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Bioelectric effect and bacterial biofilms. A systematic review

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Abstract

Bacteria growing in biofilms cause a wide range of human infections. Biofilm bacteria are resistant to antimicrobics at levels 500 to 5,000 times higher than those needed to kill non-biofilm bacteria. In vitro experiments have shown that electric current can enhance the activity of some antimicrobial agents against certain bacteria in biofilms; this has been termed the “bioelectric effect”. Direct electrical current has already been safely used in humans for fracture healing. Application of direct electric current with antimicrobial chemotherapy in humans could theoretically abrogate the need to remove the device in device-related infections, a procedure associated with substantial morbidity and cost. In this article, we review what has been described in the literature with regards to the bioelectric effect.

Keywords

Bioelectric effect; Biofilm

BIOFILM-RELATED INFECTIONS

The epidemic bacterial diseases that occupied human attention at the turn of the last century were generally acute in nature and caused by planktonic bacteria of highly specialized pathogenic species (e.g., *Corynebacterium diphtheriae*); most of these diseases are now prevented by vaccines and/or effectively controlled by currently-available antimicrobial agents (18). In their place, chronic bacterial diseases (which are poorly responsive to antimicrobics), have emerged (1). Some of the most refractory modern bacterial diseases are those associated with medical devices (e.g., joint replacements and other orthopedic instrumentation, prosthetic heart valves, pacemakers, intraventricular cardiac assist devices, automated implantable cardioverter defibrillators, urinary tract catheters and stents, peritoneal dialysis catheters, central venous catheters, neurovascular shunts, synthetic vascular grafts and stents, artificial voice prostheses, intrauterine devices) (2). When the surfaces of medical devices become the foci of device-related bacterial infections, the associated microorganisms grow in well-developed, adherent biofilms (1). It has been estimated that two thirds of human bacterial infections may involve biofilms (3).

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Microorganisms growing in biofilms on medical devices are protected from killing, to a large extent, by innate host defenses and the bactericidal activity of antimicrobial agents, a type of resistance unique to biofilm-associated bacteria and distinct from conventional antimicrobial resistance (4). Bacteria in biofilms exhibit dramatically reduced (i.e., 500-5000 times) susceptibility to killing by antimicrobial agents as compared to free-floating (planktonic) cells of the same microorganism (5, 6). The resistance that bacteria exhibit when they grow in biofilms is not due to "classic" genetic mechanisms (i.e., gene mutation, genetic exchange), but is instead determined by peculiarities of biofilm growth. A variety of potential mechanisms implicated in biofilm resistance to antimicrobial agents have been proposed (2) including: restricted penetration through the biofilm matrix, antimicrobial destroying enzymes, quorum sensing signaling systems, existence of altered growth rate (persister cells) inside the biofilm, stress response to hostile environmental conditions, and overexpression of genes.

From the experience of clinical practice, it is known that device-related infections are highly refractory to antimicrobial therapy. Currently, a commonly applied therapeutic approach for implant related infections includes removal of the implanted biomaterial (1). Given the failure of conventional antimicrobics in the management of most biofilm-associated infections, novel and innovative therapeutic and preventive approaches are warranted.

Electrical current and bacteria

Bacterial cells depend on physical phenomena such as membrane potentials for their basic metabolic activity (7). It has been shown that external fields can affect the alpha-helix content and orientation of membrane proteins in eukaryotic cells, and the electrophoretic mobilities of bacterial membrane proteins (7). Moreover, electric fields and currents can influence the organization of biological membranes, metabolic and developmental processes within both prokaryotic and eukaryotic cells, and even the shape of cells, cell behavior and the dimensions of the bacterial glycocalyx (8). Directional growth in response to electric fields (galvanotropism) is well-known amongst eukaryotic cells as diverse as fibroblasts, neurons, algae, and fungal hyphae (9). This mechanism may involve differential stimulation of wall growth in both anode- and cathode-facing regions, modulating wall growth spatially (9).

The antibacterial activity of electric current has been previously demonstrated against *Escherichia coli* in salt solutions (10), *Staphylococcus aureus* in agar (11), normal flora on human skin (12), *E. coli*, *Proteus* species and *Klebsiella pneumoniae* in synthetic urine (13), and *E. coli*, *S. aureus* and *Bacillus subtilis* in water (14, 15).

The mechanism of the antibacterial activity of electric current has been variously suggested to result from toxic substances produced as a result of electrolysis (e.g., H_2O_2, oxidizing radicals, chlorine molecules), oxidation of enzymes and coenzymes, membrane damage leading to leakage of essential cytoplasmic constituents, and/or decreased bacterial respiratory rate (16).

According to several studies, the efficacy of biocides (17) and antibiotics (18) in killing biofilm bacteria can be radically enhanced if these agents are used within a low-intensity DC electric field; this has been termed the "bioelectric effect". Costerton et al showed in 1994 (19) that the efficacy of certain antimicrobial agents could be increased through the application of weak electric fields. In this study, it was shown that with the combined application of direct current electric fields of about 1.5 to 20 V/cm^2 (current densities of about 15×10^{-6} to 2.1×10^{-3} A/cm^2) and tobramycin, the concentration of antimicrobial needed to exhibit activity against biofilm bacteria fell out 1.5 to 4.0 times compared to that needed against planktonic bacteria. Jass et al were the first to report the bioelectric effect

using antimicrobials other than aminoglycosides (20). This group demonstrated that an electrical current could enhance the activity of some antimicrobials (i.e., ciprofloxacin and polymyxin B) but not of others (i.e., piperacillin) against *P. aeruginosa*. Wellman's studies (21) indicate that a dose response may exist for the level of antimicrobial plus electrical field, since enhanced killing was seen at 5 mg of tobramycin per liter and 1 mA of current, but no enhanced killing was recorded at 1 mg/liter and 1 mA of current. Similar findings were presented with regards to the amount of current (21). These authors also suggested that there might be a level of current above which the bioelectric effect ceases. However, dose-response curves for both the antimicrobial agent and the current flow were not established.

Bioelectric effect mechanism of action

Much has been hypothesized to explain the mechanism of action of the bioelectric effect; however, a satisfactory explanation remains to be formulated. Some of the hypothetical mechanisms that have been suggested include reduction of biofilm capacity for binding the antimicrobial agent (17), increased membrane permeabilization (18), electrophoretic augmentation of antimicrobial transport (18), increased bacterial growth due to electrolytic generation of oxygen (and subsequently enhanced susceptibility to antimicrobials) (22, 23), electrochemical generation of potentiating oxidants (19), increased convective transport due to contraction and expansion of the biofilms (24), increased transport through electroosmosis, physical removal of the biofilm with electrolytically generated bubbles, and enhanced susceptibility due to a temperature increase inside the biofilm (Fig. 1).

a. Reduction of the biofilm capacity for binding to the antimicrobial agent— The matrix in which biofilm cells are enclosed may, in some cases, bind antimicrobial agents before they reach their target cells. Some authors have hypothesized that if an electrical current disrupts charges in the matrix, this might allow penetration of antimicrobial agents (17).

b. Membrane permeabilization and electrophoretic augmentation of antimicrobial agent transport—Electroporation-like mechanisms have been suggested as a mechanism of action of the bioelectric effect (19). Electroporation is a significant increase in the electrical conductivity and permeability of a cell plasma membrane caused by an externally applied electrical field. It is used in molecular biology as a way of introducing DNA into cells. Pores are formed when the voltage across a plasma membrane exceeds its dielectric strength. These pores formed by the electrical pulse reseal after a short period of time. In the same way, the bioelectric effect may depend largely on electrophoretic forces that allow antimicrobial agents to overcome diffusion barriers that would otherwise limit their access to their targets within bacterial cells (17, 19, 25).

c. Electrolytic generation of oxygen—The bioelectric effect could be related to electrolysis, with resultant increased delivery of oxygen to the biofilm, which might overcome biofilm biomass and cell wall barriers, as well as increase the metabolic activity and growth rate of the contained bacteria (20, 23). Reduced antimicrobial susceptibility of biofilm bacteria has been associated with localized oxygen depletion within biofilms (26, 27), and with an increased expression of the extracellular polysaccharide which mediates bacterial cell to cell adhesion (13). Production of free oxygen by electrolysis might overcome the two phenomena. However, the mechanism by which oxygen might enhance biofilm susceptibility remains to be definitively established. Stewart et al, proposed that oxygen might reach toxic levels, making the bacteria more susceptible to the antimicrobials; alternatively, increased delivery of oxygen could enhance growth of biofilm cells, overcoming reduced susceptibility associated with slow growth (23). If biofilm resistance to antimicrobials is due to oxygen deprivation (e.g., it is well know that aminoglycosides are

less active under anaerobic conditions than under aerobic conditions) inside the biofilm, then an increase of the concentration of oxygen could make biofilm cells more susceptible to aminoglycosides. Stewart et al showed that when oxygen was sparged into a *P. aeruginosa* biofilm exposed to antimicrobial (but not electrical current), there was a significant enhancement of tobramycin efficacy (23). This enhancement was about two-thirds of that obtained when biofilm was exposed to electrical current and tobramycin.

d. Electrochemical generation of potentiating oxidants—The voltage generation of ions might be the cause of the bioelectric effect. However, Costerton et al presented experiments which indicate that this is probably not the case, on the basis of the absence of antimicrobial activity immediately downstream of an electrified chamber (19). Some studies have reported that electrical current applied alone does not result in discernible killing (17-20, 22); however, other studies have reported some effect of the electrical current alone when applied to biofilms (23, 28).

Bioelectric effect in vitro *studies*

A number of *in vitro* models have been described to test the bioelectric effect (Tab. I). Although the bioelectric effect was initially described for *P. aeruginosa* and biocides (isothialazone 1.5%, dimethyl ammonium chloride 50%, and glutaraldehyde 25%) (17), most subsequent studies have been done with antimicrobial agents. Khoury et al (18) used an electric modified Robbins device to demonstrate that the *in vitro* killing of *P. aeruginosa*, *E. coli*, and *S. epidermidis* biofilms by tobramycin could be enhanced by direct electric current delivered for 12 hours. Steel studs connected to a direct current electric source constituted one electrode. A platinum wire set in a groove in the bottom of the flow chamber constituted the other. The application of electric current (15-400 $\mu A/cm^2$) alone showed no effect on biofilms of *E. coli* or *S. epidermidis* and a single log decrease of *P. aeruginosa* biofilms. When biofilm bacteria were treated with antimicrobial agents alone (0.5 to 35.0 times the minimum inhibitory concentration), no effect on biofilms was noted. When tobramycin (2.5 μg/mL) was combined with electric current, all of the *S. epidermidis* biofilm bacteria were killed in 8 hours (a greater than 3 log decrease compared to bacterial cells exposed to tobramycin alone). When tobramycin (8 μg/mL) was combined with electric current, all of the *P. aeruginosa* bacteria biofilms were killed in 12 hours (a 4-log decrease compared to bacterial cells exposed to tobramycin alone). Ciprofloxacin (1.25-5.0 μg/mL), in combination with direct electric current, also demonstrated activity against *P. aeruginosa* biofilms.

Costerton et al (19) grew *P. aeruginosa* biofilms on stainless steel electrodes in a Perspex flow chamber for 24 hours. It was demonstrated that the combination of tobramycin (5 μg/mL) and direct current (1.7 mA/cm^2) administered for 48 hours increased the *in vitro* killing of the bacteria 4-5 log orders (compared with tobramycin alone or electric current alone). Importantly, these investigators showed that the *in vitro* bioelectric effect applied to all areas of the active electrodes and to the surfaces of conductive elements lying within the electric field but not themselves functioning as electrodes.

Jass et al (22) used an electrical colonization cell to study the effect of tobramycin on *P. aeruginosa* biofilms suspended on one side of a dialysis membrane between two electrodes, thereby avoiding electrochemical and mechanical disturbances, yet remaining in the path of the electric current. Electric currents of up to 20 mA/cm^2 delivered for 12 hours did not prevent biofilm formation or have any detrimental effect on an established biofilm. Tobramycin (10 μg/mL) alone did not affect the biofilm, but its antimicrobial action was enhanced nearly 2 log orders by 9 mA/cm^2 electric current. In a follow-up manuscript (20), they used the same model to study the effect of ciprofloxacin (5 μg/mL) in the presence of 0

or 9 mA/cm^2 current density on *P. aeruginosa* biofilms. Ciprofloxacin alone reduced the biofilm population; in the concomitant presence of the electrical current, the population was further reduced.

Wellman et al (21) grew mixed-culture biofilms of P. *aeruginosa* and *K. pneumoniae* for 7 days on polycarbonate coupons. Experimental chambers were built from FisherBrand five-slide, 50 gauge polypropylene slide transporter boxes modified to allow a pathway for nutrient support medium flow, and for placement of 22 gauge platinum wire electrodes at either end of the chamber. Delivery of the combination of direct electric current (1 mA) and tobramycin (5 μg/mL) for 24 hours resulted in an increase in the *in vitro* killing of the bacteria of 6 to 8 log orders (compared with tobramycin alone). Little killing was observed with tobramycin alone; a 1 log reduction in viable cell numbers with current alone was noted.

Stewart et al (23) placed *P. aeruginosa* biofilms grown for 3 days on polycarbonate slides in rectangular treatment chambers and delivered direct electric current (2 mA) through the chamber by means of a circuit containing a current controller and two stainless-steel wires at opposite ends of the long axis of the treatment chamber. When treated with tobramycin (5 μg/mL) for 24 hours, *P. aeruginosa* biofilms exhibited a 3 log reduction in viable cell numbers whereas a 5 log reduction was measured in a planktonic culture. When direct current was applied with tobramycin, biofilm killing increased by a further 3 log orders.

Wattanakaron et al (29) demonstrated electrical enhancement of *Streptococcus gordonii* biofilm killing by gentamicin in an *in vitro* model. The experimental methods were as described by Stewart et al (23), except that the biofilms were grown for 6 days. In this model, electric current (0.4 mA/cm^2) flowed approximately parallel to the substratum to which the biofilm was attached. When treated with gentamicin (2 μg/mL) for 24 hours, *S. gordonii* biofilms exhibited a 1 log reduction in viable cell numbers whereas a 5 log reduction was measured in a planktonic culture. When direct current was applied during gentamicin treatment, biofilm killing increased by a further 4 log orders. Electrical current alone caused a 2 log reduction in viable cell numbers.

Pickering et al (30) investigated the *in vitro* effect of a pulsed electromagnetic field on the activity of gentamicin or vancomycin in the treatment of five-day-old *S. epidermidis* biofilms grown on the tips of stainless-steel pegs. The biofilms were exposed to varying concentrations of antimicrobic in microtiter trays at 37°C and 5% CO_2 for 12 hours with or without a pulsed electromagnetic field. Exposure to a pulsed electromagnetic field increased the activity of gentamicin against the five-day biofilms of *S. epidermidis*. In three of five experiments there was reduction of at least 50% in the minimum biofilm inhibitory concentration. In a fourth experiment there was a 1 to 2 log reduction in colony count on exposure to 256 times the MIC of gentamicin and pulsed electromagnetic field. Analysis of variance confirmed an effect by a pulsed electromagnetic field on the activity of gentamicin ($p<0.05$). Importantly, however, no significant bioelectric effect was observed with vancomycin in this model.

Caubet et al (31) demonstrated electrical enhancement of *E. coli* biofilm killing by gentamicin or oxytetracycline in an *in vitro* model. They used *E. coli* grown for 24 hours on glass slides in rectangular treatment chambers and delivered direct electric current (200 mA) through the chamber by means of a standard constant current generator and two stainless-steel electrodes at opposite ends of the long axis of the treatment chamber. When treated with gentamicin (5 μg/mL) or oxytetracycline (50 μg/mL) for 24 hours, *E. coli* biofilms exhibited a 2.11 and 1.90 log reduction in viable cell numbers. When direct current was applied during gentamicin or oxytetracycline treatment, biofilms exhibited a 4.27 and >5.15

log reduction in viable cell numbers. Electrical current alone caused a 0.91 log reduction in viable cell numbers.

Our group demonstrated electrical current-mediated enhancement of the *in vitro* bactericidal activity of erythromycin (2 μg/mL), daptomycin (2 μg/mL) or moxifloxacin (4 μg/mL) against methicillin resistant *Staphylococcus aureus* (MRSA) biofilms (32). However, the activity of linezolid (16 μg/mL) or minocycline (4 μg/mL) against MRSA biofilms was not enhanced by electrical current. We designed a model that permitted us to study the interaction between the biofilm itself, the electric field, and the antimicrobial agent. An eight-channel current generator/controller and eight chambers delivering a continuous flow of fresh media with or without antimicrobial agents and/or electrical current (20, 200 or 2,000 mA) via graphite or stainless steel electrodes to biofilm-coated Teflon coupons was used. This technology was used to extensively assess whether the *in vitro* enhancement of killing of biofilm-associated *P. aeruginosa* and *S. epidermidis* by electric current plus aminoglycoside, quinolone, or tetracycline antimicrobics was generalizable to antimicrobial agents representing a variety of other antimicrobial classes, and to MRSA. Results of our experiments indicate that the enhanced activity of antimicrobial agents by electrical current against biofilm organisms may not be a generalizable phenomenon across microorganisms and antimicrobial agents (unpublished data).

Electrode composition may have an impact on bioelectric effect. Stainless steel electrodes have been most commonly studied (29, 31), but carbon, platinum and gold electrodes have also been used (13, 18, 32, 33). Using the previously described *in vitro* model, we demonstrated that electrode composition plays a role in the observed *in vitro* bioelectric effect. We studied the *in vitro* enhancement of bactericidal activity of rifampin by electrical current against MRSA biofilms using two different electrode materials. Rifampin combined with electrical current (2000 μA) delivered by stainless steel electrodes demonstrated a 3.5-4.4 log reduction of MRSA biofilms. However, a lesser effect (2.1-2.4 log reduction) was observed when electrical current was delivered by graphite electrodes (33).

Effect of electrical current alone on bacterial biofilms

Electrical current alone has been shown to have a bactericidal effect when applied as a 10 μA DC current for 16 hours to bacteria or human skin or on agar plates (11, 12). Davis et al (13, 34-36) reported that, in medium containing chloride ions, planktonic cells of *E. coli*, *P. aeruginasa*, *Proteus mirabilis* and *Candida albicans* were killed by electric fields and current densities similar to those used by Costerton et al in his first report about the bioelectrical effect (15 $\mu A/cm^2$ to 2.1 $\mu A/cm^2$) (19). Davis et al attributed the killing of these planktonic cells to iontophoresis, in which the accretion of metal ions on or in the bacterial cell is responsible for the effect (34, 35).

The development of biofilm-related infections begins with the adhesion of the microorganism to the biomaterial surface, mediated by the Van der Waals forces, acid base interactions and electrostatic forces. The electrostatic force between bacteria and the biomaterial is generally repulsive since almost all biomaterial surfaces are negatively charged, as are bacterial cells (37). It has been proposed that repulsive forces can be enhanced by the application of electric current, provoking surface detachment of bacterial biofilms (38, 39).

Poortinga et al (38) have demonstrated that it is possible to stimulate *Streptococcus oralis* detachment from conducting indium tin oxide by applying electrical currents of 10 $\mu A/cm^2$. Almost total cleaning of anodic and cathodic surfaces could be achieved, even in the presence of an adsorbed conditioning film. In their study, an ionic strength-dependent transfer of electrons during an initial bacterial adhesion mechanism, that had to be reversed

in order for detachment to occur, was proposed. Van der Borden et al (40) demonstrated that a variety of initially adherent *Staphylococcus* strains, isolated from biomaterial-related infections, could be stimulated to detach from surgical stainless steel by the application of low electrical DC currents (25-125 μA). This current-induced detachment of initially adhering bacteria from stainless steel surfaces not only involved detachment, but also the prevention of re-deposition of detached bacteria (40). It was shown that under high -flow conditions, detached bacteria were more readily transported away from the surface than under low-flow conditions, making re-deposition unlikely (40). In a follow-up manuscript (41), the effect of DC electrical currents (60 and 100 microamps) and block currents (60 and 100 μA with a 50% duty cycle, 1 Hz) against biofilms in the late stages of formation was studied. The block currents yielded higher detachment percentages than DCs due to the electro-osmotic fluid flow. Bacteria remaining on the surface after current application were less viable than they were prior to the current application, as demonstrated by confocal laser scanning microscopy (41).

Van der Borden et al designed an experimental infection model in which three percutaneous stainless steel pins were implanted in the tibia of goats and colonized with S. *epidermidis* (42). One pin was subjected to electric current while the other pin was used as a control (the third implanted pin was used for frame support). Infection developed after 21 days in 89% of the control pin sites, whereas only 11 % of the pin sites in the current group showed infection.

Potential applications of the bioelectric effect in the human setting

As we have shown, electrical currents may be potentially applied in the human setting either alone (the “electricidal effect”) or combined with antimicrobial agents (the bioelectric effect). Both approaches need more *in vitro* studies as well as studies in experimental models before they can be translated to clinical practice.

DC currents have been used clinically to drive chemotherapeutic molecules into solid tumors (43), and antibiotic molecules into the inner ear and other tissues (44). The obvious human application of the bioelectric effect could be in the management of infections associated with orthopedic hardware. In addition to systemic delivery of antimicrobics, local delivery of antimicrobics (e.g., in polymethylmethacrylate) also deserves further study as concerns the bioelectric effect. Furthermore, implantable devices could be accessed to produce effective electric fields to enhance the perioperative use of antimicrobials to kill developing bacterial biofilms, thereby preventing device-related infections.

Issues concerning electric current mediated tissue toxicity, delivery systems for electric current, and electrode geometry would need to be addressed before it could be applied in a human setting. Ideally, if the bioelectric effect is applied to human infections, the electric current should be delivered in a non-invasive (e.g., transcutaneous) or minimally invasive (e.g., subcutaneous) fashion. Attaching wires directly to the surface of foreign bodies is not ideal since the wires themselves may be a conduit for microorganisms. According to some authors, the bioelectric effect requires a current flow, not just an electrical field. Stewart et al reported that when electrodes were placed outside the treatment chamber to create essentially the same electric field, but with zero current, the electrical enhancement of killing was completely eliminated (23). However, Pickering et al (30) investigated the *in vitro* effect of a pulsed electromagnetic field on the efficacy of tobramycin and vancomycin against *S. epidermidis* biofilms on the tips of stainless-steel pegs. As it is described in their study, exposure to a pulsed electromagnetic field increased the activity of gentamicin but not vancomycin against *S. epidermidis* biofilms.

CONCLUSIONS

The pathogenesis of a wide variety of human infections, including device-related infections, as well as infections not associated with devices, is now recognized to relate to the presence of bacteria in biofilms. It has been estimated that two thirds of human bacterial infections involve biofilms. Existence within a biofilm represents a basic survival strategy for bacteria, within which they are protected from environmental influences and exhibit resistance to therapeutic levels of antimicrobial agents. Bacteria in biofilms exhibit dramatically reduced (i.e., by several log orders) susceptibility to killing by antimicrobics as compared to planktonic bacteria. The threat of such devastating bacterial infections is a serious problem that limits the current and future development of medical devices. *In vitro* experiments have demonstrated that direct electric current substantially enhances the activity of certain antimicrobial agents (i.e., aminoglycosides, quinolones, tetracycline, erythromycin, daptomycin, moxifloxacin or polymyxin B), against some biofilm-associated bacteria (*P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. epidermidis*, MRSA, *S. gordonil*), rendering biofilm bacteria susceptible to antimicrobial levels active against non-biofilm (planktonic) bacteria. The significance of the bioelectric effect is that it affords a potential means to overcome the reduced susceptibility of biofilm microorganisms. Although the mechanism of the bioelectric effect remains unclear, it is interesting because it may facilitate the design of technological applications to improve treatment of biofilm-related infections. Whatever the mechanism of the bioelectric effect may be, it is clear that electric currents may enhance the efficacy of certain antimicrobial agents in killing biofilm bacteria. Furthermore, it has been shown how the application of direct electric current alone can provoke the surface detachment of bacterial biofilms. Some questions about the bioelectric and electricidal effects need to be addressed. It is not clear which electric parameters are more important (e.g., electric field strength, current density, time of application). Moreover, the optimal antimicrobial concentration to achieve the maximum effect remains to be defined.

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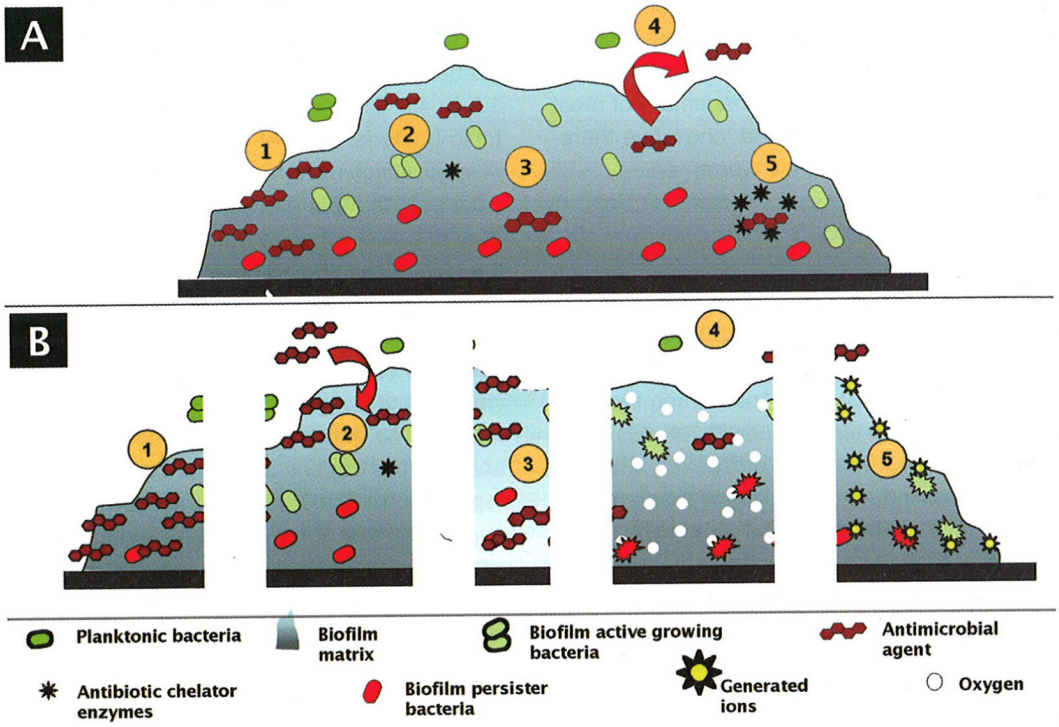

Fig. 1.

A) Some proposed biofilm-associated resistance mechanisms: (1) antimicrobial agents may be trapped and destroyed by enzymes in the biofilm matrix; (2) *antimicrobial agents may fail* to penetrate beyond the surface layers of the biofilm; (3) antimicrobial agents may not be active against non-growing microorganisms; (4) expression of biofilm specific resistance genes (e.g., efflux pumps); (5) *stress response* to *hostile environmental conditions.*
B) *Some proposed bioelectric effect mechanisms:* (1) *reduction of the biofilm capacity for binding* to *the antimicrobial agent;* (2) electrophoretic augmentation of the antimicrobial agent transport; (3) *membrane permeabilization;* (4) *electrolytic generation of oxygen;* (5) electrochemical generation of potentiating oxidants.

TABLE I

SUMMARY OF THE BIOELECTRIC EFFECT *IN VITRO* STUDIES

Method	Biofilm substrate	Microorganism	Electrical current	Electrode materials	Antimicrobial agent	Exposure time (h)	Effect	Reference
Electric modified Robbins device	Stainless steel	*Pseudomonas aeruginosa*	2.1 mA/cm^2	Stainless steel and platinum	Isothialazone 1.5%, Dimethyl ammonium chloride 50% and Glutaraldehyde 25%	24	3-6 log reduction	(17)
Electric modified Robbins device	Stainless steel	*P. aeruginosa* *P. aeruginosa* *Escherichia coli* *Staphylococcus epidermidis* *Candida albicans*	15-400 μA/cm^2	Stainless steel and platinum	Tobramycin (5-100 μg/mL) Ciprofloxacin (1.25-5.0 μg/mL) Tobramycin (10-100 μg/mL) Tobramycin (2.5-100 μg/mL) Cycloheximide (100 μg/mL)	12	4- 6 log reduction	(18)
Two electrodes inside a Perspex flow chamber	Stainless steel	*P. aeruginosa*	1.7 mA/cm^2	Stainless steel	Tobramycin (5 μg/mL)	24-48	4-5 log reduction	(19)
Two electrodes inside a Perspex flow chamber	Dialysis membrane	*P. aeruginosa*	9 mA/cm^2	Stainless steel	Tobramycin (10 μg/mL) Ciprofloxacin (5 μg/mL) Polimixin B (20 μg/mL) Piperacillin (40 μg/mL)	12	2 log reduction 0.5-1 log reduction No reduction	(22) (20)
Experimental chambers	Polycarbonate	*P. aeruginosa and Klebsiella pneumoniae*	1 mA	Platinum	Tobramycin (5 μg/mL)	24	6-8 log reduction	(21)
Experimental chambers	Polycarbonate coupons	*P. aeruginosa*	2 mA	Stainless steel	Tobramycin (5 μg/mL)	24	6 log reduction	(23)
Experimental chambers	Polycarbonate coupons	*P. aeruginosa*	2 mA	Stainless steel	Tobramycin (5 μg/mL)	24	5-6 log reduction	(28)
Experimental chambers	Polycarbonate	*Streptococcus gordonii*	0.4 mA/cm^2	Stainless steel	Gentamicin (2 μg/mL)	24	4-5 log reduction	(29)
Microliter trays	Stainless steel	*S. epidermidis*	Pulsed electromagnetic field	No electrodes	Gentamicin (256 times the MIC)	12	1-2 log reduction	(30)
Experimental chambers	Glass	*Escherichia coli*	200 mA Radio frequency current at 10 MHz	Stainless steel No electrodes	Gentamicin (5 μg/mL) Oxytetracycline (50 μg/mL) Gentamicin (5 μg/mL) Oxytetracycline (50 μg/mL)	24	4.27 log reduction > 5.15 log reduction 3.4 log reduction 2.8 log reduction	(31)
Experimental chambers	Teflon	Methicillin resistant *Staphylococcus aureus*	20, 200, 2000 mA	Graphite	Erythromycin (2 μg/mL) Daptomycin (2 μg/mL) Moxifloxacin (4 μg/mL) Linezolid (16 μg/mL) Minocycline (4 μg/mL) Rifampin (4-32 μg/mL)	24	1-2 log reduction 0-1 log reduction	(32)
			2000 mA	Graphite Stainless steel		24	2.1-2.4 log reduction	(33)

Method	Biofilm substrate	Microorganism	Electrical current	Electrode materials	Antimicrobial agent	Exposure time (h)	Effect	Reference
							3.5-4.4 log reduction	