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The Roleof Low Level Laser Therapy (820nm) on full –thickness skin graft in Rabbits

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By

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Lecture

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بسم الله الرحمن الرحيم

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ

يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُلْ رَبِّ زِدْنِي عِلْمًا ﴿١١٤﴾

صدق الله العظيم

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Certificate of Supervisor

I certify that the research entitled (The Role of Low Level Laser Therapy (820nm) on full – thickness skin graft in Rabbits) was prepared under my supervision at the college of veterinary medicine / University of Al-Qadissiya .

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Certificate of Department

We, head of dept. of Int. and prev. med. , certify that (Ahmad Kamal Hamza) is adequate for the debate of graduation project of Bachelor degree in science in veterinary medicine

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21.4.2016

الإهداء

الى من هما سر وجودي .....

ابي .... امي

الى من هم سندي في الحياة...

الى من هم رفاق مشواري ....اصدقائي و صحبتي...

الى من هم لهم الفضل في اناره طريقي...

اساتذتي الافاضل..

شكر وتقدير

اتقدم بجزيل الشكر والتقدير لعماده كليه الطب البيطري وخاصه عميد الكليه المحترم لتوجيهاته وتشجيعه المستمر للبحث العلمي....

ولايفوتني ان اتقدم بالشكر الجزيل والعرفان لفرع الجراحة والتوليد الذين كانو عونا لي.....

كان لاستاذي المشرف د. مسلم فاهم ديوان دورا عظيما في تصميم وانجاز البحث فله مني كل الحب والتقدير لمابذله من جهد لن انساه ابدا.....

كما اتقدم بجزيل الشكر والعرفان لكل اساتذتي واصدقائي ....

Ahmed Kamal

;]

Summary

summary

The study aim to evaluate the effect of 820nm wave length low–level laser therapy (LLLT) on free full-thickness homograft skin healing in rabbits model . Eight adult male rabbits were randomly divided into two equal groups . Rabbit were anesthetized by IM injection of ketamine (75mg/k.g B.W) , xylazine (10mg/k.g B.W) mixture . the dorsal of animal was prepared for aseptic surgery , and full thickness square incisions 4 cm . that the skin was removed and replaced by skin from another rabbit and viceversa and suture. Then immediately after surgery first group treated by close-contact pulsed gallium- aluminum arsenide laser (GaALAs) on the skin graft(with energy density 8J/cm and wave length of 820nm, power output (1000mw) , pulsing rate 146 hz.) and this treatment repeated daily for seven successive days . second group (control group) left without treatment . specimens taking was carried out 7 and 14 days after surgery and send to the pathology laboratory.

The laser treated group at days 7 were observed the healing of wound was better than of control group . which characterized by narrow scar and collagen deposition. However the laser treated group at days 14 was showed that perfuse of granulation tissue which characterized by proliferation of fibroblast when compared with control group .

In conclusion the laser wave length (820 nm) at 8J/ cm2 have beneficial effect on acceleration the healing of surgically induced skin homograft in rabbit by increase of granulation tissue and formation of new blood vessels , compare with control group .

LIST OF CONTENTS

CHAPTER ONE

Introduction

Introduction

Skin is the largest apparatus that its function is protection body from mechanical damage and chemical exposure. This organ experiences most damage such as loss of and regeneration throughout its life. Surgical treatment and plastic surgery has been used to resolve the defect and restoration its function and is the choice treatment. (Swaim , 2003) . The wound healing is a multi stage phenomenon that requires the activation , recruitment of numerous cell types as keratinocytes , endothelial cells, fibroblast and inflammatory cells , and macrophage which appear to be central to this process (Rodero and Khostrotehrani , 2010) . Damage to any tissue triggers a cascade of events that lead to rapid repair of the wound .

If the tissue is skin , then repair involves re-epithelization, formation of granulation tissue and contraction of underlying connective tissue . This concerted effort by the wounded cell layers is accompanied by a robust inflammatory response , in which first neutrophils and then macrophage , and mast cells emigrate from nearby tissue and from the circulation , this inflammatory response is crucial for fighting infection

(Marten and Leibovich, 2005) .

The remodeling phase involves formation of new collagen . The amount of collagen secreted determines the tensile strength of the wound (Blee *et al.*, 2002) . proliferative phase are fulfilled, unknown stop signals induce are differentiation of fibroblasts, keratinocytes, and endothelial cell, so that the accelerated proliferation and micration normalizes. Gradually the provisional collagen (TypeIII) is replaced by more stable collagen (Type I) that is produced by fibroblastes and is deposited in a physiological alignment. Thus, the healing wound gains increased wound tensile strength ,the collagen fibres contract so that the wound tissue shrinks (Tomasek *et al.*, 2002 ; Wrobel *et al* ., 2002) . With time , the fibrils become more organized in a manner that maximizes strength

against physiologically relevant stress forces on skin . The processes that underlie ECM remodeling are most active during first 6 months post wound less so the subsequent 6 months , and minimally (although not zero) there after (Monaco and Lawrence , 2003) . Mast cells accumulated at the wound edge may participate in tissue remodeling in the late phase of wound healing (Iba *et al.,* 2004) .

Low levels of laser or non-coherent light termed low level light therapy (LLLT) have been reported to accelerate some phases of wound healing (Demidova – Rice *et al.,* 2007) . In past low level laser therapy use in the united states to investigational use only . Yet LLLT is used clinically in many other areas including Canada, Europe, and Asia for treatment of various neurologic, chiropractic, dental, and dermatologic disorders (Posten *et al.,* 2005) . In wound healing, laser therapy can increase number of vessels, it can stimulate proliferation of fibroblasts and increase its number, can promote process of collagenization (Nasirian and Nasirian , 2012) . Although molecular mechanism of LLLT has not been elucidated completely, the anti-inflammatory effect of laser irradiation may be one of the most important therapeutic effects on many injuries and disorders including wound healing (Yasukawa *et al.*, 2006) . Mitochondria are believed to be the primary target of photo radiation since they contain cytochrome c oxidase (CCO) is a component of cellular respiratory chain which acts as light receptor or photo acceptor which activates a cascade of cellular respiratory effects upon the absorption of photon (Zungu *et al.*, 2009) .

Aim of study

The aim of present study is to evaluate the effect of 820 nm (8J/cm) low level laser on full- thickness homograft skin healing in rabbit .

CHAPTER TWO

Literatures of Review

Literatures review

The full-thickness homograft (allograft) are tissue transplanted from one individual to another of same species also as one of the most practical and choice method of treatment of these lesions that accelerates healing of lesions and makes appropriate and acceptable appearance (Bohling and Swaim , 2012) . Skin has 2 layers , epidermis and dermis . epidermis through its rapid mitotic activity and thick layer of keratin at its surface , allows quick wound healing and provides ahighly resistance barrier to foreign invasion . The dermis provides abase for hair growth,sweat and oil production , thermoregulation and sensation to be effectively carried out (McGraw *et al.*, 2007) .

This barrier is avery complex system formed by atremendous number of inter-related components . These compenents are mainly localized in the stratum corneum and consists of protein-enriched cells which include corneocytes with cornified envelope and cytoskeletal elements ,as well as corneodes mosomes and the second component of sc is lipid enriched intracellular domains the nucleated epidermis also contributes to the barrier through tight , and adherens junctions (Proksch *et al.*, 2008) . Healing is involve the interaction between many cell types, their cytokines, mediators and the extra -cellular matrix .during inflammatory phase platelets , neutrophils ,macrophages and lymphocytes migrate to wound.the proliferative phase showns an increase in fibroblasts and macrophages and finally during the remodeling phase , fibroblasts helps recreate the extra-cellular matrix and deposite collagen (Kirsner *et al.*, 2003) . It is acomplex process that can be deivided into at least three continuous and overlapping processes :an inflammatory reaction, aproliferation process leading to tissue restoration, and eventually tissue

remodeling (Li *et al.*, 2007) . The inflammatory stage begins with platelet aggregation and formation of the fibrin clot , which reestablishes hemostasis and provides an extracellular matrix for cell migration (Diegelmann and Evans , 2004) . This phase is triggered by avariety of mediators released from injured tissue cells and capillaries, activated platelets and their cytokines and the by –products of hemostasis (Strodlbeck , 2001) .

proliferative phase are fulfilled, unknown stop signals induce are differentiation of fibroblasts, keratinocytes, and endothelial cell, so that the accelerated proliferation and micration normalizes. Gradually the provisional collagen (TypeIII) is replaced by more stable collagen (Type I) that is produced by fibroblastes and is deposited in a physiological alignment. Thus, the healing wound gains increased wound tensile strength ,the collagen fibres contract so that the wound tissue shrinks (Tomasek *et al.*, 2002 ; Wrobel *et a*l ., 2002) .

Low level laser therapy

Low level laser therapy (LLLT) is a form of phototherapy used to promote wound healing in different clinical conditions . The LLLT at an adequate wavelengath, intensity, and dose, can accelerate tissue repair (Hawkins and Abrahamse , 2006) . Laser is a form of light or infrared radiation both of which are electromagnetic radiations . In contrast , to non-laser radiation which is made up of divergent and generally multiple wave lengths , laser beam is monochromatic , coherent and collimated . Because of these properties , laser became an important tools in the scientific community (Reddy , 2004) . LLLT monochromatic light has been shown to cause avariety of effects on irradiated cells, depending on the state of those irradiated cells (Tafur and Mills, 2008) . It has proved to be very efficient in speeding and

improving the quality of healing in pathological conditions of diverse etiologies (Zunggu *et al* ., 2009) , also appears to have an anti-inflammatory effect, as evidenced by the reduction in neutrophil infiltrate (Lopes *et al.,* 2010) . The LLLT emission increased tissue oxygenation, morphofunctional activity, and substantial expansion of the microcirculation bed and inturn accelerated the restoration of the functional features of the injured area (Ihsan, 2005) .

Laser stimulation shortened the inflammatory phase as well as accelerated the proliferative and maturation phase ,and postitively stimulated the regeneration of injured epidermis and the reparation of injured striated muscle (Gal *et al.,* 2006) . It may reduce pain related to inflammation by lowering levels of prostaglandin E_2 ,TNF-α ,cellular influx of neutrophil granulocytes oxidative stress, edema, and bleeding (Bjordal *et al.,* 2006).

LLLT accelerates wound healing by stimulating the biological activities and differentiation of fibroblast and by reduceing the inflammatory process (Araujo *et al.,* 2007) . All laser treatment led to marked improvements in dermal layer thickness and collagen fiber density , and increase in fibroblast number and hydroxyproline content. Hydroxyporline is amajor component of collagen and rarely found outside collagen (Liu *et al.,* 2008) . LLLT in rat significantly increase the wound closure and the inhibitory effect of the LLLT on microbial flora is minimal (Ezzati *et al.,* 2010). It can be stated that all the cells participating in immune system reaction and in tissue regeneration showed higher levels in the animals treated with low energy laser, demonstrating the stimulative effect of this type of radiation.The erythrocytic indices showed higher levels of erythrocyte counts and average erythrocyte volume which suggests better oxygen input into the tissues as a result of biostimulation (Calin *et al.,* 2010) . LLLT increase

the number of intact mast cells during the inflammatory and proliferative phase of healing , and decreased the total number of mast cells during the remodeling phase (Vasheghani *et al.,* 2008) . Mast cell can secrete angiogenic factors such as vascular endothelial growth factor (VEGF) .

Irradiation promoted VEGF production by mast cells in metalloproteinase-9- dependant manner , irradiation promoted migration of mast cells from bone marrow to the ischemic site . after irradiation increase recruitment and activation of mast cells alters the ischemic micro environment and promotes vascular regeneration (Heissig *et al.,* 2005) . Laser therapy stimulate lymphocytes to produce angiogenic factors that can modulate endothelial cells proliferation (Agaiby *et al.,*2000) .

CHAPTER THREE

Materials and Methods

Materials and Methods

3-1-Materials

3-1-1 Experimental animal :

Eight adult male rabbits weighting between(750-850g) and aged about (6- 8) months. Animals were randomly divided into two groups and each group has four animals .The animals were kept in metallic cages with a metal top, in normal laberatory conditions and were fed a solid diet and water.

3-1-2 Apparatus:

3-1-2-a: Diode laser:

The laser device (Omega Laser Systems Limited,UK) emits a wave lengths under 1000nm utilizing red light (600nm to 820nm) and infrared (over 780nm).The power, pulsing frequency and programming is set via the control unit and the frequency laser pulse is varied (2.5Hz-10KHz).The Omega XP Clinic laser system It is manufactured in England. Laser are Gallium Aluminum Arsenide Lasers, (GaALAs),in multi-wave length probes.

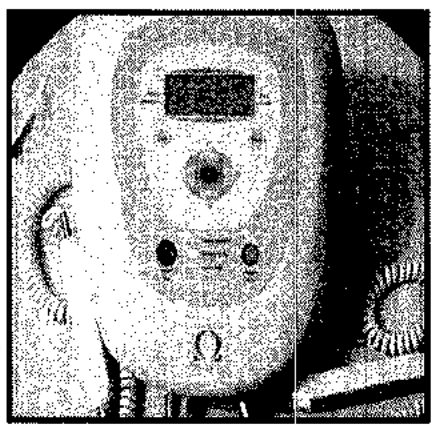


Fig.(1)diode laser device

3-1-3 Anesthesia of animals

The rabbit were anesthetized with ketamine (75mg/kg B.w) and xylazine (10mg/kg B.w) using as mixtured and given intramuscular injection .

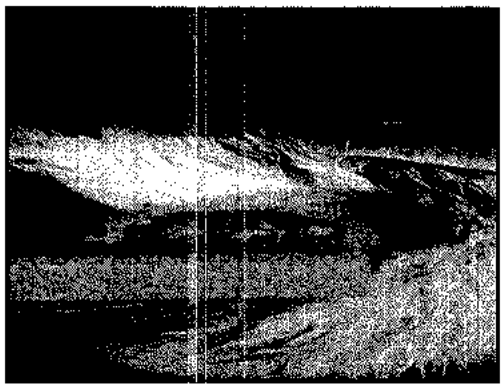
3-2-Methods

3-2-1 Surgical procedure

After general anesthesia the site of incision was prepared for aseptic surgery (clipping ,shaving and apply antiseptic by gause saturated with 70% alcohol) in back of animal .To induced the square incision 4 cm that full- thickness skin was removed fig(2) . then replaced by skin graft from another rabbit and viceversa and suture incision by non absorbable suture (0# silk) fig (3) .

Fig(3) suture of skin graft

Fig(2) induce of incision



3-2-2 Irradiation of the animal by diode laser

Treatment was started in all groups directly after surgical procedure and continued for 7 days . The process of irradiation including treatment the graft skin only, the diode laser used with a wave length of (820 nm), power out put (1000 mw), pulsing rate (146), and spot size (4 cm) . For G1we used energy density (8J/ cm), for 5 second . The laser irradiation process was performed by restraint

of the rabbit and keeping diode head of the laser device closely to the wound surface fig. (4) . The specimens was collected from the wound area including a part of the skin edge on the 7and 14 days post wound.

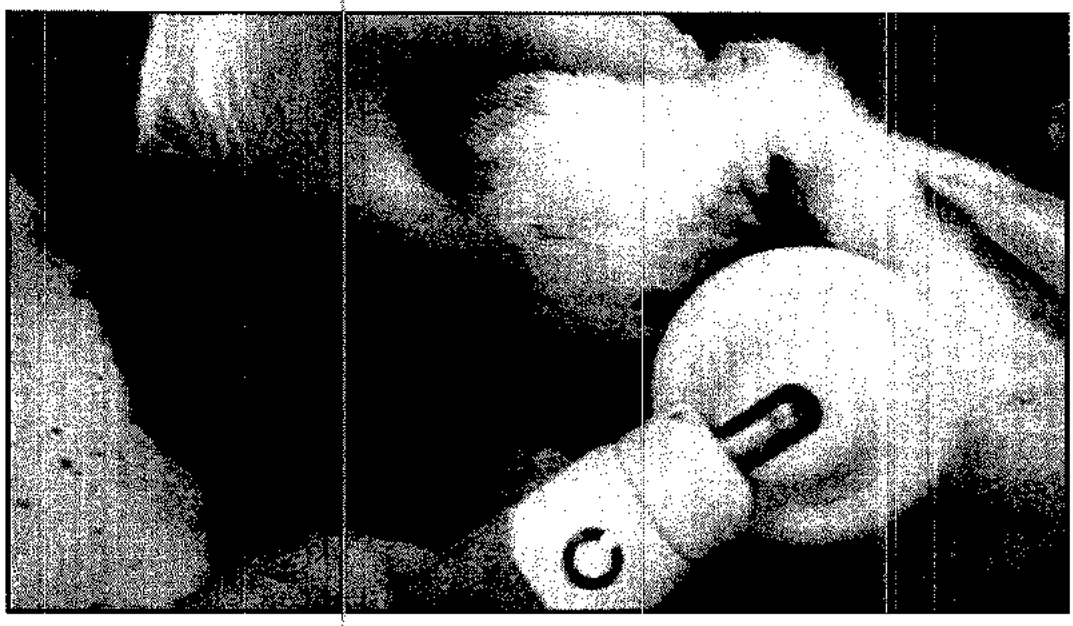


Fig (4) the process of irradiation by laser

3-2-3 Experimental group (control group)

Four male rabbit used as control group . Doing incision with skin graft and not exposure to the laser, then at 7and 14 days taken specimen and send to histological evaluation .

3-2-4 Preparation of specimens for histological examination

The animals were anesthetized by general anesthesia . 1cm of full thickness skin were taken at 7 and14 days post wound from all groups ,then the specimens were preserved in 10% buffer formalin solution and send to histological laboratory for evaluation .

CHAPTER FOUR

Results

Results

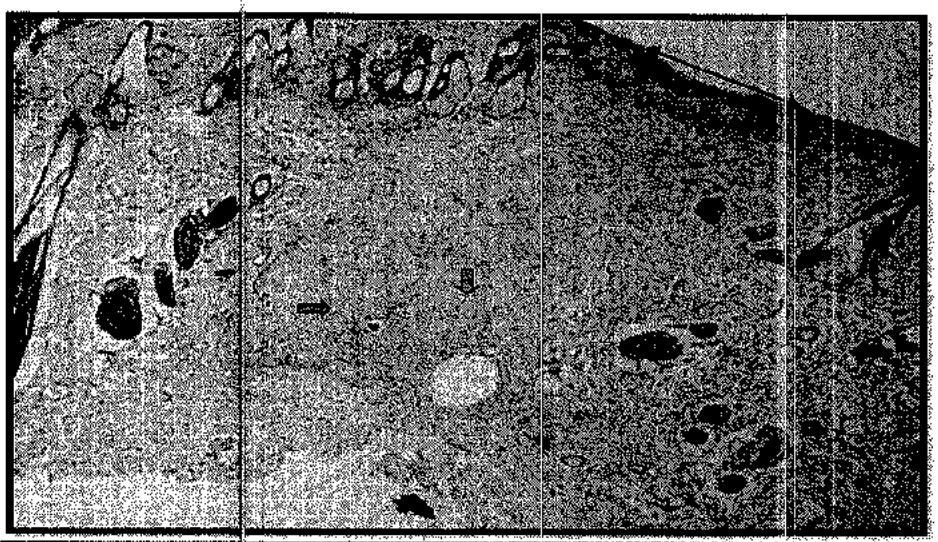
On day 7 in the treated group (8J/cm), the histological assessment showed significant changes, particularly wound healing . there were marked contraction and scar tissue was small (narrow scar), also there were proliferation of endothelial cell to form new blood vessels and wound become more small than control group (fig. 5) .

After 14 days post skin graft in treated group (8J/cm), marked higher of granulation tissue which characterized the wound with formation of new blood vessels, also presence of fibroblast to form fibrosis (fig. 6)

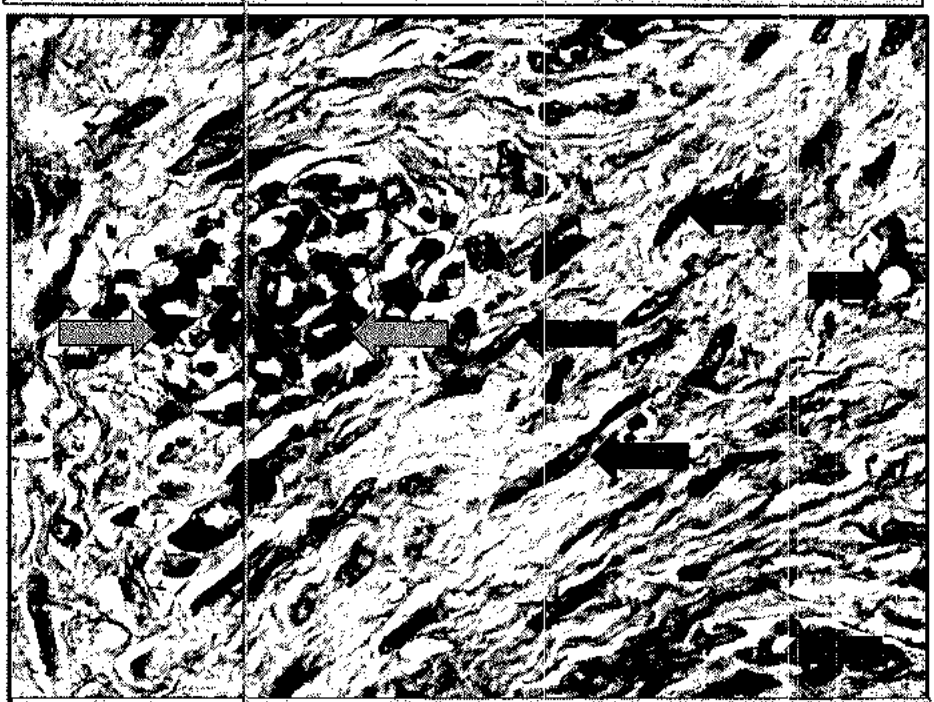
.

While the histological analysis for the control group (without treated) on day 7 showed wide scar tissue and presence of hemorrhage in the dermas (fig. 7).

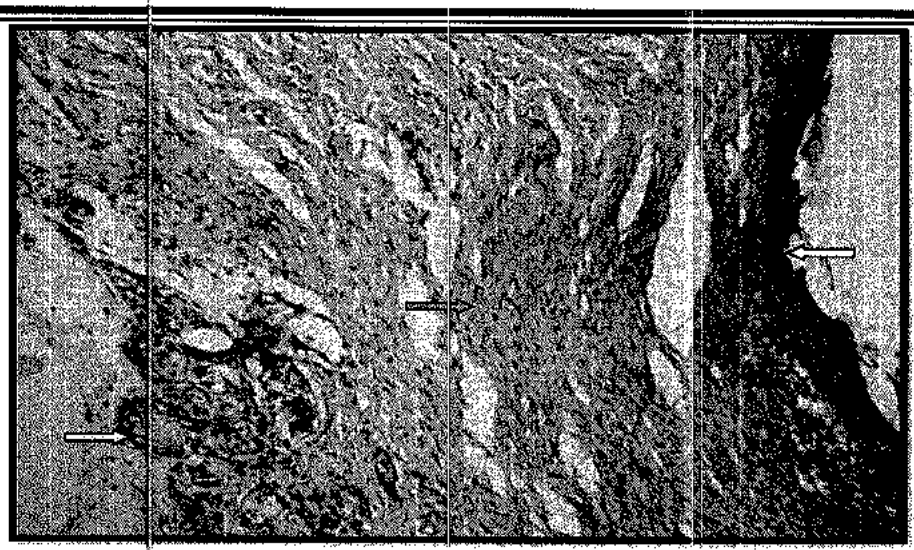
After 14 days post skin graft in control group, there were marked irregular and dgeneration fiber . also infiltration of inflammatory of cells, with presence of hemorrhage (fig. 8) .



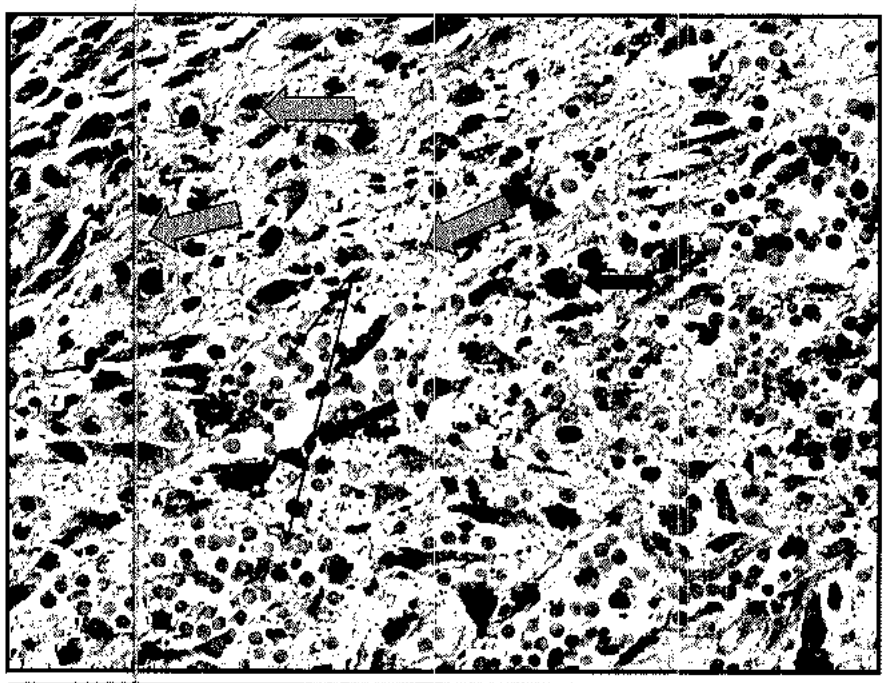
Figure, 5 : laser treated (8J/cm^2). Histopathological section of skin graft 7 days post-graft show narrow scar tissue (double head arrow) . There was presence of newly blood vessels (red arrow), . 50X H&E.



Figure, 6: laser treated (8J/cm)). Histopathological section of skin graft 14 days post-graft there is presence of granulation tissue which characterized by proliferation of fibroblast to form fibrosis (green arrows) and presence of blood vessels (blue arrows) also there is smaller of inflammatory cells (red arrows).10XH&E



Figure, 7: control group. Histopathological section of skin graft 7 days post-graft show wide scar tissue, with epithelial invagination (white arrow). Entity of granulation tissue which involved profuse fibrous connective tissue . There is hemorrhage in the dermis (yellow arrow) 50X H&E.



Figure, 8: control group. Histopathological section of skin graft 14 days post-graft show there is marked irregular arrangement with severe degeneration of fibers (red arrows) also there is hemorrhage between fiber (thin arrows) also infiltration of inflammatory cells (green arrows). 10XH&E

CHAPTER FIVE

Discussion

DISCUSSION

The healing process of large defect is very problematic and clinicians and researchers have paid attention to manage of this old and powerful competitor in plastic surgery field.

The results of this study revealed that of treated group in the 7 day is marked contraction and scar tissue was small (narrow scar), also there were proliferation of endothelial cell to form new blood vessels and this result is accord with Gal *et al.,* (2009) who find that at 7 days in this period remodeling and reorganization of extra cellular matrix (ECM) is characterized , thus the scar is formed and a mild regress of vessels in the granulation tissue is seen , also he found that LLLT is able to accelerate wound healing without enlarge area of the scar tissue, and this result was consistent with de Melo *et al.,* (2011) also documented that, since no sign of excessive collagen synthesis is observed, indicate that this protocol successful in improving wound healing without increasing scar formation .

We, too, found that there is formation of new blood vessels . This result is companionable with Calin *et al.,* (2010) who reported the presence of scar connective tissue made of young collagen, with lacking hair follicules in the deep dermis, and blood vessels are formed increasing the circulation of blood . in this group irradiation was efficient for increasing wound healing , may be due to the enhancement of vascular perfusion (Corazza *et al.,* 2007) . laser photo-biomodulation can activate the local blood circulation and stimulate proliferation of endothelial cells (Schindl *et al.,* 1999; Garavello *et al.,* 2004) .

In 14 days found higher of granulation tissue which characterized the wound with formation of new blood vessels, also presence of fibroblast to form fibrosis this results corresponded with (Byrnes *et al.,* 2004) . Whom find that the use of LLLT for treatment of wounds result in a thick epithelial layer that

completely covered the wound tissue, the granulation tissue was thick and composed primarily of fibroblast and collagen, and a moderate of neovascularization, acceleration of the wound healing process with laser can be explained by a higher collagen synthesis from fibroblast , and numerous vascular proliferation in the connective tissue, coupled with higher mitotic activity of the epithelial cells . this result is accorded with doNasciment *et al.,* (2004) who find the use of lower intensity laser resulted in an accentuated amount of granulation tissue being associated with increased neovascularization and hyperemia, this granulation tissue is characterized by intense proliferation of mature fibroblast distributed in a dense connective tissue when compared to control .

Chapter six

Conclusions and Recommendations

Conclusions

1- LLLT can speed up the healing process in full-thickness of homograft skin .

2- LLLT (820 nm ; 8J /cm) dose have beneficial effect during of the inflammatory , proliferation and maturation phases of homograft healing .

Recommendations

In view of present study, recommend the following

1- studying the effect of low level laser therapy on xenograft healing .

2- studying the effect of 8J/cm dose of laser on induced burning and treated by graft .