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ABSTRACT: Currently, electrical stimulation is an accepted method used clinically to promote chronic wound healing. A literature review revealed that similar therapeutic current has been shown to suppress growth of common wound pathogens in vitro and in vivo. To date, little has been reported on the factors contributing to the antibacterial effects of microamperage direct current (μADC) stimulation. The purpose of this project was to investigate the role of electric field strength, current density, pH, and type of electrode used in vitro, to gain a better understanding of how these factors contribute to inhibiting growth of select wound pathogens. μADC was applied via silver electrodes at amplitudes ranging from 26 μA to 800 μA in an in vitro system consisting of Staphylococcus aureus *and* Pseudomonas aeruginosa. *Results suggest that transmission of μADC by silver wire inhibits bacterial growth around the anode, and that the area of inhibition is directly proportional to the size of the electrode used. Current amplitude (as a function of electric field strength and current density) and pH did not seem to cause the antibacterial effects observed in this study.*

INTRODUCTION

Clinically, wound healing is impeded when infection is present. The use of electrical stimulation to inhibit or destroy wound pathogens in vitro and in vivo has been documented. Rowley[1] produced bactericidal effects in vitro on *Escherichia coli B* growth rates using cathodal μADC. Rowley and colleagues[2] demonstrated a similar effect using 1000 μA of direct current (DC) on rabbit skin wounds infected with *Pseudomonas aeruginosa*. In another report by Barranco and coworkers,[3] the use of cathodal DC produced a decrease in *Staphylococcus aureus* growth rates in infected rat and rabbit femurs after 1 hour of electrical stimulation. More recently, Kincaid and Lavoie[4] reported that the growth of three microorganisms commonly found in human wounds was inhibited in vitro at both the anode (positive) and cathode (negative) when exposed to high-voltage pulsed current for 2 hours at 250 V. Unfortunately, this voltage amplitude would be intolerable if used on infected wounds in humans.[4] Inhibition of bacterial activity at both the anode and cathode has also been reported for pulsed DC.[5]

Wheeler and associates[6] used continuous cathodal μADC to suppress proliferation of pathogens in clinically noninfected human pressure ulcers during the first 3 days of treatment, and also began and continued cathodal treatment of infected ulcers until resolution of infection was determined by pathogen-free cultures. The authors proposed two mechanisms by which cathodal DC stimulation decreased pathogens. First, they postulated that continuous cathodal DC bombarded organisms with electrons that continually excited cell membranes. They suggested that this stimulation depletes the bacterial substrates and results in death of the organism. The second mechanism they proposed was disruption of intracellular metabolic processes.

Galvanotaxis, or the attraction of cells to the anode or cathode, has been reported in a number of in vitro studies. Macrophages migrate toward the anode,[7] while neutrophils migrate toward both the anode and the cathode.[8,9] However, Dineur[10] and Monguio[8] have reported that leukocytes migrate toward the cathode in regions where infection or inflammation is present. Perhaps the documented antibacterial effect of continuous cathodal μADC is the result of Galvanotaxic attraction of phagocytic macrophages and leukocytes to infected tissues rather than from detrimental effects of pathogens caused by electrolysis or altering the tissue pH.[2]

Other in vitro and in vivo studies have reported that metallic electrodes have antibacterial properties, especially when electrical current is passed through the electrode.[11-23] Guffey and Asmussen[5] used a DC generator to deliver 1 mA to 10 mA of DC, pulsed at 100 pps to *Staphylococcus aureus* in vitro for two 30-min sessions through a stainless steel electrode. They reported that zones of bacterial inhibition occurred with current amplitudes of 5 mA and 10 mA at both the anode and cathode. Barranco et al[3] have reported that a positively charged silver electrode demonstrates excellent inhibitory capacity and negligible toxic effects compared with electrodes made of surgical stainless steel, pure platinum, and gold. Parcilleux and Sicard[12] demonstrated that the lethal effects of alternating current on *Escherichia coli* is mainly due to the toxicity of metal ions from metallic electrodes and not from the alternating current. Other authors have reported that antibacterial activity occurring in the presence of an anode and cathode results from metallic Ag^+ cations deposited in the medium by low levels

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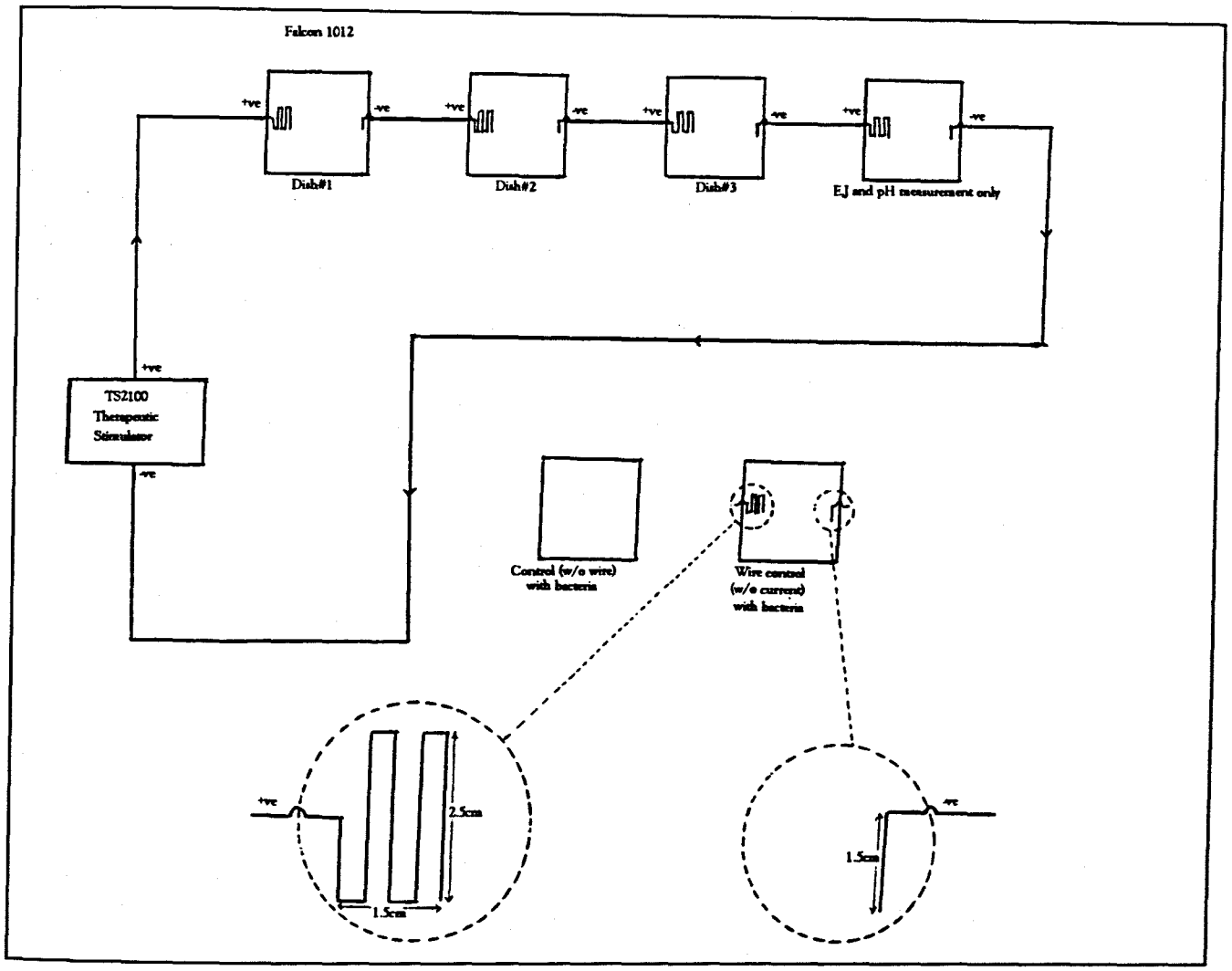

FIGURE 1. Schematic of experimental set up.

of direct current.[11-23] Collectively, these latter reports suggest that the use of μADC delivered with silver electrodes may be clinically beneficial in killing bacteria in the treatment of infected or contaminated wounds.

To date, little has been reported on the factors contributing to the antibacterial effects of μADC stimulation. The literature does not describe the mechanisms by which electrical stimulation suppresses bacterial growth. Kincaid and Lavoie[4] have proposed that the antibacterial effect may be due to pH changes in the medium surrounding the electrodes, whereas others have suggested it is due to the current density[5] and/or the electrodes used.[11-23] The relationship between current density and the zone of inhibition of bacterial growth also needs to be resolved. These uncertainties need to be clarified for a better understanding of the use of μADC for its antibacterial effect in the treatment of infected or contaminated wounds. Therefore, the purpose of this project was to investigate the role of pH, electric field strength, current density, and type of electrode used in vitro to gain a better understanding of the contributions these factors make on inhibiting growth of selected wound pathogens.

METHODS

The μADC device used in this study was the TS 2100 Therapeutic Stimulator.* This device, when used in the DC mode, delivers constant current ranging from 100 μA (0.1 mA) to 5000 μA (5 mA). Sterile, pure silver wires† were positioned as shown in Figure 1. The positive electrode was configured into a 1.5 cm "square," and the negative electrode was simply a straight wire, 1.5 cm in length.

Staphylococcus aureus (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were grown for 18 to 24 hours at 37°C in tryptic soy broth.†† Each suspension was diluted to a final concentration of 1 x 10^7 colony-forming units per mL. Molten tryptic soy agar, of sufficient quantity to cover the electrodes, was inoculated with the organism, poured into a sterile petri dish, and allowed to

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harden. A square petri dish, was used instead of the classical round dish because equipotential lines that represent the electric field in the culture medium are less distorted.

For test runs, wires in the dishes were connected in series by alligator-clip leads to the TS 2100 Therapeutic Stimulator. Currents of 26 μA, 100 μA, 300 μA, 500 μA, and 800 μA were applied to the test organisms for a 30-min duration by passing current through the culture medium. Each of the two bacteria was tested in three separate culture dishes at each individual current level. Each dish was then incubated at 37°C for 18 to 24 hours. The current of 26 μA was obtained by a simple current division method. The other current levels have been used in other studies to determine the effect on wound healing.[6]

After incubation, each culture was observed for clear zones of growth inhibition surrounding the anode and cathode, the inhibition (clear) zone paralleling the wire electrode was noted, and photographs were taken of observed inhibition zones. Subcultures from the zone of inhibition were checked for sterility by transferring the medium with a sterile scalpel into tubes of sterile tryptic soy broth and incubating them at 37°C for 18 to 24 hours. Visible turbidity was used as an indication of growth.

Changes in pH (range = 3.0 - 12.0) of the culture medium were determined by using pH paper touched to the solid surface of uninoculated media at each 10-min interval during the 30-min test run. Another pH check was carried out on inoculated plates after incubation. Determination of the actual field strength and current density was made using a multimeter. Voltage drops across the electrodes were also determined with a multimeter by measuring the voltage across the culture media between the terminals. All measurements were made on an uninoculated culture medium to prevent contamination of the test organisms.

Control plates (seeded plates with and without wire) were incubated without current exposure to determine whether the wire itself would have an antibacterial effect. Corrosion and media discoloration were noted by visual examination. Gas formation was considered to be present when bubbles were noted around the electrodes during stimulation.

RESULTS

Table 1 shows both the electric field strength (E theoretical) and current density (J theoretical) to be much lower than the electric field strength (E measured), E (electrode), and current density (J measured) respectively.

Table 2 shows that the anodic silver electrode produced growth inhibition zones at all current amplitudes below 500 μA. It was found that the area of the inhibition was directly proportional to the size of the electrode used. At 800 μA, inhibition zones occurred at both the positive and negative electrodes. There was no observable corrosion or discoloration of media at either electrode. Gas formation did occur at the cathode when the current levels were 300 μA and above. As for the silver wire control, pure silver wire by itself was found to exhibit no antibacterial activity. The μADC was found to be bacteriostatic with *Staphylococcus aureus*, and bactericidal with *Pseudomonas aeruginosa*. The pH was found to be acidic at the anode, and basic at the cathode at the end of the 30-min run time. It was also found that the higher the current level used, the more acidic the media became around the anode and more basic around the cathode. However, the pH value reverted back to pH 8.5 after 24 hours of incubation, regardless of the pH obtained during the earlier 30-min test run.

TABLE 1. Summary of electric field strength and current density measurement.

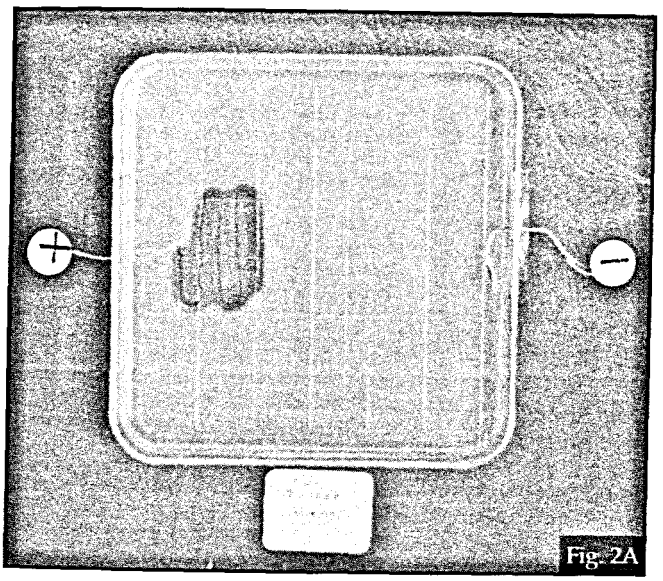
	I = 26 μA	I = 100 μA	I = 300 μA	I = 500 μA	I = 800 μA
E (Theoretical) mV/cm	0.92	3.53	10.6	17.6	28.2
E (Measured) mV/cm	38.01	32.02	29.48	30.75	30.02
E (Electrode) mV/cm	147.0	163.0	185.0	213.0	228.0
J (Theoretical) mA/cm²	7.2×10^{3}	0.028	0.083	0.139	0.222
J (Measured) mA/cm²	0.299	0.252	0.232	0.242	0.236

E (Theoretical): Value obtained via equation; E = (current introduced * resistivity)/(cross sectional area).
E (Measured): Actual value recorded with Fluke 8062A multimeter.
E (Electrode): Value obtained by measuring the voltage across the culture media divided by distance between the terminals.
J (Theoretical): Value obtained via equation; J = σE, where E is E (Theoretical).
J (Measured): Value obtained via equation; J = σE, where E is E (Measured).
E: Electric field strength.
J: Current density.
σ: Conductivity of media.

DISCUSSION

Our observations suggest that electric field strength values obtained by measuring the voltage directly across the culture media and divided by the distance between the terminals (E electrode) are not accurate because the value obtained is usually much higher than the measured and theoretical value. The electric field, E (measured), yields more accurate information because it takes into consideration the interface resistance problem at the electrode. It is suggested that a four-electrode device[24] method be employed to provide a better estimation of the conductivity of the culture media, because this method as demonstrated by Plonsey[24] minimizes the culture media-electrode interface impedance problem. Because current density is directly proportional to the product of conductivity and electric field strength (J = σE), better estimation of the conductivity by using the four-electrode device results in better estimation of the current density.

A zone of inhibition was consistently located at the anode only, and can be attributed to the presence of Ag^{+} activated by the μADC.[11,12,22] The dimensions of inhibition zones are not reported because, in a pilot study, we found them to be proportional to the area of the electrode used. In the study reported here, the size of the electrode was held constant from plate to plate.



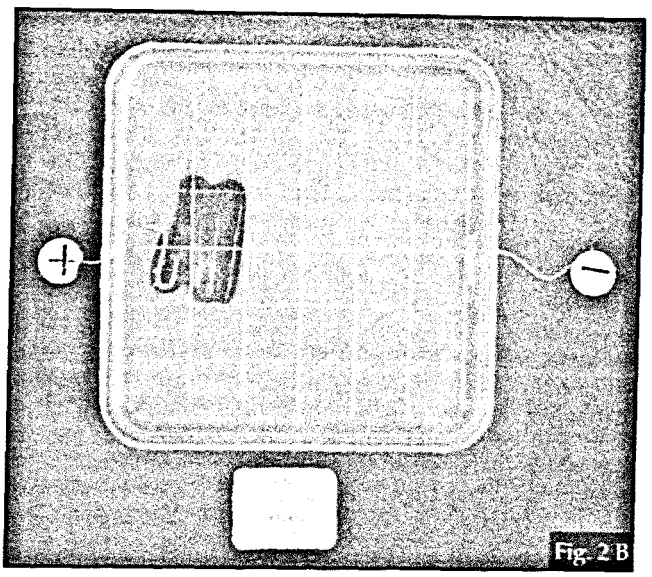


FIGURE 2. Culture dishes showing anodic inhibitory zones at 26 μA (A) and 100 μA (B).

TABLE 2. Summary of various testings.

		I = 26 μA		I = 100 μA		I = 300 μA		I = 500 μA		I = 800 μA	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Inhibition zone	PSI	+++	-	+++	-	+++	-	+++	-	+++	+
	SP2	+++	-	+++	-	+++	-	+++	-	+++	+
Corrosion	PS1	-	-	-	-	-	-	-	-	-	-
	SP2	-	-	-	-	-	-	-	-	-	-
Discoloration of medium	PS1	-	-	-	-	-	-	-	-	-	-
	SP2	-	-	-	-	-	-	-	-	-	-
Gas formation	PS1	-	-	-	-	-	+	-	++	-	+++
	SP2	-	-	-	-	-	+	-	++	-	+++
Antibacterial effect	PS1	Bactericidal		Bactericidal		Bactericidal		Bactericidal		Bactericidal	
	SP2	Bacteriostatic		Bacteriostatic		Bacteriostatic		Bacteriostatic		Bacteriostatic	
Wire control (w/o current)	PS1	-	-	-	-	-	-	-	-	-	-
	SP2	-	-	-	-	-	-	-	-	-	-
pH0	PS1	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
	SP2	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
pH30	PS1	6.8	8.0	6.4	8.0	6.4	9.5	6.4	11.0	5.5	12.0
	SP2	6.8	8.0	6.6	8.0	6.4	9.5	6.4	10.5	5.5	12.0
pH24	PS1	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
	SP2	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5

PS1: Pseudomonas aeruginosa (ATCC 27853).
SP2: Staphylococcus aureus (ATCC 25923).
pHO: pH value before electrical stimulation.
pH30: pH value after 30 minutes of electrical stimulation.
pH24: pH value after 24 hours of incubation.
+: Positive result.
-: Negative result.
Pos: Positive electrode.
Neg: Negative electrode.

Neither current amplitude nor current density seems to play an important role in inhibition zone size, at least in this experimental setting. One can see from representative photographs (Figure 2) taken of the cultures that by increasing the current delivered to the medium, the size of the inhibition zone does not increase accordingly. The limiting factor on inhibition zone size is the limited distance the Ag^+ can penetrate into the culture medium. These results agree with Berger et al[22] and Marino et al,[21] who showed that Ag^+ did not migrate more than 1 cm from the electrode. Thus, silver wire carrying current depositing Ag^+ is the contributing factor to bacterial inhibition and not current amplitude. This explains why bacterial growth was inhibited only when silver wire was carrying current; silver wire not carrying current did not inhibit either of the bacteria studied. An interesting observation was noted when the current was 800 μA; inhibition zones occurred at both the anode and cathode. The reason for inhibition at the cathode is unknown, although Spadaro et al[17] associated this phenomenon with gas evolution at the cathode.

There was no observable corrosion or discoloration of culture media at any current amplitude at either electrode. However, gas formation did occur at the cathode when the current levels were 300 μA and above. The content of the gas was not analyzed; however, in an aqueous solution, bubbles at the cathode consist of hydrogen gas.[25] This response was observed for both cultures used.

The μADC was found to be bacteriostatic with *Staphylococcus aureus* and bactericidal with *Pseudomonas aeruginosa*. One can only speculate that this might be due to their different cell wall structures since *S. aureus* is Gram positive, with a thick peptidoglycan cell wall, and *P. aeruginosa* is Gram negative, with a thin cell wall containing little peptidoglycan. Perhaps the silver ions can penetrate the thin cell wall of *P. aeruginosa* and bind to cell components (eg, proteins, DNA[17]) more easily than they can penetrate a thicker cell wall. This binding may alter the bacterial cell structure, ultimately causing cell death. For *S. aureus*, perhaps silver ions bind only to the external cell wall causing inhibition of growth. Once exposed to favorable conditions, the *S. aureus* was observed to resume growth.

The pH was found to be acidic at the anode and basic at the cathode at the end of the 30-min test run. The magnitude of the pH change depended upon the amount of current introduced. That is to say, the higher the current, the more acidic the medium became around the anode and the more basic it became around the cathode. As long as bacterial growth activity was present, the pH value reverted back to 8.5 after 18 to 24 hours incubation regardless of the pH during the 30-min test. This pH change is due to metabolic endproducts produced when bacteria use media substrates. The uninoculated culture medium remained acidic around the anode and basic around the cathode. Because the zone of inhibition was limited to the anode, we can only speculate that pH is not the contributing factor to the inhibition of bacterial growth.

CONCLUSION

Based upon observations made in this investigation, transmission of μADC by silver wire seems to arrest bacterial growth around the anode. However, more tests need to be conducted to verify the bactericidal or bacteriostatic effect on Gram-negative versus Gram-positive bacteria. Current amplitude (as a function of electric field strength and current density) and pH did not seem to cause the antibacterial effects seen in this study, but we cannot eliminate these as contributing factors. Other studies have included measurements of the inhibition zones, but none have attributed zone size to the size of the electrode.[1,22,25] We have found that inhibition zone size can be adjusted by altering the size of the electrode.

This investigation provides strong evidence that the mechanism for bacterial growth inhibition is the electrically repelled silver ion at the anode. This study suggests that μADC applied to infected chronic wounds via a silver-coated electrode may suppress bacterial growth, which in turn would enhance the wound healing process. Further tests are needed to verify whether this in vitro effect can be reproduced in vivo.

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