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Low Frequency Ultrasonic Versus Microcurrent Effect on Tissue Healing After Tendon Suture

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ABSTRACT

Background: *Tendon injury has poor healing process. Therapeutic US has a positive role to play in providing the growth factors, also Microcurrent therapy has been used to increase the rate of healing.*

Purpose: *this study was conducted to investigate the difference between high frequency ultrasound versus microcurrent therapy on the tissue healing of Achilles tendon after suture.*

Methods: *Thirty female albino rats were surgically transected Achilles tendons, were randomly and assigned into three groups with ten rats in each group (group I served as a control, group II was treated with high frequency US and group III was treated with microcurrent). The treatments were administered with ultrasound and microcurrent day other day starting immediately from the day after injury for 5 weeks. Wound size measurements were evaluated till complete healing.*

Results: *Both low frequency ultrasonic and microcurrent have a significant effect on decreasing wound size where the mean value of the wound treated with low frequency ultrasonic decreased from 0.2 mm ± 0.03 to 0.0 mm in the fifth time of measurement while the wound size of the control group decreased from 0.2 mm± 0.08 mm in the fifth time of measurement. Comparing wound size in Micro-current group among the times of measurements Vs the control has shown a significant decrease of wound size where the mean value decreased from 0.2 mm ± 0.06 to 0.0 mm in the fifth time of measurement. The t test of group II has shown significant decrease in wound size measurement where the t value and P value showed that it was significant during 3rd and 5th times of measurement. The t test of group III has shown significant decrease in wound size measurement where the t value and P value showed that it was significant during 2nd, 3rd and 5th times of measurement.*

Conclusion: *low frequency ultrasonic has more significant effect thanmicrocurrenton decreasing wound size after tendon suture.*

Keywords: *Ultrasound, Microcurrent, Achilles tendon, Tissue healing.*

INTRODUCTION

Tendons are metabolically active tissues requiring vascular supply but, in some (Achilles tendon, tibialis posterior and supraspinatus), hypovascular or watershed areas have been identified [(1)]. Tendons receive their blood supply from three main sources: the intrinsic systems at the myotendinous junction and osteotendinous junction, and the extrinsic system through the paratenonor the synovial sheath [(2,3).]

Tendon degeneration may lead to reduced tensile strength and a predisposition to rupture. Indeed, histological evaluation of ruptured Achilles tendons has demonstrated greater degeneration than was found in tendons that were chronically painful as a result of an overuse injury[(4).] The golden aim of the repair of tendon injury is to achieve a permanent repair that could reestablish the smooth mobility of the regenerating tendon and to have significant tensile strength loads.[(5,6).]

The healing process is a balance between the intrinsic and extrinsic healing processes. The mechanism for achieving this balance may lie in the differential ability of the cells to respond to local factors[(7).] The penetration (or transmission) of ultrasound (US) is not the same in each tissue type, it is clear that some tissues are capable of greater absorption of US than others. Generally, the tissues with the higher protein content will absorb US to a greater extent, then tissues with high water content and low protein content absorb little of the US energy (e.g. blood and fat) while those with a lower water content and a higher protein content will absorb US far more efficiently. Tissues can be ranked according to their relative tissue absorption and this is critical in terms of clinical decision making [(8).]

During the proliferative phase (scar production) US also has a simulative effect (cellular up regulation), though the primary active targets which are now the fibroblasts, endothelial cells and myofibroblasts cells[(8,9,10).] It also enhance fibroplasia and collagen synthesis[(11,8).] (Therapeutic US has a positive role to play in providing the growth factors [(12,13).]

Micro electrical current stimulation (MES) is a physical therapy modality providing electric current in millionths of an ampere.[(14)]MES appears to play a significant role in the healing process, as it can promote healing in variety of bone and skin lesions. The evidence for other tissues is encouraging but presently scant. MES uses electric currents similar to those produced by the body during tissue healing. It may be particularly beneficial where endogenous healing has failed [(15).] An effective mean of promoting tendon healing either an US or electrical stimulation will be the concern of this study.

METHODOLOGY

Instrumentation:

- Ultrasound apparatus: Sonopuls 464 ultrasound unit manufactured by Enraf-Holland; pulse repetition rate 100 Hz; pulse duration 2 ms; model 1464-900; serial number 9-043
- MES apparatus was used to deliver microcurrent stimulation, model: EMSI-4250, serial no.:A358612, made in Taiwan.

- Plastic sheet: was used to measure the wound size and wound total circumference, before MES application and every session during the treatment period.
- Q tips to measure depth of the wound.

Surgical Procedures

Following the protocol of Sharifi et al., (2007)[16].

Preparation of the animals

In preparation for surgery, the rats were fasted 12 hours for food while water was withheld 3 hours only before the operation. Immediately before the surgery the hair was removed from the site of the operation, at the posterior and medial aspects of the hind limb using hair removal cream, the remaining hair was short cut using hair scissor. Each rat was weighed before the operation to determinate the dose of anesthesia.

Anesthesia

On the day of surgery each rat was weighed and anesthetized by general anesthesia using intramuscular injection of ketamine hydrochloride (35 mg/kg body weight) (Ketalar (Parke _Davis SA Barcelona _Spain) and Xylazin hydrochloride (5 mg/kg body weight) (Rompun 2% (Bayer Leverkusen, Germany).

Surgical Technique

All surgical techniques were done under complete standard sterile conditions. The animal was immobilized on the surgical table in a prone position. Achilles tendon of right hind limb of each rat was exposed and isolated, through about 3cm of incision at the hind limb. Achilles tendon was sharply transected with a scalpel, about 1cm apart from calcaneal insertion Each end of the served Achilles tendon was approximated and immediately sutured by 4/0 proline (Ethicon, NJ, USA) using modified Kessler suture technique. The skin was then left opened. Afterward; the operated hind limb was immobilized using plaster of Paris cast leaving opening for E.S.

Immobilization

After tenotomy and suture, the served hind limb was immobilized in a plaster of Paris cast. The cast extended from mid-thigh to the toes, with the knee in flexion and ankle held in 45 degree of planter flexion to make the calf muscles in shortened position. A window was done at the site of tenotomy for wound dressing and MES application in the treatment groups

All animals were returned back to cages and were injected intra-muscularly by prophylactic antibiotic.

Treatment procedures

- Before treatment the skin was cleaned and any growing hair was removed to decrease the electrical resistance of the skin over the site of the electrode placement.
- Ultrasound was applied from the first day post injury and continued throughout the study at a frequency of three sessions per week for 5 weeks. Ultrasound was applied on the site of injury with frequency 1MHZ and intensity of 0.5 W/cm2 for 5 minutes.

MES was applied from the first day post operative and continued throughout the study at a frequency of 3 sessions per week. MES was applied transcutaneously.

- Micro-current electric stimulator, model: EMSI-4250 (made in Taiwan) was used for the treatment. The active electrode (cathode) (1.0 x 1.0 cm) was placed over the tendon injury site, while the inactive electrode (anode) was placed proximally on the thigh region of the same side, approximately 3 cm apart. Clip electrode were used.
- Intensity 100 micro ampere , frequency 10 Hz ,duration 30 min, pulse width 50 ms, and constant mode of application was used in the study.
- Only one person was responsible to provide treatment for all animals, to standardize the handling process.

Wound size measurements:

By using clear and sterilized plastic sheet to measure the wound length x width x depth, to get the total wound size, Wound circumference had taken by multiplying length x width x depth of the skin wound of all groups (both study groups and control group). This procedure took place before starting the treatment protocol, immediately after the surgical procedure was performed, and it was repeated each session during the treatment period throughout the study.

RESULTS

The data presented in table (1) showed the mean and the standard deviation of the wound measurements of the area of tenotomized tendon of the study group which received low frequency ultrasound were lower than the control group which did not receive the ultrasound treatment.

The wound size measurements every other day between low frequency ultra sound and control groups showed significant decrease in wound size till complete healing, where the mean size decreased from 0.2388 ± 0.08206 to 0.0. Application of ANOVA showed significant decrease of wound size, where F value was 0.503 and p value was ($P \leq 0.02$) as shown in table (1 and 2). Application of post hoc test showed a significant decrease in wound size during all periods of measurements except the first period of measurement (before treatment)

Table (1): Wound size measurement through treatment periods between control group low ultrasound and Micro-current group (mean ± standard deviation).

Times of measurements	**Control Group** X±SD	low ultrasound group X±SD	Micro-current **group** X±SD
1st	0.236±0.041	0.2388 ±0.08206	0.213±0.0612
2nd	0.177±0.034	0.1280* ±0.05098	0.148±0.026
3rd	0.146±0.033	0.0554* ±0.02984	0.064* ±0.0182
4th	0.11±0.032	0.0213* ±0.0238	0.0386* ±0.0152
5th	0.04±0.021	0.0*	0.0*

*significant at ≤ 0.05

Table (2) ANOVA between low frequency ultra sound and control

Comparison	Degree of freedom	F value	P value
Group 2 Vs control	29	0.503	≤ 0.02

Table (3): Application of Analysis of Variance between Micro-current group during periods of measurements.

Micro-currentvs Control g	**Degree of freedom**	**F value**	**P value**
1st	29	0.3571	0.7029
2nd	29	3.506	0.0443*
3rd	29	21.923	< 0.0001*
4th	29	0.5182	0.6014
5th	29	33.058	< 0.0001*

*significant <0.05

Comparing wound size in low frequency ultrasonicgroup among the times of measurements Vs the control has shown a significant decrease of wound size where the mean value decreased from 0.2 mm ± 0.03 to 0.0 mm in the fifth time of measurement while the wound size of the control group decreased from 0.2 mm± 0.08 mm in the fifth time of measurement. The t test has shown significant decrease in wound size measurement where the t value and P value showed that it was significant during 3rd and 5th times of measurementas shown in table**(4).**

Table (4): t-test between low frequency ultrasonic group and Control group among evaluation periods

low frequency ultrasonicvs Control g	**Degree of freedom**	**T value**	**P value**
1st	9	0.6331	0.24520
2nd	9	3.531	0.5312
3rd	9	6.000	< 0.0001*
4th	9	0.6432	0.2843
5th	9	7.83	< 0.0001*

Comparing wound size in Micro-current group among the times of measurements Vs the control has shown a significant decrease of wound size where the mean value decreased from 0.2 mm ± 0.06 to 0.0 mm in the fifth time of measurement while the wound size of the control group decreased from 0.2 mm± 0.08 in the fifth time of measurement. The t test has shown significant decrease in wound size measurement where the t value and P value showed that it was significant during 2nd, 3rd and 5th times of measurements shown in table **(4).**

Table (4): t-test between Micro-current group and Control group among evaluation periods

Micro-current vs Control g	**Degree of freedom**	**T value**	**P value**
1st	9	0.8771	0.3920
2nd	9	2.631	0.0169*
3rd	9	8.000	< 0.0001*
4th	9	0.7902	0.4397
5th	9	10.83	< 0.0001*

*significant at <0.05 ,t:test ,p: probability.

Morphological changes:

Achilles tendon morphology:

The normal Achilles tendon of female rat which was not subjected to the experiment showed normal parallel arrangement of acidophilic collagen bundles separated by flattened Fibrocytes with dark flattened nuclei.

Group I (After one week):

One week following injury of Achilles tendon of untreated group (group I) revealed few collagen bundles arrangement with fibroblasts migrated to the area of injury with newly formed blood capillaries. In contrast, Achilles tendon of rats treated with low frequency ultrasound (group II), showed more abundant irregular arranged collagen bundles with numerous fusiform fibroblasts at the site of injury. Inflammatory cells and new blood vessels are also seen.

In cathodal treated achilles tendon of the same group, an apparent increase in the amount of regenerating collagen bundles was observed. Fibroblasts with pale oval nuclei and fibrocytes with dark flattened nuclei were aligned on the regenerating collagen bundles, infiltrating cells were scattered in-between the collagen bundles.

Two weeks following injury, the Achilles tendon in the untreated group (group II) showed collagen bundles invading the injured area with cellular infiltration and blood vessels. In contrast, rat Achilles tendon of (group II) treated with low frequency ultrasound showed denser collagenous bundles filling the injured area with areas of heavy cellular infiltration.**Fig (1)**.

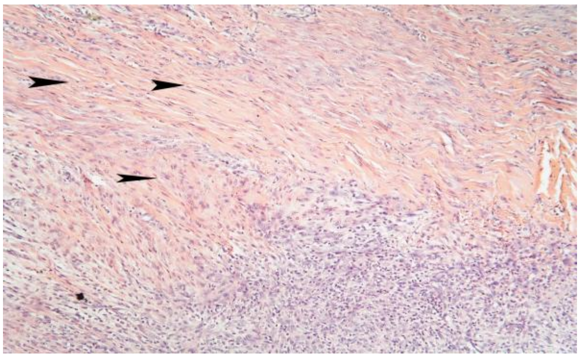

Fig (1): photomicrograph of Achilles tendon of female rat in group II after two weeks showing condensed collagen bundles (arrow head) filling most of injured area with cellular infiltration. (H&EX100)

Two weeks following injury Cathodal treated achilles tendon of the same group showed regularly arranged regenerated acidophilic collagen fibers. Few Infiltrating cells were observed.**Fig (2).**

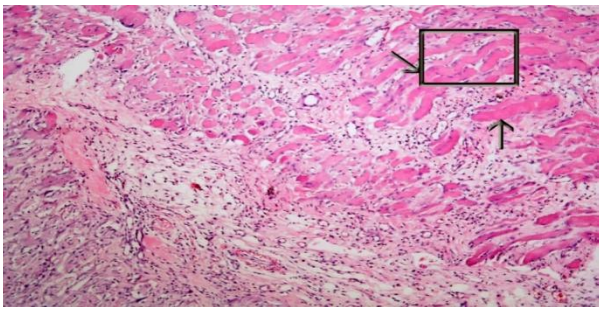

Fig.(2): Photomicrograph of a section in cathodal treated Achilles tendon in group III showing regularly regenerated acidophilic collagen bundles (arrow) (H & E, x100).

Five weeks following injury sections in Achilles tendon in untreated group (group I) showed collagen bundles (dense but in different directions), abundant fibroblasts with few fibrocytes, with also some blood vessels are still seen. While group II showed parallel arrangement of the dense collagen bundles with less spacing and more fibrocytes.(**Fig. 3).**

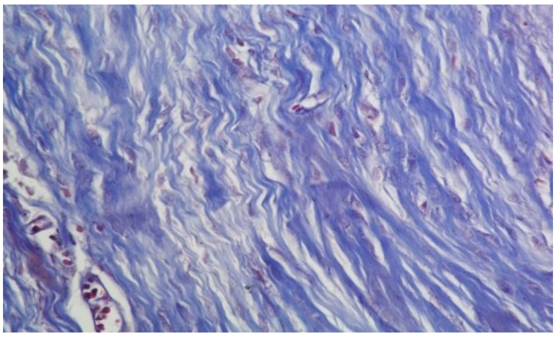

Fig (3): photomicrograph of Achilles tendon of female rat in group II treated with low frequency US for four weeks showing more regular arrangement of collagen bundles with less spacing. (masson's X400).

Regularly arranged collagen bundles intermingled with many fibroblasts were recorded in group III**.** regularly arranged collagen bundles occupied most of the field**(Fig4).**

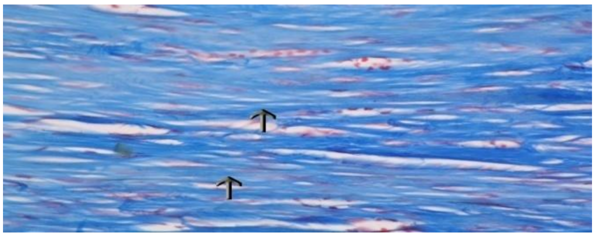

Fig. (4): Photomicrograph of a section in cathodal treated Achilles tendon demonstrating regularly arranged collagen bundles occupying most of the field (arrow) (Masson's Trichrome, x400).

DISCUSSION

The results of this study showed significant decrease of wound size among the low frequency ultrasonic and Microcurrent groups compared with the control group. Application of Bonforrini multiple trial test showed significant decrease in wound size during second, third, and fifth periods of measurements. Group (II): showed that wound size has shown a significant decrease from 0.2 mm ± 0.03 to 0.0 mm during three weeks where P value was< 0.0001. On the other hand, in the control group, the wound size decreased from 0.2 mm± 0.08 where P value was < 0.0001 but this takes longer period than the experimental group.

Selection of ultrasound application parameters is based on the desired effect and the location and density of the tissue to be treated. These decisions are best made by the physician and the therapist experienced in performing therapeutic ultrasound. Common indications for high frequency ultrasound therapy include treatment of tendon injuries and short-term pain relief. Ultrasound has also been shown to promote healing of some acute bone fractures, venous and pressure ulcers, and surgical incisions. However, conventional therapeutic ultrasound can cause burns or endothelial damage if applied incorrectly.

Recently, low frequency ultrasound was tested and introduced to the market. The motivation of looking for alternative ultrasound parameters was due to the fact that application of high-frequency US in clinical medicine is limited due to tissue heating. Thus, using low-frequency US with less tissue heating, thereby acting as a "slow release" mechanism, may become the standard care in treating slow-to-heal lesions, skin ulcers and nonunion fractures. In addition it may be able to facilitate protein secretion and enzymatic reactions.

Based on the results of Marie et al., (2008)who reported that, low frequency ultrasound energy are thought to be primarily attributed to the mechanisms of cavitations and micro streaming. *Cavitations* involve the creation and oscillation of microscopic bubbles that concentrate acoustic energy into shearing and micro streaming fields. The movement and compression of these bubbles can cause changes in cellular activities(17) *Furthermore, Micro streaming* is the physical forces of sound waves that are capable of displacing ions and small molecules. The mechanical pressure applied by the micro streaming wave produces a unidirectional movement of fluid along and around cell membranes. Based on laboratory studies on both *vivo* and *vitro*, the combination of cavitations and micro streaming, which occur more frequently with low frequency ultrasound, appear to provide a mechanical energy capable of altering cell membrane activity and, therefore, cellular activity.

In contrast, Watson (2008)showed that enhancement of wound healing using the properties of high ultrasound suggested that the higher the US frequency, the greater the absorption rate; raising the temperature above normal thermal levels by a few degrees may be attributed to numbers of beneficial physiological effects(8).

In this study, the wound size measurements were taken every other day for low frequency ultra sound and control groups. It showed significant decrease in wound size till complete healing, where the mean wound size decreased from 0.2388 mm ± 0.08206 to 0.0 (P value≤ 0.02), and application of post hoc test showed the significant decrease were in all times of measurement except the first period of measurement (before treatment). One of the objectives of the use of therapeutic ultrasound in wound healing is to reduce the inflammatory phase and accelerate the migration and proliferation of fibroblasts, which are critical to the healing process. The results of this study went in accordance with several studies, that showed the same effect of low frequency ultrasound on the wound healing (18,19,20).

Low-frequency ultrasound therapy is a modality used to promote healing in chronic wounds by cleansing and maintenance debridement to remove yellow slough, fibrin, tissue exudates, and bacteria. The mechanisms by which low frequency ultrasound energy stimulates wound healing have been previously described (21).

Breuing et al., (2005)provided further support on the effectiveness of low frequency ultrasound therapy in a group of 17 patients with acute and chronic wounds of varying etiology. These patients also received adjunct wound therapy, which included moist dressings, alginate and Panafil. A total of nine wounds (47%) healed primarily or were sufficiently debrided to receive skin grafting, while six wounds (29%) achieved greater than 50% healing. The remaining 2 wounds achieved 20% and 30% healing of the original wound area. It should be noted that due to the varying etiology of wounds within this patient cohort, the frequency of treatment varied substantially, ranging from twice / week to fortnightly sessions and the average number of treatments per wound ranged from 6 to 15 over the 3-month period[22].

In this study, group(III): showed that wound size has shown a significant decrease from 0.2 mm ± 0.06 to 0.0 mm during two weeks where P value was< 0.0001. On the other hand, in the control group, the wound size decreased from 0.2 mm± 0.08 mm where P value was < 0.0001 but this takes longer period than the experimental group.

It was suggested that, electrode placement significantly affected field strength in the target tissue. Furthermore, the presence of many surrounding structures reduces field strength in the target tissue considerably. These factors should be taken into account when establishing protocols for electrical current based therapeutic devices once it has been proven that these devices are clinically effective. It was concluded that tissue composition and electrode placement of electrical therapeutic devices strongly affect the effective field strength in the target tissue. Knowledge about these effects were necessary for the further assessment of these devices with respect to their potential value for clinical use[23].

There was another point with respect to the direction of current application. Physiological electric fields can also control the direction of cell migration [24]. In general, cells orientated parallel to an electric field will retract, and reorient perpendicular to the electric field [25]. After injury tenocyte proliferation and collagen synthesis were randomly organized, and may result in the formation of inferior tissue.

In an attempt to identify the optimal method for applying microcurrent in this study totenocytes in culture, the results revealed that the direct contact between the electrodes and the culture medium or the cells themselves resulted in extensive cell death. It was suggested that, this phenomenon could be a result of electrolysis, whereby dissolved positive and negative ions in the medium were discharged at the cathodal and anodal electrodes, respectively, and was probably mediated through formation of H_2O_2 at the anodal electrode. The use of bridges to allow indirect current transmission avoided this. However, only the paper bridges worked satisfactorily. The agarose bridges most likely had an intrinsic resistance that was high enough to prevent sufficient current to generate a biological effect to pass to the cells.[26].

Similar results was reported by Mehmandoust at al., (2007)that, the use of positive polarity augments the migration and proliferation of epithelial cells and therefore hasten wound closing. It was suggested that, the wound closing in a shorter periods was due to the antibacterial effect of negative polarity and the epithelialization effect of positive polarity[27]. Also Mehmandoust et al.,(2007)added that some useful effects of negative polarity include killing microorganisms, removing necrotic tissues due to decreasing pH of the wound environment, limiting protein molecule infiltration and therefore limiting the formation of edema in the injury site, and increasing fibroblast cell proliferation and collagen synthesis[27].

This study was in agreement with Rowley et al., (1974) who study that the use of negative polarity for the first 3 days and positive polarity for the remaining days throughout the healing period. As the negative polarity has antibacterial effects[28]. In contrast, Bayat et al., (2006)Reported that, in both vivo and vitro studies the application of positive polarity has some useful effects, including destruction of microorganisms, increased migration and proliferation of the epithelial cells, increased attraction of macrophage cells to the wound site, and better stimulation of endogenous currents of the wound[29].

It was known from the past that cathode was superior to anode in wound healing process. Robert I. and Pcker,[30] reported that, the cathodal (negative) current has been shown to be successful in stimulating bone deposition and repair if applied at the fracture site as an indwelling electrode. Consistent with this empirically successful clinical approach to stimulating bone repair is the observation that injury to bone produces negative voltage-potential gradients in the area of injury relative to the undamaged bone. Short-lived potential differences are also induced by stressing the bone with a mechanical load. Areas of compressive stress are electronegative relative to the unloaded portion of the long bone.

In addition PoltawskiL, and Watson Tadded that MES promotes healing in variety of bone and skin lesions. The evidence for other tissues is encouraging but presently scant. MES uses electric currents similar to those produced by the body during tissue healing. It may be particularly beneficial where endogenous healing has failed[31].

The effect of the electrical stimulation on biological structures was related to the current flow through the target tissue. This study clearly demonstrates that the effective field strength in the target tissue may vary greatly with the plane in which the current is applied and with the tissues located between electrodes and target tissue. Main conclusion from this study is therefore that the current output of any therapeutic device has to be considered a very unreliable indicator of actual field strength in the target tissue, and hence of potential therapeutic effect. We did not vary the direction of the current, i.e. the positions of the negative and positive electrodes. To the authors' knowledge there was no data on possible semi-conductivity of biological tissues, but it is an item that might be addressed in future studies.[26]

Regarding the morphological changes

Andres and Murrell 2008 reported that the healing process is a balance between the intrinsic and extrinsic healing processes. The mechanism for achieving this balance may lie in the differential ability of the cells to respond to local factors[7].

In 2003 Joan and his colleagues reported that, the phases of wound healing are overlapping, but are described in a linear fashion for the purpose of clarity. They suggested that the five phases that characterize wound healing include (1) homeostasis, (2) inflammation, (3) cellular migration and proliferation, (4) protein synthesis and wound contraction, and (5) remodeling[32].

In the present study, group (II) during the first two weeks post injury, the wound was heavily populated by inflammatory cells and vasculature; as the healing progressed fibroblasts and endothelial cells were predominated; mainly after the 3rd week fibroblasts became inactive and changed into fibrocytes. After the 4th week fibrocytespredominanted.

In the current study the morphometric results regarding the ratio between fibroblasts &fibrocytes showed significant increase in the numbers of fibroblasts which are responsible for collagen production and healing process in response to US in low subgroup when compared to both untreated and high subgroups after 1st two weeks of the study. This is in accordance with previous work of Yeung et al., 2006 who found an improvement in the quantity of fibroblasts and collagen alignment in Achilles tendons of rats treated with low ultrasound after the 3rd week of this study there was significant increase in the fibrocytes numbers in low subgroup when compared to both untreated and high subgroups[(33).]

In group, (III) (Two weeks following injury), H&E stained sections showed thatcollagen bundles were disrupted, irregularly arranged and infiltrated with active fibroblasts that exhibited pale oval nuclei .Mononuclear cell infiltration was clearly evident in between the bundles together with congested capillaries, these changes were gradually replaced by acidophilic regularly arranged collagen bundles that occupied most of the field. Resident and quiescent fibrocytes with dark flattened nuclei were aligned in- between these bundles and with gradual disappearance of the inflammatory cells and congestion.

Fereshte, 2007added that the negative polarity (cathode) exhibited other useful effects in means of killing microorganisms, removing necrotic tissues due to decreasing pH of the wound environment, limiting protein molecule infiltration and therefore limiting formation of edema in the injury site and increasing fibroblast cell proliferation and collagen synthesis[(34)].

Going along with results of the present study, it was reported by Sharifi et.al, 2007thatincreased blood supply enhanced the rate of protein synthesis in fibroblasts responsible for collagen production, therefore, an adequate blood supply is necessary for tendon healing[(16).]

CONCLUSION

Both low frequency ultrasonic and microcurrent electrical stimulation is an effective mean for promoting wound healing. Despite many studies on wound healing, the electrical stimulation is still in its infancy, an important parameter of electrical stimulation in wound healing is the type of applied polarity. The present study revealed the following findings. that "low frequency ultrasonic has more significant effect than microcurrenton decreasing wound size after tendon suture".

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