Study the Effect of Illumination Time Parameter of Diode Laser on Wound Healing Process

Assist. Prof. Dr. Munqith S. Dawood
Head of Medical Engineering Department
College of Engineering / Nahrain University

Assist. Lect. Saif Dawood Salman
Biomedical Engineering Department / Al-Khawarizmi College of Engineering/Baghdad University

Abstract

In this study we introduce a solution for wound healing controlling therapy by using a monochromatic pulse diode laser light 650 nm, 50% duty cycle and 1 MHz.

Many studies proved last years a positive effect of diode laser light application in wound healing process.

In this paper we study the effect of time illumination parameter on healing process. For this kind of therapy, a 650 nm diode laser used. Its original electronic circuit drive was improved to give pulsed output laser of 50% duty cycle, 1 MHz.

Twenty male mice, three months old were usd in this study (photo-biostimulation). The wound was made on bilateral back sides of each mouse, two sessions of illumination carried everyday for 12 and 18 minutes during 15 days time. This study was compared with our previous study set for 5 minutes for one session therapy in 1st and 2nd day of healing process.

Introduction

Low-level laser therapy (LLLT) of injuries or wounds is based on the application of some visible and near infrared lights to improve soft tissue healing and relieve both acute and chronic pain [1].

Light is transmitted through the skin's layers (the dermis, epidermis and the subcutaneous tissue or tissue fat under the skin) at all wavelengths in the visible range. However, light waves in the near infrared ranges penetrate deepest than of all light waves in the visible spectrum [2, 3].

The main reason for using the radiation in the red and NIR spectral region is the fact that hemoglobin does not absorb these wavelengths so light can penetrate deep into living tissue [1,4].

The effect of semiconductor laser was seen in the enhancement of tissue metabolism and stimulating the processes of regeneration and epithelization of tissue. Unlike the He: Ne laser, semiconductor laser exerts a less pronounced stimulating effect on the maturation of granulation tissue, at the same time its stimulating action on the protective force of the organism, including that on the immune and phagocytic systems is more pronounced. Besides the semiconductor laser capable of penetrating the tissue in depth about 7 mm [5, 6].

The LLLT has beneficial effects on tissue healing and pain relief, because it stimulates the production of basic fibroblast growth factor (BFGF), which supports fibroblast proliferation and differentiation. Fibroblasts irradiated by low dose LLLT show increase of cell proliferation and enhance the production of BFGF. Also LLLT increases the motility of human epidermal keratinocytes. [7, 8, 9].

This study used laser diode of wavelength (650 nm) because this wavelength is easily absorbed by the fibroblast and therefore have a good stimulatory effect for wound healing or superficial conditions. For deep injuries it has less efficiency [2].

Materials and methods

❖ Animals

Thirty male mice with age ranging between four to six months used in this study. The mice were kept in plastic cages; two animals per cage, they were housed and provided with normal standard diet and water, cages were cleaned daily.

They were divided into three groups, ten animals for each group. The first group was treated by CW diode laser, the second group was treated by pulse diode laser, and the third group was considered as control.

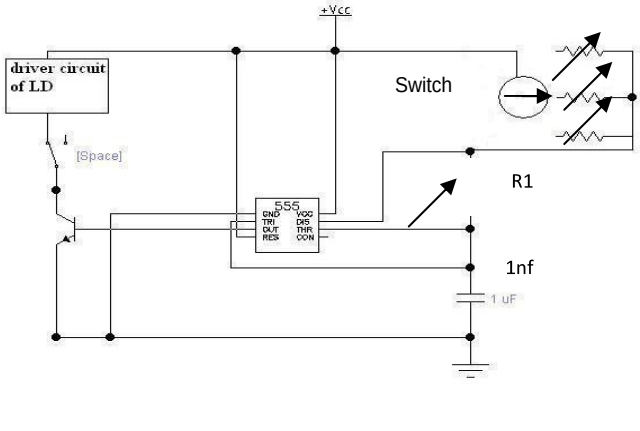
The backs of the animals were cleaned and shaved. Two wounds measuring about 3 cm in length were created at the end of the spinal column on the both sides of the back of each

animal. For the sake of histological examination, small piece of the wound area of 2×2 cm was taken by a sharp scissor. These pieces were divided into two parts, one was kept in a fixative and the other part was directly sectioned (after fixation) by using freezing microtome.

❖ Laser source

The study was performed by using pulse red diode laser. The laser was of 5 mW maximum output power and average power approximately 2.5 mW and 650 nm wavelength. The spot radius was about 5 mm (area=0.785cm^2). The power density was 3.185 mW/cm^2 for both treated groups. For group A, the energy was 30 J and the dose was 38.2 J/cm^2. For group B the energy was 45 J and the dose was 57.3 J/cm^2.

The internal electrical circuit of this CW laser was improved in the laboratory to supply pulse laser. The parameters of the pulse laser like pulse rate, duration and duty cycle may be changed. In this study the pulses were adjusted to be of maximum output average power of 2.5 mW, frequency 1 MHz and duty cycle 50%. The improved circuit is shown in the figure below



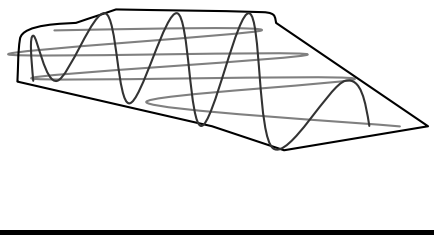
The improved circuit of the pulse diode laser driving circuit

❖ Treatment Experimentation

Both first (group A) and second (group B) of the wounds were irradiated by the pulses of low level diode laser. The third group was not illuminated by laser irradiation. It is the control group.

The first and second sets of mice were irradiated two times per day for 15 days after inducing injury; the first dose of irradiation immediately after inducing the wound and the second dose was given after 12 hours of inducing the wound. The laser beam was scanned on the wound's margins as shown in figure below for 12 minutes per session for the 1st set of mice (group A) and 18 minutes per session for the 2nd set of mice (group B).

The wound-healing rate was observed histologically daily for both the irradiated and control group at equal successive periods. The observations started from day one post irradiation till day 14 after inducing the wound.



Laser treatment of scanning path wound bed with single diode probe

❖ Histological examination

For histological study of the regenerative process of the wound tissue sample were cut from the wounded area of both irradiated groups (A&B) at equal successive period. The wound-healing rate was observed daily by taking samples from day one post irradiation till day 15 after inducing wound.

The back of the animals were cleaned and shaved, small piece of the wound area of 2×2 cm were taken by a sharp scissor. These

pieces were divided into two parts, one was kept in a fixative and the other part was directly sectioned (after fixation) by using freezing microtome.

Fifty serial sections per animal were obtained; those sections were arranged in special racks and were kept in the refrigerator for 24 hours. Then the sections were stained (after fixation) with Haematoxylin and Eosin staining.

Results

The results of LLLT in our study are shown in the following figures. These results and figures are selected out of 70 figures to show the rate of healing process. About 500 slides were prepared to follow the process of healing of these wounds by using frozen sectioning method.

Examining the slides of injury in day two after pulse diode laser treatment (group A) showed approaching and alignment of the edge of the wound with epidermis formation and inflammatory cells at the site of the wound induced everyday from inducing wound.

Re-epithelization took place on the surface of the wound and epidermal layer started to appear in group (A) between day 3 and day 4 with number of small hail follicles as shown in figure (1) and figure (2).

Complete wound healing, in group (A), with formation of continuous dermal layer of their epidermis and appearance of hair follicles with no dermal reaction seen at the 5^{th} and 6^{th} day of treatment. Collagen fibers and some of skin appendices can be also seen in this stage as shown in figures (4, 5).

While in day 5 for group (B), the re-epithelization took place but still there are inflammatory cells infiltrating the area of the wound, as shown in figure (3). On the other hand the healing process continued by its owns in the control group, where the basal layer of the epidermis started to appear just at day 9 after injury, that the basal layer of the epidermis being formed bellow the crust and inflammatory cells still infiltrating the dermal area.

While in group (B), the dermal papilla starts to appear at day 8 and 9 and small hair follicles could be seen as shown in figures (6, 7).

On the other hand for control group the dermal papilla starts to appear just at day 14 and also still no hair follicles could be seen at this point

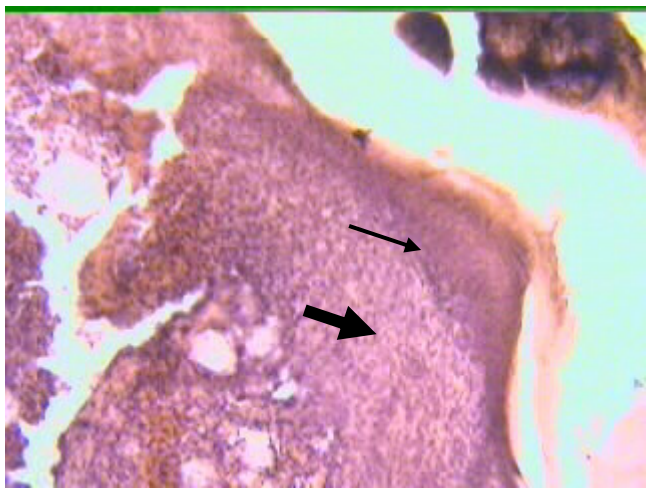


Figure (1): epidermis formation at the site of injury with inflammatory cell reaction still taking place at the dermis (DAY 3 - pulse DL - group A - H&E - × 50, frozen section).

Figure (2): site of wound after healing ↑ and starting of appearance of small hair follicles at wound site (DAY 4 - pulse DL - group A - H&E - × 50, frozen section).

Figure (3): complete healing of the epidermis with keratin formation and crust and small hair follicles can be seen at the site of injury ↑. Dermis still shows some inflammatory cell ↑. (DAY 4 - pulse DL - group B - H&E - ×50, frozen section).

Figure (4): complete wound healing at day 5 with small hair follicles and thin epidermis with few collagen fibers (DAY 5 - pulse DL - group A - H&E - × 50, frozen section).

Figure (5): complete wound healing at day 6 with no scar formation with small hair follicles and thin epidermis with no scar formation (DAY 6 - pulse DL - group A - H&E - × 50, frozen section).

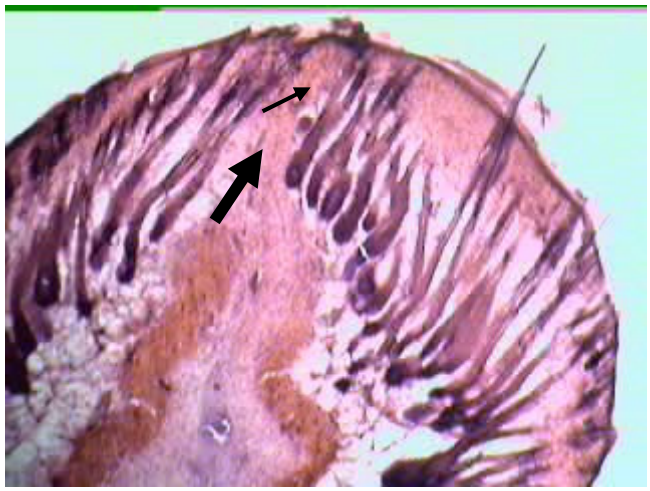


Figure (6): site of wound after healing and starting of appearance of hair follicles and very thin scar can be seen (DAY 6- pulse DL - group B - H&E - × 50, frozen section).

Figure (7): complete wound healing at day 8 with many hair follicles and thin Epidermis (DAY 8 - pulse DL - group B - H&E - × 50, frozen section).

Discussion

In our study we have found that the healing process started by clot formation at the site of wound. In regards for wound contraction, it was found that the group (A) of wounds seems to contract more than that in group (B) as shown in figures (2 and 3).

The daily examination of healing process of both groups shown that after 72 hours of injury the wounds showed closed and formation of epidermal layer.

In a study done by Hopkins *et al.*, 2004, [10]. It was found that LLLT is an effective form of treatment for enhancing wound contraction of partial thickness type. And continued for the first four days, after which wound surface area showed remarkable reduction. All types of inflammatory cells started to infiltrate the wound at day two on ward in treated groups while at day three in control group.

Re-epithelization and epidermal formation started at the third day onward and complete layers of a thin type of skin was able to be seen by day five in group (A), while a complete layer of skin closing the wound surface was seen in group (B) at days 4-5 as in figure (1, 2 and 3).

In control group we did not see the crust till day 7 with formation of the basal layer of the epidermis as shown in our previous study.

Re-epithelization and epidermal formation started for day three onward and complete layers of a thin type of skin was able to be seen by day five in pulse laser diode (LD) treated group, while a complete layer of skin closing that wound surface was seen in CW LD treated group by day 7-9, while in control by day 14 [11].

Bisht *et al.*, 1999 [5], which used He:Ne laser on wound irradiated daily for 5 min and found early epithelization and increased fibroblastic reaction in the irradiated wounds.

New small hair follicles started to appear at days 3-4 in group (A) as in figure (2) and complete healing process with the appearance of new hair follicles was in day 6 as shown in figure (5), while in group (B) the appearance of hair follicles was delayed till day 4-5 as shown in figure (3). For control group no hair follicles seen till day 14.

In group (A), the complete wound healing with formation of continuous layer of epidermis and skin appendages was seem by 6^{th} day, while in group (B) it was seen after days 8-9. This indicates that group (B) needs longer time for wound healing, properly due to its lower effects on cell proliferation and vascularization.

Complete wound healing with formation of continuous layer of epidermis and skin appendages was seem in pulse laser diode treated group by days 9-12, while in continuous laser diode treated group seen after days 14-16. This indicates that continuous laser therapy group has a delayed period in wound healing properly due to its decrease affects of cell proliferation and vascularization [11].

In our study the scar formed in group (A) animals was small and fine with fully regenerated area both in epithelial layer and skin appendages and disappear at day 6, while in the group (B) it seems to be larger with smaller number of hair follicles at the area and disappear at day 8-9. For control group the scar seems even larger than the laser treated group.

The thermal injury to the surrounding normal tissue produced by the laser increase the risk for scar formation, and this injury may be reduced with pulse Co2 laser in a study done by Garrett *et al.*, 2002 [12]. Histological comparison showed less thermal injury and scar formation in the vocal folds treated with the pulse Co2 laser than with the CW Co2 laser [12].

Conclusion

From the results we found out that the pulse diode laser of 680 nm wavelength, 50% duty cycle and pulse repetition frequency 1MHz has more positive effect on enhancing the process of wound healing. The wound healing process needs 3 days longer for group (B) which was illuminated for 18 minutes per session than group A which was illuminated for 12 minutes per session and 11 days longer for the control group of wounds was found.

The hair follicles in group (B) need two days more than in group (A), while the control groups need seven days more for the hair appearance.

No scar formation after the healing process in treated groups of wounds. The effect of laser therapy is biostimulated effect rather than heating effect of the biological cells.

References:

1. Al-Magsosy R.A., "design and construction of a system to study the effect of laser radiation on the absorbtion of antibiotics in blood", Engineering college in Al-Nahrain University, M.Sc. thesis, 2005.
2. Herascu N, Velciu B, Calin M, Savastru D, Yalianu G "Low Level Laser Therapy LLLT Efficacy in Post-Operation Wounds" Photomed Lase Surg.,23(1):2005.
3. Walsh L.J., “Soft tissue applications”, Australian Dental Journal, 42 :(4):247-54, 1997.
4. Joseph Niamtu, III. DDS, "Clinical Applications of the 532nm Diode Laser fot the treatment of Facial Telangiectasia and Pigmented Lesions: Literature Review, History, and Discussion of clinical Experience", The American Journal of Cosmetic Surgery, Vol.18, No. 2, 2001.
5. Bisht D., Mehrtra R.,Singh P.A. "Effect of Helium-Neon Laser on Wound Healing", Indian Journal of Experimental Biology 37(2): 187-189 1999.
6. Husegin D., Balag H. And Mehmet K. "A Comparison Study of The Effects of Electrical Stimulation and Laser Treatment on Experimental Wound Healing in Rats", Journal of Rehabilitation Research and Development, 2004.
7. Hopkins JT, McLoda TA, Seegmiller JG and David Baxter G. "LLLT

Facilities SuperficialWound Healing in Human", Ath1 Train, 2004.
8. Laurence Rosenberg, MD, " Wound Healing and Growth Factors" University of Alabama at Birmingham Hospital, 2004.
9. Kandela S. A., Kantrajian A. H., "Laser light in enhancing wound healing rate", Journal of Nahrain University, Vol. 2 (4), 1998.
10. Hopkins JT, McLoda T A, Seegmiller J G, and David Baxter G., "LLLT facilitates superficial wound healing in human", Athl Train, 2004.
11. Saif Dawood Salman, Munqith S D, May F Al-Habeeb, " A HISTOLOGICAL STUDY OF THE EFFECT OF LOW LEVEL LASER THERAPY ON WOUND HEALING", Engineering college in Al-Nahrain University, M.Sc. thesis, 2006.
12. Garrett CG, Reinisch L, "New generation pulsed carbon dioxide laser: comparative effects on vocal fold wound healing", Ann Otol Rhinol Laryngol, Jun; 111(6): 471-6, 2002

دراسة تأثير عامل مدة الإضاءة على عملية شفاء الجروح باستخدام ليزر ثنائي الوصلة

أ.م.د.منقذ سليم داود
رئيس قسم الهندسة الطبية
كلية الهندسة – جامعة النهرين

م.م. سيف داود سلمان
قسم هندسة الطب الحياتي
كلية هندسة الخوارزمي – جامعة بغداد

الخلاصة:

أثبتت الدراسات المختلفة في السنوات الأخيرة التأثير الايجابي لضوء الليزر ثنائي الوصلة في شفاء الجروح.

في هذا البحث تمت دراسة عامل مدة الإضاءة على عملية شفاء الجرح باستخدام ليزر ثنائي الوصلة بطول موجي ٦٨٠ نانومتر حيث تم تغيير دائرته الالكترونية الأصلية للحصول على نبضات ذات ٥٠%دورة عمل و تكرار ١ ميكاهرتز.

استخدم لهذا الغرض عشرون فأراً بعمر ٣ أشهر لدراسة تأثير التحفيز الضوئي على عملية شفاء الجروح التي استحدثت على ظهر كل فأر من الجهتين و عرضت للإضاءة بالليزر يومياً لمدة ١٢، ١٨ دقيقة لحين شفاء الجرح تماماً و قورنت نتائج هذا البحث مع دراسة أخرى سابقة لنا تم فيها الإضاءة باستخدام نفسه لمدة ٥ دقائق ليومين فقط بعد حصول الجرح و حصلنا على نتائج ايجابية في تسريع شفاء الجروح.