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# Antibacterial Activity of Positive and Negative Polarity Low-Voltage Pulsed Current (LVPC) On Six Typical Gram-Positive and Gram-Negative Bacterial Pathogens of Chronic Wounds

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## ABSTRACT

The positive effect of electrical stimulation (ES) on wound healing has been shown in vitro and in vivo. On the basis of increased blood flow, protein denaturation, and stimulation of cellular defense, an antibacterial effect of ES is to be expected. Although the antibacterial effect of ES already has been demonstrated in vitro, little attention has been paid to the direct antibacterial effect of changing polarity of the applied current. The aim of this study was to investigate the antibacterial effect of positive and negative monophasic low-voltage pulsed current on typical Gram-positive and Gram-negative pathogens of chronic wounds. Using the Dermapulse®-System, three Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) and three Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia faecium*) organisms were tested against positive and negative polarity low voltage pulsed current. All tested organisms were significantly reduced by ES. The reduction differed significantly between positive polarity and control and negative polarity and control, with the highest log10 reduction factor (RF) achieved with positive polarity. Using positive polarity, the maximum RF was measured for *E. coli* (median log10 RF 0.83; 25th percentile 0.59, 75th percentile 0.98) and the lowest for *S. epidermidis* (median log10 RF 0.20; 25th percentile 0.17, 75th percentile 0.24). Yet, there was no significant difference with positive ES against Gram-positive or Gram-negative organisms.

Electrical stimulation (ES) has been used for many medical applications, including accelerating wound healing. ES for treatment of wounds refers to the application of an electrical current through electrodes placed directly onto the skin in close proximity to the wound. ES has been used in wound healing since the 1960s. Its positive effect on the healing of wounds has initially been shown in vitro. Later, positive effects on wound healing were demonstrated both in vitro and in animal experiments.[1–5] In the past decade, they have also been shown in patients with chronic wounds.[6–8] Moreover, there have been reports on single treatments of burns,[9] malum perforantes,[5] and keloids.[10] In a randomized double-blind placebo-controlled multicenter study, it was shown that the healing time of Stage III chronic decubitus ulcers was twice as fast with ES compared with no ES.[11] Currently, use of ES is considered medically justifiable as a treatment of Stage III or Stage IV pressure ulcers, arterial ulcers, diabetic ulcers, and venous stasis ulcers when a 30-day trial of conventional wound management failed and when the procedure is performed in a medically supervised setting.[12] When discussing ES, however, it is important to distinguish the type of electricity and the waveform used for the protocol. The type of electricity transferred is controlled by the electrical source. On the basis of the type of current, ES can be categorized as (a) alternating current (AC), (b) high-voltage pulsed current (HVPC), and (c) low-intensity direct current. Although there are many waveforms available in electrotherapy equipment, the one that has the most detailed and consistent evaluation in vitro, in animal studies and in controlled clinical trials, is monophasic HVPC. HVPC devices also allow selection of polarity and variation in pulse rates both of which seem to be important in wound healing.[13]

With negative polarity of the electrode, ES has been shown to have the following effects: stimulation of granulation tissue, increased blood flow with decrease of edema and necrotic tissue, proliferation of fibroblasts, production of collagen, and migration of neutophils and epidermal cells. With positive polarity of the electrode, ES stimulates epithelialization, induces blood clotting and blockage of small blood vessels, denatures proteins,

reduces mast cells in the wound, and induces migration of macrophages into the wound bed.[13] Kloth and McCulloch[14] have pointed out that changing polarity of the electrode is especially important when healing of the wound has slowed down or comes to a stand-still. Usually treatment is commenced with negative polarity of the electrode for the first 7 days and is then continued for at least 3 days of positive polarity or alternating polarity daily.[15,16]

On the basis of increased blood flow, protein denaturation, and stimulation of neutrophils and mast cells, a direct and indirect antibacterial effect of ES is to be expected. Although the antibacterial effect of ES already has been demonstrated in vitro against *Staphylococcus aureus*,[17]*Escherichia coli*, and *Pseudomonas aeruginosa*,[18] little attention has been paid to the direct antibacterial effect of changing polarity of the applied current, apart from one study investigating four different ES conditions against one microorganism.[17]

The aim of this study was thus to investigate the antibacterial effect of positive and negative monophasic low voltage pulsed current (LVPC) on typical Gram-positive and Gram-negative bacterial pathogens of chronic wounds.

# MATERIALS AND METHODS

## Test bacteria

As typical pathogens of wound infections, *E. coli* (ATCC 11229), methicillin-sensitive *S. aureus* (ATCC 6538), and *P. aeruginosa* (ATCC 15442) were tested; as representatives for colonization of chronic wounds (i.e., ulcus decubitus), *Enterococcus faecium* (ATCC 6057), *Klebsiella pneumoniae* (neonatal skin isolate IHU 05/2036), and *Staphylococcus epidermidis* (adult skin isolate, IHU 04/1853) were used.

## Application device

The hydrogel electrodes (Type WoundEL®, Gerromed, Hamburg, Germany) were composed of a moderately absorbing hydrogel surface (also suitable for application directly onto the wound), a medium layer of conducting thin silicone–carbon and a cover layer made of polyurethane foam. For the test, the electrodes were cut into small pieces of 2.5 cm × 2.5 cm (6.25 $cm^2$). The specific electro-physical properties of the test model are shown in **Table 1**.

**Table 1.** Electrical operating characteristics of the Dermapulse® device

| **Physical parameter** | **Characteristics** |
|---|---|
| Pulses | Monophasic, square-wave shaped |
| Pulse rate–Hz (pulses per second) | 128 Hz |
| Polarity–positive or negative | Selectable |
| Charge per pulse | Max 5.9 µC |
| Wave-length | 140 µ/seconds |
| Intensity | 42 mA |

## Test procedure

To simulate a wound without influence of antibacterial effects caused by humoral and cellular human defense mechanisms, sterile 100% cotton patches 2.5 cm × 2.5 cm (6.25 $cm^2$) were used. Three hundred microliters of each bacterial suspension (concentration $1.2 \times 10^4$ colony forming units (CFU)/mL)[19,20] were pipetted onto a cotton patch and placed onto a 58 cm × 40 cm × 4 mm-sized sterile stainless steel panel. The cotton patches were covered with the electrodes and a sterile glass slide to improve contact between the electrode and the cotton patches. Then, four of the six electrodes were connected to the Dermapulse® electro-stimulation

device (Kinberg Enterprises Ltd., Richmond, BC, Canada) and a current with an intensity of 42 mA and a pulse rate of 128 Hz was set for 30 minutes. This procedure simulates the clinical application.

To determine differences in polarity, two of the four connected electrodes in each test were connected with the positive and two with the negative poles. As negative controls, the two remaining cotton patches were covered with electrodes as described but not electrically connected. After 30 minutes of ES, the electrodes were removed and each patch was vortexed for 60 seconds in 20 mL of Casein soja pepton bouillon (CSL, Oxoid, Wedel, Germany). 34.5 µL of the suspension were plated onto Columbia blood agar (Oxoid, Hampshire, UK) by a spiral plater (Meintrup DWS Laborgeräte, Laehden, Germany) and incubated at 36°C for 24 hours.

### Statistical design and calculation

Inter-test reliability was determined by repeating each experiment at least 32 times. All counts were then averaged to obtain a value for the number of surviving bacteria expressed as CFU using a repeated measures analysis of variance (ANOVA). The log10 reduction factor of CFU reduction factor (RF), after exposure to positive polarity ES and negative polarity ES was calculated as log10 CFU control (no ES) minus log10 CFU test. To compare differences in bacterial reduction, a two-sided *T*-test for independent samples for significant difference testing was used. All statistical tests were set at a power of 0.8 and $\alpha \leq 0.05$ using SPSS for Windows® (SPSS for Windows ® version 12.0, SPSS Inc., Chicago, IL).

## RESULTS

The antibacterial effects of positive and negative polarity ES against *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, and *E. faecium*, represented by the log10 RF of CFU, are show in **Table 2**.

**Table 2.** Log10 RF of positive and negative polarity ES against tested microorganisms

| **Bacteria** | ***S. aureus*** | | ***S. epidermidis*** | | ***P. aeruginosa*** | | ***E. faecalis*** | | ***K. pneumoniae*** | | ***E. coli*** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Polarity** | **+** | **–** | **+** | **–** | **+** | **–** | **+** | **–** | **+** | **–** | **+** | **–** |
| Maximum | 0.69 | 0.29 | 0.33 | 0.27 | 0.5 | 0.30 | 0.59 | 0.44 | 0.45 | 0.36 | 1.30 | 0.31 |
| Minimum | 0.09 | 0.05 | 0.07 | 0.02 | 0.15 | 0.03 | 0.09 | 0.01 | 0.15 | 0.01 | 0.22 | 0.03 |
| Mean | 0.35 | 0.15 | 0.21 | 0.17 | 0.33 | 0.16 | 0.34 | 0.15 | 0.28 | 0.13 | 0.77 | 0.16 |
| Standard Deviation | 0.14 | 0.06 | 0.06 | 0.07 | 0.10 | 0.06 | 0.14 | 0.14 | 0.73 | 0.08 | 0.28 | 0.08 |
| Median | 0.34 | 0.15 | 0.20 | 0.17 | 0.34 | 0.17 | 0.33 | 0.10 | 0.27 | 0.12 | 0.83 | 0.16 |
| $25^{th}$ Percentile | 0.26 | 0.09 | 0.17 | 0.13 | 0.25 | 0.12 | 0.23 | 0.02 | 0.25 | 0.07 | 0.59 | 0.10 |
| $75^{th}$ Percentile | 0.38 | 0.20 | 0.24 | 0.22 | 0.41 | 0.22 | 0.43 | 0.28 | 0.33 | 0.18 | 0.98 | 0.22 |
| Interquartile range | 0.12 | 0.11 | 0.07 | 0.09 | 0.16 | 0.10 | 0.19 | 0.26 | 0.08 | 0.11 | 0.39 | 0.12 |

No antibacterial effects were found in the control cultures that contained bacteria but were not subjected to ES. Compared with no ES, all tested organisms were significantly ($p$<0.01) reduced by positive or negative polarity ES. However, the reduction differed significantly ($p$=0.02) between positive and negative polarity, with the highest log10 RF achieved with positive polarity. Using positive polarity, the maximum RF was measured for *E. coli* (median log10 RF 0.83; 25th percentile 0.59, 75th percentile 0.98) and the lowest for *S. epidermidis* (median log10 RF 0.20; 25th percentile 0.17, 75th percentile 0.24). Yet, there was no significant difference of positive ES against Gram-positive ($p$=0.35) or Gram-negative ($p$=0.71) organisms (**Figure 1**).

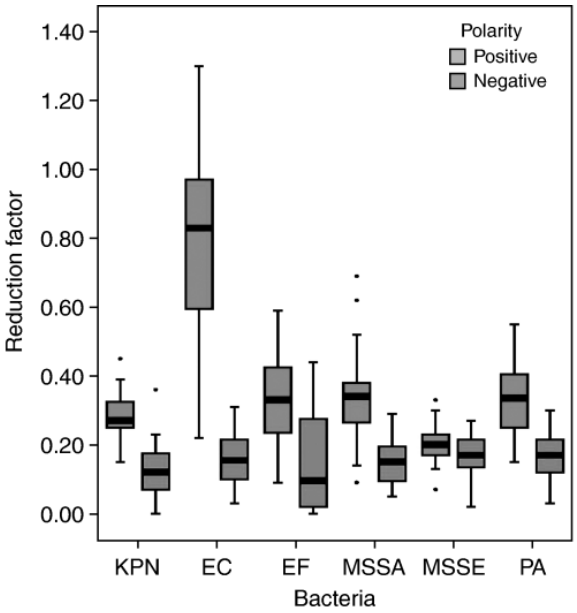


**Figure 1** Median reduction factors of the log10 RF (together with 25th and 75th percentile and minimum and maximum) obtained by ES with negative (green) and positive (blue) polarity of the electrodes and distribution on cotton patch for different Gram-positive and Gram-negative bacterial species. KPN, *Klebsiella pneumoniae;* EC, *Escherichia coli;* EF, *Enterococcus faecium;* MSSA, Methicillin-sensitive *Staphylococcus aureus;* MSSE, Methicillin-sensitive *Staphylococcus epidermidis;* PA, *Pseudomonas aeruginosa*.

# DISCUSSION

The use of ES in promoting wound healing is based on the theory that it may (a) increase ATP concentration in the skin, (b) increase DNA synthesis and attract epithelial cells and fibroblasts to wound sites, (c) accelerate the recovery of damaged neural tissue, (d) reduce edema and increase blood flow, and (e) inhibit growth of possible pathogens. Hence, the effect of ES is due to a combination of different physical and biochemical processes, which can reestablish the normal repair potential of the skin and support the natural wound healing process. Furthermore, it limits excessive inflammation and increases the synthesis of collagen in the wound.[21,22] The direct antibacterial effect of ES is supported not only by in vitro studies,[1,5,17,18,23] but also by the observation that ES has an indirect positive effect on wound healing, especially of chronic wounds. This is plausible since every infection delays wound healing by exacerbated release of bacterial toxins and proinflammatory mediators. In vivo, additional factors influence the positive effect on wound healing and indirect antibacterial action, e.g., chemotaxis of neutrophils and macrophages, increased blood flow, and alteration of membrane potentials.[13]

Our study showed that ES has a direct antibacterial effect on Gram-positive and Gram-negative tested bacteria; however, this effect is much lower as compared with wound antiseptics.[24] The highest log10 RF was observed for *E. coli*, a Gram-negative isolate frequently found in decubital ulcera around the sacrum. Since the mid 1990s, it has been believed that Gram-positive bacteria appear to be more “resistant” to ES than Gram-negative

bacteria, such as *P. aeruginosa*.[13,25] It was hypothesized that the enhanced "resistance" of Gram-positive bacteria may perhaps be because of their cell wall composition, and therefore it was assumed that ES may have a lower antibacterial effect on Gram-positive bacteria.[17] Yet, we were not able to confirm that there was a significant difference overall between Gram-positive and Gram-negative organisms.

Furthermore, we could demonstrate that positive polarity has a higher antibacterial effect than negative polarity. This observation is in contrast to the only study also investigating the effect of positive and negative polarity ES against *S. aureus*.[17] Recently, Merriman et al. have investigated the inhibitory effect of four different ES current types (ES-continuous microamperage direct current, HVPC, low-voltage monophasic milliamperage pulsed current, and low-voltage biphasic milliamperage pulsed current) on bacterial growth in vitro. The authors found an inhibitory effect for HVPC and continuous microamperage direct current at both poles, while no effects were found for low-voltage monophasic milliamperage pulsed current or low-voltage biphasic milliamperage pulsed current at either pole. The authors concluded that an effect could be observed for stimulation type, but not for polarity, and consequently, they did not discuss this aspect further. In much older studies other authors, on the other hand, also noted that positive polarity exhibited a higher antibacterial effect than negative polarity. The primary investigation of Barranco et al.[23] focused on the comparison of several types of electrodes (silver, platinum, stainless steel, and gold) using an in vitro system. In a subset analysis, they found that the positive silver electrode provided the highest level of antibacterial effect and the lowest level of toxicity. The reason for the difference in results through changing polarity is unknown. Its importance in vivo is uncertain to date. Under clinical conditions, ES is applied using negative polarity during the first seven days of therapy followed by at least 3 days of positive or daily alternating electrode polarity. The reasons for this are the better effects of negative electrode polarity on blood flow, the attraction of neutrophils, which support wound debridement, anti-inflammatory activity, and edema reduction. It can be assumed that, despite the lower antibacterial effects of negative polarity, this polarity will additionally support the *in vivo*-induced indirect antibacterial effects.

Our study has several limitations. One limitation pertains to the possible influence of different doses and different application times on bacterial elimination. Dose–time dependencies are well known for antimicrobial compounds. The presented data using an intensity of 42 mA at a pulse rate of 128 Hz for 30 minutes cannot rule out possible differences in antibacterial effects using other settings. We decided to test the application device's maximum possible intensity of positive and negative polarity at 42 mA for 30 minutes application time, which is recommended by the manufacturer. A second limitation is the use of a cotton patch for simulation of a wound. Previous in vitro studies have used culture plates[1,5,17,18] and one study[23] used fluid of the anode bath to assess viable bacterial counts. In this respect, we believe that a cotton patch is a better surrogate of wound tissue than culture plates or fluid of an anode bath. However, using a cotton patch is debatably less advantageous than using organic material like contaminated skin biopsies, which would perhaps have been a better alternative. Regrettably, at the moment there is no generally accepted in-vitro wound model. Such a model, however, would have the advantage that several studies on antibacterial efficacy of various wound therapies could be conducted generating comparable data. Because additional experiments are needed to support a possible antibacterial role during wound treatment, establishing a standardized in vitro test protocol would be of value for the scientific community.

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