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Abstract

The use of electrical stimulation (ES) should be considered for treating nonhealing pressure ulcers (PUs), but optimal ES wound treatment protocols have yet to be established. A randomized, controlled, double-blind clinical study was conducted to evaluate the effects of cathodal and anodal high-voltage monophasic pulsed current (HVMPC) on periwound skin blood flow (PSBF) and size reduction of Stage 2 to Stage 4 PUs of at least 4 weeks' duration. Persons >18 years of age, hospitalized with neurological injuries, at high risk for PU development (Norton scale <14 points; Waterlow scale >15 points), and with at least 1 Stage 2 to Stage 4 PU were eligible to participate in the study. Persons with necrotic wounds, osteomyelitis, electronic or metal implants in the PU area, PUs in need of surgical intervention, acute wound inflammation, diabetes (HBA1c >7%), diabetic neuropathy, cancer, and/or allergies to standard wound treatments were excluded. Patients were randomly assigned to 1 of 3 groups: anodal (AG), cathodal (CG), or placebo (PG) ES. All groups received individualized PU prevention and standard wound care. In the PG, sham ES was applied; the AG and CG were treated with anodal and cathodal HVMPC, respectively (154 µs 100 Hz; 360 µC/second; 1.08 C/day), 50 minutes per day, 5 days per week, for a maximum of 8 weeks. PSBF was measured using laser Doppler flowmetry at baseline, week 2, and week 4, and wound surface area measurements were obtained and analyzed using a digitizer connected to a personal computer. Data analysis utilized the maximum-likelihood chi-squared test, the analysis of variance Kruskal-Wallis test, the Kruskal-Wallis post-hoc test, and Spearman's rank order correlation. Nonlinear approximation based on exponential function was used to calculate treatment time needed to reduce the wound area by 50%. In all tests, the level of significance was set at $P \leq .05$. Of the 61 participating patients, 20 were in the AG (mean age 53.2 ± 13.82 years), 21 in the CG (mean age 55.67 ± 17.83 years), and 20 in the PG (mean age 52.5 ± 13.18 years). PUs (baseline size range 1.01 cm^2 to 59.57 cm^2; duration 4 to 48 weeks) were most frequently located in the sacral region (73.77%) and classified as Stage 3 (62.29%). PSBF at week 2 was significantly higher in the AG and CG than in the PG ($P < .05$). Week 4 differences were not statistically significant. Wound percentage area reduction calculated at week 8 for the AG (64.10% ± 29.22%) and CG (74.06% ± 23.23%) were significantly different from PG ulcers (41.42% ± 27.88%; $P = .0391$ and $P = .0024$, respectively). In both ES groups, PSBF at week 4 and percent wound surface area reductions between weeks 4 and 8 were positively correlated, but only the AG correlation was statistically significant ($P = .049$). In this study, both ES modalities improved blood flow and wound area reduction rate. Studies examining optimal ES treatment times for healing to occur, the effect of comorbidities and baseline wound variables on ES outcomes, and the nature of the relationship between blood flow and healing are necessary.

Keywords: randomized controlled trial, pressure ulcer, wound healing, electrical stimulation, skin blood flow

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Key Points

- A randomized, controlled clinical study was conducted to examine the effect of 2 types of electrical stimulation (ES) — cathodal and anodal high-voltage monophasic pulsed current — on periwound skin blood flow and size reduction of Stage 2 to Stage 4 pressure ulcers (PUs) as compared to control (sham) stimulation.
- Sixty-one (61) patients with neurological impairment (61 ulcers) received 1 of the 3 interventions 50 minutes per day, 5 days per week, for a maximum of 8 weeks.
- The majority of patients had a Stage 3 PU in the sacral area for the previous 10 to 13 weeks.
- At week 2, blood flow was significantly higher in both ES groups than in the control group, and wound area reduction was significantly greater after 8 weeks.
- Studies to examine optimal ES treatment times for PU healing to occur and to increase understanding about the relationship between blood flow and healing are needed.

Clinical practice guidelines[1,2] suggest electrical stimulation (ES) should be considered for treating recalcitrant Stage 2, Stage 3, and Stage 4 pressure ulcers (PUs). The recommendation is based on A-level strength of evidence from controlled trials on PUs in humans.[1]

ES influence on blood flow. A common factor that may prevent or delay wound healing is compromised perfusion of the wound and periwound tissues. In a preclinical, experimental study with 10 healthy volunteers (age 20 to 40 years), Petrofsky et al[3] investigated whether skin blood flow changes induced by ES could be attributed to the electrical current effect on nitric oxide (NO) that is known to dilate blood vessels. From the first part of the study,[3] the authors concluded a 4-minute application of biphasic sine current in the thigh area significantly increased skin blood flow compared to its pre-ES level ($P < .01$). In the second part of the study, biphasic sine current and iontophoresis with a NO synthetase inhibitor (N-nitro-L-arginine methyl-ester) were applied simultaneously to the same part of the body in the same patients. A significant reduction in skin blood flow ($P < .01$) was observed. The authors concluded the increase in skin blood flow in the first part of the experiment was caused by the release of NO induced by ES.

A randomized, double-blind, controlled, placebo clinical study by Mohajeri-Tehrani et al[4] (N = 20) investigated the effect of low-intensity direct current on the release of plasma vascular endothelial growth factor (VEGF) and NO in patients with diabetic foot ulcerations. After 12 ES sessions, a significantly higher level of NO was noted in blood plasma in the ES-treated patients compared to patients in the control (placebo) ES group ($P = .04$). A randomized, single-blind, controlled clinical trial by Asadi et al[5] showed ES significantly increased wound-fluid levels of hypoxic inducible factor-1α and VEGF while decreasing wound surface area after 12 sessions of ES with low-intensity direct current applied at sensory level. *In vitro* studies by Zhao et al[6] and Bai et al[7] have shown that an electric field (75 to 200 mV/mm) induced by a direct current, comparable in amplitude to the measurable current of injury at a wound, caused a direct release of VEGF by vascular endothelial cells[6] and induced directional migration, orientation, and elongation of endothelial cells, vascular fibroblasts, and smooth muscle cells.[7] It also was shown that vascular endothelial cells migrate toward the cathode, as opposed to vascular fibroblasts and smooth muscle cells that migrate toward the anode.

The cited preclinical[3,6,7] and clinical[4,5] studies have demonstrated that direct[4-7] and biphasic pulsed[3] currents increase the release of NO, dilating blood vessels,[3,4] stimulating the release of angiogenetic factors (VEGF[4-6] and of hypoxic inducible factor-1α[5]), and induce reorientation, elongation, and migration of endothelial cells as well as vascular fibroblasts and smooth muscle cells,[7] all of which exert a positive influence on wound healing.

Clinical evidence supporting the use of high-voltage monophasic pulsed current (HVMPC) for wound treatment. Chronic wounds treated with ES utilize subsensory amplitudes of direct and pulsed currents with amperage below 1 mA (so-called microcurrents)[8,9] as well as sensory stimulation below the muscle contraction threshold. Sensory ES involves the application of HVMPC[10-18] and low-voltage monophasic[19-21] and biphasic[22-24] pulsed currents. The authors of critical and systematic reviews published in 2014[25] and 2017[26] concluded both high-voltage and low-voltage

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pulsed currents can produce consistently positive results in patients with chronic wounds.

Several clinical studies compared HVMPC to standard wound care (SWC) alone[12,15,16] or SWC + sham ES[10,11,13,17,18] to treat PUs,[10,11,15-18] venous leg ulcers,[12,14] and diabetic foot ulcers.[13] The main results and methodology of HVMPC in PU treatment studies are summarized in Table 1. The outcomes of the cited studies[10,11,15-18] indicated PU area decreased more in the SWC + ES groups than in controls treated with HVMPC.

In reviews of clinical[27,28] and epidemiological studies,[29] wound closure was the crucial endpoint in evaluating treatment efficacy. However, clinical studies utilizing ES rarely last long enough for closure to be achieved. Only 1 study[10] involved treatment of PUs with HVMPC until full closure. In trials that terminated before wounds were closed, the percentage wound area reduction from baseline at weeks 4, 6, or 12 of treatment was a crucial indicator of treatment efficacy.[11-18] The time during which PU surface area decreases from baseline by at least 50% is also important.[27-29]

Three (3) clinical studies evaluated changes in wound surface area (WSA) and periwound skin blood flow (PSBF) in ulcers that received 2 and 4 weeks of ES with sensory level low-voltage, biphasic, pulsed currents.[22-24] To address a gap in the research literature, this clinical study was designed assess the effect of cathodal and anodal high-voltage HVMPC on PSBF and WSA reduction in Stage 2 to Stage 4 PUs in adult persons with neurological injuries.

Methods

Study design. This prospective, randomized, blind, controlled, clinical trial was designed to compare PSBF around the PU area after 2 and 4 weeks of treatment as well as WSA reduction during 8 weeks of treatment between 3 groups of patients receiving SWC plus cathodal ES, anodal ES, or sham ES, respectively. Parallel treatment of patients in all 3 groups and equal numbers of patients in the groups were assumed.

Ethical approval. Ethical approval was granted by the Academy Bioethics Commission.

The trial was prospectively registered with the Australian-New Zealand Clinical Trials Registry (ANZCTR): ANZCTRN 12615001281583.

Study enrollment and criteria. Patients screened for the study were treated as inpatients at 1 rehabilitation center between December 1, 2015, and January, 30, 2017. Their eligibility to participate was assessed by their physician using the following criteria: neurological injuries (spinal cord injury, ischemic stroke, and/or blunt trauma to the head); age 18 years or older; high risk of PU development (<14 points on the Norton scale and >15 points on the Waterlow scale); and Stage 2, Stage 3, or Stage 4 PU of at least 0.5 cm^2 in size and at least 4 weeks' duration located on the pelvic girdle or lower extremities.

Because it was initially planned that only patients with spinal cord injuries would be enrolled in the trial, this inclusion criterion was stated in the ANZCTR registration form. However, because the number of qualifying patients in the rehabilitation center turned out to be insufficient for statistical analysis, patients with brain injuries from a cerebral stroke or skull trauma also were included. These changes were reported to the ANZCTR. Patients who could not receive ES (ie, persons with cancer, electronic implants, metal implants, osteomyelitis in the PU area, tunneling, necrotic wounds, and PUs requiring surgical intervention), patients with acute inflammation in the wound area, and patients with conditions impeding wound healing such as diabetes (HbA1c >7%), critical wound infection, allergies to standard wound treatment, and/or alcoholism were excluded from the study.

Randomization. Patients were informed in writing by the research manager about the aim and course of the study and that they could withdraw from the study at any time without having to state a reason and without any consequences for their further treatment. Patients who consented to participate in the trial (or whose legal guardians gave consent for their inclusion) were randomly assigned to 3 groups.

The study was initially designed to involve 3 groups of 15 people. Accordingly, a person independent of the trial was given 3 sets of 15 slips of paper that were marked with letters A, B, and C denoting group assignment: A for the SWC + anodal HVMPC group, B for the SWC + cathodal HVMPC group, and C for the SWC + sham ES group. The person, who was not aware of what the letters meant, inserted the slips into 45 computer-generated, randomly drawn envelopes. Once sealed, the envelopes were delivered to the main investigator in charge of allocating patients to groups. Before the trial commenced, the envelopes were opened 1 at a time in the presence of a physiotherapist and the patient concerned was directed to the appropriate group. Because more persons volunteered to participate in the study than originally planned, 3 additional sets of 6 envelopes were prepared for each group and the randomization of patients proceeded as described. The final sample consisted of 61 patients. The increased number of participants diminished the risk of study bias.

Blinding. All patients, medical personnel, and researchers were blinded as to what type of ES was being applied to individual patients (anodal ES, cathodal ES, or sham ES). The exceptions were the main investigator and the principal physiotherapist who set the equipment to apply active or sham ES. The person responsible for statistical analysis also was blinded.

Study variables. Demographic information on the patients enrolled in the study was obtained from standardized participant interviews, physical examinations, the results of additional examinations, and the history of concomitant diseases. Study variables for assessing wounds, PU risk, and patients' nutritional status were collected with paper-pencil instruments and transferred to a data sheet. Other data, such as patient case history, blood cell count results, and previous

Table 1. Summary of controlled clinical high-voltage monophasic pulsed current (HVMPC) studies[a] in patients with pressure ulcers (PUs)

	Kloth and Feedar[10] (1988)	Griffin et al[11] (1991)	Houghton et al[15] (2010)	Franek et al[16] (2012)	Polak et al[17] (2016)	Polak et al[18] (2017)
Total number of patients	16	17	34	50	49	63
Number of patients (ES group)	9	8	16	26	25	CESG=23/C+AESG=20
Treatment						
ES group	SWC+ES	SWC+ES	SWC+ES	SWC+ES	SWC+ES	SWC+CES/SWC+C+AES
Control group	SWC+sham ES	SWC+sham ES	SWC	SWC	SWC+sham ES	SWC+sham ES
ES groups:						
Mean patient age (years)	70.1	32.5	50.3	59	79.92	CESG = 79.35/C+AESG 79.65
Stage of PU	4	2–4	2–4	2–3	2–3	2–4 in both ES groups
Mean baseline WSA (cm²)	4.08	2.34	3.48	4.54	10.58	CES = 9.59/C+AES = 7.37
Mean duration of PU	Not reported	4.5 weeks	1.2 years	3.17 months	2.54 months	CES 2.41 mo/C+AES 2.65mo.
HVMPC methods:						
Positioning of electrodes:						
Treatment	On PU	On PU	On PU	On PU	On PU	On PU
Reference	30cm from PU	Distally from PU	At least 20cm from PU	At least 20cm from PU	At least 20cm from PU	At least 20cm from PU
Polarity of treatment electrode	Anode (reversed if healing progress was not observed)	Cathode	Initially cathode, then reversed weekly	Cathode 1-2 weeks, then anode	Cathode	CESG – cathode C+AESG – cathode for first week, than anode
Pulse duration/ frequency	100 µs/105 pps	Not reported /100 pps	50 µs / 100 pps for first 20 min; 10 pps for next 20 min	100 µs/100 pps	154 µs/100 pps	154 µs/100 pps
Electric charge	342 µC/s	500 µC/s	Not reported	Not reported	250 µC/s	250 µC/s
Duration of ES	45 minutes	60 minutes	40 minutes	50 minutes	50 minutes	50 minutes
Frequency of ES	1 a day/5 a week	1 a day/7 a week	8 a day/7 a week	1 a day/5 a week	1 a day/5 a week	1 a day/5 a week
Total time of ES per week	3.75 hours	7 hours	37 hours	4.16 hours	4.16 hours	4.16 hours
Period of treatment	7.3 weeks	20 successive days	12 weeks	6 weeks	6 weeks	6 weeks
Main outcomes:						
ES group (ESG)	100% PUs closed	WSA ↓ 80%	WSA ↓ 70% Closed: A: 100 % Stage 2 PUs; B: 33.3% Stage 3-4 PUs	WSA ↓ 88.9%	A: WSA ↓ 88.31 % closed: B: 45% Stage 2 PUs C: 17.65% Stage 3 PUs	A: CESG – WSA ↓ 82.34% A: C+AESG – WSA ↓ 70.77 % closed; B: 47.8% PUs in CESG B: 45% in C+AESG

(continued)

Table 1. Summary of controlled clinical high-voltage monophasic pulsed current (HVMPC) studies[a] in patients with pressure ulcers (PUs)

	Kloth and Feedar[10] (1988)	Griffin et al[11] (1991)	Houghton et al[15] (2010)	Franek et al[16] (2012)	Polak et al[17] (2016)	Polak et al[18] (2017)
Control group (CG)	WSA ↓ 28.9% over a mean period of 7.4 weeks	WSA ↓ 52%	WSA ↓ 36% Closed: A: 100% Stage 2 PUs; B: 7.1% Stage 3-4 PUs	WSA ↓ 44.4 %	A: WSA ↓ 54.65 % Closed: B: 35.29% Stage 2 PUs C: 6.25% Stage 3 PUs	WSA ↓ 40.53% Closed: B: 0% PUs
Level of significance (P)	Not reported	*P*(ESG:CG)=.05	A: *P*(ESG:CG) =.048 B: *P*(ESG:CG) =.62 C: *P*(ESG:CG) =.55	*P*(ESG:CG) =.00003	A: *P*(ESG:CG) =.046 B: *P*(ESG:CG) =.74 C: *P*(ESG:CG) =.60	A: *P*(CESG:CG)=.0006; A: *P*(C+AESG:CG) =.0124; A: *P*(CESG:C+AESG)=.9932; B: *P*(CESG:CG) = .013; B: *P*(C+AESG:CG) = .045; B: *P*(CESG:C+AESG)=.48

[a] *double-peaked impulses; amperage at sensory level*
ES=electrical stimulation; PU=pressure ulcers; SWC=standard wound care; WSA=wound surface area; ESG=electrical stimulation group; CG=control group; CESG=cathodal ES group; C+AESG=cathodal + anodal ES group; mo=months; no=number

treatment results, were obtained from the electronic hospital database. The information then was entered to a computer database enabling data analysis.

Wound assessment. Before treatment, patients' wounds were examined by a physician. Patients with PUs covered with eschar and with wounds showing signs of acute inflammation were excluded from the study. Patients with a PU covered with slough or with PUs in the granulation and epithelialization phases were included in the study. Wound depth was determined by a physician based on the criteria developed by the National Pressure Ulcer Advisory Panel[1] (Stage 2 PUs = partial-thickness loss of the dermis presenting as a shallow open ulcer with a red pink wound bed, no slough; Stage 3 PUs = full-thickness tissue loss and subcutaneous fat possibly visible but not the bone, tendon, or muscles; and Stage 4 PU = full-thickness tissue loss, muscle/bone exposed).

PU risk. Patients' risk of PU development was assessed with the Norton scale[30] and the Waterlow scale[31] with respect to factors such as patient gender and age, body composition and weight, mobility, concomitant diseases (primarily central nervous system injuries, diabetes, anemia), history of smoking, appetite and level of nourishment, and medications used (mainly anti-inflammatory drugs, cytostatics, and steroids).

Nutritional status. In assessing patients' nutritional status, malnutrition was defined as a state resulting from lack of intake or uptake of nutrition that leads to altered body composition (decreased fat free mass) and body cell mass leading to diminished physical and mental function and impaired clinical outcome from disease.[32] The primary diagnostic criteria of malnutrition included body mass index (BMI) <18.5 kg/m^2 as indicated by the underweight definition created by the World Health Organization or combined weight loss and reduced BMI.[33]

To determine patients' nutritional status, their blood samples also were tested for markers of metabolic disorders, anemia, thyroid dysfunction, impaired glycemic control, dehydration, protein deficit, hypoalbuminemia, vitamin level, indicators of inflammation (C-reactive protein), and nitrogen balance.[34,35] Diet was reviewed to assess intake of healthy and nonhealthy nutrients and fluid losses. Nutritional status was quantified by means of the Nutritional Risk Score (2002).[36]

Anemia. Anemia was diagnosed as hemoglobin level <13.4 g/dL in men and <12 g/dL in women. In cases where mean corpuscular volume (MCV) erythrocyte was <80 fl, blood iron concentration was determined. When MCV erythrocyte was above 100 fl, blood B_{12} concentration was tested.

Interventions. All patients in the cathodal ES, anodal ES, and placebo groups received SWC (prevention measures, wound care, and physical treatment) under the supervision of the physician and the principle investigator following best practices.[1,2,37] Each patient was assessed by an interdisciplinary team consisting of a physician, a nurse, a physiotherapist, and a dietitian.

Individualization. The team developed individual wound prevention and treatment programs in consideration of patients' needs regarding PU prevention and nutritional intervention, the optimization of the wound dressing protocol, and incontinence management. To protect the trial participants from developing more PUs, pressure-redistribution surfaces, foam devices, and pillows were used. Patients who were immobile were repositioned by a nurse or physiotherapist at least every 2 hours, and persons who could move were instructed to change position as often as they could. Malnourished patients received individual nutritional support. They were assisted during meals by a nurse or a medical assistant who made sure the quantity and quality of food and liquids they ingested followed the dietitian's guidelines. Nutritional supplementation with proteins, vitamins, and minerals was administered when necessary. Patients who did

not consume sufficient amounts of food received enteral or parental nutrition.[1]

Wound care protocol. Wounds were regularly assessed by the physician throughout the period of the study with a view to selecting topical treatments to appropriately address moisture control, bacterial burden, and debridement needs. When wound infection was suspected, a swab was taken to identify the bacteria and prepare an antibiogram.

Before ES was applied, necrotic tissue was removed from PUs with surgical/sharp, conservative sharp, or enzymatic debridement.

A moist wound environment was maintained consistently with hydrogel, hydrocolloid, or alginate dressings. Dressing type depended on the PU stage, phase of healing, the amount of wound secretion, and pain severity. Wounds covered with slough and granulating wounds were covered between ES procedures with alginate dressings (high or moderate exudate levels; surface and deep wounds) and hydrocolloid dressings (moderate or low exudate levels; surface wounds). Granulating and epithelializing wounds with minimal exudate were covered between ES procedures with hydrocolloid or hydrogel dressings.

Infected wounds were washed with antiseptics that included Octenilin Wound Gel (Schülke, Norderstedt, Germany); Octenisept solution (Schülke); and/or Actolind W Solution/Gel (Polvet Healthcare Teodorowski SJ, Laziska, Gorne, Poland). PUs also were washed with Kodan Tinktur forte solution (Schülke) and Skinsept (Ecolab, Monheim am Rhein, Germany). According to the information provided by the manufacturers of these antiseptic agents, none of them is cytotoxic to healthy cells.

Patients with elevated leukocyte levels received antibiotics selected according to the results of microbiological culture and sensitivity tests. All immobile patients received low-molecular-weight heparin.

Anodal ES group (AG). Patients in the AG were administered SWC and anodal HVMPC energy. The device used to deliver HVMPC was the Intelect Advanced Combo unit, Model 2771 (Chattanooga Group, Vista, CA) with 2 independent electrical circuits, of which only 1 was active. The device generated a twin-peak monophasic pulse (154 µs) consisting of 2 77-µs exponential pulses in rapid succession. Pulse frequency was set at 100 pps and voltage above 100 V for amperage of 0.36 A that did not elicit motor reactions. The electrodes delivered a 360 µC per second charge (1.08 C per day). Patients participated in 5 50-minute, once-a-day sessions held weekdays (Monday through Friday). All patients had a personal set of conductive carbon-rubber electrodes. During the procedure, the treatment electrode (5.0 cm x 10.0 cm) was placed on the wound and the return electrode (10.0 cm x 10.0 cm) was attached to healthy periwound skin at least 20 cm from the PU. Both electrodes were separated from the tissue by aseptic gauze pads saturated with physiological saline.

Cathodal ES group (CG). HVMPC protocol in the CG was almost the same as the AG protocol; the only difference was cathodal (not anodal) stimulation was used. The protocol of HVMPC in both ES groups was based on methods used in earlier clinical trials using anodal[10] and cathodal[11,14,17,18] ES in patients with PUs[10,11,17,18] and venous leg ulcers.[14]

Placebo ES group (PG). This group received SWC and sham ES. The arrangement of electrodes during the procedure was the same as in the ES groups. The monitor of the ES unit displayed all parameters, but because the electrodes were connected to the inactive electrical circuit current energy was not delivered to wounds.

The main physiotherapist connected the electrodes and selected the polarity of the treatment electrode. The procedure was performed in an inconspicuous manner so neither the patient nor the members of the medical team could see whether real or sham ES was applied. In the active ES groups, voltage was set above 100 V (the same value was displayed on the monitor for patients receiving sham ES), which did not cause muscle contractions, only weak tactile sensations. Because most patients in the groups had tactile sensory impairments and did not feel the current, patients in the sham ES group did not know they were not receiving treatment. All treatment sessions had the same duration and frequency (50-minute sessions, once a day, 5 times a week) and followed the same protocol whether sham or active ES was applied.

The electrodes were sterilized before and after each session in an approved disinfectant solution (Incidin Liquid and Sani-Cloth Active, Ecolab). As soon as the procedure ended, patients' wounds were thoroughly washed with a 0.9% sodium chloride solution and covered with SWC dressings as described earlier.

The trial design assumed wounds would be monitored for 8 weeks in all groups, representing the average length of stay in the facility. Patients hospitalized longer than 8 weeks were to be treated and monitored for wound healing as before. In patients with more than 1 PU, all wounds were treated, but only the deepest PU (the most advanced stage) was included in the study analysis.

Measures/data collection.

PSBF. PSBF was measured using a laser Doppler imager (PERIFLUX 5000, Perimed, Järfälla, Sweden) linked to a personal computer with the PeriSoft software for Windows (version 25.5; Galen Ortopedia Sp z o.o., PL, Bierun, Poland) that also was used for making computations and storing the results. A single point, infrared (IR), laser blood flow probe was used; it was calibrated before each measurement. To ensure the stability of readings, the laser was warmed for 15 minutes before measurement commenced. The probe was attached to the skin using a small piece of adhesive tape. Over the 4 weeks of treatment, 3 blood flow tests were conducted: before the first treatment session, at week 2 (after 10 ES procedures), and at week 4 (after 20 ES procedures). All measurements were conducted in the patient's room, usually in an ambient

Table 2. Wound outcome calculations

	Indicator	Formula	Abbreviation/description
Equation 1	Percentage change in PSBF at week 2 from baseline (%)	PSBF2-0 = (PSBF2 – PSBF0) x 100%/PSBF0	PSBF2-0 – Percentage change in PSBF at week 2 from baseline (%) PSBF2 – PSBF at week 2 [perfusion units] PSBF0 – PSBF at baseline (perfusion units)
Equation 2	Percentage change in PSBF at week 4 from baseline (%)	PSBF4-0 = (PSBF4 – PSBF0) x 100%/PSBF0	PSBF4-0 – Percentage change in PSBF at week 4 from baseline (%) PSBF4 – PSBF at week 4 (perfusion units) PSBF0 – PSBF at baseline (perfusion units)
Equation 3	Relative WSA at a given week of treatment (cm^2)	$WSA_{rel}(t) = WSA(t)/WSA(t=0)$	$WSA_{rel}(t)$ - relative WSA (cm^2])WSA (t) – WSA at given week's end (cm^2) WSA (t=0) – baseline WSA (cm^2)
Equation 4	Nonlinear approximation exponential function used to calculate T½	$WSA_{rel}(t) = WSA = 2^{-t/T_{1/2}}$	$WSA_{rel}(t)$ - relative WSA area (cm^2) t – week of treatment T½ – approximate time that WSA would need to decrease by half.
Equation 5	Percentage WSA reduction at week 8 (%)	PAR 8 = (WSA 0 – WSA 8) x 100%/ WSA 0	PAR 8 - percentage WSA reduction at week 8 (%) WSA 0 – initial WSA (cm^2) WSA 8 – WSA at week 8 (cm^2)
Equation 6	Percentage WSA reduction at week 2 (%)	PAR 2 = (WSA 0 – WSA 2) x 100%/ WSA 0	PAR 2 - percentage WSA reduction at week 2 (%) WSA 0 – initial WSA (cm^2) WSA 2 – WSA at week 2 (cm^2)
Equation 7	Percentage WSA reduction at week 4 from week 2 (%)	PAR 2-4 = (WSA 2 – WSA 4) x 100%/WSA 2	PAR 2-4 percentage WSA reduction at week 4 from week 2 (%) WSA 2 – WSA at week 2 (cm^2) WSA 4 – WSA at week 4 (cm^2)
Equation 8	Percentage WSA reduction at week 8 from week 4 (%)	PAR 4-8 = (WSA 4 – WSA 8) x 100%/WSA 4	PAR 4-8 percentage WSA reduction at week 8 from week 4 (%) WSA 4 – WSA at week 4 (cm^2) WSA 8 – WSA at week 8 (cm^2)

PSBF=periwound skin blood flow; WSA= wound surface area

temperature of 21° C to 22° C. Patients could assume a position that was comfortable for them (supine or sidelying). In preparation for the measurement of skin blood flow, the skin around the wound first was washed with aseptic fluid. Using the patient's head as the 12 o'clock reference point, the laser probe was attached to wound edges at 4 points (superior, inferior, right, and left side of the wound). At each point, blood flow was measured for 20 seconds. The temperature under the probe was maintained at all times at a constant level of 33° C. The authors of other clinical trials used a similar protocol to measure skin blood flow.[38]

Wound measurements. Wound (cm^2) measurements were taken and area calculated at baseline and after each week of therapy. If a PU closed before week 8 ended, the day it closed was recorded. The WSA was determined by tracing the wound shape onto acetate sheets and from the sheets onto rigid, transparent film for measurement with a planimeter. Measurements were processed by a digitizer (Mutoh Kurta XGT; ALTEK Information Technology Inc, (Spokane, WA) connected to a personal computer with the C-GEO software (version 4.0; Nadowski SoftLine, PL, Tychy, Poland) that also was used for making computations and storing the results. Measurement errors caused by irregular wound shapes ranged from 2.7% (for PUs of 70 cm^2 in size) to 37.9% (PUs <1 cm^2). The method used to estimate wound size has been presented in an earlier study.[16]

Primary outcome. The primary outcome of the trial was PSBF at weeks 2 and 4 of treatment and between-group differences in the flow. To compare the study groups, percentage changes in PSBF at weeks 2 (PSBF 2-0) and 4 (PSBF 4-0) were calculated using equations 1 and 2 described in Table 2.

Secondary outcomes. The secondary outcome was the rate of change in wound area. It was estimated as the nonlinear approximation of the time during which PU area would decrease from baseline by 50% (T½). First, the nonlinear approximation of the relative wound area in each week of treatment ($WSA_{rel}[t]$) was calculated to ensure the comparability of WSA change rates regardless of treatment length

(see equation 3 in Table 2). For the sake of illustration, the relative wound area of 20 cm^2 at week 0 (baseline) was calculated as $WSA_{rel}(0) = WSA\ (0)/WSA\ (t=0) = 20\ cm^2/20\ cm^2 = 1$. For a wound area of 15 cm^2 at week 1, the relative area is given by $WSA_{rel}(1) = WSA\ (1)/WSA\ (t=0) = 15\ cm^2/20\ cm^2 = 0.75\ cm^2$. In the next step, the nonlinear approximation was performed using the exponential model presented as equation 4 in Table 2. Percentage wound area reduction at week 2 and 8 also was calculated (see Table 2). Finally, the number of PUs closed during the 8-week study and duration of time to closure for ulcers that healed were analyzed, and correlations between PSBF and changes in wound area were examined.

Statistical analysis. All statistical analyses were performed by a person blinded to the ES devices using Statistica software (version 13.0, StatSoft Polska Sp. z o.o, Krakow, Poland). In all tests, the level of significance was $P \leq .05$.

Group sizes for the trial were determined through a pilot of this study during which 3 groups of 4 patients with PUs were treated with AG, CG, and PG. At week 2, percentage changes in PSBF from baseline were calculated and compared between pairs of groups: AG and PG and CG and PG. The greatest standard deviation of PSBF2 calculated for the groups (92.38%) and the smallest between-group difference for PSBF2 (75.00%) indicated that statistically significant between-group differences (at $P < .05$) in PSBF2 could be obtained with groups of at least 12 participants. Taking advantage of the fact that the number of patients willing to participate in the study was greater than originally planned, enrollment continued and the final sample was enlarged to 61 patients to reduce the risk of error if some of them dropped out before the end of treatment.

Because PSBF values obtained at the end of treatment were considerably different from those calculated with the pilot study data, the relative values of PSBF were subjected to statistical analysis to minimize the risk of baseline interpatient differences biasing the results of the main study. Relative values also were used to estimate changes in WSA at week 8 (PAR8) and the amount of time necessary for WSA to decrease by half ($WSA_{rel}[t]$) (nonlinear approximation) and to calculate correlations between PSBF and percentage reductions in wound area between weeks 0 and 2 (PAR 0-2), 2 and 4 (PAR 2-4), and 4 and 8 (PAR 4-8).

To retain data of all randomly allocated participants, an intent-to-treat analysis was performed. Data that were not available were approximated using an exponential regression function written as $WSA = b \exp(-at)$, where WSA is wound surface area; b and a are respectively, the regression constant and the exponential regression coefficient calculated for each patient using WSA (cm^2) obtained over the period of treatment; exp is the exponential regression function with a base of $e \approx 2.718282$ (the Euler's number); and t is the week of treatment. The function allows WSA decreases[39] to be described and can be calculated with data from at least 3 weeks. The exponential correlation coefficient proved negative for each patient and higher than 0.9 for the absolute WSA.

Patient characteristics were tested for normal distribution using the Shapiro-Wilk W-test, which showed their distribution was not normal. The Levene test revealed heterogeneity of variance. Despite the absence of normal distribution and because of low absolute values of skewness and kurtosis (<2.5), a mean was used as the central value and standard deviation as a measure of dispersion.

The homogeneity of patients' characteristics between groups was assessed using the maximum-likelihood chi-squared test, the analysis of variance (ANOVA) Kruskal-Wallis test, and the Kruskal-Wallis post-hoc test.

To compare test and control groups' mean PSBF2, PSBF4, and PAR8, the ANOVA Kruskal-Wallis test and the Kruskal-Wallis post-hoc test were employed. The nonlinear approximation was calculated using the exponential function (equation 4, see Table 2).

PSBF and PAR were tested for correlation with Spearman's rank order correlation.

The following correlations were calculated between:

1) skin blood flow at baseline (PSBF0) and the percentage change in wound surface area noted at week 2 of treatment (PAR 2; equation 6 in Table 2);
2) skin blood flow at week 2 (PSBF2) and a change in wound surface area from week 2 to week 4 (PAR 2-4; equation 7 in Table 2); and
3) skin blood flow at week 4 (PSBF4) and change in wound surface area from week 4 to week 8 (PAR 4-8; Equation 8 in Table 2).

Results

Of the 73 persons screened for the trial, 12 failed to meet the inclusion criteria. The other 61 persons were randomly assigned to groups AG (20), CG (21) and PG (20). Between 4 and 8 weeks of treatment, 19 individuals (31.15%) dropped out from the trial. A statistical analysis was applied to all data obtained, including data from patients who dropped out between weeks 4 and 8. The flow of participants through the trial is illustrated in Figure 1.

Baseline patient and wound characteristics.

Sample characteristics. Of the 61 patients enrolled in the trial, 27 were women (44.26%) and 34 were men (55.74%), age range 22–78 years. The risk of PU development, assessed with the Norton and Waterlow scales, was <14 points and >15 points, respectively, for all patients; 10 patients (16.39%) were obese (BMI >30) and 16 (26.23%) were considerably underweight (BMI <18.5); 45 patients (73.77%) were immobile and needed assistance to change positions; 23 (37.70%) smoked cigarettes before they became ill; and 35 (57.38%) were malnourished and administered nutrition therapy. Twenty-seven (27) patients (44.26%) had spinal cord injury, 32 (52.46%) had experienced a cerebral stroke, and 3 (4.92%) had a head injury. Tetra- or quadriplegia was diagnosed in 21 (34.43%) patients, 19 (31.15%) had paraplegia, and 20 (32.79%) had hemiparesis. Nineteen (19) patients

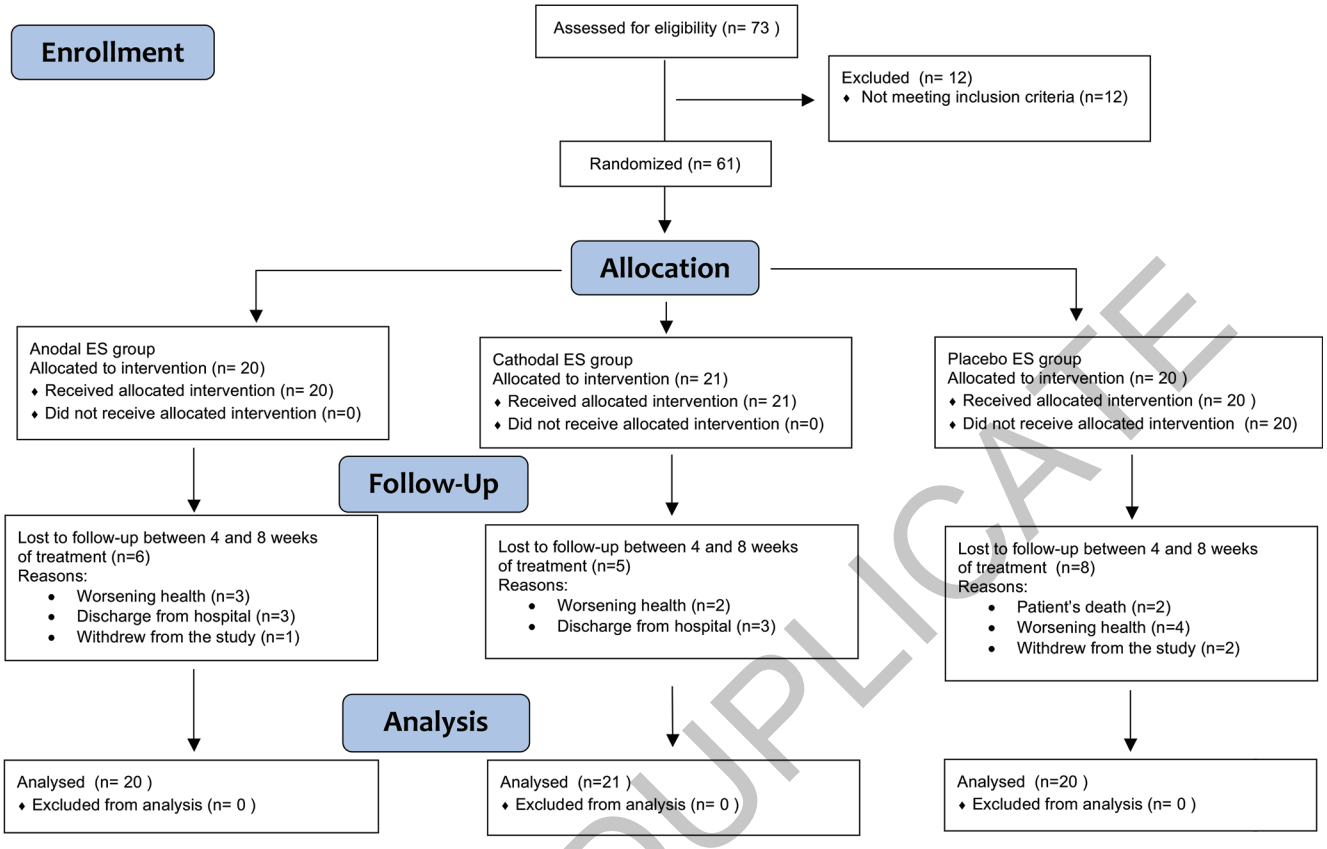

Figure 1. Flow diagram of the study.

(31.15%) had type 2 diabetes and 26 (42.62%) had anemia. The 61 PUs treated as part of the protocol ranged in size from 1.01 cm^2 to 59.57 cm^2; 9 were Stage 2 (14.75%), 38 were Stage 3 (62.29%), and 14 were Stage 4 (22.95%). Most PUs were located in the sacral region (45; 73.77%); 7 (11.45%) were located on the ischial tuberosity or the trochanter and 9 (14.75%) on the lower extremities (lower leg and foot). Forty (40; 65.57%) patients had more than 1 PU. The duration of the study PUs varied from 4 to 48 weeks. At baseline, most PUs (41; 67.21%) had started to granulate, 16 (26.23%) wounds were covered with slough, and in 4 wounds (6.56%) reepithelialization occurred. Between ES treatments, hydrogel dressings were applied in 38 (62.3%) patients, hydrocolloid dressings in 16 (26.2%) patients, and alginate in 7 (11.5%) patients. PSBF measured at baseline ranged from 9.33 to 86.08 perfusion units, and blood flow in healthy skin approximately 20 cm to 30 cm from the wound edges was 4.3 to 34.92 perfusion units.

According to the baseline demographic and wound characteristics of the patients (see Table 3), the groups were not significantly different for any of the characteristics considered ($P > .05$).

Anodal ES group characteristics. The AG included 20 patients (8 women, 12 men, average age 53.2 ± 13.82 years). Mean PU risk on the Norton and Waterlow Scales was 10 ± 2.63 and 31.3 ± 6.96, respectively. Two (2; 10%) patients were obese (BMI >30), 6 (30%) were underweight (BMI <18.5), 15 (75%) were immobile and needed assistance to change position, 8 (40%) smoked cigarettes, and 11 (55%) were malnourished and were administered nutrition therapy. Nine (9) patients (45%) had spinal cord injury, 10 (50%) had experienced a cerebral stroke, and 1 (5%) had a head injury. Six (6; 30%) patients had tetra- or quadriplegia, 7 (35%) had paraplegia, and 6 (30%) had hemiparesis. Five (5) patients (25%) had type 2 diabetes and 9 (45%) had anemia. The patients had a total of 20 PUs, mean size 17.88 ± 16.68 cm^2: 2 PUs were Stage 2 (10%), 13 Stage 3 (65%), and 5 Stage 4 (25%). Most PUs were located in the sacral region (15; 75%), 2 (10%) were located on the ischial tuberosity or the trochanter, and 3 (15%) on the lower extremities (lower leg and foot). The mean duration of the PUs was 13.9 ± 11.21 weeks. Twelve (12; 60%) patients had more than 1 PU. At baseline, most PUs (13; 65%) started to granulate, 6 (30%) were covered with slough, and reepithelialization occurred in 1 wound (5%). Between ES treatments, hydrogel dressings were applied in 13 (65%) patients, hydrocolloid dressings in 4 (20%), and alginate in 3 (15%). The mean PSBF was 46.42 ± 20.42 perfusion units, and blood flow in healthy skin approximately 20 cm to 30 cm from the edges of the wound was 12.53 ± 7.13 perfusion units.

Table 3. Baseline patient and pressure ulcer characteristics (N=61)

Variable	Anode ES group	Cathode ES group	Placebo ES group
Sample size (n)	20	21	20
[a]Gender (n): Female/Male	8 (40%)/12 (60%)	11 (52.38%)/10 (47.62%)	8 (40%)/12 (60%)
[b]Age [years]:			
Mean (SD)	53.20 (13.82)	55.67 (17.83)	52.50 (13.18)
Median/Lower - upper quartile	53.5/47-61	55/53-70	53/49-61
[a]Body mass index (BMI) (n, %):			
BMI >30;	2 (10%)	6 (28.57%)	2 (10%)
BMI <18.5	6 (30%)	5 (23.81%)	5 (25%)
[a]Norton scale (points)			
Mean (SD)	10 (2.63)	9.72 (2.31)	10.05 (1.96)
Median/Lower–upper quartile	10/8-12	10/8-12	10/8.5-12
[a]Waterlow scale (points)			
Mean (SD)	31.3 (6.96)	30.5 (6.83)	31.8 (8.13)
Median / Lower - upper quartile	30/27-37	30.5/25-34	32.5/25-37.5
[a]Etiology of neurological injury (n, %):			
Spinal cord injury	9 (45%)	8 (38.10%)	10 (50%)
Cerebral stroke	10 (50%)	13 (61.90%)	9 (45%)
Head injury	1 (5%)	1 (4.76%)	1 (5%)
[a]Tetra/Quadriplegia (n, %)	6 (30%)	6 (28.57%)	9 (45%)
[a]Paraplegia (n, %)	7 (35%)	7 (33.33%)	5 (25%)
[a]Hemiparesis (n, %)	6 (30%)	8 (38.10%)	6 (30%)
[a]Concomitant diseases (n, %)			
Diabetes (HbA1c <7%)	5 (25%)	8 (38.10%)	6 (30%)
Anemia	9 (45%)	11 (52.38%)	6 (30%)
[b,c]Malnourished (n, %)	11 (55%)	11 (52.38%)	13 (65%)
[a,b]WSA of PUs (cm^2)			
Mean (SD)	17.88 (16.68)	19.25 (16.47)	25.12 (17.42)
Median / Lower–upper quartile	10.68 /4.76-22.08	14.31 5.05-26.35	21.66/9.66-42.38
[b,c]Skin blood flow (perfusion unit)			
In the ulcer area			
Mean (SD)	46.42 (20.42)	37.37 (21.50)	45.61 (15.73)
Median/Lower–upper quartile	49/37.68-56	30.85/15.66-60.67	44.81/34.22-55.89
In healthy skin 30 cm from PU			
Mean (SD)	12.53 (7.13)	12.07 (7.52)	13.76 (4.54)
Median/Lower - upper quartile	10.64/8.01-14.41	10.26/6.23-17.15	13.82 / 10.23-16.36
[b,c]Duration of PUs (weeks):			
Mean (SD)	13.90 (11.21)	11.62 (8.98)	10.85 (8.59)
Median /Lower–upper quartile	9/8–13	8/8-12	8/6-12
[a]PU depth/stage (NPUAP staging, n, %)			
Stage 2	2 (10%)	4 (19.05%)	3 (15%)
Stage 3	13 (65%)	12 (57.14%)	13 (65%)
Stage 4	5 (25%)	5 (23.81%)	4 (20%)

Table 3. Baseline patient and pressure ulcer characteristics (N=61)

Variable	Anode ES group	Cathode ES group	Placebo ES group
[a]Location (n, %)			
Sacrum	15 (75%)	16 (76.19%)	14 (70%)
Ischial tuberosity or trochanter major	2 (10%)	2 (9.52%)	3 (15%)
Lower leg or foot	3 (15%)	3 (14.29%)	3 (15%)
[a]PU characteristic/stage of healing (n, %)			
Slough present	6 (30%)	4 (19.05%)	6 (30%)
Granulating	13 (65%)	16 (76.20%)	12 (60%)
Reepithelializing	1 (5%)	1 (4.76%)	2 (10%)

[a]chi-squared
[b]ANOVA Kruskall-Wallis Test
[c]post-hoc Kruskal-Wallis Test
In all tests, the between-group differences were not statistically significant (P >.05)
SD=standard deviation; ES=electrical stimulation; WSA=wound surface area; PU=pressure ulcer;NPUAP=National Pressure Ulcer Advisory Panel

Cathodal ES group characteristics. The CG included 21 patients (11 women, 10 men, average age 55.67 ± 17.83 years). The mean PU risk per the Norton and Waterlow Scales was 9.72 ± 2.31 and 30.5 ± 6.83, respectively. Six (6) patients (28.57%) were obese (BMI >30) and 5 (23.81%) were underweight (BMI <18.5). Eighteen (18) patients (85.71%) were immobile and needed assistance to change position, 6 (28.57%) smoked cigarettes, and 11 (52.38%) were malnourished and were administered nutrition therapy. Eight (8) patients (38.10%) had spinal cord injury, 13 (61.90%) had experienced a cerebral stroke, and 1 (4.76%) had a head injury. Six (6) patients (28.57%) had tetra- or quadriplegia, 7 (33.33%) had paraplegia, and 8 (38.10%) were diagnosed with hemiparesis. Eight (8) patients (38.10%) had type 2 diabetes and 11 (52.38%) had anemia.

The average size of the 21 PUs was 19.25 ± 16.47 cm^2; 4 were Stage 2 (19.05%), 12 were Stage 3 (57.14%), and 5 were Stage 4 (23.81%). At baseline, most PUs were located in the sacral region (16; 76.19%), 2 (9.52%) were on the ischial tuberosity or the trochanter and 3 (14.29%) on the lower extremities (lower leg and foot). The mean duration of the PUs was 11.62 ± 8.98 weeks; 14 patients (66.67%) had more than 1 PU. Most PUs (16; 76.19%) started to granulate, 4 (19.05%) wounds were covered with slough, and in 1 PU (4.76%) reepithelialization occurred. Between ES treatments, hydrogel dressings were applied in 11 (52.38%) patients, hydrocolloid dressings in 7 (33.33%) patients, and alginate in 3 (14.29%) patients. The mean PSBF was 37.37 ± 21.50 perfusion units, and blood flow in healthy skin approximately 20 cm to 30 cm from the edges of the wound was 12.07 ± 7.52 perfusion units.

Placebo ES group characteristics. The PG included 20 patients (8 women and 12 men, average age 52.50 ± 13.18 years). Mean PU risk as per the Norton and Waterlow Scales was 10.05 ± 1.96 and 31.8 ± 8.13, respectively. Two (2, 10%) patients were obese (BMI >30) and 5 (25%) were underweight (BMI <18.5); 13 (65%) were immobile and needed assistance to change position, 9 (45%) smoked cigarettes, and 14 (70%) were malnourished and were administered nutrition therapy. Ten (10) patients (50%) had spinal cord injury, 9 (45%) had experienced a cerebral stroke, and 1 (5%) had a head injury. Nine (9; 45%) patients had tetra- or quadriplegia, 5 (25%) had paraplegia, and 6 (30%) had hemiparesis. Six (6) patients (30%) had type 2 diabetes and 6 (30%) had anemia.

The mean size of the 20 PUs was 25.12 ± 17.42 cm^2; 3 were Stage 2 (15%), 13 Stage 3 (65%), and 4 Stage 4 (20%). Most were located in the sacral region (14; 70%), 3 (15%) on the ischial tuberosity or the trochanter, and 3 (15%) were on the lower extremities (lower leg and foot). The mean duration of the PUs was 10.85 ± 8.59 weeks; 14 patients (70%) had more than 1 PU. At baseline, more than half of the PUs in the study (12; 60%) started to granulate, 6 (30%) were covered with slough, and in 2 PUs (10%) reepithelialization occurred. Between ES treatments, 14 patients (70%) received hydrogel dressings, 5 (25%) received hydrocolloid dressings, and 1 (5%) received alginate. Mean PSBF was 45.61 ± 15.73 perfusion units, and blood flow in healthy skin approximately 20 cm to 30 cm from the edges of the wound was 13.76 ± 4.54 perfusion units.

Primary outcome. The cumulative change in PSBF flow after 2 weeks of treatment (PSBF2) was 109.52% (95% confidence interval [CI]: 44.59-174.45) for the AG, 131.54% (95% CI: 60.28-202.80) for the CG, and 35.83% (95% CI: 14.63-57.02) for the PG (see Table 4). The CIs of the mean PSBF2 values overlapped between AG:PG and CG:PG, but the mean PSBF2 calculated for the PG was smaller than the smallest CIs for the AG and the CG, showing that the results obtained for AG and CG groups were statistically more significant than those obtained for the PG. These results were supported by the ANOVA Kruskal-Wallis and post-hoc Kruskal-Wallis tests. The ANOVA Kruskal-Wallis test showed changes in PBSF were significantly different among the 3 groups (AG, CG, PG; $P = .0129$). The post-hoc Kruskal-Wallis test showed statistically significant differences between the AG and PG

Table 4. Periwound skin blood flow (N=61)

Group	Periwound skin blood flow			Percentage change in periwound skin blood flow	
	[Perfusion unit]	At week 2	At week 4	[%]	Between baseline and week 4
Anode ES group/AG N=20	46.42 (20.42) 27.59–47.16	79.82 (27.37) 67.01–92.63	64.66 (23.83) 53.51–75.81	109.52 (138.73) 44.59 – 174.45	89.30 (144.25) 21.79–156.81
Cathode ES group/ CG N=21	37.37 (21.50) 27.58–47.16	68.30 (30.75) 54.31–82.30	51.33 (27.83) 38.66–63.95	131.54 (156.55) 60.28 – 202.80	88.25 (153.90) 18.19–158.30
Placebo group/PG N=20	45.61 (15.73) 38.24–52.97	59.77 (25.58) 47.80–71.75	56.54 (16.62) 48.76–64.32	35.83 (45.29) 14.63 – 57.02	34.53 (44.08) 13.90–55.16
Level of significance				[a]*P*(AG:CG:PG)=.0129; [b]*P*(AG vs CG)=.9999; [b]*P*(AG vs PG)=.0470; [b]*P*(CG vs PG)=.0152;	[a]*P*(AG:CG:PG)=74 [b]*P*(AG vs CG)=.9999; [b]*P*(AG vs PG)=.9999; [b]*P*(CG vs PG)=.9999

[a]*ANOVA Kruskal-Wallis Test.* [b]*post-hoc Kruskal-Wallis Test; ES=electrical stimulation*

Table 5. Nonlinear exponential approximation of time necessary for wound area to decrease by half from baseline (N=61)

Group		$T_{1/2}$	Confidence interval		Significance level of the differences $T_{1/2}$ between groups
			-95%	+95%	
Anode ES group	AG	4.30	0.202	0.262	*P*(AG vs CG)>.05
Cathode ES group	CG	3.86	0.233	0.284	*P*(AG vs PG)≤.05
Placebo group	PG	9.86	0.087	0.116	*P*(CG vs PG)≤.05

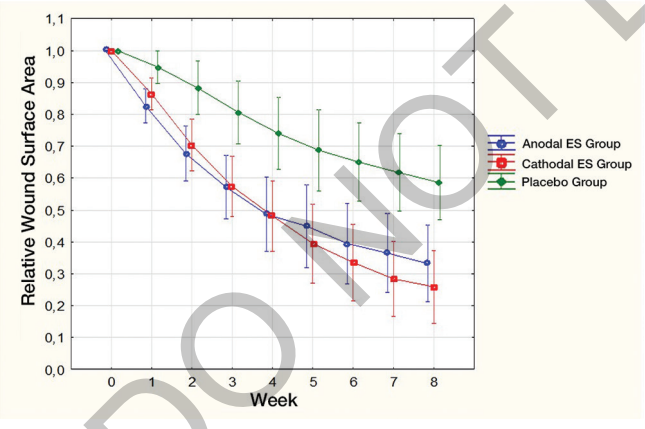

Figure 2. Nonlinear exponential approximation of the length of time necessary for wound area to decrease by half from baseline (dots correspond to observed mean values of relative wound surface area; whiskers represent 95% confidence interval). The length of treatment necessary for wound surface area to decrease from baseline by half (T1/2) was 4.30 weeks in the anodal group (AG), 3.86 weeks in the cathodal ES group (CG), and 9.86 weeks in the sham group (PG). The analysis of confidence intervals for T1/2 showed that the lengths were significantly different between the AG and PG (*P* <.05) and between the CG and PG (*P* <.05).

groups (*P* = .0470) and CG and PG groups (*P* = .0152). The AG and CG were not found to be significantly different from each other (*P* = .9999) (see Table 4).

The cumulative change in PSBF after 4 weeks of treatment (PSBF4) was 89.30% (95% CI: 21.79-156.81) for the AG, 88.25% (95% CI: 18.19-158.30) for the CG, and 34.53% (95% CI: 13.90-55.16) for the PG. Differences among AG, CG, and PG were not statistically significant: the ANOVA Kruskal-Wallis test showed *P* = .74, and the post-hoc Kruskal-Wallis tests showed that in AG versus CG *P* = .9999; in AG versus PG *P* = .9999; and in CG versus PG *P* = .9999 (see Table 4).

Secondary outcomes. The nonlinear approximation of treatment results showed that to decrease WSA from baseline by half (T½) would require 4.30 weeks of treatment (95% CI: 0.20-0.26) in the AG, 3.86 (95% CI: 0.23-0.28) in the CG, and 9.86 (95% CI: 0.09-0.12) in the PG. The analysis of CIs for T½ showed that the periods were statistically significant different between the AG and the PG and between the CG and the PG (*P* <.05) but not between the AG and the CG (see Table 5 and Figure 2).

The cumulative percentage area reduction after 8 weeks of treatment (PAR8) was 64.10% (95% CI: 50.42-77.78) for the AG, 74.06% (95% CI: 63.48-84.63) for the CG, and 41.42% (95% CI: 28.37-54.47) for the PG. The PAR8 for CG was significantly higher statistically than that obtained

Table 6. Pressure ulcer size (n=61)

Group	Mean (SD) 95% Confidence interval		
	Wound surface area (cm^2)		Percentage area reduction (%)
	Before treatment	At week 8	At week 8
Anode ES group/AG N=20	17.88 (18.68) 9.13–26.62	8.32 (11.76) 2.82–13.82	64.10 (29.22) 50.42– 77.78
Cathode ES group/ CG N=21	19.25 (16.47) 11.76–26.75	6.46 (8.97) 2.37–10.54	74.06 (23.23) 63.48–84.63
Placebo group/PG N=20	25.12 (17.42) 16.96–33.27	16.38 (13.90) 9.88–22.89	41.42 (27.88) 28.37 – 54.47
Level of significance			[a]*P*(AG:CG:PG)=.0026; [b]*P*(AG vs. CG)=.9999; [b]*P*(AG vs. PG)=.0391; [b]*P*(CG vs. PG)=.0024

[a]ANOVA Kruskal-Wallis Test; [b]post-hoc Kruskal-Wallis Test

Table 7. Correlationsa between periwound skin blood flow (PSBF) and percent wound area reduction (PAR) (N=61)

Variables	N	R	*P*
Anode ES group			
PSBF at week 0: PAR 2 (%)	20	-0.433	.036
PSBF at week 2: PAR2–4 (%)	20	0.122	.608
PSBF at week 4: PAR 4–8 (%)	20	0.389	.049
Cathode ES group			
PSBF at week 0: PAR 2 (%)	21	0.103	.656
PSBF at week 2: PAR 2-4 (%)	21	-0.064	.784
PSBF at week 4: PAR 4–8 (%)	21	0.318	.161
Placebo ES group (control)			
PSBF at week 0: PAR 2 (%)	20	0.064	.788
PSBF at week 2: PAR 2–4 (%)	20	-0.139	.557
PSBF at week 4: PAR 4–8 (%)	20	-0.120	.612

[a]Spearman's rank correlation; N=number; R=correlation coefficient; P =level of significance
PAR 2 percentage wound area reduction at week 2 in relation to baseline; PAR 2–4 percentage wound area reduction at week 4 in relation to week 2;PAR 4–8 percentage wound area reduction at week 8 in relation to week 4

for the PG, as indicated by nonoverlapping CIs of the mean PAR8 values and results of the post-hoc Kruskal-Wallis test ($P = .0024$). The CIs for the mean PAR8 values overlapped between AG:PG, but the mean PAR8 for PG was smaller than the smallest confidence intervals for AG, meaning that results obtained for the AG were also statistically more significant than those obtained for the PG. The finding was supported by the post-hoc Kruskal-Wallis test ($P = .0391$). No statistically significant differences were noted between the AG and the CG ($P = .9999$) (see Table 6).

Over 8 weeks of treatment, 4 of the 20 (20%) PUs in the AG, 7 of 21 (33%) in the CG, and 2 of 20 (10%) in the PG closed. Differences among the groups were not statistically significant: for AG versus PG, $P = .3721$; for CG versus PG, $P = .0645$; and for AG versus CG, $P = .3331$. Average duration of treatment to wound closure was 6.7 weeks (95% CI: 5.04-8.36) in the AG, 7.38 weeks (95% CI: 5.72-9.04) in the CG, and 6.05 weeks (95% CI: 4.92-7.18) in the PG. Differences among the groups (AG:CG:PG) were not statistically significant ($P = .5111$).

In the AG, PSBF at baseline (PSBF0) was negatively correlated with percentage wound area reduction after 2 weeks of treatment (PAR 0-2; $P = .0361$), meaning that greater baseline PSBF was associated with smaller reduction in wound area over the first 2 weeks of therapy. A positive correlation between PSBF at week 2 (PSBF2) and week 4 (PSBF4) and percentage wound area reduction between weeks 2 and 4 (PAR 2-4), as well as between weeks 4–8 (PAR 4-8), was indicative of a positive relationship between greater PSBF and a smaller reduction in wound area. In the AG, positive correlation between PSBF4 and PAR 4 to 8 was statistically significant ($P = .049$; see Table 7). In the CG, positive correlation (although not statistically significant [$P = .161$]) was established between PSBF at week 4 and PAR 4–8. For PG, no positive correlations between blood flow at weeks 2 and 4 and PAR 2–4 and PAR 4–8 were determined (see Table 7).

No adverse effects of applying HVMPC were observed in this study.

Discussion

Statement and principal findings. The trial has shown that in patients with neurological injuries an 8-week treatment program consisting of SWC plus anodal HVMPC and/or SWC plus cathodal HVMPC can increase PSBF and reduce PU surface area more significantly than SWC alone. PUs treated with SWC plus anodal and cathodal ES decreased in size by half, significantly faster than when only SWC was provided.

In this trial, increases in PSBF measured at weeks 2 and 4 of treatment were not significantly different between groups receiving anodal and cathodal ES, nor was percentage wound area reduction significantly different between groups after 8 weeks of treatment. This implies anodal and cathodal HVMPC have a similar effect on PSBF and PU area reduction. In both groups receiving HVMPC, the amount of blood flow in wound edges and WSA decrease were positively

correlated with each other. In the group treated with anodal HVMPC, a positive correlation between the amount of blood flow after 2 weeks of treatment and WSA decrease between weeks 2 and 4 was observed. In both ES groups, the amount of blood flow at week 4 of treatment positively correlated with WSA decrease between weeks 4 and 8. In the anodal ES group, the correlation was statistically significant. In the control group, positive correlations between the amount of blood flow after weeks 2 and 4 of treatment and WSA decrease between weeks 2 and 4 and 4 and 8 were not noted. More research is necessary to determine the exact role of wound blood flow changes observed with HVMPC and between anodal and cathodal HVMPC in PU wound healing.

The primary research outcome in this study was percentage change in PSBF after 2 and 4 weeks of treatment. After 2 weeks of applying HVMPC, PSBF increased in the AG by 109.52% and in the CG by 131.54%. Both rates were significantly higher than in the PG (35.83%). Between weeks 2 and 4 of treatment, PSBF slightly decreased in all groups studied, but after 4 weeks it was still higher than at baseline by 89.3% in the AG, 88.25% in the CG, and 34.53% in the PG. No clinical studies were found that compared how anodal and cathodal HVMPC influences PSBF and area reduction of PUs (or other chronic wounds).

Several clinical studies[22-24] on wounds of mixed etiology (including PUs and diabetic foot ulcers) showed biphasic currents increase PSBF and reduce wound area, results similar to those obtained in the present study. In these studies,[22-24] researchers applied biphasic charge-balanced sine wave current (30 Hz; 250 µs; 20 mA) for 30 minutes once a day, 3 times a week, for 4 weeks. Current was delivered via electrodes attached to the opposite wound edges. In these 3 studies,[22-24] as well as in the present study, current amplitude was set at sensory level without eliciting muscle contractions.

In the randomized clinical study by Lawson and Petrofsky,[22] ES was applied to 2 groups of 10 patients with Stage 3 and Stage 4 chronic wounds of mixed etiology. The groups included patients with type 2 diabetes (mean age 64.7 years; mean ulcer duration 10.6 months) and nondiabetic patients (mean age 55.3 years; mean ulcer duration 12.7 months). Both groups received ES. After 2 weeks of treatment, PSBF measured before an ES procedure in patients with diabetes was 35% greater than at baseline and significantly greater than in nondiabetic patients whose PSBF did not change over that period (P <.003). At week 4 of treatment, PSBF before ES in patients with diabetes was 21% greater than at baseline and in nondiabetic patients an increase of 18% was noted (P >.05). PSBF in patients with diabetes measured during ES was significantly greater than in nondiabetic patients after both 2 and 4 weeks of treatment. At week 2, the PSBF in patients with diabetes increased by 215%, as opposed to nondiabetic patients' whose PSBF did not change significantly (P <.003). PBSF measured at week 4 in patients with diabetes was 87% higher compared with only 6% in nondiabetic patients (P <.003). Lawson and Petrofsky[22] also noted that the 4-week healing rate for patients with diabetes was 70.0%, while the healing rate for the other patients was 38.4%, a statistically significant difference (P <.01).

Suh et al[23] conducted a pilot study without a control group where 18 persons (10 men, 8 women, mean age 35.7 years) with chronic ulcers of mixed etiology (mainly Stage 3 and Stage 4 PUs and diabetic ulcers; mean area 10.7 cm^2) unhealed after 26.1 months' duration received ES. Twenty (20) minutes before each ES procedure, the wound and periwound skin were warmed to 37° C using thermal energy from an IR heat lamp. This temperature was maintained until the ES procedure ended. After 4 weeks of treatment, a mean increase in PSBF recorded 20 minutes after the lamp was switched on was 9.3% (P >.05); the application of ES resulted in a significant increase in PSBF by an average of 15.6% (P <.05) after 30 minutes. During the 4 weeks of treatment, wound measurements decreased by 43.4% (P <.05). The authors also observed that PSBF increased from the beginning and well past the

middle of the study (2 weeks) (P <.05) but decreased toward the end of treatment (statistically, the changes were not significant;P >.05).

In a longitudinal, randomized, clinical study by Petrofsky et al,[24] 20 patients (mean age 48.4 years) with nonhealing diabetic foot ulcers (mean duration 38.9 months) were randomly divided into 2 groups. Groups were treated with local dry heat (heat group; n = 10; mean WSA was 28.2 cm^2) or local dry heat + ES (heat + ES group; n = 10; mean WSA was 24.1 cm^2). In both groups, local heating was provided by an IR lamp positioned 5 cm above the wound to warm the wound area to 37° C. Before treatment, the average blood flow was greatest in the center of the wounds. The average blood flow for all participants in both groups was 112.3 flux in the outside of the wound, 224.3 flux on the edge of the wound, and 385.7 flux in the center of the wound. On the first day of the study, the average blood flow from all 3 areas increased from baseline by 102.3% in the heat group and by 152.3% in the heat + ES group. By the last day of the study, the average blood flow in all 3 areas had decreased by 54.5% in both groups. Blood flow differences between the groups were not significant, but percentage wound area reduction in the heat + ES group at week 4 was significantly greater than in the heat group (68.4% and 30.1%, respectively; P <.05).

The authors of the cited studies[22-24] reported blood flow was greater after weeks 2 and 4 of treatment than at baseline. As in the current study, blood flow was at its highest at week 2 and then decreased. The authors also stated that after 4 weeks of treatment with biphasic current surface area of mixed etiology, wound size decreased from 38.4% to 70%. In the current study, 8 weeks of treatment with anodal and cathodal HVMPC reduced wound area by 64.10% and 74.06%, respectively.

In the cited studies,[22-24] blood flow was measured using laser Doppler imaging and computerized image analysis. A study on healthy people by Wikstrom et al[40] found both laser Doppler flowmetry and intravital video microscopy to be useful in studying the microcirculation in the healthy skin and in the skin around experimental blister wounds. In the present study, Doppler flowmetry was used; skin temperature under the probe head was 33° C. In the cited studies,[22-24] skin temperature during ES procedures and PSBF measurements was 37° C. In the study by Petrofsky et al[38] among healthy men, biphasic square wave ES (30 Hz; 250 µs; 15 minutes; 15 mA) increased skin blood flow when skin temperature was maintained at 30° C and 40° C, but at 20° C no significant change in skin blood flow was observed.

The secondary research outcome in the current study was the healing rate and percentage decrease in PU surface area, which also was used by the authors of other clinical studies evaluating the efficacy of wound treatment.[10-18] The percentage reduction in wound area noted at week 8 was significantly greater in the AG and CG than in the PG (64.10% in the AG, 74.06% in the CG, and 41.42% in the PG). The authors found the approximate length of treatment necessary to decrease area in Stage 2 to Stage 4 PUs by 50% was 4.30 weeks in the AG and 3.86 weeks in the CG — in both scenarios, times significantly shorter statistically than in the PG (9.86 weeks).

Thus far, no clinical studies have compared the influence of anodal and cathodal HVMPC or other electrical currents on the healing of PUs or other types of wounds. However, the results of the existing clinical research show both anodal and cathodal HVMPC promote the healing of PUs, which supports present results.[10,11,17,18]

In the study by Kloth and Feedar,[10] anodal stimulation with HVMPC + SWC (9 patients) decreased the surface area of Stage 4 PUs by an average of 44.8% a week. Wounds closed completely over a period of 7.3 weeks. In the control group (7 patients) that received sham ES + SWC, wound area increased by 28.8% over a mean period of 7.4 weeks.

In Griffin et al,[11] HVMPC + SWC decreased the area of 8 Stage 2 to Stage 4 PUs by 80% after 3 weeks of cathodal ES, a result that was significantly greater than in the control group (sham ES + SWC; 9 patients), where PUs decreased by an average of 52% (P <.05).

In the study by Polak et al,[17] 6 weeks of cathodal HVMPC + SWC (25 patients) decreased the area of Stage 2 to Stage 3 PUs by 88.31%, also significantly better than in the control group (sham HVMPC + SWC; 24 patients) where wound area decreased by an average of 54.65% (P = .046). The Polak et al[17] study also showed that in the group treated with cathodal HVMPC + SWC, 45% of Stage 2 PUs and 17.65% of Stage 3 PUs closed after 6 weeks of treatment compared with 35.29% of Stage 2 PUs and 6.25% of Stage 3 PUs in the control group (sham HVMPC + SWC). The results were not significantly different between the groups (P = .74 and P = .60, respectively).

In an additional study by Polak et al,[18] the surface area of 23 Stage 2 to Stage 4 PUs decreased after 6 weeks of cathodal HVMPC + SWC by an average of 82.34%. This result was significantly greater than in the control group (sham HVMPC + SWC), where average decrease in WSA was 40.53% (P = .0006). Cathodal HVMPC was therapeutically as effective as 1 week of anodal stimulation followed by 5 weeks of cathodal stimulation that reduced WSA by 70.77% (P = .9932). WSA decrease induced by anodal plus cathodal stimulation also was significantly greater than in the control group (P = .0124). In the group treated with cathodal HVMPC + SWC, 47.8% of Stage 2 to Stage 4 PUs closed over 6 weeks, compared with 45% of Stage 2 to Stage 4 PUs (P = .48) in the group receiving anodal plus cathodal HVMPC + SWC. In both ES groups, the percentage of PUs that closed was significantly greater than in the control group, where not a single PU closed (P = .013 and P = .045, respectively).

Implications for clinicians and policymakers. In designing the protocol for the application of HVMPC, the authors referred to solutions used by other authors.[10-18] After sterilizing, the treatment electrode was placed on the wound and

the return electrode on intact periwound skin at least 15 cm from the wound edge. Both electrodes were separated from the tissue by sterile gauze pads, which were moistened with physiological saline to improve electrical conductivity and maintain a moist wound environment.

Previous and present research showed HVMPC with twin-peaked pulses (50-154 µs, 100 pps) used to treat PUs,[10,11,15-18] venous leg ulcers,[12,14] and diabetic foot ulcers[13] is therapeutically efficient. In the study, 0.36 A and an electric charge of 360 µC/sec (1.08 C/day) in the voltage range from 100 to 150 V was applied; in other studies, the electrical charge ranged from 250 – 500 µC/sec (0.89 – 1.78 C/day).[10,11,17-20,41] Following the protocols used by other researchers,[10-18] the amperage used in the present study could be detected only by cutaneous receptors.

Most authors[10-12,14,16-18] applied HVMPC to wounds for 45 minutes to 60 minutes, once a day, 3 to 7 days a week, so treatment time ranged from 2.25 to 7 hours per week. The sessions in this study were similar in duration (50 minutes, 5 days/week; total treatment time of 4.16 hours of a week).

The results of preclinical ES reports[7,42-44] note the polarity of the treatment electrode is important in managing chronic wounds, but this clinical trial appears to be the first to compare PUs treated with anodal versus cathodal HVMPC. More clinical research is necessary to determine how the polarity of the treatment electrode influences wound healing. More research is also necessary to determine whether anodal or cathodal HVMPC accelerates wound healing by stimulating blood flow in the wound area. Future clinical trials also should investigate the influence of electrical currents on the concentrations of pro- and anti-inflammatory factors such as cytokines and growth factors in wounds.

Limitations

This study has several strengths. First, the research team (physicians, nurses, physiotherapists), the person in charge of measuring WSA, and the statistician were blinded as to treatment provided. Second, the participants were hospitalized at the same rehabilitation center, making it possible for the medical staff to supervise the uniform application of PU prevention measures and treatments and to ensure the ES protocol was observed at all times. Third, wound sizes were measured based on valid and reliable acetate tracings. Fourth, all patients completed at least 4 weeks of treatment, so PSBF4 and PAR4 could be calculated and compared for all of them. Finally, the intent-to-treat analysis employed

the exponential regression function, which allows WSA decreases to be precisely represented, to approximate the likely treatment results between weeks 4 and 8.

However, the study was not without limitations. A major limitation of the study is that the period of treatment was insufficient for all PUs to close. Consequently, length of treatment (ie, optimal treatment time of anodal or cathodal HVMPC to facilitate complete closure of Stage 2 to Stage 4 PUs) could not be determined. The blinding rate of patients and assessors was not assessed. Another limitation is the relatively high dropout rate between weeks 4 and 8 (31.15%); some patients were discharged from the hospital to be treated at home and a number of others were moved to other wards for treatment for concomitant diseases. This thwarted the monitoring of the healing of their PUs. The PU prevention and treatment program for all 3 groups generally followed the same best practice recommendations,[1,2,37] but its specific solutions addressed the needs of individual patients. Finally, the sample size in each group was too small to control for the potential effect of baseline variables such as ulcer depth/stage and start of treatment wound characteristics on study outcomes.

Conclusion

This study demonstrated that anodal and cathodal HVMPC with double-peaked impulses (154 µs; 100 pps; above 100 V; 360 µC/sec; 1.08 C/day) administered 50 minutes a day, 5 times a week, can be used in clinical practice to improve PSBF and promote healing of Stage 2 to Stage 4 PUs in patients with neurological injuries. These results are consistent with those obtained by other researchers who also reported HVMPC can improve the healing of chronic wounds, including PUs. However, this is the first study to document that type of ES (anodal or cathodal) did not affect wound blood flow and wound size reduction in patients with PUs.

Future clinical trials are necessary to elucidate the nature of the relationship between the stimulation of wound blood flow following anodal and cathodal HVMPC and change in wound area. ■

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