Anti-Biofilm Activity of Electrical Current in a Catheter Model

Paul Voegele,¹ Jon Badiola,¹ Suzannah M. Schmidt-Malan,¹ Melissa J. Karau,¹ Kerryl E. Greenwood-Quaintance,¹ Jayawant N. Mandrekar,² Robin Patel¹,³#

¹Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,
²Division of Biomedical Statistics and Informatics, Department of Health Sciences Research,
³Division of Infectious Diseases, Department of Medicine

Mayo Clinic, Rochester, MN, 55905

#Correspondence to:
Robin Patel, M.D.
Division of Clinical Microbiology
Mayo Clinic
200 First St S.W.
Rochester, MN 55905
507-538-0579
507-284-4272 (fax)
Email: patel.robin@mayo.edu
ABSTRACT

Catheter-associated infections are difficult to treat with available antimicrobial agents because of their biofilm etiology. We examined the effect of low amperage direct electric current (DC) exposure on established bacterial and fungal biofilms in a novel experimental in vitro catheter model. *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida parapsilosis* biofilms were grown on the inside surfaces of PVC catheters after which 0, 100, 200 or 500 µA of DC was delivered via intraluminally-placed platinum electrodes. Catheter biofilms and intraluminal fluid were quantitatively cultured after 24 hours and 4 days of DC exposure. Time- and dose-dependent biofilm killing was observed with both amperages and durations of DC administration. 24 hours of 500 µA sterilized the intraluminal fluid for all bacterial species studied; no viable bacteria were detected after treatment of *S. epidermidis* and *S. aureus* biofilms with 500 µA for 4 days.
Catheter-associated infections, including catheter associated urinary tract infection (CAUTI) and catheter related blood stream infection (CRBSI), are associated with morbidity, mortality, and expense, often requiring catheter removal. The pathogenesis of these infections relates to the presence of biofilms on the surface of the catheters.

Compared with planktonic (i.e., free floating) forms, microorganisms in biofilms exhibit increased resistance to host immunity and antimicrobial therapy (1). Proposed mechanisms underlying biofilm-associated antimicrobial resistance include limited penetration through or neutralization of antimicrobials within biofilms (2-3), subpopulations of resistant phenotypes, referred to as ‘persister’ cells (4-5), and dormant stationary phase zones within biofilms (4, 6-7).

As a result, most conventional systemically-administered antimicrobial agents have little ability to cure catheter-associated infections. Catheter removal is necessary in the majority of cases, typically in conjunction with systemic antimicrobial treatment. Strategies to control biofilms, such as coating catheters with silver ions, chlorhexidine or minocycline and rifampin have been proposed (8-12), and catheter lock solutions, using conventional antimicrobial agents or antiseptics, have shown activity against catheter-associated biofilms (13-19). However, none of these strategies has solved the clinical challenge of catheter-associated infections, underscoring the need for new approaches.

We previously described an anti-biofilm strategy we termed the electricidal effect. Biofilms of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* on Teflon discs were exposed to 20, 200 or 2,000 µA direct current (DC) for up to seven days which resulted in time- and dose-dependent anti-biofilm effects, as measured by decreases in...
viable cell numbers (20). Subsequent studies confirmed the microbicidal activity of continuously
and intermittently applied electrical current against established biofilms of several bacterial and
fungal species in vitro and in animal models (21-25).

A potential avenue to deliver electrical current is to administer it to the lumen of
catheters. This location of biofilm formation in CRBSI and CAUTI provides a site targetable
using electrical current. Based on our prior work, we hypothesized that DC delivered via
intraluminally-placed electrodes would provide an anti-biofilm strategy targeting intraluminal
biofilms. This approach could limit the use of antibiotics as well as the replacement of infected
catheters. In this study, we examined the effect of different amperages and delivery durations of
DC on established intraluminal biofilms of four bacterial and one fungal species in a novel in vitro catheter model.
MATERIALS AND METHODS

Microorganisms. *Staphylococcus epidermidis* Xen43, *Staphylococcus aureus* Xen30, *Pseudomonas aeruginosa* Xen5, *Escherichia coli* IDRL-7029 and *Candida parapsilosis* IDRL-7250 were studied. The Xen strains were generous gifts of Perkin Elmer Caliper Life Sciences (formerly Xenogen Corporation), Waltham, MA, and the IDRL isolates were clinical isolates collected at Mayo Clinic, Rochester, MN.

Catheters. 6 mm inner diameter 28 Fr PVC Thoracic Catheters (Atrium Medical Corporation, Hudson, NH) were cut to a length of 45 mm and sterilized using ethylene oxide. Polyoxymethylene plastic caps were used to seal the catheter bottoms and tops; platinum electrodes (50 mm in length and 1.6 mm in diameter) were inserted through and held in place by the latter (Figure 1).

Biofilms. Microorganisms were subcultured from frozen aliquots onto BBL™ Trypticase™ Soy Agar with 5% sheep blood plates (TSA II, Becton Dickinson, Franklin Lakes, NJ) and incubated at 37°C overnight. One to three colonies were added to 2.5 ml of trypticase soy broth (TSB) and grown for 1-3 hours at 37°C on an orbital shaker to reach a 0.5 McFarland standard. 100 µl of the 0.5 McFarland solutions were added to 0.9 ml TSB in sterile PVC catheters capped at one end, placed into a sterile glass box and incubated on an orbital shaker at 37°C for 24 hours. TSB was then removed using a pipette and replaced with 1.1 ml phosphate buffer.

Phosphate buffer. Phosphate buffer (1X) was prepared with 426 mg Na₂HPO₄, 205 mg KH₂PO₄, 640 mg glucose, and 1 liter distilled water, filter sterilized, and stored at 4°C. The stock phosphate buffer was diluted to 3% in sterile water for each experiment.
Electrical treatment. Electrical current was applied using an 8-channel computer controlled current generator designed by the Mayo Clinic Division of Engineering (Rochester, MN) via anode and cathode electrical hooks connected to the electrodes. Catheters were treated with 0, 100, 200 or 500 μA DC current for either 24 hours or 4 days, with testing performed in triplicate.

Biofilm and planktonic cell densities. Biofilm and planktonic cell densities were determined by quantitative culture. Intraluminal phosphate buffer was quantitatively cultured to obtain planktonic cell densities. To obtain biofilm densities, caps were aseptically removed from the catheters, which were gently rinsed in sterile saline and placed into test tubes containing 5 ml of sterile saline. Biofilms were removed by vortexing for 30 seconds, sonication for 5 minutes and vortexing again for 30 seconds; the resultant fluid was quantitatively cultured. Colony forming units (cfu) were counted after 24 to 48 hours. Biofilm reduction was expressed subtracting the mean log_{10} cfu/cm^2 of exposed catheters from that of non-exposed catheters.

Statistical methods. Comparison among 4 current levels (0, 100, 200, 500 μA) was first performed using the Kruskall Wallis test. If the results were significant, further comparisons were performed in a pairwise manner (0 vs 100 μA, 0 vs 200 μA, 0 vs 500 μA, 100 vs 200 μA, 100 vs 500 μA, 200 vs 500 μA) using the Wilcoxon rank sum test. No adjustment was performed for multiple comparisons due to small sample sizes. All tests were two-sided; p-values less than 0.05 were considered statistically significant. Analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina).
RESULTS

Results are shown in Figure 2. We detected statistically significant differences between no electric current exposure and electric current exposure of 200 µA and higher for all microorganisms studied (p≤0.02). Higher amperage yielded greater reductions of biofilm viability at all times studied. Time-dependent reductions in viable biofilm cells were observed, with lower viable cell counts when electric current was applied for longer periods of time. No viable cells were detected when *S. aureus* or *S. epidermidis* biofilms were exposed to 500 µA for 4 days. 5.2 to 5.5 log₁₀ cfu/cm² reductions were observed when *P. aeruginosa* and *E. coli* biofilms were exposed to 500 µA for 4 days (p<0.02). *C. parapsilosis* biofilms were more resistant, with a maximum 3.2 log₁₀ cfu/cm² reduction achieved after 4 days of 500 µA treatment (p, 0.01). Significant biofilm reductions were also observed with 200 µA for 24 hours, ranging from 1.4 to 2.1 log₁₀ cfu/cm² (p<0.02). 100 µA applied for 24 hours (and 4 days) reduced *E. coli* biofilms and planktonic cells (p≤0.02); 100 µA applied for 4 days reduced *S. epidermidis* biofilms (p, 0.01). A greater biofilm effect was measured with 500 compared with 200 µA (p<0.05) for all organisms and durations, except for *C. parapsilosis* after 24 hours and *S. epidermidis* and *P. aeruginosa* after 4 days, possibly due to the small sample size. Likewise, 500 days of application (p≤0.049), except for *S. aureus* and *S. epidermidis* planktonic cells with 4 days of exposure. Comparison between 100 and 200 µA showed less marked differential reductions of biofilms and planktonic cells, however a significant difference in effect was measured for 200 compared to 100 µA for both planktonic cells and biofilms at 24 hours and 4 day for *P. aeruginosa* (p≤0.049).
Generally, greater reductions were observed with planktonic cells compared to biofilms. No viable planktonic cells were observed after exposure to 500 µA at any time point for all bacterial species studied. However, viable planktonic *C. parapsilosis* cells were found after 24 hours of exposure to 500 µA (2.4 log_{10} cfu/ml reduction; p, 0.01). Exposure to 200 µA achieved reductions ranging from 1.7 to 6.2 log_{10} cfu/ml (p<0.02) after 24 hours and from 3.6 to 7.0 log_{10} cfu/ml (p<0.02) after 4 days for all bacterial species. Planktonic cell reductions ranging from 1.1 to 4.4 log_{10} cfu/ml were measured after 4 days of exposure to 100 µA for the bacterial species. Again, *C. parapsilosis* was more resistant, with just a 0.7 log_{10} cfu/ml reduction after 4 days of 100 µA exposure (p=0.16).
In this study, we demonstrated that DC reduces staphylococcal, E. coli, P. aeruginosa and C. parapsilosis biofilms on the intraluminal surface of catheters in a time- and dose-dependent manner. The most dramatic effects (i.e., no detectable viable cells) were observed when S. epidermidis and S. aureus biofilms were exposed to 500 µA DC for 4 days, although large reductions (≥5.0 log_{10} cfu/cm²) were also observed when P. aeruginosa and E. coli biofilms were exposed to 500 µA DC for 4 days. Reductions of more than 1.0 log_{10} cfu/cm² were observed with C. parapsilosis exposed to 200 or 500 µA, however, the degree of reduction was less compared to that observed with the other organisms studied. Exposure to 100 µA yielded biofilm reductions for S. epidermidis (after 4 days), as well as E. coli and P. aeruginosa (after 24 hours). Overall, these results show that electrical current, applied via intraluminal electrodes, has a marked effect on microbial biofilms on catheter surfaces.

The underlying mechanism of the effect observed is not fully understood. Oxidative stress (26-30), damage to the cell walls (31-32), changes in pH (33-34) and formation of hypochlorous acid by electrolysis (33) have been proposed. The results generated herein support an electrochemical mechanism. Detachment promoted by enhanced repulsive forces between microorganisms and surface materials may also play a role (31, 35-38).

Although our results are consistent with previous data showing a bactericidal effect of DC against sessile and planktonic cells, previous studies either used different electrode positioning, focusing on biofilms grown on discs placed between two electrodes (22, 25, 39), or investigated the effects of custom-fabricated electrically conductive catheters on bacterial colonization in agar plates (40). In this study, electrodes were simply placed into the lumen of commercially available catheters on which biofilms had been grown. Theoretically, this could be
adapted to clinical settings by introducing electrodes into infected catheters, without the need of expensive changes to the design of available catheters.

Limitations of this study relate to the methodology employed and ability to extrapolate our findings. The phosphate buffer did not resemble physiological body fluids such as blood or urine. Chlorine, which is abundantly present in body fluids and which might enhance the effect by formation of hypochlorous acid (33), was not added to the study buffer (but might have been present in small quantities as a result of having grown biofilms in TSB). Catheter materials like silicone or latex, which are widely used in urinary or venous catheters, might behave differently with regards to biofilm growth and/or reduction achieved by electric current, compared to the PVC catheters used in this study. It also has to be considered that in this model electrodes were placed intraluminally, not affecting the outer catheter surface. In vivo biofilms may grow on the outer and inner surfaces of infected catheters (41-42). Interestingly, studies using electrified catheters with comparable electrode positioning showed reduced encrustation by *Proteus mirabilis* biofilms at the catheter eyelet region in vitro (43), and reduced microbial populations associated with catheter-associated urinary tract infections in vivo (44-45). Finally, safety issues need to be addressed to use this strategy in a clinical setting. The use of low dose electric current within the urinary tract appears to be safe; a study of electrified catheters in sheep resulted in no physical or chemical changes of urine or the tissues of the urinary tract using an amperage of 400 µA (44). Similarly, an electrified urinary catheter trial in humans did not show adverse effects or evidence of catheter damage (45). Possible adverse reactions of electric current delivered into intravascular catheters, including cardiac arrhythmias, hemolysis and thrombus formation, require further investigation.
In conclusion, our results demonstrate that biofilms in catheters can be reduced using low dose DC. Although further *in vitro* and *in vivo* studies are needed, this strategy might be useful to combat clinically challenging catheter-associated infections.
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DISCLOSURES

The authors report no conflicts of interest in this work.
REFERENCES


Figure 1. Catheter biofilm model. PVC catheters (inner diameter 6 mm) were cut to a length of 45 mm; polyoxymethylene plastic caps were used to seal the bottom of the catheters and were placed in catheter tops to hold platinum electrodes (50 mm in length and 1.6 mm in diameter) in place.
Figure 2. Results of quantitative cultures of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida parapsilosis* biofilms on PVC catheters and planktonic cells in the study buffer after 0, 100, 200 and 500 µA DC exposure for 24 hours and 4 days. The x-axis represents hours (h) and days (d) of DC exposure. The y-axis shows results of quantitative cultures in log_{10} cfu/cm² for biofilm and log_{10} cfu/ml for planktonic cultures. Error bars indicate standard deviation. The dashed line indicates the limit of detection. *Statistical significance (p<0.05) compared to 0 µA.*