# Prevention and Control of Bacterial Infections Associated with Medical Devices

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**Bacteria that grow in association with medical devices always form slime enclosed biofilms, within which they are protected, to a large extent, from the bactericidal activity of chemical biocides and antibiotics. Mature biofilms (>7 days) are demonstrably resistant to 500–5,000 times the concentrations of these agents than are necessary to kill free floating (planktonic) cells of the same organism. The authors have discovered that this well established inherent resistance of biofilm bacteria to antibacterial agents can be completely obviated if these agents are applied to these adherent populations within an electric field. The killing of biofilm bacteria by antibiotics can be dramatically enhanced by relatively weak electric fields (1.5 V/cm and 15 $\mu A/cm^2$) that, in themselves, have no deleterious effects on these slime protected populations adherent to plastic or metal surfaces. This bioelectric technology can readily be used to enhance the preimplantation sterilization of medical devices by biocides. The authors suggest that it may also be used to control biofilm formation and consequent infection by electrically enhanced perioperative antibiotic prophylaxis and by electrically enhanced penetration of antibiotics to kill the biofilm bacteria that form the inherently resistant nidus of chronic device related infections. *ASAIO Journal* 1991; 38: M174–M178.**

The direct examination of a large number of natural and industrial ecosystems has detected the pronounced tendency of bacteria to grow in slime enclosed biofilms adherent to available surfaces.[1] Perhaps we should not have been surprised when similar direct observations of material from chronic diseases showed the same tendency of bacteria to form slime enclosed microcolonies and biofilms, because many modern pathogens are essentially environmental species.[2] Similarly, we should have expected that biofilms would be detected, as they have been, on the plastic and metal surfaces of medical devices that were the foci of device related infections.[3,4] Electron and light microscopy led us to visualize a random distribution of bacterial cells in a homogeneous exopolysaccharide matrix,[2] but recent observations of living hydrated biofilms by confocal scanning laser microscopy[5] have clearly shown that biofilm bacteria live in microcolonies surrounded by a dense slime matrix, and that the biofilm is traversed by less dense "water channels" within the matrix **(Figure 1)**. Clearly, biofilm bacteria live in functional "niches" that are profoundly different from the bulk fluids of these ecosystems, and they respond to these altered environmental conditions by triggering important phenotypic variations.[2]

Clinicians have long realized that exacerbations of device related bacterial infections could be controlled by antibiotic therapy but that these chronic infections cannot usually be fully resolved until the device in question is removed.[6] This inherent resistance of biofilm bacteria to antibiotics and to biocides has been demonstrated and quantified in a large number of *in vitro* and *in vivo* animal studies.[7–9] We can now conclude that biofilm bacteria are resistant to all antibacterial agents tested to date at levels 50–500 times higher than those that kill planktonic cells of the same strain. Older biofilms (circa 7 days) are even more resistant to antibiotics and may require 5,000 times the minimal bactericidal concentration to kill all of their slime protected organisms.[10] This perception was confirmed when viable biofilm cells were discovered on the Jarvik heart and disseminated in the tissues of patients who had been treated with the maximum tolerable doses of modern antibiotics for extended periods of time.[11] The mechanism of this inherent resistance of biofilm bacteria to antibiotics is not well understood, but speculation centers on the limitation of diffusion by the polyanionic matrix layer and on phenotypic adaptations to biofilm growth that alter the metabolic targets of these antibacterial agents.[2]

Biofilm bacteria are also effectively protected from host defense mechanisms, as is attested to by the remarkable persistence of device related infections; specific antibacterial antibodies and activated white blood cells have both been shown to be ineffective in killing these slime protected cells.[12,13] The host defenses of the rabbit peritoneum are sufficiently vigorous to withstand challenge by $1 \times 10^6$ planktonic cells of *Pseudomonas aeruginosa*, but these humoral and cellular mechanisms cannot clear even $1 \times 10^3$ cells of the same strain if they are allowed to form biofilms on plastic surfaces before implantation.[14] These data constitute a useful object lesson because it is clear that the placement of a medical device that has been colonized with a bacterial biofilm before implantation will always lead to an

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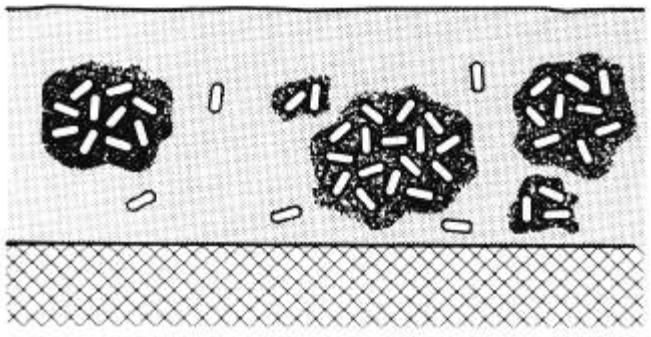

**Figure 1.** Diagram of the structure of a living biofilm as revealed by confocal scanning laser microscopy showing discrete bacterial microcolonies surrounded by exopolysaccharide slime and traversed by water channels.

overt device related infection.[14] Prophylaxis in device related infections is especially important because of the persistent and devastating consequences that follow when a bacterial biofilm becomes established and cannot be resolved by host defenses or antibiotic therapy.

Because we perceived the frequent failure of the conventional attack on biofilm bacteria that uses targeted antibiotics as its primary, essentially biochemical weapon, we resolved to try to overcome what may be an access problem by a combined physicochemical approach that uses these same charged molecules within an electric field that may affect their distribution within biofilms. We chose a direct current (DC) field similar to that widely used to accelerate bone healing and tissue repair and to drive chemotherapeutic agents into solid tumors and antibiotics into the inner ear.[15-18] The electric fields used in the present study were much weaker than those used to kill planktonic bacterial cells by electric activity alone and substantially less than those used to kill planktonic bacterial cells by iontophoresis.[19,20,21] In the present study, our emphasis is on the killing of bacterial cells within slime enclosed biofilms by the combined activity of antibiotics and electric fields.

## Materials and Methods

In this study, we have used the modified Robbins device (MRD), a perspex block with a central flow channel (**Figure 2A**) in which studs with a surface area of 0.5 $cm^2$ are exposed to the flowing fluid.[7] We used stainless steel studs and modified the MRD by adding a platinum wire set in a groove in the bottom of the flow channel (**Figure 2B**) to produce the electric MRD (e-MRD). The steel studs were connected to a DC electric source and constituted one electrode, while the platinum wire constituted the other electrode; polarity was reversed every 64 sec. Voltage was monitored and field strengths were seen to vary between 1.5 and 20.0 V/cm, while current densities varied between 15.0 and 400.0 $\mu A/cm^2$.

Biofilms were produced in the e-MRD by passing fluid from batch cultures of bacteria and fungi through the e-MRD at 60 ml/hr using a peristaltic pump. The strains of *P. aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Candida albicans* used in this study were fresh clinical isolates obtained from the diagnostic laboratories of the Foothills Hospital (Calgary, Alberta, Canada). Fresh isolates were used because strains that have been transferred repeatedly in the laboratory tend to lose their ability to form biofilms.

The number of living biofilm bacteria was determined by removing two studs from random locations and detaching and dispersing the adherent cells by scraping, vortex mixing, and sonication before duplicate plating to determine colony forming units (CFU).[7] These quantitative recovery methods were used to monitor the formation of biofilms on the studs as batch cultures were passed through the e-MRD and to determine the extent to which biofilm cells were killed by antibiotic/electric treatments. After biofilm formation was complete, control experiments were conducted in which sterile medium was passed through the colonized e-MRD. In other experiments, sterile medium containing antibiotic was passed through the colonized e-MRD, and both sterile medium and sterile medium containing antibiotic were passed through the colonized e-MRD with the application of a continuous DC electric field.

## Results

We were able to establish biofilms on the studs of the e-MRD using the three bacterial species and the single fun-

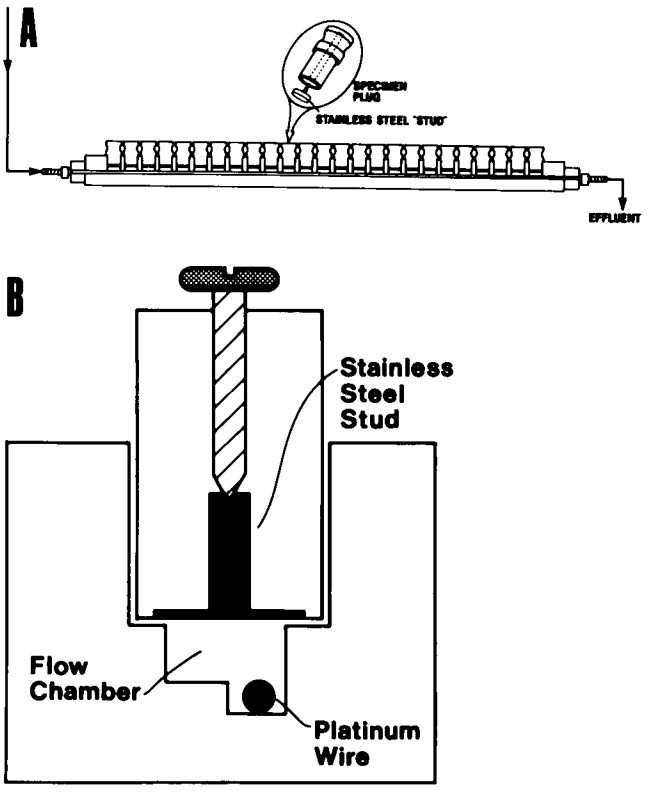


**Figure 2.** (A) Diagram of a modified Robbins device (MRD) in which 25 specimen plugs expose stainless steel studs at the top of a flow channel through an elongated perspex block; (B) Cross-sectional diagram of an electrically conductive modified Robbins device (e-MRD) in which a platinum wire constitutes one electrode and a stainless steel stud constitutes the other.

gal species, and these biofilms continued to increase in cell numbers (CFU) in control experiments in which sterile medium was passed through the colonized e-MRD (data not shown). When sterile medium was passed through the colonized e-MRD while the electrodes were activated to produce an electric field, biofilms of *S. epidermidis*, *E. coli*, and *C. albicans* showed no change in the number of living adherent bacteria (CFU), and biofilms of *P. aeruginosa* showed a decrease of only one order of magnitude (data not shown). When colonized e-MRDs were treated with antibiotics in the absence of an electric field, no significant decreases were seen in the number of living biofilm cells at antibiotic concentrations between 0.5 and 35.0 MIC **(Figures 3 and 4)**, but decreases of up to two orders of magnitude were seen at antibiotic concentrations of 40.0–100.0 MIC in the case of *P. aeruginosa*. In these and other experiments, antibiotics must usually be used at concentrations of 500–5,000 minimum inhibitory concentration (MIC) to produce complete killing of biofilm bacteria.[7,15]

When antibiotics were used to treat colonized e-MRDs in the presence of an electric field, we noted dramatic decreases in the concentrations of those agents necessary to kill biofilm cells completely. When 1.25 MIC of tobramycin (2.5 mg/L) was used to treat an e-MRD colonized by biofilm cells of *S. epidermidis* **(Figure 3)**, all of these adherent cells were killed at 8 hr. Biofilm cells of *C. albicans* were completely killed in 12 hr by 0.1 MIC of cycloheximide, an agent that is not notably effective against this fungal pathogen. Biofilm cells of *P. aeruginosa* were completely killed in 12 hr by 8 MIC (8 mg/L) of tobramycin in the presence of the electric

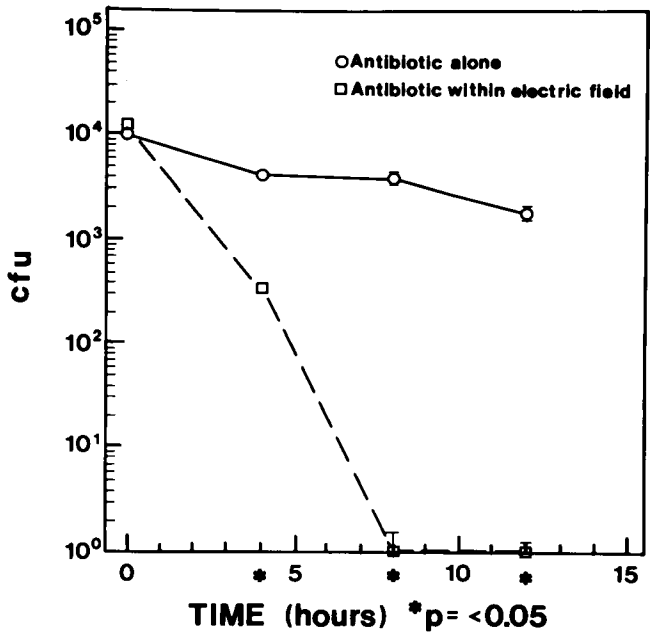


**Figure 3.** *S. epidermidis* biofilm + tobramycin 2.5 mg/L. Graphic representation of the killing of biofilm cells of *S. epidermidis* by exposure to 2.5 mg/L of tobramycin (1.25 minimum inhibitory concentration) within an electric field. Vertical bars represent standard deviations and asterisks indicate probability at the p = <0.05 level in Figures 3–5.

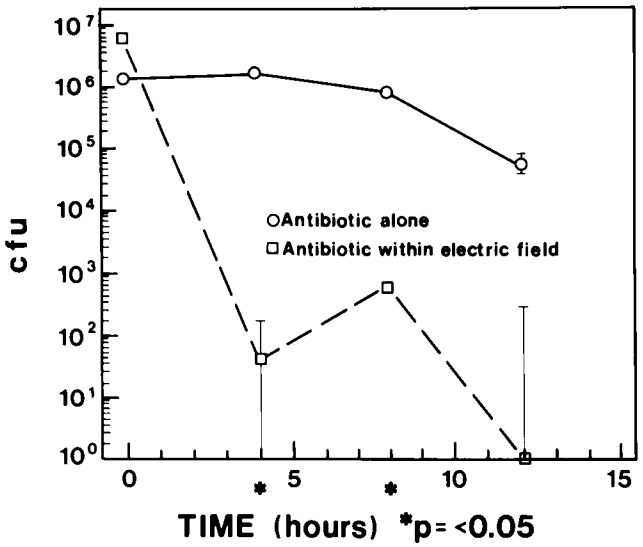


**Figure 4.** *P. aeruginosa* biofilm + tobramycin 8 mg/L. Graphic representation of the killing of biofilm cells of *P. aeruginosa* by exposure to 8 mg/L (clinically attainable concentration) of tobramycin (8 MIC) within an electric field.

field **(Figure 4)**; killing the biofilm cells of this organism was even more rapid and clear-cut **(Figure 5)** when the concentration of this antibiotic was raised to 40.0 MIC. **Table 1** lists all of the organisms used to form biofilms in this study and the antibiotic agents that were used at various concentrations to treat these developed biofilms. All of these organisms were susceptible to all of these agents at concentrations as low as 0.1–5.0 MIC, if the antibiotics were applied in the presence of an electric field. Wide ranging preliminary experiments in which antibiotics of several classes have been used to treat biofilm cells of 12 species of gram negative

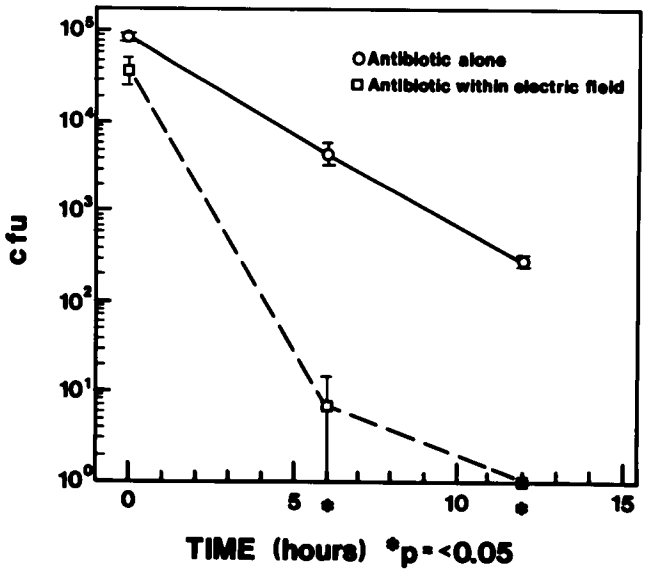


**Figure 5.** *P. aeruginosa* biofilm + tobramycin 40 mg/L. Graphic representation of the killing of biofilm cells of *P. aeruginosa* by tobramycin in the presence and absence of an electric field.

**Table 1. Representative Data from Tests of the Susceptibility of Biofilm Bacteria to Antibiotics**

| Organism | Antibiotic | Minimum Inhibitory Concentration (mg/L) | Concentration of Antibiotic Used | Minimum Inhibitory Concentration Used | Biofilm Kill with Electric Field Alone | Biofilm Kill with Antibiotic Alone | Biofilm Kill with Antibiotic in Electric Field |
|---|---|---|---|---|---|---|---|
| *P. aeruginosa* (Strain 1) | Tobramycin | 1.0 | 5.0 | 5.0 | – | – | + |
| | | | 8.0 | 8.0 | – | – | +* |
| | | | 40.0 | 40.0 | – | – | +† |
| | | | 100.0 | 100.0 | – | – | + |
| *P. aeruginosa* (Strain 2) | Tobramycin | 2.0 | 10.0 | 5.0 | – | – | + |
| | | | 50.0 | 25.0 | – | – | + |
| | | | 100.0 | 50.0 | – | – | + |
| *E. coli* | Tobramycin | 2.0 | 10.0 | 5.0 | – | – | + |
| | | | 50.0 | 25.0 | – | – | + |
| | | | 100.0 | 50.0 | – | – | + |
| *S. epidermidis* | Tobramycin | 2.0 | 2.5 | 1.25 | – | – | +‡ |
| | | | 5.0 | 2.5 | – | – | + |
| | | | 10.0 | 5.0 | – | – | + |
| | | | 50.0 | 25.0 | – | – | + |
| | | | 100.0 | 50.0 | – | – | + |
| *P. aeruginosa* | Ciprofloxacin | 0.5 | 1.25 | 2.5 | – | – | + |
| | | | 2.5 | 5.0 | – | – | + |
| | | | 5.0 | 10.0 | – | – | + |
| *C. albicans* | Cycloheximide | 1,000.0 | 100.0 | 0.1 | – | – | + |

* Time course data in Figure 4.
† Time course data in Figure 5.
‡ Time course data in Figure 3.

bacteria, 5 species of gram positive bacteria, and 1 fungal species, indicate that the dramatic enhancement of the efficacy of these agents by electric fields is a general phenomenon.

## Discussion

An ecologic examination of the data from direct examination of the growth of bacteria in numerous natural, industrial, and medical ecosystems clearly leads us to the conclusion that most of these organisms live in slime enclosed biofilms adherent to available surfaces.[1,2] Because many common bacteria (e.g., *P. aeruginosa*) grow and even predominate in all three types of ecosystems, it is logical that they would employ the same basic growth strategy: most of their cells grow in protected biofilms that periodically shed smaller numbers of planktonic cells that can colonize new surfaces.[2] The epidemic diseases that occupied human attention early in this century were acute diseases caused by planktonic cells of highly specialized pathogenic species. Most of these diseases are now prevented by vaccines and effectively controlled by antibiotics. In recent decades, we have encountered many more bacterial diseases of compromised patients[6]; these chronic diseases are caused by biofilm cells of common environmental organisms (e.g., *P. aeruginosa*) that are not prevented by vaccines and respond poorly to treatment with antibiotics.[11] Some of the most refractory of modern bacterial diseases are associated with medical devices, and these pathogens have been clearly shown to form biofilms within which they are inherently resistant to antibiotics[6,7,10] and host defense factors.[14] The threat of devastating bacterial infections remains perhaps the most serious problem that limits the current and future development of medical devices.[3]

Antibiotic molecules have generally unimpeded access to their specific targets in bacterial cells that are growing planktonically in laboratory media or in body fluids; agents developed in the laboratory have been used successfully to treat these acute infections. Direct observation of bacteria growing on the surfaces of medical devices has shown that they grow predominantly in biofilms and that these biofilm bacteria are highly protected from the essentially chemical attack of antibiotics.[3,4] Biofilm bacteria do not always cause overt symptoms, as when they colonize a vascular catheter but do not cause a bacteremia[22]; however, they comprise a bacterial nidus that is protected from antibiotic therapy. Once a nidus of bacterial colonization has developed into an overt, chronic, device related infection, current therapeutic strategies are usually ineffective until the device (and the biofilm) is removed.

We have discovered the "Achilles' heel" of the bacterial biofilm in that we have found that we can obviate the inherent resistance of biofilm bacteria to chemical agents (biocides and antibiotics) by the use of an electric field to produce the bioelectric effect.[23] Most workers have attributed the resistance of biofilms to antibiotics to the failure of these agents to penetrate the biofilm matrix[2]; preliminary evidence on the mechanism of the bioelectric effect suggests that the electric field may drive charged molecules through this matrix by electrophoresis.[23] Additionally, there is some preliminary evidence that biofilm bacteria are rendered more permeable by some form of low voltage electroporation within the electric field, both because there is some leakage of cytoplasmic constituents and because some organisms within biofilms are made susceptible to agents (e.g., cycloheximide) that will not even kill planktonic cells (e.g., *Candida albicans*). Whatever the mechanism of the bioelec-

tric effect may be, it is clear that electric fields dramatically enhance the efficacy of biocides and antibiotics in killing biofilm bacteria.

The bioelectric effect can be produced by field strengths as low as 1.5 V/cm and current densities as low as 15.0 $\mu A/cm^2$; the effect operates on biofilms anywhere in the electric field as effectively as it does on biofilms growing directly on one of the electrodes. Because the bioelectric effect appears to operate mainly by overcoming permeability problems by directed electrophoresis, it has already been shown to enhance the efficacy of all biocides and antibiotics tested to date against biofilm cells of all bacteria and fungi tested to date.[23] The bioelectric effect does not appear to be agent specific or organism specific; we assume that it will facilitate the killing of all pathogenic cells, including protozoa and viruses, by agents that normally have difficulty in penetrating accretions of biofilm matrix, host products, and/or general organic detritus (e.g., tissue fragments in dental drills).

We anticipate that this new bioelectric technology will be used immediately to enhance the efficacy of biocides in sterilizing contact lenses, delicate medical instruments (e.g., endoscopes), and the lubricating solutions used in metal rolling and metal cutting operations. In these cases, low voltage fields can be used to reduce the concentration of biocides necessary to kill planktonic and biofilm bacteria by at least 1,000 times. Next, we propose to produce very weak electric fields in transcutaneous catheters that are rapidly colonized by biofilm bacteria and to develop bioelectric antibiotic treatment strategies to sterilize these devices *in situ*.[22,24,25] Finally, we propose to develop implantable devices that can be accessed to produce effective electric fields to enhance the perioperative use of antibiotics to kill developing bacterial biofilms and prevent device related infections. If device related infections develop because of contamination during surgery, or because of the hematogenous spread of bacteria,[6] they can be treated by bioelectric technology if a sufficient field can be generated by direct connection to an electric source or by electromagnetic induction. The electromagnetic induction of fields sufficient to produce the bioelectric effect may facilitate effective antibiotic treatment of non-device related chronic bacterial diseases such as prostatitis, osteomyelitis, and cystic fibrosis pneumonia. Currently, antibiotic therapy is ineffective in treating these diseases because of permeability limitations; i.e., the pathogens live in slime enclosed microcolonies and biofilms.[26-28]

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