International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:4, No:9, 2010

# The Effect of Electrical Stimulation Intensity on VEGF Expression and Biomechanical Properties during Wound

M R Asadi, G Torkaman, M Hedayati

***Abstract*—**We evaluated the effect of sensory (direct current (DC), 600μA) and motor (monophasic current, pulse duration 300μs, 100 Hz, 2.5-3mA) intensities of cathodal electrical stimulation (ES) current to release VEGF and biomechanical properties of wound. 54 male Sprague-dawley rats were randomly assigned into one control and two experimental groups. A full thickness skin incision was made on animals' dorsal region. The experimental groups received ES for 1h/day and every other day. VEGF expression was measured in skin on the 7th day after surgical incision and tensile strength was measured on 21st day. On the 7th day, the values of skin VEGF in the sensory group were significantly greater than those of the other groups ($p < 0.05$). Sensory and Motor intensity stimulation, can not improve the biomechanical properties of the repaired wounds.

It seems the mechanical environment induced by sensory and motor intensity of electrical stimulation, could not simulate the role of normal daily stress and strain to maturation of collagen fibers and their cross links. Further work is needed to determine the relationship between VEGF expression after ES and its effect on tensile strength of healed wound.

***Keywords*—**Biomechanical properties Direct current, Monophasic current, Skin, VEGF

## I. Introduction

DIRECT and indirect evidence implicates that VEGF is a significant factor in wound healing immediately after injury and stimulates wound healing via multiple mechanisms including collagen deposition, angiogenesis and epithelialization [1].

There are evidences that electrical stimulation can enhance the release of VEGF in wound site [2]. Some studies demonstrated the electrical stimulation could induce the release of VEGF in rat skeletal muscles, both when electrical stimulation caused muscle contraction and following sub-threshold stimulation, which did not induce contraction. Zhao et al. [3] reported that applied electric fields (EFs) of small physiological magnitude directly stimulate VEGF production by endothelial cells in culture without the presence of any other cell types. Talebi et al. showed that the cathodal stimulation increased the number of macrophages and fibroblast cells as compared to the control [4]. Therefore, it seems that applying the cathodal electrical stimulation on the wound site can induce the release of more VEGF and also applying of motor intensity of cathodal ES can increase the tensile strength of healed wound.

This study was conducted to investigate the role of sensory (600 μA) and motor (threshold of contraction) intensities of cathodal current on releasing VEGF and enhancement of tensile strength in wound healing.

M. R. Asadi was with Physical Therapy Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, I.R.Iran. (e-mail: reza asadi <reza.as12@gmail.com).

G. Torkaman is with with Physical Therapy Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, I.R.Iran. (Corresponding author to provide phone: +98 21 82884509; fax: +98 21 88006544; e-mail: torkamg@modares.ac.ir).

M. Hedayati is with Prevention & Treatment of Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, I.R.Iran. (e-mail: hedayati@erc.ir).

## II. Materials and Methods

In this investigation, we used 54 healthy, male Sprague-Dawley rats (Razi Vaccine and Serum Research Institute Karaj, Tehran, Iran) weighing 250 to 300 g. The study was approved by the Ethical Commission of Tarbiat Modares University.

After weighing, we anesthetized the animals using a mixture of xylazine hydrocholoride (20mg/mL, Alfasan, Woerden-Holland) and ketamine hydrochloride (100 mg/mL, Alfasan, Woerden-Holland) (xylazine: ketamine ratio of 1:9 ml and dose of 1 mL/kg). The hairs on the middle of the back of each rat were shaved and the area was cleaned with Betadine® antiseptic solution. Following the sterilization, we made a 2.5 cm longitudinal full-thickness incision, in the craniocaudal direction, at a distance of 1 cm from the spine on the right side of the paravertebral region.

Animals were randomly divided into one control and two experimental groups (motor and sensory electrical stimulations). Each group included 18 animals consisting of 8 rats that were studied for measuring VEGF expression on 7th day and 10 animals for performing uniaxial tensile test on 21st day. Treatment began 24 hours after injury. An active treatment electrode (1 × 3 cm) was placed on the incision wound area and a passive indifferent electrode (2 × 4 cm) was placed on the opposite side of the paravertebral region, at the highest part of the back. In the experimental groups, the polarity of the active treatment electrode was negative (cathode) during applied protocol. In one of the experimental groups (sensory ES group), we applied microamperage DC ES with an intensity of 600 μA, for 1 h/day, every other day, for 7 or 21 days. In the other experimental group (motor ES group),

we applied monophasic pulsed current with an intensity enough to elicit a visible minimum contraction (about 2.5-3 mA), pulse duration 300μs, frequency 100 Hz, for 1 h/day, every other day, for 7 or 21 days. In the control group, electrodes were similarly placed on the wound site but no current was applied.

*A. Tissue preparation for VEGF level determination and biomechanical testing*

On the 7th day post-injury, eight rats in each group were euthanized by chloroform inspiration and skin strips were removed along incision including 3 mm from the edges and were used as tissue samples for skin VEGF protein examination.

Tissue samples were homogenized in phosphate-buffered saline (PBS) containing anti-protease (PMSF, EDTA, Aprotinin, for each 100 mg of tissue, 1mL buffer). The homogenates were centrifuged at 12000 rpm for 15 min at 4 ºC. The supernatant was collected and stored at -80 ºC until used. VEGF was determined with an enzyme-linked immunosorbent assay (ELISA) kit (R & D systems, Minneapolis, MN, USA).

For performing uniaxial tensile test, the tissue samples, which were 6 cm long and 3 cm wide, were taken vertically to the initial incision.

The uniaxial tensile test was performed by tensiometer (model Z 2.5; Zwick Gmbh & Co, Ulm-Einsingen,Germany). The rate of the tensile test was 20 mm/min.After the test, load-deformation and stress-strain curves were obtained.

## III. Statistical analysis

Kolmogorov-Smirnov test demonstrated that the VEGF values and stress, E modulus, strain, and area under load-deformation curve had a normal distribution in all of the groups ($p>0.05$). $P<0.05$ was considered statistically significant.

## IV. Results

On the 7th day post-injury, the VEGF level (mean ± SEM) in the sensory, motor and control groups was 4.43±0.97, 1.74±0.59, and 1.14±0.46 pg/mg, respectively (Fig 1). ANOVA revealed significant difference among the groups on day 7 ($p = 0.01$). Tukey test demonstrated that the values of VEGF protein in the sensory group was significantly greater than those in the motor and control groups on day 7 ($p= 0.03$ and $p = 0.01$, respectively), but differences between the motor and the control groups were not significant ($p = 0.8$). After 21 days, the biomechanical parameters of wound consisting of stress, E modulus, strain, and area under load-deformation curve showed no statistical differences between groups (table1).

## V.DISCUSSION

In full-thickness wounds, maximal VEGF protein is found between the 3rd and the 7th days after wounding, coincident with the early stages of angiogenesis [1]. Level of skin VEGF protein in the sensory group was significantly greater than that in the motor and control groups by the seventh day ($p < 0.05$).

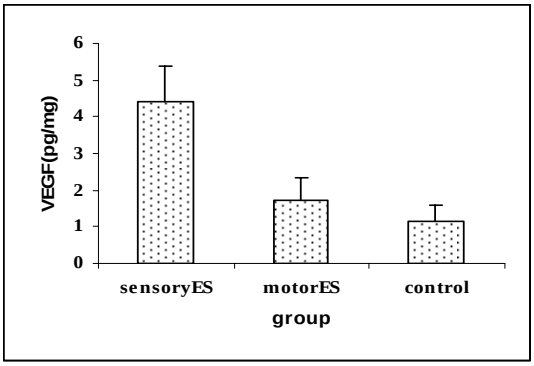


Fig. 1 VEGF protein level in skin wound on the 7th day after incision as Mean ± SEM

TABLE I BIOMECLANICAL PARAMETERS ON THE 21ST DAY FOR THREE GROUPS AS MEAN±SD

| group | Stress(N/mm$^2$) | Strain(%) | E modulus(N/mm$^2$) | Area under curve(N*mm) |
|---|---|---|---|---|
| Sensory ES | 0.64±0.32 | 31.49±7.16 | 2.68±0.72 | 140.7±66.87 |
| Motor ES | 0.63±0.19 | 28.65±10.1 | 2.98±0.92 | 126.69±54.83 |
| Control | 0.59±0.27 | 32±11.07 | 2.5±0.68 | 136.24±72.88 |

Morris et al. [2] reported that application of pulsed DC electrical stimulation with 110 μs pulse width significantly increased VEGF level on the 14th day compared to pulsed DC electrical stimulation with 5 μs pulse width. They applied ES to ischemic full-thickness wound in ears of rabbit.

The mechanisms by which ES induces the release of VEGF in wound site are still not known well.

In the wound healing process, VEGF is produced by endothelial cells, fibroblasts, platelets, neutrophils, keratinocytes, and macrophages [1]. In vitro studies have reported that fibroblasts, neutrophils, and keratinocytes migrated toward cathode (negative polarity) in electric fields. Talebi et al. showed that the cathodal stimulation increased the number of macrophages and fibroblast cells as compared to the control group [4]. Also, it claimed that low intensity current may resemble the natural electrical field current created following injury, thus it can enhance galvanotaxis (directional migration of various types of cell) [5]. Therefore, it seems that the sensory ES can facilitate cell migration rather than (such as neutrophils, macrophages, fibroblasts and keratinocytes) to the wound site, thus it can induce the release of more VEGF in wound site as compared with motor intensity ES and control groups.

Although our previous study showed increase of fibroblasts and collagen deposition after applying cathodal microampeage direct current [4], but based on obtained results of this research, it appeared that, sensory and motor ES have not had a significant effect on biomechanical findings of the wound. It should be notes that the tensile strength of the

healing wound depends on not only to amounts of the collagen fibers, but rather to collagen orientation, formation of cross links between the filaments, and maturation of collagen fibers. In other words, the increase of electrical stimulation intensity could not affect the VEGF expression and biomechanical properties of full-thickness wound.

The increase of skin VEGF level by sensory ES on the 7th day post injury concurs with early stages of angiogenesis, thereby sensory ES may be more effective at promoting angiogenesis in wound healing process.

Further work is needed to determine the relationship between VEGF expression after the sensory and motor ES and its effect on collagen synthesis, and improvement of biomechanical parameters during wound healing.

## VI. Conclusion

In summary, this study demonstrated a more expression of skin VEGF on the 7th days after application of sensory ES. Using sensory or motor stimulation could not improve the biomechanical properties of wound. Further work is needed to determine the relationship between VEGF expression after ES and its effect on tensile strength of healed wound.

## Acknowledgment

This material was based on work supported by Iran National Science Foundation resources (grant 87040798), and the facilities of Tarbiat Modares University, Tehran, I.R.Iran. The authors have declared that no competing interests exist.

## References

[1] P. Bao , A.Kodra , M.Tomic-Canic , M.S. Golinko, H.P. Ehrlich, H. Brem," The role of vascular endothelial growth factor in wound healing", J Surge Res. vol. 153, no. 2, pp. 347-358, 2009.

[2] K.A. Morris, M.F. McGee, J.J. Jasper, K.M. Bogie. "Evaluation of electrical stimulation for ischemic wound therapy: a feasibility study using the lapine wound model", Arch Dermatol Res. Vol. 301, no. 4, pp. 323-327, 2009.

[3] M. Zhao, H. Bai, E. Wang, J.V. Forrester, C.D. McCaige, " CD. Electrically stimulation directly induces pre-angiogenic responses in vascular endothelial cells by signaling through VEGF receptors", J cell Sci. no. 117, pp. 397-405, 2004.

[4] G. Talebi, G. Torkaman, M. Firoozabadi, S. Shariat, " Effect of anodal and cathodal micro-amperage direct current on the skin wound healing: A biomechanical and histological study", Journal of biomechanics, no. 40(s2), p. s665, 2007.

[5] L.C. Kloth, J.M. McCulloch, "Promotion of wound healing with electrical stimulation", Adv Wound care. Vol. 9, no. 5, pp. 42-45, 1996.G. O. Young, "Synthetic structure of industrial plastics (Book style with paper title and editor)," in *Plastics*, 2nd ed. vol. 3, J. Peters, Ed. New York: McGraw-Hill, 1964, pp. 15–64.