Acceleration of skin wound healing by application of galvanic stimulation in rabbits

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Abstract

The study was designed to investigate the effect of galvanic stimulation on skin wound healing in rabbits. Thirty male adult New Zealand rabbits were utilized and randomly divided in to equal main two groups(control and treated). Each group was divided into three subgroups(5 animals of each). A full-thickness skin incision was made on the dorsum of each rabbit, and sutured by simple interrupted suture with 4/0 silk suture. Incisions in the treated group were exposed to galvanic stimulation(2.21-2.93µA, 29V. and 0.4 pulse duration)in a dose of 42833x10-8 watt/cm2(power) by application of the two electrical electrods cranial and caudal to the incision.Daily application of galvanic was used for 3,7 and 14 successive days for 30 minutes. Skin wound samples were taken after 3,7, and 14 days post wounding for histopathological evaluation of the healing process. The study reveal early formation of dense collagen fibers with the thickening of the epidermal layer adjacent the site of the wound at 3 days p.w., and complete reepithelialization and disappear of signs of inflammation at 7 days p.w. incontrast to incomplete healing and presence of inflammation in control group.In conclusion ,the galvanic stimulation accelerate the wound healing by early granulation tissue formation and reepithelialization and reducing the signs of inflammation.

Introduction

Electrical stimulation of wound healing(ES) is defined as the use of an electrical current to transfer energy to a wound. The type of electricity that is transferred is controlled by the electrical source ,or application of electric current from electrodes placed directly within a wound or on skin in close proximity to it, thereby creating an electrical current that passes through the wound(1). Essentially, delivery of ES to a wound requires a device that can pass an electrical current across the wound bed. This is usually achieved by placing one electrode in contact with the wound bed and another in contact with the periwound skin close to the wound margin. The galvanic current often mean using of direct current. Historically, the term galvanic used to describe the use of uninterrupted direct-current. High-volt pulsed galvanic electrical stimulators are considered to be useful in acute injuries associated with major tissue trauma accompanied by bleeding or swelling. The direct current creates an electrical field over the treated area that, theoretically, changes blood flow. The using of two pads(positive and negative pads)in galvanic stimulation gives two different effects. The positive pad like ice, causing reduced circulation to the area under the pad and an associated in reduction of swelling, while the negative pad act like heat, causing increased circulation and reportedly speeding healing(2).The electrical stimulation differently affects on each phase of wound healing, starting with the stimulation of the inflammatory phase, in which, increasing of wound blood flow can help in the removal of debris by phagocytosis; in addition, enhances tissue oxygenation, also initiates the wound repair process by its effect on the current of injury, and reduces edema by reducing microvascular leakage, also attracts and stimulates fibroblasts and epithelial cells and solubilizes blood products including the necrotic tissue (3).Employment of Direct current (DC)in experimental models in vivo and in vitro can produce undesirable electrothermal, electrochemical as well as the desired electrophysical effects. An electrothermal effect is the process in which heat is released due to an electric current passing through a conductor and is governed by Joule's law. This effect can be minimised by reducing the current and treatment time, thus avoiding thermal damage. Electrochemical effects describe the chemical reaction that takes place between the electrodes and stimulation bath/ tissues which may result in pH changes or release of electrode by-products which can cause chemical burns, blisters or be highly toxic to cells and tissues(1). The purpose of this study was to explore the histological effect of Galvanic direct current on the wound healing processes.

Materials and Methods

Thirty adult male New Zealand rabbits, aged (4-6) months, and weighted 1±0.300kg were used in this study. Bedding and environmental conditions were similar among all animals. The rabbits were randomly subdivided into two equal groups, first group (Galvanic group); contains 15 rabbits, second group (control group); consists of 15 rabbits.General anesthesia were used to prevent animal's pain and movement of animals. During the procedure of experiment, using of a mixture of xylazin 2٪* (5mg/kg B.w) and ketamine* (40mg/kg B.w) by intramuscular injection. The device (electrical stimulator)* generates electrical impulses that are sent through electrodes placed over the wound. It is a safe non – invasive and has no side effects. This therapeutic stimulator used for different purposes, the Galvanic, Interrupted Galvanic, Faradic and surge Faradic Currents.The site of incision was prepared for aseptic surgery (clipping , shaving and disinfection) and apply antiseptic. Scalpel skin incision of 2 cm length at a distance of 1cm from the spine involving all skin thickness were made paravertebrally in the dorsal aspect of each animal. The skin incision were closed by simple interrupted 0/4 silk suture.In treated group fifteen rabbits were used in this group which was divided into three subgroups. five rabbits of each, were received 30 minutes of treatment (2.21-2.93µA, 29V. and 0.4 pulse duration)in a dose of 42833x10-8 watt/cm2(power) per day for 3,7 and 14 successive days. Stimulation was done immediately after finishing surgical operation then each day in same time, the process of Stimulation was involving fixing of the animal on the special restraint. The anode electrode was placed on the wound area(cranial border) and the cathode was placed on the opposite-site shaved area(caudal border), 2 cm proximal from the wound. Biopsies were collected from the stimulated wound, at 3, 7 and 14 days. Fifteen rabbits were regarded as a control group.In which the incision wound was not treated by galvanic. Biopsy from each wound was excised and removed (3, 7 and 14 days) for histopathological examination.Biopsies were taken under general anesthesia 1cm biopsy with full skin thickness were taken. The specimen was preserved in 10% formalin solution for histological evaluation. The remaining skin wound was sutured by silk suture (0/3) .

Results

The histopathological changes of the closed back skin wound (sutured) after 3 days post wounding (p.w) in male rabbits with galvanic field stimulation group reveal an accumulation of huge dense collagen fibers (thin and thick)with less hemorrhage and less edema.Early primitive granulation tissue filling the wound center were seen during this period. Thickening of the epidermal layer adjacent the site of the wound, with discrete epithelial cells distributed in the wound center (Fig.1). The fibroblast begin to stuck and crowded, with formation of new blood vessels but in less numbers. The inflammatory signs that include congestion of blood vessels and presence of neutrophile and macrophage were fade. Trace congested blood vessels were seen, few macrophage, no neutrophile with less edema. The dermis still suffering from mild necrosis while the sebaceous gland in the adjacent epidermis was still unaffected.In contrast, untreated skin wound at the same period (3 days p.w), the wound center were seen not filled completely with granulation tissue. The hemorrhage and edema were the dominant finding observed, with thin collagen fibers infiltrating with inflammatory cells and migrating epithelial cells under large and thick scab(Fig.2).The treated sutured skin wound at the 7 days post wounding show, completely disappear of signs of inflammation, (no edema, no inflammatory cells, no congested blood vessels). Complete and thick epidermal layers connecting the two sides of incision, while the epidermal layer seen extending deep in the epidermis(Fig.3). Between the two toungs of the epidermal layer, thick scar tissue fill the incision line. The collagen fibers reduced in number, the fibroblast become accumulated and more dense and converted to myfibroblast. No granulation tissue, and no newly blood vessels were seen(Fig.4). In contrast the untreated skin wound sections at the same time (7 days p.w) shown, presence of thick scab over the wound, the dermal layer filled mostly with collagen while the epidermal layer still unregenerated, there was hemorrhage, edema, presence of inflammatory cells, collagen fibers, newly granulation tissue formation, with the epithelial cells satellite in the epidermal space. There were flattening of the epithelial layer adjacent the site of incision(Fig.5).The histopathological sections of treated group at the 14 day post wounding show complete filling of the wound with flattened epithelial layer like the normal adjacent epithelial layer, with clear visible basement membrane(Fig.6). The center of incision were obliterated and filled with thin strip of mature scar tissue have less collagen and less number of fibroblast. Most of the fibroblast converted to myofibroblast .The center of the wound become narrow, and the collagen fibers take one direction. In contrast the untreated sections show fade signs of inflammation with less congestion of blood vessels, there was more granulation tissue filling the center of wound, and the epithelialization was incomplete(Fig.7).

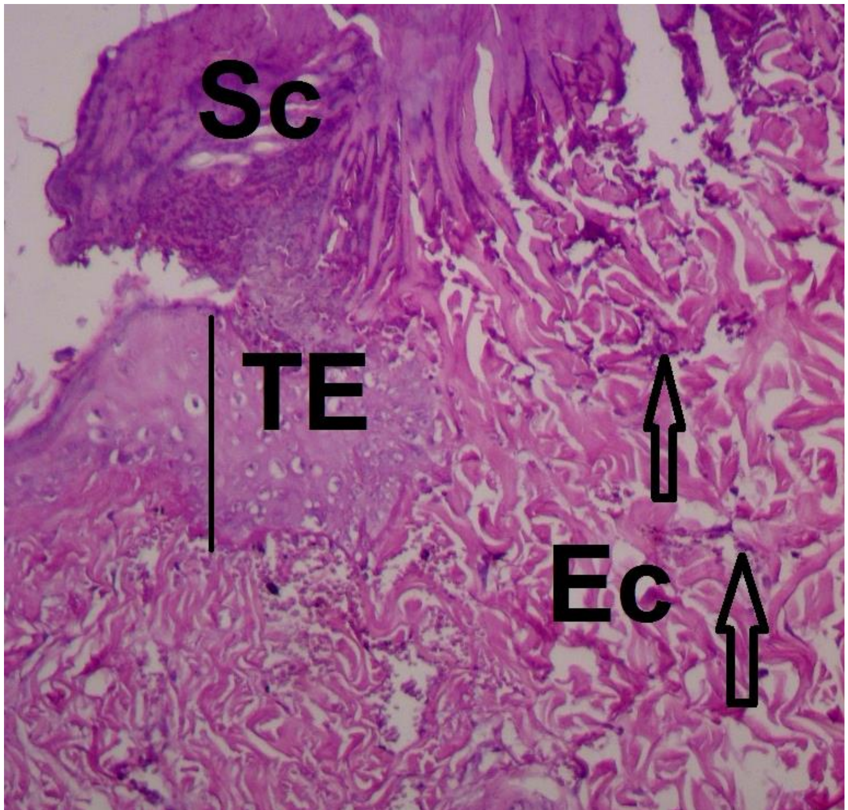

Fig.(1): 3 days p.w. galvanic treated skin section revealed thickening of the epidermal layer adjacent the site of the wound(TE) with discrete epithelial cells migrated into the connective tissue(Ec),Scab was seen over the wound(Sc). H&E, 100X.

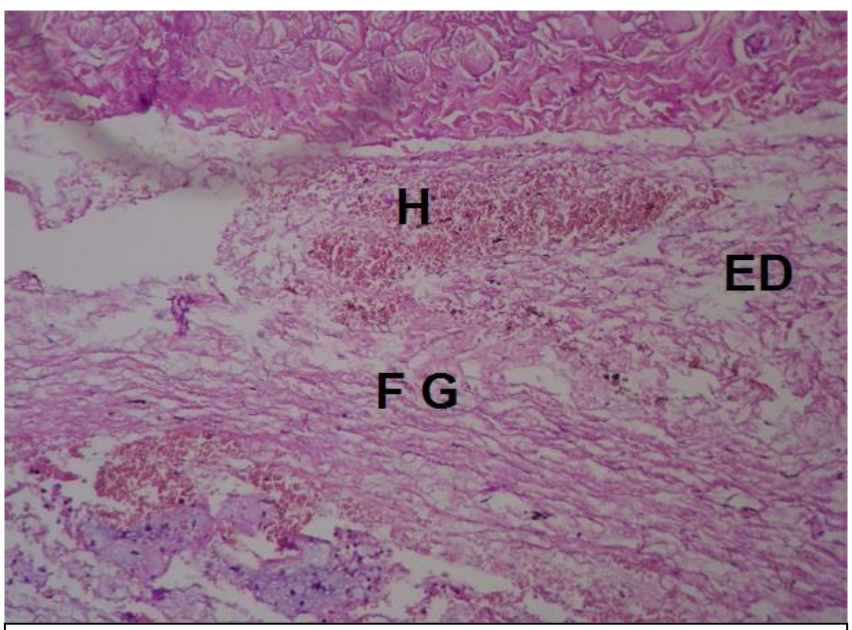

Fig.(2): 3 days p.w. contol skin section, there are hyperemia is observed at the surface(H), edema(ED) & wound center were seen not filled completely with granulation tissue(FG). H&E, 100X.

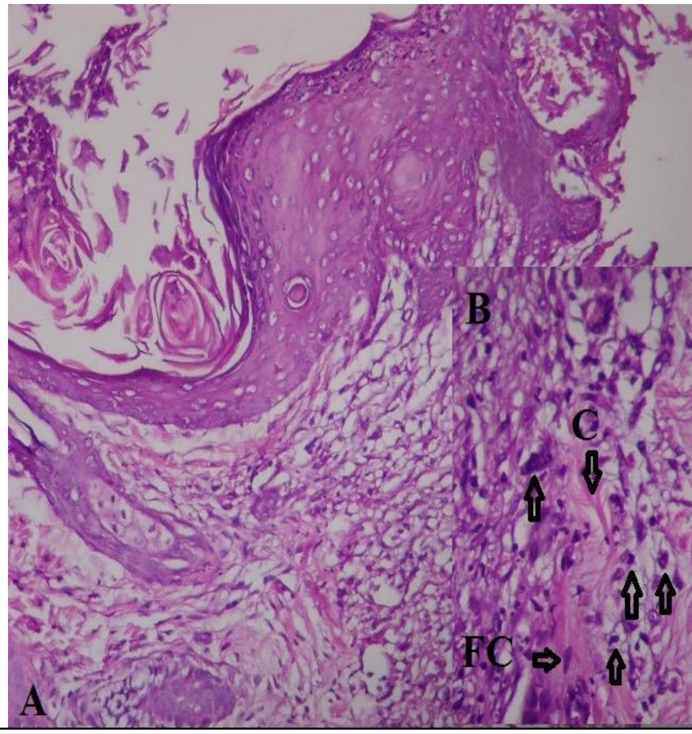

Fig.(3): 7 days p.w. galvanic treated skin section show complete regeneration of epidermal layer(A)H&E.40X. The fibroblast become accumulated and more dense(B)H&E.100X, with accumulation of scar tissue in the incision line note the scar tissue consists of collagen fibers (C) and fibroblast cells (FC).

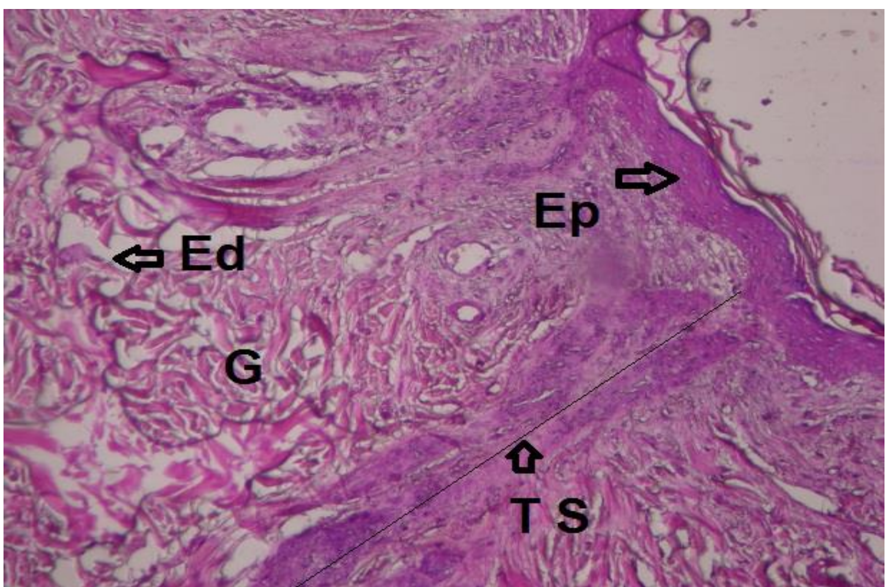

Fig.(4): 7 days p.w. galvanic treated skin section show the epidermal layer was extending very deep in the supporting stroma(TS). It observed complete reepithelization below the scab, mild edema(ED) and granulation tissue in the dermis(G). H&E, 100X.

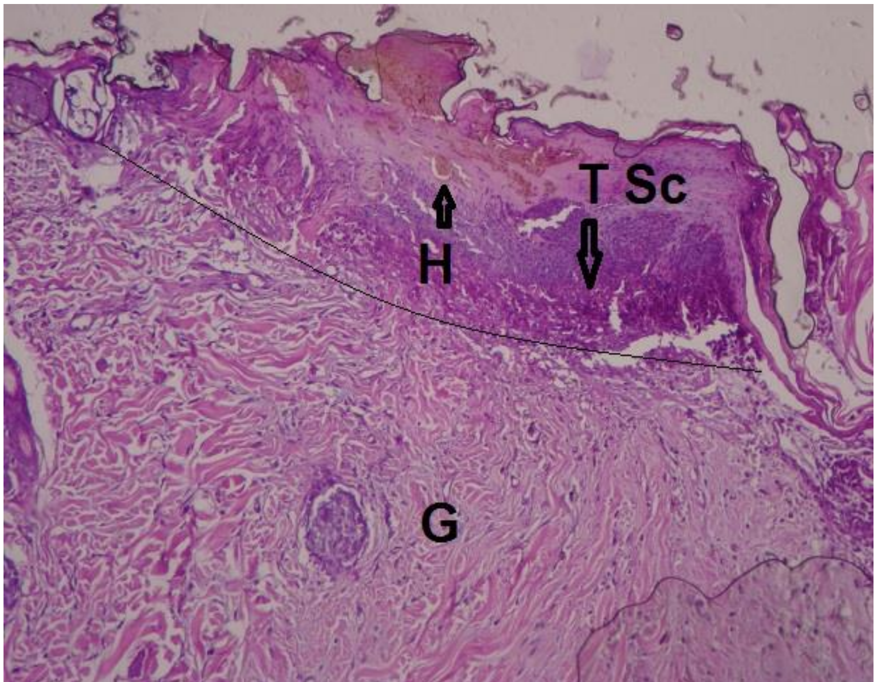

Fig.(5): 7 days p.w. control skin section, there are scab tissue over the wound(TSC), with accumulation of collagen fibers in the dermis (G). H&E, 100X.

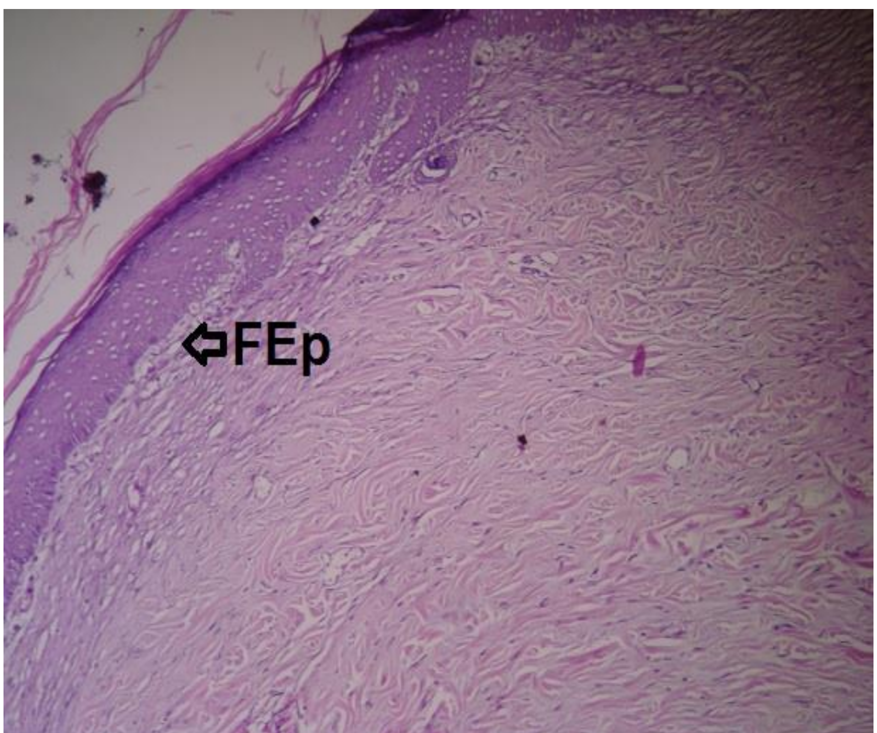

Fig.(6): 14 days p.w. galvanic treated skin section revealed the collagen fibers take one direction with flat epidermal layer (FEp). H&E, 100X.

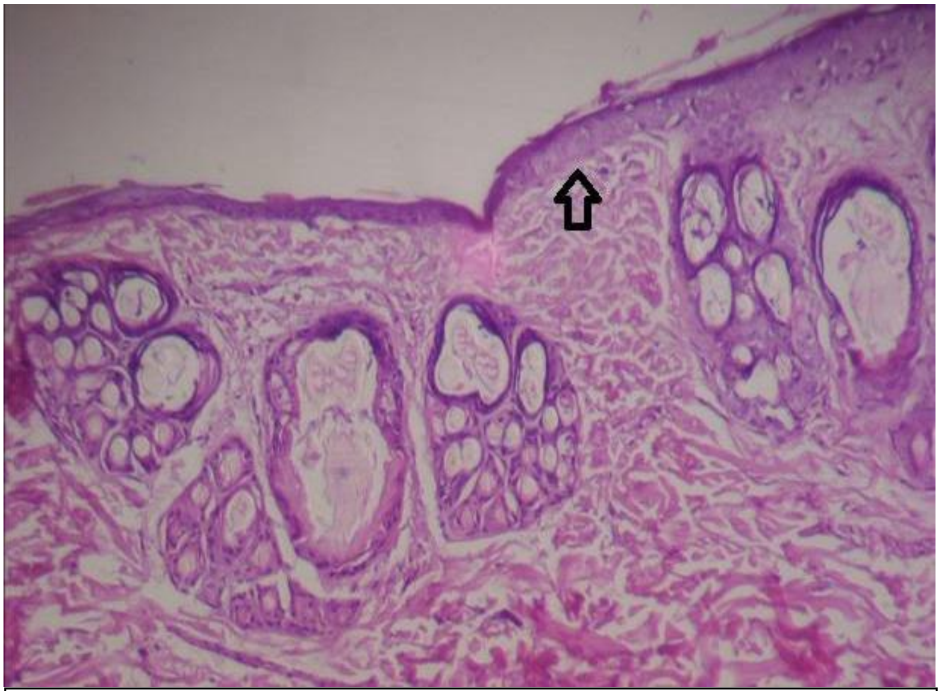

Fig.(7). 14 days p.w. control skin section revealed that the epidermal layer become flat and even. but the dermal layer still incompletely healing. H&E. 100X.

Discussion

This study was designed to investigated the effect of galvanic stimulation causing stimulation of wound healing. In which important findings concerning wound surface area were obtained on days 3,7, and 14 by application of electrical stimulation. Studies have been published on the effects of high voltage electrical stimulation on wound healing process, many of them either had a poor sample size or were poorly controlled (4).With galvanic field stimulation group, there were earlie accumulation of huge dense collagen fiber with less edema and primitive granulation tissue filling the wound center after 3 days. This result was agreement with Demir et al 2004(5) who found that the use of negative polarity current for first 3 days caused increase in collagen synthesis in wound healing and a greater decrease on the wound surface with respect to control group. Also we found the fibroblasts were found early stuck and crowded with formation of new blood vessels in 3 days post wounding in contrast to the control. This result was accord with Bayat et al.,2006(11) who found in this period the mean number of fibroblast and blood vessel were higher than those of control group. Less edema was found. This result was consistent with Reed,1988(12) and Thornton et al.,1998(13) who attributed due to the submotor levels of cathode limited edema formation by blocking macromolecular leakage from microvessels.During this period, early granulation tissue filling the wound center, presence of neutrophil and macrophage as well as signs of congestion of blood vessels were decreased and this was companionable with Demir et al., 2004 (5) who found that electrical stimulation shortens the inflammatory stage and hastens healing progression into the proliferation stage. Also in this period there was obvious thickening of the epidermal layer adjacent the site of the wound with discrete epithelial cells distributed in the wound center. This was consistent with Bayat, 2006(11) and Mehamodont, 2007(14) who declare that anodal stimulation suggested to facilitate migration and proliferation of epithelial cells , So improving wound closure.In this period there was a trace congestion of blood vessels were seen and this was compatible with Kloth 1995 (10)who found that application of positive current to wound which enhanced decrease vascular congestion.The application of a negative direct current, with respect to control group, cause grater and faster decreases of wound surface(5), and this accord with our study, when using two polarities on the skin , as well as same results get when using a positive polarized current and reported that after 4 days, wound epithelialization occurred faster in the stimulated animal group than in control group(6). The explanation of this may return to the macrophage cells that play important roles during the inflammatory phase of healing migrate toward the cathode(7), whereas neutrophils migrate toward both the anode and cathode(8,9), while Kloth,1995(10), have reported that leukocytes migrate toward the cathode which suggests a link between chemically mediated events and electrical responsiveness .The treated wound at the 7 days p.w. show completely disappearance of the signs of inflammation. This result supported by Talebi et al.,2007(15), who found reduction of the inflammatory stage causing faster beginning of proliferation stage .In this period the epidermal layer was thick, the scar tissue fill the incision line. This agreed with Mertiz et al.,1993(16), who declared epidermal cells migration macroscopically for (7 days)stimulation of induced wound . The collagen fibers reduced in number in this period. This result contradicted with Talebi et al.,2008(17), who found the cathodal stimulation increased the collagen density of wound tissue and this may attributed to the application of cathode alone on the wound in gninea pigs, while we use two electrodes together on the wound in the rabbit.The fibroblast seen accumulated and more dense and converted to myofibroblast early within the 7 days compared with the control at the same period. This result consistent with Castillo et al.,1995(18), found an increase of fibroblast number within the wound after pulsed electric stimulation.Also the thick scar tissue fill the incision line early in this period and this agreed with Peters et al.,2001(19), Houghton et al.,2003(20), found there was improving the strength and quality of scar formation.In 14 days post wounding the wound was completely covering with epithelial layer similar to the adjacent normal tissue with clear basement membrane. This result was accord with Alvarez et al.,1983(6), Brown and Gogia 1987(21), whom found that the current cause important epithelialization more rapidly compared with control group as well as Kloth and McCulloch,1996(22), Ojingwa and Isseroff 2003(1), who found that the effect of the current cause attraction and greater proliferation of epithelial cells.

Conclusions

To better understand the affecting mechanisms of electrical stimulation on the wound healing process, we further suggest measuring electrical potential at the wound site throughout healing and evaluating biochemical and histological factors as well as we suggest that endogenous potentials of injured skin be studied in infectious and chronic conditions. However, further investigations are required to address the stimulation effects in animals suffering from conditions (e.g., diabetic mellitus) that seem to retard wound healing and required to address the stimulation effect in other modalities in animals.

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تعجيل التئام الجروح الجلدية باستخدام التحفيز الكهربائي (كلفانيك) في الارانب

عباس علي حسين ثاير علوان عبد

الخلاصة

الهدف من هذه الدراسة هو تقييم تأثير التحفيز الكهربائي (كلفانيك)على عملية التئام الجروح في جلد الارانب. اجريت الدراسة على 30 ارنب نيوزيلندي ذكر بالغ قسمت عشوائيا وبالتساوي الى مجموعتين (السيطرة والمعاملة). كل مجموعة قسمت الى ثلاثة مجاميع ثانوية (5 حيوانات لكل مجموعة). تم عمل جرح على جلد منطقة الظهر لكل ارنب وخياطته باستخدام الخياطة المتقطعة البسيطة بخيط حرير حجم 0/4 .خضعت الجروح في مجموعة المعاملة الى التحفيز الكهربائي (2.21- 2.93µA, 29V. and 0.4 pulse duration in a dose of 42833x10-8 watt/cm2(power)) باستخدام اقطاب كهربائية امام وخلف الجرح. استمر التطبيق اليومي للتيار الكهربائي للأيام 3 ، 7 و 14 متتالية ولمدة 30 دقيقة في كل مرة . لغرض اجراء الدراسة النسيجية اخذت عينات نسيجية من منطقة الجرح في الايام (3 - 7- 14)بعد العملية.اظهرت النتائج بان الياف الكولاجين الكثيفة تكونت مبكرا مع تثخن طبقة الادمة المجاورة لمنطقة الجرح خلال الايام الثلاثة بعد الجرح، ورجوع الطبقة الطلائية بشكل كامل مع اختفاء علامات الالتهاب خلال سبعة ايام بعد الجرح، وبالمقارنة، كان هنالك شفاء غير كامل ووجود الالتهاب في مجموعة السيطرة .نستنتج من هده الدراسة امكانية تسريع شفاء الجروح بواسطة التكوين المبكر للنسيج الحبيبي وإعادة النسيج الطلائي وتقليل علامات الالتهاب عن طريق استخدام التحفيز الكهربائي.