EFFECT OF LOW LEVEL LASER AND CHITOSAN POWDER ON THE ACCELIRATION OF WOUND HEALING IN RABBITS

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ABSTRACT

Open wounds have lost the barrier that protects tissues from bacterial invasion and allow for the escape of vital fluids. The purpose of this study is to evaluate the enhancing of therapeutic effect of Low Level Laser (LLL) and chitosan on the acceleration of open wound healing. Forty two adult rabbits were used. They divided into three equal groups (I, II and III). Wound was produced at the dorsal region by remove all thickness of skin at width of 3.5cm and of length 4 cm. Group I was left without treatment, while chitosan powder and laser therapy were used in group II and III respectively. Results of two groups were compared with control group. Histpathologicl results at the period of 3, 7, 14, 21 and 28 days post operation reflected the presence of large number of fibroblast with formation of new blood vessels, also the collagen fiber become dense and regular in 14 days post operation in irradiated groups. In 21days there was formation of new and normal epidermal closed the rupture area of III group.

INTRODUCTION

In full thickness skin wounds dermal regeneration usually fails, resulting in scar formation and wound contraction. The biological processes of wound healing include cell regeneration, cell proliferation and collagen production. Healing wound requires a well orchestrated coordination between many specialized cell types to restore a structural and functional integrity. Systemic treatment and local application of different chemical, materials or physical methods will improve wound healing [(1)].

The easy process ability of chitosan coupled with its versatile properties makes it an attractive material for various medical applications. Chitosan has been extensively used as

a wound and burn dressing material due to its easy applicability, oxygen permeability, water absorptive, haemostatic property, and ability to induce interleukin-8 from fibroblasts, which is involved in the migration of fibroblasts and endothelial cells [2, 3]. The chemical structure of chitosan which is similar to that of hyaluronic acid, suggested that it would act as a wound healing agent [3]. Chitosan promotes granulation and organization. Therefore, it is beneficial for healing of large open wounds in animal. The chitin and chitosan have been used as filaments, powders, granules, sponges, or composite with cotton or polyester in treatment of open wounds [4, 5].

Tissue healing is a complex process that involves local and systemic responses. The use of Low Level Laser Therapy (LLLT) for wound healing has been shown to be effective in modulating both local and systemic response [6]. Over 75 Trillion cells in a human body all need electrons to communicate with each other. Laser light delivers the required electrons directly to the cells and enhances both their ability to communicate with each other and their ability to produce ATP (energy), which they require for optimal functioning. Additionally LLLT causes the release of healing enzymes and the production of endorphins, which are natural pain killers [7, 8]. The aim of this study is to accelerate and facilitate wound healing and reduce scar formation and wound contraction of open wound by Low Level Laser and chitosan then evaluated histopathologically.

MATERIALS AND METHODS

Forty two healthy male and female rabbits, age (8-10) months, and weighted approximately 1.5kg ± 500gm were used throughout this study. Bedding and environmental conditions were similar among all animals. The animals were divided into 3 equal groups (I, II, III). The dorsal back area was prepared surgically the animals were injected by Acepromazine maleate at a dose 0.5mg /kg b.w. as premedication, then 10 minutes later to anesthetized animal injected by Ketamine hydrochloride at dose 40mg /kg b.w. and Xylazine at dose 10mg /kg b.w. intramuscular. Double wound were inflicted on the dorsal skin of each animal, by removing all thickness of skin at width of 3.5cm and length of 4cm (Fig: 1a), after six hours of operation the wound area washed by normal saline, in group **I** regarded as a control (Non-treated). While in treated groups, after six hours of operation dealing with them as the follows, in group **II** chitosan powder (chitosan was sterilized by autoclaving for 10 minutes) 4gm was used to cover the avulsed area (Fig: 1b), group **III** treated with a gallium-aluminum-arsenide diode laser emits a wavelength of 890nm (MILTA, Moscoo 2000) (figure: 1c). The process of

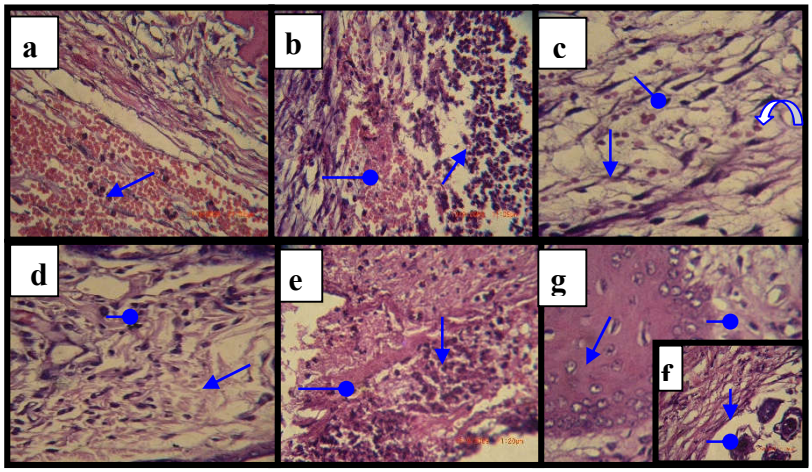
irradiation was involved by fixed the animal on the special restraint, the aperture of the head of diode laser device is fixed at the distance 0.5cm from the skin wound surface, and laser light is kept perpendicular to the wound, irradiation was done spot by spot along the wound to cover the whole area of the wound the beam size of this laser was 1cm in diameter with power 4W, frequency 50Hz the exposure time are 5 minutes once a day ten day for 2 week for receiving 5.1W/cm^2 of power density upon the wound and then reducing the power to 1w, frequency 5Hz and exposure time to 2 minutes once a day every 3day.

In all groups, we performed histopathological evaluations on the 3, 7, 14, 21 and 28 days.

(Fig: 1): induced skin wound by removal all thickness of skin [3.5 x 4 cm] (a), the wound was covered by chitosan (b), diode laser device (c).

RESULTS

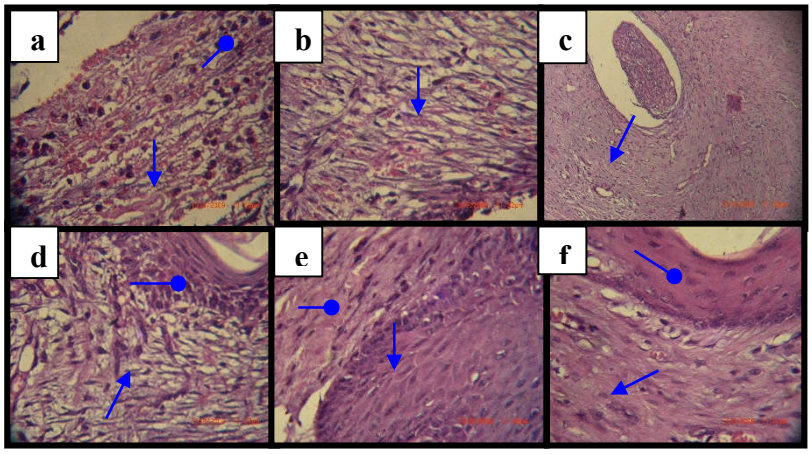
The histopathological finding of group **I** at days three post operation shows hemorrhage with inflammatory cell infiltration mainly neutrophils as well as congested blood vessels in the gap (Fig: 2a). At seven days the gap contains necrotized neutrophils together with hemolysis and granulation tissue form under dermis tissue (Fig2b) and hemolysis was seen between muscle fiber. In days 14 there is irregular fibrous connective tissue proliferation with congested blood vessel was seen in gap with mononuclear cell infiltration (Fig: 2c). At days 21 post operation showed dense fibrous connective tissue infiltrated with mononuclear cell filled the gap together with few blood vessels and modulate thickness of epithelial layer (Fig: 2d), congested blood vessel with sever neutrophils infiltration was seen between muscle fiber show's Zinker necrosis (Fig: 2e). While at day 28 shows lesion characterized by presence of space contains debris materials surrounding by fibrous connective tissue (fig: 2f) and there is dense moderates cellular fibrous connective tissue with few mononuclear replaced the gap and cover by very thickness cellular epidermal layer (fig: 2g).



(Fig:2) Histological section in skin of rabbit on control group at:
a- 3 days post operation shows hemorrhage with inflammatory cell infiltration (arrow) (H&E 40X).
b-7 day post operation shows the gap contains necrotized neutrophil(arrow) with hemolysis RBC (arrow) (H&E 40X).
c- 14 days post operation shows irregular fibrous connective tissue proliferation(arrow) with congested blood vessel(arrow) as well as mononuclear cell infiltration (arrow) (H&E 40X).
d- 21days post operation shows dense fibrous connective tissue filled the gap (arrow) with few blood vessels and inflammatory cell infiltration (arrow) (H&E 40X).
e- 21days post operation shows congested blood vessel with sever neutrophil infiltration between muscle fiber (arrow) show's Zinker necrosis (arrow) (H&E 40X).
f- 28 days post operation shows lesion characterized by presence space contains debris materials (arrow) surrounding by fibrous connective tissue(arrow) (H&E 40X).
g- 28 days post operation shows dense moderates cellular fibrous connective tissue with few mononuclear replaced the gap(arrow) and cover by very thickness cellular epidermal layer(arrow) (H&E 40X).

In group **II** at day three post operations sever inflammatory cell infiltration mainly neutrophils with proliferation of fibroblast form few fibrous connective tissues (Fig: 3a). On day 7 the main lesion characterized by severs granulation tissue consist from proliferation of fibrous connective tissue and congested blood vessel in gap of incision with mononuclear cell infiltrated (fig: 3b). At day 14 the section revealed space contains debris material surrounding by sever thickness of fibrous connective tissue (fig: 3c) and the gap filled with granulation tissue consist from fibroblast and blood vessels with few mononuclear cell infiltrated cover by moderate thickness of epidermal layer (fig: 3d). day 21 post operation there is mature fibrous connective tissue characterized by regular their direction more collagen and few congested blood vessel present in the dermis cover by thickness epidermal layers (fig: 3e). At day 28 the section characterized by dense

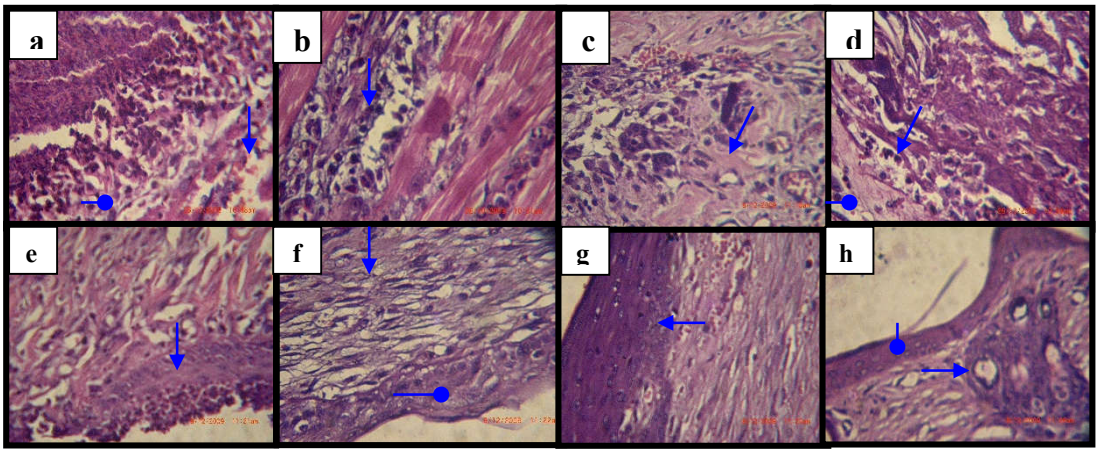
collagens, less cellular fibrous connective tissue present in the dermis covered by thick epidermal layer (fig: 3f).



(Fig: 3) Histological section in skin of rabbit on chitosan group at
a- 3days post operation shows proliferation of fibroblast with few fibrous connective tissue (arrow) as well as sever inflammatory cell infiltration (arrow) (H&E 40X).
b-7days post operation shows severs granulation tissue (arrow) with mononuclear cell infiltrated (arrow) (H&E 40X).
c-14 days post operation revealed space contains debris material surrounding by sever thickness of fibrous connective tissue(arrow) (H&E 40X).
d- 14days post operation shows the gap filled with granulation tissue with few mononuclear cell infiltrated (arrow) cover by moderate thickness of epidermal layer (arrow) (H&E 40X).
e- 21days post operation shows mature fibrous connective tissue characterized by regular their direction present in the dermis (arrow) cover by thickness epidermal layers (arrow) (H&E 40X).
f- 28 days post operation shows dense collagens, less cellular fibrous connective tissue present in the dermis(arrow) covered by thick epidermal layer (arrow) (H&E 40X).

In three days of group **III** the gap area containing cellular debris neutrophils infiltration, congested blood vessel with proliferation of few fibroblast and production of fibrous connective tissue (fig: 4a) congested blood vessel with neutrophils infiltration was seen between muscle fiber (fig: 4b). At day 7 the main lesions characterized by filled the gap of wound by a thickness granulation tissue (fig: 4c) and infiltrated with few mononuclear cell and proliferation of epithelial cells form a thick layer extended under the cellular debris in the gap area (fig: 4d) together with congested blood vessel and proliferation of fibroblasts with mononuclear cell infiltration was seen between muscle fiber (fig: 4e). In day 14 the gap are filled with regular less cellular collagens fibrous connective tissue contain congested blood vessel and cover by moderate thickness of epidermal layer (fig: 4f) with formation of moderate hair follicle. At day 21 presence dense regular less cellular, more collagen fibrous connective tissue with congested blood vessel are seen in dermis covered by thick epidermal layer with rete ridge (fig: 4g) while

at day 28 post operation most of granulation tissue in the dermis converted with scar tissue which characterized by dense less cellular fibrous connective tissue contain hair follicle and cover by normal epidermal layer with show rete ridge (fig: 4h).



(Fig: 4) Histological section in skin of rabbit on laser group at:

a- 3days post operation show the gap aria containing cellular debris, neutrophils infiltration, new capillary blood vessel formation (arrow)and fibrous connective tissue (arrow) (H&E 40X).

b- 3days post operation shows congested blood vessel with neutrophils infiltration was seen between muscle fiber (arrow) (H&E 40X).

c- 7days post operation shows dense granulation tissue (arrow) with moderate cellular debris in the gap and infiltrated with few mononuclear cells (H&E 40X).

d- 7 days post operation shows congested blood vessel and proliferation of fibroblasts form fibrous connective tissue (arrow) with mononuclear cell infiltration was seen between muscle fiber(arrow) (H&E 40X).

e- 7 days post operation shows filled the gap of wound by a thickness granulation tissue infiltration with few mononuclear cells and proliferation of epithelial cells form a thick layer extended under the cellular debris in the wound area (arrow) (H&E 40X).

f-14 days post operation shows the gap are filled with regular less cellular collagens fibrous connective tissue (arrow) contain congested blood vessel and cover by moderate thickness of epidermal layers(arrow) (H&E 40X).

g- 21 days post operation shows dense, regular, less cellular, more collagen fibrous connective tissue with congested blood vessel are seen in dermis covered by thick epidermal layer(arrow) with rete ridge (H&E 40X).

h- 28 days post operation shows most of granulation tissue in the dermis converted with scar tissue and cover by normal epidermal layer (arrow) with show rete rideg with moderate hair follicles formation(arrow) (H&E 40X).

DISCUSSION

The histopathological results shown clear promote of healing in treated groups in comparison with control group, the histopathological examination observed that good response of groups III may be related to stimulation of inflammatory cell or activation of the chemo tactic factor by irradiation with this dose this observation was agreed with Petrova[9] who mentioned that under LLLT the high phagocytic activity of macrophages

was observed as early as 6 hours. LLLT can facilities wound healing may be due to that acute inflammatory is resolved more rapidly and the proliferation phase of healing begins earlier [10], so the LLLT decreased the inflammatory reaction of wound healing.

The good response and fast healing of wound in group II could be due to the chitosan enhances of the function of inflammatory cells such as poly morphonuclear leucocytes (PMN) and macrophages these were revere by histopathology findings which observed a large number of inflammatory cells in the wound area[11]. During the stage of healing some study revealed to the presence of neutrophils at the site of injury during 48-96 hours and reach's a peak in the 72 hours and these cells have important role in healing during phagocytes and then lyses and inter the cement material [12, 13]. These results come in agreement with this study.

The present study revealed that wound healing in group II is vastly enhanced where in group III is vaster and better enhanced and takes dramatically less time to complete when compared with control group. The cause of enhancement of healing, first of all is the increase of the proliferation of the epithelial cells, fibroblast, local microcirculation and increase of collagen syntheses by fibroblast following laser irradiation treated [14].

In general fibroblast is known to be essential in healing of tissue injuries including surgical wounds, the epithelialization and granulation tissue formation was created in the repair stage; fibroblast begin to synthesize collagen and ground substances[7, 14]. The laser treated group (III) in comparison with other groups shown higher numbers of fibroblast proliferation in early stage these observations confirms the result achieved by Baxter et al.[8] who indicated a possibility that laser induced fibroblast proliferation during healing mechanism. The effect of laser stimulation of fibroblast on wound regeneration is by maintaining a high mitotic activity of the fibroblast in the later healing period [15], who demonstrated that LLLT preferentially stimulates resting cells rather than proliferating ones.

The good microscopic results in group II could be to the increase of collagen production by fibroblast this is attributed to effect of chitosan that enhance the job of mononuclear cells through production of leukotrien-B4 which derived from arachnoid acid and production of interleukin-8 which promote the fibroblast [3, 16], these results are in agreement with the present study.

In the present work the histopathological examination revealed that the proliferation of the epithelial cells appear in day seven post operation in the group III faster than the group II and, control group. This results come in agreement with [16, 17] which report that LLLT

induced faster epitheliazation that culminated in better formation of epidermis and this come in agreement with this study which formation of normal epidermis and disappear of scar. The enhancement of collagen syntheses that may be due to light energy is observed by endogenous chromospheres in the mitochondria and used to synthesize ATP. The resulting ATP is then used to power metabolic processes; synthesize DNA, RNA, proteins, enzymes, and other biological materials needed to repair or regenerate cell and tissue components, rapid mitosis or cell proliferation and restore homeostasis[6, 15, 18]. Among improved micro-vascular circulation, reduced inflammation and increased cell energy in the form of ATP (adenosine triphosphates) may be worked together in low powered laser irradiation stimulated hair growth[19, 20], this come in agreement of this study.

The treatment groups (II, III) had greater wound contraction than the control group; the greatest difference between groups in wound contraction was seen on laser group (III) treatment. Several theories may help explaining the enhanced wound contraction observed here. In vitro studies have shown an increase in fibroblast proliferation after irradiation, suggesting that LLLT therapy may facilitate fibroplasia during the repair phase of tissue healing [1, 18]. Facilitated wound contraction may also be supported by the work of Ribeiro et al. [21] who reported that laser irradiation transforms fibroblasts into myofibroblasts, myofibroblasts are directly involved in granulation tissue contraction, and increased numbers could lead to facilitated wound contraction. The rate of wound contraction was proportional to cell numbers that begin from the healthy tissue surrounding the wound and often increasing its surface area [14, 17, and 22].

CONCLUSION

In this study found that LLLT and chitosan (III and II) to be effective in open wounds which appeared better regeneration and faster to restore a structural and functional integrity as compared to the control group.

تاثير الليزر الواطئ الطاقة ومسحوق الكايتوزان على تسريع شفاء الجروح في الارانب

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الخلاصة

تفقد الجروح المفتوحة المانع الذي يحمي الأنسجة منِ الغزو الجرثومي وخروج سوائل الجسم الحيوية. إنّ هدف هذه الدراسة هو تقييم تأثير العلاج بالليزر الواطئ الطاقة (Diode laser)، الكايتوزان والعلاج بالليزر الواطئ الطاقة

مع الكايتوزان على تعجيلِ شَفَاء الجروحِ المفتوحة. استخدم في هذه الدراسة أثنان وأربعون أرنباً بالغاً قسمت إلى ثلاثة مجاميع متساوية (I, II, III) احدثت الجروح في المنطقة الظهرية بازالة كل سمك الجلد بعرضِ 3.5 سنتيمتر وطول 4 سنتيمتر. المجموعة الى تركت بدون علاج بينما المجموعة المعالَجة بمسحوقَ الكايتوزان والليزر الواطئ الطاقة استخدم في المجموعة III و IIعلى التوالي. نتائج المجموعتان قورنت بمجموعة السيطرة، نتائج الفحص النسجي المرضي خلال(3, 7, 14, 21, 28) يوم بعد العملية اظهرت تكوين وتكاثركثيف للارومات الليفية وتكوين اوعية دموية جديدة، وايضاً حدث تكاثر كثيف ومنتظم لالياف الكولاجينِ في 21 يوم بعد العملية في المجموعة المعالجة بالليزر.

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