Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(1): 25-32. **OPEN ACCESS**

Low-energy Gallium Arsenide laser versus polarized light on healing of full-thickness wound in rats

Hesham Galal Mahran El-Sayed*[1], Ali Abd El-Monsif Thabet[1], Mohamed Mohamed Ibrahim Ali[1] and Mohamed Raafat Abdalla[2]

[1]Physiotherapy and Rehabilitation Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Mecca, **Saudi Arabia.**
[2]Department of Pharmacology & Toxicology, College of Pharmacy, Umm Al Qura University, Mecca, **Saudi Arabia.**

***Correspondence: heshammahran75@yahoo.com** **Received 01-01-2020, Revised: 19-01-2020, Accepted: 20-01-2020 e-Published: 30-01-2020**

Objective: To compare between Gallium Arsenide Laser and Polarized Light effects on Full-Thickness wound healing in rats Methods: Sixty mature male albino rats were included. While rats were under anesthesia, area of skin with five-centimeter square (2×2.5cm) was excised from all rats nape, and then rats were assigned directly into three groups equal in number; Group A) received low-intensity laser (GAlAs Laser, 830 nm), Group B) received polarized light (400–2000 nm). Irradiation and illumination starting immediately after surgery as wound irradiation by laser or wound illumination by polarized light occur at an equal distant point from each wound and both groups received total energy of 20 joules and at a rate of three times per week and for two weeks for both light techniques. Group C: received no treatment. Outcome measures: Wounded area and contraction rate measured after 7 days, and 14 days of treatment. Results: In both GAlAs Laser group and polarized light group, there was a better significant reduction of WSA along with better contraction rate than those in the control group, also, significantly, there was a difference between both light groups effects on wound recovery. Conclusion: Both GAlAs Laser and polarized light with the total received the energy of 20 joules have a facilitating effect on wound healing in addition, polarized light had a better wound healing effect than GAlAs laser.

Keywords: Wound, Rats, Laser, Polarized light

INTRODUCTION

The laser biological effects cannot belong to the heating effect as there is no thermal effect for a therapeutic laser on tissues (Basford, 1995). The magnitude of the laser effects based on the cells' physiological state or the clinical condition of the pre-irradiation state and this gives the evidence that the biomodulation effect may not always observable (Karu, 1989). Regarding therapeutic doses, many previous studies reported that therapeutic laser with low doses has positive effects on living cells (Van, 1992, Reddy et al.,1998). Controversy, a laser with higher doses has no positive effects on tissues (Van, 1992, In de Braekt et al.,1992, Pogrel et al., 1997, Sommer et al., 2001). The wavelength of the laser is characterized by monochromaticity and coherence, so laser has a highly focused intensity (Van, 1992, Pinheiro et al., 2000, Pinheiro et al., 2000 a,b). Studies reported that maximum cell proliferation occurs after 24 post-laser irradiation but as time increased while maintaining the same output power, cell stimulation decreased (Kreisler et al., 2002). in the same ratio, the amount of energy within the tissue will increase, so tissue wit larger volume will receive doses within the therapeutic range (Tuner and Hode, 2004).In a correct treatment protocol, tissue healing responds to the coherence of light, but no response to incoherent light (Nicola J and Nicola E, 2002, Rosner et al.,1993). In spite of the reduction of coherence with more tissue penetration by laser, there is enough coherence to induce a positive effect (Tuner and Hode, 2004). Light from alternative sources has been used to enhance healing of the wound, polarization is considered as laser light characteristic which a cause of the biomodulation, so, biomodulation of biological tissues may attribute to other sources of polarized light (Pogrel et al., 1997, Fenyo, 1984).

Polarized light therapy is a modern therapeutic approach, in which linear polarized polychrome light was utilized, in Polarized light, the beam is concentrated and focused beam as parallelism moves of light waves. Contrary to normal light, when waves open in every direction, common light refraction through special mirrors and filtration through a specific system produces polarized light (Monstrey et al., 2002a). polarized light has beneficial biological effects to improve cell membrane function and increase the production rate of mitochondria adenosine triphosphate. Furthermore, it decreases inflammation, improves microcirculation and tissue oxygenation, facilitates fibroblast propagation, collagen composition, and accelerated epithelization. By improving these functions, this approach has led to the improvement of the wound healing process (Monstrey et al., 2002b).

The present study aimed to evaluate and compare the healing process of wounds exposed to Gallium Arsenide Laser with 830 nm or polarized light (Bioptron System) with 480 - 3400 nm with a total energy of 20 Joules for bot lights.

MATERIALS AND METHODS

Animals

Sixty young mature male albino rats were housed in a condition of the same temperature and brightness of environment in the Laboratory of Animal Experimentation at the Medicine Faculty at the University of Um Al Qura. Rats have cared according to the Guide of Experimental Animals Care and Use of Umm Al-Qura University, KSA, and International Principles associated with Animal Research were followed in the whole study. Each rat was lived in an individual cage, was fed a registered pelted diet and had drinking water throughout the full time of the study.

Creating a wound model

The skin area on the upper back of each rate was prepared as it was saved and cleaned by alcohol 70% then under anesthesia, the surgical excisional procedure was performed to create a uniform area of wound approximately five-centimeter square (2.5x2 cm).

Research design:

The study was a randomized controlled study as rats were randomly assigned straight into 3 groups.

Treatment intervention

Laser group (1):

Twenty rats were irradiated by beam of Gallium Arsenide Laser, (830 nm, BTL 5000, UK) as the manufacturer's instructions were followed using the subsequent parameters; 4 Jouls /cm^2, the total energy of 20 Joules, three times per week for two weeks, and scanning method was used with perpendicular alignment at distant of 10 cm from the area of the wound.

Polarized light group:

Twenty rats were illuminated by polarized light (Bioptron, 480–3400 nm, AG, made in Switzerland) as the manufacturer's instructions were followed. In treatment procedure, The Bioptron device head was aligned perpendicular at distant of 10 cm from the area of the wound, the polarization degree was above 95%, polarized light intensity was around forty mW/cm2 which gave an average fluence of 2.4 Joules/cm2 every minute, each session duration was 8 min.and 20 sec with total energy of 20 Joules and the treatment frequency was 3 times per week, and for 2 weeks.

Untreated (control) group:

Twenty rats had no treatment.

Measurement and evaluation:

Wound area and contraction rate measurement:

A sterilized transparency metric grid of double-layered sheet 16 × 16 squares (1 cm2 each) was applied directly over the wound and a fine tip marker was used to trace wound outline. HP LaserJet p2030 equipped-scanner was used for scanning traced sheets to be saved on personal computers, opened on photoshop Adobe

Photoshop CS6 program and the pixel numbers in scanned traced wound area were counted in addition number of pixels per centimeter square were estimated. Then, dividing total pixel numbers in the wound by the number of pixels in the square centimeter gave the wound area in centimeter square (Li et al.,2012).

-The rate associated with wound closure was calculated utilizing the following formula:

[(Area of 1^{st} day− Area of X days) /Area associated with 1^{st} day] × 100.

-In each group, the wound area plus the rate of contraction for each rat had been measured two times; after 7 days, and 14 days of treatment.

Statistical analysis

The outcomes were collected and examined using the SPSS program (V.16) as means plus standard deviations were determined in addition, as parametric procedures, one way ANOVA in addition to repeated measures were utilized to compare mean values among groups as well as within a group respectively. A probability value less than (0.05), the differences between means had been defined as significant differences (Riedel, 2005).

RESULTS

Wound Surface Area (WSA) and the rate of contraction:

The results considered in **table 1** and in **figure 1** show a highly significant wound surface shrinking within all groups throughout different measurement phases (p-value < 0.0001) for all measures, as data were analyzed by repeated-measures ANOVA.

Table 1: Comparison of wound surface area (WSA) and healing rate mean values after (1st, and 2nd weeks) within a group and between groups.

Mean ± SD	**Day(0)**	**1^{st} week**		**2^{nd} week**		**P-value**
	WSA (cm^2)	WSA (cm^2)	Healing Rate (%)	WSA (cm^2)	Healing Rate (%)	
PL. G.	5.11±0.01	2.61±0.49	48.75±9.94	0.1±0.04	97.9±0.83	0.0001
GL.G.	5.10±0.08	2.89±0.41	43.48±7.39	0.25±0.14	94.9±2.85	0.0001
Control G.	5.16±0.13	3.33±0.31	35.37±6.2	0.37±0.10	92.71±2.08	0.0001
P value	0.41	0.001		0.0001		
Post Hoc test		1**vs**2=(0.13) 2**vs**3=(0.017) 1**vs**3=(0.0001)		1**vs**2=(0.003) 2**vs**3=(0.015) 1**vs**3=(0.0001)		
F value	0.90	8.5		17.32		

PL.: Polarized light GL.: Gallium Arsenide Laser
PL. group: (1): PL. group, (2): GL.group, (3): Control group.
(1) vs. (2): Comparison between PL.group and GL.group group.
(2) vs. (3): Comparison between GL.group and control group.
(1) vs. (3): Comparison between PL.group and control group.

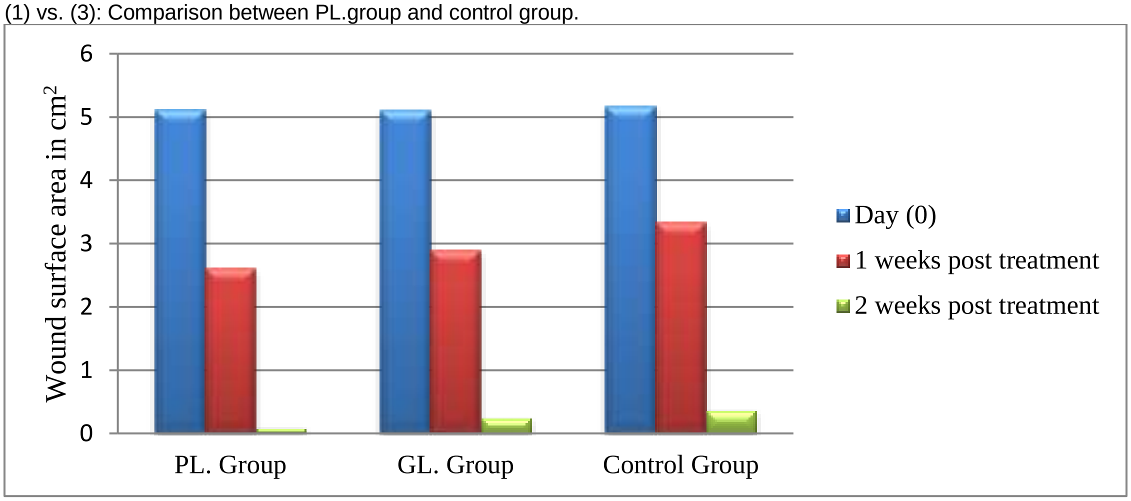

Figure 1: Comparison of wound surface area mean values within each group at different treatment phases

At day (0), there were no statistically significant differences between groups as (p-value equal to 0. 41) (An area of each wound was approximately 5 cm^2).

After 1st week, the results in **table 1** and **figure 2** show that the mean value of WSA in polarized light (PL.) group was (2.61±0.49) with contraction rate mean of (%48.75±9.94), and it was (2.89±0.41) for gallium arsenide light (GL.) group and with contraction rate mean of (%43.48±7.39) while it was (3.33±0.31) for control group with contraction rate mean of (%35.37±6.2), analysis of outcomes by one-way ANOVA, revealed that there were statistically significant differences between groups as (p-value equal to 0.001), additional analysis by Post Hoc test provided that there were no differences between the mean of W.S.A or contraction rate in PL. group and parallel measures in GL.group as p-value was (0.13), while, significantly, there were differences between; the mean of W.S.A or contraction rate in PL. group and parallel measures in control group and the mean of W.S.A or healing rate in GL. group and in control group after 1stweek as p-value were (0.0001), and (0.017) respectively.

After 2nd week, **table 1** and **figure 2** show that; mean of the WSA in PL. group was (0.1±0.04) cm2, with contraction rate mean of (%97.9±0.83) while the mean of the WSA was (0.25±0.14) cm2 with contraction rate mean of (%94.9±2.85) in GL. group and it was (0.37±0.10) cm2 and contraction rate mean of (%92.71±2.08) in control group; the analysis of these data revealed that there was a significant difference between the groups (p-value = (0.0001). Detailed analysis of the data revealed that; there were significant differences between; the mean of W.S.A or rate of healing in the PL. group and that in GL. Group, the mean of W.S.A or contraction rate in the PL. group and that in the control group and the mean of W.S.A or contraction rate in the GL. group and that in the control group as p values were (0.003), (0.0001) and (0.015) respectively.

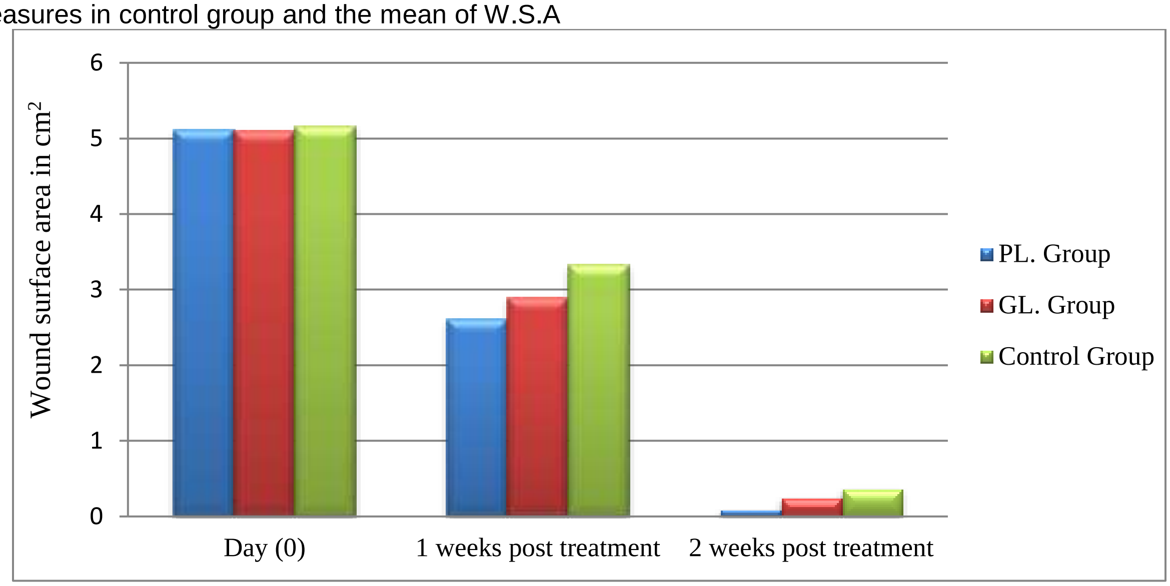

Figure 2: Comparison of wound surface area (WSA) mean values between groups at different treatment phases.

DISCUSSION

In this study, a uniform surgical wound model was created in rats in order to compare between the effects of Gallium Arsenide laser and polarized light on healing of full-thickness wound, and the results of the study elicited that there were significant wound contractions and better healing rate in PL. group and in GL. group when respectively compared to measures in control group after 1st and 2nd weeks as p-value was < (0.05) for all measures. these results may attribute to the efficient healing effect of both approaches; Gallium Arsenide laser and polarized light at a total energy of 20 Joules and the results were consistent with following previous studies:

Polarized Light and Wound:

Electric and magnetic forms of polarization are the main causes of therapeutic effects light, not its wavelength (Nicola J and Nicola E, 2002). After a few millimeters of penetration, the polarization is lost and the peak penetration is continued produced by collagen fibers through polarized light scattering (Anderson, 1991), polarization in a Linear direction can penetrate 1.2 millimeters of human skin with no polarization loss (Tuchin, 2000).

Polarized light which is emitted from an incoherent source of light is like a low power laser in bio- stimulating living tissues. 480-700 nm of visible light and 700-2000 nm of infrared light are combined in a Bioptrona lamp, it is similar to low power laser as it is a light source with low power, but it is different in that it is incoherent and polychromatic light. Both of the electromagnetic spectrum parts present in this polychromatic light source is responsible for photo bio-stimulating effects through different mechanisms. at different cellular levels, the cascade of metabolic events starts and leads to the same final photoresponse. Visible light absorption stimulates mitochondria which is one of the main visible light effects, a sequence, which results in cell energy increased, nucleic acid synthesis activated, and wound repaired (Tuner and Hode, 2004).

The cellular membrane bilipid layer is affected by a polarized light as the lipids end polarized and directed to the polarization source and its structures changed. The cellular membrane reorganization occurred as a result of energy transfer from the lipids to proteins and this affects all regulation processes of the membrane (Fenyo, 1984, Kubasova et al.,1988).

A significant beneficial effect of polarized light on wound healing has been confirmed in many studies as it has been demonstrated that polarized light speeds up wound contraction, reduces exudation, improves scar tissue formation quality, fastens the wound closure and enforces wound tensile strength. From different studies, different Polarized light effects have been proved; it enhances humoral and cellular defense mechanisms in human being, stimulates release of cytokines, and growth factors, and increases collagen formation. Also, the use of polarized light may also stimulate peripheral vasodilatation, improve blood circulation to the skin and deliver good oxygenation and nutrients to the wound site (Bolton,1992, Melo et al.,2001).

Gallium Arsenide Laser and wound:

The laser bio modulation effects are depended on the theory that photo acceptor elements, such as hemoglobin, oxyhemoglobin, melanin, and cytochrome c oxidase, engulf light energy then the energy is converted to chemical energy inside the cell (Iordanou, 2002). Cytochrome m. oxidase as one of the photo acceptor elements accepts photons and stimulates changing in the redox of mitochondria and facilitates ion movement across the internal membrane of mitochondria as well as speed up ATP formation (Fukuda,2010). Also, intracellular calcium (Ca^{2+}) increases and enhances DNA and RNA formation, and followed by activating intracellular indicators cascade (Houreld, 2014). Which represented by promotes DNA duplication, speeds up protein formation, facilitates the release of different cytokines and enhances oxidative stress regulation, (Karu, 1999, Silveira et al., 2009) All these events lead to of different cell types modulation which is responsible for steps of tissue rebuilding (Alves, 2013), Which include fastening of fibroblast divisions (Bayat and Azari, 2010), promoting of angiogenesis (Bayat and Azari, 2010, Bates–Jensen,1997), changes in cytokines activity (Schubert, 2001), and helping fibroblasts transforming into myofibroblasts (Bayat and Azari, 2010).

Many studies investigated the effect of low power lasers on normal wound healing as well as on delayed wound healing, animals and human were included in studies and they reported that low power lasers speed up wound closure, stimulate fibroblasts replication, promote endothelial cells blood vessel growth, increase tensile strength, decrease inflammation, and induce synthesis of collagen in normal cutaneous wounds, (Bates–Jensen,1997, Schubert, 2001), ulcers (Schubert, 2001), superficial thickness wounds in healthy humans, (Hopkins et al.,2004), and in rats, (Bayat and Azari, 2010, Monici et al.,2008).

Despite a positive bio-modulatory effect for both therapies using the same total dose (20 Joules) as irradiated or illuminated rats showed a more wound shrinking in comparison to untreated rat, the study results at the end of 1st week, also founded that there was slight difference between two groups receiving therapies in form of better wound contraction in PL. group than in GL. group but not reach a significant level as p-value was (0.13), while at the end of 2nd-week the difference between two groups increased and reached a significant level in form of better wound contraction and lesser wound areas in the PL. group than in GL. group as the p-value was (0.003).

The above results are aligned with previous reports, which stated that subjects who were irradiated by laser light had a fewer number of myofibroblasts (Fisher et al.1983, Luomanen et al., 1988), while subjects who were illuminated by polarized had more numbers of myofibroblasts (Antonio, 2005). Previous wound healing studies found that there is a linear relationship between myofibroblasts numbers and the amount of wound contractions as a smaller number provides smaller contraction and vice versa (de Freitas, et al., 2002, Pinheiro, 1993).So the previous study may confirm and explain our study results as the better wound healing in a polarized light group compared to the laser group which represented by larger wound contraction may be attributed to larger myofibroblasts number. In spite of the causes for that result maybe not completely clear, responsibility for that result may be related to some properties which distinguish two lights such as monochromaticity and coherence (Antonio, 2005). In most studies, the use of coherent and incoherent lights has different impactions on tissues (Tuner and Hode, 2004), and in polarized light, as the light penetration depth increases within the tissues, the light polarization degree decreases (Nicola J and Nicola E, 2002). While in laser light, the penetration depth is power independent and laser biological effect relies on the power value and maximum penetration which may affect the intensity effect of laser light on deep tissue. So The laser therapy effect may be detected in 4 cm deeper. While non-coherent polarized light effects may be observed in the form of more superficial biostimulation as provide faster epithelialization (Tuner and Hode, 2004).

CONCLUSION
Both Gallium Arsenide Laser and Polarized Light with total light energy of 20 joules speed up full-thickness wound healing in addition polarized light has been found to have more better wound healing effect than GAlAs laser.

CONFLICT OF INTEREST
The authors declared that present study was performed in the absence of any conflict of interest.

ACKNOWLEGEMENT
We would like to thank Physiotherapy Department members as well as animal house workers in Medicine Faculty at University of Umm Al Qura, Mecca, KSA. for their cooperation and assistances.

AUTHOR CONTRIBUTIONS
HGM designed and performed the experiments and also wrote the manuscript, AAT and MMIA performed animal treatments, MRA performed data analysis, designed experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES
Alves AC, Vieira R, Leal-Junior E, dos Santos S, Ligeiro AP, Albertini R, et al. Effect of low-level laser therapy on the expression of inflammatory mediators and on neutrophils and macrophages in acute joint inflammation. Arthritis Res Ther. 2013; 15: R116.

Anderson RR. Polarized light examination and photography of the skin. Arch. Dermatol. 1991;127:1000-1005.

Antonio Luiz B. Polarized Light (400–2000 nm) and Non-ablative Laser (685 nm):A Description of the Wound Healing Process Using Immunohistochemical Analysis

Photomedicine and Laser Surgery. 2005; 23(5): 485–492.

Basford J R.. Low-intensity laser therapy: still not an established clinical tool. Lasers Surg. Med.1995;16:331-342.

Bates–Jensen B M. The Pressure Sore Status Tool: a few thousand assessments later. Adv.Wound Care.1997; 10:65–73.

Bayat M, Azari A, Golmohammadi, M G. Effects of 780-nm low-level laser therapy with a pulsed gallium aluminium arsenide laser on the healing of a surgically induced open skin wound of rat. Photomed. Laser Surg.2010;28:465–470.

Bolton P, Dyson M, Young S. The Effect of Polarized Light on The Release of Growth Factors From The U-937 Macrophage-Like Cell Line. Laser Therapy. 1992; 4(1): 33–37.

de Freitas AC, Pinheiro AL, de Oliveira MG, et al. Assessment of the behavior of myofibroblasts on scalpel and CO2 laser wounds: an immunohistochemical study in rats. J. Clin. Laser Med. Surg. 2002;20: 221–22515. Fenyo M. (1984). Theoretical and experimental basis of biostimulation. Optics Laser Technol.1984;16: 209-215.

Fisher SE, Frame JW, Browne RM, et al. A comparative histological study of wound healing following CO2 laser and conventional surgical excision of canine buccal mucosa. Arch. Oral Biol.1983;28:287–291.

Fukuda TY, Tanji MM, Jesus JF, Sato MN, Duarte AJ, Plapler H. Single session to infrared low level diode laser on TNF-alpha and IL-6 cytokines release by mononuclear spleen cells in mice: a pilot study. Lasers Surg Med. 2010; 42: 584–588.

Hopkins JT, Mcloda TA, Seegmiller JC, Baxter GD. Low-level laser therapy facilitates superficial wound healing in humans: a triple-blind, sham-controlled study. J. Athl. Train.2004; 39: 223–229.

Houreld NN. Shedding light on a new treatment for diabetic wound healing: a review on phototherapy. ScientificWorldJournal. 2014;2014:398-412.

In de Braekt M M, van Alphen F A, Kuijpers-Jagtman A M, et al. Effect of low-level laser therapy on wound healing after palatal surgery in beagle dogs. Lasers Surg. Med.1991;11: 462.-470.

Iordanou P, Baltopoulos G, Giannakopoulou M, et al. Effect of polarized light in the healing process of pressure ulcers. Int. J. Nurs. Pract. 2002;8:49–55.

Karu T I. Photobiology of low-power laser effects. Health Phys. 1989; 56:691.704.

Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J PhotochemPhotobiol B. 1999; 49: 1–17. pmid:10365442.

Kreisler M, Christoffers AB, Al-Haj H, et al. Low-level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts. Lasers Surg. Med. 2002;30: 365-369.

Kubasova T, Fenyo M, Somosy Z, et al. Investigations on biological effects of polarized light. Photochem. Photobiol.1988; 48:505-509.

LI, PN, H LI, M L WU, SY WANG, QY KONG. A cost-effective transparency-based digital imaging for efficient and accurate wound area measurement. PLoS ONE. 2012;7:1–8.

Luomanen M, Lehto V P, and Meurman J H Myofibroblasts in healing laser wounds of rat tongue mucosa. Arch. Oral Biol.1988;1:17–23.

Melo CA, Lima AL, Brasil IR., et al. Characterization of light penetration in rat tissue. J. Clin. Laser Med. Surg. 2001;19:175-179.

Monici M, Cialdai F, Fusi F, Romano G, Pratesi R. Effects of pulsed Nd: YAG laser at molecular and cellular level. A study on the basis of Hilterapia.Energy for Health. 2008;3.

Monstrey S, H Hoeksema, K Depuydt, G Van Maele, K Van Landuyt, P Blondeel, The effect of polarized light on wound healing.Eur. J. Plastic Surg. 2002; 24:377–382.

Monstrey S, H Hoeksema, H Saelens, K Depuydt, M Hamdi, K Van Landuyt, A conservative approach for deep dermal burn wounds using polarized-light therapy.Br. J. Plast. Surg. 2002; 55: 420–426.

Nicola JH, Nicola E M D.Wavelength frequency and color: absolute in relative concepts? J. Clin. Laser Med. Surg.2002; 20:307-311.

Pinheiro A L, Vieira A L, Rolim A B. et al. Effects of LLLT on the proliferation of HEP2 cells. In: Bios 2000, San Jose. Lasers in Dentistry VI. Bellingham, WA: SPIE. 2000; 75-81.

Pinheiro AL. Comparison of tissue damage and healing in scalpel and CO2 laser mucosal wounds [Doctoral dissertation]. Birmingham, UK: University of Birmingham.Antonio Luiz B et al. Polarized Light (400–2000 nm) and Non-ablative Laser (685 nm): A Description of the Wound Healing Process Using Immunohistochemical Analysis Photomedicine and Laser Surgery. 1993;23(5): 485–492.

Pinheiro, ALB, Nascimento, SC, and Vieira, ALB. Does LLLT stimulate laryngeal carcinoma cells? An in vitro study. Braz. Dent. J. 2002;13:109-112.

Pinheiro ALB, Nascimento SC, Vieira ALB, et al. Effects of low-level laser therapy on malignant cells: in vitro study. J. Clin. Laser Med. Surg. 2002;20:23-26.

Pogrel M A, Chen, J W, Zhang K. Effects of low energy gallium-aluminum-arsenide laser irradiation on cultured fibroblasts and keratinocytes. Lasers Surg. Med. 1997; 20: 426-432.

Reddy G K, Stehno-Bittel L, Enwemeka, C S. Laser photostimulation of collagen production in healing rabbit Achilles tendons. Lasers Surg. Med. 1998; 22:281-287.

Riedel F, Philipp K, Sadick H, Goessler U, Hormann K, Verse T. Immunohistochemical Analysis of Radiation-induced Non-healing Dermal Wounds of the Head and Neck. In vivo. 2005;19: 343-350.

Rosner M, Caplan M, Cohen S, et al. Dose and temporal parameters in delaying injured optic nerve degeneration by low energy laser irradiation. Lasers Surg. Med.1993; 13;611-617.

Schubert V. Effect of phototherapy on pressure ulcer healing in elderly patients after a falling trauma.A prospective, randomized, controlled study. Photodermatol.Photoimmunol.Photomed. 2001;17:32–38.

Silveira PC, Silva LA, Fraga DB, Freitas TP, Streck EL, Pinho R. Evaluation of mitochondrial respiratory chain activity in muscle healing by low-level laser therapy. J PhotochemPhotobiol B. 2009; 95: 89–92.

Sommer AP, Pinheiro AL, Mester A R et al. Biostimulatory windows in low-intensity laser activation: lasers, scanners, and NASA's light-emitting diode array system J. Clin. Laser Med. Surg.2001;19:29-33.

Tuchin VV. Tissue Optics Light Scattering Methods, and Instruments for Medical Diagnosis. Bellingham WA: SPIE. 2000.

Tuner J, Hode L, 2004.Laser Therapy: Clinical Practice and Scientific Background. Tallinn: Prima Books AB.

Van Breugel H. Power density and exposure time of He-Ne laser irradiation are more important than total energy dose in photo-biomodulation of human fibroblasts in vitro. Lasers Surg. Med. 1992; 12:528-537.