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Effect of Light Emitting Diode Therapy (LEDT) on Wound Healing

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ABSTRACT

Objective: *The aim of this study* aimed to asses the effects of Light Emitting Diode Therapy (LEDT) on wound healing in rats using different wavelengths of LEDs: 680, 730, 950 nm simultaneously.

Background Data: Light Emitting Diode Therapy (LEDT) developed from Sim-Med Ltd; using GaAs semiconductor as the diode for delivering light deep into tissues to promote wound healing and tissue growth. We review in this paper and present our data of LED treatment for wound healing to rats.

Material and Methods: To perform this study we used variety of wavelengths, power density, and energy density parameters for each wound in 2groups of rats (104 rats), also we used lens to maximize the power and reduce the exposure time in the second experiment.

Results: we found that LED with three wavelengths (680, 730, 950 nanometer) increase wound healing process in rats in first experiment more than second experiment which used lens to maximize the power.

Conclusion: The results of the study demonstrated that the LED with three wavelengths (680, 730, 950 nanometer) simultaneously with contact control increase the wound healing of the rats. The results also showed that LED has large spot size than the laser so LED is more suitable than laser for large wounds, moreover the combined wavelengths of laser optimal for wound healing cannot be efficiently produced, and the size of wounds which may be treated by lasers is limited.

Introduction

Wound healing is a complex and dynamic cascade of events initiated by injury. This response to injury is guarded by many factors as innate host immune response, infection, kind of tissue, nutrition, immunity, general health, etc…

Many studies using different types of medications to aid healing of different stages, also Laser has an important role for improve healing process.

Nowadays, Light Emitting Diodes (LEDs) are fast becoming the light source of choice for medical application. LED therapy has been performed to investigate the influence of LEDT on the healing process of wounds or lesions.

The researchers reported that light therapy as LEDT has a biostimulative effect and greatly enhance the natural wound healing process.

This will save valuable time and resources for both patients and health care facilities.

Furthermore, improved wound healing will reduce the risk of infection for the patient, decrease the amount of costly dressings required, and more quickly return the patient to a preinjury/ illness level of activity; also laser light has been widely acclaimed to speed wound healing of ischemic, hypoxic, and infected wounds [1] .LEDs provide low energy stimulation of tissues that result in increased cellular activity during wound healing [2]; these activities include collagen production and angiogenesis [3].

LEDs stimulate the basic energy processes in the mitochondrial (energy compartments) of each cell particularly when near-infrared light is used to activate the wavelength sensitive constituents inside (chromospheres, cytochrome systems). Investigations into low-energy stimulation of tissues by LED have shown increased cellular activity during wound healing, including increased collagen production and angiogenesis.

Optimal light wavelengths proven in prior studies of laser and LED light [2, 3, and 4] to speed wound healing include 680,730, and 880nm these wavelengths can be produced accurately be NASA LEDs which have a band width of 25nm.

Wound healing has three phases: First is inflammatory phase; then cells proliferating and finally there is remodeling of tissue [2, 3].

The depth of near-infrared light penetration into human tissue has been measured spectroscopically [2,3and5] spectra taken from the wrist flexor muscles in the forearm and muscles in the calf of the leg demonstrate that most of the photons at wavelengths of 630-800nm travel approximately 23cm through the skin surface (light input) and muscle, exiting at the photon detector.

A series of experiments has recently been completed using LED for enhancement of cell growth. The cells were exposed to the LED light at combined wavelengths and individual wavelengths (680,730, and 880 nm), energy density of 4 and 8 J/cm^2 and intensity of 50 mw/cm^2. They demonstrated that a cell growth increase of about 140 % over untreated controls, particularly at 8J/cm2 [6-17].

There are various factors in the LED therapy that must be understood to efficiently evaluate it. Some of these factors are: the voltage drop across LED, optimal drive current, the correct emitted colour (or wavelength) and radiation pattern of the light source (LED or Laser light).

The use of polychromatic LED as alight source in photo therapy is in its infancy, so stricter guidelines to improve LED manufacturing standards and quality control should be imposed. Further studies that are directed at finding the optimum wavelength and light dose combination need to be done augment efficacy, concurrent with filling gaps in our knowledge about the mechanisms of its effect [18].

LED Therapy

LEDT is the application of light to injuries and lesions to stimulate healing in tissues. Moreover it is used to resolve inflammation, reduce pain, increase quality and strength of tissue repairs and resolve infection. LEDs stimulate the basic energy processes in the mitochondria (energy compartments) of each cell particularly when near-infrared light is used to activate sensitive constituents inside (chromospheres, cytochrome systems).

1-The effects of LE D (similar to effects of LLLT) are photochemical, not thermal.

2-Photons enter the tissue and are absorbed in the mitochondria and at the cell membrane.

3-The photon energy is converted to chemical energy within the cell, in the form of ATP.

4-This LED stimulation vitalizes the cell by increasing the mitochondrial ATP production and Cell membrane permeability alters.

5-These physiological changes affect macrophages, fibroblasts, endothelial cells, mast cells, bradykinin and nerve conduction rates and more.

6-High ATP leads to cell stimulation and proliferation.[19]

Aim of work

To asses the effect of LEDT on wound healing using different wavelengths of LEDs: 680, 730, 950 nm simultaneously.

Materials and Methods

1- Experimental Animals;

One hundred and four healthy male rats with average (300-350 gm) divided into 2 pilot trials and 2 experiments. All rats were fed balanced diet and housed in standard rat cages under good hygienic measures, food and water were ad. Libitum.

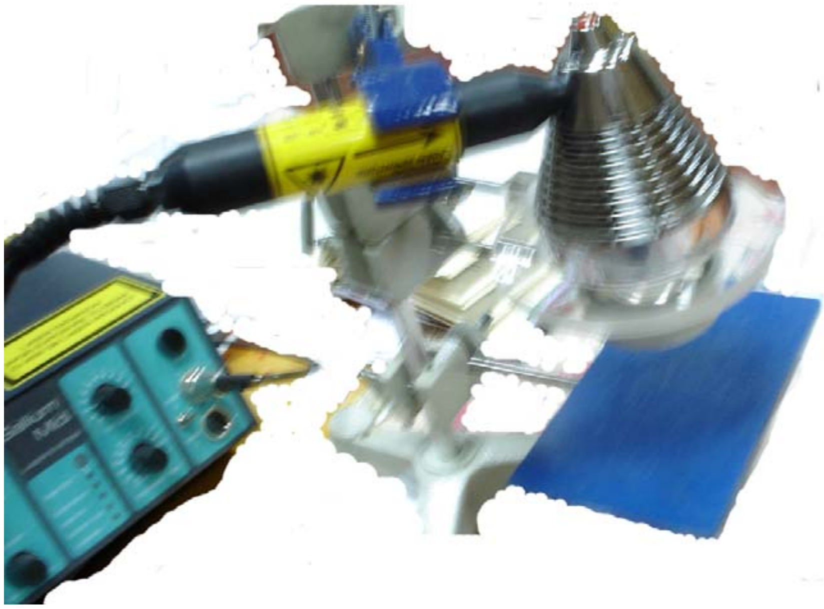
2- Light Emitting Diode (LED) Apparatus;

The LED used in this work was the Gallium Midi Models (801/ 802) are manufactured in the United Kingdom by: Sim-Med Ltd; using GaAs semiconductor as the diode with 3 wavelengths (670, 730, and 950 nm), 33 clusters=35 mw.

1- 12 cluster×670nm=10 mw, 2- 13cluster×730nm=10mw and 3- 8 cluster× 950 nm=15 mw. We used lens to maximize the power and reduce the exposure time in the second experiment.

(Fig. 1) LED Apparatus with 33 clusters used in our experiment

(Fig. 2) LED application, contact procedure 1st experiment



(Fig. 3) LED application, non contact procedure 2nd experiment with external lens

3-Experimental design

The parameters of first experiment: Frequency was 20 Hz (position 3)**,** Time of exposure :8 minutes**,** Power density :10 mw/cm^2**,** Energy density : 5j/cm^2**,** Duration :14 days**,** Number of sessions/week------5 sessions (1, 2,3,4,5 days postoperative)**,** Technique : contact.

1st Exposure of LED: Immediately after biopsy; In this Experiment 24 rats were allocated into four groups**:** G_1 (6 rats treated with LED), G_2 (6 rats treated with LED+ panthenol), G_3 (6 rats treated with panthenol) and G_4 (6 rats control)

*All rats photographed after: (3, 6, 8, 10, 12, 14 days post wound induction)

The parameters of second Experiment: Frequency was 20Hz (poison: 3), Time of exposure: 90sec, Power D:75mw, Energy D : 5Jcm2, Duration :21 days, Number of sessions/week =5 sessions /week (1,2,3,4,5,8,9,10,11,12,15,16,17,18,19 days post operative), Technique :non contact (1.5cm away)

$$\text{Energy density (J/cm}^2\text{)} = \frac{\text{power (w)} \times \text{time (s)}}{\text{Area (cm}^{2)}} \quad [137]$$

5j/cm^2 = 75x10^{-3}mw/cm^2 x T , then T =5 x 10^{-3} / 75 =90 sec.

*1st Exposure of LED: Immediately after biopsy; In this Experiment 40 rats were divided into four groups: G_1 (10 rats treated with LED), G_2 (10 rats treated with LED + Panthenol), G_3 (10 rats treated with Panthenol only) and G_4 (10 rats control).

*All rats photographed after: (3, 6, 8, 10, 12, 14, 18, days post wound induction).

External lens was supplied to maximize the power and reduce the exposure time with specific characteristics as follow :Convex lens, Focal lens =1.5 cm, Diameter= 5.5 cm, Wavelength transmission= 350 nm to 1100 nm, Material= BK-7.

4-Wound induction

The rats were intramuscularly anesthetized with 1mg/100g of ketamar (ketamine HCL-10 mg/mL) together with 0.3 mg/100g of Zylaject (xylazine, 20 mg/mL).

After anesthesia, the dorsal region of the rat was shaved and paint with petadine.

The wounds were induced through circular blade using punch biopsy of 9 mm on the dorsal region of the upper back of each rat but the wound area 1cm^2 (Because the skin is stretched during the operation to 1cm^2), all wounds were left open without suture or cover, and each rat was kept in special cages.

LED irradiation and photography were also performed under the same dose of anesthesia.

LED energy was delivered above the center of wound (Fig. 3.4) and the adjacent normal tissue for 8 min through the cluster which was contact to the Wound in the 1st experiments but in the 2nd experiment, the cluster was held vertically about 1.5 cm a part from the surface of irradiated area where the LED pass through the external lens. The first exposure by LED was performed immediately after the biopsy of both 1st and 2nd experiments.

The wounded areas were examined every day and the clinical observations were recorded.

The wounded areas in both treated and control groups were determined and photographed at the same time.

<u>5- Methods of assessment:</u>

*Gross observation, photography and microscopical examination.

The microscopical findings were recorded for different treated groups and control one.

*Photography was applied through fixed digital camera; the treated rats and control one were photographed simultaneously.

*Regarding to Histological examination the Samples from wounds were collected by scarifying the rats from both control and treated groups, after 3, 7, 10, 14 days post operative in the 1st experiment while in the 2nd experiment, after 4, 6, 8, 10,12, 14, 18 days post operative.

*The samples were taken and fixed immediately in neutral buffered formalin 10 %,dehydrated in ascending grades of alcohols (50%, 70%, and 100%) then cleared in zylien two changes, imbedded in paraffin wax blocked and sectioned at 4-6 microns by rotatory microtome.

* The samples after that stained by H & E, and specifically by massons trichrom stain.

RESULTS

In the 1st experiment, after 10 sessions of LED (14 days) G1 and G2 healed completely; G3 had not healed completely but showed more advanced healing process than G4 ;while G4 showed a poor degree of cicatrisation when compared to wounds G1, G2, G3.

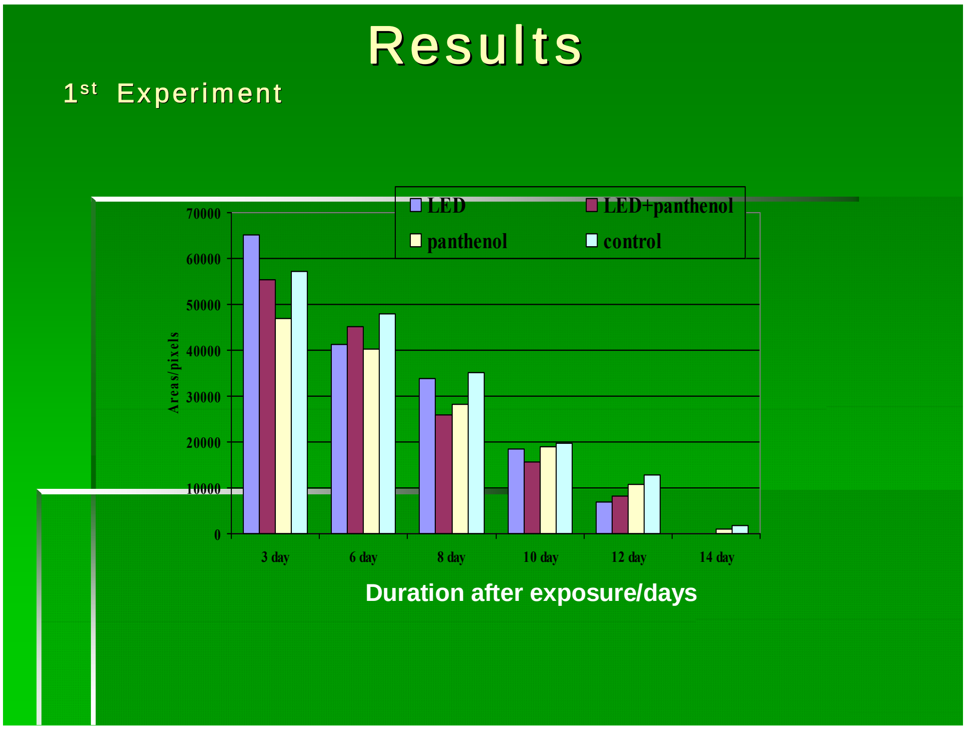


Plate 1.

In the 2nd experiment

After 15 sessions of LED (21 days) G3 treated with Panthenol only had showed more advanced healing process than G1, G2, G4 but not healed completely, G1 and G2 showed more advanced healing process than G4 , while G4 showed a poor degree of healing.

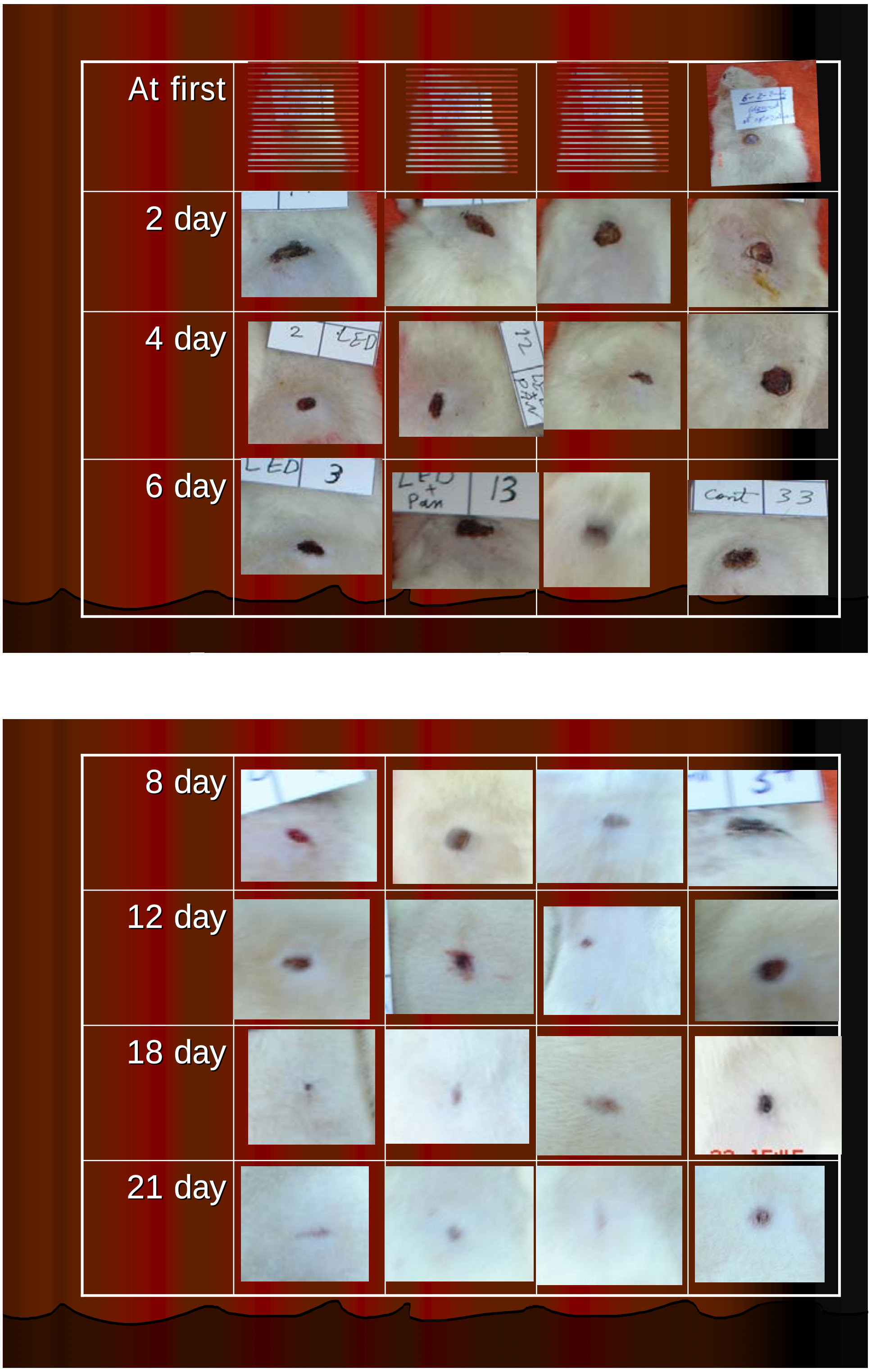


Plate 4.

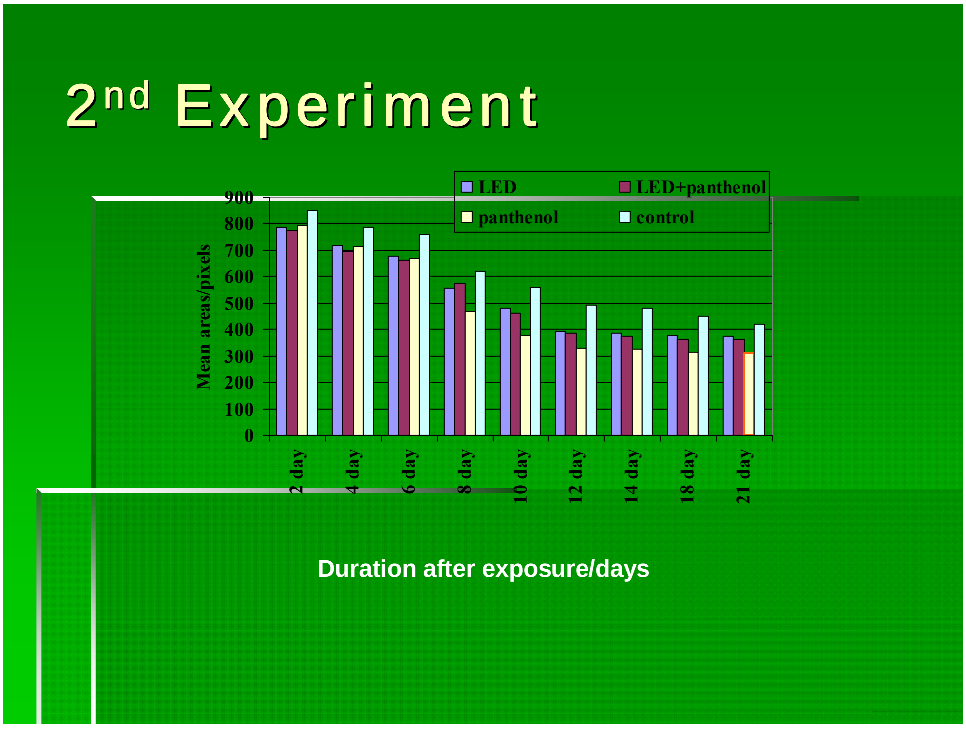


Plate 5.

DISCUSSION

Our study suggest potential for using LED light at 680, 730 and 950 nm simultaneously and in combination with panthenol ointment in several regimes for acceleration of wound healing process in rats .

To assess the role of different LED parameters in the process of wound healing we designed several experimental regimes by changing the selected parameters and techniques. Irrespective of the affected tissue, the wound healing process follows a conserved sequence of events which overlap in time, including inflammation, proliferation, and tissue remodeling.

The inflammation phase lasts 3 to 10 days and characterized by blood vessel contraction and exudation of edema rich with fibrin and inflammatory cells (neutrophils & macrophages).The fibrin- fibronectin matrix is degraded by inflammatory cells. Fibroblasts synthesis fibronectin, interstitial collagen and glycosamine glycans (GAGs) to form new fibrovascular connective tissue (granulation tissue, 2-4 days) after injury [21, 22,23].

The proliferation phase (3-14 days) is characterized by the formation of granulation tissue in the wound. Granulation tissue consists of a combination of cellular elements including fibroblasts and inflammatory cells, along with new capillaries embedded in a loose extracellular matrix of collagen, fibronectin and hyaluronic acid. Fibroblast first appears in significant numbers on the 3rd day post injury and achieves peak numbers around the 7th day . Synthesis of type 1 collagen is maximal between 5-7 days. Re-epithelialization at wound edges begins within 24 hours and achieves the maximum proliferation at 24-72 hours. Collagen synthesis was proceeding followed by capillary resorption and disappearance of fibroblasts.

Regarding to maturation phase, the total amount of collagen reaches the maximum at 2 to 3 weeks but collagen remodeling continues over months to years. The earliest collagen fibers are thin and unorganized, becoming thickened, cross-linked, and parallel to skin tension. As new collagen is formed, abnormal or damaged collagen is broken down by collagenases and proteases produced by fibroblasts, macrophages and inflammatory cells. Proteoglycans, responsible for water storage in the healing wound and the water is reabsorbed as the wound heals. The final product of the healing process is scar which is relatively a vascular and a cellular mass of collagen [24].

Our finding was in accordance to the work done by NASA Marshall Space flight center who used NASA LED (680, 730, 880 nm). They concluded that the use of NASA LED greatly enhance the natural wound healing process and more quickly return of the patient to a pre injury / illness level of activity. They were referring the enhancement in healing process to increased cellular activity including increased fibroblast proliferation, growth factor syntheses, collagen production and angiogenesis [25].

Another study by NASA concluded that the use of NASA LED for light therapy alone, and in conjunction with hyperbaric oxygen, will greatly enhance the natural wound healing process; The cell growth increased 150-200% over untreated controls, increase DNA synthesis e.g. increase cellular growth rate [26].

Also our findings were in accordance with the concept of Whellan, 1993-1999, et al; who demonstrated that the cell growth was increased about 140% over untreated control; On using LED Light at combined wavelengths (680, 730, 880nm); with energy density of 4 and 8 J/cm^2 and intensity of 50mw/cm^2 [6-17].

In our study in the 1st experiment; LED with wavelengths (670, 730, 950 nm) was delivered to the wound 5 times per week with energy density 5J/cm^2. The healing process appeared histologically improved as early as 3 days post treatment by using LED. This was apparent in this stage by dense proliferation of granulation tissue and epithelial cells at the wound margins; Also subsiding of inflammatory phase (edema, congestion and neutrophils). The histological evaluation revealed that there was a distinctive progressive improvement in wound healing in group treated with LED by the 10th and 14th day post treatment. Complete epithelialization and remodeling were marked in the LED treated group by the 10th day while the gap is still observed in the rest of treated group (G_2, G_3) and control group until the end of the experiment (14days). The G_2, G_3 showed marked proliferating phase while the control group showed the inflammatory phase.

The biochemical mechanism by which LED enhances the process of wound healing is not known, but the current theory is that the infrared light is absorbed by some photoreceptors, which then trigger a cascade of reactions in the cell. The major biological photoacceptors in the near-infrared range have been determined to be hemoglobin, myoglobin, and cytochrome oxidase. LED treatment effectively energized the cells by stimulating their cytochrome oxidase and triggered a cascade of cellular and molecular events that have significant biological benefits [27].

The marked wound healing improvement in the group treated with LED (In the pilot trials and 1st experiment) goes with the finding of Philyeon Lee, 1993 et al; who reported that biostimulation effects of infrared LLLT (GaAs) in the samples of rats led to significantly increase the rate of wound closure in the LLL irradiation group. They proved that the speed of inflammation due to bacterial infection was significantly decreased in the LLL irradiation group. It is confirmed that the acceleration of healing in the infected lesion following GaAs LLLT indicates that increasing the cellular activity due to the biostimulation effect of LLLT [28].

Our findings were in accordance to the work done by (Farouk. AA) who postulated that polychromatic LED therapy affects burn healing in a dose dependent manner. They explained that the doses of 5 and 10 J/cm^2 were barely effective [29].

In the 2nd experiment, our concept was increasing the energy density by reducing the exposure time from 8 minutes to 90 sec, so we used a converging lens 1.5 cm away from the wounded area.

We use also wound healing accelerator (panthenol ointment) for achievement of combined action. By gross and histological observations, we found good healing process in the group received the combined treatment LED + Panthenol (G_2).

The healing was preceded by the time and increasing the number of sessions compared with the other control group. The complete epithelialization was noticed 12 days after receiving 10 sessions in the combined regime (LED + Panthenol); In this group maturation and remodeling were visible after 14 days post treatment while the gap is visible in control and panthenol treated group.

The improvement of healing was comparable in the group treated with LED (G_1) and group treated with LED & panthenol (G_2) at 14th, 18th day but the progress was visible in the group received combined treatment (G_2).

These parameters represented a compromise between power and time of exposure to obtain the desired effect on acceleration of wound healing.

In the present study, although there was improvement in wound healing comparing the treated groups and control one, the completion of healing is retarded in 12, 14 days. The improvement in wound healing was noticed in the group received LED either alone or combined with panthenol.

The group treated with LED showed decreased number of polymorphs (neutrophils) and this may be attributed to the effect of LED as bactericidal.

Also the stimulatory effect of LED was mentioned by many authors as Karu T. (1999-2004) who reported that using different wavelengths (760nm and 633nm) can produce very different effects within the cell, depending upon the order in which each is applied, due to the interaction of these wavelengths with different photoreceptors [30].

LED irradiance, at power density (10 and 12mw) or laser irradiance at power density (100mn) has pronounced decreasing effect on bacterial count for treated semen samples [19].

Several studies have demonstrated that gram-positive bacteria are susceptible to photo inactivation [31].

In conclusion the complete epithelialization and remodeling were observed as early as 7th day in the 1st and 2nd trial while complete healing observed by the 10th day post wounding in the 1st experiment and occurred by the 14th day post wounding in the second experiment.

The variation in the energy density, number of sessions and duration of each session might be contributed in the time of healing completion. The energy density was 4J/cm^2 in the 1st and second trial and the session duration was 7 minutes while the number of sessions was 4/week in the 1st trial and 2sessions/day in the 2nd trial. The energy density was 5J/cm^2 and the sessions were 5/week in the 1st and 2nd experiment while the session duration was 8 minutes in the 1st experiment and 90 second in the 2nd experiment.

On determination of an optimal dose for acceleration of wound healing by some investigator who used diode- Laser systems 815nm, the best results was obtained at the 3rd day post treatment when they used fluence 145J/cm^2 and 3second for the time of exposure [32].

Other investigators used LED with 670nm in daily treatment of wound by fluence of 4J/cm^2 for 14 days on group of 10 animals. They concluded that using gene discovery technique, one can begin to understand the biochemical mechanisms that are triggered by the LED and playing a role in enhancing the healing process.

In the 1st experiment: the technique of exposure by LED was contact while in the 2nd experiment was non contact.

In the contact treatment procedures, the most important modes of light interaction with tissue are absorption and scattering.

Absorption results in transformation of light energy into some other forms of energy, while scattering procedure is changes in the direction of propagation of light. The relative degree of absorption and scattering that occurs in particular situation is dependent upon the type of tissue through which the light is passing as well as the wavelength of incident light.

In the non contact treatment a percentage of incidents light are reflected from tissue surfaces which reduce the energy delivered to tissues and in turn the potential efficiency. Experience has shown that treatment without control contact can be therapeutically ineffective. To avoid this, all treatments have been carried out with contact control, which ensures accurate and as a consequences, optimal treatment. The high power density of light emitting diode ensures short treatment time.

Our study in the second experiment was designed to determine the influence of increasing power density of LED photobiostimulation on wound healing using a collimating lens.

The results of this study was less than that of the experiment number one, the explanation of the presented results could be due to the passage of LED through the chromatic lens; Although this lens increase the power density, it leads to separation of the three wavelengths to different points and minimizing the healing effect of LED.

Although the results of this experiment was satisfactory but it was less than the results of NASA which used LED for wound healing where the healing was very fast (about 48 hours), this may be due to:

(a) The wavelengths of NASA were (680, 730, 880nm) while the wavelengths of our experiment were (670, 730, 950 nm).

(b) The NASA used an achromatic lens to make the three wavelengths homogenous & simultaneously emitted and reach the wound area at one point but this advantage was absent in our experiment.

(c) The NASA used hyperbaric oxygen with LED during the treatment sessions.

(e) The advantage of NASA results also may be due to the difference of dosages, frequencies and durations of treatment.

The researchers reported that LEDT has a biostimulative effect and greatly enhance the natural wound healing process. Furthermore, improved wound healing will reduce the risk of infection, decrease the amount of costly dressings required N and more quickly return an individual to a preinjury level of activity.

(Conlan, MJ; Rapley et al. Biostimulation of wound healing. 23, 492- 496 (1996).

Optimal wavelengths of laser and LED light proven to speed wound healing include 680,730, and 880nm, which have a band width of 25nm.

(Abergel, R. P; et al. Biostimulation of wound healing by lasers experimental approaches in animal models and in fibroblast cultures; J. Dermal. Surg. On col 13, 127, 133 1997).

CONCLUSION

The results of the study demonstrated that the LED with three wavelengths (670, 730, 950 nanometer) simultaneously with contact control increase the wound healing in the rats.

The results also showed that LED has large spot size so LED is suitable for large wounds.

The results after using the lens (in the 2nd experiment) was not satisfactory, (undesired effect for healing).

The use of panthenol as a growth accelerators have no promising effect either alone or combine with LED.

Still, it is more or less unknown which wavelengths and doses are optimal. Simply, too little researches were done on the effects of LEDT.

It would be valuable to find out which parameters are good for what condition.

More researches are needed to optimize the parameters of LED for wound healing & also to know the exact biochemical effect on different cells during wound healing process.

N. B.

The results after treating the *human wounds* by LED were more effective especially in wounds (126). Some authors studied the effect of polychromatic light-emitting diodes (LED) in burn healing of non-diabetic and induced diabetic rats [128] ,they concluded that polychromatic LED significantly stimulate burn healing in the non diabetic rats than diabetic one.

*** Clinical human cases**

First case: Male 55 years, diabetic

Diagnosis: Ingrowing Nail of right big toe lead to Granuloma about 6months then eliminated surgically but still unhealed although he had treated by different traditional types of treatment. We applied LED Treatment for 24 days through 12 sessions (day after day) with power; 10mw, 8 minutes, contact technique and frequency 10kH

Second case:- Male 57 years old, diabetic patient.

Excised 2nd toe of the right foot after gangrene (Plate 6)

Treatment by LED during 25 days through 11 sessions (day after day) with power, 10mw, 8 minutes, contact technique and frequency 10kH during 7sessions and 2 kHz during 5sessions.

Third case:- Male 70years old

Diagnosis: acute burn by boiling water (9.3cm x 5.6cm).

Treatment by LED during 12 days through 6 sessions (day after day)with power,10mw, 2 minutes, contact technique and frequency 2.5Hz.

Forth case:- Female 33 years.

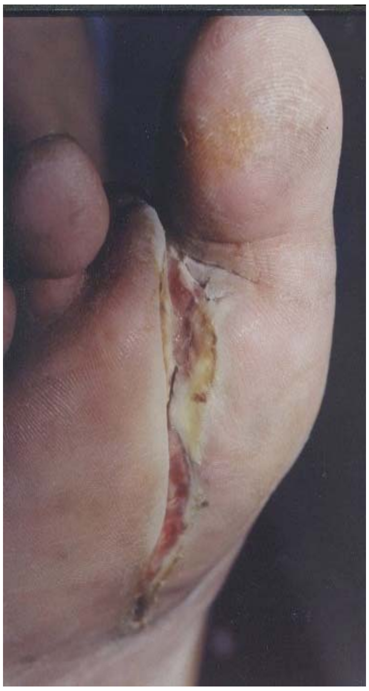
Leg ulcer at the dorsum of the foot (7.6cm x 8.8cm), diabetic, Vasculitis.

Treatment by LED during 40 days through 16 sessions,(2times/week);

Power 10mw, 12 minutes, contact technique and frequency 10 kHz.

The results of the clinical human cases

In the first case, (Granuloma) healed completely after receiving 12sessions of LED in 24 days. The second case (gangrene) healed about 90% after receiving 11sessions of LED within 25 days. The 3rd case (acute burn) healed completely after 6sessions within 12days. The 4th case, partially healed after 40 days, area of the ulcer decreased about 2cm in its diameter.



After the operation 10day.

After 25 days and 11sessions.

Plate (6)

Discussion

The results after treated the human wounds by LED were more effective specially in burns . Some authors studied the effect of polychromatic light-emitting diodes (LED) in burn healing of non-diabetic and induced diabetic rats [18] ,they concluded that polychromatic LED significantly stimulate burn healing in the non diabetic rats than diabetic one.

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