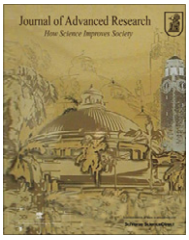
Journal of Advanced Research (2012) 3, 109–117

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

Polarity effect of microcurrent electrical stimulation on tendon healing: Biomechanical and histopathological studies

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Received 12 November 2010; revised 3 May 2011; accepted 8 May 2011
Available online 11 June 2011

KEYWORDS

Microcurrent electrical stimulation;
Tendon;
Healing;
Polarity;
Biomechanical testing

Abstract The purpose of the current study was to investigate the effect of microcurrent electrical stimulation (MES) applied with different polarity on the biomechanical properties of injured tendons and to correlate results with histopathological studies. Ninety six male white New Zealand rabbits were used in the study. Six rabbits were kept as normal group with intact tendons and the remaining 90 rabbits with their right Achilles tendons tenotomized, sutured and immobilized. After that rabbits were allocated into equal three groups; cathodal, anodal and control. Each group was further subdivided into three subgroups according to the study period; 3, 5 and 8 weeks. There were significant increases of all biomechanical measurements for cathodal and anodal groups than those of control group at all study periods. Furthermore there were significant increases of all biomechanical measurements in the cathodal group more than the anodal group at the 3 week period, while there was significant increase of the anodal group more than the cathodal at 5 and 8 week periods. The histopathological findings supported the biomechanical results. Tendons in cathode group showed better healing picture compared to those of anodal group at third week. While tendons in the anodal group showed better improvement at the 5 and 8 week. MES improved the healing

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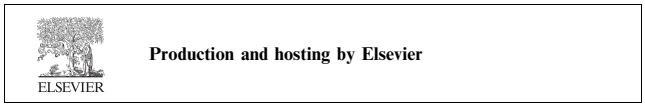
Peer review under responsibility of Cairo University.
doi:10.1016/j.jare.2011.05.004



process of tendon and the polarity of MES could be an important factor to be considered in treating tendon injuries.

Introduction

Injury of the dense connective tissues as tendons and ligaments, either from acute trauma or repetitive strain lesions results in protracted periods of disability. The resolution of such injuries often fails to restore the normal morphologic and functional characteristics of the tissue structure and therefore, either compromises the future performance of the individual or predisposes to an increase risk of recurrent injury [1,2]. Tendons are characterized by their slow rate of healing and much debate has been aroused concerning the intrinsic capacity of tendons to heal after injury. Tendons have shown a capacity for healing, either alone or in conjunction with extratendinous structures. Intrinsic healing results in improved biomechanics and has less complication. In particular, a normal gliding mechanism within the tendon sheath is preserved. It was suggested that a means of enhancing intrinsic repair mechanisms would be highly desirable [3–6].

MES is a low-intensity current that delivers monophasic or biphasic pulsed microamperage currents usually between 1 microampere (μA) and 1000 μA. MES is thought to mirror the body's own natural current as so, it has the privilege of using electric currents similar to those produced by the body during tissue healing and it may be a particularly beneficial where endogenous healing has failed [7,8]. It was reported that MES plays a significant role in enhancing the healing process of tissue healing [9]. The proposed mechanisms by which MES produced its effect are, increasing adenosine triphosphate (ATP) concentration, promoting amino acid uptake, and enhancing protein synthesis in human fibroblasts [4,8,10].

Regarding the effect of MES on tendon healing, many studies have been conducted using variable current parameters, and demonstrated that MES improves tendon healing [4,11–15]. The effect of MES may be related to the selected treatment parameters as current intensity, current density and polarity [11,12,15]. An important parameter of electrical stimulation in healing is the type of applied polarity which may affect protein synthesis, cell migration, growth of bacteria, electrotaxis, inflammation, edema, and also the processes of bioelectric events of injury [10,16–19]. Some studies have reported significant improvement of tendon healing using negative polarity [11,12], while others reported significant improvement using positive polarity [13,14].

So, despite the presence of many studies on the effect of MES on tendon healing, more comparative studies are needed to compare and standardize the ideal polarity at each stage of tendon healing. Therefore, the present study investigated the effect of MES with different polarity on the biomechanical and histopathological properties of surgically repaired rabbits Achilles tendon at different stages of healing.

Material and methods

Animal model

The ethical committee of the Faculty of Physical Therapy, Cairo University approved this study. Ninety six, 4–6 months old male New Zealand White rabbits, with average weight 2–2.5 kg, were used in this study. The rabbits were purchased from the Rabbit Production Unit, Faculty of Agriculture, Cairo University. The animals were housed individually in a standard rabbit cage of $15 \times 20 \times 20$ cm. (The size of the cages did not allow them full activity such as running, but they could move around freely.) The rabbits were kept at the same conditions of temperature (about 20 °C), humidity (50%) and light (a 12 h light/dark cycle), and subjected to comprehensive veterinary care. Tap water and balanced diet were given ad libitum throughout the study. The rabbits were assigned to normal group served as a basic reference and three studied groups. The normal group included six rabbits with intact tendons five of which were used for biomechanical measurements and one was processed for histopathological studies. The remaining ninety rabbits, their right Achilles tendons were tenotomized, sutured and immobilized. After that, rabbits were randomly divided into equal three groups ($n = 30$ in each) by a technician not involved in the surgery. The studied groups were cathodal, anodal and control and each group was further subdivided into three subgroups according to the study period; 3, 5 and 8 weeks ($n = 10$ in each). In each subgroup, 7 of the tendons were used for biomechanical measurements and 3 were processed for histopathological studies. Tendons in the cathodal and anodal groups were treated with MES while those of the control group did not receive MES treatment.

Surgical procedures

In preparation for surgery, food was withheld 12 h while water was withheld 3 h 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 rabbit was weighed before the operation for the determination of the dose of anesthesia. The rabbits were anesthetized by general anesthesia using combination of 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).

All surgical techniques were done under sterile conditions according to the following steps (Fig. 1). The animal was immobilized on the surgical table in a side lying position. The right Achilles tendon was exposed and dissected using a longitudinal incision of about 3 cm in length on the medial aspect of the leg extending from just above the heel to the middle of the leg. Achilles tendon was sharply transected with a scalpel, about 1 cm apart from calcaneal insertion. To standardize the injury mode in both groups, a complete surgical transection of the Achilles tendon was performed. After that both ends of the severed Achilles tendon were approximated and sutured by 4/0 Proline (Ethicon, NY, USA) using modified Kessler suture technique. The skin was then closed by interrupted silk sutures. Afterward; the operated limb was immobilized using Plaster of Paris cast with the knee in flexion

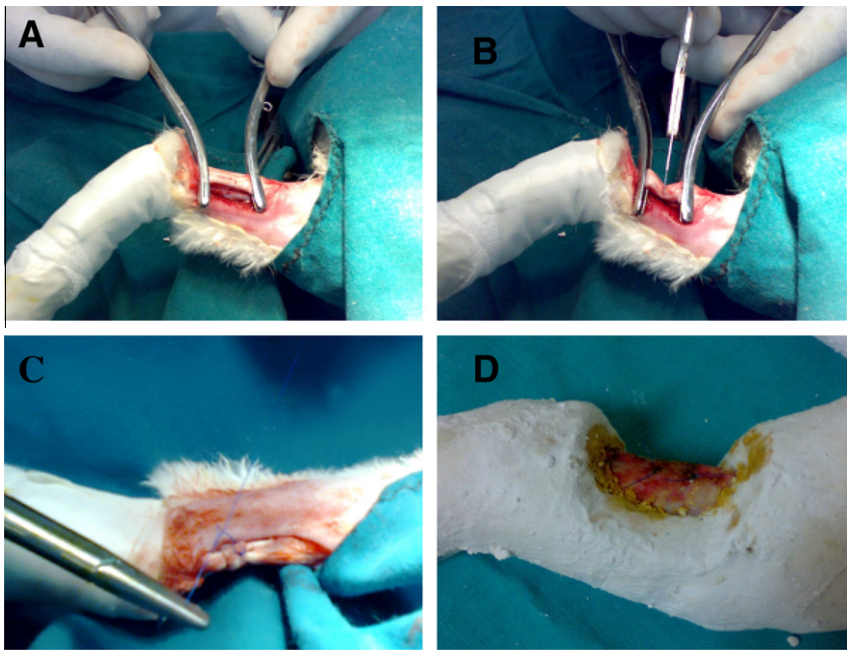


Fig. 1 (A) The right Achilles tendon after dissection, (B) tenotomy, (C) repair and (D) skin closure and immobilization with window at tenotomy site.

and ankle held in 45° of plantar flexion so that the calf muscle was in a shortened position [20]. A window was done at the site of the tenotomy for wound dressing and MES application. All rabbits were returned back to their cages and were fed ad libitum with prophylactic antibiotic to their drinking water. On the sixth postoperative day, all cast were removed and unlimited movements of the rabbits within cages were permitted.

Microcurrent electrical stimulation application

Rabbits in both anodal and cathodal groups were treated transcutaneously at the tenotomy site using MES according to a treatment regimen of 6 sessions/week on a daily basis from the first day post surgery and for the entire duration of the study (3, 5 and 8 weeks). A Trio 300 electric stimulator (ITO, Tokyo, Japan) was used to deliver MES. The following parameters were used; intensity 100 $\mu A/cm^2$, pulse frequency 10 Hz, pulse width 50 ms, with a duration 30 min [8,13,14]. The polarity of the active electrode was positive for anodal group and negative for the cathodal group. The device was calibrated using EZ Digital 60 MHz Analog Oscilloscope OS-5060A (EZ Digital Co. Ltd., Gyeonggi, Korea). 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. As shown in Fig. 2 during treatment, each rabbit was positioned relaxed on his side and two disposable electrodes (ECG electrodes Ag/Ag Cl (Leonhard Lang Gmbh, Innsbruck, Austria), were used. The active electrode (1.0×1.0 cm) was placed over the tendon injury site, while the inactive electrode was placed proximally on the thigh region of the same side, approximately 3 cm apart.

Tendons harvesting

According to the assigned time of each group, the cast was removed and the animals were weighted. The right Achilles

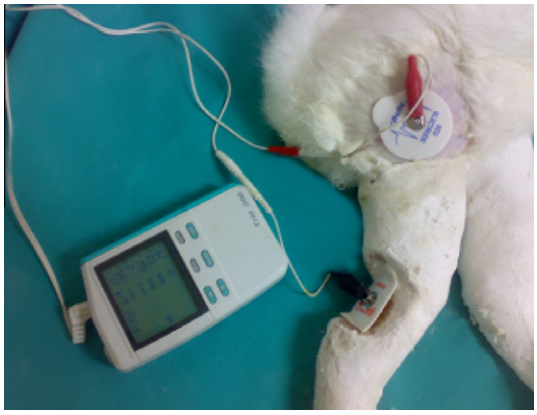


Fig. 2 Application of MES using active electrode at the tenotomy site and ground electrode proximally placed.

tendons were exposed under general anesthesia as previously described. The tendons were freed carefully from the surrounding and the sutures were carefully removed before tendon excision. The excised tendons were assigned for biomechanical or histopathological studies. For tendons used for biomechanical measurements, Sharp transverse cuts were made part of the calcaneal bone below and fleshy muscles above were incised to give stability and prevent slack of the tendon during measurements. After removal, those tendons were preserved in saline 9% concentration and freezed at −70 °C until biomechanical tests were performed [21]. For tendons used for histopathological studies, sections were cut and fixed in 10% neutral buffer formalin for routine processing.

Biomechanical measurements

The Biomechanical analysis was made at the Cellulose and Paper Department, National Research Center, Dokki, Cairo,

Egypt. The tensile machine Lloyd instruments LR10K (Lloyd Instruments Ltd, West Sussex, UK) was used to measure biomechanical properties of the tendons. A load deformation curve and other biomechanical parameters were obtained, including: load at break in Newton (N) (amount of load applied beyond which the tendon will fail), stiffness in Newton/millimeter (N/mm) (resistance to deformation), ultimate tensile strength in Newton (N) (maximum stress that tendon can withstand while being pulled before necking), elastic modulus in Newton/millimeter^2 (N/mm^2) (the slope of the stress strain curve in the elastic deformation region) and work done in milli Joule (mJ) (the amount of energy transferred by a force acting through a distance) [22].

Each tendon was clamped at each end of serrated grips; jaws secured the calcaneus at one end and the musculotendinous junction at the other. The musculotendinous junction end of the Achilles tendon was fixed between two pieces of sandpaper and was mounted and secured with quick-setting superglue (Aron Alpha, Toagosei Co Ltd, Tokyo, Japan). The system was loaded to 250 N load cells. Each tendon was loaded to failure (till tendon rupture) at a constant crosshead speed of 50 mm/min. The specimen was kept moist throughout testing using normal saline to avoid tensile strength changes associated with drying.

Histopathological study

Specimens were fixed in 10% neutral buffered formalin for one week, dehydrated in alcohol, cleaned in Xylol and embedded in paraffin. The blocks were cut at 6 μm thickness and the sections were stained with (Hematoxyline and Eosin H&E) for histological examination [23].

Statistical analysis

Statistical analysis was performed using "SPSS" for windows evaluation version 15.0. According to the experimental design, the study included five dependent variables which were the measured biomechanical parameters and two independent variables which were time and MES. The biomechanical results were presented in the form of mean, standard deviation (SD) and the percentages of these measures in relation to that of the normal intact Achilles tendons. Factorial ANOVA was used to determine the effect of time and MES and a Post –hoc test (LSD) was then used to determine differences between weeks 3, 5, 8 and the differences between control, cathodal and anodal groups. Significance level was set at (0.05).

Results

Biomechanical results

The results of all biomechanical parameters of the tenotomized and repaired tendons in the three experimental groups were found to be lower compared to those of the normal intact tendons with the highest percent of improvement recorded from the three studied groups at week 8 for all the biomechanical measures (Tables 1 and 2).

Load at break

Effect of time: Load at break differ significantly throughout the study periods (3, 5, 8 weeks) within each groups ($P = 0.000$). In the three studied groups, load at break at week 8 was significantly higher than those of weeks 3 and 5 ($P = 0.000$), and at week 5 load at break was also significantly higher than week 3 ($P = 0.000$) (Table 3).

Effect of MES: As shown in Table 4, load at break values of the cathodal and anodal groups at weeks 3, 5, 8 was significantly higher than the control group ($P = 0.000$) and that of the cathodal group was significantly higher than that of the anodal group at weeks 3 ($P = 0.04$) and anodal group was significantly higher than cathodal group at weeks 5 and 8 ($P = 0.01$, and 0.001 respectively).

Stiffness

Effect of time: Regarding changes across study period, Stiffness at week 8 was significantly higher than those of weeks 3 and 5 ($P = 0.000$), and at week 5 also was significantly higher than week 3 ($P = 0.000$) (Table 3).

Effect of MES: As shown in Table 4, stiffness values of the cathodal and anodal groups at weeks 3, 5, 8 were significantly higher than the control group ($P = 0.000$) and that of the cathodal group was significantly higher than that of the anodal group at weeks 3 ($P = 0.04$) while at weeks 5 and 8 that of the anodal group were significantly higher than cathodal group ($P = 0.000$).

Ultimate tensile strength (UTS)

Effect of time: Regarding changes across study period, UTS at week 8 was significantly higher than those of weeks 3 and 5 ($P = 0.000$), and at week 5 also was significantly higher than week 3 ($P = 0.001$, 0.003, and 0.004 for control, cathodal and anodal groups respectively) (Table 3).

Effect of MES: As shown in Table 4, UTS of the cathodal and anodal groups at weeks 3, 5, 8 were significantly higher than the control group ($P = 0.000$) and that of the cathodal group was significantly higher than that of the anodal group at weeks 3 ($P = 0.02$) while at weeks 5 and 8 that of the anodal group were significantly higher than cathodal group ($P = 0.006$ and 0.000 respectively).

Elastic modulus

Effect of time: Elastic modulus of the three groups at week 8 was significantly higher than those of weeks 3 and 5

Table 1 Biomechanical values of normal group.

	Load at break (N)	Stiffness (N/mm)	UTS (N)	Elastic modulus (N/mm^2)	Work done (mJ)
Mean	215.66	124.95	301.21	54.84	2093.00
SD	9.72	5.78	15.68	4.64	74.65

SD = standard deviation, N: Newton, UTS: ultimate tensile strength, N/mm: Newton/millimeter, mJ: milli Joule

Table 2 Biomechanical measurements of the studied groups through the study period.

	Third week			Fifth week			Eighth week		
	Control	Cathodal	Anodal	Control	Cathodal	Anodal	Control	Cathodal	Anodal
Load at break (N)									
Mean	56.18	84.28	78.27	66.32	95.44	103.09	86.33	131.53	141.37
SD	5.22	4.44	5.25	5.09	7.84	7.02	5.05	10.08	6.38
%	26%	39%	36%	31%	44%	48%	40%	61%	67%
Stiffness (N/mm)									
Mean	22.44	35.91	32.98	35.01	53.63	59.05	58.24	80.03	85.75
SD	1.37	1.17	1.31	2.86	2.81	2.19	6.06	4.241	2.80
%	18%	28.7%	26%	28%	43%	47%	40%	64%	68%
UTS (N)									
Mean	68.26	98.61	90.97	122.19	141.16	150.3	159.1	206.37	218.9
SD	3.76	5.78	4.74	8.54	4.93	9.14	7.60	10.37	8.22
%	23%	32.7%	30%	41%	47%	50%	53%	68%	73%
Elastic modulus (N/mm^2)									
Mean	8.00	17.29	12.86	14.14	22.29	26.71	22.71	42.14	47.29
SD	1.91	2.36	1.86	4.63	4.23	2.13	3.45	5.39	4.42
%	14.5%	31.5%	23.4%	25.7%	40.6%	48.7%	41.4%	76.8%	86.2%
Work done (mJ)									
Mean	450	833.00	757.5	511.70	948.00	1103.2	658.7	1099.5	1270
SD	54.91	56.72	52.47	61.64	66.67	52.87	81.52	85.38	49.38
%	21.5%	40%	36.1%	24.4%	45.2%	52.7%	31.4%	52.5%	60.6%

SD: standard deviation, %: percentage to corresponding normal, N: Newton, UTS: ultimate tensile strength, N/mm: Newton/millimeter, mJ: milli Joule

Table 3 Comparison of the biomechanical measurements across study time within the study groups.

Treatment time	Control group			Cathodal group			Anodal group		
	Mean Diff.	SE	Sig.	Mean Diff.	SE	Sig.	Mean Diff.	SE	Sig.
Load at break (N)									
Week 3 vs week 5	10.14	2.9	0.000*	11.16	2.9	0.000*	24.28	2.9	0.000*
Week 3 vs week 8	30.15	2.9	0.000*	47.25	2.9	0.000*	63.1	2.9	0.000*
Week 5 vs week 8	20.01	2.9	0.000*	36.09	2.9	0.000*	38.28	2.9	0.000*
Stiffness (N/mm)									
Week 3 vs week 5	12.57	1.4	0.000*	17.72	1.4	0.000*	26.07	1.4	0.000*
Week 3 vs week 8	33.8	1.4	0.000*	44.12	1.4	0.000*	52.77	1.4	0.000*
Week 5 vs week 8	23.23	1.4	0.000*	26.40	1.4	0.000*	20.98	1.4	0.000*
UTS (N)									
Week 3 vs week 5	53.93	3.2	0.000*	42.09	3.2	0.000*	59.40	3.2	0.000*
Week 3 vs week 8	90.89	3.2	0.000*	107.76	3.2	0.000*	127.25	3.2	0.000*
Week 5 vs week 8	36.96	3.2	0.000*	65.21	3.2	0.000*	68.55	3.2	0.000*
Elastic modulus (N/mm^2)									
Week 3 vs week 5	6.14	1.93	0.002*	5.00	1.93	0.01*	13.86	1.93	0.000*
Week 3 vs week 8	14.71	1.93	0.000*	24.86	1.93	0.000*	34.43	1.93	0.000*
Week 5 vs week 8	8.57	1.93	0.000*	19.86	1.93	0.000*	20.57	1.93	0.000*
Work done (J)									
Week 3 vs week 5	61.71	6.005	0.000*	115	6.005	0.000*	345.71	6.005	0.000*
Week 3 vs week 8	208.71	6.005	0.000*	266,57	6.005	0.000*	512.43	6.005	0.000*
Week 5 vs week 8	147	6.005	0.000*	151.57	6.005	0.000*	166.71	6.005	0.000*

Mean diff: mean difference, SE: standard error of the mean difference, N: Newton, UTS: ultimate tensile strength, N/mm: Newton/millimeter, J: Joule.
* Significant difference.

($P = 0.000$), and at week 5 also was significantly higher than week 3 ($P = 0.002$, 0.01, and 0.000 for control, cathodal and anodal groups respectively (Table 3).

Effect of MES: As shown in Table 4, the values of the elastic modulus of the cathodal and anodal groups at weeks 3, 5, 8 were significantly higher than the control group ($P = 0.000$)

and that of the cathodal group was significantly higher than that of the anodal group at weeks 3 ($P = 0.02$) while at weeks 5 and 8 that of the anodal group were significantly higher than cathodal group ($P = 0.02$ and 0.01 respectively).

Work done

Effect of time: As presented in Table 3, Work done by the tendons in the three groups at week 8 was significantly higher than those of weeks 3 and 5 ($P = 0.000$), and at week 5 was significantly higher than week 3 ($P = 0.000$).

Effect of MES: The work done by tendons of the cathodal and anodal groups at weeks 3, 5, 8 were significantly higher than the control group ($P = 0.000$) and that of the cathodal group was significantly higher than that of the anodal group at weeks 3 ($P = 0.000$) while at weeks 5 and 8 that of the anodal group was significantly higher than cathodal group ($P = 0.000$) (Table 4).

Histopathological results

The normal rabbit Achilles tendon consisted of closely packed bundles of collagen fibers with relatively few fibrocytes which were aligned with the collagen fibers along the longitudinal axis of the tendon (Fig. 3).

Week 3: Regarding the control non treated tenotomized and repaired tendons, the microscopic findings revealed less organized fibroploriferative changes with poorly aligned collagen bundles. Inflammatory tissue reaction with mononuclear cells (macrophage) infiltrations is clearly noticed. (Fig. 4A). While that of the cathodal group, revealed well developed granulation tissue with a properly aligned pattern of collagen bundles (Fig. 4B). Tendons in the anodal group showed less-organized fibroploriferative changes with poorly aligned collagen bundles, Inflammatory tissue reaction with notice of the newly formed blood vessels and few numbers of inflammatory cells. (Fig. 4C).

Week 5: Histological changes of the control tendons showed high cellularity in relation to the fibrils. Many attempts to form bundles with parallel fibers were observed but still

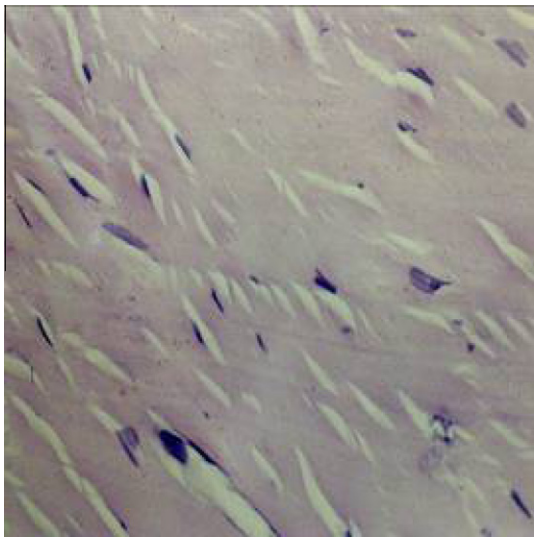


Fig. 3 Normal tendon consisting of mature compact bundles entangling compressed few fibrocytes (H&E 400×).

Table 4 Comparison between studied groups at different treatment time.

Compared groups	Third week			Fifth week			Eighth week		
	Mean Diff.	SE	Sig.	Mean Diff.	SE	Sig.	Mean Diff.	SE	Sig.
Load at break (N)									
Cathodal vs anodal	6.01	2.9	0.04*	−7.56	2.9	0.01*	−9.84	2.9	0.001*
Cathodal vs control	28.10	2.9	0.000*	29.12	2.9	0.000*	45.20	2.9	0.000*
Anodal vs control	22.09	2.9	0.000*	36.77	2.9	0.000*	55.04	2.9	0.000*
Stiffness (N/mm)									
Cathodal vs anodal	2.93	1.4	0.04*	−5.42	1.4	0.000*	−5.72	1.4	0.000*
Cathodal vs control	13.47	1.4	0.000*	18.62	1.4	0.000*	21.79	1.4	0.000*
Anodal vs control	10.54	1.4	0.000*	24.04	1.4	0.000*	27.51	1.4	0.000*
UTS (N)									
Cathodal vs anodal	7.64	3.2	0.02*	−9.21	3.2	0.006*	−12.55	3.2	0.000*
Cathodal vs control	30.35	3.2	0.000*	18.97	3.2	0.000*	47.22	3.2	0.000*
Anodal vs control	22.71	3.2	0.000*	28.18	3.2	0.000*	59.77	3.2	0.000*
Elastic modulus (N/mm^2)									
Cathodal vs anodal	4.43	1.93	0.02*	−4.43	1.93	0.02*	−5.14	1.93	0.01*
Cathodal vs control	9.29	1.93	0.000*	8.14	1.93	0.000*	19.43	1.93	0.000*
Anodal vs control	4.86	1.93	0.02*	12.57	1.93	0.000*	24.57	1.93	0.000*
Work done (J)									
Cathodal vs anodal	75.43	6.005	0.000*	−155.29	6.005	0.000*	−170.43	6.005	0.000*
Cathodal vs control	383	6.005	0.000*	436.29	6.005	0.000*	440.86	6.005	0.000*
Anodal vs control	307.57	6.005	0.000*	591.57	6.005	0.000*	611.29	6.005	0.000*

Mean diff: mean difference, SE: standard error of the mean difference, N: Newton, UTS: ultimate tensile strength, N/mm: Newton/millimeter, J: Joule, Sig. significance level.
* Significant difference.

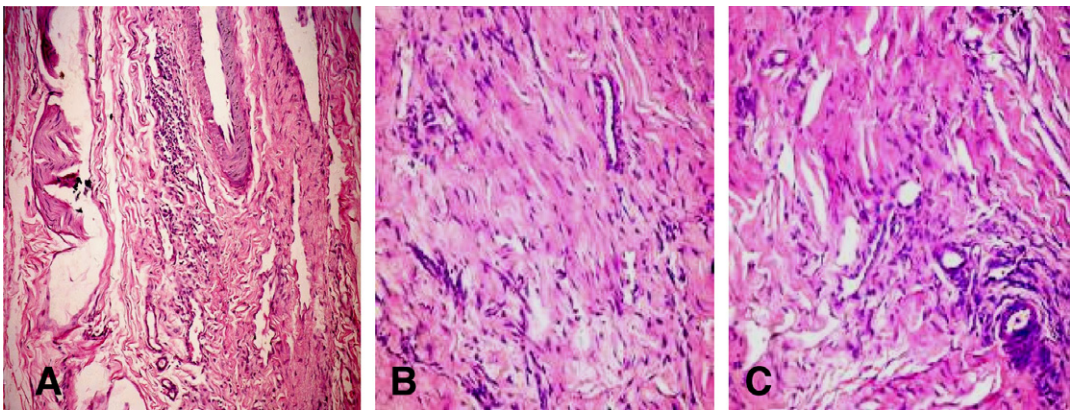


Fig. 4 Photomicrograph of a three week neotendon (H&E 200×). (A) Untreated control showing less-organized fibroploriferative changes with poorly aligned collagen bands, inflammatory tissue reaction with mononuclear cells infiltrations is clearly noticed. (B) Photomicrograph of cathodal group showing well-developed granulation tissue with a properly aligned pattern of collagen bands. (C) Photomicrograph of anodal treated tendons showing well-organized fibroploriferative changes. Inflammatory tissue reaction with notice of the newly formed blood vessels and few numbers of inflammatory cells.

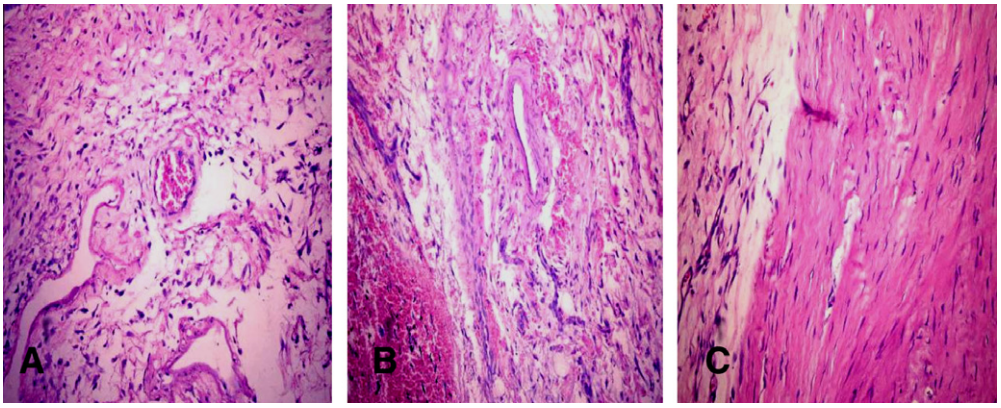


Fig. 5 Photomicrograph of a five weeks neotendon (H&E 200×). (A) Untreated neotendon showing high cellularity in relation to the fibers. Notice attempts to form bundles with parallel fibers but still in disarray. (B) Photomicrograph of cathodal MES showing cellular neotendon, small blood vessels and collagen fibers appears scattered and in loose bundles. Notice foreign body granulomatous reaction. (C) Anodal MES showing mature collagen fibers with fibrocystes in-between.

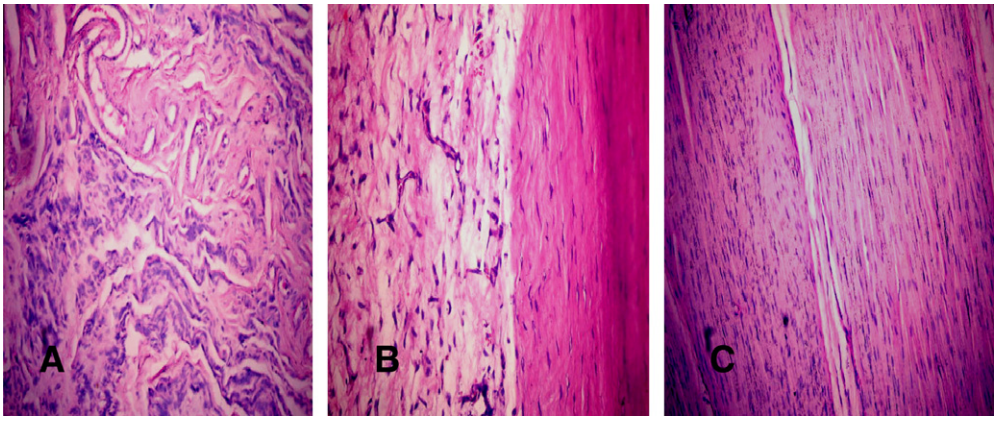


Fig. 6 Photomicrograph of an eight week neotendon (H&E 200×). (A) Photomicrograph of untreated tenotomized left Achilles tendon showing poorly aligned collagen bundles. Inflammatory tissue reaction is observed. (B) Photomicrograph of cathodal MES stimulation showing diminished granulation tissue with formation of properly aligned mature collagen bundles. (C) Photomicrograph of Anodal MES stimulation showing closely packed collagen bundles with compressed fibrocytes. Both of them are well oriented along the longitudinal axis of the tendon.

disarray (Fig. 5A). Regarding the cathodal group, tendons showed better healing picture than control group with cellular neotendon, newly formed small blood vessels and collagen fibers that appeared in loose bundles. Obvious foreign body granulomatous reaction could be seen (Fig. 5B). The Anodal group, showed the best healing picture with spindle shaped fibrocytes arranged parallel to the longitudinal axis of the collagen fibers which form compact bundles (Fig. 5C).

Week 8: Light microscopy of untreated tenotomized right Achilles tendon showed poorly aligned collagen bundles, inflammatory tissue reaction could be noticed (Fig. 6A). Regarding the Cathodal group, the right Achilles tendon showed diminished granulation tissue with formation of properly aligned mature collagen bundles (Fig. 6B). While tendons in the anodal group, showed closely packed collagen bundles with compressed fibrocytes. Both of them are well oriented along the longitudinal axis of the tendon (Fig. 6C).

Discussion and conclusion

The ultimate aim in treatment of tendon injury is to achieve anatomical and functional healing [22]. Recently MES has gained considerable attention for stimulating soft tissues repair as wounds, bones, tendons and ligaments and promising results have been reported [9,10,17–19].

In this study, the results demonstrated that both cathodal and anodal MES could improve the mechanical properties of surgically repaired rabbits Achilles tendons at third, fifth and eighth weeks post-injury when compared with the controls. This was also proved by the presented histopathological findings as tendons in the cathodal and anodal groups showed less prominent inflammatory reactions with better aligned collagen fibers which were organized in parallel bundles. The biomechanical properties of tendons were reported to be directly related to the amount and orderly orientation of collagen fibers which are responsible for transmitting the force generated by the tendon to bone [24].

The biomechanical testing of the regenerating tendons is considered as one of the criteria to judge the degree of tendon healing, greater tensile strength and load at break means increased ability to perform movement. While higher stiffness, elastic modulus and work done means increase of the ability to withstand load for a longer period of time before sniping [20,24].

The improvement in both the biomechanical properties and healing process recorded in both MES groups could be explained by the previously reported physiological effects of MES that related to enhancement of the intrinsic healing of the tendon include promoting ATP production, increasing amino acid uptake, enhancing active secretion of tenocytes and increasing collagen synthesis [4,8,9,11]

Furthermore the results of the current study shed a light on the role of polarity of MES as a parameter during stimulation of tendon healing throughout the different healing periods. According to the biomechanical and histopathological findings, cathodal MES showed significant improvements than anodal MES in the 3-week, while anodal MES showed more significant improvements in the 5 and 8 weeks.

It was reported that the regenerating Achilles tendon undergoes different stages of healing and each stage involves a different set of cellular events [23]. Furthermore, it was suggested that microcurrent applications are believed to be effective by influencing and modifying cellular processes and activity. Employing different levels of current, frequency and polarity have been shown to have diverse effects upon different cell groups [9,25].

Cathodal stimulation was suggested to promote and attract macrophages [26]. During the first stage, macrophages play a prominent role in healing. Not only do macrophages debride the injury site via phagocytosis, they facilitate angiogenesis, migration of fibroblasts to the site of injury, and their proliferation prior to collagen synthesis. Thus, although fibroblasts are dominant and produce the collagen of tendons, their metabolic process may be remarkably impaired in the absence of macrophages that initiate the sequence of events that precede their migration [27]. The previous explanation may explain the significant higher values of cathodal than anodal during the 3 week period.

On the other hand, anodal stimulation was suggested to facilitate migration and proliferation of epithelial cells so improving wound closure [10,18]. Regarding tendon healing, MES with positive polarity was suggested to accelerate the process of tendon repair resulting in stronger tendons with reduced contracture formation [13]. It was also reported that tendons treated with anodal MES had higher breaking strength than control which means that tendons became stronger and can withstand higher loads before breaking [14]. This might explain the significant improvement of both biomechanical properties and healing picture of the healed tendons treated with anodal MES in the anodal group.

Most of the studies conducted on the effect of MES on tendon healing used single polarity Some reported that cathodal MES could enhance tendon healing [11,12], while others reported positive results with anodal MES [13,14]. Up to our knowledge, only one study was conducted by Owoeye et al. [14] were comparing the cathodal and nodal MES on tendon healing. The findings in our study regarding anodal MES agree with them but contradict their result regarding cathodal MES. In this study, authors found no significant effect for the cathodal than control. However, the authors used implanted electrode with stainless which might have affected the outcome also they used pulsed galvanic current in the form of twin spike not in the form of rectangular which may be a factor to be considered. It was suggested that the waveform to be rectangular that resemble body activity [8].

According to the experimental design of the study, the plaster casts were removed at sixth day postoperative which allowed early mobilization without any tendon rupture or recorded drawback of the results. MES mimic endogenous electrical signal that guide cellular behavior which results in stimulating intrinsic capacity of tendon to heal with minimal complications [4,7]. So we can suggest that with MES application to the surgically repaired tendons, safe early mobilization could be allowed. Early cast removing and functional loading were reported to augment the healing strength of the experimentally tenotomized Achilles tendons and to reduce the complications of prolonged immobilization [20,28]

The intensity and pulse frequency of MES used in the study were chosen according to previous studies which suggested the optimal range for the best biological effect of microcurrent therapy [8,13,14].

One limitation to this study was that, for standardization, we induced complete surgical transection of the Achilles

tendons. The healing of surgically induced wound may differ from a tendon ruptures due to stress or loading. So this issue could be studied in future research.

So it can be concluded that, for improving the healing of surgically repaired rabbits tendons, application of cathodal MES in the early stage could result in more beneficial effects on biomechanical and histopathological properties rather than anodal MES, while anodal MES application could produce better results than cathodal later at late stage of healing. So, in light of the present study, it may be germane to adjust the MES polarity differently for the different stages of healing to obtain optimal effects. Further studies investigating the effects of combination of cathodal polarity of MES at early phase of tendon healing, then switching to anodal polarity are needed.

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