Systemic Evaluation of Electrical Stimulation for Ischemic Wound Therapy in a Preclinical *In Vivo* Model

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Objective: In a systematic preclinical investigation of ischemic wound healing, we investigated the hypothesis that electrical stimulation (ES) promotes the healing of ischemic wounds.

Approach: The effects of varying clinically relevant ES variables were evaluated using our modified version of the Gould F344 rat ischemic wound model. Stimulation was delivered using the novel lightweight integrated, single-channel, current-controlled modular surface stimulation (MSS) device. Stepwise variation allowed the effects of five different stimulation paradigms within an appropriate current density range to be studied. Within each group, 8–10 animals were treated for 28 days or until the ischemic wounds were healed and 5 animals were treated for 12 days. Eight rats received sham devices. A quantitative multivariable outcomes assessment procedure was used to evaluate the effects of ES.

Results: Ischemic wounds treated with a decreased interpulse interval (IPI) had the highest rate of complete wound closure at 3 weeks. Wounds treated with decreased pulse amplitude (PA) had a lower proportion of closed wounds than sham ischemic wounds and showed sustained inflammation with a lack of wound contraction.

Innovation: Our systematic study of varying ES paradigms using the novel MSS device provides preliminary insight into potential mechanisms of ES in ischemic wound healing.

Conclusion: Clinically appropriate ES can more than double the proportion of ischemic wounds closed by 3 weeks in this model. Ninety percent of wounds treated with a decreased IPI healed by 21 days compared with only 29% of ischemic wounds treated with decreased PA, which appears to inhibit healing.

INTRODUCTION

WE INVESTIGATED THE PRELIMINARY hypothesis that electrical stimulation (ES) promotes the healing of chronic ischemic wounds by restoration of the normal wound healing process using the Gould F344 rat ischemic wound model. The model was implemented with a wearable

untethered modular surface stimulation (MSS) device, programmable to deliver a variety of clinically relevant stimulation paradigms. The goal of our study was to carry out a systematic preclinical investigation of ischemic wound healing employing a therapeutic ES delivery system in a standardized animal model.

The central theory underlying ES for wound therapy is that discontinuity in the skin due to a wound will create a “current of injury”, with the wound bed having very high resistance to electrical current compared with intact skin. In a chronic wound, the normal healing process is disrupted, the wound remains in an inflammatory stage, tissue formation is impaired, and there is little or no reepithelialization. Application of an electrical field across the wound is thought to overcome the high electrical impedance of the open wound bed and thus promote healing of chronic wounds. However, this straightforward explanation belies the complexity of the wound healing milieu.

Clinical studies have typically utilized direct current (DC) or pulsed DC ES, but the selection of waveforms and other stimulation parameters has varied in both between and within studies.^{1–4} Indeed, almost every clinical study has used a different stimulation paradigm with no consistent rationale for selection of variables. Thus, a wide range of stimulation parameters and waveforms have been reported in the literature and clinical outcomes, such as increased regional tissue oxygenation and successful wound healing, have been variable. A 2001 meta-analysis found that determination of an optimal ES protocol was hampered by lack of consistency among studies.¹ An update by the Cochrane Review in 2012 found no new clinical trials to update their meta-analysis.⁵

Underlying the clinical challenge of providing effective ES for chronic wound therapy is an incomplete understanding of the physiological pathways by which ES may alter the state of chronic wounds to promote healing. Previous *in vitro* work has shown that in acute scratch tests, ES can increase cell motility.⁶ However, the clinical wound environment is much more complex than a monoculture.

CLINICAL PROBLEM ADDRESSED

Chronic nonhealing wounds continue to occur at an unacceptably high rate, with skilled clinical management requiring repeated visits to the outpatient clinic and often rehospitalization, frequently for prolonged periods. There is limited information available on clinical treatment costs, however, the U.S. healthcare system costs for chronic wound treatment have been estimated to be \$6 to \$15 billion per year.⁷ The failure of a wound to heal thus not only impacts the individual but also places a significant burden on healthcare systems.

Among the plethora of treatment options, ES has been proposed as a suitable intervention for over

150 years.⁸ It is one of only two adjunctive therapies with Level A support in the current Clinical Practice Guidelines.⁹ Despite a long-standing awareness of ES in wound therapy, this adjunctive therapeutic modality is still not standard in healthcare. ES for wound therapy continues to be viewed by clinical providers as experimental, investigational, or unproven.^{10,11} Clinical experience and reported studies have shown variable levels of success in achieving wound closure using ES, which tempers enthusiasm. The variability in outcomes is understandable given the variety of ES options that have been reported in clinical studies. There is a need for preclinical testing using *in vivo* animal models together with a systems approach to investigate the mechanisms at all levels and provide further understanding so that effective ES therapy can be optimized.

MATERIALS AND METHODS

The effects of varying clinically relevant ES variables on wound healing were evaluated using our modified version of the Gould F344 rat ischemic wound model, which elegantly prevents wound healing by contraction and facilitates studies of chronic wound healing that are reproducible, cost-effective, and simple to perform.¹² The model has been optimized for the F344 strain and the delay in ischemic wound healing shown to be exacerbated by age.¹³

Selection and delivery of stimulation variables

Potential combinations of clinically relevant ES variables are nearly infinite and a wide range has been used both *in vitro* and *in vivo*.^{14,15} Previous studies were reviewed and ES variables collated, specifically, pulse width (PW), pulse amplitude (PA), and interpulse interval (IPI). Stimulation variables were selected to provide target mean tissue current densities up to $12 \mu\text{A}/\text{cm}^2$, a range that positively impacts *in vitro* fibroblast proliferation¹⁶ and *in vivo* wound healing activity.¹⁷ Stimulation was delivered using the MSS device, which contains all the components of a single-channel, current-controlled stimulation system within a lightweight, flexible, independently powered portable device.¹⁸ The programmable MSS allowed a range of ES waveforms to be tested using the same physical hardware. Individual stimulation waveform components were varied in a stepwise manner to determine the effects of individual waveform components within an appropriate current density range, as shown in Table 1. The MSS remained *in situ* for up to 7 days, thus reducing the number of dressing changes.

Table 1. Stimulation groups and electrical stimulation variables applied

Stimulation Group	Amplitude (mA)	Width (μ s)	Interpulse Interval (ms)	Current Density (μ A/cm ²)	Number of Wounds	
					POD28	POD12
Sham ischemic	0	0	0	0	16	
1: Central	4	100	50	5.3	18	10
2: pulse amplitude ↓	2	100	50	2.7	16	10
3: pulse amplitude ↑	6	100	50	8.0	20	10
4: pulse width ↑	4	150	50	8.0	16	10
5: interpulse interval ↓	4	100	40	6.7	22 ^a	

^aNinety percent of all wounds healed by 21 days.

↑, increased; ↓, decreased; POD, postoperative day.

Creation of wound model and delivery of electrotherapy

Longitudinal studies of ES were carried out using 6-month-old male F344 rats (350–450 g). The MSS was applied to ischemic wounds created using the Gould protocol modified for use in a device-based intervention.

A standardized preoperative skin preparation and surgical procedure were employed as described in Henzel *et al.*¹⁹ Specifically, to minimize the impact of known stressors, multiple acclimatization measures were implemented, including pre-surgical acclimation to single housing, flavored children's ibuprofen diluted 3.5 mg/mL in sterile water for adequate analgesia, and Prang (BioServ, Frenchtown, NJ) for adequate hydration. Animals were also habituated to wearing a protective heavy rip-stop nylon jacket (Harvard Apparatus, Holliston, MA) for 5 days before surgery. The total acclimation period was 26–28 days. All procedures and experimental protocols were approved by the LSCDVAMC and CWRU Institutional Animal Care and Use Committees.

All procedures were performed under inhalational isoflurane-induced anesthesia using an aseptic surgical technique to minimize the risk of postsurgical wound infection. One day before ischemic flap surgery, the dorsum of the animal was clipped and treated with a depilation product (Sally Hansen Spray-On Shower-Off Hair Remover—Extra Strength; Coty, Inc., New York, NY), providing a smooth surface to promote device adherence. Skin preparation 1 day before surgery provided time for recovery of observed cutaneous erythema after hair removal. At surgery, the skin was prepared by cleansing with 1% chlorhexadine gluconate solution alternating with alcohol for 6 min to remove any residue, which could impair device adherence. Flap dimensions (26 mm × 100 mm) were drawn over the dorsum of the rat and centered over

the spine using a sterile surgical marker. Four 6 mm wounds were created using a punch biopsy and placed symmetrically about the midline at a distance of 5.0 cm from the base of the scapula. Wounds were laterally separated such that two wounds were centered over the flap region and two over normal dermis 1 cm lateral to the bipedicle flap.¹² The full-thickness punch wound included the skin and underlying panniculus carnosus, but avoided the anterior muscle fascia. The biopsied tissue was removed by dissection in a plane between the panniculus carnosus and fascia. A dorsal, bipedicle flap was raised deep to the panniculus carnosus. Sterilized, nonreinforced, medical grade silicone sheeting 10 mil thickness (Sil-Tec; Technical Products, Inc., Decatur, GA), precut to flap dimensions, was placed under the flap and sutured to adjacent skin edges using interrupted nonabsorbable sutures. Attachment to this fixed substrate inhibits wound contraction and prevents readherence or reperfusion of the flap from the underlying tissue. This model thus maintains prolonged ischemia in the wounds created over the flap.

The effects of five different stimulation paradigms delivered for 12 and 28 days were studied (Table 1). Within each group, 8–10 animals were treated for 28 days or until the ischemic wounds were healed (defined by epidermal closure) and five animals were treated for 12 days. To provide a negative control, eight rats from different groups received sham devices that delivered no stimulation to the ischemic wounds. Nonfunctional devices were assigned by the engineer assembling the devices. The technicians working directly with the animals and assessing the wounds were therefore blinded to the treatment received.

The MSS was applied to fit lengthwise over the flap area and provide an occlusive dressing. The electrode layout directly stimulates the controlled ischemic wounds. Tegaderm™ (3M Health Care, St. Paul, MN) with supplemental cyanoacrylate VetBond tissue adhesive (3M Health Care) was applied as a secondary occlusive dressing to protect the device. To prevent the rat from removing the device, a protective jacket was applied 1 day postsurgery. Delivery of stimulation was initiated using the MSS at a continuous 10% duty cycle immediately following creation of the ischemic wounds.

Ketoprofen (2 mg/day) was administered subcutaneously at surgery and for 3 days postsurgery. Antibiotic prophylaxis was provided by ampicillin, 0.5 mg in normal saline, administered subcutaneously at surgery and for 10 days postsurgery. Postoperatively, flavored ibuprofen water and Prang

were provided *ad libitum* to ensure analgesia and hydration.

Devices were changed under isoflurane anesthesia weekly following surgery. For the long-term (28 day) studies, device changes occurred on postoperative day (POD) 7, 14, and 21, or until wounds healed. For the shorter (12 day) groups, there was a POD7 change only. The flexible electrode substrate and coin battery were discarded after 7 days of use. Other electronic components were transferred to a new flexible substrate and reused after STERRAD sterilization.

Wound assessments

A quantitative multivariable outcomes assessment procedure was used to evaluate the effects of ES. The postoperative wound healing progress was monitored at weekly device changes. Regional ischemia was monitored by measurement of transcutaneous oxygen (T_cPO_2) using the Radiometer TCM4 monitoring system (Radiometer America, Inc., Westlake, OH). Sensing electrodes were placed on the ischemic flap just caudal of the wounds and on normal skin on the left haunch. T_cPO_2 was measured for 15 min, following stabilization of T_cPO_2 levels. Progressive changes in wound size, indicative of wound healing, were determined using high-resolution digital photography.

At completion of the stimulation protocol, a final *in vivo* assessment of wound healing status was carried out. Tissue was then harvested from the ischemic and control wounds. A 6 mm biopsy punch was used to obtain a full sample at each wound site. Wound tissue samples were sectioned, slightly offset of center in the direction of hair growth. For each animal, one section from a control wound and one from an ischemic wound were fresh frozen in the Tissue Freezing Medium (Triangle Biomedical Sciences, Inc., Durham, NC) before immediate storage in a -80°C freezer. The remaining samples were placed in paraffin cassettes and immersed in formalin for 24 h for tissue fixation before storage in 70% ethanol in a refrigerator before further processing. Following tissue harvest from all wounds, the rat was euthanized using a 1 mL transcardial injection of sodium pentobarbital.

Data analysis

Transcutaneous oxygen. Continued tissue ischemia was assessed by comparing mean T_cPO_2 at the caudal ischemic flap to control sites on normal skin.

High-resolution digital images. The wound area was calculated from high-resolution digital images

using Photoshop CS5.1 (Adobe Systems Incorporated, San Jose, CA) (Fig. 1).

Wound histology. Hematoxylin and eosin (H&E) staining of fixed wound tissue was used to evaluate multiple aspects of wound healing, specifically, wound closure, inflammation, angiogenic activity, fibrosis (scar formation), and collagen fiber organization (Fig. 1). These variables were graded to provide a semiquantitative analysis of the wound healing activity. All the H&E-stained wound tissues were evaluated by a single observer (K.H.), who was fully blinded to the intervention status of the sections and a composite wound histology (CWH) score was developed. Variables were rated on a scale from 0–5, where 0=absence of desirable activity and 5=maximal desirable activity. The absence of angiogenic activity was coded as -1 since this was a critical variable.

Immunohistochemical analysis. Immunohistochemical analysis (Fig. 1) of fresh frozen wound tissue was performed to detect the proangiogenic activity (vascular endothelial growth factor [VEGF]) and early-stage macrophage activity (cluster of differentiation 68 [CD68]).

The VEGF A-20 rabbit polyclonal primary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) diluted 1:100 in phosphate-buffered saline (PBS) was used with a biotinylated goat anti-rabbit immunoglobulin G (IgG) secondary antibody (#BA-1000; Vector Laboratories, Burlingame, CA) diluted 1:200 in PBS with 1.5% normal goat serum. The same dilution was used for negative control (IgG2a) slides. Antibody binding was highlighted using an avidin–biotin complex (#SP-2001; Vector Laboratories) and the 3,3'-diaminobenzidine (DAB) substrate (#SK-4100; Vector Laboratories). Hematoxylin (#H-3401; Vector Laboratories) was applied to counterstain and slides were coverslipped using Permount (Fisher Scientific, Pittsburgh, PA).

Immunofluorescence techniques. Immunofluorescence techniques to detect epithelial cell activity (epithelial cell adhesion molecule [EpCAM]) and superoxide production (dihydroethidium [DHE]) were applied to fresh frozen wound tissue.

EpCAM is a monomeric transmembrane glycoprotein expressed in the epithelium and required for development of epithelial integrity. EpCAM (EBA-1) mouse monoclonal IgG₁ primary antibody (sc-66020; Santa Cruz Biotechnology, Inc.) diluted 1:100 in a normal goat serum blocking solution was used with an Alexa Fluor® 488 fluorescent conjugate goat anti-mouse IgG1 secondary antibody

Figure 1. Wound images and sections (all sections $10\times$). **(A)** Control wound at POD12. **(B)** Electrical stimulation-treated ischemic wound at POD28. **(C)** Sham ischemic wound at POD28. | |, wound margins. Note that wound margin could not be delineated on sham ischemic wounds. H&E, hematoxylin and eosin; POD, postoperative day; VEGF, vascular endothelial growth factor. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

diluted 1:1,000 in the normal goat serum blocking solution. Slides were coverslipped using Fluormount-G.

DHE is a lipophilic cell-permeable compound, which detects reactive oxygen species (ROS) released in the inflammatory response. In the cytosol, DHE exhibits blue fluorescence, however, once oxidized to form the DNA-binding fluorophore ethidium/oxyethidium, it stains the cell nucleus red. A standard staining protocol was employed using $30\mu\text{M}$ of DHE solution in PBS and coverslipping with Fluormount-G (Fisher Scientific).²⁰

Quantitative immunofluorescence analysis. Quantitative immunofluorescence analysis was applied to EpCAM and DHE signal in the wound regions. These stains are nonstoichiometric and do not follow the Beer-Lambert law. Stain intensity does not cor-

relate with the magnitude of activity, however, intensity thresholding provides a relative measure of area or extent of activity.^{21,22} Relative signaling in the wound area was calculated using Photoshop intensity thresholding by segmenting and outlining the wound area. A signal-positive area within the wound was then determined relative to the total wound area.

Statistical analysis

Wound status was assessed using a systems approach. Control wounds in the rat model healed very rapidly. POD28 ischemic wounds were therefore compared with both POD12 and POD28 control wounds. Data could not be assumed to be normally distributed; thus, the Mann-Whitney test was applied to determine significant differences between treatment and control groups at POD12 and POD28, or when all ischemic wounds were healed.

The Bonferroni correction was applied to correct for repeated testing. This conservative correction ensures that only truly significant differences are reported. In the current analyses, a minimum significance level of $p < 0.01$ was used.

RESULTS

Transcutaneous oxygen

Baseline T_cPO_2 was 26 ± 7 mmHg on normal skin. Control T_cPO_2 was elevated on POD1 and POD7 compared with the preoperative baseline, but was not significantly different from baseline on POD14, POD21, and POD28. T_cPO_2 of the ischemic flap was decreased to 6 ± 3 mmHg at POD7 compared with control locations and remained low throughout the postoperative period.

Complete wound closure

Complete wound closure at POD21 was determined (Fig. 2). Group 5 ischemic wounds had the highest rate of complete wound closure at POD21. Group 2 had a lower proportion of closed wounds than sham ischemic wounds.

Relative wound size

The relative wound size as a percentage of the original wound was determined for open wounds at

Figure 2. Percentage of wounds closed at POD21. ↑, increased; ↓, decreased. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 3. Relative wound size over time. Error bars show standard deviations. ** $p < 0.01$. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

each time point. Figure 3 shows relative wound size for ischemic wound groups that healed most effectively, together with control and sham ischemic wounds. A low proportion of sham ischemic wounds had closed by POD21, and those wounds that remained open increased in mean size. Control wounds contracted rapidly. At 12 days, they were significantly smaller ($p < 0.01$) than ES-treated ischemic wounds. However, continuous ES treatment at a 10% duty cycle for 14 days and more produced relative wound closure comparable to control wounds.

H&E histological analysis

H&E histological analysis showed that all ES-treated ischemic wounds exhibited increased blood vessel formation in the wound bed and decreased contraction relative to the highly contracted control wounds in the rat model. Control wounds had a median CWH score of 19 at POD28 (range 11–20). Sham ischemic wounds had a median CWH score of 14 (range 7–18). Median CWH scores for ES-treated ischemic wounds varied from 9 to 18 (Fig. 4).

The two ES groups with the highest proportion of closed wounds had slightly different CWH scores over time; Group 1 (central stimulation) had significantly lower CWH scores than POD28 control wounds ($p < 0.01$), with high early-stage fibrosis and low late-stage wound contraction. In comparison, the Group 5 wounds treated with a decreased IPI did not have significantly different CWH scores relative to control wounds at any time point. Histological analysis showed that blood vessel formation and fibrosis at POD21 were moderately increased in these wounds.

Figure 4. Composite wound histology (CWH) score. Error bars show standard deviations. ** $p < 0.01$. Key: See Fig. 2. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

The ES ischemic Group 2 (decreased PA) had the poorest healing rate and also had a CWH score lower than the sham ischemic wounds. This score was significantly lower ($p < 0.01$) relative to control wounds at POD28, and showed sustained inflammation with a lack of wound contraction.

Immunohistochemical staining

Immunohistochemical staining showed that the VEGF signal was very low within the wound area for control wounds as early as POD12. ES-treated wounds showed VEGF activity within the wound bed at POD12 even in closed wounds. By POD28, this had decreased to similar levels as for POD12 control wounds. Immunohistochemistry (IHC) also showed that control wounds had low to no CD68 signal at POD28, while the CD68 signal in closed ES-treated ischemic wounds was moderate within

the wound area, but low in the margins. Most sham ischemic wounds were open at POD28 and had increased macrophage signals at the wound surface and throughout the putative wound area.

Quantitative immunofluorescence analysis

Quantitative immunofluorescence analysis (Fig. 5) indicated that the relative EpCAM activity was insignificantly lower for control wounds than for the majority of ES-treated ischemic wound groups at POD12. At POD28, the relative EpCAM activity area decreased for control and ES-treated ischemic wounds, apart from those treated with the Group 1 (central) ES paradigm, which had a significantly higher relative EpCAM area than control levels ($p < 0.01$).

In control wounds, the relative DHE activity decreased significantly between POD12 and POD28. Conversely, the ES-treated ischemic wound groups tended to have an increase in relative DHE area over time, apart from the group treated with the Group 1 (central) ES paradigm, which had a significantly higher relative DHE area than control levels ($p < 0.01$).

DISCUSSION

Twenty years ago, electrotherapy was specifically recommended for the treatment of severe, unresponsive (Grade III or IV) pressure ulcers.²³ There have been many clinical reports of the technique^{24,25} and various groups have attempted to consolidate these findings, leading to three separate meta-analyses of this treatment modality.^{1,2,26} Examination of the treatment patterns summarized in the report shows that ES parameters are

Figure 5. Relative immunofluorescence marker activity in wound area. Error bars show standard deviations. ** $p < 0.01$ versus POD28. DHE, dihydroethidium; EpCAM, epithelial cell adhesion molecule. Key: See Fig. 2. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

often incompletely reported in clinical studies. When reported, the stimulation parameters and treatment paradigms for pulsed DC stimulation therapy are highly variable.^{4,5,14} The Cochrane Review in 2010 concluded that there is still no strong basis for efficacy of ES. Medicare provides coverage for the use of electrotherapy only when all other types of therapy have failed²⁷ and no device has yet been approved by the Food and Drug Administration specifically for chronic wound therapy. These limitations reflect the underlying deficit in the understanding of the physiological mechanisms, which is an essential precursor to optimization of clinical therapy. Selection of optimal treatment parameters for delivery of effective ES for chronic wound therapy needs to be guided by an understanding of the underlying physiological effects.^{28,29}

In the normal healing wound, the angiogenic activity is high during the early stages of healing and decreases as healing progresses to the tertiary remodeling phase. The healing process is inhibited in the ischemic wound, with these wounds often being described as “stuck” in the inflammatory phase of wound healing. Macrophages are important at all stages of wound healing,³⁰ specifically, the CD68 macrophage signal is indicative of the early-stage wound healing response.³¹ DHE superoxide is indicative of ROS activity and is essential for effective wound healing although it appears to have a dual role. It may play a role in reducing wound infection; however, extended elevation may also lead to deterioration of tissue quality.³²

In vitro and clinical studies concur in the finding that maintenance of a physiologically moist wound environment is essential for wound healing in general.^{33–36} For electrotherapy, in particular, it is essential to maintain tissue current. Currently, electrotherapy for chronic wounds uses disposable surface electrodes placed around the wound area at each treatment session and removed post-treatment. This introduces the potential for nonuniform treatment delivery due to errors in repeatability of electrode placement and increases infection risk due to repeated wound exposure. Frequent exposure of the wound area disrupts the moist wound environment and increases the risk of the wound bed drying out. The MSS developed by our group is a fully integrated programmable surface ES system that facilitates delivery of ES direct to the wound area for up to 7 days without disrupting the wound bed. Thus, the MSS enables ES to be delivered consistently, while maintaining the moist wound environment.

Using the MSS also allowed standardized and reliable delivery of therapy in a systemic preclinical study of clinically relevant ES variables. All

wounds were assessed using the same quantitative *in vivo* and postmortem techniques to determine information on the effect of varying ES paradigms on the overall wound healing process.

By POD28, control wounds were in the tertiary remodeling phase and had low angiogenic and macrophage activity. Sham ischemic wounds at this time point still had increased macrophage signals throughout the putative wound area. Overall, closed ES-treated wounds still appeared to have some inflammatory activity at POD28, indicative of a delayed healing process.

Wounds treated with Group 2 (low PA) stimulation had a lower percentage of closed wounds at POD21 than sham ischemic wounds (Fig. 1) with less than 30% closed. Histological assessment showed that even when closed, these wounds had poor tissue quality, with high fibrosis and little or no angiogenesis.

Ischemic wounds treated with Group 1 (central), Group 3 (increased PA), and Group 4 (decreased PW) stimulation showed similar proportion of closed wounds at POD12 (Fig. 1). This implied that with respect to the critical clinical outcome of wound closure, these treatment groups all healed similarly. Immunofluorescence analysis revealed some differences in healed tissue quality. Group 1 stimulation produced high sustained levels of angiogenesis and ROS/DHE at POD28, both of which were significantly greater than control wounds, implying delayed progression to the tertiary stages of wound healing and maturation.

Ninety percent of wounds treated with Group 5 (decreased IPI) stimulation healed by POD21. Both angiogenesis and fibrosis were slightly greater in these wounds at POD21 compared with control wounds at POD28, implying an active healing process.

Our findings imply that ischemic wounds treated with ES exhibit active but altered healing pathways. Furthermore, different aspects of wound healing are impacted by varying ES paradigms. ES paradigms that produce higher current density appear preferential, implying that microcurrent ES would be less effective.

INNOVATION

Insurance coverage for ES as a primary modality for chronic wound care is not routinely provided²⁷ and recent reports have highlighted the continued need to determine mechanisms to optimize outcomes.³⁷ Our systematic study of varying ES paradigms using the novel MSS integrated surface stimulation device provides preliminary insight into

potential mechanisms of electrotherapy in ischemic wound healing. We found that clinically appropriate ES can more than double the proportion of ischemic wounds closed by POD21 in the rat ischemic wound model. Conversely, low-amplitude stimulation appears to inhibit ischemic wound healing.

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AUTHOR DISCLOSURES AND GHOSTWRITING

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ABOUT THE AUTHORS

Jennifer K. Graebert, BS, is a Research Assistant in Dr. Bogie's laboratory and was respon-

KEY FINDINGS

- A valid rat ischemic wound model refined to enable reliable systemic evaluation of ES variables delivered by the MSS and standardized multi-variate outcomes measures has shown that clinically appropriate ES can more than double the proportion of ischemic wounds closed by POD21.
- Varying ES paradigms have different impacts on the rate and quality of ischemic wound healing.
- Ninety percent of ischemic wounds treated with ES with a decreased IPI healed by 21 days in this model compared with only 29% of ischemic wounds treated with decreased PA.

sible for the overall day-to-day conduct of the study, including tissue preparation, collation, and processing of experimental data. **M. Kristi Henzel, MD, PhD**, is currently an SCI Physiatrist at the Cleveland VA Medical Center. Dr. Henzel completed her fellowship as a Quality Scholar in Spinal Cord Injury Research at the Louis Stokes Cleveland Veterans Affairs Medical Center in Dr. Bogie's laboratory. **Kord S. Honda, MD**, is the Director of Dermatopathology at the University Hospitals Case Medical Center and holds an appointment as an Assistant Professor in the Department of Pathology at the Case Western Reserve University. **Kath M. Bogie, DPhil**, is a biomedical engineer whose research interests focus on translational research, particularly, in the prevention and treatment of wounds, from biomolecular techniques to clinical assessment and medical device development. Dr. Bogie is a Principal Investigator with the Advanced Platform Technology Center and holds an appointment at the Case Western Reserve University in the Department of Orthopedics.

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Abbreviations and Acronyms

CD68 = cluster of differentiation 68
 CWH = composite wound histology (score)
 DAB = 3,3'-diaminobenzidine
 DC = direct current
 DHE = dihydroethidium
 EpCAM = epithelial cell adhesion molecule, also known as EBA-1
 ES = electrical stimulation
 H&E = hematoxylin and eosin (stain)
 IgG = immunoglobulin G
 IHC = immunohistochemistry
 IPI = interpulse interval
 MSS = modular surface stimulation
 PA = pulse amplitude
 PBS = phosphate-buffered saline
 POD = postoperative day
 PW = pulse width
 ROS = reactive oxygen species
 T_cPO₂ = transcutaneous oxygen
 VEGF = vascular endothelial growth factor