

# **Wound Healing as Well as Fibroblasts and Neutrophil Numbers in a Skin Exposed to Infrared and Electrical Stimulation**

*Shaheen E. Jaafar*

*Department of Medical Laboratory, College of Technical - Kirkuk*

Accepted:2011/3/8, Received:2010/6/1

## **Abstract**

**Objective:** To investigate wound healing as well as fibroblasts and neutrophil numbers on a limited skin field which is previously exposed to combined Infrared and Electrical stimulation.

**Methods:** 20 male rabbits were randomly assigned to two groups each one of 10 animals. The experimental group was exposed to combine Infrared (IR) and Electrical Stimulation (ES) at dorsal area for three days respectively, in day four an incision was made in the center of exposed skin then sutured. A biopsies were taken at 24,48,72 hours, week and two weeks after surgery for counting of fibroblasts and neutrophil numbers as well as for histological observation. The same procedure was made for control group without exposing to radiation and stimulation.

**Results:** There was statistically significant difference in fibroblasts and neutrophil numbers between two groups ( $p < 0.01$ ). The number of these cells were higher in experimental group than control in all periods. Histological observation revealed good orientation of fibroblasts and collagen fibers in experimental group as well as excellent wound healing.

**Conclusion:** This study suggested that application of IR combined with ES on limited skin field have beneficial effects on fibroblasts and neutrophil numbers of a wound created in same exposed field with good wound healing.

## **Introduction**

The use of Infrared (IR) radiation in the treatment of variety of medical conditions has been studied for long time (Abou-Hala *et al.*, 2007). IR radiation is that invisible portion of the electromagnetic spectrum adjacent to the long wavelengths, or red end, of the visible light range that extend up to the microwave range (Hideyoshi, 2003).

This phototherapeutic phenomenon has been reported particularly by physicians performing phototreatment of poorly healing wounds. Regeneration and microcirculation processes are stimulated not only in the irradiated lesions, but are also seen in distant non-irradiated lesions in the same patient; simultaneously, improvement of patients' immune, metabolic and hormonal status can be registered. Local changes are usually associated with the direct action of light on skin cells.

The systemic mechanisms of photobiomodulation remain, however, non-elucidated. (Samoilova *et al.*, 2004). Radiation therapy has profound effects, both acute and long-term, on skin and connective tissues. Radiation therapy also affects the time course and end result of wound healing, and the risk of postoperative complications (Wang *et al.*, 2006). It has been reported that wounds combined with whole body irradiation heal slowly, but the mechanisms are not fully clarified (Qu *et al.*, 2004).

On the other hand, electrical stimulation of very low amplitude and frequency modulation has become an increasingly popular treatment modality. This form of stimulation has been referred to as microamperage electrical stimulation (MES). MES is defined as stimulations with a very low frequency (1 Hz or less) and low intensity or amplitude (1–1,000  $\mu$  A) (Mohammad, 2006), in addition, numerous morphological and functional effects of electric stimulation have been identified, both at the cellular and at the tissue level (Sumano *et al.*, 2002). Surface stimulation studies have shown that Electrical Stimulation (ES) can produce positive short-term changes in tissue health variables such as regional blood flow and pressure distribution (Bogie *et al.*, 2000). Many aspects of treatment with ES have been studied. Several randomized controlled trials (RCTs) have evaluated ES with varying protocols using different currents and voltages for the healing of pressure sores, venous stasis ulcers, arterial ulcers, surgical wounds, and diabetic foot wounds (Hayes, 2003). Radiation, however, impairs wound healing (Schaffer *et al.*, 2007). Many studies have showed negative effects of radiation such as gamma ray and external beam radiation of wound healing when body or limited part of body exposed to these radiations (Qu *et al.*, 2004; Dubin *et al.*, 2000). No one study to date however examined the beneficial or harmful effect of IR or ES or combination between them on healing of wound in field that previously exposed to these stimulators. The investigation into the effect of combined IR and ES on local skin field to the healing of wounds that are created in a same exposed field, as well as on fibroblasts and neutrophil numbers was the main goal of this study.

## **Material and methods**

We used 20 male rabbits, weighing mean of (1,500-1,900), with age 5-6 months. Animals were housed individually with access to food and water for a week before trial. All animals injected with penicilline streptomycine 0.75 ml\ day for three days as a protective dose.

## **Exposure and surgical technique**

The dorsal skin area of 10 rabbits clipped and shaved ( about 10 cm<sup>2</sup>) the field was exposed to an infrared radiation (IR) for 5 minutes, the distance between IR lamp and the field was approximately 35cm, and the

exposure area was 5x5cm<sup>2</sup>. Immediately after that an electrodes were placed on sterile pads moisted with water and placed on shaved area, we applied electrical stimulation(ES) at frequency 0.5 Hz current intensity for 5 minutes, this procedure was applied for 3 days frequently\*. After 24 hr from last application of combined IR and ES, the animals were anesthetized with 50 mg/kg ketamine hydrochloride mixed with 35 mg/kg xylazine intramuscularly. The area that have been received the stimulators was sterilized by a pad of cotton immersed in ethanol alcohol (70%) for 5 minutes, the pad removed and the area swabbed with povidone iodine for further sterilization. An incision about 3cm was made in the centre of exposed skin area then sutured using interrupted suture pattern. The wound covered with sterile bandage for protection. The same surgical procedures were made in control group (10 rabbits) without application of the stimulators.

### **Biopsy collection**

The biopsy collected at 24,48,72 hours, week and two weeks after the surgery both in experimental and control group (two animals for each period). The biopsies fixed in 10% formalin solution at least 24hr for tissue processing (clearing, infiltration and embedding in paraffin wax), the sections of block were done in microtome for cutting about 5 microns, the slides stained with hematoxyline-eosin for histological examination.

### **Counting technique**

Counting of neutrophils and fibroblasts was expressed per microscopic field. The number of these cells were counted in 10 microscopic fields for each specimen using ALTAY microscope at magnification of 40x.

### **Statistical analysis**

To make comparison between experimental and control group in cell numbers and also to observe the differences of cell numbers during different periods, we used F test with ANOVA table.

## **Results**

### **Statistical analysis of cells**

The mean number of fibroblasts and neutrophils were higher in experimental group than those in control, and by using (F test) relying on ANOVA table, there was a significant difference in fibroblasts and neutrophil numbers between experimental and control group at  $p < 0.01$  at all periods 24, 48, 72hrs, week and two weeks. (Table1 and 2).

**Table (1): ANOVA (for fibroblasts number and different periods)**

**A=Group factor with two levels (experimental and control).**

**There is a significant differences between the levels of factor A,**

**\*\* p <0.01**

**B= Period factor with five levels (24,48,72 hours, week and two weeks).**

**There is a significant differences between the levels of factor B,**

**\*\* p <0.01**

s.v	d.f	s.s	M.S
Total	239	11248.9	—————
	9	4379.4	—————
treatment	1	1825.6	1825.6 **
A	4	1764.3	441.1**
B	4	789.5	197.4
A x B	230	6869.6	
Error			

**Table (2): ANOVA (for neutrophils number and different periods)**

**A= Group factor with two levels (experimental and control).**

**There is a significant differences between the levels of factor A,**

**\*\* p <0.01**

**B= Period factor with five levels (24,48,72 hours, week and two weeks).**

**There is a significant differences between the levels of factor B,**

**\*\* p <0.01**

s.v	d.f	s.s	M.S
Total	239	1502.8	—————
	9	941.5	—————
treatment	4	228.4	228.4 **
A	1	477.7	119.4**
B	4	235.4	58.8
A x B	230	56.3	2.4
Error			

s.v = source of variation

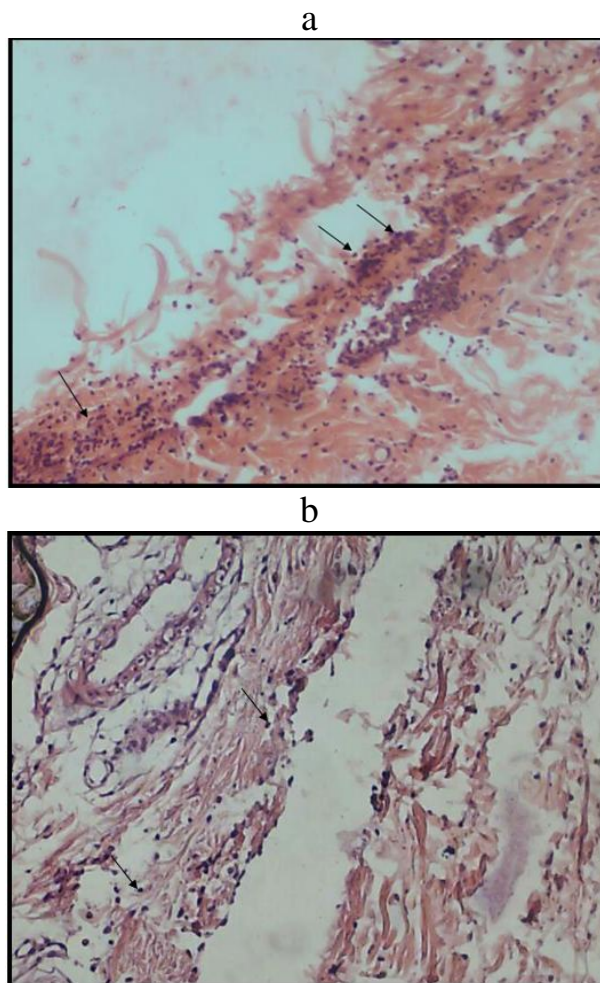
d.f = Degrees of freedom

s.s = sum of square

M.S = Mean square

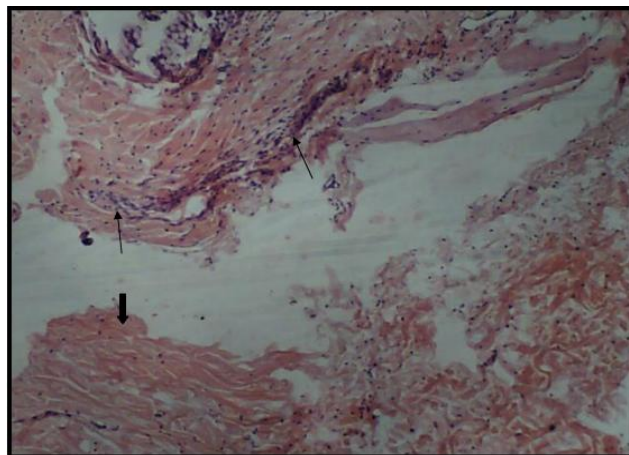
### **Histological observations**

At 24, 48 and 72 hours, histological evaluation revealed the inflammatory phase during the first three days after surgery with the culmination between one and two days in both groups but the appearance of neutrophils in experimental group in the margin of incision was more obvious than control. Fig.(1).



**Fig. (1): Skin wound 24 hours (H&E, 10x). Showing the infiltration of neutrophils to incisional area. Marked neutrophils number in experimental group (a) more than control group(b). (arrows)**

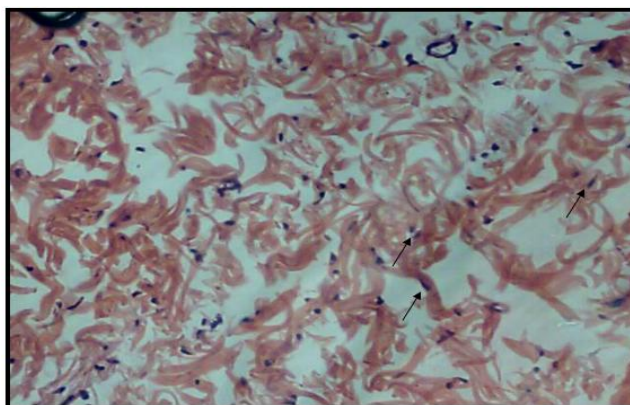
By one day after surgery only a limited number of fibroblasts were present near the incisional space in both groups. However, two days after surgery fibroblasts were increased in number near the incisional space. Proliferation of fibroblasts and new endothelial cells with characteristic circular nuclei, which forms granulation tissue, was found in this time interval. At 48 to 72 hours appearance of young fibroblasts in experimental group started with disoriented collagen fiber product in the margins of the incision and fibroblast number were more than in control group at the same periods. (Fig. (2)).



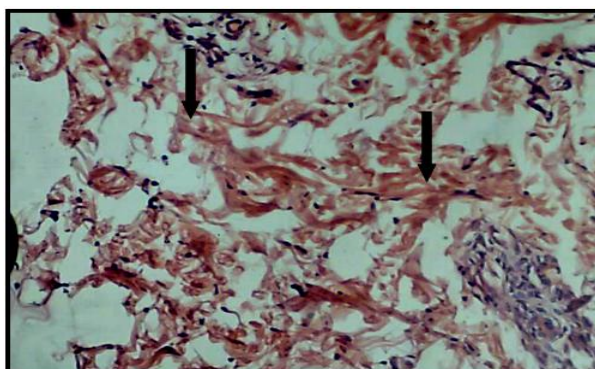
**Fig.(2): Skin wound in experimental group after 72 hours (H&E, 10x). There are infiltration of neutrophils (thin arrows), with increasing in fibroblasts cell (thick arrow)**

After week the collagen fibers become more abundant and more clear and density in experimental group compared with control group. (Fig 3,4).

a

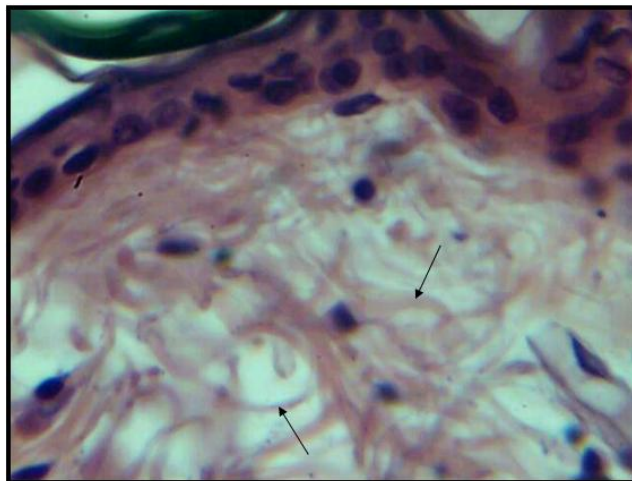


b



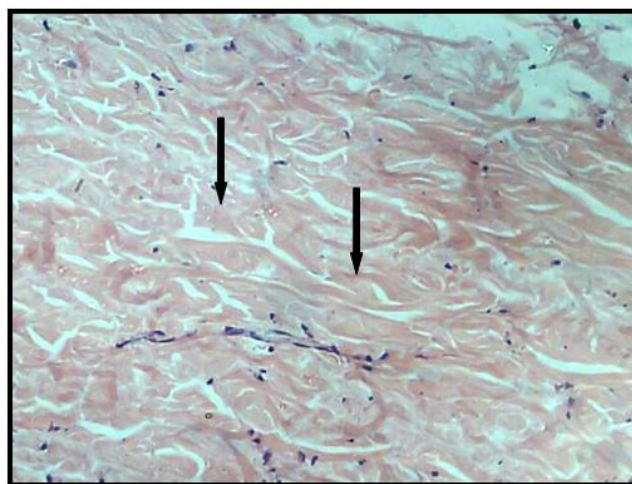
**Fig .(3): Skin wound after one week (H&E, 40x). Showing the density and well orientation of collagen fibers in experimental group(a) (thin arrows), while collagen fibers in control group are less density with less orientation (b)(thick arrows).**





**Fig. (4): Skin wound of experimental group after one week (H&E 100x).Showing collagen fibers formation and fibroblasts cell. (arrows)**

Continuous accumulation of collagen, proliferation of fibroblasts were found in experimental group at two weeks and was more obvious than control group Fig.(5) and the leukocyte infiltration decreased, vascularity are disappeared. The number of neutrophils lowered at week and two weeks which replaced by fibroblasts.



**Fig.(5): Skin wound in experimental group after 2 weeks. (H&E 40x). There are tidily orientation of collagen fibers with abundant density (arrows).**

## **Discussion**

In a study by (Sachik *et al.*, 2006) who used infrared irradiation on dorsal rat skin in different doses and then created wounds in same skin area, he observed activation of inflammatory cells, fibroblasts as well as enlargement of fibroblasts and collagen fibers, the same results that we observed by using infrared with electrical stimulation.

Because of many studies have been used combination between two stimulators and the results were significant, such as combination between

visible light and IR , microwave and IR and between ultrasound with certain light wave lengths (Samoilova *et al.*, 2004; Sumano *et al.*, 2002& Schramm *et al.*, 2003), also because the ES can influence the proliferative and \or migratory capacity of epithelial and connective tissue cells and can affect orientation, migration and proliferation of cells, which are key importance for healing, such as fibroblasts and kiratonocytes (Sumano *et al.*, 2002; Robinson, 1985; Sheridan *et al.*, 1996 & Pullar *et al.*, 2001), we decided to combine between these stimulators (ES and IR).

No one study applied infrared combined with electrical stimulation to observe their biological effects on tissue, therefore we used minimum electrical stimulation pulse(0.5 Hz) for 5 minutes exposure time, and we also select short exposure time (5 minutes) of IR in each session, concerning about the action of each other.

Local changes are usually associated with the direct action of light on skin cells that their biostimulatory effect specially activating neutrophils and fibroblasts which they can perceived as heat by specialized nerve endings known as thermo receptors in the skin (Hideyoshi, 2003; Samoilova *et al.*, 2004 & Ju *et al.*, 2006) showed that infrared radiation may have proliferative effects of fibroblasts in either an in vivo or in vitro environment with beneficial effect on skin texture, and (Samoilova *et al.*, 2004) said that irradiation of a small area of the human body surface with visible or infrared (IR) laser light causes the development of both local and systemic effects. The usual duration for using infrared irradiation in the physiotherapies often 15-20minutes, cutaneous and subcutaneous temperature rise to a peak value during the first 5minutes of heating. By the time that the temperature of deeper tissues was raised to an effective level ,the superficial tissues would be damaged (King, 1982), therefore our exposure time of IR was 5 minutes in each session.

Some studies have been demonstrated the mechanism of action of ES and also IR on tissues. (Biedebach, 1989) demonstrated the action of ES which transmembrane currents open voltage-controlled calcium channels in fibroblast cell, causing ATP resynthesis, activation of protein kinase mechanisms of synthesize new cellular protein and the DNA replication necessary for mitotic cell division. While (Lubart *et al.*, 1997; Karu, 1988) said that once light at appropriate dose absorbed, the energy is stored as ATP, the form of energy that cells can use. The ATP produced may be used to power metabolic process, synthesize DNA, RNA, protein, enzymes, and other biological materials needed to repair or regenerate cell and tissue component. Depending on these mechanisms we can explain the affirmative effect of these stimulators on living tissue,in our opinion these energies have been stored as ATP and then contributed on increasing cell



numbers (neutrophils and fibroblasts) and subsequently enhanced wound healing.

Disagreement with our study, in a skin injury after whole body irradiation, a data of in a vivo showed that the amount of tissue repairing cells such as fibroblasts and macrophages, in wounds combined with whole body irradiation was significantly less than that of simple incision. (Sumano et al., 2002) mentioned that the result of direct fibroblast count showed that the quantity of fibroblasts from wounds combined with whole body irradiation (WBI) was significantly less than those from simple incision suggested that WBI had damaging effects on fibroblasts on wound, he also mentioned that Co-60 Gamma ray had damaging effects on cultured fibroblasts and that irradiation injured fibroblasts were less responsive to the stimulation of cytochrome. (Schaffer *et al.*, 2007) used Gray electron radiation at the limited dorsal skin of rats for five days and the wound created in exposed area was impaired by impairment expression of wound lymphocyte subtypes. (Dubin *et al.*, 2000) concluded that in the rat model, graft thickness and neovascularization of the AlloDerm dermal implant do not appear to be adversely affected by a field that has received external-beam radiation, and fibroblast counts decrease over time regardless of irradiation status.

Fibroblasts play critical roles in the process of wound repopulation and subsequent remodeling (Qu *et al.*, 2004) so in our study the fibroblasts number was significant and collagen orientation was obvious.

Also the accumulation of neutrophils numbers were significant in experimental group, which neutrophils are the first white blood cells population to arrive and affect the host inflammatory response during exercise and soft tissue injury. These cells have both specific and nonspecific defensive mechanisms (Timothy *et al.*, 2006).

## **Conclusion**

In conclusion, the findings of present novel study indicated that the effect of combination IR and ES seem to improve wound healing in a field that is previously exposed to these stimulators as well as activation neutrophils and fibroblast cells, further studies are required to determine the effect of these stimulators on other living tissue parameters prior to wound creation and to know the exact dose and duration of exposure time of them.

## **References**

- Abou-Hala A.Z., Barbosa D.G., Marcos R.L., Soares C.P., and Silva N.S.,(2007): Effect of the Infrared Lamp Illumination During the Process of Muscle Fatigue In Rats., *brazilian archives of biology and technology*, vol.50, No.3, pp.403-407.
- Biedebach M.C., (1989): Accelerated Healing of Skin Ulcers By Electrical Stimulation and the Intracellular Physiological Mechanisms Involved., *Acupuncture Electrotherapy Research*, vol.14, pp.43-60.
- Bogie K.M., Reger S.I., Levine S.P., and Sahgal V., (2000): Electrical Stimulation for Pressure Sore Prevention and Wound Healing ., *Journal of Assist. Technology*, vol.12, pp.50-66.
- Dubin G., Feldman M., Ibrahim H.Z., Tufanor R., Evans S.M., Rosenthal D., Wolf P.F., Weber R.S., (2000): Allograft Dermal Implant (AlloDerm) in a previously irradiated field., *Journal of Laryngoscope*, vol.110, No.6, pp.934-937.
- Hayes, W.S., (2003): *Electrical Stimulation for Treatment of Chronic Nonhealing Dermal Ulcers*. Lansdale, PA: HAYES, Inc.
- Hideyoshi T., Yoichi M., Junya U., Hideto T., Shigeru T., Hideki N., Hon K., Yoshihiko A., Tetsuo N., Takafumi O., and Yasuo K., (2003): Promotive Effect of Far-Infrared Ray on Full-Thickness Skin Wound Healing in Rats., *Experimental Biology and Medicine*, vol.228, pp.724-729.
- Ju. H. L. , Mi. R. R. and Kwang. H. L., (2006): Effects of Infrared Radiation on Skin Photo-Aging and Pigmentation., *Yonsei Medical Journal*, vol.47, No.4, pp. 485-490.
- Karu T.,(1988): Molecular Mechanism of The Therapeutic Effect Of Low Intensity Laser Radiation., *Laser Life Science*, vol.2, pp.53-74.
- King S.W., (1982): Infrared Rays–discussion on some of the issues., *The Journal of Hong Kong Physiotherapy Association*, vol.4, pp.9-12.
- Lubart R., Rriedman H., Grassman N., Cohen N., Breibart H., (1997): The Role of Reactive Oxygen Species In Photobiostimulation., *Trends in photochemistry and photobiology*, vol.4 , pp.277-2283.
- Mohammad B., (2006): Experimental Wound Healing Using Microamperage Electrical Stimulation In Rabbits., *Journal of Rehabilitation Research & Development*, vol.43, No.2, pp.219–226.

- Pullar CE., Isseroff RR., Nuccitelli R., (2001): Cyclic AMP-dependent protein kinase a plays a role in the directed migration of human keratinocytes in a DC electric field., *Cell Motile Cytoskeleton*, vol.50, No.4, pp.207–217.
- Qu J.F., Cheng T.M., Xu L.S., Shi C.M., Lin Y., Yan G.H., and Ran X.Z., (2004): A Study on the Activity of Fibroblast Cell in Connection with Tissue Recovery in the Wounds of Skin Injury after Whole-body Irradiation., *Journal of. Radiation. Research.*, vol.45, No.2, pp.341-344.
- Robinson KR., (1985): The Responses of Cells To Electrical Fields—a review., *Journal of. Cell Biology*, vol.101, No.6, pp.2023–2027.
- Sachik K, Rei O., Ritsu A., Hiko H., (2006): Intradermal Change Resulting From Irradiation With B raodband Infrared Light (Titan®), *Journal of the Japan Society of Aesthetic Plastic Surgery*, vol. 28, No. 2, pp 39-41.
- Samoilova A.K., Bogacheva N.O., Obolenskaya D. K., Blinova I. M., Kalmykova V.N., and Kuzminikh V.E.,(2004): Enhancement Of The Blood Growth Promoting Activity After Exposure of Volunteers To Visible And Infrared Polarized Light. Part I: stimulation of human keratinocyte proliferation in vitro. *Photochemical Photobiostimulation Sciences.*, vol.3, pp.96-101.
- Schaffer, M, Christina St-lten, Richard Viebahn., (2007): Role of T-Lymphocytes In Radiation-Impaired Wound Healing., *Polski Przegląd Chirurgiczny*, vol.79, No.4, pp.312-318.
- Schramm JM, Warner D, Hardesty RA, Oberg KC., (2003): A Unique Combination of Infrared And Microwave Radiation Accelerates Wound Healing ., *Journal of Plastic Reconstruction Surgery*, vol.111, No.1, pp. 66-258.
- Sheridan DM, Isseroff RR, Nuccitelli R., (1996): Imposition of A Physiologic Dc Electric Field Alters The Migratory Response of Human Keratinocytes on Extracellular Matrix Molecules., *Journal of. Invest. Dermatology*, vol.106, No.4, pp.642–646.
- SumanoH., Goiz G., and Clifford V., (2002): Use of Electrical Stimulation For Wound Healing In Dogs., *Veterinary Medicine Journal*, vol.57 , No.2.

- Timothy A. Butterfield, Thomas M. Best, Mark A. Merrick, (2006): The Dual Roles Of Neutrophils And Macrophages In Inflammation: A Critical Balance Between TissueDamage and Repair., Journal of Athletic Training, vol.41, No.4 , pp.457-465, .
- Wang J., Boerma M., Fu Q. and Hauer-Jensen M., (2006): Radiation Responses in Skin and Connective Tissues: Effect on Wound Healing and Surgical Outcome, vol.10, No.6, pp. 502-506.

## التئام الجروح وأعداد الأرومات الليفية والعدلات لمنطقة من الجلد تم تعريضها للأشعة فوق الحمراء والتحفيز الكهربائي

شاهين إكرام جعفر

قسم التحليلات المرضية، الكلية التقنية – كركوك

تاريخ الاستلام: ١/٦/٢٠١٠، تاريخ القبول: ٨/٣/٢٠١١

### الخلاصة

لغرض تقييم مدى التئام الجروح وأعداد الأرومات الليفية والعدلات في منطقة محددة من الجلد تم تعريضها مسبقاً لكل من الأشعة فوق الحمراء والتحفيز الكهربائي. تم استخدام ٢٠ أرنب ذكر قسمت لمجموعتين بواقع ١٠ أرانب لكل مجموعة. تم تعريض مجموعة التجربة على كل من الأشعة فوق الحمراء والتحفيز الكهربائي لمدة ١٠ دقائق (خمس دقائق لكل منهم) ولمدة ثلاث أيام متتالية، وفي اليوم الرابع عمل شق جراحي في المنطقة المعرضة وتم خياطتها. أخذت الخزعة النسيجية من الجروح حسب الفترات التالية ٢٤، ٤٨، ٧٢ ساعة، أسبوع وأسابيع، وبمعدل حيوانين لكل فترة لغرض احتساب أعداد الأرومات الليفية والعدلات ولغرض الملاحظات النسيجية. مجموعة السيطرة لم تعرض للإشعاع والتحفيز. أظهرت النتائج وجود فرق معنوي عند مستوى معنوية ( $p < 0.01$ ) لأعداد الأرومات الليفية والعدلات، حيث كانت نسبة هذه الخلايا أعلى في مجموعة التجربة، وأظهرت الملاحظات النسيجية عمليات الالتئام الجيدة للجروح مع التراصف الجيد للنسيج الغراوي والأرومات الليفية، وبالتالي اثر بشكل ايجابي في تسريع عمليات الالتئام. أثبتت هذه الدراسة التي يتم إجرائها لأول مرة وجود تأثير ايجابي لكل من الأشعة فوق الحمراء مع التحفيز الكهربائي المعرضين مسبقاً لمنطقة محددة من الجلد في تسريع عمليات الالتئام وزيادة أعداد خلايا الأرومات الليفية والعدلات فضلاً عن التراصف الجيد للألياف الغراوية