Richard Eloin Liebano, PT, MSc, PhD

Submitted for publication February 4, 2013.
Accepted in revised form June 21, 2013.

*Correspondence: Master's and Doctoral
Programs in Physical Therapy, Universidade
Cidade de São Paulo, São Paulo, Brazil (E-mail:
liebano@gmail.com).

Abbreviations and Acronyms

μ A = microampere

μ s = microsecond

ES = electrical stimulation

ESD = electrical stimulation day

FREMS = frequency-modulated
electrical stimulation

Hz = Hertz

IL-1 β = interleukin-1 beta

mA = milliamp

NSD = normal skin day

pps = pulses per second

TENS = transcutaneous
electrical nerve stimulation

V = volts

VEGF = vascular endothelial
growth factor

Vascular Endothelial Growth Factor Release Following Electrical Stimulation in Human Subjects

Richard Eloin Liebano* and Aline Fernanda Perez Machado

Department of Physical Therapy, City University of São Paulo, São Paulo, Brazil.

Significance: Angiogenesis is an important phenomenon involved in the healing of chronic wounds, and it is mainly mediated by the release of vascular endothelial growth factor (VEGF) from endothelial cells. Electrical stimulation (ES) is a well-documented treatment used to assist the healing of chronic wounds. Due to the importance of VEGF in the healing process, and the need to know the mechanisms of action of ES involved in the process, this report aimed to determine by a literature review whether the VEGF release occurs following ES in human subjects.

Recent Advances: The findings of this literature review suggest that ES releases VEGF, and this effect may be responsible for promoting angiogenesis after ES.

Critical Issues: Despite the findings of this literature review on the release of VEGF by ES on wound healing are promising, a large number of studies are needed to confirm such effects.

Future Directions: Further studies should be conducted to identify the best parameters and treatment schedule of ES to be used for the VEGF release.

BACKGROUND

THE WOUND HEALING process consists of complex and well-orchestrated phases that begin right after injury, and there is an interaction between several tissues and cells.^{1,2} The main events present at each phase are in the inflammatory phase, neutrophils and macrophages migrate into injury to clear the area; in the proliferative phase, there is the formation of new blood vessels that encourages the fibroblasts to proliferate, produce extracellular matrix, and regenerate epidermis; and finally, the remodeling or maturation phase occurs where the matrix is turned over and the wound undergoes contraction.¹⁻³

However, the most important events of wound healing occur in the proliferative phase, which ensures

successful closing of the wound. In the proliferative phase, there is the formation of new blood vessels by branching of existing capillaries through their bifurcation and extension, and this is important and indispensable for successful wound healing.⁴⁻⁷ This phenomenon is called angiogenesis (or neovascularization) and is mediated by some growth factors, such as the vascular endothelial growth factor (VEGF).⁴⁻¹⁰ VEGF is a cytokine that regulates the multiple biological functions of endothelial cells, enhancing the production of vasodilatory mediators, increasing vascular permeability, and acting as a chemotactic agent.^{8,9,11}

Electrical stimulation (ES) is a well-documented treatment used to assist the healing of chronic wounds.^{7,12} There are many mecha-

nisms involved in the healing of tissues promoted by ES. Intact skin has a transepithelial potential, where the stratum corneum is negative when compared with the underlying dermis. This difference of potential is also known as skin battery, and when skin is damaged, there is a movement of charged particles and creation of a current of injury. The presence of the current of injury in the injured tissue has been suggested as a trigger to tissue repair and is associated with effective wound healing. Chronic and dry wounds no longer have this electrical signal, which makes the tissue repair even more difficult. Thus, it is believed that the application of an exogenous electrical current may mimic the body's own bioelectric currents and reinitiate or facilitate the wound repair process. The ES promotes electrotaxis of neutrophils, macrophages, epidermal stem cells, mast cells, myofibroblasts, endothelial cells, fibroblasts, and keratinocytes. Electrotaxis can be defined as the attraction of electrically charged cells toward an electrical field of opposite polarity. The ES also has both bactericidal and bacteriostatic effects, increases the microcirculation, and ATP and protein synthesis, stimulates the cellular calcium influx, fibroblast proliferation, and collagen production, increases endothelial nitric oxide,^{7,11,13-16} and improves angiogenesis, which is mediated by the release of VEGF.⁷

Due to the importance of VEGF in the healing process and the need to know the mechanisms of action of the ES involved in this process, this report sought to determine by literature review whether the VEGF release occurs following ES in human subjects.

CLINICAL PROBLEM ADDRESSED

The healing process can be negatively influenced by local and systemic factors, causing delayed healing and leading patients to develop chronic wounds.¹⁰ The local factors include infection, inadequate blood flow, and inadequate nutrition, resulting in low oxygen levels and a poor inflammatory response. Sustained pressure or repeated wound stresses can also contribute to impairing of cutaneous healing. The age-related changes, hormonal disturbance, and systemic diseases are considered systemic factors. The pressure ulcers, chronic venous ulcers, and arterial and diabetic ulcers are the most common types of ulcers observed in clinical practice. These chronic ulcers are costly to treat and impair the self-esteem and quality of life of patients.

Angiogenesis is an important phenomenon involved in the healing of chronic wounds, and it is

mainly mediated by the release of VEGF from endothelial cells, fibroblasts, platelets, neutrophils, keratinocytes, and macrophages.⁷ The regulation of VEGF expression during wound healing is of considerable importance since angiogenesis appears to be inhibited in abnormally healing wounds. Although ES has been shown to be an important biophysical energy for the healing of chronic wounds and promoting angiogenesis, little is known about its effects on VEGF release in humans. This information would contribute to a better understanding of the mechanisms involved in the ES for wound healing. Moreover, the type of ES current (direct, alternating, or pulsed) and its parameters (phase/pulse amplitude, duration, and frequency) as well as charge density in wound tissues that best enhance VEGF release and wound healing still need to be determined.

RELEVANT BASIC SCIENCE CONTEXT

The wound healing process is characterized by complex phases that begin right after injury^{1,2} to restore structure and regional homeostasis.^{17,18} The classic model of wound healing is divided into three continuous and overlapping phases: the inflammatory phase, the proliferative phase, and the remodeling or maturation phase.^{2,3} During the proliferative phase, there is the formation of new blood vessels and this phenomenon is called angiogenesis. Angiogenesis is an integral part of this sequence of events involved in wound healing and is mediated by VEGF.^{19,20} The VEGF is one of the basic angiogenesis regulators because it stimulates migration, proliferation, and formation of endothelial cells^{8,9,17} and has a crucial role in the healing of wounds.¹⁸

A large number of nonpharmacologic interventions have been used to facilitate the wound healing process. Such treatments are usually well tolerated by patients and do not produce systemic side effects. Although there are clinical studies showing the beneficial effects of some biophysical energies on wound healing, including low-level laser therapy, light-emitting diodes, whirlpool, therapeutic ultrasound, ultraviolet radiation, ES, pulsed shortwave, and magnetic therapies, the mechanisms by which they may enhance tissue repair still need to be better understood.¹² Among these energies, ES is one of the most studied, and since the number of published, successful clinical trials has increased appreciably during the past 3 decades, the use of ES for the treatment of chronic soft tissue wounds has become more widely accepted in many countries.¹²

The ES is a therapeutic modality, which is non-pharmacologic, noninvasive, low cost, easy to use, and widely applied in clinical practice.^{12,21,22} The ES has been used to assist the healing of acute and chronic wounds in humans and animals.^{7,13,20}

Experimental studies involving animal models have showed that ES increases the release of VEGF in the wound site (Fig. 1).^{20,23} Morris *et al.* applied monophasic square-wave pulsed current to ischemic wounds in the ear of rabbits with fixed pulse amplitude (11 mA), but with two different pulse durations (110 and 5 μ s).²³ They found that the VEGF level was significantly higher for the 110 μ s pulse duration, because this group demonstrated more effective angiogenesis on the 14th day following injury. Asadi *et al.* verified that the cathodal electrical sensory stimulation (direct current, 600 μ A) is more effective than the cathodal motor ES (monophasic pulsed current, pulse duration 300 μ s, 100 Hz, 2.5–3.0 mA) in promoting wound healing of the full-thickness skin incision on the dorsal region in rats because the skin VEGF levels were higher in the group that received sensory stimulation on the 7th day.²⁰

Experimental wound healing studies *in vitro* and in animal models are located at the base of the evidence pyramid. Nevertheless, they are still important because they provide relevant information based on tissue analyses that are not always possible to perform in humans. Moreover, in experimental models, researchers can test the influence of different electrical parameters on wound healing and cytokine release. Although the pig skin is

similar to human skin and considered the ideal model for studying cutaneous wound healing, for logistical and economic reasons, a larger number of studies using rats as sample are present in the literature.

Therefore, although it is known that the use of ES promotes the release of VEGF in animals, it is deemed important to know the effect of ES on VEGF release in humans, since clinical trials will allow the applicability of the observed results in clinical practice.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

ES as an adjunctive therapy for acute and chronic wound healing dates back to the 1700s.¹⁷ The reports published in the seventeenth century record the use of electrostatically charged gold leaf applications to skin lesions associated with small-pox to facilitate healing. Since then, studies have been performed to understand the mechanisms involved and the effects of different parameter settings of ES for wound healing. The aim of this study was to verify VEGF release following the ES in human subjects. After an extensive literature review, only three clinical trials could be included in this study. These studies found in the literature are summarized below.

Ferroni *et al.* (2005) proposed a pilot study to verify the effect of software-controlled electric impulses on circulating the VEGF levels in patients with dystrophic ulcers due to peripheral arterial

Figure 1. The wound healing process begins immediately after an injury. The most important events of wound healing occur in the proliferative phase. In the proliferative phase, there is the formation of new blood vessels, called angiogenesis. This phenomenon is mediated by some growth factors, such as the vascular endothelial growth factor (VEGF). The electrical stimulation (ES) promotes electrotaxis of endothelial cells and stimulates the VEGF release. Therefore, ES may improve angiogenesis and contribute to a faster wound repair. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

disease.¹¹ Nine patients (seven males and two females, mean age 67 years), affected by dystrophic ulcers due to peripheral arterial disease, were selected. Patients received one session of low voltage software-controlled pulses, for 10 min, with the following parameters: frequency ranging from 1 to 420 pps, phase duration from 1 to 9 μ s, and amplitude of 30 to 120 V (100 μ A max). Samples of peripheral venous blood were withdrawn from each patient. The serum and plasma VEGF levels were determined by enzyme immunometric assay on all samples obtained before (baseline), during, and after the treatment. ES resulted in maximum VEGF expression during the first 10 min, followed by a rapid decrease in VEGF levels toward the baseline value. Indeed, an immediate rise of the mean serum VEGF level was observed in samples obtained during the treatment, reaching a peak at 7 min from its start. There was also an increase in the serum tumor necrosis factor- α and interleukin-1 beta levels during the treatment.

This was a very interesting study showing the release of VEGF with the use of ES. Presumably, the VEGF release would activate endothelial cells improving wound healing especially in patients with ischemic diseases. However, some details about the ES were not clear. The authors did not describe the waveform delivered by the electrical stimulator, and they used a specific software that varied pulse patterns according to the patient's response. Moreover, the placement of electrodes is not described, making difficult the reproduction of their results. Another possible limitation is related to the time of VEGF peak. The VEGF peak was observed at 10 min of treatment. Further studies with longer treatment times are needed to determine the optimal treatment time for the VEGF release.

Bevilacqua *et al.* aimed to compare the effects of frequency-modulated electrical stimulation (FREMS) to transcutaneous electrical nerve stimulation (TENS) on the VEGF release in type 2 diabetic and nondiabetic subjects.²⁴ Twenty human subjects, including ten diabetic patients with diabetic polyneuropathy (mean age 52 years) and ten nondiabetic subjects (mean age 37 years), were recruited. All the subjects underwent TENS for 10 min, followed by an interval of 30 min without the ES, after this, the FREMS therapy was applied for 10 min via two electrodes applied over the forearm volar surface. FREMS consisted of sequences of monophasic compensated negative potential electrical pulses, with sharp spikes and asymmetrical shapes. Peak amplitude ranged from 0 to 255 V; pulse frequency ranged from 1 to 50 Hz, and pulse duration ranged from 10 to 40 μ s. Blood

samples for plasma VEGF measurements were obtained from the contralateral arm through an intravenous indwelling catheter at different time points during 70 min at 30, 20, and 10 min before; 0 and 10 min during the TENS application; 0, 10, 20, and 30 min without the ES; 0 and 10 min during the FREMS treatment; and 0, 10, 20, and 30 min without the ES again. The VEGF rise in plasma observed in response to FREMS administration was not observed during the TENS application in both nondiabetic and diabetic subjects. The VEGF concentrations during TENS were similar to those observed during the intervals before the application of the electrotherapies. The conclusion was that the VEGF release during FREMS may help explain the positive effects on nerve conduction velocity in diabetic polyneuropathy and that this effect was possibly mediated by favorable effects on *vasa nervorum* microangiopathy.

In this study, the application order was not randomized, which could create an order bias. Thus, it can be argued whether FREMS is really more effective than TENS for the VEGF release or if this effect was because FREMS was always applied after TENS. Moreover, the TENS parameters, such as pulse duration, frequency, and pulse amplitude, were not described.

Sebastian *et al.* showed for the first time the effect of a degenerate waveform *in vivo* by comparing cutaneous wound healing in human volunteers with and without the ES. Twenty healthy volunteers (11 males and 9 females) were subjected to a punch biopsy.¹³ The volunteers were subjected to a nonintervention and an ES treatment period. A punch biopsy of full-thickness skin was obtained from the upper arm on day 0 (normal skin day-NSD0) and from the same wound site on day 14 (NSD14). Subsequently, a punch biopsy was taken from the contralateral upper arm on the same day as NSD14 (electrical stimulation day-ESD0), which was then treated with ES for the next 14 days before the second punch biopsy sample from the same wound site on day 14 (ESD14). The initial punch biopsy was 5 mm, and the second was 6 mm through the full-thickness skin. A near-completed inflammatory stage of wound healing occurred in ESD14 compared to NSD14. VEGF was assessed by immunohistochemistry and showed significant expression in ESD14 (66%) and NSD14 (38%), compared to 24% in normal skin. VEGF was mainly expressed by keratinocytes and dermal fibroblasts, endothelial cells, and macrophages. The cutaneous wounds that received the ES displayed accelerated healing reflected by the reduced inflammation, enhanced angiogenesis, and advanced remodeling phase.

This is an interesting and well-written article; nevertheless, there is a lack of details of electrical current used. The authors did not explain the parameters of the degenerate waveform.

In summary, there are wide variations in the type, dose, frequency, and method of delivery of the ES. The findings of this literature review based on the release of VEGF by the ES on wound healing are promising, but still inconclusive, because the clinical trials are still insufficient to confirm such effects. The reported studies demonstrate great variability in the parameters of the ES application for the VEGF release, leading to an inability to generate sufficient evidence to support any one standard therapeutic approach.²⁵

INNOVATION

In contrast with a larger number of experimental studies in animals, just a few studies showing the VEGF release in humans after ES were found. Despite the limited evidence on the release of VEGF by ES during wound healing, the results of the studies presented here are promising and suggest that further studies should be conducted to identify the best treatment schedule and optimal parameters of ES to be used for this purpose. The discovery of the optimal electrical parameters to be used for the expression and release of VEGF during the healing of the skin may lead to the development of more efficient ES devices and advances in therapeutics related to the healing of ischemic chronic ulcers.

ACKNOWLEDGMENTS AND FUNDING SOURCES

The authors gratefully acknowledge Prof. Luther Kloth from the Marquette University for his suggestions on this study. The authors have not received funding for this work.

TAKE-HOME MESSAGE

Despite the wide use of ES for wound healing, only 3 studies were found showing the VEGF release following ES in human subjects.

The studies were performed with patients with peripheral arterial disease,¹¹ diabetes,²⁴ and in healthy volunteers.¹³

It is suggested that the use of ES is beneficial to release VEGF to promote the healing of skin wounds, but more studies are needed to confirm the findings of the studies cited here.

AUTHOR DISCLOSURE AND GHOSTWRITING

The authors have nothing to disclose. No ghost-writer has been used to write this article.

ABOUT THE AUTHORS

Richard E. Liebano, PT, MSc, PhD, is a full professor at Universidade Cidade de São Paulo in São Paulo, Brazil. Dr. Liebano received his degree in Physical Therapy from Pontifícia Universidade Católica de Campinas. He is certified in Orthopedics and Sports Physical Therapy and Masters in Plastic and Reconstructive Surgery. His PhD in Sciences was received from Universidade Federal de São Paulo. In 2009, he did his postdoctoral fellowship in Physical Therapy and Rehabilitation Science at The University of Iowa. He has been teaching electrophysical agents for Physical Therapy students during 13 years, and his researches are performed in this area. Dr. Liebano lives in São Paulo, Brazil, and acts as an advisor for Master's and PhD students. **Aline Fernanda Perez Machado, PT**, received her degree in Physical Therapy from Universidade Cidade de São Paulo in São Paulo, Brazil. She is certified in Physical Therapy Applied to Women's Health and Aesthetic Physical Therapy. She is currently doing a Master's in Plastic and Reconstructive Surgery area at Universidade Federal de São Paulo.

REFERENCES

- Li J, Chen J, and Kirsner R: Pathophysiology of acute wound healing. *Clin Dermatol* 2007; **25**: 9.
- Kondo T: Timing of skin wounds. *Leg Med* 2007; **9**: 109.
- Diegelmann RF and Evans MC: Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci* 2004; **9**: 283.
- Demidova-Rice TN, Durham JT, and Herman IM: Wound healing angiogenesis: innovations and challenges in acute and chronic wound healing. *Adv Wound Care* 2012; **1**: 17.
- Jung M, Lord MS, Cheng B, Lyons JG, Alkhouri H, Hughes JM, *et al.*: Mast cells produce novel shorter forms of perlecan that contain functional endorepellin: a role in angiogenesis and wound healing. *J Biol Chem* 2012; **12**: 1.
- Slusarz R, Gadomska G, Biercewicz M, Grzelak L, Szewczyk MT, Roś D, *et al.*: The influence of selected demographic factors and wound location on the concentration of vascular endothelial growth factor (VEGF-A) in the wound healing process after neurosurgery: brief report. *Wound Repair Regen* 2012; **20**: 667.
- Bai H, Forrester JV, and Zhao M: DC electric stimulation upregulates angiogenic factors in endothelial cells through activation of VEGF receptors. *Cytokine* 2012; **55**: 110.
- Bates DO and Harper SJ: Regulation of vascular permeability by vascular endothelial growth factors. *Vascul Pharmacol* 2003; **39**: 225.

9. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, and De Bruijn EA: Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev* 2004; **56**: 549.
10. Peplow PV and Baxter D: Gene expression and release of growth factors during delayed wound healing: a review of studies in diabetic animals and possible combined laser phototherapy and growth factor treatment to enhance healing. *Photomed Laser Surg* 2012; **30**: 617.
11. Ferroni P, Roselli M, Guadagni F, Martini F, Mariotti S, Marchitelli E, *et al.*: Biological effects of a software-controlled voltage pulse generator (Phy-Back PBK-2C) on the release of vascular endothelial growth factor (VEGF). *In Vivo* 2005; **19**: 949.
12. Kloth LC: Electrical stimulation for wound healing: a review of evidence from *in vitro* studies, animal experiments, and clinical trials. *Int J Low Extrem Wounds* 2005; **4**: 23.
13. Sebastian A, Syed F, Perry D, Balamurugan V, Colthurst J, Chaudhry IH, and Bayat A: Acceleration of cutaneous healing by electrical stimulation: Degenerate electrical waveform down-regulates inflammation, up-regulates angiogenesis and advances remodeling in temporal punch biopsies in a human volunteer study. *Wound Repair Regen* 2011; **19**: 693.
14. Long H, Yang G, and Wang Z: Galvanotactic migration of EA.Hy926 endothelial cells in a novel designed electric field bioreactor. *Cell Biochem Biophys* 2011; **61**: 481.
15. Hammerick KE, Longaker MT, and Prinz FB: *In vitro* effects of direct current electric fields on adipose-derived stromal cells. *Biochem Biophys Res Commun* 2010; **397**: 12.
16. Chang PC, Sulik GI, Soong HK, and Parkinson WC: Galvanotropic and galvanotoxic responses of corneal endothelial cells. *J Formos Med Assoc* 1996; **95**: 623.
17. Stockmann C, Kirmse S, Helfrich I, Weidemann A, Takeda N, Doedens A, and Johnson RS: A wound size-dependent effect of myeloid cell-derived vascular endothelial growth factor on wound healing. *J Invest Dermatol* 2011; **131**: 797.
18. Fleischli JG and Laughlin TJ: Electrical stimulation in wound healing. *J Foot Ankle Surg* 1997; **36**: 457.
19. Wilgus TA and DiPietro LA: Complex roles for VEGF in dermal wound healing. *Invest Dermatol* 2012; **132**: 493.
20. Asadi MR, Torkaman G, and Hedayati M: Effect of sensory and motor electrical stimulation in vascular endothelial growth factor expression of muscle and skin in full-thickness wound. *J Rehabil Res Dev* 2011; **48**: 195.
21. Jones I and Johnson MI: Transcutaneous electrical nerve stimulation (TENS). *Contin Educ Anaesth Critic Care Pain* 2009; **9**: 130.
22. Liebano RE, Abila LEF, and Ferreira LM: Effect of low-frequency transcutaneous electrical nerve stimulation (TENS) on the viability of ischemic skin flaps in the rat: an amplitude study. *Wound Repair Regen* 2008; **16**: 65.
23. Morris KA, McGee MF, Jasper JJ, and Bogie KM: Evaluation of electrical stimulation for ischemic wound therapy: a feasibility study using the lapine wound model. *Arch Dermatol Res* 2009; **30**: 323.
24. Bevilacqua M, Domingues LJ, Barrella, and Barbagallo M: Induction of vascular endothelial growth factor release by transcutaneous frequency modulated neural stimulation in diabetic polyneuropathy. *J Endocrinol Invest* 2007; **30**: 944.
25. Isseroff RR and Dahle SE: Electrical stimulation therapy and wound healing: where are we now? *Adv Wound Care* 2012; **1**: 238.