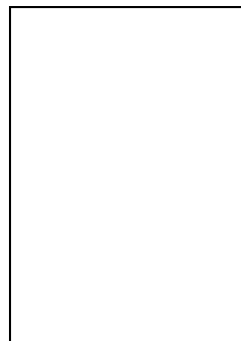


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A Histological Study of The Effect of Low Level Diode Laser Therapy on Wound Healing

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Abstract

In this study we show an approach for wound healing process based on using of low level laser therapy, in order to enhance the healing rate of wounds. The low power laser (LPL) has been introduced also to solve problems associated with scar formation after completing the healing process.

In this study the wound healing process rate was enhanced by using the illumination of 650 nm, 5 mW diode laser in CW and 2.5 mW maximum average power pulse modes with 1 MHz frequency and 50% duty cycle.

The low power laser light biostimulates the wounded area which was made on the back of many mice. The healing process was histologically studied during fourteen days to find out the differences between the laser illuminated groups of wounds and other group of wounds (control).

From the results, we found out that the pulse diode laser is more efficient than continuous diode laser in enhancing the wound healing process within shorter time.

Keywords: Laser Diode

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 both CW and pulse red diode laser. The CW laser was of 5 mW maximum output power and 650 nm wavelength. The spot radius was about 5 mm. The internal electrical circuit of this laser was improved in the laboratory to supply both CW and 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%.

Treatment Experimentation

The first group the wounds were irradiated by the continuous low level diode laser. The second group was irradiated by

pulses of the same 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; the first dose of irradiation immediately after inducing the wound and the second dose was given after 24 hours of inducing the wound. The laser beam was scanned on the wound's margins as shown in figure (1) for 5 minutes.

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.

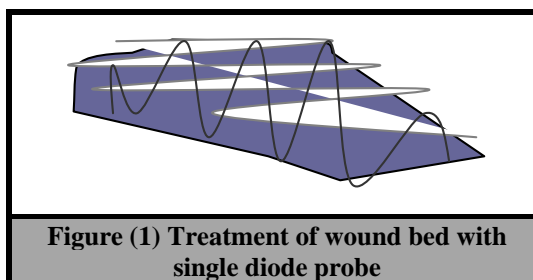


Figure (1) Treatment of wound bed with single diode probe

Results

The results of LLLT in our study are shown in the following figures. These results and figures are selected out of 25 figures to show the rate of healing process. About 200 slides were prepared to follow the process of healing of these wounds by using frozen sectioning method.

Examining the slides of injury in day one after pulse diode laser treatment showed approaching and alignment of the edge of the wound induced a day before. The site of injury showed clot and crust formation in day one and day two with decrease in the area separating between the two edges of the wound. The distance between the two edges was measured. In the pulse laser treatment it was about 2.7 mm as shown in figure (2), while in figure (3) for CW treatment the distance was wider (4.8 mm) for the first two days. For control group, the distance between the two edges about 4.9mm as in figure (4). Re-epithelization took place on the surface of the wound and epidermal layer started to appear in

pulse diode laser treated group between day 7 and day 8 with number of small hair follicles as shown in figure (5). While in day 7 for CW treated group, the re-epithelization took place but still there are inflammatory cells infiltrating the area of the wound, as shown in figure (6). On the other hand the healing process continued by its own in the control group, where the basal layer of the epidermis started to appear just at day 9 after injury, figure (7) shows the basal layer of the epidermis being formed below the crust and inflammatory cells still infiltrating the dermal area.

Complete wound healing, in the pulse diode laser treated group, with formation of continuous dermal layer of their epidermis and appearance of hair follicles with no dermal reaction seen at the 9th day of treatment. Collagen fibers and some of skin appendices

can be also seen in this stage as shown in figure (8).

While in the CW diode laser treated group, the dermal papilla starts to appear at day 12, but still no hair follicles could be seen yet as shown in figure (9).

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 as shown in figure (10).

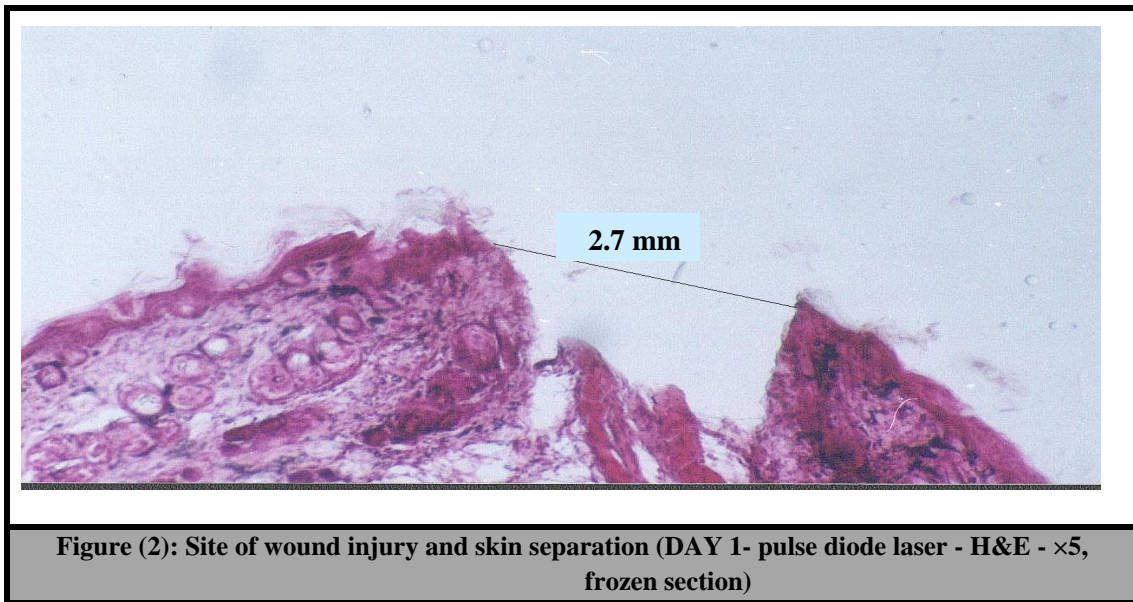


Figure (2): Site of wound injury and skin separation (DAY 1- pulse diode laser - H&E - ×5, frozen section)

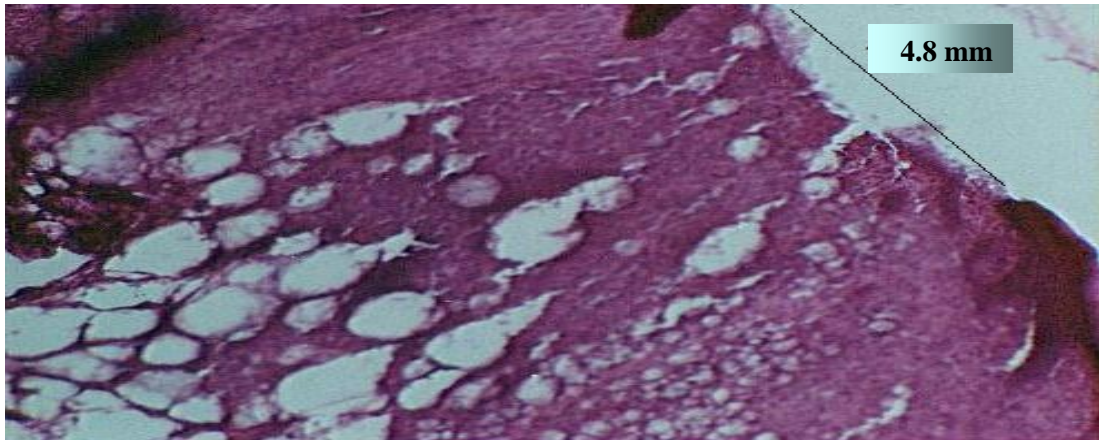


Figure (3): Site of wound showing the two edge of skin after injury (DAY 1- Continuous diode laser - H&E - $\times 50$, frozen section)



Figure (4): Site of the wound showing a wide defect, and separation between the two edges (DAY 3 - Control - H&E, frozen section).

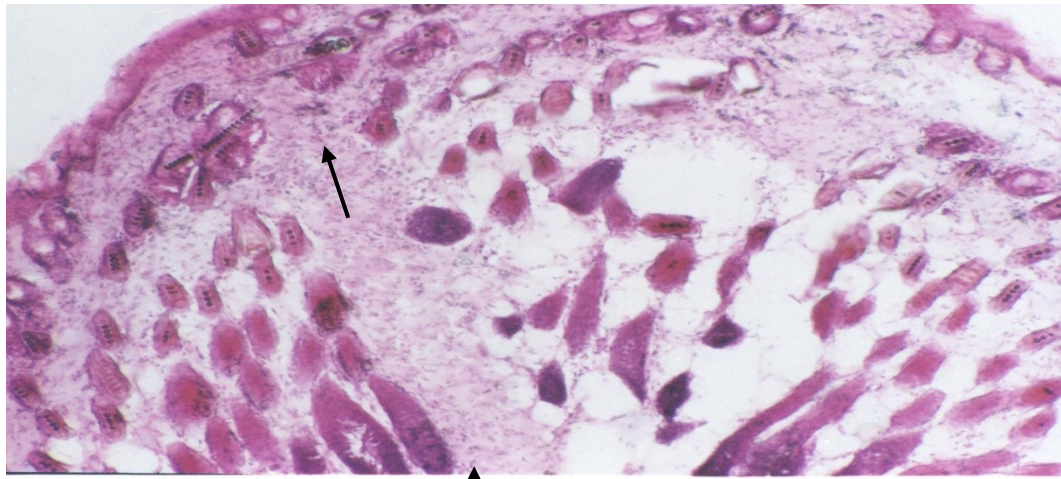


Figure (5): site of wound after healing and starting of appearance of small hair follicles (DAY 7 - pulse DL - H&E - $\times 50$, frozen section).

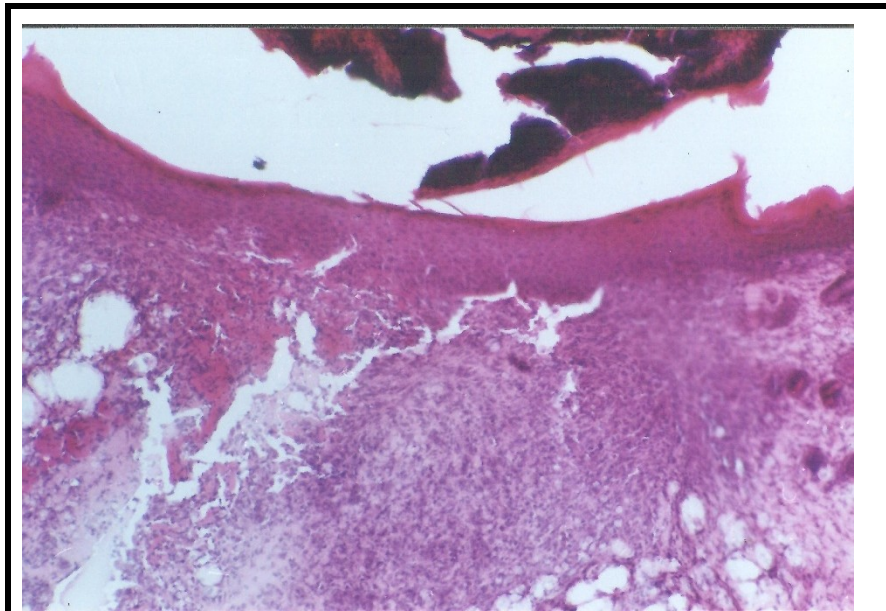


Figure (6): epidermis formation at the site of injury with inflammatory cell reaction still taking place at the dermis (DAY 7 - CW DL - H&E - $\times 50$, frozen section).

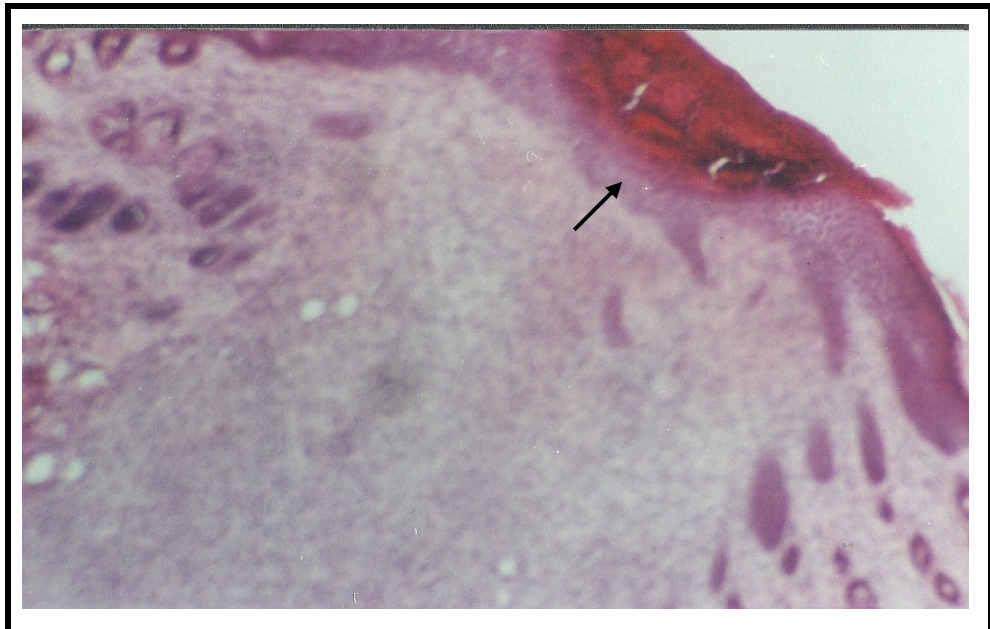


Figure (7): site of injury showing formation of the basal layer of epidermis below the crust (DAY 9 - control - H&E, frozen section).

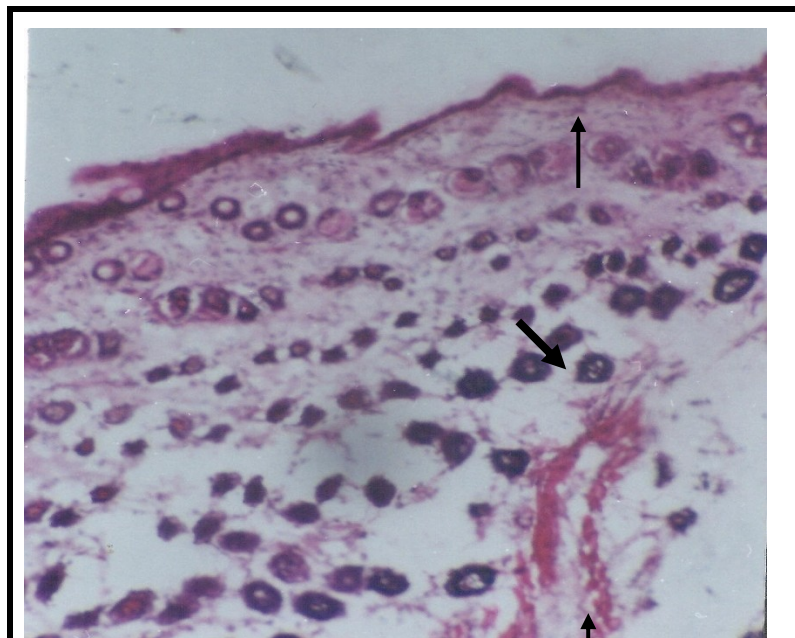


Figure (8): complete wound healing at day 9 with small hair follicles and thin epidermis with few collagen fibers (DAY 9 - pulse DL - H&E - × 50, frozen

section)

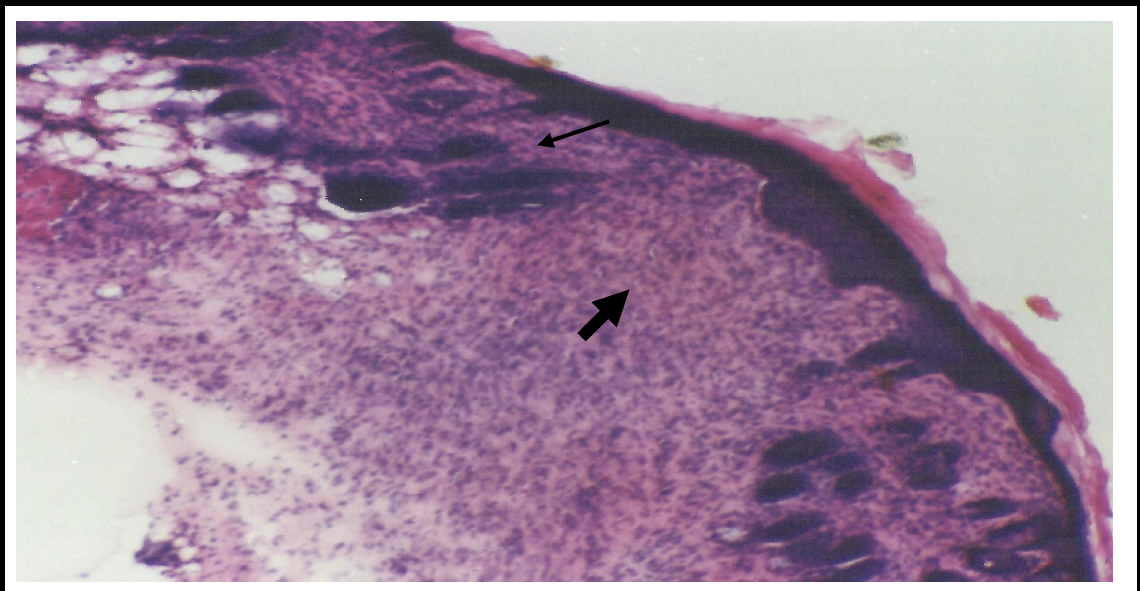


Figure (9): complete healing of the epidermis with keratin formation and no hair follicles can be seen at the site of injury ↑ Dermis still shows some inflammatory cell ↑ . (DAY 12 - CW DL - H&E - ×50, frozen section).

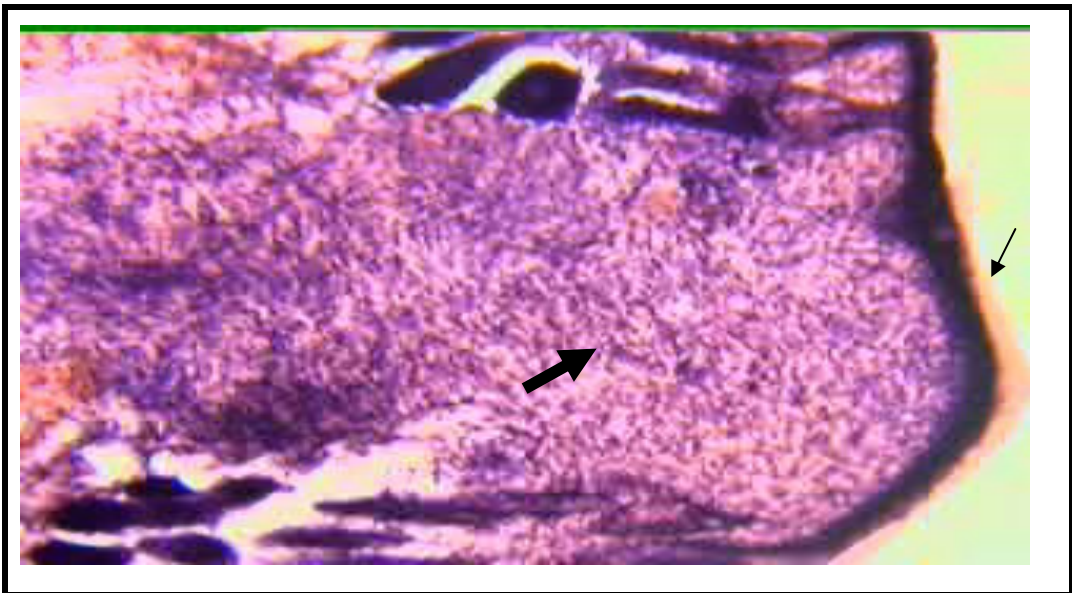


Figure (10): complete healing of the epidermis with keratin formation and no hair follicles can be seen at the site of injury. Dermis still shows some inflammatory cell . (DAY 14 - control - H&E - ×50, frozen section).

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 of wounds treated by pulse diode laser seems to contract more than that treated by continuous diode laser as shown in figures (2 and 3). We found that the clot and crust formation seems to need longer period in continuous diode laser treated group than in pulse diode laser treated group. Wound contraction seems to be not affected by the exposure of low output energy He:Ne laser radiation in a work done by Kandela and Karrarjian, 1998, [6]. Both treated and nontreated animal was followed and found that 36 hours after injury the wounds showed increase in surface area.

Both treated and nontreated animals were examined daily. We found that after 36 hours of injury the wounds showed increase in surface area.

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 pulse diode laser treated group, while a complete layer of skin closing the wound surface was seen in CW diode laser treated group at days 7-9 as in figure (6). In control group we did not see the crust till day 7 with formation of the basal layer of the epidermis as in figure (7).

Bisht *et al.*, 1999 [1], 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 7-9 in the pulse laser treated group as in figure (5) and complete healing process with the appearance of new hair follicles was in day 9 as shown in figure (9), while in the continuous diode laser treated group the appearance of hair follicles was delayed till day 12 as shown in figure (8). For control group no hair follicles seen till day 14 as in figure (10).

In pulse diode laser treated group, the complete wound healing with formation of continuous layer of epidermis and skin appendages was seen by days 9-12, while in continuous diode laser treated group seen after days 14-16. This indicates that continuous

laser treated group need longer time for wound healing, properly due to its lower effects on cell proliferation and vascularization.

In our study the scar formed in pulse diode laser treated animal was small and fine with fully regenerated area both in epithelial layer and skin appendages, while in the group treated by continuous diode laser it seems to be larger with smaller number of hair follicles at the area. 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 [7]. 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.

Conclusion

This study presents the usage of LLLT in treatment of skin wounds, by injuring the backs of the mouse, then treating them by low level laser diode radiation applied on the wounds for 5 minutes from distance 0.5 cm away from the wounds. The spot size was 1 cm². From the results we found out that the pulse diode laser has more positive effect than the continuous diode laser on enhancing the process of wound healing. The wound healing process needs 5 days longer for continuous diode laser treated group and 7 days longer for the control group of wounds. The hair follicles in CW laser treated wounds need four days more than pulse laser treated wounds, while the control groups need seven days more for the hair appearance. The scar formation after the healing process is the lowest in pulse treated groups of wounds. The effect of laser therapy is biostimulated effect rather than heating effect of the biological cells.

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

الوصلة

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الخلاصة:

أثبتت الدراسات المختلفة في السنوات الأخيرة التأثير الإيجابي لضوء ليزر 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 تمت تغيير دائرته الإلكترونية الأصلية للحصول على نبضات ذات ٥٠% دورة عمل وتكرار ١ كيلوهرتز.

أستخدم لهذا الغرض عشرون فأراً بعمر ثلاثة أشهر لدراسة تأثير التحفيز الضوئي على عملية شفاء الجروح التي استحدثت على ظهر كل فأر من الجهتين وعرضت لإضاءة بالليزر يومياً لمدة ٥، ١٢، ١٨ دقيقة لحين شفاء الجرح تماماً وقورنت نتائج هذا البحث مع دراسة أخرى سابقة لنا تمت فيها الإضاءة باستخدام الليزر نفسه لمدة خمس دقائق ليومين فقط بعد حصول الجرح وحصلنا على نتائج إيجابية في تسريع شفاء الجرح.

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