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Electrical stimulation on open wound healing in Rabbits

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Abstract

Micro-amperage electrical stimulation (MES) on the healing process of skin wound in rabbits was studied. Twenty adult New Zealand white rabbits were randomly divided into four equal groups and a full thickness skin incision was made in each rabbit. This experimental group received an MES of 1000 µA (1mA) current intensity for 30 minutes twice a day. Swelling area of the wound of experimental groups were 11.1cd ± 0.2 mm, 10.8d ± 0.1 mm and 10.7cd ± 0.1mm at days 4, 7 and15, respectively. Elevation of sutured line (mm) of experimental groups was 2.4b ± 0.1, 2.1b ± 0.1, 2.2b ± 0.1 at days 4, 7 and 15, respectively. The average healing time was 30.4b, 28.4b and 26.4b in days 4, 7 and 15, respectively, significantly different from control groups. Number of fibroblasts and blood vessels were significantly higher in the experimental group than in control group. The result indicates that the application of MES significantly enhances the wound healing in rabbits. (*Bangl. vet*. 2016. Vol. 33, No. 2, 51 – 61)

Introduction

Wound healing is regulated by extrinsic and intrinsic factors that may result in complications in healing (Hess *et al.,* 2003). Wound repair includes inflammation, angiogenesis, development of granulation tissue, and remodeling (Midwood *et al.,* 2004). Following tissue injury, demarcation current is generated that triggers biological repair process (Watson, 1994). Exogenous electrical stimuli have been shown to enhance the wound healing (Taskan *et al*., 1997; Demir *et al.,* 2004).

Electrical stimulation has been referred to as micro-amperage electrical stimulation (MES). MES is defined as stimulations with a very low frequency (1 Hz or less) and low intensity (1 – 1,000 µA) or amplitude (Friedenberg *et al.,* 1971). Application of MES accelerates bone healing (Goh *et al.,* 1988; Sharrard *et al.,* 1990); and dermal repair (Byl *et al.,* 1994; Canseven and Atalay, 1996*)*. Electrotherapy decreases edema, attracts neutrophils and macrophages, stimulates growth of fibroblasts and granulation tissue, induces epidermal cell migration, inhibits bacteria (Gentzkow, 1993), decrease the ulcer size (Griffin *et al.,*1998), and accelerates healing time (Carley and Wainapel, 1985). MES causes acceleration of cutaneous wound healing. This study was

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undertaken in light of the growing enthusiasm for MES and the paucity of supporting evidence for its effectiveness. The present study was designed to demonstrate the effectiveness of micro- amperage electrical stimulation (MES) on wound healing.

Materials and Methods

Experimental animals

A total of 20 healthy rabbits weighing from 1.8 to 2.0 kg were used. The animals were kept under standard laboratory conditions and veterinary supervision with no restrictions on water and food. Before the study the rabbits were in quarantine for three weeks. This study was conducted with approval from the ethics committee of Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Micro-amperage electrical stimulation (MES) device

A "Newstar Multiple AC/DC Adaptor™" (U.K. Reg. No. 2042653) was used to supply 1,000 μA (1 mA) current at 1.5 volt. The details of the capacity of the device were:

Conversion : AC-DC
Input : AC 220 volt, 50 Hz
Output : DC1.5V/3V/4.5V/6V/9V/12V
Current : 350 mA Max
Polarity : Reversible

Light Emitting Diode (LED) indicator with 6-way universal outputs plugs was used. 1.5 V/1 mA, a 1.5kΩ resistance was used in addition to adapt the voltage.

Setting of MES device

The "Newstar Multiple AC/DC Adaptor™" was set on the top of the Electrotherapy chamber (a plastic made multiple shelf rack fenced with a plastic net). The two plugs of the adaptor were lodged in two sockets connected with main line (220 V, 50 Hz). The adaptor was ready to supply a 1000 μA current to be applied into the wound area with two electrodes.

Experimental design

Twenty adult New Zealand white rabbits were randomly divided into four groups each containing five rabbits. Electrotherapy was applied on three groups A, B and C for 4, 7, 15 days, respectively, and the group D was kept as control. A full thickness of skin incision was made on each rabbit. The groups A, B and C received an MES of 1000 μA (1mA) current intensity for 30 minutes twice a day. The control group D received no MES treatment. Xylazine hydrochloride (Xylaxin®, 23.3 mg/mL, Indian Immunological Ltd., India) @ 6 mg/kg, Ketamine hydrochloride (G-Ketamine®, 50 mg/mL, Gonosasthya, Bangladesh) @ 50 mg/kg were used for anesthesia of the animals. Surgical wound of 3 cm length and 0.5 cm depth was made by a vertical

incision. Wound was closed with simple interrupted silk sutures 8 mm apart. Distance between needle placement and border of cutting edge was 5 mm. No antibiotic, antihistaminic or anti-inflammatory drugs were used. Swelling of wound and width of sutured area were noted up to day 42 post-operative. Elevation of sutured line was recorded to day 15 (D15). Width of sutured area was measured at the day 7 (D7) and day 15 (D15) to determine wound contraction length. Tissue samples were collected from all rabbits for histopathology.

Fig. 1: (a) Electro-stimulation device with two electrodes (b) Plastic shelf-rack with rabbit in electrotherapy chamber.

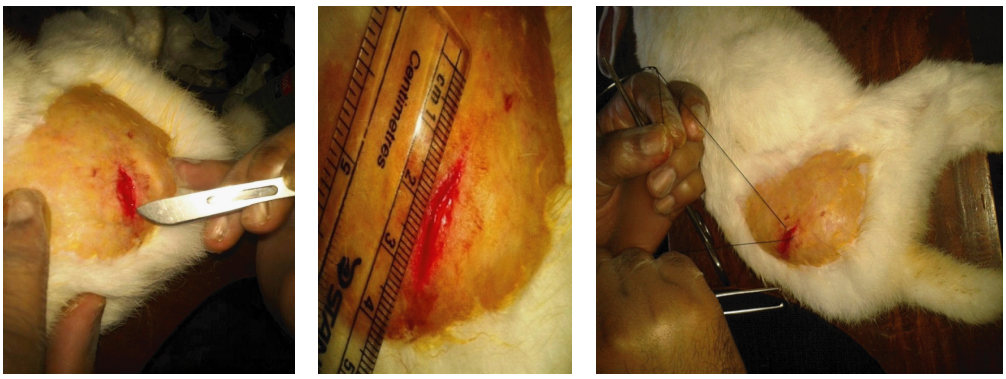


Fig. 2: Creation of fresh open wound and their management of rabbits in aseptic condition.

Table 1: Experimental design for determination of wound healing in rabbits

Groups	Duration of electrotherapy (days)	Application of MES therapy	Day of sample collection
Group A	3	+	4th day
Group B	6	+	7th day
Group C	14	+	15th day
Group D	-	-	4th, 7th and 15th day

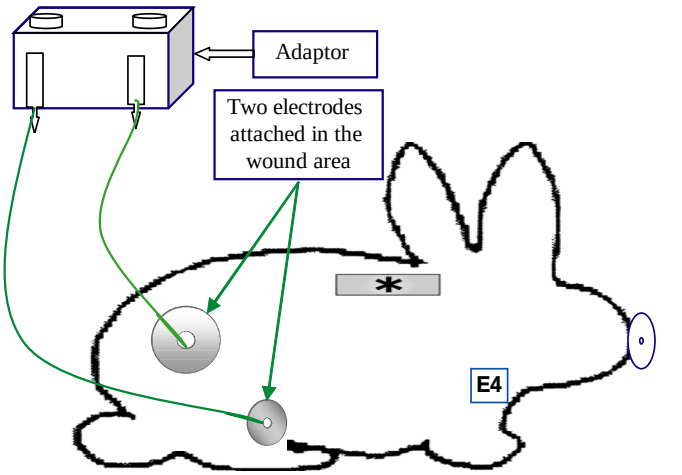


Fig. 3: Schematic view of the experimental rabbit.

Application of electrotherapy

Each experimental animal was placed in the individual electrotherapy chamber. The electrodes were kept attached with the shaved skin of rabbit using adhesive micro-pore tape. Treatment of MES was started 24 hours after surgery. Two electrodes were placed on the incision area- one on the incision and another one 5 cm away from the wound.

Observation of morphological changes

Slide calipers was used to measure (mm) swelling area, elevation of suture line, wound contraction and width of sutured area of wound to compare effects of treatments on wound healing. Swelling was observed up to three days after operation, decreased gradually from day 3 (D3). Elevation of sutured line was recorded after 7 days of surgery. Width of sutured area was measured from the day of surgical intervention at day 0 (D0), day 3 (D3), day 7 (D7), day 14 (D14), day 21 (D21) to determine wound contraction length.

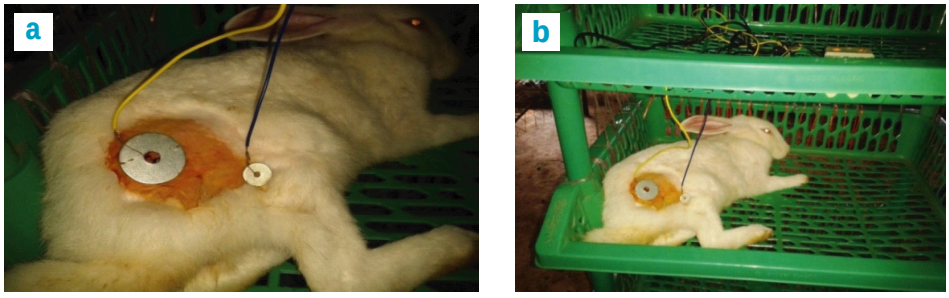


Fig. 4: Representation of the use of two electrodes (a) and one electrode (b) on the wounded area of the rabbit.

Assessment of wound colonization

Wound colonization was assessed according to exudation, purulent efflux, efflux odour, erythema and oedema. A score between 0 and 3 was given to each assessment as follows: 0: no colonization, 1: mild exudation and odour, 2: erythema, moderate purulent efflux, exudation and odour, 3: severe exudation, purulent efflux, odour, oedema and erythema.

Assessment of wound healing

This study was continued for six weeks after the creation of surgical wound. Each wound was clinically observed every three days up to six weeks. Wounds were considered to be healed when there was visible epithelialization, cicatrisation and pigmentation.

Histopathological assessment

The biopsies (1.5 cm × 1 cm) were collected from the wound areas of each experimental animal on the 4^{th}, 7^{th} and 15^{th} days after wounding using standard surgical procedure. The samples were fixed in 10% buffered neutral formalin solution more than seven days for histopathology. Histopathological slides were prepared as of Luna (1968).

Processing of tissues

Collected tissues were trimmed by scalpel and were fixed for 72 hours in 10% formalin and kept overnight in running tap water. The tissues were dehydrated in ascending grades of alcohol using 50, 70, 80, 95%. Then sections were cleaned in chloroform by two changes, for 90 and 60 minutes. The samples were embedded in paraffin wax at 56°C and paraffin block was prepared. The tissues were sectioned with a microtome at 5-µm thickness. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The sections were allowed to spread on warm water bath (45°C) and taken on grease-free glass slides. The slides containing sections were air-dried and kept in cool place. Routine haematoxylin and oeosin staining were used. The stained sections were examined under compound light microscope to evaluate tissue reaction in control and experimental groups.

Statistical analysis

All data were presented as mean ± SEM. To compare data between groups and one-way ANOVA (Analysis of Variance) was done. The data were analyzed with SPSS statistics 17.0 software. Probability $P<0.05$ was considered statistically significant.

Results and Discussion

MES accelerated the wound-healing process of incision wounds in rabbits. MES increased fibroblast counts within seven days and tensile strength within 15 days. All surgical wounds were healed at the end of six weeks.

Morphological changes

Distinct changes in the wound were demonstrated at days 3, 7, 15 and 30.

Table 2: Evaluation of morphological changes as a result of MES therapy at days 3, 7, 15 and 30

Groups	Swelling of suturing area (mm)	Elevation of sutured line (mm)	Wound contraction length (mm)
E4	11.1cd ± 0.2	2.4b ± 0.1	1.4 ± 0.2
C4	11.6b ± 0.1	3.0a ± 0.2	1.8 ± 0.2
E7	10.8d ± 0.1	2.2b ± 0.1	1.5 ± 0.1
C7	11.2bc ± 0.1	3.1a ± 0.1	1.6 ± 0.1
E15	10.7cd ± 0.1	2.2b ± 0.1	1.6 ± 0.1
C15	12.0a ± 0.11	3.3a ± 0.2	1.1 ± 0.2
Level of sig.	**	**	NS

(In the columns, dissimilar letters indicate significant differences as per DMRT)

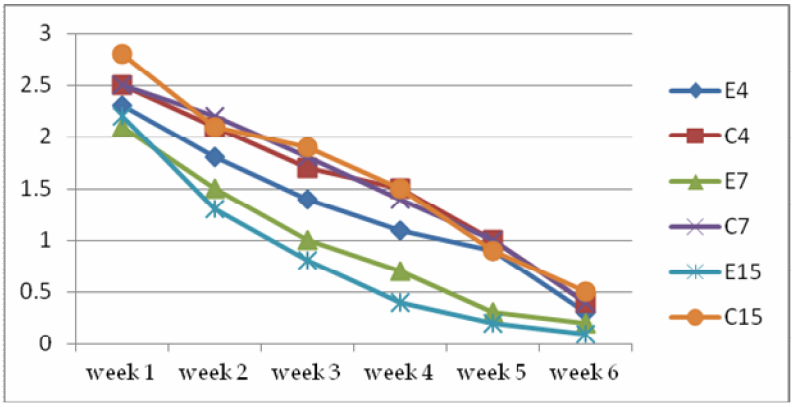
Days to healing

Application of negative polarity to the wound for the first three days and positive polarity thereafter, were observed. The negative polarity seems to inhibit the growth of bacteria (Taskan *et al.,* 1997; Demir *et al.,* 2004). The positive pole promoted the migration of skin cells toward the center of the wound, thus decreasing its healing time (Gault and Gatens, 1976).

Histopathological changes

Biopsies were focused on the presence of reactive cells as an indication of inflammation. The regeneration of epidermis, proliferation of fibrous connective tissue was observed in the normal healing process. Samples were collected on three occasions (D4, D7 and D15) postoperatively.

Wound colonization



Graph 1: Progress of colonized wounds of four groups in six consecutive weeks.

Table 3: Effect of MES on wound healing in rabbits

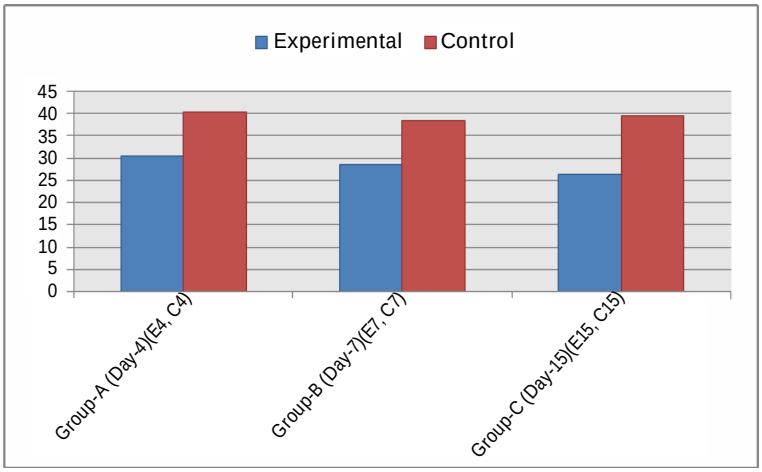
Weeks	E4	C4	E7	E7	E15	C15
1.	2.3	2.5	2.1	2.5	2.2	2.8
2.	1.8	2.1	1.5	2.2	1.3	2.1
3.	1.4	1.7	1.0	1.8	0.8	1.9
4.	1.1	1.5	0.7	1.4	0.4	1.5
5.	0.9	1.0	0.3	1.0	0.2	0.9
6.	0.3	0.4	0.2	0.4	0.1	0.5
Mean	1.3	1.5	1.0	1.6	0.8	1.6
± SE	0.3	0.3	0.3	0.4	0.4	0.4

(Level of significance is 0.375 NS)

Table 4: Time required for healing of wounds treated with electrotherapy

No. of animals	Healing time (days)					
	Group A (day-4)		Group B (day-7)		Group C (day-15)	
	E4	C4	E7	C7	E15	C15
1.	30	40	29	37	26	40
2.	31	42	27	39	25	41
3.	32	39	28	38	27	39
4.	29	41	30	40	28	38
5.	30	40	28	38	26	39
Average	30.4^{b}	40.4^{a}	28.4^{b}	38.4^{a}	26.4^{b}	39.4^{a}
SE	0.51	0.51	0.51	0.51	2.16	0.51

(Level of significance 0.0004 **In the columns, different letters indicate significant differences as per DMRT)



Graph 2: Mean days to wound healing.

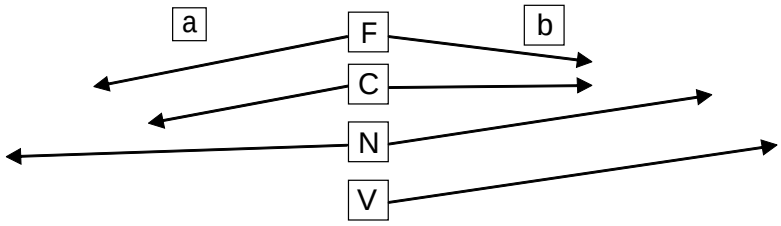


Fig. 5: Light micrographs (100×) of incision wound bed of (a) control (n = 5) and (b) experimental group (n = 5) 4 days after surgery. Marked connective tissue fibers shown in experimental group compared with control group. F = fibroblast, V = blood vessel, C = connective tissue fiber, N = neutrophil.

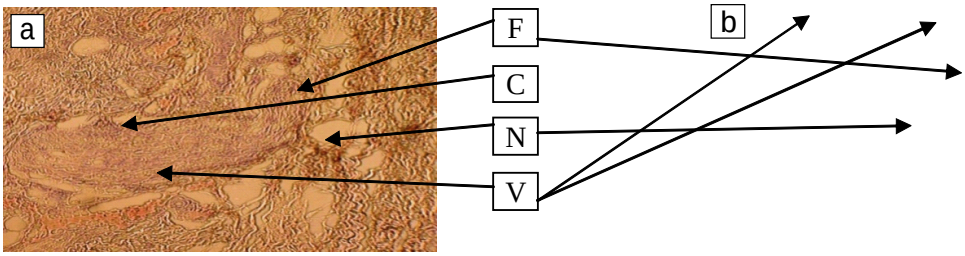


Fig. 6: Light micrographs (100×) of incision wound bed of (a) control group C7 (n = 5) and (b) experimental group E7 (n = 5) 7 days after surgery. More fibroblasts shown in experimental group compared with control group. F = fibroblast, C = connective tissue fiber, N = neutrophil.

Our results demonstrated that MES increased the number of fibroblasts at day 7 and tensile strength of collagen at day 15 compared with the control group. As fibroblasts mature, they produce a matrix through which other cells can readily migrate. From which delicate new capillaries can provide mechanical support (Gray *et al.*, 1995). During the proliferative phase of repair, fibroblasts of the granulation tissue develop into cells called "myofibroblasts," which are responsible for wound contraction (Majno *et al.,* 1979; Gray *et al.,* 1995).

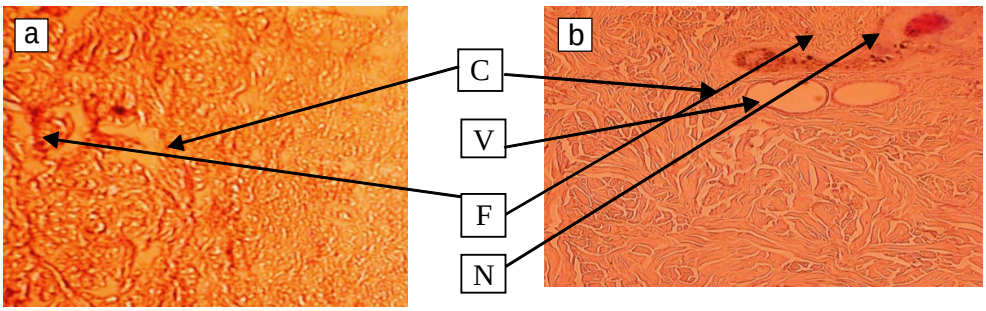
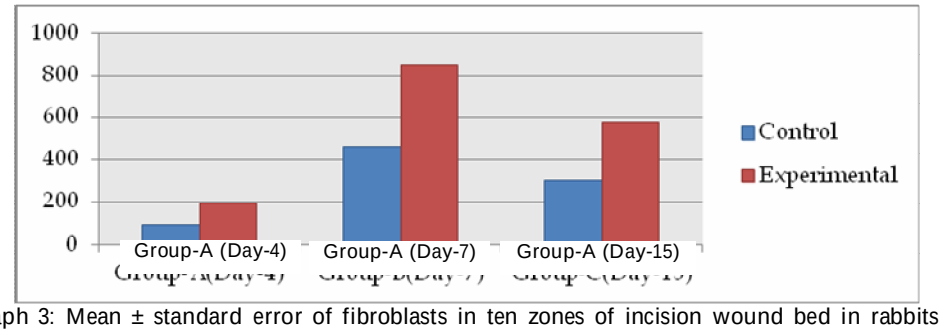
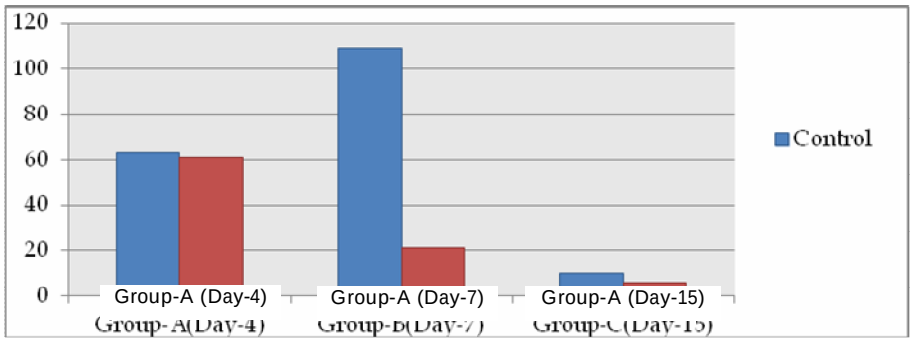


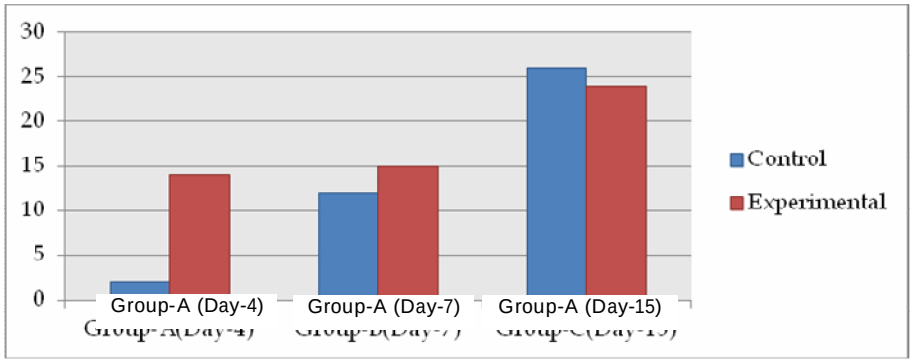
Fig. 7: Light micrographs (100×) of incision wound bed of (a) control group C15 (n = 5) and (b) experimental group E15 (n = 5) 15 days after surgery. More connective tissue fibers and more mature fibroblasts showed in experimental group compared with control group. F = fibroblast, C = connective tissue fiber, N = neutrophil.



Graph 3: Mean ± standard error of fibroblasts in ten zones of incision wound bed in rabbits of control (con) (n = 15) and experimental (Ex) (n = 15) groups at sequential intervals. Student t-test showed significant differences between control group and experimental group at day 7, P<0.01.



Graph 4: Mean ± standard error of neutrophils in ten zones of incisional wound bed in rabbits of control (con) (n = 15) and experimental (Ex) (n = 15) groups at sequential intervals.



Graph 5: Mean ± standard error of blood vessel sections in ten zones of incisional wound bed in rabbits of control (con) (n = 15) and experimental (Ex) (n = 15) groups at sequential intervals.

Day 4 after surgery

The mean number of fibroblasts and blood vessel sections of the experimental group were significantly higher than those of the control group.

Day 7 after surgery

The mean number of fibroblasts and blood vessel sections of the experimental group was higher than those of the control group. Only the mean number of fibroblasts in the experimental group increased significantly as a result of MES ($P<0.01$).

Day 15 after surgery

The mean number of fibroblasts in the experimental group was higher than that in the control group.

Conclusions

Application of MES accelerates wound healing and made a strong base by stronger scar. It may be concluded that the daily application of MES on surgically induced incisional wounds significantly accelerates the wound healing process in the rabbit skin.

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