Electrical Stimulation for Wound Healing: A Review of Evidence From In Vitro Studies, Animal Experiments, and Clinical Trials

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This article reviews theories linked to endogenous bioelectric currents and the role they may play in wound repair with further appraisal of in vitro and in vivo research related to the effects of clinically applicable electrical currents on protein synthesis, cell migration, and antibacterial outcomes. In addition, studies on the effects of electrical stimulation (ES) on skin grafts, donor sites, and musculocutaneous flaps in animals are evaluated, as well as assessments of numerous clinical reports that examined the effects of ES on angiogenesis, perfusion, $PtcO_2$, and epithelialization. Finally, a plethora of clinical trials related to the responses of chronic lower extremity wounds to ES therapy are reviewed, with emphasis on wounds caused by venous insufficiency, diabetic neuropathy, and ischemia in patients with and without diabetes mellitus. A glossary that addresses ES terminology is also included.

Key words: electrical stimulation, wound healing, wound injury current, antibacterial effects, lower extremity wounds, electrical stimulation terminology

The delivery of electrical current into refractory wounds for the purpose of enhancing tissue healing is not new. Several reports from the 17th and 20th centuries describe the use of electrostatically charged gold leaf in the treatment of skin lesions associated with smallpox[1] and wounds of various etiologies,[2-7] including ischemic and venous insufficiency ulcers of the lower extremity.[3,5,6] However, findings from these studies are indecisive. In 1850, Lente[8] was the first to report on the use of electrical stimulation (ES) to treat bone fractures. Much later, many credible experimental and clinical research reports on the use of ES to augment bone repair[9,10] resulted in the US Food and Drug Administration's granting approved labeling of electromagnetic devices for treatment of nonunion and delayed union fractures.[11-14] Since the mid-1960s, much research has been aimed at evaluating the effects of ES on healing of chronic wounds. 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. In 2002 in the United States, ES was approved for payment by the Centers for Medicare and Medicaid Services for the treatment of pressure ulcers and wounds of the lower extremity caused by venous and arterial insufficiency and diabetes that have not responded to standard wound treatment.[15]

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ENDOGENOUS BIOELECTRIC CURRENTS

In the modern age of medicine, health care practitioners from several disciplines use electricity either to treat illness or injury or to evaluate and diagnose with countless medical instruments that are electrically powered. Some common applications of therapeutic electricity include electroanalgesia for chronic pain control, pacing devices for regulating nodal activity of the heart, cochlear stimulation to aid hearing, functional ES to augment purposeful movements in people

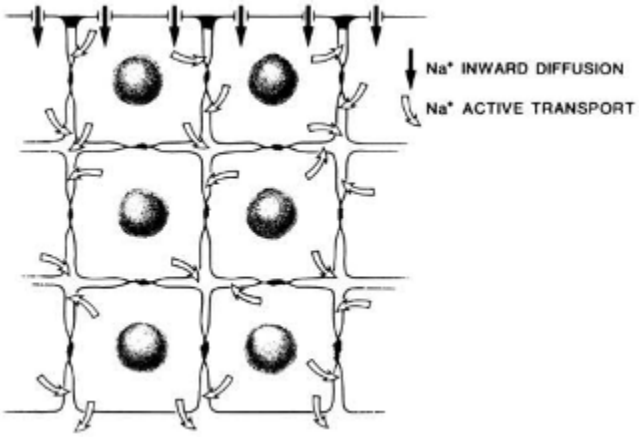

Fig. 1 Diagram of Na+ transporting syncytial epithelium.
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with paralyzed limbs, and ES to enhance wound healing. That the human body has endogenous bioelectric systems that produce electrochemical signals is clearly evident by the action potentials recorded from the heart (electrocardiogram), brain (electroencephalogram), skeletal muscle (electromyogram) and retina (electroretinogram) during electrophysiological evaluation procedures.

Cutaneous Bioelectric Currents: The Skin Battery

Measurable currents that reportedly contribute to wound healing are also found in the intact and wounded skin of humans, mammals, and amphibians.[16-21] Several investigators have reported measuring electronegative voltages from the surface of intact skin and electropositive voltages from the dermis of superficial wounds.[17,18,21] In amphibians, these measurable transepithelial potentials (TEPs) are known to occur as a result of Na^+ channels in the apical membrane of the skin's mucosal surface that allow extracellular Na^+ to diffuse to the inside of epidermal cells (see Fig. 1 from Vanable[20]). Foulds and Barker[19] measured TEPs of human skin and reported values ranging from 10 mV to almost 60 mV depending on the region measured. By placing a reference electrode in electrical contact with the dermis and a recording electrode at multiple positions on intact skin of normal human volunteers, they demonstrated the presence of a skin battery. They found that the stratum corneum of all skin sites of all subjects had an average negative potential of 23.4 mV (see Fig. 2 from Foulds and Barker[19]). The skin battery voltage effect is primarily produced by electrical activity in exocrine sweat glands.[22] Further experimental evidence supporting the existence of the skin battery has been demonstrated by applying amiloride (a compound that blocks Na^+ channels in the outer epidermal membrane) of mammalian skin. This results in a drastic reduction of TEPs across the skin, which in turn either reduces or abolishes the cutaneous bioelectric currents.[23]

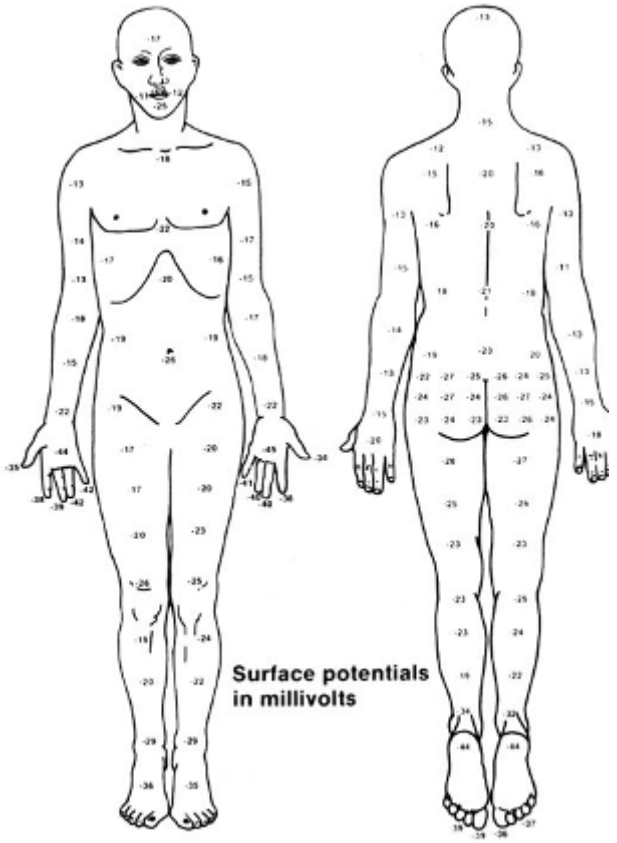

Fig. 2 Average human skin battery potential measured on a typical person aged 29 years.
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Wound Current of Injury

When a wound occurs in the skin, an electrical leak is produced that short-circuits the skin battery at that point, allowing current to flow out of the moist wound.[18,20] Natural bioelectric fields present as ionic currents in injured tissues were first demonstrated in 1830.[24] In 1843, Dubois-Reymond[25] demonstrated the existence of wound currents, which more recently were recorded up to 35 $\mu A/cm^2$ from the amputated fingers of children[17] and 10 to 30 $\mu A/cm^2$ from induced

wounds in guinea pigs.[18] This "current of injury," measurable in wounded amphibian skin[26,27] where it likely contributes to wound healing, is sustained in a moist wound environment and is shut off when a wound dries out.[28] Cheng et al[29] have demonstrated that an occlusive dressing applied to wounds in a porcine model sustained the injury current at 29.6 ± 8.6 mV for 4 days compared to a significantly lower potential of 5.2 ± 12.6 mV recorded from wounds exposed to air during the same time period. Their study provides evidence that the wound current of injury may be sustained with occlusive, moisture-retentive dressings that contribute to the enhanced healing rate that occurs under occlusion.[30,31] When a wound occurs in the skin, the measurable positive injury current flows out of the wound and is also measurable in a 2- to 3-mm margin of periwound skin. This lateral voltage gradient falls from a high of 140 mV/mm at the wound edge to 0 mV/mm just 3 mm lateral to the wound edge.[28] McGinnis and Vanable[26] have shown that currents escaping through healing wounds and their accompanying lateral voltage gradients are gradually reduced and ultimately become nonexistent due to the resistance created by newly regenerated epithelium.

EXOGENOUS ELECTRICAL CURRENTS: EFFECTS ON TISSUE CELLS IN VITRO

Considerable experimental research has contributed to the expanding body of knowledge that provides insights into the cellular and physiological mechanisms by which ES enhances wound healing. Numerous studies have investigated how cells respond when exposed to electrical currents of different amplitudes and frequencies. Some studies have reported changes in cell synthesis and metabolism, while others have observed migratory effects of cells exposed to electric fields. For the benefit of the reader who may need clarification of terms and definitions related to ES, please see the glossary in the appendix.

Protein Synthesis

Bassett and Herrmann[32] delivered a continuous electrostatic field of 1000 V/cm (see appendix) through fibroblast cultures and demonstrated a 20% increase in both DNA and collagen synthesis after 14 days. Bourguignon et al[33,34] stimulated healthy human fibroblasts in cell cultures with high-voltage pulsed current (HVPC). They suggested that the fibroblasts were induced to increase their rate of DNA and protein synthesis, the latter of which increased by 160% over controls. Maximum synthesis occurred with clinically applicable stimulus parameters of 50 and 75 V and 100 pulses per second (pps) with the cells in close proximity to the cathode. Voltages in excess of 250 V inhibited both protein and DNA synthesis. Within the first minute of fibroblast stimulation in vitro using the same stimulus parameters cited in the previous study, Bourguignon et al[35] reported an increase in Ca^{2+} uptake followed by upregulation of insulin receptors on the fibroblast membrane during the second minute of stimulation. When they added insulin to the electrically stimulated cultures, there was an immediate second increase in Ca^{2+} uptake and significant increases in both protein and DNA synthesis compared to nonstimulated cells. The significance of the latter finding is that during ES treatment of wounds, if insulin is available to bind the additional receptors, the fibroblasts will significantly increase both protein and DNA synthesis. Transforming growth factor-β (TGF-β) is also known to play an important role in collagen synthesis. Falanga et al[36] have shown that ES upregulates receptors for TGF-β on human dermal fibroblasts in culture. Fibroblasts that were exposed to 100 V and 100 pps had receptor levels of TGF-β that were 6 times greater than those of control fibroblasts.

Cheng et al[37] evaluated the effects that occurred in rat skin following microampere levels of direct current (DC) stimulation. They reported that 10 to 1000 μA applied to skin strips 0.5 mm thick at 500 μA for 2 hours in vitro increased adenosine triphosphate concentration in the skin 5-fold. They also found that 100 to 500 μA of DC increased amino acid uptake 30% to 40% above control levels and that 50 μA was required to obtain a maximum stimulation effect on protein synthesis. Other investigators have noted that fibroblasts in a 3-dimensional collagen matrix that were exposed to an electric field responded by increasing the intake of ^{3}H-thymidine.[38]

Based on the findings of these in vitro studies, possible mechanisms by which ES enhances soft tissue healing include triggering of the opening of voltage-sensitive calcium channels in the fibroblast plasma membrane. Subsequently, upregulation of insulin and TGF-β receptors on the cell surface may cause increased rates of collagen and DNA synthesis, the latter of which suggests that fibroblasts are stimulated to proliferate.

Cell Migration

Several investigators (Table 1) have reported that cells involved in wound healing migrate toward the anode or cathode of an electric field delivered into the cell cultures.[39-47] This phenomenon known as

Table 1. Galvanotaxic Theory During Phases of Healing

Phase of Healing	Effects	Cells (Polarity)	Current (Polarity)	Investigator
Inflammatory	Phagocytosis and autolysis	Macrophage (–)	DC (+)	Orida et al[39]
		Neutrophil (–)	DC (+)	Fukushima et al[41]
		Neutrophil (–)	PC (+)	Eberhardt et al[50]
		Activated neutrophil (+)	DC (–)	Dineur[42]/Monguio[40]
Proliferative	Fibroplasia	Fibroblast (+)	PC (–)	Bourguignon et al[33-35]
			DC (–)	Canaday and Lee[43]
			DC (–)	Erickson and Nuccitelli[44]
			DC (–)	Yang et al[45]
Remodeling	Wound contraction	Myofibroblast (+)	PC (–)	Stromberg[48]
	Epithelialization	Keratinocyte (+)	DC (–)	Nishimura et al[46]
		Epidermal (–)	DC (–)	Cooper et al [49]
			PC (–/+)	Mertz et al[51]
			PC (+)	Greenberg et al[107]

DC = direct current; PC = pulsed current.

galvanotaxis is the attraction of positively or negatively charged cells toward an electric field of opposite polarity. For example, macrophage cells that play important roles during the inflammatory phase of healing migrate toward the anode,[39] whereas neutrophils migrate toward both the anode and cathode.[40,41] However, Dineur[42] and Monguio[40] have reported that leukocytes migrate toward the cathode in regions where there is infection or inflammation, which suggests a link between chemically mediated events and electrical responsiveness. There is considerable evidence that the fibroblast migrates toward the cathode.[33,34,43-45] With respect to healing of the skin, investigators have shown that exogenously applied electric fields of the same magnitude as those found in mammalian wounds direct the migration of human keratinocytes toward the cathode.[46,47] Two other studies have also provided information related to galvanotaxis of cells involved in wound repair.[48,49]

Two studies investigated the effect of ES on cell migration in vivo. Eberhardt et al[50] evaluated the effects of exogenous ES on cell composition in human skin and found that 69% of 500 cells counted 6 hours poststimulation were neutrophils compared to 45% found for control wounds. The authors proposed that the 24% difference in neutrophil percentage was due to the galvanotaxic effect created by the cutaneously applied currents. Mertz et al[51]assessed epidermal cell migration macroscopically for 7 days following two 30-minute sessions of monophasic pulsed current stimulation of induced wounds in an ovine model. They observed that wounds treated with the cathode on day 0 followed by the anode on days 1 to 7 demonstrated 20% greater epithelialization compared to wounds treated with either positive (+9%) or negative (–9%) polarity alone. In addition, they observed that alternating polarity daily inhibited epithelialization by 45%.

There are several potential mechanisms by which ES facilitates cell migration in vivo by galvanotaxis. A cell may detect an electric field by electrophoretic movement of proteins within the plasma membrane. For instance, epidermal growth factor receptors have been shown to move to the cathode side of keratinocytes exposed to a DC electric field.[52] Other intracellular sites the electric field may perturb to effect galvanotaxis are localized membrane depolarizations that result in changes in calcium ion fluxes,[53] changes in cell shape and cytoskeletal reorganization,[54-58] and activation of protein kinases.[59,60] As previously mentioned, the weak electric fields used to cause galvanotaxis of cells in cell culture or the currents used clinically to enhance healing of chronic wounds may mimic the natural electric fields found in mammalian wounds that guide migration of keratinocytes.[46,47] As a matter of fact, cell migration findings from some of the in vitro and in vivo studies previously cited have been used as the basis for selecting the anode or cathode in the clinical treatment of wounds with ES.

ANTIBACTERIAL EFFECTS OF ES IN VITRO AND IN VIVO

In a recent position document titled *Wound Bed Preparation in Practice*, Falanga emphasized the acronym TIME, which addresses the 4 components of wound bed preparation: *T*issue management, *I*nflammation and *I*nfection control, *M*oisture balance,

and *E*pithelial (*E*dge) advancement.[61,62] Although none of these components can be singled out as being more important than the other, reducing the bacterial burden and thereby allowing chronic inflammation to subside and preventing infection is clearly recognized as an important requirement in the management of chronic wounds. Traditionally, systemic antibiotic treatment is always indicated for cellulitis, lymphangitis, and osteomyelitis.[61] In wounds that exhibit local signs of infection, antiseptic agents designed to sustain slow-release formulations of iodine or silver have been found to reduce bacterial burden safely and efficiently.[63] Unlike topical antibiotics that may cause allergic reactions, inhibit healing, or cause resistant strains of bacteria,[64] antiseptic agents such as cadexomer iodine and silver compounds that are delivered to the wound under controlled conditions are effective against a wide spectrum of organisms and do not impair healing, and to date, there are no reports of allergic reactions or bacterial resistance.[65,66]

The antibacterial effects of ES have been studied both in vitro and in vivo, and the results summarized in Table 2 indicate that ES may impose a bacteriostatic or bactericidal effect on microbes that commonly colonize or infect wounds. Using milliampere levels of alternating current (AC) and microampere (<1.0 mA) and milliampere levels of cathodal DC delivered through platinum electrodes, Rowley[67] observed, as would be expected, that in vitro growth rates of *Escherichia coli* were affected very little or not at all by AC while a significant bacteriostatic effect occurred with DC. Rowley indicated that the decrease in growth rate with DC was not due to a pH change since the cells were maintained in a buffered condition. Wolcott et al[68] observed that human wounds initially colonized with *Pseudomonas* and/or *Proteus* organisms were pathogen free following several days of treatment with microampere levels of cathodal DC. Motivated by these findings, Rowley et al[69] demonstrated a bacteriostatic effect after delivering 1.0 mA of cathodal DC for 72 hours to rabbit cutaneous wounds infected with *Pseudomonas aeruginosa*. Barranco et al[70] subjected cultures of *Staphylococcus aureus* to DC current at amplitudes of 0.4, 4.0, 40, and 400 µA with stainless steel, platinum, gold, and silver electrodes. They found that the silver anode electrode had excellent growth inhibitory capacity on *S aureus* at 0.4 and 4.0 µA with negligible toxic effects from electrode corrosion, gas production, or pH changes. Marked growth inhibition occurred with the other 3 electrodes at 400 µA, but at this amplitude, undesirable pH shifts, electrode corrosion, gas formation, and media discoloration were noted. Subsequent to this report, numerous studies established that antibacterial activity occurred in the presence of silver cations deposited in vivo or in vitro by low levels of DC.[71-78] In other in vitro studies, investigators found that 100 µA of DC delivered to cell cultures via a silver wire anode had a bacteriostatic effect on gram-positive bacteria, whereas the same current amplitude and polarity produced a bactericidal effect on gram-negative bacilli.[79,80] The authors suggested that differences in cell wall composition may have been a determining factor in the effectiveness of the electrically mediated silver antisepsis. Other investigators have compared in vitro antibacterial effects of HVPC and DC and found that HVPC applied at 50 to 800 mA and 100 pps for 30 minutes had no inhibitory effects on *S aureus*, whereas both anodal and cathodal continuous DC applied at 1, 5, and 10 mA did inhibit *S aureus* growth.[81] The findings from the latter study suggest that the mechanism by which DC kills bacteria is through electrochemical pH changes that occur at both poles, specifically an alkaline pH at the cathode and an acid pH at the anode. Electrochemical pH changes have not been shown to occur at the anode or cathode when HVPC is applied to human tissues for 30 minutes.[82] However, when Kincaid and Lavoie[83] evaluated antibacterial effects of HVPC in vitro, they observed pH changes only at the cathode at a dosage of 500 V and at both the anode and the cathode at 250 V. Szuminsky et al[84] attempted to identify the mechanisms by which HVPC applied at 500 V causes bacterial killing in vitro. They observed bactericidal effects at both poles but were unable to determine whether the killing effect was due to the direct action of the current on the organisms, electrophoretic recruitment of antimicrobial factors, local heat generation, or pH changes. Although both of the latter studies demonstrated antimicrobial effects in vitro, it is unlikely that the high voltages used would be tolerated if applied to wounds of human subjects. Interestingly, research has shown that antibiotic effectiveness against biofilm cells is increased in the presence of a weak, intermittent electrical field.[85,86]

In summary, numerous in vitro and in vivo studies have demonstrated that microampere levels of DC either kill or inhibit proliferation of common wound pathogens. Since there are many silver-impregnated dressings currently available that passively deliver silver ions to wounds with the intention of reducing the bacterial burden, one might speculate that the efficacy of such dressings may be enhanced by actively repelling the silver ions into the wound with anodal DC.

Table 2. In Vitro and In Vivo Studies on the Antibacterial Effects of Electrical Stimulation

Reference	Study Type	Pathogen(s)	Current Type	Stimulation Parameters	Polarity/Effect	Electrode Type	Growth Rate
Rowley[67]	In vitro	*Escherichia coli*	DC	mA = 1.0, 14, 140	Cathode None	Platinum	Bacteriostatic
			AC	Frequency = 1, 10, 30, 60 mA = 15 or 30		Platinum	No bacteriostatic effect
Rowley et al[69]	In vivo rabbit	*Pseudomonas aeruginosa*	DC	mA = 1.0	Cathode None	Copper mesh in gauze	Bacteriostatic
Baranco et al[70]	In vitro	*Staphylococcus aureus*	DC	µA = 40 and 400	Anode (negligible gas and pH Δ)	Silver, platinum, gold, stainless steel	Bacteriostatic
Deitch et al[71]	In vitro	*P aeruginosa* *S aureus* *Candida albicans*	Not stated	1-4 V	Not stated	Silver nylon cloth	Bacteriostatic
Thibodeau et al[75]	In vivo	Oral bacteria	DC	5.0 µA for 20 min	Anode None	Silver	Bacteriostatic
Ong et al[79]	In vitro	*S aureus*	DC	µA = 26, 100, 300, 500, 800	Anode	Silver wire	Bactericidal
		P aeruginosa					Bacteriostatic
Laatsch et al[80]	In vitro	Gram positive	DC	µA = 100	Anode Cathode	Silver wire	Bacteriostatic
		Gram negative				Silver nylon	Bactericidal
Kincaid and Lavoie[83]	In vitro	*S aureus* *E coli*	HVPC	V = 150, 200, 250, 300	Anode (toxic end products)	Stainless steel	No bacteriostatic effect
		P aeruginosa		Frequency = 120 pps	Cathode		Bacteriostatic
Szuminsky et al[84]	In vitro	*E coli* *Klebsiella*	HVPC	V = 500	Anode (gas and pH Δ)	Stainless steel	All inhibited at both poles
		P aeruginosa *S aureus*		Frequency = 120 pps	Cathode (gas and pH Δ)	Stainless steel	

DC = direct current; AC = alternating current; HVPC = high-voltage pulsed current.

ELECTRICAL STIMULATION RESEARCH ON ACUTE WOUNDS IN ANIMALS

Numerous animal studies have evaluated a variety of tissue and cellular responses following delivery of ES into traumatically induced wounds or their periwound tissues. The most noteworthy response reported is an increase in tensile strength following treatment with cathodal DC at current levels often considerably less than 1.0 mA.[87-91] Other studies demonstrated fibroblast proliferation in the wound along with collagen deposition.[92-96] Investigators have also reported that anodal HVPC enhanced epithelialization more rapidly than the cathode but had no effect on wound tensile strength.[51,97,98] Three other studies demonstrated that submotor levels of cathodal HVPC limited edema formation by blocking macromolecular leakage from microvessels.[99-101]

Effect of ES on Skin Grafts, Donor Sites, and Musculocutaneous Flaps

Several studies have investigated the effects of ES on skin grafts, donor sites, skin and musculocutaneous flaps, and angiogenesis in animal models.

Chu et al[102] investigated the effects of weak anodal DC (20-40 μA) delivered through silver nylon dressings for 5 days in a guinea pig model on (1) healing of partial-thickness scald burns, (2) split-thickness grafts taken from these wounds when healed, and (3) the resulting donor sites. Scald wounds in 180 animals treated with weak DC reepithelialized by 12 days postburn, whereas only 20 of 40 animals with control wounds that received sham DC had reepithelialized by 16 days postburn. Split-thickness grafts taken from the healed scald wounds showed more rapid revascularization with DC treatment than did control grafts. Grafts and donor sites treated with DC showed more rapid reepithelialization, decreased contraction, improved hair survival, and decreased dermal fibrosis compared to controls not treated with DC. Only donor wounds treated with DC could be repeatedly harvested as donor sites for successful split-thickness autografts. The authors proposed that DC treatment might limit the extent of tissue destruction as evidenced by DC-treated wounds having less inflammation, granulation tissue, and fibrosis than control wounds.

Politis et al[103] also used microampere DC to determine if ES could improve the posttraumatic quality of dermis and epidermis in full-thickness skin grafts in rats. Using an ES device that delivered 4.5 μA of DC for 3 days, they studied the effects of 3 surgically implanted electrode configurations: anode on top of the graft, cathode on top of the graft, and an inactive electrode on top of the graft. Quantitative and histologic assessment on the seventh postoperative day revealed the presence of necrotic skin in 80% to 90% of graft surface areas in animals treated with cathodal stimulation and control animals that received no current. In animals treated with anodal DC, only 50% of the graft area was necrotic, and the significantly thicker dermis had multilayered patches of intact epidermis.

Two other studies investigated the effect of ES on the survival of ischemic skin[104] and musculocutaneous flaps.[105] Im et al[104] stimulated the ischemic central portion of bipedicle skin flaps in pigs with a monophasic pulsed current (PC) at 35 mA, 128 pps, and a pulse duration of 140 μs for 30 minutes twice daily for 9 days following skin flap elevation. The skin flaps were stimulated with the cathode on postoperative days 1 to 3, with the anode on days 4 to 6 and the cathode on days 7 to 9. Two control pigs received sham ES treatment, and 2 others received no treatment. The length of viable flap and the extent of skin necrosis were measured on postoperative day 21. The mean area of skin flap necrosis was 28% in control animals and 13.2% in ES-stimulated animals ($P < .001$). The authors proposed that the initial 3 days of cathodal treatment might have prevented severe ischemia by blocking sympathetic vasoconstriction and also might have negated ischemia reperfusion that could have occurred in the transition zones of the skin flap. They also theorized that anodal stimulation of the flap in the later stages of tissue repair might have prevented tissue injury by scavenging superoxide radicals.

The other study that evaluated the effect of ES on flaps used a PC device (transcutaneous electrical nerve stimulation) with an unspecified waveform and parameters that are customarily used to suppress musculoskeletal pain.[105] In that study, 10 groups of rats received different current amplitudes (mA) and pulse frequencies for postoperative treatment of musculocutaneous flaps. A highly significant difference ($P < .001$) was noted between the group with the highest percentage of flap survival (94.6%) that received high-intensity (20 mA), high-frequency (80 pps) stimulation delivered to the base of the flap for 3 days and the other groups. Also a significant difference ($P < .001$) in flap survival occurred when high-intensity (20 mA) treatment was compared with low-intensity (5 mA) treatment. Flap survival was not related to the ES frequency used. In summary, the evidence cited from animal studies suggests that ES facilitates survival of failing skin grafts and musculocutaneous flaps. Clinical studies are needed to substantiate the findings from these animal studies.

CLINICAL RESEARCH

Augmentation of Wound Angiogenesis by ES

Two studies have reported increased blood flow secondary to increasing capillary density in human wounds treated with ES. Junger et al[106] reported a mean increase of 43.5% in capillary density in venous leg ulcers of 15 patients whose wounds had not improved after several months of standard care. They treated the wounds with monophasic PC for 30 minutes daily for a mean of 38 days. The monophasic PC with a 140 μs pulse duration delivered a weak DC component having an average current of 630 μA at 128 pps or 315 μA at 64 pps. For the first 7 to 14 days, they delivered 630 μA of current to the wounds via the cathode then switched the treatment electrode polarity to positive for 3 to 10 days. After this time, the polarity was changed back to negative. When the wound had made significant clinical progress toward healing, they reduced the current amplitude to 315 μA. Capillary density as observed by light microscopy improved from a prestimulation baseline of 8.05 capillaries/mm^2 to 11.55 capillaries/mm^2 poststimulation ($P < .039$). In addition, the investigators also measured $PtcO_2$ in the periwound skin prior to and following the ES treatments. They found that oxygen tension increased from 13.5 to 24.7 mm Hg, respectively (normal is >40 mm Hg), and that skin perfusion increased as determined by laser Doppler fluxmetry.

Angiogenesis was also observed to increase in a pilot study by Greenberg et al.[107] They used the same pulsed ES device used in the study by Junger et al[106] to evaluate the effects of polarity on epithelialization and angiogenesis in burn wounds of pigs. They found that reepithelialization began 2 days earlier in wounds treated with the anode when compared with the results of cathode-treated wounds and control wounds. In addition, prominent neovascularity was seen on day 10 in wounds treated with negative versus positive polarity. The finding of earlier reepithelialization agrees with findings of Mertz et al[51] described earlier, who observed that pig wounds treated with the cathode on day 0 and the anode on days 1 to 7 enhanced epithelialization by 20% compared to wounds treated with either positive or negative polarity alone. The enhancement of epithelialization with positive polarity also supports the galvanotaxis theory and the studies that have reported enhanced epidermal cell migration toward the anode.[49,51,107]

Improvement of Tissue Oxygenation With ES

There is growing evidence from human subject studies that ES facilitates a temporary increase in local tissue oxygen tension. It is commonly recognized that cells involved in tissue repair require oxygen to function most efficiently. Cells become inefficient in hypoxic tissue environments and die in anoxic environments. While oxygen is needed for the survival of cells involved in wound healing, bacterial cells, which have detrimental effects on wound-healing processes, are adversely affected by elevated levels of tissue oxygen. Indeed, a reduction in tissue oxygen partial pressure decreases resistance to infection by impairing oxidative killing of bacteria by neutrophils.[108]

Gagnier et al[109] assessed the effects of ES on the transcutaneous partial pressure of oxygen ($PtcO_2$) in 30 individuals with spinal cord injury (SCI). Ten patients were assigned to each of 3 groups that received ES either by a positive or negative monophasic paired-spiked waveform or by a symmetric biphasic square waveform. All 3 groups received submotor stimulation. Thirty minutes before ES, during 30 minutes of ES, and 30 minutes after ES was stopped, the $PtcO_2$ was recorded and compared with the prestimulation baseline. The $PtcO_2$ increased considerably compared with prestimulation values in each of the 3 groups both during and after ES. However, the differences in $PtcO_2$ changes found among the 3 different ES waveform groups were not statistically significant. The authors suggested that all 3 waveforms and the protocol they described could be used with SCI subjects to increase local $PtcO_2$ to facilitate wound healing.

To further study the effects of ES on cutaneous oxygen levels, Dodgen et al[110] enrolled 10 diabetic patients and 20 age-matched normal subjects to participate in 3 sessions of ES. They delivered current from monophasic, paired spikes through the cathode placed over the gastroc-soleus muscle group at submotor stimulus amplitude. They also delivered an asymmetric biphasic (balanced) waveform via the cathode placed over the gastroc-soleus with the amplitude set just below muscle contraction or adequate enough to elicit a 1+ level contraction. Transcutaneous oxygen levels ($PtcO_2$) were measured by oximetry for 30 minutes prior to ES, during a 30-minute ES session, and for 30 minutes after the session. The older normal subjects demonstrated increased $PtcO_2$ following 30 minutes of ES regardless of the waveform or level of stimulation used. This increase continued for 30 minutes after ES ended. On the other hand, diabetic subjects showed no significant increases in $PtcO_2$ following 30 minutes of ES but did show significant increases in $PtcO_2$ 30 min-

utes after ES ended. Perhaps the delayed response in the diabetic subjects may be attributed to neuropathic changes compromising sympathetic vasomotor control and/or to sensory nerve dysfunction compromising conduction of sensory afferent impulses. In a study by Peters et al,[111] diabetic patients with impaired vascular function who had 1 foot and distal leg treated with subsensory ES delivered through a silver mesh sock for 60 minutes on 2 consecutive days did not have the delayed response to an increase in $PtcO_2$ reported by Dodgen et al.[110] On the contrary, patients in the former study showed a significant increase in perfusion in the stimulated extremity, reflected by a significant increase in $PtcO_2$ after 5 minutes of ES. These results suggest that ES increases cutaneous oxygen saturation secondary to increasing local perfusion in diabetic subjects. Hence, ES may be useful in augmenting wound healing in diabetic and other patient populations, such as the elderly and persons with SCI, known to have difficulty healing chronic wounds (eg, pressure ulcers and leg ulcers due to vascular compromise).

The effect of ES on $PtcO_2$ has also been studied in individuals with SCIs. It is widely accepted that SCI patients have an altered autonomic nervous system. Some evidence also suggests that a decrease in the number of adrenergic receptors in the skin may occur below the level of the spinal cord lesion.[112] The reduced number of adrenergic receptors could in turn cause abnormal vascular responses in the skin below the level of SCI. Other investigators have determined that the $PtcO_2$ in the skin over the sacrum[113,114] in the supine position and the tibia[115] is lower in persons with SCI than in able-bodied individuals. This evidence indirectly suggests that the abnormal vascular responses in the skin below the level of the spinal cord lesion may reduce cutaneous blood flow, thereby lowering tissue oxygenation and predisposing the tissues to pressure ulcer formation.

Mawson and associates[116] specifically investigated the effect of ES on $PtcO_2$ in the sacral skin of SCI patients at high risk of pressure ulcer development in this area. The objective of their study was to determine whether HVPC stimulation could increase sacral skin $PtcO_2$ in SCI persons lying prone and supine. The normal range for $PtcO_2$ is 60 to 100 mm Hg.[114] In one group of 3 subjects (2 incomplete quadriplegics and 1 complete paraplegic), they applied ES with subjects lying prone for two 60-minute sessions a few days apart. The cathode was placed at spinal level T6, and the anode was placed at L2. During the first session for each subject, ES parameters were set at 50 V and 10 pps. During the second session, parameters were set at 75 V and 10 pps. Following a 5-minute baseline recording, $PtcO_2$ was recorded at 5-minute intervals during each 60-minute stimulation period and during a 20-minute poststimulation period. For all 3 subjects in the prone-lying position, they found that stimulation with HVPC led to a sustained, dose-related increase in $PtcO_2$ at the sacrum. The increase was more dramatic in 2 subjects with baseline $PtcO_2$ values at or below the lower end of the normal range. The authors noted that stimulation with 100 V had no additional incremental effect on $PtcO_2$ levels above that achieved with 75 V. In a second group of 29 SCI subjects lying supine, HVPC was applied with the cathode (polarity assumed) positioned at spinal level T6 and the anode at T12. Prior to ES, $PtcO_2$ was recorded from sacral skin at the end of a 15-minute baseline period. The ES parameters used included 75 V, 10 pps delivered for 30 minutes followed by a 15-minute poststimulation period of 15 minutes. After 30 minutes of ES, $PtcO_2$ increased 35%, from a baseline of 49 mm Hg to 66 mm Hg ($P < .00001$). This level fell slightly to 63 mm Hg by the end of the 15-minute poststimulation period. The investigators hypothesized that ES may be able to prevent development of pressure ulcers by restoring sympathetic tone and vascular resistance below the level of the cord lesion, resulting in an increase in perfusion to the cutaneous capillary beds.

CLINICAL TRIALS INVOLVING LOWER EXTREMITY CHRONIC WOUNDS

Chronic wounds of the lower extremities due to venous and arterial insufficiency, diabetes mellitus, atherosclerosis, or small-vessel disease affect nearly 1% of the general population and up to 10% of people who are in health care facilities.[117] Seventy percent to 90% of leg amputations are due to vascular ulcers, and foot ulceration and infection are primary causes of hospitalization among individuals with peripheral vascular disease due to diabetes mellitus.[118] Chronic wounds owing to venous insufficiency represent between 70% and 90% of chronic lower extremity ulcers. In the United States, costs related to the management of these wounds on an outpatient basis have been reported to be as high as $2500 per ulcer for a 4-month period.[119] A variety of therapeutic interventions are available to treat wounds of the lower extremity including topical and systemic antibiotics, topical antiseptics and dressings, compression bandages, hyperbaric oxygen, negative pressure wound therapy, biologically engineered skin substitutes, growth factors, and electrical stimulation.

Many reports uphold the use of ES for enhancing the healing of chronic wounds.[68,120-151] Numerous random-

ized controlled clinical trials have demonstrated that ES combined with standard wound care improves the healing rate of chronic pressure ulcers more than standard wound care alone.* Twenty-two other studies have investigated the effects of ES on lower extremity wounds. Of these studies, 6 included ulcers due to mixed etiologies.[68,124,128,136-138] The remaining studies included ulcers caused by venous insufficiency,[106,139-141] wounds related to nonischemic diabetic neuropathy,[142-145] and diabetic ischemic wounds.[111,133,146,147,149-152]

ES Studies on Venous Insufficiency Wounds

In reviewing the clinical studies related to treatment of recalcitrant human wounds with ES, one finds that many of them are designed to compare healing of wounds treated with active ES plus standard care, against healing of control wounds treated with placebo ES plus standard care. In other studies, patients whose wounds were treated with ES served as their own historical controls. Regardless of study design, most treatment protocols applied ES to wounds only for 30 to 60 minutes, 5 to 7 days per week. During the remaining 23 hours of each protocol day, investigators were ethically bound to provide local wound treatment consisting of standard wound care alone.

The clinical research support for the use of ES in augmenting the rate of healing of venous ulcers is limited. Assimacopoulos[139] was the first to report using ES (microampere DC) on venous leg ulcers of 3 individuals whose wounds had not responded to previous treatments. He suggested that ES might be beneficial in managing these wounds. In a study of wounds having mixed etiologies, Wolcott et al[68] also used microampere DC to treat venous leg ulcers on 15 patients. They reported that after 6 weeks of daily ES treatment that totaled 6 hours, the mean healing rate per week was 14.4%, which resulted in a mean volume reduction of 85%. As previously mentioned, Junger et al[106] investigated the effect of ES on wound healing and angiogenesis. They treated 15 venous leg ulcers that had failed to show significant evidence of healing with standard compression therapy over a mean period of 79 months. After a mean of 38 days of wound treatment with daily ES for 30 minutes, the mean ulcer area decreased 63% ($P < .01$) from 16 cm^2 to 6 cm^2. Katelaris et al[140] reported negative outcomes on healing of venous leg ulcers assumably because they treated the wounds with a combination of ES and povidone iodine, which is known to have cytotoxic effects. Franek et al[141] enrolled 79 patients into a study that compared the effects of HVPC, topically applied medications, and the Unna boot on the healing of chronic venous leg ulcers. In addition to being treated with one of these interventions, wounds of all patients were managed with dressings and compression bandaging. They randomized 65 patients to have their ulcers treated either with HVPC (n = 33) or topical medications (n = 32). A subset of 14 patients who served as controls had their ulcers treated with the Unna boot. At the outset of the study, all groups were identical with respect to patient and wound characteristics. HVPC was delivered directly to wounds through saline moist gauze for 50 minutes 6 days per week for an average of 7 weeks. Initial polarity of the treatment electrode was negative (1 to 3 weeks) to rid the wound of slough and pus, after which the polarity was switched to the anode. All groups showed a significant decrease in wound size compared to baseline measurements ($P < .001$). The rate of wound area change was greatest in the group treated with HVPC, but there were no statistically significant differences between the groups. The rate of pus clearance and the degree of granulation tissue development after 2 weeks was significantly greater for wounds treated with HVPC ($P < .003$). The authors concluded that HVPC was an efficient treatment for the enhancement of venous leg ulcer healing. In another recent study designed as a randomized, double-blind prospective clinical trial, Houghton et al[138] separated 27 subjects with 42 chronic leg ulcers (wound age longer than 3 months) into subgroups according to primary etiology of the wound (diabetic, venous insufficiency, arterial insufficiency). They then randomly assigned the patients to wound treatments with active HVPC (150 V, 100 pps, 100 μs pulse duration) or sham HVPC for 45 minutes, 3 times weekly for 4 weeks. Negative polarity of the active electrode placed on saline moist gauze over the wound was used throughout the 4-week treatment period. During the days when wounds were not treated with ES, they were treated with standard care based on wound etiology. The results for all wounds demonstrated that active HVPC applied over the 4-week period reduced the wound surface area to nearly one half of its initial size, which was more than 2 times greater than occurred in wounds treated with sham ES ($P < .05$). After the 4-week protocol, in 7 patients with bilateral venous ulcers, there was also a statistically significant difference in wound size between ulcers treated with active ES and sham ES ($P < .05$). Using the Pressure Sore Status Tool, the investigators also compared wound appearance between pretreatment, posttreatment, and a 1-month follow-up assessment. They found that active ES produced a statistically significant

References 120, 121, 125-127, 129, 130, 135.

improvement in wound appearance compared with sham-treated wounds ($P < .05$).

ES Studies on Wounds Caused by Nonischemic Diabetic Neuropathy

Electrically induced acceleration of the closure of wounds caused by nonischemic diabetic neuropathy has been demonstrated in 4 studies, including 2 randomized controlled clinical trials.[142-145] Alon et al[142] used HVPC to treat 15 neuropathic diabetic foot ulcers and reported that 12 wounds (80%) healed completely in a mean period of 2.6 months with anodal stimulation applied for 1 hour, 3 days a week. In a randomized controlled trial, Lundeberg et al[143] evaluated the effect of biphasic asymmetric PC on wound healing. Sixty-four patients with chronic diabetic neuropathic foot ulcers were randomized to receive either active ES (parameters not given) or sham control ES for 20 minutes twice a day for 12 weeks in addition to standard wound care. Polarity of the treatment electrode was changed each session. After 12 weeks, there was a statistically significant treatment effect based on the closure of 42% of wounds in the active ES group compared to 15% of the controls ($P < .05$). Baker et al[144] conducted a randomized trial involving 80 individuals with diabetes and 114 open wounds. Wounds were randomized to be treated either with symmetrical or biphasic asymmetrical PC plus standard care or with standard care alone. The authors demonstrated that both waveforms combined with standard care enhanced the wound-healing rate by nearly 60% over control wounds treated with only standard care. One other randomized, double-blind, placebo-controlled, 12-week trial investigated the effect of HVPC as an adjunct to healing diabetic foot ulcers.[145] Forty patients with diabetic foot ulcers and loss of protective sensation due to neuropathy were randomized to active HVPC and sham HVPC. At the outset of the study, there were no significant differences between active and sham ES groups in patient characteristics and clinical variables. Active (subsensory) ES was delivered to the ipsilateral lower extremity (leg segment) at 50 V, 80 pps, and pulse duration of 100 μs via a Dacron-mesh silver nylon stocking worn nightly for 8 hours. Protocol adherence was stratified into compliant patients who used the ES device for 20 hours or more a week on average and noncompliant patients who used the ES device less than 20 hours per week. Following 12 weeks of the research protocol, 65% of the wounds in the active ES group closed compared with 35% of wounds in the sham ES group ($P = .058$). Regarding compliance, significant differences were found among patients in the active ES group (71% closed) compared with 50% closed among noncompliant patients in the same group. In the sham ES group, 39% of compliant patients' wounds closed compared with 29% of noncompliant patients' wounds ($P = .038$). The authors concluded that ES enhances the healing of diabetic foot ulcers when used adjunctively with weight off-loading and local wound care.

ES Studies on Lower Extremity Ischemic Wounds

Several clinical trials designed to evaluate the effects of ES on lower extremity wounds caused by ischemia have reported positive outcomes. In a case report, Thurman and Christian[146] attributed healing of a purulent septic abscess on the foot of a 43-year-old woman with juvenile-onset diabetes mellitus to HVPC treatment. Cutaneous electrodes were applied adjacent to the abscess, and mild pulsating muscular contractions were elicited twice daily. The authors reported that blood flow increased, the abscess resolved, and the wound went on to heal, averting possible amputation of the distal extremity. In 1995, Debreceni et al[147] reported results of treating 24 individuals (10 diabetic) with chronic ischemia of the lower extremities with biphasic symmetrical PC. Of the 24 subjects, 12 had ischemic ulcerations on the distal legs and feet, and 6 either had beginning or advanced distal gangrene. All subjects had been treated with antiplatelet drugs, pentoxifylline, and vasodilating drugs over a period of 5 to 6 years. Under this treatment regimen, all of the subjects were experiencing progressive deterioration of their ischemic lower limbs. Although subjects were continued on their drug therapies, ES was administered in addition for 20 minutes daily with 1 electrode applied over the peroneal nerve near the head of the fibula with the other electrode placed between the first and second metatarsals of the involved extremity. The pulse frequency was set at 1 to 2 pps at an amplitude of 15 to 30 mA, which elicited rhythmical, painless muscle contractions between the electrodes. Over a period of 1 year, 20 of 24 subjects reportedly made significant progress, including the disappearance of ischemic pain, halting of gangrenous progression, and complete healing of ulcerations. In addition, following ES, pain-free walking distance increased from a mean of 87.5 m to 421.25 m ($P < .001$), and oxygen saturation measured on the toes increased from 73.46% to 95.46% ($P < .05$). The latter effect may have occurred as a result of improved cutaneous microcirculation during ES. In support of this response, Kaada et al[148] have shown that the concentration of vasodilation inducing vasoactive intestinal polypeptides increases in the plasma during ES.

As mentioned earlier in the section on enhancement of tissue oxygenation, Peters et al,[111] using transcutaneous oximetry and laser Doppler flowmetry, demonstrated a significant increase in perfusion in the lower extremities of diabetic patients with impaired vascular function who had 1 foot and distal leg treated with subsensory ES delivered through a silver mesh sock for four 60-minute periods. In patients with transcutaneous oximetry values <40 mm Hg, a significant but transient increase in tissue oxygenation occurred during the first 5 minutes of ES. These results suggest that ES increases cutaneous oxygen saturation subsequent to increasing local perfusion in diabetic subjects.

Since 2002, Goldman et al[149-152] have published 4 interesting articles related to salvaging the ischemic lower extremities with HVPC stimulation. In a case series, they instructed 6 adult patients to use HVPC at home to treat their critically ischemic (defined as $TcPO_2$ < 10 mm Hg), nonsurgical, malleolar, or inframalleolar wounds, which had a mean $TcPO_2$ of 2 ± 2 mm Hg at the wound edge. After ES began, periwound $TcPO_2$ increased exponentially until it exceeded 20 mm Hg about 40 days into the protocol and the rate of healing turned positive. The researchers suggested that cutaneous microcirculation improved secondary to a statistically significant increase in mean $TcPO_2$ of periwound skin to 33 ± 18 mm Hg. Wounds of 4 patients healed after 207 days of ES, and 2 patients underwent amputation.[149] In another report, they used HVPC to reverse a rapidly expanding ischemic, cutaneous gangrene on the left posterior calf of a patient with end-stage renal disease.[150] Hypoxia was verified by $TcPO_2 \leq$ 20 mm Hg with calf periwound $TcPO_2$ of 20 mm Hg and 12 mm Hg at the heel. To the patient's calf wound, necrotic heel, and fourth toe, home caregivers applied cathodal HVPC 1 hour daily, 5 to 7 days per week at 150 V and 100 pps. While not receiving ES, the lesions were treated with standard care for ischemic wounds. Both the left calf and heel wounds closed 250 and 234 days, respectively, after beginning ES therapy. During the extended treatment period, the transition to positive healing rate occurred coincident with an increase in periwound $TcPO_2$ from 20 to 50 mm Hg (calf) and 15 to 50 mm Hg (heel). In a 5-year retrospective observational study, Goldman et al[151] continued their investigations to determine if HVPC augments ischemic wound healing and increases periwound perfusion. The study was conducted on successive patients with ischemic lower extremity wounds who were poor candidates for revascularization. One group of 11 patients had HVPC applied directly to their wounds at >100 V, 100 pps, 1 hour daily in addition to 23 hours of standard wound care. A second group of 11 patients with ischemic wounds had their wounds treated with standard care alone. Outcome measures included the planimetry of wound areas, digital wound appearance, and $TcPO_2$ monitoring of microcirculation. The group treated with HVPC plus standard care had smaller wound areas from weeks 20 through 52 after the start of treatment compared with the group that received standard care alone ($P < .05$). One year after initiating treatment, 90% of HVPC-treated wounds were healed, compared with 29% of the wounds that were treated with standard care alone. For the HVPC group, maximum $TcPO_2$ improved from 6 ± 8 mm Hg at baseline to 26 ± 20 mm Hg ($P < .05$). These results suggest that HVPC facilitates microcirculation and the healing of ischemic wounds.

Continuing their clinical research to determine if HVPC augments ischemic wound healing and increases periwound microcirculation, Goldman et al[152] conducted a prospective, randomized, single-blinded, sham-controlled clinical pilot study on a homogenous subset of patients with infrapopliteal ischemic wounds. For the purpose of their study, they defined ischemia as periwound $TcPO_2$ <20 mm Hg, which they considered the threshold below which healing is not favorable. Eight patients were enrolled with ischemic wounds at or below the knee, periwound $TcPO_2$ <20 mm Hg, with wounds open for at least 4 weeks before enrollment in the study and arteriosclerotic disease confirmed by magnetic resonance angiography, pulse volume recording, or angiogram. Patients were randomized to have their wounds treated with active or sham HVPC. Active HVPC or sham HVPC was applied at home 1 hour per day, 7 days per week, for 14 weeks. Wounds were monitored at regular intervals for wound area, wound appearance, and microcirculation, which was measured by $TcPO_2$ and laser Doppler flow. After 4 weeks, wounds treated with sham HVPC increased in area by 50%, which was expected since ischemic wounds tend to increase in size. During the same period, wounds treated with active HVPC underwent a significant decrease in size ($P < .05$). After week 4, wounds in both groups demonstrated positive healing rates, but the healing rate in the control group continued to lag behind the healing rate for the active HVPC-treated wounds during the remainder of the 14-week period.

Based on the present evidence from clinical trials, ES used adjunctively with standard care is reported to enhance the healing of lower extremity wounds of venous, arterial, and neuropathic etiologies. Table 3 summarizes several of the clinical studies in which ES has been used to assess its effect on healing chronic wounds of the lower extremity.

Table 3. Studies of Lower Extremity Wounds Treated With Electrical Stimulation

Reference	Dressing, Current Dosage, and Polarity	Study Design	Wound Diagnosis	Current Type and Patient Study Group(s)	Number of Patients or Wounds	% Patients or Wounds Healed/Time	Other Results Provided by Authors
Wolcott et al[68]	200-800 µA 6 h/d for 0.8-15.4 wk; switched polarity cathode anode cathode	Case series	Mixed	DC	75	40/9.6 wk	Healing rate/wk: 53 paraplegics = 9.3%, 5 venous disease = 14.4%, 15 arterial disease = 14.0%
		Embedded RCT	Mixed		Bilateral wounds		
				DC	8	95/15.4 wk	Healing rate/wk: 13.4%
				Control	8	32/15.4 wk	Healing rate/wk: 5.0%
Junger et al[106]	630 µA at 128 pps or 315 µA at 64 pps	Case series	Venous	PC with some net DC	15	After 38 d, mean ulcer area decreased 63% ($P < .01$) from 16 cm^2 to 6 cm^2	Capillary density increased from 8.05 capillaries/mm^2 to 11.55 capillaries/mm^2 ($P < .039$)
Peters et al[111]	Dacron silver mesh sock	RCT	Diabetic PVD and non-PVD	HVPC subsensory	11 active ES	Not studied	Subjects with PVD had significant increase in perfusion and $TcPO_2$ after 5 min of ES
					8 sham ES	Non-PVD	
Houghton et al[138]	2 groups: (1) standard wound care based on wound etiology plus active HVPC at 150 V, 100 pps, 45 min, 3 times/wk for 4 wk; cathode on wound (2) sham HVPC plus standard wound therapy based on wound etiology	Double-blind RCT	Diabetic venous arterial ulcers of the lower extremity	HVPC	21	0/4 wk	% decrease in wound size: 44.3%/4 wk
				Sham HVPC	22	0/4 wk	16.0%/4 wk

(continued)

Table 3 (continued)

Reference	Dressing, Current Dosage, and Polarity	Study Design	Wound Diagnosis	Current Type and Patient Study Group(s)	Number of Patients or Wounds	% Patients or Wounds Healed/Time	Other Results Provided by Authors
Assimacopoulos[139]	50-100 μA/cathode	Case series	Venous	DC	8	100/30 d	Biopsy 1 yr after healed: dense hyalinized collagen
Franek et al[141]	3 groups: (1) moist wound therapy plus 100 V, 100 monophasic pps, 50 min/d × 6 d × 7 wk; cathode 3 wk, anode 4 wk (2) topical medications 6 wk (3) Unna boot 5.5 wk	RCT	Venous leg ulcers	HVPC			After 2 wk, granulation tissue development was significantly greater for group 1 than for groups 2 and 3
				Group 1	33	41/7 wk	
				Group 2	32	65/6 wk	
				Group 3	14	76/5.5 wk	
Alon et al[142]	Anodal stimulation applied for 1 h, 3 d/wk	Historical controls	Diabetic neuropathic ulcers	HVPC	12	80%/2.6 mo	None reported
Lundeberg et al[143]	Biphasic pulses at 80 pps, 1-ms PD; current amplitude to tingling paresthesia for 20 min twice a day	Double-blind RCT	Diabetic ulcers	PC	32	42/12 wk	% ulcers healed: 2 wk = 0% ES vs 4% sham; 4 wk = 12% ES vs 7% sham; 8 wk = 25% ES vs 11% sham; 12 wk = 42% ES vs 15% sham
	Controls: moist wound therapy			Control (sham)	32	15/12 wk	

Baker et al[144]	Moist wound therapy (assumed) plus: (1) asymmetric biphasic PC, submotor paresthesia, 50 pps, 100 µs PD (2) symmetric biphasic PC, submotor paresthesia, 50 pps, 300 µs PD (3) microcurrent, 1 mA, 1 pps, 10 µs PD	RCT	Diabetic ulcers	PC		Not reported	Healing rate/wk: 11 ulcers were treated under both control and ES protocols; 9.7% as controls, 43.3% with ES
				Group 1	29		
				Group 2	24		
				Group 3	20		
				Control	19		
Peters et al[145]	2 groups: (1) off-loading plus active cathodal HVPC at 50 V and 80 pps for 10 min followed by 8 min of 8 pps repeated for 8 h at night for 12 wk via Dacron mesh sock (2) sham HVPC plus off-loading	Double-blind RCT	Diabetic foot ulcers	HVPC	18	65/12 wk	Stratification by compliance: significantly more compliant patients in both groups healed than noncompliant patients
				Sham HVPC	17	35/12 wk	
Debreceni et al[147]	ES plus vascular drugs, ES 20 min/d; 1-2 pps at 15-30 mA for 1 y	Historic controls	24 ischemic LEs; 12 ischemic ulcers; 6 LE gangrene	PC biphasic symmetrical	24 with ischemic LEs; 10 were diabetic	After 1 y, 20/24 reported decreased ischemic pain; halted progress of gangrene or healed ulcers	For 5-6 y prior, all subjects treated with pentoxifylline and vasodilator or antiplatelet drugs, none of which had positive effects

(continued)

Table 3 (continued)

Reference	Dressing, Current Dosage, and Polarity	Study Design	Wound Diagnosis	Current Type and Patient Study Group(s)	Number of Patients or Wounds	% Patients or Wounds Healed/Time	Other Results Provided by Authors
Goldman et al[149]	Standard wound care plus 80-330 V to sensory threshold, 80 to 100 pps, 1 h/d, 7 d/wk for 1-9 mo	Case series; historical controls	Diabetic ischemic foot ulcers	HVPC	6	4/7.2 mo, 2 had amputation	Mean $TcPO_2$ before HVPC was 2 mm Hg; after HVPC began, it was 33 mm Hg indicating increased perfusion
Goldman et al[150]	Standard wound care plus 150 V, 100 pps cathodal ES 1 h × 4 or more d/wk at home for 250 d	Single case	Diabetic, expanding right calf gangrene; $TcPO_2 < 20$ mm Hg	HVPC	1	Wound healed after 250 d of ES at home	$TcPO_2$ increased from 20 mm Hg to 50 mm Hg indicating increase in perfusion
Goldman et al[151]	Standard wound care plus ES at > 100 V, 100 pps, 1 h/d or standard wound care alone	5-yr retrospective, observational study	Inframalleolar ischemic wounds	HVPC	11 ES	90/12 mo	For ES group, $TcPO_2$ improved from 6-26 mm Hg ($P < .05$)
					11 controls	29/12 mo	
Goldman et al[152]	Standard wound care plus active or sham ES at home 1 h/d for 14 wk; active cathodal ES at 100 pps, sensory threshold or 360 V	14-wk prospective pilot	Infrapopliteal ischemic wounds	HVPC	4 ES, 4 standard care	ES wound decreased in size, and microcirculation improved compared to wound treated with standard care	Measured by laser Doppler, $TcPO_2$ improved significantly at weeks 8 ($P < .01$) and 12 ($P < .05$)

DC = direct current; RCT = randomized controlled trial; PC = monophasic or biphasic pulsed current; HVPC = high-voltage pulsed current (twin-peaked monophasic PC); ES = electrical stimulation; PVD = peripheral vascular disease; PD = pulse duration; LE = lower extremity.

APPENDIX
Glossary: Electrical Stimulation Terminology and Types of Therapeutic Currents

Reviewers and readers of the wound-healing literature related to electrical stimulation (ES) often state that they are perplexed by the different types of current and stimulation parameters described in publications, which makes it difficult to evaluate studies and draw conclusions related to efficacy. A primary reason for this confusion may stem from a lack of standardization of ES terminology. To ease the reader's bewilderment, terminology related to ES and the types and characteristics of therapeutic electrical currents are presented here as adapted from a monograph published by the Section on Clinical Electrophysiology of the American Physical Therapy Association.[153]

Electrical Terms and Definitions

Charge: Electrical charge is a fundamental property of matter. Matter either has no net charge (electrically neutral) or is negatively or positively charged. The fundamental particle of negative charge is the electron (e–). Charge is measured in specific quantities of electrons called coulombs (Q). The quantity of charge delivered to tissues by ES is measured in microcoulombs (μQ).

Charge Density: This is a measure of the electrical charge present on the surface of a treatment electrode and is inversely related to electrode size. For wound treatment, since the amount of charge on the electrode surface area is relatively small, charge density is likely to be expressed as $\mu Q/cm^2$.

Electrodes: Electrodes are the conductive elements of an electrical circuit that are applied to the body for the purpose of transferring electrical charge into the tissues. For delivery of current into tissues, a minimum of 2 electrodes is required. The negative electrode or cathode (–) attracts positive ions (cations), while the positive electrode or anode (+) attracts negative ions (anions) in the tissues. Electrodes consist of carbonized silicon, conductive polymers, or aluminum foil placed in contact with saline moist gauze.

Polarity: Polarity is the property of having 2 oppositely charged poles or electrodes. At any given time while current is flowing, one electrode is relatively more positive while the other is relatively more negative. When the cathode and anode have sufficient charge, they may cause undesirable electrochemical burning of tissues due to pH changes of NaOH and HCl, respectively.

Electrical Circuit: An electrical circuit used for wound-healing treatment consists of at least 2 lead wires, one of which is connected to the cathode terminal and the other connected to the anode terminal of an ES device. The patient end of each lead is connected to an electrode that is applied to the patient.

Voltage: The electrical force capable of moving electrons or ions between 2 points of a conductor is the voltage or potential difference between the 2 points. The voltage between the 2 points (eg, 2 electrodes on the body) is created by the separation of charges between them, such that one electrode has an excess of negatively charged electrons or ions compared with the other. The 2 electrodes are polarized with respect to one another, one being negative and the other positive.

Current: The rate of flow of charged particles (electrons or ions) through a conductive medium past a specific point in a specific direction constitutes electrical current. Current flow in a metal wire occurs as a result of the flow of electrons, whereas current flow in tissues is carried by ions (eg, Na^+, K^+, Cl^-). The unit of measure of current is the ampere (A), which represents the movement of 1 coulomb of charge per second. Exogenous currents used for wound treatment that are intended to mimic bioelectric tissue currents may be delivered to the tissues either in the milliampere (mA) or microampere (μA) range of current amplitude. When a unidirectional current flows in the circuit, positive-charge carriers in the tissues (Na^+, K^+, or H^+) and cells (fibroblast and activated neutrophil) migrate toward the cathode while negative charge carriers (Cl^-, HCO_3^-, P^-) and cells (epidermal, macrophage, neutrophil) migrate toward the anode.

Resistance: As electrons and ions (charged particles) flow in metallic and biological conductors, respectively, their movement is impeded by collisions with other charged carriers and by the inherent properties of the substance. Thus, resistance is the opposition to the flow of current. A conductor's resistance is 1 Ω if a potential difference of 1 V causes 1 A to flow through it. This is one form of the Ohm Law, which states R = V/I.

Waveform: A waveform is a visual representation of voltage or current on an amplitude-time plot. A waveform represents a picture of an electrical event that begins when the current or voltage leaves the zero (isoelectric) baseline in one direction then, after a finite time, either returns to and stops at the same baseline (monophasic waveform) or crosses the baseline in the opposite direction and ends when the voltage or current returns again to the baseline (biphasic waveform).

Phase: This term describes an electrical event that begins when the current (or voltage) leaves the isoelectric line and ends when it returns to the baseline.

Phase/Pulse Duration: Phase duration is the time in microseconds or milliseconds between the beginning and the end of 1 phase of a pulse. Pulse duration is the time in microseconds or milliseconds between the beginning of the first phase and the end of the second phase that may include the interphase interval within 1 pulse.

Pulse Frequency: This term describes the number of pulses per second (pps) for a pulsed current or the number of cycles per second for alternating current.

Types and Characteristics of Therapeutic Currents

Although there are 2 basic types of currents, which include direct current (DC) and alternating current (AC), a third type of current (pulsed current [PC]) has been adopted by the Section on Clinical Electrophysiology (SCE) of the American

Physical Therapy Association[153] as an additional therapeutic current. The reason the SCE adopted PC is to provide clearer descriptions of pulsed waveforms that are delivered by the majority of electrotherapeutic devices used by clinicians. The adoption of PC is not meant to imply that there is an additional type of basic current.

Direct Current: DC (sometimes referred to as *galvanic current*) is the continuous, unidirectional flow of charged particles for 1 second or longer. In the tissues, the direction of DC flow is determined by the polarity selected, with negatively charged ions moving toward the anode and positively charged ions moving toward the cathode.[153] Once selected, electrode polarity remains constant until it is changed manually on the ES device. Continuous DC has no pulses and therefore no waveform. When DC is delivered to a solution or to tissues containing electrolytes, the charged ions (Na^+, K^+, Cl^-) are attracted to the cathode and anode, respectively. At the cathode, Na^+ reacts with H_2O to form NaOH and H_2, while at the anode, Cl^- reacts with H_2O to form HCl and O_2. The caustic products of NaOH and HCl that form at the tissue surface of the cathode and anode, respectively, can cause electrochemical burning seen as blistering. When DC is used to treat wounds, care must be taken to avoid undesirable responses by delivering less than 1.0 mA to the electrodes. Current amplitudes under 1.0 mA are in the microampere (μA) range.

Alternating Current: AC is the continuous bidirectional flow of charged particles in which a change in direction of flow occurs at least once every second. An AC waveform is represented by 1 cycle, which describes an electrical event that begins when the current or voltage leaves the zero (isoelectric) baseline in one direction then crosses the same baseline in the opposite direction to end when the current or voltage returns again to the baseline. When available from an ES device, the most common AC waveform is the sine wave, in which both phases of the cycle are charge balanced so there is no electrode polarity. Unlike PC, AC has no off time interval between phases of adjacent cycles.

Some authors have erroneously indicated that current delivered by transcutaneous electrical nerve stimulation (TENS) devices represents AC.[122,123,132,143] In reality, ES modalities classified as TENS devices by the US Food and Drug Administration deliver trains of isolated electrical events (pulses) that are either monophasic or biphasic PC, not AC.[153] Since only DC and PC have been used in clinical wound-healing studies and because AC is not used clinically for wound healing, it is not discussed in this review.

Pulsed Current: PC is the brief unidirectional or bidirectional flow of charged particles (electrons or ions) in which each pulse is separated by a longer off period of no current flow. Thus, each pulse is an isolated electrical event separated from each of a series or train of pulses by a finite off time. PC is described by its waveform, amplitude, duration, and frequency. PC can have 2 waveforms: monophasic or biphasic. A monophasic pulse represents a very brief movement of electrons or ions away from the isoelectric line, returning to the zero line after a finite period of time (less than 1.0 second). When the duration of a monophasic pulse is less than 1.0 second, the current is not DC because it does not cause electrochemical changes in the tissues. Monophasic PC waveforms that have been described in the clinical wound-healing literature include the rectangular waveform[106,124-126] and the twin-peaked waveform of high voltage PC.[†] High-voltage PC (HVPC) typically has very short-duration (2-20 μs) twin triangular pulses that have single-phase charges on the order of 1.6 μQ.[153] Because HVPC is unidirectional, one may incorrectly assume that this type of current is galvanic or DC that causes caustic skin and wound tissue damage secondary to pH changes. However, investigators have demonstrated that pH changes do not occur in human skin following 30 minutes of HVPC stimulation.[82]

The biphasic PC waveform also represents a very brief duration of movement of electrons or ions. However, in this case, the pulse is bidirectional and consists of 2 phases. One phase leaves the isoelectric line and after a brief finite time returns to baseline. Then, without delay (or in some waveforms, a few microseconds delay), the second phase leaves the isoelectric line in the opposite direction and after a brief time returns to baseline. The biphasic waveform may be asymmetric or symmetric about the isoelectric line. In the symmetric biphasic waveform, the phase charges of each phase are electrically equal or balanced; therefore, there is no polarity. Asymmetric biphasic waveforms may be electrically balanced or unbalanced. Biphasic symmetrical (charge balanced)[130,144] and asymmetrical (charge balanced)[139] waveforms have been described in recent clinical wound-healing literature.

[†]*References 124, 125, 127-129, 131, 133, 138, 141, 142, 149-152.*

REFERENCES

1. Robertson W. Digby's receipts. Ann Med Hist 1925;7(3):216.
2. Kanof N. Gold leaf in the treatment of cutaneous ulcers. J Invest Dermatol 1964;43:441-4.
3. Wolf M, Wheeler P, Wolcott L. Gold-leaf treatment of ischemic skin ulcers. JAMA 1966;196:105-8.
4. Smith K, Oden P, Blaulock W. A comparison of gold leaf and other occlusive therapy. Arch Dermatol 1967;96:703-5.
5. Chick N. Treatment of ischemic and stasis ulcers with gold leaf and polyethylene film. J Am Geriatr Soc 1969;17:605-8.
6. Risbrook A, Goodfriend S, Reiter J. Gold leaf in the treatment of leg ulcers. J Am Geriatr Soc 1973;21:325-9.
7. Harris D, Keefe R. A histological study of gold leaf treated experimental wounds. J Invest Dermatol 1969;52:487-91.
8. Lente F. Cases of united fractures treated by electricity. N Y State J Med 1850;5:5117-8.
9. Fukada E, Yasuda I. On the piezoelectric effect in bone. Nippon Seirigaku Zasshi 1957;12:1158-62.

10. Becker R, Spadero J, Marino A. Clinical experiences with low intensity direct current stimulation of bone growth. Clin Orthop 1975;124:75-83.
11. Bassett C. The development and application of pulsed electromagnetic fields (PEMFS) for un-united fractures and arthrodeses. Orthop Clin North Am 1984;15(1):61-87.
12. Brighton C, Pollak S. Treatment of recalcitrant nonunion of the tibia with a capacitively coupled electric field: a preliminary report. J Bone Joint Surg 1985;67(4):577-85.
13. Mammi G, Rocchi R, Cadossi R, et al. Effect of PEMF on the healing of human tibial osteotomies: a double-blind trial. Clin Orthop 1993;288:246-53.
14. Bodamyali T, Bhatt B, Hughes J, et al. Pulsed electromagnetic fields simultaneously induce osteogenesis and upregulate transcription of bone morphogenic proteins 2 and 4 in rat osteoblasts in vitro. Biochem Biophys Res Commun 1998;250:458-61.
15. Centers for Medicare and Medicaid Services. Electrostimulation for wounds: decision memorandum (#CAG-00068N). Centers for Medicare and Medicaid Services, 2002. Available from: URL: http://cms.hhs.gov/coverage/8b3-ii3.asp#P256-48704.
16. Borgens R, Vanable J Jr, Jaffee L. Bioelectricity and regeneration: I. Initiation of frog limb regeneration by minute currents. J Exp Zool 1977;200:402-17.
17. Illingsworth C, Barker A. Measurement of electrical currents emerging during the regeneration of amputated finger tips in children. Clin Phys Physiol Meas 1980;1:87-89.
18. Barker A, Jaffee L, Vanable J Jr. The glabrous epidermis of cavies contains a powerful battery. Am J Physiol 1982;242:R258-366.
19. Foulds L, Barker A. Human skin battery potentials and their possible role in wound healing. Br J Dermatol 1983;109:515-22.
20. Vanable J Jr. Integumentary potentials and wound healing. In: Borgans R, et al, editors. Electric fields in vertebrate repair. New York: Alan R. Liss; 1989. p. 183.
21. Cunliffe-Barnes T. Healing rate of human skin determined by measurements of electrical potential of experimental abrasions: study of treatment with petrolatum and with petrolatum containing yeast and liver abstracts. Am J Surg 1945;69:82-7.
22. Wilcott R. Adaptive value of aromal sweating and epidermal mechanism relating to skin potential and skin resistance. Psychophysiology 1966;2:249-54.
23. Eltinge E, Cragoe E Jr, Vanable J Jr. Effects of amiloride analogues on adult *Notophthalmus viridescens* limb stump currents. Comp Biochem Physiol 1986;89A:39-44.
24. Matteucci C. Lectures on the physical phenomena of living beings. In: Pereira J, editor. Carlo Matteucci, 1811-1868. London: Longman, Brown, Green and Longmans; 1847. p. 435.
25. DuBois-Reymond E. Vorlaufiger abrifs einer untersuchung uber den sogenanten froschstrom und die electromotorischen fische. Ann Phys U Chem 1843;58:1-4.
26. McGinnis M, Vanable J Jr. Voltage gradients in newt limb stumps. Prog Clin Biol Res 1986;210:231-8.
27. Stump R, Robinson K. Ionic current in *Xenopus* embryos during neuroulation and wound healing. Prog Clin Biol Res 1986;210:223-30.
28. Jaffe L, Vanable J. Electrical fields and wound healing. Clin Dermatol 1984;2(3):34-44.
29. Cheng K, Tarjan P, Oliveira-Gandia M, et al. An occlusive dressing can sustain natural electrical potential of wounds. J Invest Dermatol 1995;104(4):662-5.
30. Alvarez O, Mertz P, Eaglstein W. The effect of occlusive dressings on collagen synthesis and re-epithelialization in superficial wounds. J Surg Res 1983;35:142-8.
31. Winter G. Epidermal regeneration studies in the domestic pig. In: Maibach H, Rovee D, editors. Epidermal wound healing. Chicago: Year Book Medical Publishers; 1972. p. 71-112.
32. Bassett C, Herrmann I. The effect of electrostatic fields on macromolecular synthesis by fibroblasts in vitro [Abstract]. J Cell Biol 1968;39:9.
33. Bourguignon G, Bourguignon L. Electric stimulation of protein and DNA synthesis in human fibroblasts. FASEB J 1987;1(5):398-402.
34. Bourguignon G, Bergouignan M, Khorshed A, et al. Effect of high voltage pulsed galvanic stimulation on human fibroblasts in cell culture. J Cell Biol 1986;103:344a.
35. Bourguignon G, Wenche J, Bourguignon L. Electric stimulation of human fibroblasts causes an increase in Ca2+ influx and the exposure of additional insulin receptors. J Cell Physiol 1989;140(2):397-85.
36. Falanga V, Bourguignon G, Bourguignon L. Electrical stimulation increases the expression of fibroblast receptors for transforming growth factor-beta. J Invest Dermatol 1987;88:488-92.
37. Cheng N, Van Hoof H, Bockx E, et al. The effects of electric currents on ATP generation, protein synthesis, and membrane transport in rat skin. Clin Orthop 1982;171:264-72.
38. Cheng K, Goldman R. Electric fields and proliferation in a dermal wound model: cell cycle kinetics. Bioelectromagnetics 1998;19:68-74.
39. Orida N, Feldman J. Directional protrusive pseudopodial activity and motility in macrophages induced by extra-cellular electric fields. Cell Motil 1982;2:243-55.
40. Monguio J. Uber die polare wirkung des galvanischen stromes auf leukozyten. Z Biol 1933;93:553-9.
41. Fukushima K, Senda N, Inui H, et al. Studies of galvanotaxis of leukocytes. Med J Osaka Univ 1953;4(2-3):195-208.
42. Dineur E. Note sur la sensibilities des leukocytes a l'electricite. Bulletin Seances Soc Belge Microscopic (Bruxelles) 1891;18:113-8.
43. Canaday D, Lee R. Scientific basis for clinical application of electric fields in soft tissue repair. In: Brighton C, Pollack S, editors. Electromagnetics in biology and medicine. San Francisco: San Francisco Press; 1991.
44. Erickson C, Nuccitelli R. Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. J Cell Biol 1984;98:296-307.
45. Yang W, Onuma E, Hui S. Response of C3H/10T1/2 fibroblasts to an external steady electric field stimulation. Exp Cell Res 1984;155:92-7.
46. Nishimura K, Isseroff R, Nuccitelli R. Human keratinocytes migrate to the negative pole in direct current electric fields comparable to those measured in mammalian wounds. J Cell Sci 1996;109:199-207.
47. Sheridan D, Isseroff R, Nuccitelli R. Imposition of a physiologic DC electric field alters the migratory response of human keratinocytes on extracellular matrix molecules. J Invest Dermatol 1996;106(4):642-6.
48. Stromberg B. Effects of electrical currents on wound contraction. Ann Plast Surg 1988;21(2):121-3.
49. Cooper M, Schliwa M. Electrical and ionic controls of tissue cell locomotion in DC electrical fields. J Cell Physiol 1985;103:363.
50. Eberhardt A, Szczypiorski P, Korytowski G. Effect of transcutaneous electrostimulation on the cell composition of skin exudate. ACTA Physiol Pol 1986;37(1):41-6.
51. Mertz P, Davis S, Cazzaniga A, et al. Electrical stimulation: acceleration of soft tissue repair by varying the polarity. Wounds 1993;5(3):153-9.
52. Fang K, Ionides E, Oster G, et al. Epidermal growth factor receptor relocalization and kinase activity are necessary for directional migration of keratinocytes in DC electric fields. J Cell Sci 1999;112:1967-78.
53. Bedlack R Jr, Wei M, Loew L. Localized membrane depolarization and localized calcium influx during electric field-guided neurite growth. Neuron 1992;9(3):393-403.

54. Soong H, Parkinson W, Sulik G, et al. Effects of electric fields on cytoskeleton of corneal stromal fibroblasts. Curr Eye Res 1990;9(9):893-901.
55. Onuma E, Hui S. Electric field-directed cell shape changes, displacement, and cytoskeletal reorganization are calcium dependent. J Cell Biol 1988;106(6):2067-5.
56. Onuma E, Hui S. The effects of calcium on electric field-induced cell shape changes and preferential orientation. Prog Clin Biol Res 1986;210:319-27.
57. Onuma E, Hui S. A calcium requirement for electric field induced cell shape changes and preferential orientation. Cell Calcium 1985;6(3):281-92.
58. Luther P, Peng H, Lin J. Changes in cell shape and actin distribution induced by constant electric fields. Nature 1983;303(5912):61-4.
59. Baker L, Peng H. Tyrosine phosphorylation and acetylcholine receptor cluster formation in cultured *Xenopus* muscle cells. J Cell Biol 1993;120(1):185-95.
60. Peng H, Baker L, Dai Z. A role of tyrosine phosphorylation in the formation of receptor clusters induced by electric fields in cultured *Xenopus* muscle cells. J Cell Biol 1993;120(1):197-204.
61. Falanga V. Wound bed preparation: science applied to practice. In: European Wound Management Association (EWMA), editor. Position document: wound bed preparation in practice. London: MEP Ltd; 2004. p. 2-5.
62. Schultz G, Sibbald G, Falanga V, et al. Wound bed preparation: a systemic approach to wound management. Wound Repair Regen 2003;11(2 Suppl):S1-28.
63. Drosou A, Falabella A, Kirsner R. Antiseptics on wounds: an area of controversy. Wounds 2003;15(5):149-66.
64. Degreef H. How to heal a wound fast. Dermatol Clin 1998;16(2):365-75.
65. Apelqvist J, Ragnarson T. Cavity foot wounds in diabetic patients: a comparative study of cadexomer iodine ointment and standard treatment. An economic analysis alongside a clinical trial. Acta Derm Venereol 1996;76:231-5.
66. Ug A, Ceylan O. Occurrence of resistance to antibiotics, metals and plasmids in clinical strains of *Staphyloccus* spp. Arch Med Res 2003;34(2):130-6.
67. Rowley B. Electrical current effects on *E. coli* growth rates. Proc Soc Exp Biol Med 1972;139:929-34.
68. Wolcott L, Wheeler P, Hardwicke H, et al. Accelerated healing of skin ulcers by electrotherapy: preliminary clinical results. South Med J 1969;62:795-801.
69. Rowley B, McKenna J, Chase G, et al. The influence of electrical current on an infecting microorganism in wounds. Ann N Y Acad Sci 1974;238:543-51.
70. Barranco S, Spadero J, Berger T, et al. In vitro effect of weak direct current on *Staphylococcus aureus*. Clin Orthop 1974;100:250-5.
71. Deitch E, Marino A, Malakanok V, et al. Electrical augmentation of the antibacterial activity of silver nylon. Proceedings of the 3rd Annual BRAGS; 1983 Oct 2-5; San Francisco.
72. Deitch E, Marino A, Gillespie T, et al. Silver nylon: a new antimicrobial agent. Antimicrobial Agents Chemother 1983;23:356-9.
73. Marino A, Deitch E, Albright J. Electric silver antisepsis. IEEE Trans Biomed Eng 1985;32(5):336-7.
74. Colmano G, Edwards S, Barranco S. Activation of antibacterial silver coatings on surgical implants by direct current: preliminary studies in rabbits. Am J Vet Res 1980;41(6):964-6.
75. Thibodeau E, Handelman S, Marquis R. Inhibition and killing of oral bacteria by silver ions generated with low intensity direct current. J Dent Res 1978;57:922-6.
76. Alvarez O, Mertz P, Smerbeck R, et al. The healing of superficial skin wounds is stimulated by external electrical current. J Invest Dermatol 1983;81(2):144-8.
77. Falcone A, Spadero J. Inhibitory effects of electrically activated silver material on cutaneous wound bacteria. Plast Reconstr Surg 1986;77(3):445-58.
78. Becker R, Spadero J. Treatment of orthopedic infections with electrically generated silver ions. J Bone Joint Surg Am 1978;60(7):871-81.
79. Ong P, Laatsch L, Kloth L. Antibacterial effects of a silver electrode carrying microamperage direct current in vitro. J Clin Electrophysiol 1994;6(1):14-8.
80. Laatsch L, Ong P, Kloth L. In vitro effects of two silver electrodes on select wound pathogens. J Clin Electrophysiol 1995;7(1):10-5.
81. Guffey J, Asmussen M. In vitro bactericidal effects of high voltage pulsed current versus direct current against *Staphylococcus aureus*. J Clin Electrophysiol 1989;1:5-9.
82. Newton R, Karselis T. Skin pH following high voltage pulsed galvanic stimulation. Phys Ther 1983;63(10):1593-6.
83. Kincaid C, Lavoie K. Inhibition of bacterial growth in vitro following stimulation with high voltage, monophasic pulsed current. Phys Ther 1989;69(8):651-5.
84. Szuminsky N, Albers A, Unger P, et al. Effect of narrow, pulsed high voltages on bacterial viability. Phys Ther 1994;74:660-7.
85. Costerton B, Dirckx P. Antibiotic effectiveness is increased in the presence of even a weak, intermittent electrical field. The Center for Biofilm Engineering, Montana State University, Bozeman. Available from: URL: http//www.erc.montana.edu. Accessed January 13, 2005.
86. McLeod B, Dirckx P. The combination of electricity plus antibiotic is more effective against biofilm cells than either is alone. The Center for Biofilm Engineering, Montana State University, Bozeman. Available from: URL: http//www.erc.montana.edu. Accessed January 13, 2005.
87. Assimacopoulos D. Wound healing promotion by the use of negative electric current. Am Surg 1968;34(6):423-31.
88. Bigelow J. Effect of electrical stimulation on canine skin and percutaneous device: skin interface healing. In: Brighton CT, Black J, Pollack SR, editors. Skin interface healing and electrical properties of bone and cartilage. New York: Grune & Stratton; 1979. p. 289.
89. Carey L, Lepley D. Effect of continuous direct electric current on healing wounds. Surg Forum 1962;13:33-5.
90. Castillo E, Sumano H, Fortoul T, et al. The influence of pulsed electrical stimulation on the wound healing of burned rat skin. Arch Med Res 1995;26(2):185.
91. Konikoff JJ. Electrical promotion of soft tissue repairs. Biomed Eng 1976;4:1-5.
92. Smith J, Romansky N, Vomero J, et al. The effect of electrical stimulation on wound healing in diabetic mice. J Am Podiatr Assoc 1984;74(2):71-5.
93. Cruz N, Bayron F, Suarez A: Accelerated healing of full-thickness burns by the use of high voltage pulsed galvanic stimulation in the pig. Ann Plast Surg 1989;23(1):49-55.
94. Taskan I, Ozyazgan I, Tercan M, et al. A comparative study of the effect of ultrasound and electrostimulation on wound healing in rats. Plast Reconstr Surg 1997;100:966-72.
95. Dunn M, Doillon C, Berg R, et al. Wound healing using a collagen matrix: effect of DC electrical stimulation. J Biomed Mater Res 1988;22(A2 Suppl):191-206.
96. Bach S, Bilgrav K, Gottrup F, et al. The effect of electrical current on healing skin incision. Eur J Surg 1991;157:171-4.
97. Brown M, Gogia P. Effects of high voltage stimulation on cutaneous wound healing in rabbits. Phys Ther 1987;67:662-7.
98. Brown M, McDonnell M, Menton D. Electrical stimulation effects on cutaneous wound healing in rabbits. Phys Ther 1988;68:955-60.

99. Reed BV. Effect of high voltage pulsed electrical stimulation on microvascular permeability to plasma proteins: a possible mechanism in minimizing edema. Phys Ther 1988;68:491-5.
100. Taylor K, Mendel F, Fish D, Hard R, Burton H. Effect of high voltage pulsed current and alternating current on macromolecular leakage in hamster cheek pouch microcirculation. Phys Ther 1997;77(12):1729-40.
101. Thornton R, Mendel F, Fish D. Effects of electrical stimulation on edema formation in different strains of rats. Phys Ther 1998;78(4):386-94.
102. Chu C-S, McManus A, Mason A Jr., Okerberg C, Pruitt B Jr. Multiple graft harvestings from deep partial-thickness scald wounds healed under the influence of weak direct current. J Trauma 1990;30(8):1044-9.
103. Politis M, Zanakis M, Miller J. Enhanced survival of full-thickness skin grafts following the application of DC electrical fields. Plast Reconst Surg 1989;84(2):267-72.
104. Im J, Lee W, Hoopes J. Effect of electrical stimulation on survival of skin flaps in pigs. Phys Ther 1990;70(1):37-40.
105. Kjartansson J, Lundeberg T, Samuelson U. Transcutaneous electrical nerve stimulation (TENS) increases survival of ischaemic musculocutaneous flaps. Acta Physiol Scand 1988;134:95-9.
106. Junger M, Zuder D, Steins A, et al. Treatment of venous ulcers with low frequency pulsed current (Dermapulse): effects on cutaneous microcirculation. Der Hautartz 1997;18:879-903.
107. Greenberg J, Hanly A, Davis S, et al. The effect of electrical stimulation (RPES) on wound healing and angiogenesis in second degree burns. Proceedings of the 13th Annual Symposium on Advanced Wound Care; 2000 Apr 1-4; Dallas, TX.
108. Sheffield C, Sessler D, Hopf H, et al. Centrally and locally mediated thermoregulatory responses alter subcutaneous oxygen tension. Wound Repair Regen 1996;4:339-45.
109. Gagnier K, Manix N, Baker L, et al. The effects of electrical stimulation on cutaneous oxygen supply in paraplegics. Phys Ther 1988;68(5):835-9.
110. Dodgen P, Johnson B, Baker L, et al. The effects of electrical stimulation on cutaneous oxygen supply in diabetic older adults [Abstract]. Phys Ther 1987;67(5):793.
111. Peters E, Armstrong D, Wunderlich R, et al. The benefit of electrical stimulation to enhance perfusion in persons with diabetes mellitus. J Foot Ankle Surg 1998;37(5):396-400.
112. Rodriguez G, Claus-Walker J, Kent M, Stal S. Adrenergic receptors in insensitive skin of spinal cord injury patients. Arch Phys Med Rehabil 1986;67:177-113.
113. Bogie K, Nuseibeh I, Bader D. Transcutaneous gas tension in the sacrum during the acute phase of spinal cord injury. Proceedings of the Institute of Mechanical Engineers, Part H. J Engr Med 1992;206:1-6.
114. Mawson A, Siddiqui F, Connolly B, Sharp C, Summer W, Biundo J Jr. Sacral transcutaneous oxygen tension levels in the spinal cord injured: Risk factors for pressure ulcers? Arch Phys Med Rehabil 1993;74:745-51.
115. Patterson R, Cranmer H, Fisher S, Engel R. The impaired response of spinal cord injured individuals to repeated surface pressure loads. Arch Phys Med Rehabil 1993;74:947-53.
116. Mawson A, Siddiqui F, Connolly B, et al. Effect of high voltage pulsed galvanic stimulation on sacral transcutaneous oxygen tension levels in the spinal cord injured. Paraplegia 1993;31:311-9.
117. Callam M, Ruckley C, Harper D, et al. Chronic ulceration of the leg: extent of the problem and provision of care. BMJ 1985;290:1855-6.
118. American Diabetes Association. Diabetes: 1996 vital statistics. Alexandria (VA): American Diabetes Association; 1996.
119. Falanga V. Venous ulceration. J Dermatol Surg Oncol 1993;19:764-71.
120. Gault W, Gatens P. Use of low intensity direct current in management of ischemic skin ulcers. Phys Ther 1976;56(3):265-9.
121. Carley P, Wainapel S. Electrotherapy for acceleration of wound healing: low intensity direct current. Arch Phys Med Rehabil 1985;66:443-6.
122. Barron J, Jacobson W, Tidd G. Treatment of decubitus ulcers: a new approach. Minnesota Med 1985;68(2):103-6.
123. Stefanovska A, Vodovnik L, Benko H, et al. Treatment of chronic wounds by means of electric and electromagnetic fields. Part 2. Value of FES parameters for pressure sore treatment. Med Biol Eng Comput 1993;31(3):213-20.
124. Feedar J, Kloth L, Gentzkow G. Chronic dermal ulcer healing enhanced with monophasic pulsed electrical stimulation. Phys Ther 1991;71(9):639-49.
125. Gentzkow G, Pollack S, Kloth L, et al. Improved healing of pressure ulcers using Dermapulse, a new electrical stimulation device. Wounds 1991;3(5):158-70.
126. Gentzkow G, Alon G, Taler G, et al. Healing of refractory stage III and IV pressure ulcers by a new electrical stimulation device. Wounds 1993;5(3):160-72.
127. Akers T, Gabrielson A. The effect of high voltage galvanic stimulation on the rate of healing of decubitus ulcers. Biomed Sci Instrum 1984;20:99-100.
128. Kloth L, Feedar J. Acceleration of wound healing with high voltage, monophasic, pulsed current. Phys Ther 1988;71(4):503-8.
129. Griffin J, Tooms R, Mendlus R, et al. Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. Phys Ther 1991;71(6):433-42.
130. Baker L, Rubayi S, Villar F, et al. Effect of electrical stimulation waveform on healing of ulcers in human beings with spinal cord injury. Wound Repair Regen 1996;4:72-9.
131. Fitzgerald G, Newsome D. Treatment of a large infected thoracic spine wound using high voltage pulsed monophasic current. Phys Ther 1993;73(6):355-60.
132. Karba B, Vodovnik L. Promoted healing of chronic wounds due to electrical stimulation. Wounds 1991;3(1):16-23.
133. Jacques P, Brogan M, Kalinowski D. High-voltage electrical treatment of refractory dermal ulcers. Physician Assist 1997(March):84-97.
134. Wood J, Evans P, Schallreuter K, et al. A multi-center study on the use of pulsed low intensity direct current for healing stage II and III decubitus ulcers. Arch Dermatol 1992;129:999-1009.
135. Frantz R. The effectiveness of transcutaneous electrical nerve stimulation (TENS) on decubitus ulcer healing in adult patients. In: Funk S, Tornquist E, Champagne M, Copp L, Wiese R, editors. Key aspects of recovery: improving nutrition, rest, and mobility. New York: Springer; 1990. p. 197-205.
136. Mulder G. Treatment of open skin wounds with electrical stimulation. Arch Phys Med Rehabil 1991;72:375-7.
137. Cukjati D, Robnik-Sikonja M, Rebersek S, et al. Prognostic factors in the prediction of chronic wound healing by electrical stimulation. Med Biol Eng Comput 2001;39:542-50.
138. Houghton P, Kincaid C, Lovell M, et al. Effect of electrical stimulation on chronic leg ulcer size and appearance. Phys Ther 2003;83(1):17-28.
139. Assimacopoulos D. Low intensity negative electric current in treatment of ulcers of leg due to chronic venous insufficiency: preliminary report of three cases. Am J Surg 1968;115:683-7.
140. Katelaris P, Fletcher J, Little J, et al. Electrical stimulation in the treatment of chronic venous ulceration. Aust N Z J Surg 1987; 57:605-7.
141. Franek A, Polak A, Kucharzewski M. Modern application of high voltage stimulation for enhanced healing of venous crural ulceration. Med Eng Phys 2000;22:647-55.

142. Alon G, Azaria M, Stein H. Diabetic ulcer healing using high voltage TENS [Abstract]. Phys Ther 1986;66:775.
143. Lundeberg T, Eriksson S, Malm M. Electrical nerve stimulation improves healing of diabetic ulcers. Ann Plast Surg 1992;29(4):328-31.
144. Baker L, Chambers R, DeMuth S, et al. Effects of electrical stimulation on wound healing in patients with diabetic ulcers. Diabetes Care 1997;20(3):405-12.
145. Peters E, Lavery L, Armstrong D, et al. Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial. Arch Phys Med Rehabil 2001;82:721-4.
146. Thurman B, Christian E. Response of a serious circulatory lesion to electrical stimulation: a case report. Phys Ther 1971;51:1107-10.
147. Debreceni L, Gyulai M, Debreceni A, et al. Results of transcutaneous electrical stimulation (TES) in cure of lower extremity arterial disease. Angiology 1995;46:613-8.
148. Kaada B, Olsen E, Eielsen O. In search of mediators of skin vasodilation induced by transcutaneous nerve stimulation: III. Increase in plasma VIP in normal subjects in Raynaud's disease. Gen Pharmacol 1984;15:107-13.
149. Goldman R, Brewley B, Golden M. Electrotherapy reoxygenates inframalleolar ischemic wounds on diabetic patients. Adv Skin Wound Care 2002;15(3):112-20.
150. Goldman R, Brewley B, Cohen R, et al. Use of electrotherapy to reverse expanding cutaneous gangrene in end-stage renal disease. Adv Skin Wound Care 2003;16(7):363-6.
151. Goldman R, Brewley B, Zhou L, et al. Electrotherapy reverses inframalleolar ischemia: a retrospective, observational study. Adv Skin Wound Care 2003;16:79-89.
152. Goldman R, Rosen M, Brewley B, et al. Electrotherapy promotes healing and microcirculation of infrapopliteal wounds: a prospective pilot study. Adv Skin Wound Care 2004;17:284-90, 292-4.
153. American Physical Therapy Association. Electrotherapeutic terminology in physical therapy. Alexandria (VA): APTA; 2001.