, especially after long periods of time **(15).**

This conclusion became quite popular and unfortunately is often repeated even in recent publications. Recent evidence from our laboratory and others disapproved the erroneous conclusion mainly because (1) the authors did not directly estimate the numbers of injured neurons that regenerated into the distal nerve stump and those that reinnervated denervated muscles and (2) counting nerve fibers in the distal nerve stump overestimates the numbers of regenerated nerve fibers because many daughter axons arise from single regenerating nerve fibers in the proximal nerve stump and may be erroneously counted as representative of the individual nerve fibers in the proximal stump **(15).**

In several experiments, many authors studied the process of axonal regeneration after immediate and delayed repairs of nerve injury. Further, quantitative methods of counting the motoneurons that regenerated their axons into distal nerve stumps and of counting the number of reinnervated motor units in the target muscles were used to assess the capacities of motoneurons to regenerate their axons and to reinnervate muscle **(12).**

Using a cross-suture technique in rats, which allows for independent study of the effects of delayed reinnervation of the distal nerve stump (termed chronic or prolonged denervation) and delayed neuronal regeneration to their targets (termed chronic or prolonged axotomy), the numbers of regenerated motoneurons were evaluated using the back-labeling technique whereby retrogradely transported fluorescent dyes are applied to the cut ends of reinnervated distal nerve stumps which label the motoneurons that regenerated their axons **(11).**

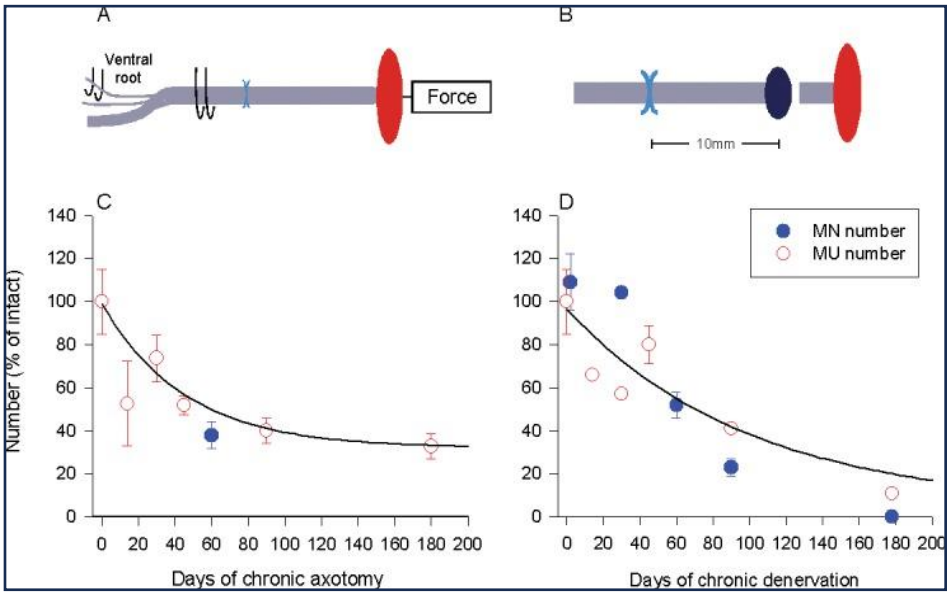

Figure (4): Regenerative capacity declines with time due to prolonged axotomy and Schwann cell denervation. In rats, we experimentally prolonged either (A) the duration of time during which motoneurons were prevented from regenerating their axons (chronic axotomy; by delaying the suture of the proximal nerve stump to a freshly denervated distal nerve stump) or (B) the denervation of Schwann cells in the distal nerve stumps (chronic denervation; by delaying the suture of a freshly cut proximal nerve stump to a nerve stump that was chronically denervated prior to nerve repair). The capacity of the neurons for regeneration after chronic axotomy of motoneurons or chronic denervation of Schwann cells and target muscles was determined either by (a) calculating the number of reinnervated muscle units (the nerve and the muscle fibers that the one motoneuron supplies) using force measurements in response to stimulation of single axons and the muscle nerve or (b) counting back labelling motoneurons that had regenerated their axons successfully by application of a retrograde dye to the regenerating axons in the nerve stump distal to the site of nerve repair. The evaluations of regenerative success obtained by the methods of motoneuron counts and counts of motor units were in good agreement demonstrating the progressive decline in regenerative capacity as a function of (C) chronic axotomy of the motoneurons and (D) chronic denervation of the Schwann cells **(15).**

This way, it is possible to enumerate the number of motoneurons that regenerated their axons in the ventral horn of the spinal cord after terminal experiments. The number of motor units can be estimated by applying supramaximal stimulation to individual ventral roots and measuring total muscle force **(11).**

Then many authors teased out ventral root fibers and stimulated them individually to determine motor unit force. The number of reinnervated motor units was then calculated by a division of muscle force by motor unit force **(14).**

Chronic schwann cell denervation:

The provision of the growth-supportive environment by SCs is intimately related to the loss and timely reestablishment of axonal contact with the cells. SCs whose reinnervation by regenerating axons is delayed are said to be chronically denervated and the deleterious effect of chronic denervation on SCs worsens as the duration of chronic denervation is prolonged **(13).**

In many experiments, to test the effect of chronic denervation, the common peroneal (CP) branch of the sciatic nerve is cut and its reinnervation is delayed for periods of 1-6 months prior to cross-suturing the proximal stump of a freshly cut tibial (TIB) nerve into the chronically denervated distal CP nerve **(16).**

This way, the effect of chronic CP denervation on the regeneration of freshly injured TIB motoneurons can be directly estimated. Using this paradigm, chronic denervation reduced the number of motoneurons that were back labeled with fluororuby dye that was applied to the distal nerve stump 10 mm from the cross-suture site to less than 10% of the number that regenerated after immediate suture of nerve stumps ***(10).***

There was excellent correspondence between this proportion of motoneurons that regenerated their axons into the chronically denervated nerve stump and the proportion of freshly axotomized motoneurons that regenerated and reinnervated the denervated muscle after 4 to 6 months **(18).**

The reduced number of motoneurons that did regenerate their axons through the chronically denervated SCs not only reinnervated the denervated muscle fibers but also reinnervated up to 3 times the number of muscle fibers that they normally do **(12).**

Since this enlargement of the reinnervated motor units constitutes the maximum sprouting capacity of the motoneurons, it follows that it is not the inability of the chronically denervated muscle fibers to accept reinnervation that limits functional recovery after chronic nerve injuries **(17).**

The sustained capacity of denervated muscle to accept reinnervation was also demonstrated by the parallel recovery of both muscle weight and isometric force. Nonetheless, isolation of the effects of SC denervation from muscle denervation showed that chronically denervated muscle fibers did not fully recover their former size, arguing that limited numbers of satellite cells are incorporated into each atrophic muscle fiber to recover muscle fiber cross-sectional area **(16).**

Hence, progressive deterioration of the growth-supportive capacity of SCs in the distal nerve stumps plays a primary role in poor functional recovery after nerve injury and the role of muscle atrophy is secondary. It was striking that the long-term chronically denervated SCs maintained their capacity to remyelinate the fewer axons that regenerated, particularly in view of the progressive regression of the capacity of the denervated SCs to sustain their growth-permissive phenotype and the progressive decline in numbers of growth-supportive SCs in the chronically denervated distal nerve stumps **(11).**

A clinical correlate of experimental cross-suture paradigm is the injuries to large nerve stumps in which SCs of the distal nerve stumps are left chronically denervated due to the slow rate of regenerating axons **(11).**

The longer it takes for regenerating axons to reinnervate SCs in the distal nerve stumps, the more prolonged the period of chronic denervation is during which the SCs do not have contact with axons and the higher the likelihood that their capacity to support regenerating axons is impaired. This is partly why reinnervation and subsequent functional recovery in muscle targets located close to regenerating motoneurons are better than in more distally placed muscle targets **(12).**

Chronic neuronal axotomy:

Injured neurons whose axons may or may not be regenerating but have not made target connections are said to be subjected to chronic axotomy. For example, after brachial plexus injury, the injured neurons have to regenerate over a long distance before they can reinnervate some of the denervated muscles and are thereby being subjected to chronic axotomy **(18).**

To test the effect of chronic axotomy, the cross-suture technique is used by using the 2 branches of rat sciatic nerve. The TIB branch of the sciatic nerve is cut, and its regeneration is delayed for periods of 1-6 months prior to cross-suturing the proximal stump of the chronically axotomized proximal stump of the TIB nerve into a freshly cut distal CP nerve ***(10).***

This way, the effect of chronic TIB axotomy on the reinnervation of a freshly injured distal CP nerve can be directly estimated. Using this paradigm, the number of motoneurons that regenerated their axons fell progressively as a function of prolonged time of chronic axotomy to ~37% of those that regenerated without the effect of chronic axotomy **(13).**

This reduced capacity to regenerate after chronic axotomy is significant, especially when combined with the deleterious effect of chronic denervation, both of which occur concurrently particularly after injuries to large nerve trunks such as the brachial plexus. The progressive decline in regenerative capacity of the chronically axotomized motoneurons was paralleled by a decline in the upregulated RAGs **(14).**

The dramatic negative effect of reduced regenerative capacity of the chronically axotomized motoneurons did not affect the ability of the neurons to reinnervate up to 3 times as many muscle fibers as normal, the fewer motoneurons enlarging their motor units and the number of muscle fibers supplied by each reinnervating motoneuron **(11).**

Thus, chronic axotomy impairs the regenerative capacity of motoneurons but does not impair the capacity of the smaller number of regenerated motor axons to make functional connections with denervated muscle fibers **(17).**

Staggered axon regeneration and misdirection of regenerating axons:

One of the key prerequisites for successful functional recovery is that regenerating axons regenerate into the correct endoneurial tubes that direct them back to their original target organs. However, regenerating axons encounter a significant delay at the injury site and traverse the injury site into the distal nerve stumps in a staggered fashion (staggered axonal regeneration) **(16).**

Indeed, the crossing of regenerating axons occurs slowly prior to the entry of the regenerating axons into the distal nerve stumps. It is only once the axons enter the distal stumps that they regenerate at the slow rate of transport of 1-3 mm/d **(18).**

Functional outcomes after axonal injuries are best after nerve crush injuries in which the endoneurium remains anatomically intact all the way to the target, and axonal sprouts of the regenerating unit are contained within the original endoneurium, so the regenerating axons are led back to their original targets **(12).**

With time, reinnervated muscle fibers display the normal mosaic distribution of muscle fiber types, motor unit and muscle isometric forces recover fully, the original number of functional motor units is restored, and the numbers and diameters of the myelinated nerve fibers return to normal. Nerve injuries that disrupt the endoneurial tubes (such as axonotmetic or neurotmetic injuries) whereby regenerating axons have to find their original endoneurial tubes introduces potential for errors during the regenerative process **(18).**

Axonal sprouts emanating from the proximal nerve stump may enter several different endoneurial tubes with target destinations that they did not formerly supply as in the case of motor axons that regenerate within pathways leading to the skin rather than muscle **(11).**

Experiments that applied retrograde dyes to regenerated axons of transected sciatic and facial nerves in rats directly demonstrated the misdirection of regenerating motor axons from several different motoneuron pools to muscles that the motoneurons did not formerly supply **(14).**

In cases where the nerves supplied muscles with antagonistic actions, axonal misdirection was associated with inappropriate movements, quite consistent with findings that flexor and extensor motoneurons sustain their normal but inappropriate pattern of firing when they are directed to inappropriately reinnervate extensor and flexor muscles, respectively ***(10).***

This misdirection of regenerating injured axons plays an important role in reducing functional recovery after nerve injuries. There are some exceptions to the misdirection of regenerating axons even after a transaction injury. These include several peripheral nerve trunks whose branches innervate skeletal muscles and sense organs in the skin and the joints **(13).**

Examples include the femoral nerve with two branches, the muscle branch containing motor and sensory nerves to the quadriceps muscle, and the other pure sensory saphenous nerve branch to the skin. Although reinnervation of motor and sensory nerve branches is initially random, the motoneurons that progressively regenerate their axons across the suture site send their axons into the appropriate motor branch **(11).**

This preferential motor reinnervation emerged in parallel with the progressive or staggered regeneration of motor axons into the distal nerve stumps. An unusual acidic glycan associated with myelin profiles of motor but not sensory mouse axons that is recognized by a monoclonal antibody L2/HNK-1 may be one mechanism of the preferential reinnervation of appropriate pathways **(12).**

The differential neurotrophic factor profile in SCs derived from sensory and motor axons with the BDNF/NT4/5 profile is also likely to play an important role in preferential reinnervation of motor pathways **(10).**

Introduction of the art of microsurgery in peripheral nerve problem and the establishment of the principle of tension free repair brought several new approaches to brachial plexus reconstruction, especially when dealing with supraclavicular lesions with multiple avulsions **(19).**

In developing countries studies revealedthat nerve injuries are most common in theupper extremities in both children (78.36%)and adults (63.54%). The common causes ofnerve injury in children were as follows:obstetric lesions (46.78%), iatrogenic lesions(16.95%), traffic accidents (15.7%), and sharp lacerations (12.8%), whereas the commonestcause of nerve injury in adults was due tosharp lacerations (27.57%), followed byiatrogenic lesions (25.67%), and trafficaccidents (23.77%) **(20).**

Recently, new promising ways of neurotization using only a part of the donor nerve have been published. Transfer of the triceps motor branches of the radial nerve to the axillary nerve was performed to restore deltoid

muscle function and appears to be safe and effective. The functional loss relative to the triceps, with a single nerve transferred, is negligible because of compensation by the remaining heads **(21).**

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