Electrical methods of controlling bacterial adhesion and biofilm on device surfaces

David Freebairn, David Linton, Eileen Harkin-Jones, David S. Jones, Brendan F. Gilmore, Sean P. Gorman

Authors Names and Affiliations

David Freebairn School of Pharmacy, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom

Tel: +44 (0) 77 45 20 8149

Email: dfreebairn01@qub.ac.uk

Dr. David Linton The Institute of Electronics, Communications and Information Technology (ECIT), Queen's University Belfast, Northern Ireland Science Park, Queen's Road, Queen's Island, Belfast BT3 9DT, United Kingdom

Tel: +44 (0) 28 90 97 1761

Email: d.linton@ee.qub.ac.uk

Professor Eileen Harkin-Jones School of Mechanical and Aerospace Engineering, Queen's University Belfast, Ashby Building, Stranmillis Road, Belfast BT9 5AH, United Kingdom

Tel: +44 (0) 28 90 97 4490

Email: e.harkinjones@qub.ac.uk

Professor David S. Jones School of Pharmacy, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom

Tel: +44 (0) 28 90 97 2011

Email: d.jones@qub.ac.uk

Dr. Brendan F. Gilmore (author for correspondence) School of Pharmacy, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom

Tel: +44 (0) 28 90 97 2305

Email: b.gilmore@qub.ac.uk

Professor Sean P. Gorman Dean's Office (Medicine, Health and Life Sciences), Queen's University Belfast, 71 University Road, Belfast, United Kingdom

Tel: +44 (0) 28 90 97 5177

Email: s.gorman@qub.ac.uk

Summary

This review will summarize the significant body of research within the field of electrical methods of controlling the growth of microorganisms. We examine the progress from early work using current to kill bacteria in static fluids to more realistic treatment scenarios such as flow-through systems designed to imitate the human urinary tract. Additionally, the electrical enhancement of biocide and antibiotic efficacy will be examined alongside recent innovations including the biological applications of acoustic energy systems to prevent bacterial surface adherence. Particular attention will be paid to the electrical engineering aspects of previous work, such as electrode composition, quantitative electrical parameters, and the conductive medium used. Scrutiny of published systems from an electrical engineering perspective will help to facilitate improved understanding of the methods, devices and mechanisms that have been effective in controlling bacteria, as well as providing insights and strategies to improve the performance of such systems and develop the next generation of antimicrobial bioelectric materials.

Keywords

Bioelectric effect, electricidal effect, electrophoresis, iontophoresis, surface attachment, biofilm, indwelling medical device, conducting polymer.

Introduction

The Problem With Biofilms

Bacterial biofilms are ubiquitous in nature and are known to form rapidly on the surfaces of indwelling medical devices (IMDs) such as urinary catheters and endotracheal tubes. It is estimated that they are responsible for 65% of all human microbial infections [1-4] with treatment costs in excess of one billion dollars per year in the USA [5,6]. The key pathogens responsible for device-related infections are *Staphylococcus epidermidis* and *Stapylococcus aureus*, as well as *Escherichia Coli* in urinary tract infections (UTIs) [1,7]. Biofilms are also present in engineered systems where they are a major cause of microbially-induced pipeline corrosion, and a scourge to the oil, paper, energy, and water treatment industries, contributing to a total annual cost to society worldwide which is estimated to total hundreds of billions of dollars [8-14]. This enormous cost provides a major impetus for the development of advanced electrical solutions to prevent or reduce biofilm formation.

Bacteria exist in two basic forms, planktonic (or free-floating) bacterial cells, which are capable of rapid spread and cellular division, and sessile, or surface attached cells characterized by their slow-growth and perseverance. Such cells will aggregate and enter a reduced metabolic state, exhibiting an altered (or 'biofilm') phenotype and form architecturally complex, structured communities of bacteria encased in a self-produced extracellular polymeric matrix known as a biofilm on virtually any surface with which they come into contact [4,15,16]. The biofilm phenotype also confers the advantage of significantly enhanced tolerance to antimicrobial agents, predation and immune clearance. In fact, biofilms have been shown to be resistant to antibiotics in concentrations 500 to 5,000 times those required to kill planktonic cells of the same species [4,16-21]. Worse still, small surviving populations known as persister cells [15,22] withstand antibiotic treatment and can rapidly repopulate the surface thus acting as a nidus of recurrent infections, such as UTIs. Some possible explanations of the mechanism of this resistance of biofilm cells to antibiotics include the difficulty of overcoming the

chemical diffusion barrier posed by the glycocalyx, the interaction of exopolymer with antibiotics, and the slow-growth and therefore reduced metabolic activity of sessile cells [15,16]. It is beyond the ability of traditional antibiotics alone to control biofilm-related chronic or device-associated infections and to that end a combination of treatments would appear to be the best solution to this problem; for example, low-electrical currents applied in combination with antibiotics has been shown to be effective [23-30].

Electrical Methods of Controlling Bacteria

The observation that electrical current has the ability to effect a bactericidal activity is a longstanding one, having been reported as early as 1919 [31] when the sterilization of milk using a low alternating current was first demonstrated. In 1965, Rosenberg *et al.* found that platinum electrodes immersed in a medium would inhibit the process of cell division in *Escherichia coli* when a low frequency alternating current was applied [32]. Various other reports thereafter have documented the 'iontophoretic' (therapeutic use of electric current) killing of planktonic bacteria, and it was thought that electrochemical products formed at the metal electrodes were most likely to be responsible for this effect [33]. Over the last few decades, a number of small incremental steps have been taken to develop this field of research towards the vision of a viable infection-resistant medical device system. Davis *et al.* first described the electrical killing of bacteria in both a static fluid phase and a flowing fluid phase resembling the dynamic flow and stasis observed in catheterized individuals [34]. Ten years later, a modified Robbins device (MRD) was used to apply a low-strength electric field together with a range of industrial biocides against *Pseudomonas aeruginosa* biofilms [35]. Results from this study first demonstrated that the application of an electric current can reduce the very high concentrations of antimicrobials needed to kill biofilm bacteria to levels close to those needed to kill planktonic bacteria of the same species [23-30,35]. This electrical enhancement of the efficacy of antimicrobials against biofilm bacteria is now known as the "bioelectric effect". In 1994, Costerton *et al.* expanded this work to highlight the possibility of using the bioelectric effect to prevent and treat device-related bacterial infections. In 2004, Caubet *et al.* applied a radio frequency alternating current to biofilms instead of

the usual direct current giving rise to a new bioelectric effect for which all previously proposed explanations were no longer applicable. It has been widely reported that whilst an electric current enhances the efficacy of the antimicrobial treatment of biofilms, the same electric current without an antimicrobial agent has no significant impact on the numbers of viable biofilm bacteria. However, in the last few years, studies of the long-term exposure of biofilms to a low intensity electrical current have shown that prolonged exposure in the absence of antimicrobial agents resulted in a marked decrease in the viability of a number of biofilm strains [4]. This recent development, known as the electricidal effect, goes contrary to the findings of many previous reports which stated that electric current alone is not effective in killing biofilm bacteria and provides fresh incentive to explore the possibility of designing a new generation of antimicrobial surfaces and devices.

Most research efforts to date involving electrically induced bacterial eradication have focused on delivering quantifiable reductions in the number of various species of bacteria adhering to surfaces in a range of custom-designed electrical chambers or modified flow cells. Various aspects will need to be addressed in order to translate the bioelectric effect into the realms of both clinical practice and commercialization. To date, few attempts have been made to reproduce promising *in vitro* bacterial killing results on human patients, or to reproduce such results on the surfaces of relevant materials such as siliconepolymers [36,37,38] that might actually be employed in the manufacture of medical devices. Furthermore, in order to prevent and treat UTIs using electrical current, a set of optimal treatment parameters must be defined targeting the key pathogens responsible, namely *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Proteus mirabilis.* Bringing together all of these elements in a strategic, cohesive manner should facilitate the design of a device that could drastically reduce the epidemiology of a wide range of the most damaging hospital acquired, device-associated infections, as well as systems that could prevent and treat biofilms in industrial settings, and engineered systems, thereby facilitating significant productivity gains and improved efficiency. Additionally, the application of electrical therapy would facilitate the treatment of infection with the

device still in place, avoiding the trauma associated with device removal; this is in itself responsible for substantial morbidity, cost and mortality in some cases [39].

Expert Review Structure

There are two key strategies to consider when combating biofilm device-related infection, prevention and cure. Electrical techniques could be used to stop the initial deposition of extracellular polymer matrix or alter surface charges, preventing surface adherence or bacterial growth in the first place and stopping biofilm formation from ever occurring. This strategy would avoid the heavy cost and damage incurred by biofilm-related infection. Another approach involves reduction and eradication of the biofilm in situations where it has already been established on the device surface. The review will therefore be divided into two distinct parts, the killing of planktonic bacteria (more akin to the prevention of initial surface adherence) and the killing of biofilm bacteria. Literature on planktonic bacteria will be further subdivided to assess the effects of direct current (DC) systems, alternating current (AC) systems, radio frequency (RF) systems and other more advanced electrical treatment stimuli. Anti-biofilm approaches will be reviewed chronologically, describing what key advances were made over a short timeframe within a framework of (often) collaborative research efforts.

Electrical Control of Planktonic Bacteria

Direct Current Control

The earliest work in this field may be attributed to a number of attempts to sterilize milk and drinking water by passing a small electric current through them [31,40,41]. Antibacterial effects have also been shown to arise from high-frequency discharges as well as high voltage sparks [42,43,44]. However, the

use of high voltages would clearly not be feasible for industrial applications or for *in vivo* antibacterial treatment.

Electrically Induced Bactericidal Effects of Silver

In 1974, Spadaro *et al.* examined how the use of low electric currents with a range of metal electrodes could counter bacterial growth in a liquid broth medium [33]. In this set of experiments, it was found that a silver anode would have the greatest inhibitory effect on bacterial multiplication and in fact, silver would continue to have this effect after current flow ceased. Bacterial culture plates were prepared by mixing bacteria in broth medium with agar and the electrodes used were pure silver wires (2 cm long, 0.4mm diameter) which emerged through the side of the plates to contact the agar, which would itself act as the electrical current path. Of all the metal electrodes tested after 24 h of treatment at the lowest current levels (0.4 – 4 μA, 1 V), only the silver anode and gold cathode produced notable inhibitory clear-zones, with the silver anode inhibiting all organisms tested. Electrical treatment was either applied over 24 h during incubation or for the next 24 h following 24 h of normal incubation. The greater the current, the faster the anodic inhibition and within 4 h, the 4 μA, 40 μA and 400 μA (3 V) anodes could reduce bacterial levels by up to 5 orders of magnitude representing the majority of the overall inhibitory effect observed. At the higher current ranges (40 to 400 μA), pH shift was measured at both electrodes except for silver. This, coupled with electrode corrosion would be used to explain any bacteriostatic action in other electrodes. The silver ion was suggested as the reason for such a strong bactericidal effect and other research on the antibacterial effects of silver sulfadiazine would appear to support this [45,46]. Electrically injected silver ions in this experiment were shown to be at least as effective as an antibiotic. Significant inhibition observed at the gold cathode for 4 μA may be attributed to the formation of electrochemical products, and it was suggested that the efficacy of both this and the silver anode could relate to their position in the periodic table as previously reported [32]. In 1976, Berger *et al.* presented further evidence for the antibacterial properties of silver ions with low electric currents [47]. In culture plate experiments, four hours of treatment produced a large growth inhibitory zone at the anode with currents of just 0.4 μA. The silver anode caused *Staphylococcus aureus* to have

abnormal mesosomes (invaginations of the plasma membrane) when treated with this low electric current, and blocked protein production in *Escherichia coli* leading to suggestions that the inhibitory effect of silver ions may in fact occur as a result of interference with the cell plasma membrane. However, the antibacterial activity of silver ions is now well understood; DNA molecules in *Escherichia Coli* and *Staphylococcus aureus* become condensed and lose their replication abilities as a reaction against the denaturation effects of silver ions, and silver ions interact with thiol groups in bacterial proteins thereby inducing their inactivation [48,49].

Electrical Control of Bacteria in a Urinary Catheter Model

UTIs are the most common hospital acquired infections, and the majority of them are catheter associated urinary tract infections (CAUTIs) [50]. Insertion of such a foreign device into the urinary tract negates many of the body's natural defense mechanisms that are in place to guard against bacterial colonization. Human urine is a good culture medium for many strains of bacteria, whilst the catheter material provides bacteria with an inert surface on which to colonize [51]. Therefore, if the electrical killing of bacteria is to be implemented *in vivo*, then the treatment of UTIs obtained as a result of catheterization must be one of its' foremost applications.

Model catheter systems and other unique chambers have been employed in a number of experiments in order to mimic the dynamic flowing fluid phase observed in catheterized patients, presenting a much more realistic scenario than that of applying current to bacteria in a static fluid, or on a tissue or material surface. One report suggested that the use of a silver-powder coated catheter could prevent catheter associated UTIs [52], but in 1982, Davis *et al.* became the first group to demonstrate the electrical treatment of bacteria in a flowing system [34]. Preliminary tests in this study described an iontophoretic model, which compared various metal electrodes (0.2 mm diameter) immersed in a static fluid medium of inoculated heart infusion (HI) broth providing an electrical pathway for treatment. Results showed that gold wire electrodes with a constant current of 400 μA (3.2 V) were most effective, proving lethal

against a number of microorganisms, most notably *Escherichia coli*, *Streptococcus faecalis*, and *Staphylococcus aureus*. Applying this current to mammalian tissue culture cells was found to slow their growth and caused notable morphological changes such as rounding of the fibroblasts without killing the cells themselves. It should be noted that although silver was previously determined to be the most effective antibacterial electrode [33,47], Davis and co-workers found that bubbles would form on silver wires immersed in the static fluid phase, which contributed to their disintegration after just a few hours of passing an electric current.

In the flow system, two 0.2 mm gold wire electrodes were inserted through the catheter wall on opposite sides acting as an anode and cathode. Samples were removed both adjacent to the point of electrode insertion and at a site 10 cm downstream. HI broth again acted as the medium, however, in this study media flowing over the electrode was pumped through the catheter tubing using a peristaltic pump. A constant current of 400 µA flowing through a gold wire was capable of killing *Escherichia coli* (5×10^6 bacteria per ml) in the region directly adjacent to the wire in addition to inhibiting growth over a longer time period (>50 h) at a distant site located downstream from the wire. At higher inoculum densities (1×10^7 bacteria per ml) or lower flow rates (20 ml/h or less), rapid bacterial growth resulted and electric current proved ineffective at controlling proliferation. Since bactericidal effects were also seen in samples taken 10 cm downstream from the electrode after much longer periods, one assumption from this study is that the electrical impact on bacteria in flowing media still occurs after the application of electrical treatment has passed.

In later experiments, platinum electrodes with 400 µA of constant current would be used to safely reduce bacterial populations in catheterized sheep [53]. Platinum wires (0.2 mm diameter) were used due to a combination of their superior longevity which was necessary for the 21 day catheterization period and also for their ability to effectively kill bacteria when carrying a low electric current (as shown in synthetic urine [54]). Wires were threaded out of the catheter lumen and through the catheter wall;

constant current sources were used to treat the bacteria-rich urine from the sheep's infected bladder as it passed through the system. Bacterial concentrations were reduced to the order of 10^3 to 10^4 bacteria per ml over the entire 21-day period of treatment with the electrified device, well below the level of 10^5 bacteria per ml that would constitute a UTI, with *Escherichia coli* being the predominant microbe in urine. All bacterial reductions were achieved without inducing any notable physical or chemical alterations to the urine and significantly, without having any adverse impact on the tissues of the sheep urinary tract. As a result of these findings, it was suggested that the use of such a device *in vivo* could be extended to humans to safely prevent the onset of nosocomial catheter-associated UTIs via "iontophoresis", that is, the use of current to produce the ions of soluble salts.

Each of the aforementioned fluid systems raises a number of important questions about the conductivity of urine and various genera of bacteria. To date, the resistance of bacterial colonies in growth media has not been measured. If such measurements were performed with sufficient accuracy, they would enable us to determine how much current is actually flowing through the bacteria themselves compared with surrounding media and surfaces. This is significant as fundamental electrical principles state that electrical current will flow through the path of least resistance. In experiments to date it is unclear whether or not the resistance of the bacteria is lower than that of the liquid medium it is immersed in. Therefore if the bacterial resistance is higher than that of its medium, then the bacteria will not experience any direct effects of electrical current as it instead flows through the surrounding medium. It may be that the current does not directly impact bacteria, and any effects are related to voltage. Nevertheless, it is difficult to imagine how a system incorporating wires into a catheter would be feasible *in vivo* given that the objective is not only to prevent and treat infection, but to be as minimally invasive to the patient as possible.

Bacterial Behavior Under the Influence of Electrical Currents

It has been reported that both cocci and rod-shaped biofilm bacteria on surfaces can be controlled under the influence of an electric current to form ordered clusters, a phenomenon termed 'controlled electrophoretic deposition'. Furthermore, it was shown that the spacing between such clustered bacteria can be controlled by changing either the current density or the ionic strength of the media [55-59].

Poortinga *et al.* [59] used a flow chamber consisting of two parallel plates 0.6 mm apart, with the bottom plate acting as an anode and the top as the cathode. The anode was a 21 cm^2 plate of surgical stainless steel whereas the cathode consisted of an indium-tin oxide (ITO), DC-sputtered glass plate. For higher current densities (>50 $\mu A/cm^2$), bacteria were immobilized on the surface of the anode and when suspensions of high ionic strength (10^{-3} M KNO_3 and above) were used, bacteria were immobilized regardless of how little current was applied. Bacteria were observed to form clusters of varying sizes under different ionic strengths and currents as bacteria interacted with each other over a distance of several bacterial diameters – clusters would disband when the current was switched off. Bacteria within clusters were packed densely together and quite evenly spaced. Additionally, under the influence of alternating currents with voltages greater than 3 V, rod-shaped bacteria aligned instantaneously parallel to the direction of the field. It is possible that this ability to control the deposition of bacteria on surfaces using an electric current could enable the design of new biotechnology and devices with biomedical applications by creating controlled biofilms for bioreactors or biosensors as well as protective coatings of probiotic bacteria on IMD surfaces.

Bacterial Surface Detachment Using Electric Currents

Low-electric currents ranging from 15 µA up to 125 µA (1.5-1.7 V) have been used to successfully detach bacterial strains of *Staphylococcus epidermidis* and *Staphylococcus aureus* from surgical stainless steel anode surfaces within a parallel plate flow chamber [60]. Bacteria were introduced to the chamber in a flowing suspension of physiological ionic strength and following adherence to the stainless steel surface; the suspension was switched for a solution of specified ionic strength containing

no bacteria. Staphyloccocal detachment was observed for all strains tested. Increasing ionic strength of the suspension significantly increased initial detachment rates observed over the 2.5 h treatment period. However, when applying a current initial detachment rates were most effectively curtailed when using low ionic strengths of suspension media in combination with 125 µA currents.

The flow chamber implemented by van der Borden and colleagues consisted of two parallel plates 0.6 mm apart in a set-up almost identical to that used by Poortinga and co-workers [59]. Wires were attached to each of these surfaces using silver epoxy paint. Currents of varying intensity were applied between the plate electrodes for a period of 2.5 h. The number of adhered bacteria was quantified using a camera mounted on a 40 x zoom metallurgical microscope by grabbing digital images of the anode plate which could then be analyzed to determine the number of CFUs per unit area.

The DVLO (Derjaguin, Landau, Verwey and Overbeek) theory describes how bacteria interact with charged surfaces in a liquid medium through a combination of attractive van der Waals forces, repulsive surface electrostatic forces and acid-base interactions [61]. By changing the ionic strength of the suspension media or by applying an electric current, the electrostatic interactions described can therefore be altered to assist with the detachment of adhered bacteria by overcoming the aforementioned attractive forces. Biomaterial implants or IMDs provide passive surfaces for bacterial adhesion leading to the onset of device-related infection. The most common pathogen involved is *Staphylococcus epidermidis* [62,63]. The results of this study would seem to suggest that the application of electric currents to a range of medical implants such as fixation frames and bone screws used in orthopedic surgery could prove an effective preventative measure against such infection, eliminating the requirement to replace the implant – a process which will usually cause a large amount of trauma to the patient.

Soon after, van der Borden *et al.* [64] modified this experiment and demonstrated that similar results can be achieved using alternating currents (AC) with a range of different low frequencies (0.1-2 Hz) and pulse widths up to 50%. A square wave current of 100 μA was capable of detaching 76% of adhering Staphyloccoci from stainless steel. At 5% pulse width, no detachment occurred. Increasing the pulse width increased the detachment rate for both 60 and 100 μA currents up to 50%. At 2 Hz and a 50% pulse width, 100μA of current was capable of causing a 60-fold decrease in the number of viable bacteria compared to controls. It was suggested that alternating currents create an osmotic fluid flow due to the movement of hydrated ions, thereby creating an additional force to stimulate attachment, which explains the increased detachment percentages over DC in a minority of cases. Ordinarily though, fewer electrons are pumped through the system when under the influence of an alternating current and therefore this lower rate of charge transfer would seem to explain the generally lower detachment rates recorded for square wave currents compared with DC. AC requires that less power is dissipated by the skin than DC and could therefore prove a more feasible treatment in a clinical setting as they would cause less trauma for the patient.

The same group would later show that DC current applied for 6 h was much more effective at detaching 200 minute biofilms (grown on the electrode surface by circulating tryptic soy broth for 200 minutes following the aforementioned rinsing of planktonic bacteria with buffer) from stainless steel surfaces than AC applied for the same duration [65]. Results have demonstrated that direct currents are effective at and much better than alternating currents for detaching *Staphylococcus epidermidis* biofilms on orthopedic implants. Such findings would appear to indicate that clinical applications of the bioelectric effect might be better served by utilizing DC rather than AC signals, except in such cases where DC would induce unacceptable trauma for the patient.

Alternating Current Control

In 1965, Rosenberg *et al.* [32] found that platinum (and other group VIIIb compound) electrodes immersed in a medium would inhibit the process of cell division in *Escherichia Coli* when a low frequency alternating current was applied in the presence of oxygen (2 V peak-peak at a frequency of 500Hz proved most effective). Interestingly, whilst cell division was inhibited under electrolysis, the filamentous growth of bacteria was unaffected and reached up to 300 times the normal length. It should be noted that the electrodes were described as half-cylindrical mesh electrodes built into the chamber whilst the growth medium was mainly composed of glucose with a small dilution of Magnesium Chloride providing electrical conductivity. The chamber had a resistance of 6 Ohms ensuring that current would follow a path through the culture medium rather than up the chamber walls. As the oscillator frequency was increased, so the effectiveness of causing filamentous growth decreased until, at 6 kHz and above, no effects could be detected. Qualitative results were obtained under microscopic examination of the effluent of the chamber. UV, temperature, pH and magnesium concentration were all monitored and could be eliminated as possible explanations of the filamentous growth and inhibition of cell division. It was suggested that metallic oxidation compounds were created in the medium under electrolysis and as such, certain metal ions belonging to the family of group VIIIb transition metal compounds were responsible for these effects.

Bactericidal Effects of Electrically Induced Chlorides

It was later reported that the interaction of chlorides with a 50 Hz alternating current (10-200 mA) has a strong antibacterial effect on *Escherichia coli*, and this led to suggestions that such currents might be used to achieve bacterial killing in other chlorine-containing media such as polluted natural waters [66]. The 50 Hz AC source supplied two flat electrodes (stainless steel and platinum both worked) immersed in a bacterial suspension within a 0.75 ml plastic cell. The viability of *Escherichia coli* would decrease as the current passing through the liquid medium increased from a minimum killing current of 25 mA and beyond 60 mA, there was less than a 0.01 % survival rate. Lethal effects were indirect with no more than 10 s of electrical treatment producing a residual toxic effect in the media that would last for

up to 30 minutes. By repeating the experiment for a range of different bacterial suspension media, it was concluded the production of chloride ions via iontophoresis was an essential factor in the electrical killing of microorganisms. Both this and Rosenberg's study [32] would seem to suggest that the chlorine ion improves the conductivity of a suspension medium.

Alternating Electrical Treatment Fields

Alternating electric fields have been used to disrupt cancer cell replication both *in vitro* and safely *in vivo* using ceramic insulated electrodes to avoid the production of toxic by-products associated with metal electrodes [67,68]. Based on this work, an attempt was made to enhance the inhibition of planktonic bacteria using alternating electric fields safe to human cells both with and without an antimicrobial agent, and to model the field in order to determine the optimal parameters for enhancing this effect [69].

Two pairs of flat metal electrodes were positioned perpendicular to each other within a glass petri dish in order to generate electric fields at 90º to each other through the inoculated growth media (both with and without an antibiotic) in the central well of the dish (FIGURE 1). The electrodes were insulated from the media by a ceramic material with a sufficiently high dielectric constant to yield an electrode capacitance of 10 nF. They were connected to a radio frequency amplifier activated by a sine wave function generator and the entire system was placed within a Faraday cage. Field generation (swept from 100 kHz to 50 MHz) was switched between two perpendicular directions every 300 ms by alternately activating the two pairs of perpendicular electrodes in order to minimize the creation of thermal gradients that could affect bacterial growth. Temperature at the center of the chamber was continuously monitored using a thermocouple to account for any heating effects. Electric field intensity was measured with a shielded coaxial probe with two exposed tips 1 cm apart, together with an oscilloscope. The complete system was named "AMFields" (antimicrobial fields).

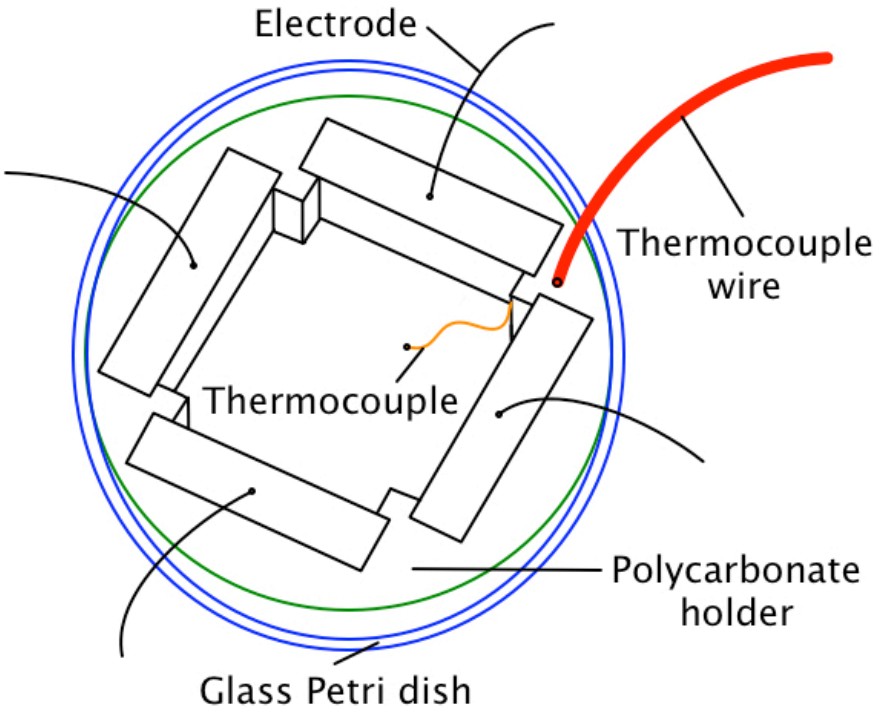

Figure 1. Modified glass petri dish from the AMFields (Antimicrobial Fields) system. Adapted from [69].

The greatest reduction in *Staphylococcus aureus* biofilms occurred after 2 h of treatment at 10 MHz reducing the amount of bacterial growth to less than 60% of controls. 10 MHz fields were also most effective at reducing *Pseudomonas aeruginosa* growth after 2.5 h of treatment, yielding reductions to less than 80% of controls. The combination of AMFields and antibiotics was found to produce an additive bioelectric effect. As previously reported, the low-intensity of electric fields used (2 to 4 V/cm) rule out electroporation [29] as a cause of bactericidal activity, and the insulated electrodes meant that the possibility of iontophoresis could be ignored. An explanation of the inhibitory effects observed was based on an earlier proposal that the fields exert unidirectional dielectrophoresis forces on the polarized parts of dividing cells causing movement towards the furrow [67,68]. To assess how such effects might apply to bacteria, a finite-element mesh method was used to simulate the field distribution for 10 MHz frequencies, modeling the field inside a single dividing bacterium. The simulation found that the forces exerted by the electric field inside a dividing bacterium would be sufficient to cause distortion and movement of particles inside the bacterium at frequencies effective against bacterial growth. With electrostatic forces directed towards the furrow, it is also possible that it causes interference with the

structural integrity of the cell membrane. It should be noted that the use of DC currents to treat bacterial infections in patients has the potential to produce toxic substances, such as metal ions, whereas the use of ceramic electrodes combined with high-frequency electric fields would mitigate any such potential toxicity issues. The system was also used recently to successfully inhibit bacterial growth *in vivo* in the lungs of mice [70]. The author also notes that whilst bacteria are remarkably adept at developing resistance to antimicrobial agents, it is still not known whether or not they possess the same ability to adapt to the inhibitory effect of electric fields and this would point would certainly merit further investigation. The efficacy of the author's system against biofilm bacteria has yet to be determined and therefore, the ability to implement AMFields on an infected IMD patient or indeed on a much larger scale in other biofilm-affected industries is a long way from realization. However, the system does show promise in preventing bacteria in the growth and attachment phases from ever reaching biofilm-related infection levels and could be eventually be implemented as such in a clinical setting.

Electrical Control of Biofilm Bacteria

The Bioelectric Effect

All of the work examined so far has looked at the eradication of bacteria in their planktonic state. However, the concentration of antibiotics and biocides required to kill bacteria residing within the biofilm matrix can be 500 to 5,000 times those needed to kill planktonic cells of the same species [17,18,21,35,71-74]. It is not known, however, whether the observed recalcitrance of bacteria in the biofilm mode of growth to antimicrobial challenge is also true for electrically induced toxicity and whether the biofilm provides a similar tolerance advantage to this approach. In 1992, Blenkinsopp *et al.* developed an electrified modified Robbins device (MRD) and used it to show that the presence of a

low-strength electric field with a low current density (EF-CD) can enhance industrial biocide efficacy against *Pseudomonas aeruginosa* biofilms [35]. As a precursor to this work, Costerton and colleagues hypothesized that the EPS matrix of a biofilm is a charged matrix responsible for binding antimicrobial agents attempting to reach target cells [75]. It was thought that it might be possible to disrupt these charges using an electric field and temporarily cease any binding of antimicrobial agents, allowing them to penetrate the matrix. The MRD is a flow through system where biofilms are grown on a series of coupons each held by coupon holders or 'plugs' situated along the length of the top of the flow chamber, which can be easily removed for analysis [76]. The MRD principle utilizes a large number of coupons (typically 12-25) along the length of its flow chamber to provide a high number of experimental replicates under flow conditions. A similar bespoke system is currently being used in our own laboratory to assess the impact of a range of electrical currents on bacterial adherence to conducting polymers (FIGURE 2). A platinum wire electrode was built into a small groove along the length of the bottom of the flow chamber acting as one electrode, whilst a number of stainless steel sample studs protruding down through the middle of each plug were converted into the other electrode. The MRDs were run in parallel for each experiment with each chamber holding 12 sample studs (surface area 0.5 cm^2). One MRD was a control, whilst the other was electrified by series connecting the screws attached to each stud and the platinum wire to a variable-voltage-and-current power source. A constant potential of 3 V was applied producing maximum field strength of ±12 V/cm^2 and current density of ±2.1 mA/cm^2. The polarity was altered every 64 s such that the electrodes were continuously alternating as anode and cathode. An electric field was induced where electric field potential would impact the flowing media and biofilm when established on the surface of the stainless steel coupons by either being directed downwards from the flat stainless steel stud surfaces to the platinum wire or vice versa in the opposite direction. A peristaltic pump controlled the flow of the inoculated M-56 nutrient medium, with or without biocide or electric current, through the MRDs at a rate of 80 ml/h (mimicking the flow of human urine).

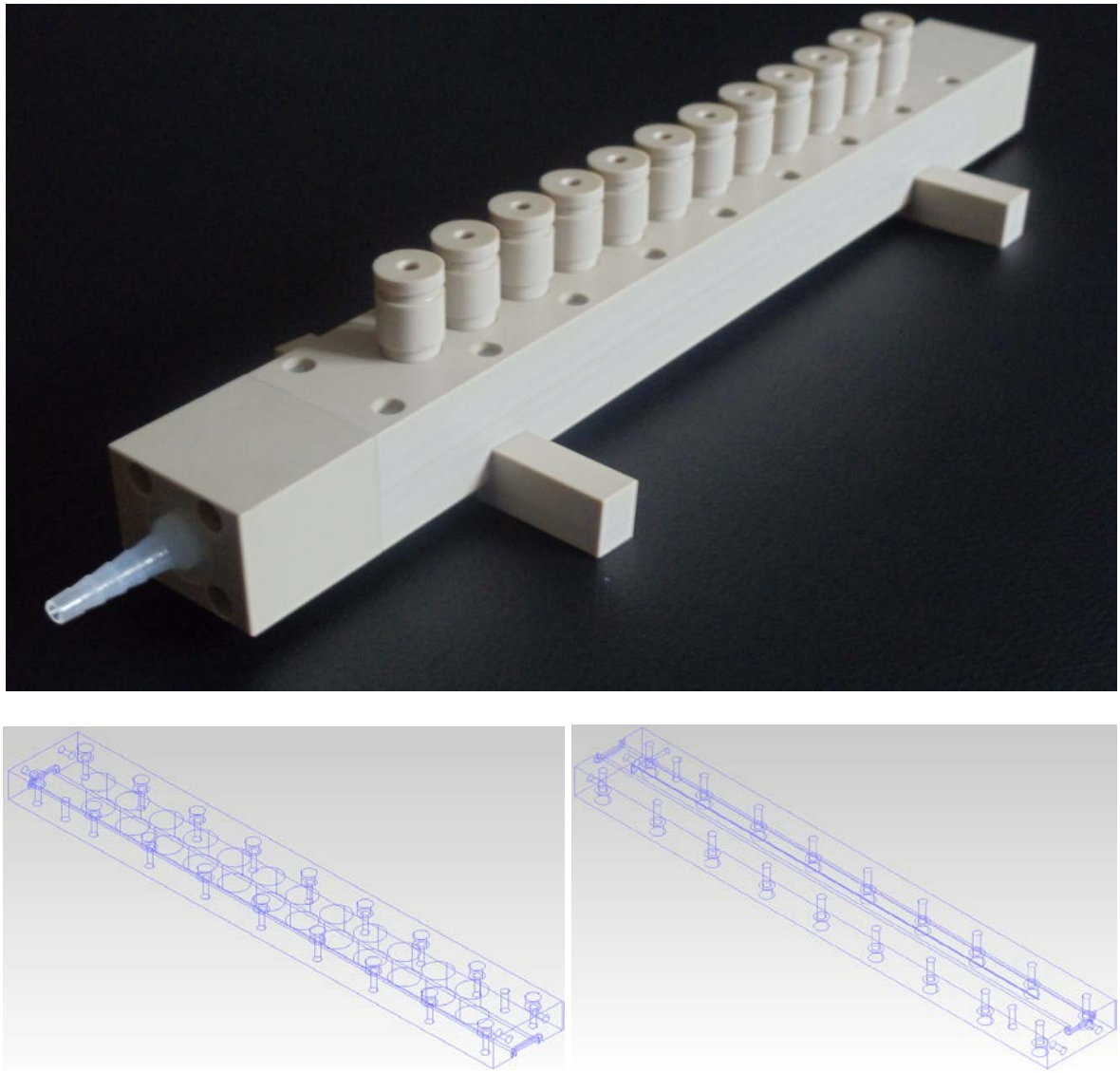

Figure 2. Electrified modified Robbins device (MRD) engineered to study the impact of electrical current on bacterial adherence to conducting polymers.

Blenkinsopp and colleagues' paper [35] represents a seminal discovery - the bioelectric effect, a synergistic effect of the enhancement of biocide efficacy with electric current was clearly demonstrated for the first time, however the definitive mechanism of action was not elucidated, although it was assumed to be related to the aforementioned hypothesis describing a charged EPS matrix [75]. Treatment with either electric field or biocide alone during the initial 24 h colonization period yielded no significant differences in bacterial concentration. However, further results from this work showed the remarkable extent of the enhancements in biocide efficacy under the influence of an electric field

as biocide concentrations lower than those required to kill free-floating planktonic cells were able to kill established 24 h biofilms of the same species over the following 24 h of electrical-biocide treatment. During this 24 h combined treatment period, significant reductions were observed in as little as 4 h depending on the biocide used with up to 6-log reductions representing a complete kill being observed in some cases after the full 24 h.

This experiment raises the question from an engineering perspective of how the biofilm matrix might be characterized electrically. A simulated electrical model of the field as it crosses the broth medium to the wire electrode would provide insights to which areas are being affected by the greatest potential on the coupon surface as well as how the combination of biocide and bacterial medium interacts with the electric field. Modification of the flow device to facilitate either *in situ* microscopy or microscopy at certain time points may provide clarification regarding the effect of this treatment on the biofilm in real time. Furthermore, the optimal electrical parameters and biocide concentrations/type remain unknown and therefore, important extensions to this research would include sweeping a broad range of frequencies, field strengths and patterns, colonization surface types, antimicrobials, and bacterial species at different stages of colony growth.

Costerton and co-workers described a three-electrode Perspex flow-cell (FIGURE 3) developed to examine the bioelectric effect with low-intensity electric fields [23]. The two exterior stainless steel plate electrodes E1 and E3 were connected together to act as the anode with E2 as the cathode for 64 s before the polarity was reversed (and reversed again continuously every 64 s), thereby helping to prevent the accretion of ions on the stainless steel surfaces. Inert nonconductive (or conductive stainless steel) materials I1 and I2 were placed between these electrodes to study the indirect effects an electric field would have on biofilms grown on their surfaces. The electrodes were connected to a DC generator adjustable up to 10 V or 50 mA. A simple-salts medium (M-56) was again inoculated with a culture of *Pseudomonas aeruginosa* and pumped through the flow cell at a rate of 60 ml/h with a peristaltic pump

such that bacterial biofilms would form on all five surfaces within the cell. The field strength applied had an intensity of 5 V/cm and an average current density of 1.7 mA/cm². Costerton *et al.* report that the degree of biofilm formation by *Pseudomonas aeruginosa* on the stainless steel elements of the flow cell was much higher than that produced in their previous study using stainless steel studs in a modified Robbins device [35]. Scanning electron-microscopy (SEM) was used to examine the effects of electrical treatment on the surface of the biofilm showing severely disrupted and cavitated biofilm structures as a result of treatment with an antibiotic in the presence of an electric field.

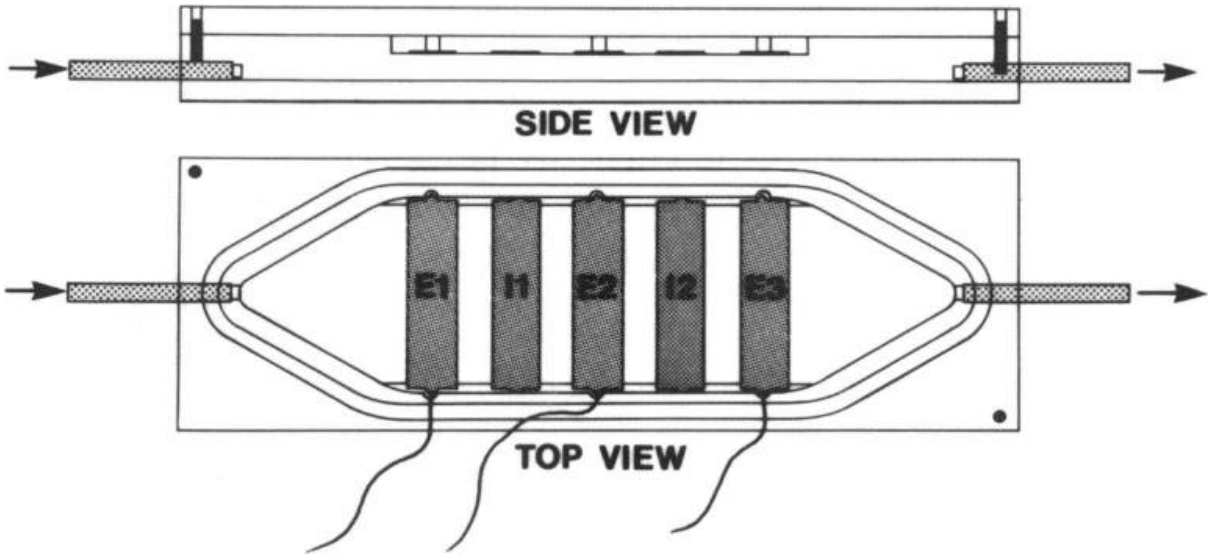

Figure 3. Three-electrode flow cell. Reproduced with permission from [23].

After 48 h of treatment with either electric field or antibiotic alone, there remained huge numbers (UTI levels) of viable bacteria on the surfaces within the flow cell. However, given that biofilm bacteria were killed by low concentrations of the antibiotic tobramycin in the presence of an electric field on all interior surfaces including the inserts, it was thought that low-intensity electric fields might be enhancing the efficacy of the antibiotic by improving its ability to penetrate the biofilm via "electrophoresis", a general term encompassing how electric fields seemingly overcome the antimicrobial diffusion barriers posed by a charged biofilm matrix as hypothesized by Blenkinsopp [35] and thereby facilitate the bioelectric effect. With the concentration of tobramycin required to kill all

bacteria in the presence of an electric field being just 5 times its minimum inhibitory concentration (MIC), the impact of the bioelectric effect's synergistic action could be quantified as being able to reduce the very high levels of antibiotic required to kill biofilm bacteria down to a mere 1.5 to 4.0 times those needed to kill planktonic cells of the same species.

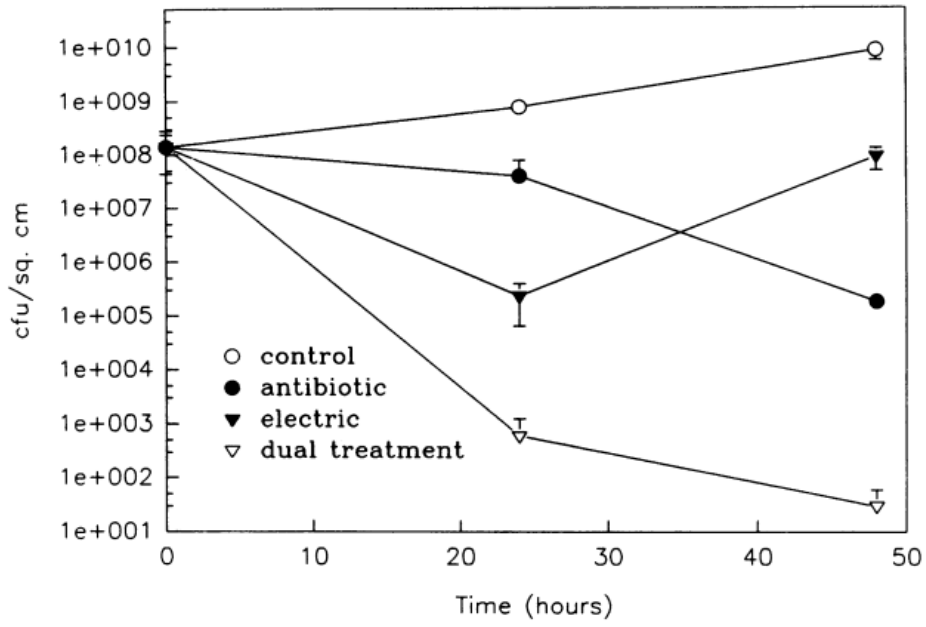

Figure 4. The electric-antibiotic bioelectric effect with tobramycin and *Pseudomonas aeruginosa.* Reproduced with permission from [23].

By quantifying the impact of local electrochemical generation of antibacterial molecules or ions on the viability of biofilm bacteria within the flow cell, Costerton and colleagues' data seemingly dispelled the possibility that the bioelectric effect was somehow dependent on the iontophoretic killing effect reported in earlier papers [32-34,53,54,66]. This study underpinned a new hypothesis that electrophoretic forces help overcome antimicrobial diffusion barriers within the charged biofilm matrix.

Current densities were measured at various points throughout the chamber, and were found to be considerably lower (50%) at the outer electrodes compared with the central electrode E2. Electrically,

this flow system was considerably more complex than in previous studies. As with their earlier work reported using the Robbins device [35], it is difficult to ascertain whether the path of current is travelling through the biofilm or is instead simply spreading in a field across the electrode surface since it is not known how resistive the biofilm is; this is an important consideration worthy of further investigation. The design of the system assumes the electrical current flows from one side to the other through the horizontal axis of the biofilm but this might not be realistic. If the electrostatics of the entire flow system were to be simulated accurately within a modeling package, then it may give a much clearer picture of how the field is interacting with certain areas of biofilm within the chamber.

Further work by this group would once again underline Blenkinsopp and Costerton's assertions that low electric currents enhance the effect of an antibiotic against biofilm bacteria [24]. All studies were performed in a minimal salts medium that excluded chloride-containing compounds to again mitigate any possibility of an iontophoretic effect explaining the antibacterial action. An electrical colonisation cell (ECC) was designed so that a biofilm could be formed on a surface distant from the electrodes, avoiding any electrochemical and mechanical disturbances by delivering only an indirect electric field effect. Biofilms were grown on a treated semi-permeable dialysis membrane, which was then suspended, equidistant between two parallel 5.31 cm^2 circular stainless steel electrodes. The ECC was divided into two chambers by the membrane, which was clamped in place by an O-ring. The electrodes were connected to a power generator to produce an electrical current through a biofilm colonized on one side of the dialysis membrane. A chemostat culture was pumped through the ECC at a rate of 50-60 mlh^{-1} for 1-2 h and then connected to a glucose MS medium to allow the formation of 12, 24 or 48 h biofilms treated with antibiotic, current, or a combination of both. The biofilms were then treated for a further 12 h with electric current supplied by the power generator that provided a constant current of 9 $mAcm^{-2}$ (polarity was changed every 32 s) in a square wave function whilst an electrolyte solution was pumped through each chamber separately at a rate of 50 mlh^{-1}.

Jass and colleagues observed that neither current alone nor standard concentrations of tobramycin alone had any effect on biofilm bacteria. However at higher antibiotic concentrations, a lethal bioelectric effect occurred in the presence of an electric field, whilst there was no significant impact on biofilms for lower concentrations (10 μgml^{-1}). This observation raised the question of whether or not the right combination of electric field intensity and antibiotic concentration would deliver an optimal killing effect. After further investigation, an interesting correlation was discovered between current density and concentration of antimicrobial required to maximize bacterial killing. It was found that a 10 μgml^{-1} concentration of tobramycin was significantly enhanced by a 9 $mAcm^{-2}$ current but not by levels above this current density. Furthermore, a 25 μgml^{-1} concentration of tobramycin was optimally enhanced by a 15 $mAcm^{-2}$ current but not by a 9 $mAcm^{-2}$ current density, whilst even greater levels of current (20 $mAcm^{-2}$) did not yield any further antibacterial enhancement. These data suggest that particular electric field strengths might combine optimally with certain concentrations of antimicrobial to produce the most effective synergistic killing effect. In further experiments using the ECC, it was demonstrated that whilst electric current clearly enhances antibiotic efficacy against biofilm bacteria, this statement only holds true for those antibiotics that are already effective against a particular species of bacteria in their planktonic mode of growth [24,25].

The ECC is a geometrically and electrically complex system designed to treat biofilms grown on a membrane distant from the electrodes using an electric field. Since the surface of the dialysis membrane upon which the biofilms are grown is positioned parallel between two plate electrodes, the electric field would have travelled through the vertical axes of the biofilm from the top to the bottom of the EPS matrix. Structurally, this may have had a drastically different effect on the biofilm morphology than in the earlier three-electrode flow cell experiment [23]. To address the problem comprehensively would require an experiment to examine microscopically the structural changes incurred by biofilms under the stress of an applied electric field *in situ*.

The proposed mechanism for bactericidal activity was that electrical current was driving charged particles (ions) into the EPS matrix by electrophoresis [23,54,77] whilst antibiotics were further driven through the membranes of individual bacterial cells; a process termed "electroporesis". The lower growth rate of biofilm bacteria residing within the EPS matrix has been widely attributed as one of the key factors responsible for their decreased susceptibility to antibiotic therapy [15,16]. Taking this into consideration, Jass *et al*. further developed a theory that since current did not appear to damage cells (under microscopy) but rather appeared to increase average cell size, the mechanism of the bioelectric effect may be that electric current increases the metabolic activity of cells (by producing more dissolved oxygen vital to their growth) within the matrix making them more susceptible to antibiotics [24]. Essentially, the slow-growing or dormant cells are given an oxygen boost to 'awaken them', enhancing susceptibility to antibiotic therapy similar to that of planktonic cells.

This theory was later developed by another group who also suggested that the increased delivery of oxygen to biofilm cells during electrolysis may be responsible for the bioelectric effect [28]. When an electric current was applied, the evolution of oxygen gas bubbles was observed in the chamber and subsequent experiments where oxygen was bubbled into the chamber together with tobramycin and no electric current revealed a 1.8-log increase in killing. The explanations for this effect included the aforementioned possibility that the metabolic activity of cells is enhanced making them more susceptible to the antibiotic [25] alongside the possibility that high concentrations of oxygen may be toxic to bacteria, supported by the observation that treatment with oxygen alone caused a small but noticeable reduction in the size of biofilm.

Wellman and colleagues demonstrated the bioelectric effect using reaction chambers built from five-slide, fifty-gauge polypropylene slide transporter boxes designed for holding a range of polycarbonate coupons with biofilms grown on their surfaces [26]. Holes drilled on either end created a pathway for nutrient flow through the chamber and platinum wire electrodes (0.63 mm diameter) were placed at

either end inside the chamber such that the positive electrode of the DC supply was situated at the influent end of the chamber. Notches were cut at each end of the chamber for electrode insertion and the wire at the influent (or positive) end was connected to an ammeter. A voltmeter was connected across the chamber. Mixed-culture biofilms of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were grown on a polycarbonate coupon within a 'RotoTorque' reactor for 7 days until the biofilm growth had reached a steady state and the biofilms were then transferred to the experimental chambers and treated with current (1 or 5 mA), antibiotic (tobramycin, 1 or 5 mg/liter), a combination of both, or neither for 24 h. The results of this experiment reinforced the assertion that there exists an optimal combination of antimicrobial and electric current for killing biofilm bacteria, even suggesting that in some cases, too much current might actually hinder the effect. Whilst Costerton *et al.* observed only 6-log reductions in biofilm bacteria using the three-electrode flow cell [23], Wellman's group noted that the experimental chamber designed by Jass and colleagues [24] showed up to 8-log increases in bacterial killing. Costerton applied an electric field that was orientated across the horizontal axes of the biofilm whereas in the studies performed by Jass, the electric field was orientated perpendicular to and hence through the vertical axes (or thickness) of the biofilm matrix. It was thought that since other factors remained constant and comparable across the two studies, the electric field orientation with respect to the biofilm's orientation might have a role in maximizing the strength of the bioelectric effect. Advanced microscopy of the biofilm structure comparing different field orientations and patterns could improve knowledge of this aspect although it could just as easily be attributed to experimental variation.

The bioelectric effect was again demonstrated by using a DC current in conjunction with gentamicin or oxytetracycline on *Escherichia coli* biofilms before the DC source was changed for a 10 MHz radio frequency current (RFC) source under the same experimental conditions [29]. A biofilm treatment chamber was developed containing two stainless steel electrodes 12 cm apart with glass biofilm supports held perpendicular such that electric field lines would pass vertically through the biofilm matrix. A peristaltic pump provided a continuous flow of M56 in the chamber (with or without the

antibiotic) over the biofilms. A custom-built RFC generator provided a root mean square RMS current of 150 mA at a frequency of 10 MHz and a power output of 5 W for 24 h of treatment.

As with DC, the RFC also enhanced the efficacy of antibiotics in a new RFC bioelectric effect. This is of particular interest since none of the proposed explanations of the DC bioelectric effect can hold true when using an RFC. Electrophoresis cannot occur at the frequency used, while electroporesis would require much higher electric fields (1,000 V/cm [69]). Additionally, iontophoresis cannot occur as the field intensities and frequencies used are non-ionizing. Caubet suggests that the RFC could have a mechanical effect on the EPS matrix wherein the radio frequencies vibrate the biofilm, weakening its structure and giving rise to a synergy phenomenon where an apparent increased fluidity of the matrix allows a better penetration of the antibiotic [15]. This would seemingly follow previous theories surrounding a charged EPS matrix [23,35] by suggesting that structural changes to the biofilm enhancing its susceptibility to antimicrobials are related to both mechanical and electrical characteristics. The way in which an electromagnetic field acts upon the polar parts of the EPS matrix via vibration energy was suggested to be comparable to other research efforts using ultrasonic frequencies to kill biofilms [78-80]. Caubet admits that at the time of reporting, attempts to model both the mechanical structure of and electric fields within biofilms were insufficiently well developed to yield any quantitative results from experimental data [29]. It was suggested that a dielectric spectroscopic analysis of the biofilm matrix over a large range of frequencies would lead to the discovery of optimal RFC relaxation frequencies for treatment of biofilms. Systems utilizing RF such as this and AMFields [69] should now be used to study their ability to combat biofilms without antimicrobial agents.

Whilst not directly related to the bioelectric effect, Stoodley *et al.* hypothesized that an electric field may cause structural changes to the biofilm [81]. The goal of this work was to microscopically observe (for the first time) a live biofilm in real time in the presence of an electric field and thereby examine

structural changes and pH changes. The complete flow cell system consisted of the polycarbonate closed-channel flow cell (0.5 cm wide, 1 cm deep) in a recycle loop (flow rate 4.5 ml/s), which included an aerated mixing chamber and recirculation pumps. Two platinum wire electrodes (100 µm diameter) were positioned across the top of the channel separated by a distance of 2 mm. The voltage produced at the electrodes was ±1.3 VDC (just below the point at which gas bubbles evolve) and the current was approximately 50 µA (current density 3.1 mA/cm²). Polarity was alternated in a square wave function at frequencies from 0.016 to 20 Hz. The system was filled with a minimal salts growth medium inoculated with 1 ml of bacterial stock culture. A mixed species biofilm (*Klebsiella pneumoniae*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa*) was allowed to grow on the the flow cell surfaces and wire electrodes for 3 days reaching a thickness of approximately 50 µm, after which experiments were performed. Structural changes to the biofilm were observed using confocal scanning laser microscopy and biofilm thickness measurements were obtained by digital image analysis.

The software measured biofilm thickness by focusing on and finding the distance from the outside edge of the wire to the outside edge of the biofilm. When a voltage was applied with oscillating polarity, the biofilm was observed to expand slightly when the wire was cathodic but contract to around 75 % of its original thickness when it was anodic. For the few seconds when the polarity was altered, contraction and expansion of the biofilm occurred at the same frequency.

PH indicators were used to monitor changes in pH during application of an electric field. Following electrical treatment, the system was alternately flushed with both alkaline and acidic solutions and under acidic conditions, a rapid contraction in biofilm thickness was observed of approximately 31%. This finding bore a striking similarity to the level of contraction observed when the wire was carrying electrical current as an anode. This correlation was then used to formulate a possible explanation of the anodic contraction wherein acid was being produced by the anodic oxidation of water lowering the pH, whilst hydroxyl ions were produced when the wire was cathodic and thereby increasing the pH. In this

experiment, biofilms were grown on wire electrodes, which meant there was a high likelihood that any bactericidal activity was caused by electrochemical reactions and it is indeed significant that no antimicrobials were used. Nevertheless, the methods used to observe any structural changes in biofilm morphology are noteworthy and the paper offers a first look at how pH changes occur under the influence of an electric current.

The Electricidal Effect

Over the short term, it has been reported that electrical treatment alone without an antibiotic has no significant effect on reducing biofilm populations [23,24,35]. However, del Pozo *et al.* recently studied the effect of biofilm exposure to electrical treatment in the absence of an antibiotic over a longer period of up to 7 days, yielding up to 6-log reductions in *Staphylococcus epidermidis* biofilms after two days at 2 mA, a phenomenon labeled the "electricidal effect" [30,82,83]. Time- and dose-dependent killing was observed for a range of different bacterial species. Indeed, these findings present an exciting new prospect where the killing of bacterial biofilms on IMD surfaces might be achieved without the aid of an antimicrobial agent in the flow device fluid stream.

Two eight-channel current controllers together with 16 polycarbonate test chambers (FIGURE 5) were used to assess the impact of using a combination of antimicrobial agents (11 variants in total) and electric currents either together or on their own against biofilms of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* grown on Teflon disks 12 mm in diameter by 1 mm thick [30]. The biofilms were grown for 36 h in a biofilm reactor prior to treatment and then transferred to the test chambers where they were continually delivered a fresh flow of media. The electrodes used were two stainless steel or graphite cylinders positioned vertically in the midline of each chamber 1 cm from each end serving as the anode and cathode. The biofilm coupons were placed vertically within a groove in the 1 cm space between the electrodes and perpendicular to them so that

only indirect field effects across the chamber would impact the biofilm. Currents of 20 µA, 200 µA or 2,000 µA were applied with or without antibiotics present in the media for a period of 24 h before the coupons were removed and viable counts were performed.

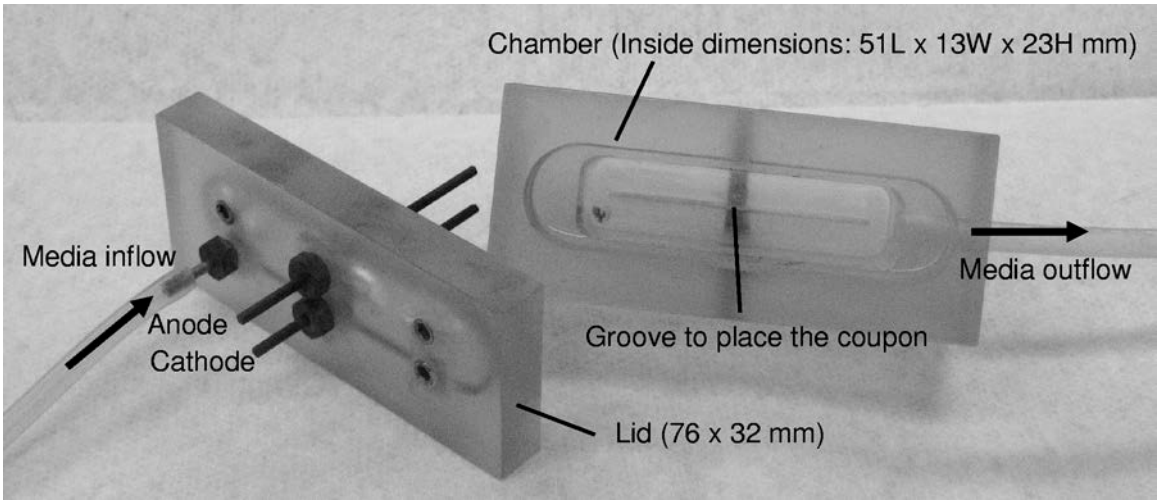

Figure 5. Polycarbonate electrical treatment chambers. Reproduced with permission from [30].

As a result of using such a broad range of microorganisms, del Pozo's key finding from this first paper using the polycarbonate treatment chambers was that the bioelectric effect cannot be generalized; it requires that specific conditions or experimental parameters are met for each bacterial species such as antimicrobial concentration and electrical current intensity [30] supporting the findings of Jass *et al.* [24,25]. Whilst there would be major concerns in using currents as high as 2 mA clinically, results obtained with 200 µA of current in combination with daptomycin and erythromycin would appear to have promise. The limitations of attaching wires to IMDs in patients are also considered, since they themselves would serve as ideal surfaces for bacterial colonization, noting that a non-invasive or minimally-invasive method of delivering the current *in vivo* must also be devised (the indirect effects witnessed in these experiments would be ideal for this). Certainly, exploring technology capable of remotely delivering electrical power would be of great merit for *in vivo* applications involving such treatments.

del Pozo repeated these experiments for prolonged periods (1, 2, 4 and 7 days) on experimental replicates that did not have an antimicrobial in the media [82]. After 2 days, 6-log reductions in the number of viable CFUs for *Staphylococcus epidermidis* biofilms and 5-log reductions in *Staphylococcus aureus* biofilms were observed at 2,000 μA of treatment. Additionally, up to 5-log reductions in *Pseudomonas aeruginosa* biofilms were observed after 7 days leading to the assertion that long-term exposure to low-intensity current has a notable effect of reducing the numbers of viable CFUs for staphylococcal and pseudomonal biofilms (the electricidal effect). The experiment also demonstrated that higher levels of treatment current and/or longer treatment periods yielded greater reductions in viable biofilm bacteria [82].

It has been suggested that the mechanism of the electricidal effect may be related to the ability of electric current to create hydrated ions, which transport water across surfaces causing a detachment force [59]. Other possible mechanisms include the potential disruption of the bacterial membrane and charged EPS biofilm matrix [23,24,27,35], as well as the enhancement of electrostatic repulsive forces between the bacteria and their colonization surface [84]. Unusual pH changes observed in this study would certainly merit further investigation as a potential mechanism and have indeed been attributed as such before [81]. It would also appear that *Pseudomonas aeruginosa* biofilms are less susceptible to electric currents than *Staphylococcus aureus* or *Staphylococcus epidermidis* biofilms. Examination of how the morphology and structure of both *Pseudomonas aeruginosa* planktonic cells and biofilms differ from those of the other species could offer clues as to which mechanisms and attributes confer enhanced tolerance. If enough comparisons of the two were performed to confirm that their electrical susceptibilities differ considerably, then new theories could be formed suggesting that certain isolated characteristics of bacterial cell walls render them more vulnerable to electrical treatment. Ultimately, it would be of great interest and value to this field to determine an optimal set of parameters for killing each of the most common biofilms responsible for nosocomial infections with electrical current alone.

The author's work only assesses the effects of DC electric fields on the biofilms. Another important extension would involve the use of AC and alternating electric fields or RF at varying intensities and frequencies to determine the optimal effects.

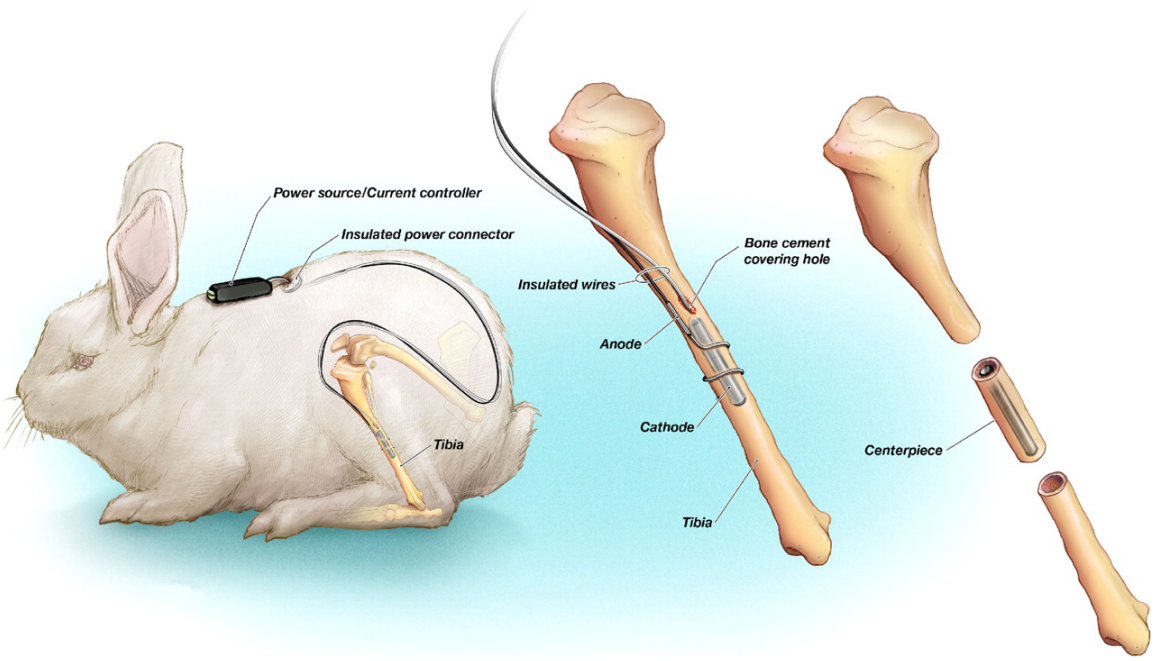

Figure 6. Chronic foreign body osteomyelitis model in rabbits. Reproduced with permission from [83].

In further studies, del Pozo and colleagues examined the feasibility of the application of the electricidal effect as an effective *in vivo* treatment [83]. A stainless steel implant was inserted into a cavity within the tibia of experimental animals (rabbits) (FIGURE 6) together with 10^4 CFU of *Staphylococcus epidermidis* and was left for four weeks to induce chronic foreign body osteomyelitis – a foreign body or IMD infection commonly caused by biofilms. Rabbits were then treated with either doxycycline or 200 μA electric current for a period of 21 days. Treatment with electrical current yielded a median bacterial concentration of 1.09 $\log_{10}$ CFU/g of bone compared with 2.55 $\log_{10}$ CFU/g for doxycycline treatment and 4.16 $\log_{10}$ CFU/g for controls leading to the assertion that electrical current is significantly more effective than intravenous doxycycline treatment, at least statistically. In 5 of 14 animals treated using doxycycline, resistance to the treatment emerged giving the electrical method

another clear advantage. It would be of great interest to examine whether or not any bacterial resistance to an antibiotic could be circumvented when administered in conjunction with an electric current (the bioelectric effect) and whether or not any resistance is developed to electrical treatments for a range of different bacterial species over a period of treatment. The authors acknowledge the enormous potential for extending this work to the treatment of biofilm infections associated with orthopedic hardware in humans, whilst commenting on limitations in the study in terms of treatment length, current intensities, and bacterial species. Additionally, the method of surgically inserting insulated wire electrodes *in vivo* is extremely invasive and potentially traumatic to the patient. The electrodes provide extra surfaces for potential bacterial colonization and as such, the study would benefit from taking a minimally invasive approach requiring redesigned hardware to administer indirect electrical therapy. The use of a battery pack to deliver continuous current could potentially be incorporated in any future IMD designs to implement bioelectric or electricidal treatments to patients *in vivo*. There should be no doubt that del Pozo's findings offer significant advancements to the field of electrical bacterial killing. It is of even greater significance however that this breakthrough biofilm treatment requiring no antibiotic could indeed bring the commercial realization of an electrical treatment method to fruition in other capital-rich sectors outside the medical arena such as the oil or water treatment industries. The reader is directed to a recent comprehensive review by del Pozo *et al.* of the literature on the bioelectric effect including a detailed digest comparing important parameters across the experiments including the electrical current used for treatment, as well as the antibiotics type and dose and the bacterial species targeted [4].

Biofilm Prevention and Control Using Acoustic Energy

Novel efforts have been made to combat device-related bacterial infections by using acoustic energy at specific frequencies to either prevent biofilm formation on surfaces or to mechanically compromise the structures of existing biofilms [85,86]. Acoustic energy holds a number of key advantages over other methods as it can prove an effective means of bypassing the conditioning-film and hence preventing

any surface adhesion occurring in the first place since the mechanical disruptions render any firm surface attachment much more difficult. Continuous ultrasonication at 500 kHz (power intensity 10 mW/cm^2) has been shown to work synergistically with the antimicrobial agent gentamicin against *Escherichia coli* and *Pseudomonas aeruginosa* biofilms by facilitating the drug's transport through the EPS matrix *in vitro* [87]. No significant changes were incurred to the *Pseudomonas aeruginosa* biofilm matrix or its bacterial cells in the absence of gentamicin [88]. The viability of *Escherichia coli* biofilms has also been significantly reduced *in vivo* using pulsed low frequency ultrasound (25% duty, power intensity 500 mW/cm^2 for a treatment period of 24 or 48 h) in combination with gentamicin but these effects were not reproducible against *Pseudomonas aeruginosa* biofilms [89-91]. In 2005, Ensing *et al.* [92] also demonstrated greater than 50% reductions in the viability of *Escherichia coli* biofilms by combining pulsed ultrasound treatment (24-48 kHz, power intensity 500 mW/cm^2) with gentamicin. Once again, no such effects were observed for *Pseudomonas aeruginosa* biofilms. It is thought that this differing level of impact for ultrasound treatment in the two species tested may be explained by the differences in the permeability of the outer membranes of the gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* [88,89,91,93-95]. Viability of the gram-positive bacteria *Staphylococcus epidermidis*, which often infects patients fitted with orthopaedic implants, was significantly reduced over 48 h in an *in vivo* rabbit model using a simultaneous combination of vancomycin and ultrasound treatments [87]. Other groups have sought to combine ultrasound treatments with proteolytic enzymes in a bid to reproduce further synergistic killing effects. 84-95% of *Escherichia coli* biofilms were eradicated by combining ultrasonication at a frequency of 40 kHz applied for 10 s with a 15 minute application of protease compared with 30% eradication using ultrasound alone [96], evidence perhaps that a synergistic kill similar to the bioelectric effect may be active in acoustic treatments. Contradictory results have also been found which reveal that low-intensity, low-frequency ultrasound (Power intensity 2 W/cm^2, frequency 70 kHz) without the aid of an antibiotic or proteolytic enzyme actually enhances the growth of a range of gram-positive and gram-negative biofilms, presumably due to ultrasound's ability to enhance oxygen and nutrient transport to cells [97].

It has been shown that high ultrasonic power density levels are the most effective at removing established biofilms, and that the extent to which those biofilms are cavitated and stripped from the surface is inversely related to the frequency applied [87,96]. However, when considering prevention of biofilm formation it appears that completely different mechanisms and hence, different levels of power intensity and frequency combine to yield the most effective results [97]. The need for a more sophisticated approach to the application of acoustic energy has already resulted in a novel solution which fine-tunes vibration energy using tiny piezo-electric elements. Hazan *et al*. developed piezo-electric elements attached to the outer surface of a catheter (FIGURE 7), which spread low-energy acoustic waves (for example 0.2 mW/cm^2) throughout the device and surrounding fluid media causing the bacteria to vibrate at the same frequency [85].

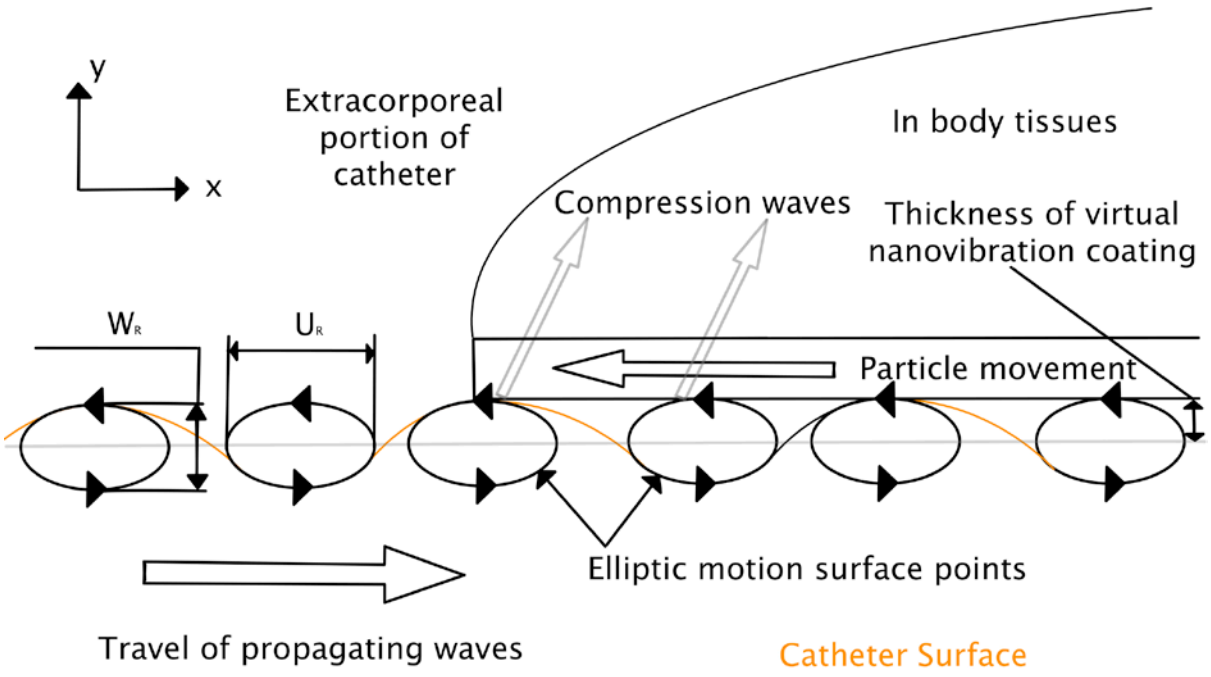

Figure 7. Dispersion of surface acoustic waves across a catheter (or other solid surfaces). Adapted from [85].

This has the effect of covering the catheter in a vibrating "coat" which facilitates biofilm prevention inspite of the usual inhibitors that develop on urinary catheters such as conditioning films encrusted with proteins, electrolytes and other organic molecules [98, 99]. The presence of the vibrating coat

resulted in extremely diminished biofilm formation for a range of microbial species including *Escherichia coli, Enterococcus faecalis, Candida albicans* and *Proteus mirabilis* over a period of 48 h. It was reported that the low power intensity levels interfered with bacterial attachment and subsequent biofilm development such that the catheter surface remained virtually clean of adherent bacteria throughout. This effect at low power intensities is analogous to the electricidal effect and its inherent advantages over the bioelectric effect in that it does not require the aid of an antimicrobial agent. Shifting to higher power intensity levels results in enhancement rather than interference of bacterial adhesion and biofilm formation meaning that precise control of the power intensity generated by the piezo-electric actuators is essential. Potentially, this is difficult to consistently achieve and would constitute a significant risk to any *in vivo* applications. Hazan *et al.* demonstrated what might be considered the most advanced step towards achieving clinical implementation using acoustic energy to date in a rabbit model where catheters utilizing piezo-electric actuators remained sterile for up to 9 days whilst all controls developed UTIs [85]. Further studies must be carried out to develop a matrix of which acoustic treatment parameters are effective at targeting a broad range of bacterial species. It would certainly appear though that in fine-tuning those parameters, power intensity is the predominant factor rather than frequency and as such, its adjustment is surely the key to effectively targeting the onset of infection. A favorable aspect of this approach is the fact that biofilm prevention is more appealing than having to treat established biofilms that would already constitute a UTI or other device-related infection. In this reviewer's opinion, this latest acoustic method can easily be considered one of the most viable means possible of producing a novel IMD that will remain infection-free throughout its period within a patient.

Expert Commentary

To date, there has been no definitive explanation for the mechanism of electrically induced reductions in bacterial bioburden (both planktonic and sessile) or for the mechanism behind the electrical enhancement of the efficacy of antibiotics, or the "bioelectric effect". Confirmation of existing hypotheses requires further experimental data and observation including live microscopic evidence of the impact of electrical fields on the biofilm *in situ* or on individual bacterial cells during successful treatments. Models (both experimental and computational) capable of predicting or elucidating the effect of electrical fields on the three-dimensional structure and architecture of the biofilm matrix are necessary to achieve this goal. Such models will be required to combine knowledge of the composition of fluids, tribology and structures within the biofilm as well as a wide range of generalized biofilm matrices to assist microscopic evidence of impact.

Key papers in this field have been revealing new information at least every few years. The reason for the relatively slow rate of publication relates to the multidisciplinary nature of an electrical approach, requiring a combination of skills including electrical engineering, mechanical engineering, physics, microbiology and materials science. Very few laboratories have managed to bring together this wide array of knowledge and abilities in a single, coordinated effort to meet the challenge of delivering a clinical product fit for commercialization. Only a group with the appropriate vision, knowledge and resources across a number of fields of expertise will be capable of developing advanced practicable electrical treatments such as an electrified IMD employing conducting polymeric materials or an industrial decontamination system capable of preventing initial bacterial growth and attachment or treating developed biofilms.

The most recent and significant advances in the field include the discovery of the "electricidal effect", a long-term reduction in the viability of biofilms using DC electric current without antimicrobials [82,83]. There should be no doubt that del Pozo's recent findings regarding the electricidal effect contribute significant advancements to the therapeutic strategy of electrical bacterial control. Biofilms

were grown on Teflon surfaces and in terms of achieving impact in the clinical environment, replicating such results experimentally against biofilms grown on the surface of conducting polymers would represent an important step towards the manufacture of a range of conducting IMDs. These IMDs, when combined with electrical currents, could prevent or treat device-related infection in a safe, cost-effective manner.

Another promising development utilizes advanced RF treatment fields to prevent initial bacterial growth (and produce an RF bioelectric effect) [29,69,70], whilst a further novel approach was developed using piezo-electric actuators to set up antimicrobial acoustic waves on IMD surfaces preventing biofilm formation *in vivo* [85]. Further work must be carried out to optimize the electric field parameters and conducting polymer combinations (such that the device surface would effectively act as the electrode) required to effectively combat the pathogens responsible for nosocomial infections such as UTIs.

Once sufficient *in vitro* testing has been performed, a fully-fledged device must be fabricated and tested using animal models before human trials can be initiated. A similar approach is required to translate the bioelectric effect into a clinical application, although we believe a better solution to the issue of both biofilm prevention and treatment can be reached without the requirement for antibiotics or industrial biocides. Research into the aforementioned RF approach requires similar fine-tuning to that of the electricidal effect to realize a clinical and commercial technology, whilst acoustic-wave research preventing initial attachment must now also be proven against established biofilms. Large-scale treatment systems utilizing RF fields would appear to have great potential in industrial applications where products such as bottles on a production line could pass through a high-frequency field generator for sterilization. Water or oil pipelines could be lined with an electrified antimicrobial material surface to counter biofilm formation, and it could even be possible to develop polymeric conductive pastes made using ionic liquids to coat all kinds of surfaces including pipeline interiors [100].

Biofilm infections are notoriously difficult to treat and in most cases it is necessary to remove the implant to cure such infections, which can cause a lot of distress to the patient [39]. A therapeutic approach without the requirement for IMD removal would therefore be most desirable. This is one of the key reasons for developing an electrical treatment to device-related infection, as well as the potential for a creating a reusable, electrically sterilized catheter whose savings would be enormous. In addition, bacteria possess the capability to adapt to antimicrobial therapy over time, meaning that a treatment regime that was once effective can be rendered useless [15]. It is unproven whether or not bacteria possess the same ability to adapt to electrical treatment and even if they could, by quickly alternating the many electrical parameters available in such treatment regimes, it is possible that they would never be given the opportunity to. Another important consideration is that any clinical treatment device must not present a risk to the patient's safety and comfort. As such, all low-electric currents and high-frequency RF fields to be implemented in human trials must undergo rigorous testing to ensure any treatment is safe and ethical (electrical currents have already been used for fracture healing) [101].

Whilst the overwhelming majority of evidence presented so far has been in support of the bactericidal potential of the DC bioelectric and electricidal effects, the application of RF or acoustic approaches may end up providing a more effective solution to the plethora of biofilm-associated device-related problems. Research currently underway in our own laboratory will look at methods of preventing initial attachment and growth and countering biofilms grown on conducting polymers using DC, AC and RF treatment sweeping a broad range of frequencies, field intensities and current types fine-tuned for optimal impact against a number of pathogens. Additionally, research into acoustic waves or vibration energy propagated across a material surface would appear to have great potential in preventing biofilm formation. It is hoped that the results of these experiments will lead to the development of a prototype urinary catheter ready for testing in clinical trials.

Five-year View

Intrinsically conducting polymers (ICPs) are organic polymers that are inherently conducting [36-38]. Their processability comes from dispersion and as such, they are in general not thermoformable as of yet for the manufacture of IMDs. Their conducting properties can be changed by organic synthesis and by advanced dispersion using nanoparticles. Limitations of conductive polymers are generally due to manufacturing costs, material variation, toxicity, poor solubility in solvents and inability to directly melt the polymer. However, when used as a very thin surface layer on a catheter for example [102], they are as effective as indium tin oxide [65]. Composite materials such as silicones mixed with silver or nickel particles could offer the best possible solution at present for implementing some of the most promising recent electrical bacterial control strategies in an IMD [30,69,85]

Silver nanoparticles embedded in a polymer matrix deposited using plasma polymerization could form a conductive polymer with inherent antibacterial properties [103]. This would maintain the antibacterial properties to planktonic bacteria close to the catheter whilst also permitting DC and RF currents to flow in the catheter surface to stop bacterial adherence and biofilm formation. It is expected that active surface layer thickness, techniques for trapping metal nanoparticles in the polymer matrix, and conductive polymer properties will have an optimum relationship that must be developed for the type of IMD required.

The Quartz Crystal Microbalance (QCM) [104,105] measures the mass per unit area by observing the change in frequency of a quartz crystal resonator. The QCM is being used in present studies in the liquid phase (taking large damping into account) to study bacterial adherence and also protein absorption. Mass density growth as low as 0.5 $\mu g/cm^2$ can be measured versus time. This shows the adherence rate for different bacterial species under a variety of growth conditions which can include

metal nanoparticles in the resonator growth medium, applied RF current through the quartz resonator using surface currents, and low frequency ultrasound (as the QCM resonance is usually in the range 4-6 MHz). The mass change is related to the frequency change using Sauerbrey's equation. The viscoelasticity of the bacterial adherence layer or established biofilm matrix can also be studied using the QCM by measuring the ratio of bandwidth to frequency over time. This will enable the change in the structure of the bacterial growth to be studied over time rather than just the mass adhered to the surface.

Another emerging strategy to combat device-related bacterial infection may eventually arise from novel research being undertaken in the cancer therapy field. Nuccitelli *et al.* [106] have used pulsed electric fields (PEFs) to cause tumor cells to shrink dramatically without the need for drugs. This essentially involves targeting cancer cells with very high voltages for very short bursts (300 ns per 20 kV/cm pulse) such that a cumulative field exposure of 120 μs over two weeks effectively reduces the melanoma by 90%. One subsequent treatment of just 210 μs causes the tumor to self-destruct, killing it completely. The author reports that the mechanism for this reduction is that the nuclei shrink and blood flow is reduced eventually stopping. Furthermore, the short overall treatment duration has only the most minimal effects on the patient's skin yielding only 3ºC increases in temperature. This increase may be regarded as minimal compared with other similar treatments, which utilize radio waves or microwaves to heat the tumor to more than 43ºC in order to kill cells via hyperthermia [106-108]. Other studies have also found methods to use PEFs to increase the permeability of the cells, facilitating the traversal of drugs to their interiors; however a drug-free method is preferable when designing an IMD [109-111]. It is possible that the application of such pulses to bacterial cells could reduce the nuclei and have similar killing effects as those exhibited in cancer cells. Adaptation of the approach developed in Nuccitelli and co-workers' experiments to target biofilm cells could provide yet another electrical strategy to control device-related infection and industrial biofilms. The obvious downside to this method stems from its inability to prevent biofilm formation in the first place since it is only an effective method of destroying existing cells.

Existing surface treatments for urinary catheters rely on embedded silver particles or antibiotic drugs. However these only work for a few hours as proteins and small sized molecules cloak the treated surface and bacterial colonies re-establish themselves. A new approach by researchers at Harvard University considers mechanical properties of the surface polymer and nanoscale surface properties to deter bacterial adherence [112]. Nanoposts on the surface of the polymer can attract or repel bacteria depending on the species of bacteria and the size, shape, and spacing of the nanoposts. Bacteria also decide which surface to adhere to depending on the mechanical stiffness, with stiffer substrates being preferred. The aim is to make the surface appear soft and 'hairy' by altering the type and spacing of the nanoposts, with nanoposts being bacterial species-specific. Manufacture would be by achieved by imprinting onto the polymer used to fabricate the catheter.

In order to use electromagnetics to control and prevent bacterial adherence and biofilm production, it will be necessary to fully characterize each bacterial species associated with UTIs. A number of techniques will be examined over the next five years. These will include determination of the electromagnetic resonance properties of individual bacterial cells or very small colonies of bacteria (10 or so individuals) with nanoprobes connected to broadband vector network analysis to study the size and activity of the bacteria when subjected to a range of frequencies (from tens of kilohertz to the gigahertz range) in one sweep and with varying power levels. This will provide a profile of each bacterial species and indicate their susceptibilities. As bacteria adapt, new strains can again be profiled to refocus the electromagnetic threat.

As well as deterring the adherence of bacteria onto urinary and endotracheal tubes, the use of conductive polymer electrodes can be used as a sensing system while still keeping costs down using cheap off the shelf components (COTS). One of the advantages of the growth in low-cost mobile telephony is that the components that can be used to build a radio frequency reflectometer are now readily available and

can be externally connected onto a modified semi-conductive disposable catheter. The reflectometer measures the incident RF power into the catheter and the reflected power coming back out of the catheter. By utilizing broadband RF stimulus and selective time domain gating controlled using digital signal processing, it will be possible to estimate the location and thickness of bacterial deposits along the length of the catheter to a position accuracy of ±3 mm and thickness accuracy down to 3 μm. The result of this will be linked wirelessly in the hospital or nursing home to central monitoring, indicating when a catheter must be changed or RF/drug therapy used to remove the bacterial growth.

In order to assess bacterial adherence and propensity to form a biofilm presently, very slow testing techniques (such as serial dilutions and viable counts) have to be used taking days to make a clear decision on the efficacy of a treatment regime. Over the next few years it is expected that single or small clusters of bacteria will be studied in advanced types of confocal microscope to assess the activity of bacteria when subjected to different DC and RF stimuli and drug treatments. Machines will be developed that can manipulate single live bacteria using nanomanipulators and apply selective stimuli to decide on species and susceptibility to external treatment. Initially this will require manual manipulation and human decision-making. With advances in microrobotics, adaptive control, nanoprobe sensors, real-time visualization and low cost parallel computing, exciting possibilities will emerge. In the five to ten year view it will be very feasible to create small low cost units that can take a bacterial specimen from a patient and identify the species and population densities of each species. This will be completed in minutes due to the prior profiling of individual bacterial species and the capacity for computing to analyze very large databases quickly. After species identification, the system will recommend the drug, ultrasound or electromagnetic combination to control and eliminate the bacterial infection in a catheter or wound.

As already mentioned basic ultrasound investigations have been used to reduce biofilm growth and adherence [85]. However so much more can be achieved with this technology. The frequencies, power

levels and pulse shapes combined with intelligent polymer materials with imprinted nanosurfaces can be focused on particular bacterial growths. Ultimately, it will be possible to use early research into microactuated surfaces to coat a polymer catheter, for example, with tiny bumps which power scavenge from a general RF field, and can be programmed remotely using an RF link, causing the surface of the catheter to take on many different forms [113]. In this way the catheter can be made to change continuously making it very difficult for bacteria to adapt. Early versions of this technology have already been explored in hypersonic wing surface drag reduction for aerospace [114]. Over five years this technology can be adapted and optimized for *in vivo* operation.

Key Issues

- Bacterial biofilms are responsible for an estimated 65% of all human microbial infections. In healthcare, biofilms present a major hygiene issue on all manner of surfaces often giving rise to illness and disease. Treatment costs are estimated to be in excess of one billion dollars per year in the USA.
- In an industrial setting, biofilms represent an expensive problem being responsible for biofouling, biocorrosion, equipment damage, and product contamination. As a major cause of pipeline corrosion they impact the oil, water treatment, and brewing industries to name but a few.
- Biofilms are widely reported to be resistant to antibiotics in concentrations 3 or 4 orders of magnitude greater than those required to kill planktonic cells of the same species.
- The preventative strategy revolves around stopping initial bacterial attachment and growth and would be preferable to having to deal with the significant cost in industry or patient trauma in a clinical setting caused by established biofilms.

- It is beyond the ability of traditional antibiotics to control biofilm-related infections alone. Additionally, the water treatment, food, drinks, and oil industries are highly reluctant to using biocides. Furthermore, treatment of device-related infection by antibiotics often requires removal of the device, which in itself is responsible for substantial patient morbidity, cost and mortality in some cases.
- An electrical method would facilitate the treatment of device infection without exposing the patient to the trauma associated with device removal. It should also be noted that whilst bacteria adapt remarkably well to antimicrobial therapy over time, it is uncertain if they possess the same ability to adapt to electrical treatment.
- Direct current (DC), alternating current (AC), and radio frequency (RF) electrical treatments as well as acoustic surface waves have all been shown to prevent initial attachment and bacterial growth both *in vitro* and in animal models *in vivo*.
- Electrical fields enhance the efficacy of antimicrobials against biofilms in a synergistic action known as the “bioelectric effect”.
- The bioelectric effect can reduce the concentration of antimicrobials needed to kill biofilms to levels lower than those required to kill free-floating planktonic cells.
- Significant advances in the field involve the discovery of the “electricidal effect”, a long-term reduction in the viability of biofilms using DC electric current without antimicrobials.
- Other promising recent developments come in the form of systems utilizing advanced RF treatment fields to prevent initial bacterial growth, and piezo-electric actuators setting up acoustic waves on IMD surfaces which have been shown to prevent biofilm formation *in vivo*.
- Future efforts should focus on the design and development of low-cost electrified devices, antimicrobial material surfaces and large-scale treatment systems employing the most novel recent advancements in the field to prevent initial bacterial surface attachment and treat established biofilms across a wide range of impacted industries encompassing healthcare, food and drinks, water-treatment, pipelines and energy.

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