The Electricidal Effect: Reduction of *Staphylococcus* and *Pseudomonas* Biofilms by Prolonged Exposure to Low-Intensity Electrical Current[▽]

Jose L. del Pozo,^{1,2} Mark S. Rouse,^{1,2} Jayawant N. Mandrekar,³ James M. Steckelberg,^{1,2} and Robin Patel^{1,2,4*}

Division of Infectious Diseases, Department of Medicine,¹ Infectious Diseases Research Laboratory,² Division of Biostatistics,³ and Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,⁴ Mayo Clinic College of Medicine, Rochester, Minnesota

Received 23 May 2008/Returned for modification 12 August 2008/Accepted 17 October 2008

The activity of electrical current against planktonic bacteria has previously been demonstrated. The short-term exposure of the bacteria in biofilms to electrical current in the absence of antimicrobials has been shown to have no substantial effect; however, longer-term exposure has not been studied. A previously described *in vitro* model was used to determine the effect of prolonged exposure (i.e., up to 7 days) to low-intensity (i.e., 20-, 200-, and 2,000-microampere) electrical direct currents on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* biofilms. Dose- and time-dependent killing was observed. A maximum of a 6-log₁₀-CFU/cm² reduction was observed when *S. epidermidis* biofilms were exposed to 2,000 microamperes for at least 2 days. A 4- to 5-log₁₀-CFU/cm² reduction was observed when *S. aureus* biofilms were exposed to 2,000 microamperes for at least 2 days. Finally, a 3.5- to 5-log₁₀-CFU/cm² reduction was observed when *P. aeruginosa* biofilms were exposed to electrical current for 7 days. A higher electrical current intensity correlated with greater decreases in viable bacteria at all time points studied. In conclusion, low-intensity electrical current substantially reduced the numbers of viable bacteria in staphylococcal or *Pseudomonas* biofilms, a phenomenon we have labeled the “electricidal effect.”

The pathogenesis of a wide variety of human infections, including device-related infections, is now recognized to be related to the presence of bacteria in biofilms. The biofilm mode of growth protects bacteria from host defense mechanisms and conventional antimicrobial agents. The development of biofilm-related infections begins with the adhesion of the microorganisms to the biomaterial surface, mediated by the Van der Waals forces, acid-base interactions, and electrostatic forces (28). Electrostatic forces between bacteria and surfaces are generally repulsive, since almost all biomaterials are negatively charged, as are bacteria (19). It has been proposed that these repulsive forces can be enhanced by the application of electrical current, which provokes the surface detachment of bacterial biofilms (26, 34, 37).

The antibacterial activity of electrical current has previously been demonstrated against *Staphylococcus aureus* and *Staphylococcus epidermidis* in agar (21, 24); the normal flora on human skin (2); *Escherichia coli*, *Proteus* species, and *Klebsiella pneumoniae* in synthetic urine (5); *E. coli*, *Staphylococcus aureus*, and *Bacillus subtilis* in water (12, 22, 23); and *E. coli* in salt solutions (27). The mechanism of the antibacterial activity of electrical current has been suggested to result from toxic substances (e.g., H₂O₂, oxidizing radicals, and chlorine molecules) produced as a result of electrolysis (21), the oxidation of enzymes and coenzymes, membrane damage leading to the leakage of essential cytoplasmic constituents (30), and/or a decreased bacterial respiratory rate (22).

The aim of the present study was to determine the effect of

prolonged exposure (i.e., up to 7 days) to low-intensity (i.e., 20, 200, and 2,000 microamperes) electrical direct current (DC) on *Pseudomonas aeruginosa*, *S. aureus*, and *S. epidermidis* biofilms.

MATERIALS AND METHODS

Microorganisms. Methicillin-resistant *S. aureus* Xen 30, *S. epidermidis* Xen 43, and *P. aeruginosa* Xen 5 (kind gifts of Xenogen Corp., Hopkinton, MA) were studied.

Semisynthetic medium. Biofilms were grown on Teflon coupons in a previously described semisynthetic medium (32) containing 426 mg Na₂HPO₄, 205 mg KH₂PO₄, 435 mg KNO₃, 32 mg MgSO₄, 32 mg CaCO₃, 6.4 mg nitriloacetic acid, 5 mg FeSO₄, 4.5 mg ZnSO₄, 0.3 mg MnSO₄, 0.09 mg CuSO₄, 0.07 mg Co(NO₃), 0.04 mg NaB₄O₇, 0.05 mg (NH₄)₂MoO₄, and 100 ml Trypticase soy broth per liter of distilled water. The solution was sterilized for 20 min at 120°C, and 640 mg of filter-sterilized glucose solution (1 mg/ml) was then added.

Substrate solution. A medium containing 12.9 mg Na₂HPO₄, 6.2 mg KH₂PO₄, and 3.0 ml Trypticase soy broth per liter of distilled water was prepared. The solution was sterilized for 20 min at 120°C, and 19.4 mg of filter-sterilized glucose solution (160 mg/ml) was then added.

Biofilm growth reactor. Biofilms were grown for 48 h on Teflon disks (12.5 mm in diameter by 1 mm in thickness) in the semisynthetic medium in a CDC biofilm reactor (Biosurface Technologies, Bozeman, MT), as described previously (13).

Biofilm treatment device. All experiments were performed with two eight-channel current generators/controllers and 16 test chambers designed and fabricated by the Mayo Division of Engineering. Each current controller was computer controlled to deliver a specified DC (20 to 2,000 microamperes) and monitor and record the voltage and current at 5-s intervals, as described previously (13). For each experiment, biofilm-covered coupons were removed from the reactor, rinsed of planktonic bacteria with 15 ml of sterile saline, and placed in a chamber in a vertical position in a plane perpendicular to the plane formed by the electrodes. Prior to each experiment, we confirmed that the density of the biofilm bacteria on one of each reactor's coupons was >5 log₁₀ CFU/cm². Substrate solution was continuously pumped through each chamber at 3 ml/h. Electrical current (0, 20, 200, or 2,000 microamperes DC) was continuously passed from the anode to the cathode in the chamber for each test. After 1, 2, 4, and 7 days of exposure, the coupons were aseptically removed from the chambers, rinsed of planktonic bacteria with 15 ml of sterile saline, and placed in sterile tubes containing 1 ml of Trypticase soy broth. Adherent biofilm bacteria were removed by vortexing and sonication, as described previously (13), and

* Corresponding author. Mailing address: Divisions of Clinical Microbiology and Infectious Diseases, Mayo Clinic, 200 First St. SW, Rochester, MN 55905. Phone: (507) 538-0579. Fax: (507) 284-4272. E-mail: patel.robin@mayo.edu.

[▽] Published ahead of print on 27 October 2008.

FIG. 1. Mean LRF (in CFU/cm²) and standard deviations (of three different experiments) for *Pseudomonas aeruginosa* (A and B), *Staphylococcus aureus* (C and D), and *Staphylococcus epidermidis* (E and F) after exposure to 0, 20, 200, or 2,000 microamperes of electrical current. Results obtained when the current was passed through stainless steel electrodes (A, C, and E) and when the current was passed through graphite electrodes (B, D, and F) are shown.

subjected to quantitative culture. The results were expressed as the mean log₁₀ CFU/cm² and standard deviation of three different experiments. The effect of the exposure was measured by using the logarithmic reduction factor (LRF) in the numbers of CFU/cm², i.e., log [(mean CFU/cm² of nonexposed coupons)/(mean CFU/cm² of exposed coupons)] (9).

Electrode composition. The electrodes were stainless steel or graphite cylinders 1.5 mm in diameter by 55 mm long, and 1 cm of electrode was extended above the chamber to connect the electrode to the current generator.

Activity of media exposed to electrical current. Substrate solution was continuously pumped through each chamber and exposed to electrical current (2,000 microamperes DC) for 24 h. The resultant effluent fluid was collected over a 24-h period, and the biofilm-covered coupons were exposed to this fluid in a biofilm treatment device without electrical current for 1, 2, 4, or 7 days. The coupons were assayed as described above.

Detection of hydrogen peroxide. A Merckoquant strip peroxide test (E. Merck, Darmstadt, Germany) was used to measure the production of hydrogen peroxide inside the treatment chambers. Peroxidase transfers peroxide oxygen to an organic redox indicator, producing a blue oxidation product. The peroxide concentration was measured semiquantitatively (0.5 to 25 mg/liter) by visual comparison of the reaction zone of the test strip with the fields of a color scale. Measurements were made every hour during the first 12 h and every 12 h thereafter for 7 days.

Detection of chlorine. A Merckoquant strip chlorine test (E. Merck, Darmstadt, Germany) was used to measure the chlorine production inside the treatment chambers. Chlorine oxidizes an organic compound to a violet dye. The chlorine concentration was measured semiquantitatively (0.5 to 20 mg/liter) by visual comparison of the reaction zone of the test strip with the fields of a color scale. Measurements were made every hour during the first 12 h and every 12 h thereafter for 7 days.

pH measurements. A pH meter (Corning Pinnacle; Cole-Parmer) was used to monitor the changes in pH inside the chambers during the application of electrical current.

Antimicrobial agents. The activities of vancomycin (Lilly & Co., Indianapolis, IN), moxifloxacin (Bayer Corp., West Haven, CT), and tobramycin (Lilly & Co) were studied against *S. aureus* (vancomycin MIC, 2 µg/ml), *S. epidermidis* (moxifloxacin MIC, ≤0.125 µg/ml), and *P. aeruginosa* (tobramycin MIC, 1 µg/ml) biofilms, respectively.

Statistical methods. All experiments were performed in triplicate. We performed a one-way analysis of variance with four levels of exposure of the coupons to electrical current (i.e., 0, 20, 200, or 2,000 microamperes) and no exposure to electrical current (LRF = 0) to determine if exposure to electrical current had any effect on the biofilms. All tests were two sided; *P* values of <0.05 were considered statistically significant. Analysis was performed with SAS software (version 9; SAS Institute, Inc., Cary, NC).

RESULTS

Electrical current-exposed biofilms. The mean LRF and standard deviation for each biofilm microorganism after exposure to electrical current are shown in Fig. 1. We detected statistically significant differences (*P* < 0.01) in the results between no electrical current exposure and any electrical current exposure for all three microorganisms studied. A higher amperage was correlated with more of a reduction of biofilm viability at all the time points studied for both electrode compositions studied. A time-dependent reduction in biofilm viability was observed, with generally lower viable cell counts observed when electrical current was applied for longer periods of time. A 5- to 6-log₁₀-CFU/cm² reduction in viable cell counts was observed when *S. aureus* or *S. epidermidis* biofilms

FIG. 2. pH measurements inside the chambers during electrical current application from time zero to day 7. Results according to different current intensities (0, 20, or 2,000 microamperes) and electrode types (stainless steel or graphite) are shown.

were exposed to 2,000 microamperes for at least 2 days. A 3.5- to 5- $\log_{10}$ -CFU/cm² reduction was observed when *P. aeruginosa* biofilms were exposed to 2,000 microamperes for 7 days.

Following the exposure of *S. aureus* or *S. epidermidis* biofilms to 2,000 microamperes delivered by stainless steel or graphite electrodes for 4 days or more, the plate counts for quantitative culture were all negative. However, bacterial growth was observed with subculture of the broth in which the coupons were incubated after the vortexing-sonication procedure.

P. aeruginosa biofilms were more resistant to electrical current. The minimum counts achieved after exposure to the three different electrical current intensities were greater than 1.75 $\log_{10}$ CFU/cm². The delivery of electrical current via stainless steel electrodes resulted in a greater decrease in biofilm viability ($P < 0.001$) than the delivery of electrical current via graphite electrodes.

Hydrogen peroxide and chlorine ion detection. Neither hydrogen peroxide nor chlorine ions were detected in any of the experiments described above.

pH measurements during electrical current application. We observed changes in pH in the chambers during electrical current application. As shown in Fig. 2, when passing 2,000 microamperes through stainless steel electrodes, we observed a change in pH, which increased from 7 to 8.5 after 15 min of electrical current delivery to almost 12 after 7 days of exposure. When using the graphite electrodes, we observed an opposite change in pH, which decreased from 7 to 4 after 15 min of electrical current delivery and which stayed at about that level for 7 days. When passing 20 microamperes through stainless steel or graphite, we did not observe any marked changes in pH. When passing 200 microamperes, we observed a great range of various pH measurements over 7 days (data not represented in Fig. 2).

Activity of media exposed to electrical current. A 1- $\log_{10}$ -unit decrease in CFU/cm² was observed when *S. aureus* or *S. epidermidis* biofilms were exposed to the broths previously exposed to electrical current (alone) for 7 days, and a 2- $\log_{10}$ -unit

FIG. 3. Mean LRFs (in CFU/cm²) and standard deviations (of three different experiments) for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* biofilms after exposure to vancomycin, tobramycin, and moxifloxacin, respectively, for 1 to 7 days compared with the values for nonexposed biofilms (No Antimicrobial).

decrease in CFU/cm² was observed when *P. aeruginosa* biofilms were exposed to the broths previously exposed to electrical current (alone) for 7 days.

Antimicrobial agent-exposed biofilms. For comparison, the mean LFR and standard deviation for each biofilm microorganism exposed to antimicrobial agents alone are shown in Fig. 3. When the colonized coupons were exposed to the antimicrobial agents in the absence of electrical current, a 2.5- $\log_{10}$ -unit decrease was observed when *P. aeruginosa* and *S. epidermidis* were exposed to tobramycin (10 µg/ml) and moxifloxacin (4 µg/ml), respectively, for 7 days. A less than 1- $\log_{10}$ -unit decrease was observed when *S. aureus* was exposed to vancomycin (32 µg/ml) for 7 days.

DISCUSSION

In this study, prolonged exposure to low-intensity electrical current in the absence of antibiotics resulted in a marked decrease in the viability of *S. aureus*, *S. epidermidis*, and *P. aeruginosa* biofilms. In our model, dose- and time-dependent effects were observed when biofilms were exposed to 20, 200, or 2,000 microamperes for 1 to 7 days. The most dramatic effect (i.e., a 4- to 6- $\log_{10}$ -CFU/cm² reduction) was observed when *S. epidermidis* biofilms were exposed to 2,000 micro-

amperes for at least 2 days. A 4- to 5-log₁₀-CFU/cm² reduction was also observed when *S. aureus* biofilms were exposed to 2,000 microamperes for at least 2 days. Less than 0.65 log₁₀ CFU/cm² of remaining biofilm was observed when *S. aureus* was exposed to 2,000 microamperes for at least 2 days or when *S. epidermidis* was exposed to 2,000 microamperes for at least 4 days. This effect was observed with both graphite and stainless steel electrodes. Such large reductions were not observed when *P. aeruginosa* biofilms were exposed to up to 2,000 microamperes for the longest time studied. However, 3.5- to 5-log₁₀-CFU/cm² reductions were observed. The results described above clearly demonstrate that electrical current can substantially reduce the viability of bacterial biofilms on a Teflon surface. Whether this effect generalizes to biofilms on others biomaterials or to other microorganisms remains to be determined.

It has previously been suggested that electrical current is not able to eradicate bacteria in biofilms but, rather, that it can act synergistically with biocides or antimicrobial agents (4, 11, 17, 18, 20), the so-called bioelectric effect. Most of the published studies have used 12 to 24 h of biofilm exposure to electrical current and/or antimicrobial agents (4, 9, 11, 13, 17, 18, 20, 32, 39, 40). In contrast, we achieved significant reductions in the numbers of viable bacteria with prolonged exposure of *P. aeruginosa*, *S. aureus*, and *S. epidermidis* biofilms to electrical current alone. To differentiate this effect from the bioelectric effect, we refer to this phenomenon as the "electricidal effect".

Bacterial detachment from stainless steel (36) and the conduction of indium tin oxide surfaces (26) by low-intensity electric DC (10 to 125 μ A) have been described. It is not known whether the electricidal effect that we describe herein relates to the detachment and/or the killing of the biofilm bacteria. Infection of the percutaneous pin sites of external fixators used in reconstructive bone surgery was demonstrated to be prevented (89% versus 11% in the treated animals) by the application of 100 microamperes electrical DC in a caprine experimental model (35).

In our study, the electricidal effect was present when electrical current was delivered via stainless steel or graphite electrodes. Other studies have used metal electrodes to demonstrate the bactericidal effects of electrical current on biofilms (4, 11, 26). Electrodes may corrode and may interfere with or affect the antibacterial activity of electrical current by the accretion of metal ions on or in bacterial cells (12). Although some studies suggest that the products of electrolysis are responsible for the bactericidal activity of the electrical current, our findings suggest that electrical current per se may have antibacterial activity. Although the mechanism of this effect remains unknown, it does not appear to be an exclusive result of the electrochemical generation of inhibitory ions, as demonstrated by exposing biofilms to broths previously exposed to electrical current. Van der Borden et al. demonstrated that more than 75% of the initially adhering staphylococci could be stimulated to detach from surgical stainless steel by the application of an electrical current of 100 microamperes (37). It was suggested that the reason for the high detachment percentage was the electro-osmotic fluid flow directed to and from the surface. The electrical current may cause the hydrated ions to move along the applied field, dragging water with them and causing an extra force that stimulates detachment (26, 38).

The mechanism of the electricidal effect could relate to the disruption of the integrity of the bacterial membrane or to the generation of chlorine, oxygen, and/or hydrogen peroxide as a result of electrolysis (21, 30, 32). However, we did not detect chlorine or hydrogen peroxide over 7 days of electrical current application. The pH changes described here may be involved in the electricidal effect as either a mechanism or a by-product. Clearly, the electrode composition affects the pH changes. It is unclear why the pH was so unstable when 200 microamperes was passed through our system. The issue of pH and electricidal effect deserves further study.

The present study tested the activity of low-intensity DC in decreasing the viability of staphylococcal and *Pseudomonas* biofilms in vitro. Low-intensity electrical currents of 20 to 2,000 microamperes reduced the bacterial biofilm loads on Teflon coupons in the absence of antimicrobial agents. Direct currents have been used clinically to drive chemotherapeutic molecules into solid tumors and antibiotics into the inner ear and other tissues (3, 10, 29). Electricity is used clinically for the treatment of bone nonunion (14–16, 31). Brighton et al. have reported that bone cells have a proliferative response to electrical stimulation (8). The obvious human application of the electricidal effect described here is in the management of infections associated with orthopedic hardware. However, this approach needs further study before it can be translated to clinical practice. The safety of the use of electrical current in humans is a clear concern. Constant DCs of 10 to 20 microamperes have been applied at the site of fractures (6, 7, 14, 16). Local treatment of malignant disease has been established with low-level DC (25, 33). Locally applied low-level electrical DC (1,000 microamperes for 30 min) was demonstrated to be an effective and safe treatment in human melanoma skin lesions in five patients (25). A patient with laryngeal sarcoma safely received electrical DC therapy of 36 C through two platinum electrodes inserted into the tumor (1). If the electricidal effect occurs in vivo, it would present some advantages compared with antimicrobial agent-based treatment, especially in the era of antimicrobial resistance. The electricidal effect affords a potential means to overcome the reduced susceptibility of biofilm microorganisms to conventional antimicrobial agents.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (grant R21AI061407).

We have no disclosures or conflicts of interest to report.

We thank Xenogen corp. for providing strains Xen 30, Xen 43, and Xen 5 and April Horne from the Mayo Division of Engineering for designing and fabricating the current generator/controllers and test chambers.

REFERENCES

1. Ando, H., K. Ito, T. Nakashima, K. Hidemura, H. Tsunoo, S. Suda, Y. Kanemitsu, T. Matsui, K. Chin, S. Saito, M. Fujiwara, H. Sekiguchi, Y. Kasai, S. Akiyama, and A. Nakao. 1999. Treatment of solid tumor by a direct electric current. *Gan To Kagaku Ryoho* 26:1856–1859.
2. Barranco, S. D., J. A. Spadaro, T. J. Berger, and R. O. Becker. 1974. In vitro effect of weak direct current on *Staphylococcus aureus*. *Clin. Orthop. Relat. Res.* 100:250–255.
3. Belehradsek, J., Jr., S. Orlowski, L. H. Ramirez, G. Pron, B. Poddevin, and L. M. Mir. 1994. Electroporation of cells in tissues assessed by the qualitative and quantitative electroloading of bleomycin. *Biochim. Biophys. Acta* 1190:155–163.
4. Blenkinsopp, S. A., A. E. Khoury, and J. W. Costerton. 1992. Electrical enhancement of biocide efficacy against *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol.* 58:3770–3773.

5. Bolton, L., B. Foleno, B. Means, and S. Petrucelli. 1980. Direct-current bactericidal effect on intact skin. *Antimicrob. Agents Chemother.* **18**:137–141.
6. Brighton, C. T., Z. B. Friedenberg, L. M. Zemsky, and P. R. Pollis. 1975. Direct-current stimulation of non-union and congenital pseudarthrosis. Exploration of its clinical application. *J. Bone Joint Surg. Am.* **57**:368–377.
7. Brighton, C. T., P. Shaman, R. B. Heppenstall, J. L. Esterhai, Jr., S. R. Pollack, and Z. B. Friedenberg. 1995. Tibial nonunion treated with direct current, capacitive coupling, or bone graft. *Clin. Orthop. Relat. Res.* **321**:223–234.
8. Brighton, C. T., W. Wang, R. Seldes, G. Zhang, and S. R. Pollack. 2001. Signal transduction in electrically stimulated bone cells. *J. Bone Joint Surg. Am.* **83A**:1514–1523.
9. Caubet, R., F. Pedarros-Caubet, M. Chu, E. Freye, M. de Belem Rodrigues, J. M. Moreau, and W. J. Ellison. 2004. A radio frequency electric current enhances antibiotic efficacy against bacterial biofilms. *Antimicrob. Agents Chemother.* **48**:4662–4664.
10. Chang, I. W., Y. B. Cho, and S. C. Lim. 1989. Iontophoretic application of cefotiam hydrochloride into inner ear through round window membrane. *Chonnam J. Med. Sci.* **2**:141–144.
11. Costerton, J. W., B. Ellis, K. Lam, F. Johnson, and A. E. Khoury. 1994. Mechanism of electrical enhancement of efficacy of antibiotics in killing biofilm bacteria. *Antimicrob. Agents Chemother.* **38**:2803–2809.
12. Davis, C. P., N. Wagle, M. D. Anderson, and M. M. Warren. 1991. Bacterial and fungal killing by iontophoresis with long-lived electrodes. *Antimicrob. Agents Chemother.* **35**:2131–2134.
13. del Pozo, J. L., M. S. Rouse, J. N. Mandrekar, M. Fernandez Sampedro, J. M. Steckelberg, and R. Patel. 2009. Effect of electrical current on the activities of antimicrobial agents against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* biofilms. *Antimicrob. Agents Chemother.* **53**:35–40.
14. Dunn, A. W., and G. A. Rush III. 1984. Electrical stimulation in treatment of delayed union and nonunion of fractures and osteotomies. *South. Med. J.* **77**:1530–1534.
15. Evans, R. O., J. A. Goldberg, W. J. Bruce, and W. Walsh. 2004. Reoperated clavicular nonunion treated with osteogenic protein 1 and electrical stimulation. *J. Shoulder Elbow Surg.* **13**:573–575.
16. Forsted, D. L., M. K. Dalinka, E. Mitchell, C. T. Brighton, and A. Alavi. 1978. Radiologic evaluation of the treatment of nonunion of fractures by electrical stimulation. *Radiology* **128**:629–634.
17. Jass, J., J. W. Costerton, and H. M. Lappin-Scott. 1995. The effect of electrical currents and tobramycin on *Pseudomonas aeruginosa* biofilms. *J. Ind. Microbiol.* **15**:234–242.
18. Jass, J., and H. M. Lappin-Scott. 1996. The efficacy of antibiotics enhanced by electrical currents against *Pseudomonas aeruginosa* biofilms. *J. Antimicrob. Chemother.* **38**:987–1000.
19. Jucker, B. A., H. Harms, and A. J. Zehnder. 1996. Adhesion of the positively charged bacterium *Stenotrophomonas (Xanthomonas) maltophilia* 70401 to glass and Teflon. *J. Bacteriol.* **178**:5472–5479.
20. Khoury, A. E., K. Lam, B. Ellis, and J. W. Costerton. 1992. Prevention and control of bacterial infections associated with medical devices. *ASAIO J.* **38**:M174–M178.
21. Liu, W. K., M. R. Brown, and T. S. Elliott. 1997. Mechanisms of the bactericidal activity of low amperage electric current (DC). *J. Antimicrob. Chemother.* **39**:687–695.
22. Matsunaga, T., S. Nakasono, and S. Masuda. 1992. Electrochemical sterilization of bacteria absorbed on granular activated carbon. *FEMS Microbiol. Lett.* **72**:255–259.
23. Matsunaga, T., S. Nakasono, T. Takamuku, J. G. Burgess, N. Nakamura, and K. Sode. 1992. Disinfection of drinking water by using a novel electrochemical reactor employing carbon-cloth electrodes. *Appl. Environ. Microbiol.* **58**:686–689.
24. Pareilleux, A., and N. Sicard. 1970. Lethal effects of electric current on *Escherichia coli*. *Appl. Microbiol.* **19**:421–424.
25. Plesnicar, A., G. Sersa, L. Vodovnik, J. Jancar, L. Zaletel-Kragelj, and S. Plesnicar. 1994. Electric treatment of human melanoma skin lesions with low level direct electric current: an assessment of clinical experience following a preliminary study in five patients. *Eur. J. Surg. Suppl.* **574**:45–49.
26. Poortinga, A. T., R. Bos, and H. J. Busscher. 2000. Controlled electrophoretic deposition of bacteria to surfaces for the design of biofilms. *Bio-technol. Bioeng.* **67**:117–120.
27. Rajnicek, A. M. 1993. Bacterial galvanotropism: mechanisms and applications. *Sci. Prog.* **77**(Pt 1-2):139–151.
28. Rijnaarts, H. H., W. Norde, E. J. Bouwer, J. Lyklema, and A. J. Zehnder. 1993. Bacterial adhesion under static and dynamic conditions. *Appl. Environ. Microbiol.* **59**:3255–3265.
29. Sersa, G., and D. Miklavcic. 1990. Inhibition of SA-1 tumor growth in mice by human leukocyte interferon alpha combined with low-level direct current. *Mol. Biother.* **2**:165–168.
30. Shimada, K., and K. Shimahara. 1985. Leakage of cellular contents and morphological changes in resting *Escherichia coli* B cells exposed to an alternating changes. *Agric. Biol. Chem.* **49**:3605–3607.
31. Srivastava, K. P., and A. K. Saxena. 1977. Fracture healing in a case of nonunion of the tibia by electrical stimulation. *Int. Surg.* **62**:35–36.
32. Stewart, P. S., W. Wattanakaroorn, L. Goodrum, S. M. Fortun, and B. R. McLeod. 1999. Electrolytic generation of oxygen partially explains electrical enhancement of tobramycin efficacy against *Pseudomonas aeruginosa* biofilm. *Antimicrob. Agents Chemother.* **43**:292–296.
33. Turler, A., H. Schaefer, N. Schaefer, M. Wagner, D. Maintz, J. C. Qiao, and A. H. Hoelscher. 2000. Experimental low-level direct current therapy in liver metastases: influence of polarity and current dose. *Bioelectromagnetics* **21**:395–401.
34. Ueshima, M., S. Tanaka, S. Nakamura, and K. Yamashita. 2002. Manipulation of bacterial adhesion and proliferation by surface charges of electrically polarized hydroxyapatite. *J. Biomed. Mater. Res.* **60**:578–584.
35. van der Borden, A. J., P. G. Maathuis, E. Engels, G. Rakhorst, H. C. van der Mei, H. J. Busscher, and P. K. Sharma. 2007. Prevention of pin tract infection in external stainless steel fixator frames using electric current in a goat model. *Biomaterials* **28**:2122–2126.
36. van der Borden, A. J., H. C. van der Mei, and H. J. Busscher. 2004. Electric-current-induced detachment of *Staphylococcus epidermidis* strains from surgical stainless steel. *J. Biomed. Mater. Res. B Appl. Biomater.* **68**:160–164.
37. van der Borden, A. J., H. C. van der Mei, and H. J. Busscher. 2005. Electric block current induced detachment from surgical stainless steel and decreased viability of *Staphylococcus epidermidis*. *Biomaterials* **26**:6731–6735.
38. van der Borden, A. J., H. van der Werf, H. C. van der Mei, and H. J. Busscher. 2004. Electric current-induced detachment of *Staphylococcus epidermidis* biofilms from surgical stainless steel. *Appl. Environ. Microbiol.* **70**:6871–6874.
39. Wattanakaroorn, W., and P. S. Stewart. 2000. Electrical enhancement of *Streptococcus gordonii* biofilm killing by gentamicin. *Arch. Oral Biol.* **45**:167–171.
40. Wellman, N., S. M. Fortun, and B. R. McLeod. 1996. Bacterial biofilms and the bioelectric effect. *Antimicrob. Agents Chemother.* **40**:2012–2014.