Review Article

Electroceutical Management of Bacterial Biofilm and Surgical Infection

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Running Title: Electroceuticals against wound biofilms

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Word count: 5249

Reference numbers: 88

Greyscale illustrations: 3

Color illustrations: 3 (online 3)

Keywords: biofilm, bioelectricity, electroceuticals, wound infection

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ABSTRACT

Significance: In the host-microbe microenvironment, bioelectrical factors influence microbial, host as well as host-microbe interactions. This article discusses relevant mechanistic underpinnings of this novel paradigm. It also addresses how such knowledge may be leveraged to develop novel electroceutical solutions to manage biofilms in the context of surgical infection.

Recent Advances: Systematic review and meta-analysis of several hundred wound studies reported a 78.2% prevalence of biofilms in chronic wounds. Biofilm infection is a major cause of delayed wound healing. In the host-microbe microenvironment, bioelectrical factors influence interactions between microbes and host.

Critical Issues: Rapid biological responses are driven by electrical signals generated by ion currents moving across cell membranes. Bacterial life, growth and function relies on a bioelectrical *milieu* which when perturbed impairs their ability to form biofilm, a major threat to healthcare. Viral stability depends on electrostatic forces. Weak electrical field strength, otherwise safe for humans, can achieve such benefit. In the host, electric field enhanced keratinocyte migration, bolstered immune defenses, improved mitochondrial function and demonstrated multiple other effects consistent with supporting wound healing. Deeper mechanistic understanding of electrical principles will inform the design of next generation electroceuticals.

Future Directions: This is an opportune moment in time as there is a surge of interest in electroceuticals in medicine. Projected to reach $35.5 billion by 2025, electroceuticals are becoming a cynosure in the global market. WHO reports that more than 50% of surgical site infections can be antibiotic resistant. Electroceuticals offer a serious alternative.

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INTRODUCTION

A recent article in *Time* magazine discusses why it is time to take electrified medicine seriously (66). Bioelectrical cues guide subcellular (virus), prokaryotic as well as eukaryotic cellular behavior(1, 48). The influence of electric principles in eukaryotic biology traverses a wide range of physical and physiological behavior in plants and animals. Electrical properties of microbial life has been leveraged to benefit humans in many ways. The use of microbial cells to produce electricity was first achieved in the early twentieth century(70). Microbial fuel cells (MFCs) rely on microbes as catalysts to generate electric power from organic matter(10). In bacterial biology, electrical principles influence fundamental processes (**Fig. 1**) including: **a.** adhesion to surfaces (electrostatic interactions)(75), **b.** cohesive interactions to build communities (matrix-eDNA, eDNA-protein, matrix-protein held together by weak physico-chemical interactions such as electrostatic forces, van der Waals interactions, hydrogen bonds and ionic forces)(46), **c.** intra and inter-species communication (ion channels)(72), and **d.** physical interactions between cells (conductive nanowires)(54). Certain plants utilize electrical activity to induce a long distance defensive signaling akin to synaptic activity in animal neurons(60). Electrostatic forces modulate the structure and function of some viral strains(77). In the animal kingdom, electrical mechanisms drive fundamental activities such as: (i) *biosensing for navigation and detection* (birds, monotremes, aquatic animals), (ii) *foraging for food* (aquatic animals, bumblebees), (iii) self-*defense* (electric eels), (iv) *neuromuscular*, *auditory* and *cardiac functioning*, and (iv) *wound healing* (eye, skin)(8, 79).

ELECTRIC FACTORS IN BIOLOGY

Interaction between electricity and physiology was established in the late 1700s. Luigi Galvani, an Italian scientist, was in an open market where he noted that lightning was able to induce twitching of frog legs on sale. Frog muscle research gave rise to the field of electrophysiology. This discipline has evolved making room for emergent areas. One such area, of central interest to the scope of this article, addresses the role of electrical factors in wound healing(64), cell migration, and management of relevant wound infection(6). In the interest of simplicity of discussion, in this work electrical factors are split into electric

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current, electric field, electrostatic forces and redox electrochemistry. During healthy living, physiological processes and mechanisms are known to be sensitive to each of these components (6, 7).

BACTERIAL BIOLOGY

The study of electrical factors in bacterial biology is deeply rooted in animal neuro-electrophysiology(57). In the 18th century, electrical stimulation experiments performed by Luigi Galvani demonstrated that the living cells utilize the flow of electrochemical species to guide biological function. In this context, ion channels, in particular, have been the subject of many lines of investigation directed to understand how the neuronal network communicates and guides processes from development to every day responses such as movement. The works of Alan Hodgkin and Andrew Huxley provided the first quantitative description of the electrical events underlying the generation of action potentials(34), thereby revolutionizing our understanding of neuronal activity. In that vein, works of Roderick MacKinnon established the fundamental importance of potassium ion channels in all life forms from prokaryotes (bacteria and archaea) to eukaryotes(63). The foundation for such work was established by research performed using simpler microbial models such as *Escherichia coli* (bacteria)(55) and *Saccharomyces cerevisiae* (yeast)(30). Bioelectricity has largely been the concern of electrophysiology, with the central focus being neuromuscular excitation leading to movement/response to external stimuli(11). Putative roles for bioelectricity in development, regeneration and wound healing have been proposed long time ago(81). Although bacteria have been used as 'tools' to dissect integral biochemical and physiological cellular bioelectric responses, particularly as they relate to gated ion channel pathways in neuro-muscular excitatory responses, a deeper understanding of these and other electro-active pathways in the bacterial/microbial lifecycle have only recently started to unfold(72). Specific questions of interest are how and why bacteria utilize bioelectrical principles for their daily functions. The quest for answers to these questions has led to the rapidly growing interest in the field of bacterial electrophysiology(53, 72, 74). In the context of biofilm mode of growth (immobilized clusters/microconsortia of synergistic bacteria encapsulated in polymeric electroactive matrix)(16), these principles seem to be amplified and guide diverse functions including

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but not limited to biofilm formation(72), intra and inter-biofilm communications(53, 72, 74) and survival in harsh environments.

Electrophysiological processes within bacterial biofilms are currently understood to function in three ways: **a.** direct electrical contact/transfer *via* nanowires and/or membrane-bound cytochromes; **b.** passive diffusion of electroactive metabolites such as flavins and phenazines; and **c.** active long-range signaling *via* voltage-gated ion channels.

a. Direct Electrical Contact/Transfer. In nature, microbial biofilms generate energy for growth by cycling carbon and other elements. For example, bacterial species such as *Geobacter* spp and *Shewanella* spp., extract and transfer electrons to insoluble and soluble electron acceptors using electro-active membrane components such as conductive appendages (e.g., pili (or nanowires)) and heme containing c-cytochromes(52). Nanowires serve as electrical conduits to extracellular electron acceptors such as insoluble metal oxides or electrodes. From a practical viewpoint, such electroactive bacterial biofilms act as electrochemical reactors in the treatment of wastes (agricultural, industrial, and human), as materials and devices for bioenergy (MFCs) and for bioremediation (52). A 2013 study was the first to demonstrate the presence of nanowires in bisphosphonate-related osteonecrosis of the jaw (BRONJ), a clinically relevant biofilm-mediated disease. The significance of this exciting observation remains to be further elucidated. Before this observation, electroactive physical structures had been primarily studied in environmental biofilm isolates(54).

b. Passive diffusion of electroactive metabolites. Some bacteria utilize soluble redox-active metabolites or capacitive particles to enable electron transfer between cells at a distance. Some examples of these metabolites include **i.** flavins (produced by *S. oneidensis*)(50), **ii.** phenazines such as pyocyanin (PYO; produced by Pseudomonas sp.) and **iii.** quinolones such as Pseudomonas Quinolone Signal (PQS). PYO is a well-known biofilm quorum sensing (QS) mediator of Pseudomonas sp. that could also enable electrical responses in biofilms. PYO enhances electric current production by mixed microbial biofilm in MFCs(73). From a clinical perspective, the redox-

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active PYO promotes virulence by impairing eukaryotic electron transport, host cellular respiration, energy metabolism and other critical cellular functions(31).

c. <u>Active long-range signaling</u>. In 2015, Prindle et al. described an ion channel-mediated electrical signaling based cell-to-cell communication process(72), that serves as a resource sharing mechanism between neighboring biofilm communities to enable survival during reduced nutrient supply. Using the *Bacillus subtilis* model system, it was demonstrated that potassium (K^+) ion channels conduct long-range electrical communications within biofilm communities that is dependent on a quorum/threshold of biofilm mass for measurable electrical oscillations. These waves form a positive feedback loop creating a wave of depolarization that coordinates metabolic states throughout the biofilm community. Interestingly, interspecies communication was noted between *Bacillus* spp and *Pseudomonas* spp, dependent on the release of K^+, as well as the membrane potential of the motile cell(36). Since Bacillus sp. are not known to have a Na^+ ion channel system, this ionic species did not have an effect on biofilm growth dynamics. However, that does not preclude the possibility of Na^+, Ca^{2+}, Cl^- and ammonium ions enabling electrical connectivity within and between bacterial species.

Bacterial electrical biomembrane – voltage gated ion channels

Electrical signaling through cellular membranes enables rapid response. In this form of communication, inducible gene expression, biochemical synthesis, specific receptors or complex signaling pathway activation are not required(72). In *Bacillus* species, the cellular machinery driving electrical communication is a voltage-gated ion channel (VGIC) specifically responsive to K^+. VGICs are multi-subunit protein complexes that undergo conformational changes in response to changes in membrane potential. Sodium (Na_v), potassium (K_v), calcium (Ca_v) and chloride (Cl^-) specific VGICs are present in microbes. Na^+, K^+ and Ca^{2+} channels have fundamental similarities in structure and function.

<u>The chemical basis of electrical signaling</u>: Rapid biological responses are typically driven by ion generated electrical currents moving across cell membranes, initiated and propagated by VGICs. VGICs contain a tetramer of transmembrane subunits or domains (S1–S6) made

up of a voltage sensor and a pore module. The S4 segment has a symmetrical arrangement of charged residues, including arginine or lysine, making this domain function as the voltage sensor of the channel(3). Upon membrane depolarization, a sliding helix mechanism drives outward movement of the voltage sensor, causing voltage-dependent activation and opening of the intracellular gate. The selectivity filter conducts hydrated ions rapidly and selectively guided by a unique negatively charged site. The collapse of an asymmetric pore caused voltage-dependent inactivation terminates ion conductance(12).

Measuring electrical activity in biofilms

Patch-clamping. The classical electrophysiological clamping setup employing glass microelectrodes is not applicable to microbes because of the size of these organisms. The patch-clamp recording method developed by Neher and Sakmann *et al*(32) overcame this shortcoming. "Giant spheroplasts" (large cytoplasmic bags devoid of cell wall) of *E. coli* were used for patching the inner membrane where the ion channels are found. Initial studies using this methodology identified mechanosensitive (MS) channels(56).

Array based measurements. Multi-electrode array (MEA) systems, previously used for studying neuronal electrical network, have been applied to study whole bacteria in biofilm communities (*Bacillus licheniformis*, *P.alcaliphila*) and planktonic growth (*E.coli* HEC30)(58). Electrical activity in the form of action potentials corresponded to maximum biofilm growth. Planktonic bacteria showed electrical activity but with significantly lower amplitude strength compared to biofilm. As bacterial cells increase in the developing biofilm, the interaction between the individual cells create a network similar to neuronal networks. It is possible that the cohesiveness within the biofilm promotes a stronger electrical activity which could play an important role in the emergence of collective behaviors such as sensing and communication with other cells for survival in a harsh environmental *milieu*.

Use of electro-responsive dyes. Radioactively labeled tetraphenylphosphonium ion (TPP$^+$) or fluorescent dyes such as Thioflavin T (ThT) can be used to measure membrane potential changes. Membrane potential-dependent protein localization also serves as a measurement for membrane depolarization(80).

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Conducting polymer-based electrochemical biosensors. Conducting polymers (CPs) are a unique category of organic polymers that exhibit electrical conductivity and redox activity(51). Some of the most commonly applied CPs, poly(3,4-ethylene- dioxythiophene) (PEDOT), poly(aniline) (PANI) and poly(pyrrole) (PPy) have low toxicity, excellent long term environmental stability in aqueous and *in vivo* environment. CPs can be doped with an appropriate antibody, oligonucleotide, enzyme, bulky dopant molecules (such as dodecyl benzene sulfonate (DBS), dodecyl sulfonate (DS), bis(trifluoromethane)sulfonimide (TFSI) or auto-doped with small mobile ions to serve as recognition elements(24). The resulting electron transfer from the dopant to the polymer serves as the transduction pathway for detection *via* potentiometric or amperometric methods . Among CPs, PANI and PPy are widely used as analytical cation, gas sensors and biosensors to varying degrees of success (**Fig.2**). PPy doped with bulky-anionic dopant such as dodecyl benzene sulfonate (DBS; (PPy(DBS)) enables the precise detection of the concentration of monovalent or divalent cations in solution and therefore is used as an electrophysiology sensor(84). The PPy(DBS) electrophysiology sensors can be directly applied to monitor biofilm ionic activity by culturing the cells directly between the electrodes in the sensor. This allows the biofilm to become a part of the *'control volume'* and ionic activity can be directly measured using methods outlined for CP electrophysiology sensors(24).

Purpose of electroactive pathways

a. Biofilm establishment and growth. Using *B.subtilis* as the model organism, independent groups have identified a role for potassium in regulating biofilm formation. Altering expression levels of surfactin, kinase and K^+ transport regulator, all of which impact K^+ intracellular levels, result in modifications in biofilm formation(49). Potassium uptake and efflux systems have also been implicated in *P.aeruginosa* biofilm formation and production of QS regulated virulence factors such as pyocyanin.

b. Bacterial adhesion and cohesion: *Electrostatic forces* enable adhesion of bacterial cells (**Fig. 3**). Studies with titanium implant surfaces in relation to oral bacteria have shown that modification of titanium implant surface significantly alters the early

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adherence of bacteria on the surface and thus biofilm formation, which eventually affect health outcomes(4). Ionic strength and pH of the suspending solution together with the potentials of bacteria and the surface drive bacterial adhesion. The resultant electric interactions play an important role in bacterial adhesion(69). The extracellular matrix, composed of extracellular DNA, polysaccharides and proteins, is essential for biofilm formation(23). Electrostatic attractive and repulsive interactions, ionic attractive forces, hydrogen bonds and Van der Waal's interactions are among the weak physico-chemical interactions that may maintain the multicellular structures that allow bacteria to co-operate metabolically and to be recalcitrant to antibiotics or immune cells(23).

c. <u>Communication</u>: Bacteria use a cell density dependent collective behavior to release chemical signals that drive survival(9). In 2017(47), it was demonstrated that artificial cells can sense and send quorum sensing molecules. Electrical signaling is recognized as an efficient cell-to-cell communication process. Ion channel based electrical signaling attracts distant motile cells based on the membrane potential and the cell's modulation of tumbling frequency. Such long range electrical signaling serves as an advanced communication mechanism, which is completely generic. Interestingly, cross species communication is thereby enabled. A question that arises here is what the long term consequences and/or benefits are of interspecies attraction and communication via electrical signaling. It also remains to be understood if the QS and electrical systems may impact each other and how that impact may be affected.

d. <u>Resource Sharing</u>: Biological systems frequently deal with resource limitations. Time-sharing is a strategy where users take turns consuming resources. In such cases, different systems may compete with each other. Glutamate starvation in *B. subtilis* biofilm communities cause collective growth-rate oscillations. A negative feedback loop guided by biomass increase leading to glutamate stress drive these oscillations. This stress, in turn, influences biofilm growth. Ion channel mediated electrical signaling coordinate this phenomenon(72). The metabolic oscillations in biofilm communities are synchronized in their growth dynamics by electrical

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signals. This further increases competition by synchronizing demand for limited nutrients.

e. <u>Flagellar Motility:</u> Transient changes in membrane potential cause motility changes. Comparable effect was demonstrated in a recent study demonstrating that K^+ signaling from the biofilm and the membrane potential of the planktonic/motile bacteria are both determinants of flagellar motility. This motility is more directional when the motile organism was further away from the biofilm (K^+ signal).

f. <u>Defense mechanisms</u>. Biofilm extracellular polymeric substance (EPS) restrict penetration of antimicrobials causing antimicrobial tolerance. EPS may also serve as a diffusion barrier to antibiotics. Extracellular DNA (eDNA) component of EPS display cation chelating properties thus inducing resistance to host-derived or therapeutic antimicrobials. Positively charged antibiotics such as tobramycin are sequestered in the biofilm periphery *via* ionic interactions with negatively charged matrix components. Tobramycin penetration into the biofilm was enhanced by the addition of cations.

g. <u>Virulence mechanisms</u>: Redox-active PYO is toxic to eukaryotic hosts[45] and other microbes. PYO induces the production of reactive oxygen species, such as superoxide anion radical, augmenting virulence[27]. PYO induces oxidative stress in cellular systems which manifests as premature cellular senescence. PYO may influence the intracellular redox state by decreasing carbon flux through central metabolic pathways[71].

EUKARYOTIC BIOLOGY

Bioelectric properties in development. Electrical fields both have been detected both extracellularly as well as intracellularly[59]. Endogenous electric fields exist within extracellular spaces and influence cell behavior in development and wound healing. Studies of amphibian (toad, axolotl) and avian (chicken) embryos demonstrate that endogenous electric fields (normal polarity and magnitude) are necessary for development of neural and other tissues. Scrambling of physiological electrical cues result in gross

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developmental abnormalities caused by interference with patterning and cell migration in the embryo(35).

Bioelectric properties of human organ systems

Neuromuscular system. Nerve fibers act as communication cables connecting and transmitting electrical impulses that guide the body's response to multiple stimuli. It is estimated that each neuron produces ~70 millivolts (mV) of electric potential, while muscle cells produce about 95mV. This potential, in the form of ATP, powers electrogenic pumps that are translated to active outputs.

Cardiac system. The sinoatrial node (SA) located in the right atrium, controls the rhythm of our heartbeat and the pumping of blood to the rest of the body. Utilizing electrical signals to set the pace, it is the body's natural pacemaker(13).

Skin. In 1849, Emil Du-Bois-Reymond first observed that the human skin was electrically active(22). This was further corroborated by Hermann and Luchsinger who demonstrated a connection between cutaneous electrical activity and sweat glands. Electrical impedance is lowest in the palms with abundant sweat ducts. A 1 Hz to 1 MHz range of electrical strength is estimated from the skin surface into the underlying dermis and subcutaneous tissue. The ability of the human skin to self-repair allows it to function as a protective barrier. The intact mammalian skin has positive transepithelial potentials (**TEP**) between 10 and 60 mV. During epithelial wound healing there is induction of electric current of magnitude of approximately 10-100 mA/cm^2 caused by trans-epidermal voltage gradient created by the epithelial sodium ion pumps. With gradual decrease in electric field strength, there is progressive coverage of wound area with epithelial cells. The presence of endogenous electric field may have critical roles in cutaneous wound healing(37). Cellular outcomes such as cell migration, cell division, leukocyte infiltration, nerve sprouting, endothelial cell remodeling and associated angiogenesis, within 500 μm -1mm of wound edge, are known to be influenced by electrical voltage gradient(7, 64).

Electric principles in cell migration. Exposure of cells to physiological electric fields affects cell orientation, migration, protein synthesis and distribution, and activation of signaling pathways such as CDC42p, Rho/Rac,PI3K/PTEN, phosphatidylinositol (PIP) (86, 88) as well

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as epithelial sodium channels(85). A local direct electric field (dc electric field) of low magnitude (10-400 mV/mm) guides the motility of living cells through a process called galvanotaxis(61). Several lines of evidence support that changes in electrical parameters may influence the function of host cells such as keratinocytes, fibroblasts, neutrophils, macrophages, lymphocytes, endothelial cells, all of which are relevant to wound healing(7). Under the influence of an electric field, changes in cell membrane plasticity, cytoskeletal rearrangements, and alterations in the interaction of the cell with its microenvironment, enable the cell to move forward. Such movement is further facilitated by electric field-induced intracellular signaling events involving several growth factors *e.g.,* epidermal growth factor, vascular endothelial growth factor,(87), hepatocyte growth factor; and protein kinases such as protein kinase C, cGMP-dependent protein kinase and mitogen-activated protein kinase (MAPK)(65). These signaling events directly regulate cell polarization and migration(7, 88).

__Immune cell function and inflammation.__ Immune cells play a major role in host defense and infection management. Electric fields stimulate immune cells function. For example, membrane-mediated Ca^{2+} signaling processes are responsive to electric fields. Neutrophils represent the first cells that arrive at the site of injury to defend the body against microbial pathogens. Application of an external electric field activates respiratory burst of neutrophils, neutrophil extension, metabolic resonance and DNA damage(42). Monocytes represent the next blood-borne immune cells that extravasate to form macrophages and migrate to the site of injury to bolster host defenses(25). The phagocytic activity of macrophages in dead cell clearance can be enhanced by external electric fields(33). Such intervention causes changes in cellular signaling *e.g.,* PI3K and ERK activation. The pattern of cytokine release thus changes as does intercellular Ca^{2+} response and actin polarization (**Fig.4**). Bioelectric modulation of ATP sensitive potassium channels influences macrophage polarization and is likely to modify macrophage plasticity.

__Wound healing.__ Endogenous electric fields (~100-200mV/mm in skin and cornea) provide directional cues to guide the tissue repair response. Electric fields guide cell migration in diverse cell types involved in the healing response including keratinocytes, macrophages, neutrophils and fibroblasts. Furthermore, supportive actions for the healing process

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including, the generation of ATP, increased secretion of collagen by fibroblasts for ECM restoration and increased blood flow and capillary density are also responsive to electric fields. Membrane receptors such as EGFR, VEGFR and integrins, integral to the wound healing process, are redistributed and activated in response to endogenous electric fields(87). Activation of any of these receptors by electric fields, trigger downstream signaling cascades directly relevant to wound healing(7, 87, 88). Recent studies testing mechanisms underlying the action of an electroceutical wound care dressing demonstrated responsiveness of key signaling pathways accelerating keratinocyte migration(7), a key cellular component in wound re-epithelialization. Electric field enhanced keratinocyte migration by three mechanisms: (i) hydrogen peroxide generation (a potent driver of redox signaling), (ii) phosphorylation of redox-sensitive insulin growth factor receptor (IGF1R), and (iii) reduction of protein thiols and increase in integrin αv expression. Electric fields also increased keratinocyte mitochondrial membrane potential supporting an energy demanding migration process. In this context therefore, exogenously applied electric fields could mimic the effect of endogenous electric field, possibly stimulating and guiding all the above cellular behaviors to enhance wound healing.

Electroceutical Wound Care Therapies. In biofilm infected cutaneous wounds, wound healing is compromised. Although the affected wound may close, barrier function of the repaired skin is deficient as measured by elevated transepidermal water loss (8, 26, 76). Treatment of wounds with electric field-based antimicrobial dressing corrected such deficiency and restored functional wound healing. Specific biofilm-repressed molecular pathways, including the adherens junction protein E-cadherin essential for *in vivo* epidermal barrier function, were rescued by such dressing. Furthermore, electric field-based wound care dressing managed biofilm-induced persistent inflammation(8). Clinical trial testing this FDA cleared dressing in a setting of burn wounds is currently in progress (NCT04079998).

Several other forms of electroceutical intervention have been tested in wound care **(Table 1)**(2, 5-7, 14, 15, 18, 20, 28, 39, 41, 43, 62, 68, 78, 82, 83)**.** Unlike electric field-based dressings discussed above, the notion of electric stimulation devices in wound care relies on direct application of electric current to stimulate the wound tissue. Most of such

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devices that rely on the application of electric current have underperformed in wound care. Such sub-optimal performance can be attributed to the lack of consideration of the complex mechanistic implications of electrical factors as addressed in this work. In wound care, tested electric stimulation devices employ a range of variables including high voltages, current, mode and length of time of application. These devices employ wired electrodes for direct application of much higher current to the wound tissue compared to the dressing discussed above. A low- or high frequency pulsed electrical current that stimulates the peripheral nerves, called TENS (transcutaneous electrical nerve stimulation), has been tested for pain control(44). The frequency rhythmic electrical modulation systems (FREMS) varies the pulse, frequency, duration, and voltage during application. The Fenzian system, an electronic biofeedback system utilizing degenerate waves (DW), has been used in the treatment of acute wound healing and treating scar problems in the skin. Pulsed current (PC) is a common mode used in electrotherapeutic trials. Short voltage PC (SVPC) devices such as Aptiva Ballet (Lorenz Therapy System) or Naturepulse (Globe Microsystems) report increase in circulating VEGF and NO in response to stimulation. Limited studies claim improved wound closure in the treatment of chronic venous and diabetic ulcers. The SIS electrotherapy device is an iontophoretic system utilizing low-intensity direct current to deliver silver ions to target sites within the body to fight infections and promote wound healing. This device claims applications for treatment of antimicrobial resistant bacterial infections as well as fungal and yeast infections.

Wireless Electroceutical Dressing (WED) is a FDA cleared *wireless* dressing with a matrix of embedded elemental silver and elemental zinc. When in direct contact with a conductive medium, redox chemical reactions drive the transfer of electrons from zinc to silver(6-8), generating an electric field at the dressing surface, which promote keratinocyte migration(7) and biofilm disruption(6, 8). When tested in a pre-clinical porcine experimental model of long-term wound biofilm infection involving an intact host immune defense system, WED was effective in preventing biofilm formation and disrupting established biofilm infection and associated pathological complications(8). Furthermore, WED effectively managed biofilm-induced persistent inflammation and promoted restoration of skin barrier function following injury(8). WED may be viewed as a first

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generation wound care dressing representing a translationally viable option to disrupt wound biofilm infection *in vivo*.

Therapies marketed as "anti-biofilm" may not necessarily by useful in fighting wound infections especially if they have been tested primarily in *in vitro* or short-term *in vivo* models(26). Such approaches are powerful in understanding microbiological processes but limited in addressing biofilm mechanisms in the context of host infection. Although the Wound Healing Society recommends the porcine model as the most relevant preclinical model of cutaneous wound healing(29), short-term infection studies even in these models disallow prolonged interaction between polymicrobial biofilm forming pathogens and host. Short-term models therefore have limited power to understand long-term clinically relevant host-biofilm interactions, inclusive of host immune system responses, that shape an acute-phase infection to a pathogenic chronic biofilm (**Fig.5**). Translational relevance of anti-biofilm therapies will be better tested in the context of live, long term immune-competent models that capture the persistent nature of biofilm infected chronic wounds(26).

Electroceuticals against Antimicrobial Resistance. Bacterial genetic mutations alter functional pathways that are targeted by traditional antibiotic therapies resulting in the evolution of: **a.** masked or decoy drug targets, **b.** drug inactivating enzymes, and **c**. drug pumping mechanisms. Because much of the effort in clinical management of infections is still dependent on pharmaceutical options, each of these pathways may be viewed as a drug-inducible loop that when treated with other drug-based strategies, results in a futile cycle, forcing the evolution and persistence of even more resilient strains with multi-drug resistance properties (**Fig.6**). Within the protected biofilm cocoons, gene exchange favors rapid transfer of such drug resistance traits. This poses a critical challenge in combating infection and warrants the development of productive non-pharmacological or combinatorial strategies to fight biofilm infection. Because electriceutical therapy is not subject to the metabolic pathways of drug resistance, it has the potential to circumvent drug resistance.

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In 1992, it was reported that weak (1.5 V/cm and 15 microA/cm2) electric fields(17, 19) could significantly enhance killing of biofilm bacteria by antibiotics. This "bio-electric effect" suggested a possible application of electrical therapeutics for antibiotic resistant bacterial strains. WED when tested *in vitro* in the context of a multi-drug resistant (MDR) strain of *P.aeruginosa*, attenuated the expression of *MexR* and *MexT* multi-drug efflux pump regulators(6). Follow up studies using a porcine wound model infected with a mixture of MDR *P.aeruginosa* and *Acinetobacter baumannii* strains, showed that WED disrupted biofilm infection by these strains(8). Other groups have tested WED against several antibiotic resistant strains in vitro and found that WED was inhibitory to almost all the strains tested(38, 40).

Electroceuticals against Viral Diseases. Electroceuticals could be a new antiviral strategy. Electrostatic forces are critical for the structure and function viral particles and could be exploited to destabilize viruses. WED was recently shown to disrupt the infectivity of some viruses i*n vitro* (e.g., Coronavirus (CoV) and Lentivirus (LV))(77). Zeta potential (electrostatic interactions in particle dispersions) determines viral particle stability(77). WED therefore was found to rapidly lower the zeta potential, possibly causing defects in viral particle stability and therefore lowering infectivity. This compelling observation warrants provides an exciting opportunity for further explorations on the use of electroceuticals as antiviral strategies.

Electroceuticals to Manage Healthcare-Acquired Infections (HAI). HAIs, also called nosocomial infections, are a pressing public health threat, estimated by the Centers for Disease Control (CDC) to affect 1 in 25 hospitalized patients on any given day. In addition to the morbidity and mortality rates associated with HAIs, there is also a heavy economic burden estimated at $28-$33 billion in excess costs. These HAIs include central line-associated bloodstream infection (CLABSI), catheter-associated urinary tract infections (CAUTI), surgical site infections (SSI) and ventilator-associated pneumonia (VAP). Several routes of transmission of the infectious agent contribute to the persistence of this problem in hospital settings including contact with contaminated surfaces such as hospital textiles (including bedding, drapery (curtains and/or privacy screens)) and scrubs among others(21). The increasing evidence of biofilms in catheters and central lines has

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necessitated the development of more sophisticated methods of sterilization and modification of medical devices in order to make it inhabitable for biofilm forming organisms(67). Several lines of research have focused on coating surfaces of catheters and central lines with various polymers, silver ions and other nanoparticles and even treatment with photodynamic therapy. However, despite these advances, the HAI problem still persists indicating a need for more effective measures in eradicating the infectious agent. In this context, electroceutical-based surface modifications may be viewed as a viable next-generation solution. For instance, coating the inner lining of catheters or central lines with electrically conductive material that generate mild electric fields could interfere with adhesion and survival of microbial pathogens. Similarly patterning hospital privacy curtains or linens with such conductive materials could make these surfaces incompatible for establishment of biofilms and thereby drastically decrease the incidence of HAIs.

CONCLUSION

Bioelectricity has largely been the concern of mammalian electrophysiology, with the central focus being neuromuscular excitation. Bacterial electrophysiology is an emergent discipline. We now know that bacterial life, growth and function relies on an intrinsic bioelectrical *milieu*, the perturbation of which could inhibit or kill these organisms. Weak electrical fields, otherwise safe for humans, can achieve such benefit, exemplifying the Arndt-Schulz rule (*for every substance, small doses stimulate, moderate doses inhibit, large doses kill)*. At the same strength that kills microbes, beneficial effects of such electroceutical has been observed in improving human keratinocyte migration – a contributor to wound closure. Electric field may stimulate immune cell function as well. Deeper mechanistic understanding of how electroceuticals may influence microbes, host and host-microbe interaction is likely to help inform the design of next generation electroceuticals aimed at prevention and management of infection. This is an opportune moment in time as there is a surge of interest in electroceuticals in medicine(66). The electroceutical market, projected to reach $35.5 billion by 2025, is rapidly becoming a cynosure in the global market. Electroceuticals broadly encompass all bioelectronic medicine that employs electrical stimulation to affect and modify functions of the body. Brain stimulation therapies, electrical muscle stimulation, cardiac stimulation therapies,

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cochlear stimulation implants and tumor treating fields in cancer are currently in medical practice. WHO reports that more than 50% of surgical site infections can be antibiotic resistant. Electroceuticals emerge as a serious alternative. Investment into advancing electroceutical management of surgical infection warrants serious consideration.

ACKNOWLEDGEMENTS

AUTHOR DISCLOSURE STATEMENT

CKS discloses financial competing interest as owner of shares of Vomaris Innovation, Inc.

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Abbreviations	
ATP	Adenosine triphosphate
BRONJ	Bisphosphonate-related osteonecrosis
CAUTI	Catheter associated urinary tract infection
CLABSI	Central line associated blood stream infection
CP	Conducting polymer
DBS	Dodecyl benzene sulfonate
DNA	Deoxyribonucleic acid
DS	Dodecyl sulfonate
DW	Degenerate waves
eDNA	Extracellular DNA
EPS	Extrapolymeric substance
FDA	Food and Drug Administration
FREMS	Frequency Rhythmic Electrical Modulation System
HAI	Hospital associated infection
MAPK	Mitogen activated protein kinase
MDR	Multi drug resistance
MEA	Multi-electrode array
MFC	Microbial fuel cell
mV	Millivolt
NO	Nitric oxide
PANI	Polyaniline
PC	Pulsed current
PEDOT	Poly(3,4)-ethylene-dioxythiopene
PIP	Phosphatidyl inositol phosphate
PPy	Polypyrrole
PQS	Pseudomonas quinolone signal
PYO	Pyocyanin
QS	Quorum sensing

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SA	Sinoatrial node
SSI	Surgical site infection
SVPC	Short voltage pulsed current
TENS	Transcutaneous electrical nerve stimulation
TEP	Transepithelial potential
TFSI	Bis(trifluoromethane)sulfonimide
ThT	Thioflavin T
TPP	Tetraphenyl phosphonium ion
VAP	Ventilator associated pneumonia
VEGF	Vascular endothelial growth factor
VGIC	Voltage gated ion channels
WED	Wireless electroceutical dressing
WHO	World Health Organization

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Table 1: Electrical treatment modalities available for wound management

Type of Electrical Stimulation	Uses for Wound Care	Limitations
Direct current (DC)	Mimics endogenous electric field[68]; stimulates migration of fibroblasts and keratinocytes[69]	High currents for extended time can cause electrochemical injury to skin; high amplitudes alkaline (NaOH) and acid (HCl) products may form at cathode/anode[68]
Monophasic pulsed current (MPC)	Mimic endogenous electric field[68]; No adverse effects on skin due to short bursts[67]	Charge must fall in a certain range to be an effective treatment[68]
Biphasic pulsed current (BPC)	Asymmetric waveform with unbalanced charge associated with positive wound healing outcomes in clinic[70]	Biphasic symmetrical (charge balanced) has no reported positive impact on clinical wound healing[68]
High voltage pulsed current (HVPC)	Bacteriostatic against *S. aureus, E. coli, and P. aeruginosa*.[71]; leads to decreased wound surface area[66]; noninvasive, painless	Fails to accelerate healing after venous surgery; only generated better results in conservative treatment of patients[65]
Pulsed electromagnetic field (PEMF)	Decreases doubling time of fibroblasts and endothelial cells[72]; Increases mitogen-activated protein (MAP) kinase activation leading to higher	Improvements in wound closure rate and re-epithelialization were not seen at later stages of healing process[74]

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levels of cell proliferation[73]

Transcutaneous electrical nerve strimulation (TENS)	Increases capillary density[75]; increases perfusion to wound site[76]; increased venous flow rate[77,78]	Disagreements in literature regarding the positive of effects of TENS on increasing blood flow and increase in skin temperature[69]
Wireless electroceutical dressing (WED)	FDA cleared disposable dressing; Bactericidal on many multidrug resistant bacteria[84]; Disrupts biofilm integrity[14]; decreases population of bacterial cells at site[14]; accelerates keratinocyte migration and wound closure[15]	May be bacteriostatic on drug resistant Enterococcus strains.

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Figure Legends
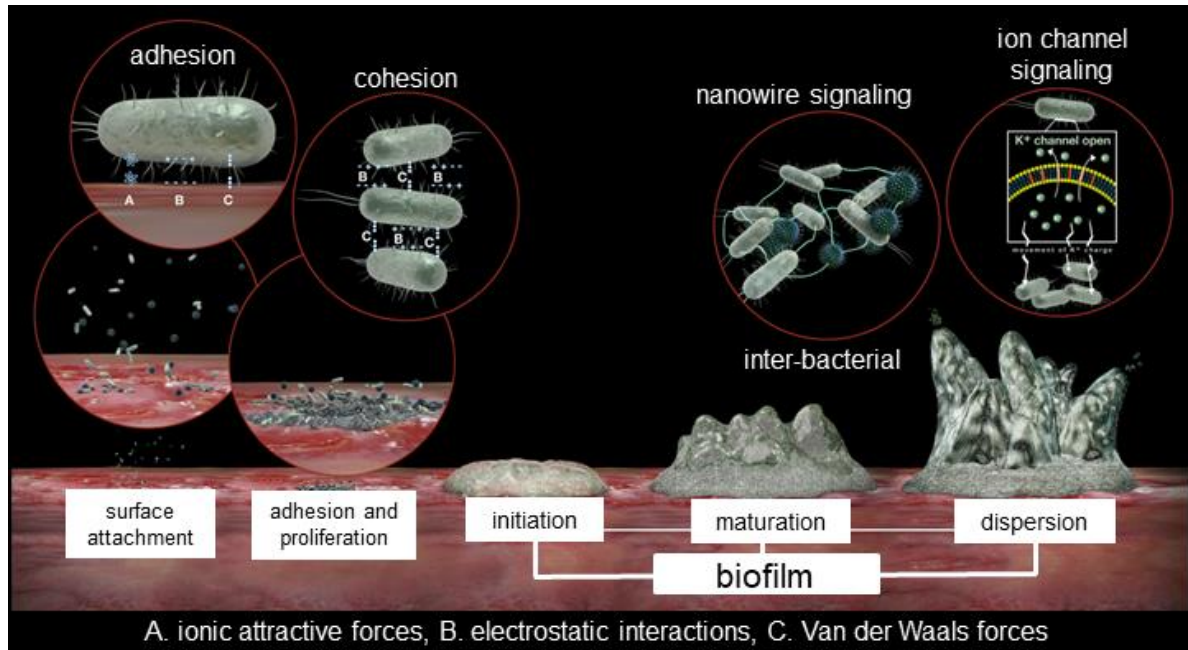
Figure 1. Electrical principles in bacterial biology. Electrical principles influence fundamental processes in bacterial biology including **a.** adhesion to surfaces (electrostatic interactions), **b.** cohesive interactions to build communities (matrix-eDNA, eDNA-protein, matrix-protein held together by weak physico-chemical interactions such as electrostatic attractive forces, repulsive forces, hydrogen bonds, van der Waals interactions and ionic attractive forces), **c.** intra and inter-species communication (ion channels), and **d.** physical interactions between cells (conductive nanowires). "**To see this illustration in color, the reader is referred to the online version of this article at www.liebertpub.com/ars"**

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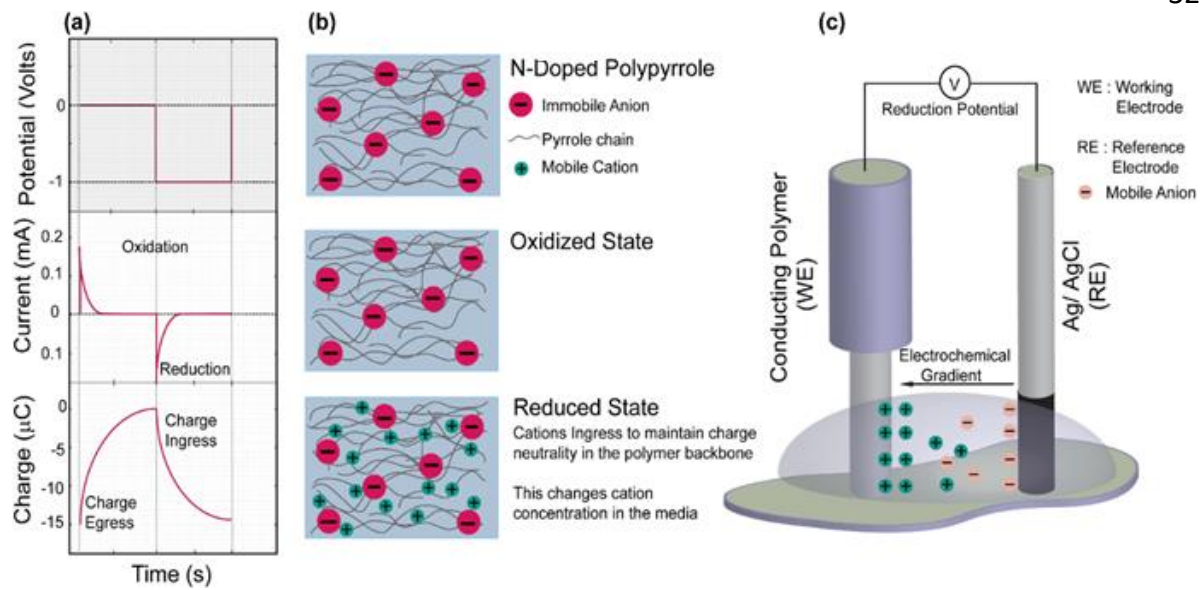
**Figure 2. Schematic of conducting polymer based electrophysiological sensor.** The sensor is constructed from PPy(DBS) electro-polymerized on a platinum (Pt) wire as the working electrode (WE), Ag/ACl as reference electrode (RE) and bare Pt-wire as counter electrode (CE) to form a three-electrode electrochemical cell in the vicinity of the tissue of interest. The sensors can be directly applied to monitor biofilm ionic activity by culturing the cells directly between the WE and RE+CE. "**To see this illustration in color, the reader is referred to the online version of this article at www.liebertpub.com/ars"**
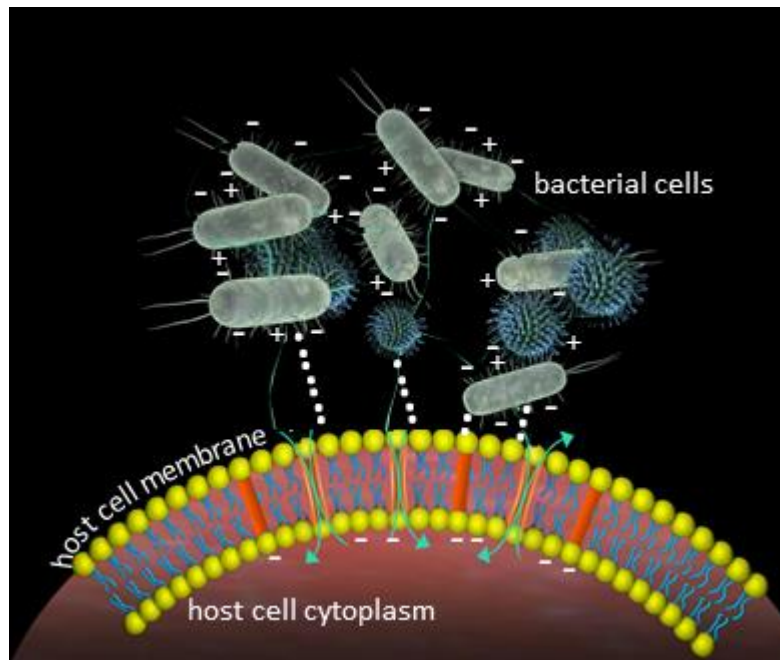
Figure 3. **Electrical principles in bacterial adhesion.** Electrostatic forces facilitate the adhesion of bacterial cells to surfaces. "**To see this illustration in color, the reader is referred to the online version of this article at www.liebertpub.com/ars"**
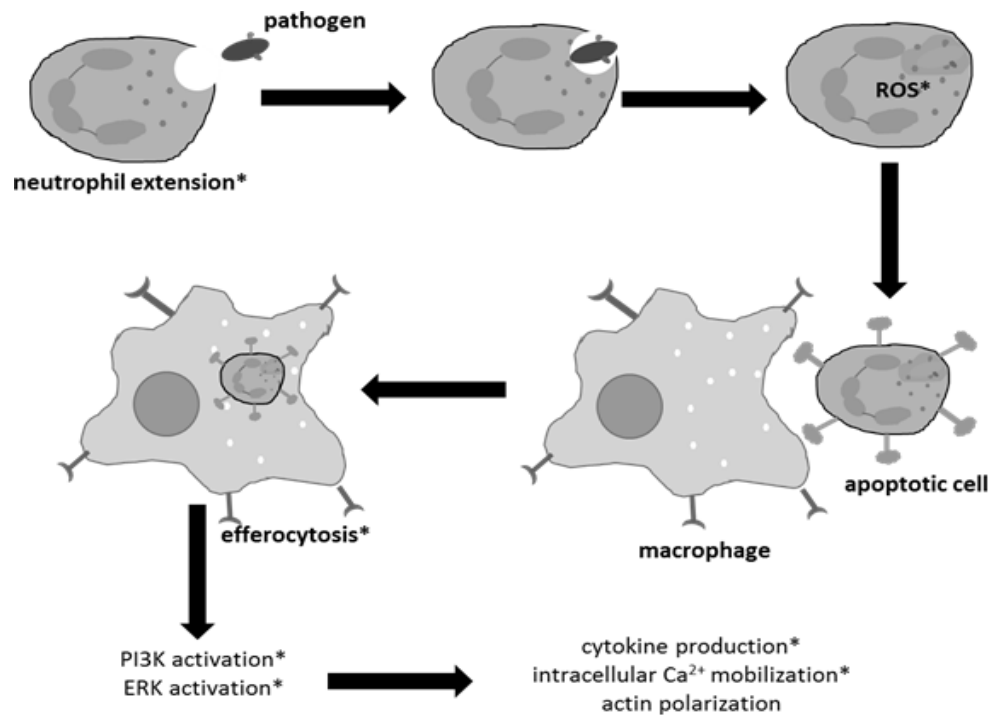
Figure 4. Effect of electric field on immune cell function. Neutrophils and macrophages play a major role in orchestrating inflammatory response. Electric fields facilitate neutrophil extension and ROS production required for killing of pathogens. Once done their cleansing task, neutrophils undergo a process of programmed cell death and are subsequently cleared by macrophages by a process called efferocytosis which is increased by electric fields and results in cellular signaling changes (PI3K and ERK activation) leading to increased cytokine production, mobilization of intracellular calcium, and actin polarization. Processes positively affected by electric field are marked with an asterisk (*).
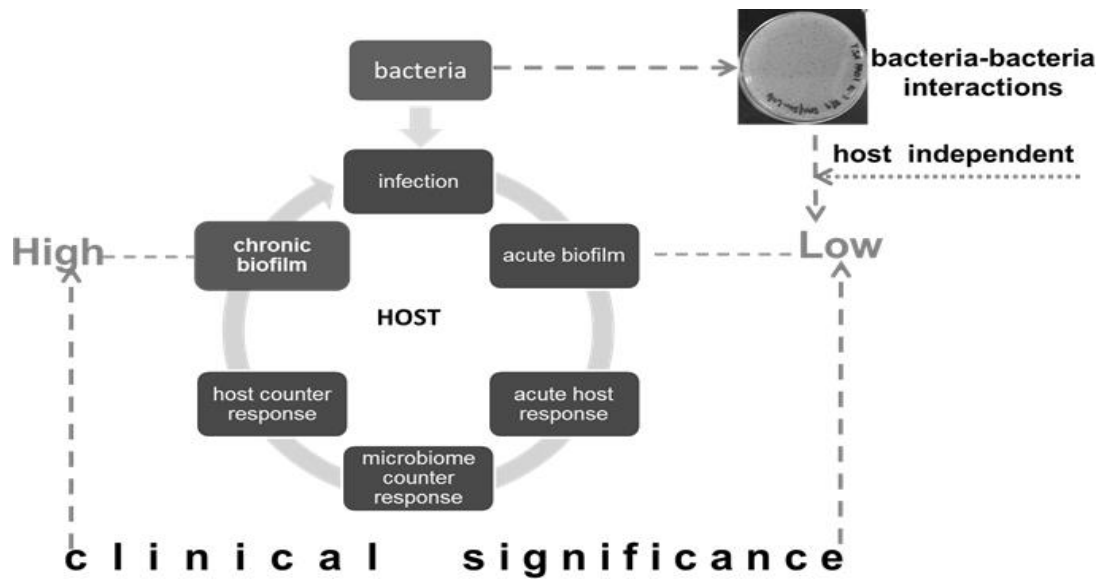
Figure 5. Biofilm and wound chronicity. Anti-biofilm therapies tested using *in vitro* biofilm models (lacking the host interaction component) and short-term *in vivo* models (lacking iterative-host interplay toward mature biofilm relevant to chronic wounds) have low translational significance. Long-term models that capture the longitudinal cascade of events culminating in a pathogenic wound biofilm are better suited to gauging the effectiveness of anti-biofilm therapies.
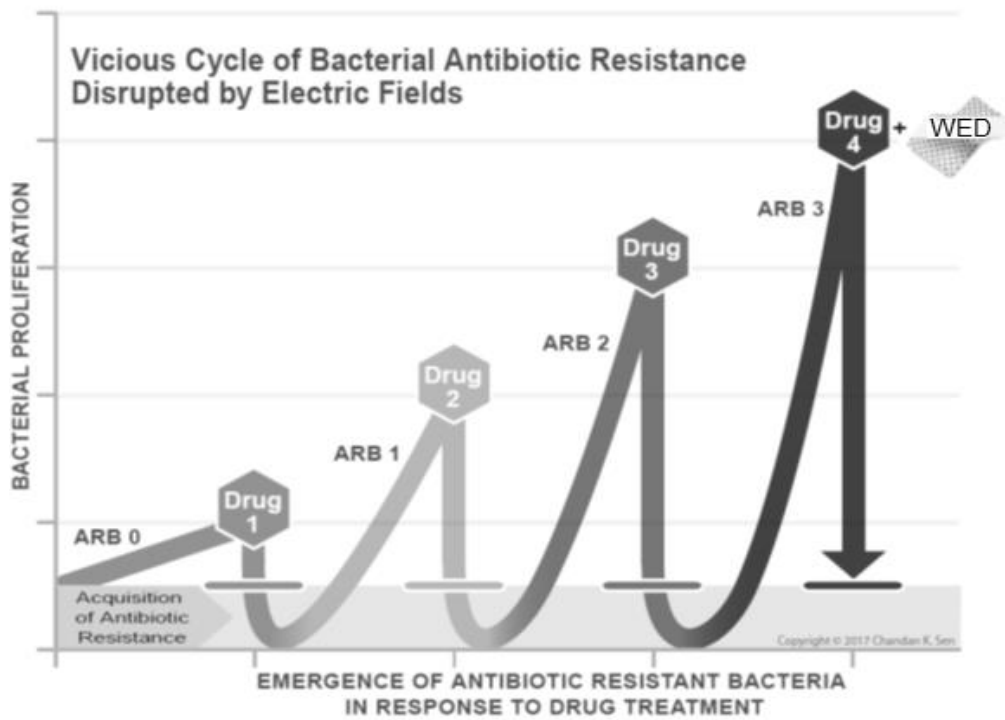
Figure 6. Combination therapy. In the current paradigm, every time a drug is developed to fight antibiotic resistance, a new Antibiotic Resistant Bacteria (ARB) colony emerges resulting in a futile cycle. A combination of pharmacological and electroceutical intervention may result in maximum growth inhibition.