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Cutaneous wound biofilm and the potential for electrical stimulation in management of the microbiome

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Cutaneous wound biofilm and the potential for electrical stimulation in management of the microbiome

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Abstract

Infection contributes significantly to delayed cutaneous wound healing which impacts patient care. External application of electrical stimulation (ES) has beneficial effects on wound repair and regeneration. The majority of studies to date have explored ES in relation to planktonic microorganisms, yet evidence indicates that bacteria in chronic wounds reside as antibiotic resistant polymicrobial biofilms, which contribute to impairing wound healing. Culture-independent sequencing techniques have revolutionised our understanding of the skin microbiome and allowed a more accurate determination of microbial taxa and their relative abundance in wounds allowing a greater understanding of the host-microbial interface. Future studies combining the fields of ES, biofilm and microbiome research are necessary to fully elucidate the use of ES in the management of wound infection.

Keywords: biofilm; electrical stimulation; microbiome; wound healing; cutaneous wounds

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Introduction

Chronic cutaneous wounds have a major impact on patients' quality of life and mobility and present a huge burden on the healthcare system. Persistent wound infection is a major contributor to delayed wound healing [1,2]. Chronic wounds can be ~~are predominantly~~ poorly managed with the prolonged use of antibiotics, leading to growing antibiotic resistance [3,4]. There is an unmet need for better non-pharmacological anti-microbial technologies in the management of chronic wound infections.

Skin is electrically charged, termed the "skin battery" [5]. Cutaneous wounds generate large and persistent endogenous electric currents and fields named the "current of injury" [5,6]. The current of injury is involved in numerous wound healing processes [7,8]. These observations support the concept ~~have led to the hypothesis~~ that applied electrical stimulation (ES) could promote wound healing by imitating the natural electrical current that occurs in cutaneous wounds ~~[8]~~. Human studies have elucidated that ES affects all the stages of wound healing ~~and its mechanism of action is likely multifactorial~~ [9,10] and specific mechanisms include ~~. Based on human *in vitro*, *ex vivo* and *in vivo* studies, the specific modes of action include:~~ anti-bacterial effects [11], down regulation of inflammation [12], increase of tissue oxygenation [13], wound blood flow [14], haemoglobin levels [14], angiogenesis [9,15], fibroblast proliferation [16] and ~~galvanotaxis~~ electrotaxis [17,18]. ES also effects collagen synthesis and wound strength ~~impacts the remodelling phase of wound healing via collagen effects (up-regulation of mature collagen synthesis and increased collagen production) leading to accelerated wound contraction and greater wound tensile strength~~ [12].

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Evidence indicates that bacteria in chronic wounds reside as highly antibiotic resistant polymicrobial biofilms rather than in their planktonic state [19-23]. Culture based techniques have significantly underestimated the number and abundance of microbial species present in these wounds [24]. Culture independent sequencing techniques have allowed more accurate determination of microbial taxa and their relative abundance in cutaneous wounds allowing a greater understanding of the host-microbial interface [25].

This comprehensive review introduces the concepts of wound biofilm and the microbiome and how these are shaping our current knowledge of cutaneous wounds. It further details the *in vitro* and *in vivo* evidence supporting the anti-microbial effects of ES on planktonic bacteria, biofilms and the microbiome. Finally, we discuss potential future perspectives in light of the above in the field of cutaneous wound infection, healing and ES.

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Cutaneous wound biofilms and microbiome

Biofilms

Acute wound infections are generally as a result of planktonic bacteria that undergo a four phase growth cycle consisting of a lag, log, stationary and death phase. However, it is the presence of microbial biofilms that are fundamental in impairing cutaneous wound healing [26-28]. In fact, 60% of chronic wounds exhibit biofilms [29]. Biofilms are defined as complex microbial communities embedded in a protective self-produced biopolymer matrix, which provides protection against antimicrobial agents and host defense mechanisms [30-32]. The ~~biofilm~~ matrix is composed of an extracellular polymeric substance, which includes bacterial carbohydrates, proteins, lipids and extracellular DNA [33,34]. This structure segregates microbes from the external environment. The components of the ~~biofilm~~ matrix provide stabilisation [35], adhesive properties [36], nutrition, hydration [37], integrity [38] and antimicrobial effects [39]. Bacteria within the biofilm communicate through quorum sensing. Quorum sensing is a cell-to-cell communication method in which bacterial cells synthesise and react to small signalling molecules. This communication coordinates the biofilm architecture, enzyme production, microbial growth rates, toxin production, species interactions, bacterial virulence and antimicrobial resistance [40-45]. Biofilm formation involves reversible followed by irreversible attachment and binding of microbes to the wound. These bacteria produce and secrete the extracellular polymeric substance, which encases the microbial colonies. As the biofilm grows and matures focal areas are freed allowing spread and further colonisation [40,46-48].

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The two main propositions suggesting the pathogenic role of wound biofilms are the specific and non-specific bacteria hypothesis ~~There are currently two main hypotheses regarding the pathogenic role of wound biofilms~~ [49]. The former implies ~~specific bacteria hypothesis suggests~~ that only a few species ~~of bacteria~~ within the ~~heterogeneous~~ polymicrobial biofilm are involved in the infectious process; ~~.~~ whereas~~Conversely,~~ the ~~non-specific bacteria hypothesis~~latter considers the bacterial composition of biofilm as a whole to constitute a functional unit, and does not examine the role of individual pathogenic bacteria alone. ~~This postulates that certain bacterial species that usually behave in a non-pathogenic manner, or at least are not capable of maintaining a chronic infection when present on their own, may co-aggregate symbiotically in a pathogenic biofilm and act synergistically to cause an infection~~ [50].

Skin microbiome

Microbes outnumber our own cells by 10 fold and the human microbiome is 100 times larger than the human genome [51]. The human cutaneous microbiome refers to the entire collection of microorganisms (bacteria, archaea, fungi, viruses, and mites) that occupy our skin. It was first defined by Ledeberg as the "ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space" [52]. The four most dominant bacterial phyla are *Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria*. The distribution of these four phyla varies significantly from tissue to tissue with *Proteobacteria* and *Actinobacteria* abundant in the skin [53]. In total at least 19 phyla are known to be part of the skin bacterial microbiome. In 2007 the National Institute of Health in the USA initiated the Human Microbiome Project. The objective was to survey and identify the microbial

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diversity that resides at different body sites, including the skin [25,54]. This influential project from 18 body sites in over 240 healthy individuals was completed in 2012 and has revealed the complex nature of the human microbial inhabitants and the incredible amount of spatial, temporal and individual variations [55]. Limited information is available regarding the skin mycobiome and virome. *Malassezia spp.* have been identified as the most abundant fungal genus in healthy skin [56]. *Propionibacterium* and *Staphylococcus* phages, human papillomaviruses, and Merkel cell polyomaviruses have been identified as common viral skin inhabitants [57-59].

Culture based methods are frequently used to isolate, identify, and study skin microbes. The sensitivity of these methods is limited thus ~~whereby~~ data using them underestimate the diversity of bacteria present [24]. Limitations of culture techniques include inability to reproduce *in vivo* conditions in the provided *in vitro* environment, and the overgrowth of rapidly dividing non-fastidious bacteria in the *in vitro* environment. Therefore, when using culture-based methods, fastidious difficult-to-grow anaerobes are often under-reported and easily cultured bacteria such as *Staphylococcus* spp. over-reported [60]. It is estimated that ~~more than~~up to 60% of bacterial species in the human microbiome are not culturable using standard methods highlighting the need for better alternative detection and identification methods [54].

Culture independent genomic sequencing techniques employed for the examination of the entire skin microbiome revolutionised our understanding of its complexity. It also highlighted the significant risk for experimental bias when using less sensitive methods [61]. For bacterial microbiome identification, these methods utilise the 16S

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ribosomal RNA (rRNA) gene, which is present in the genome of all bacteria but not in eukaryotes and amplify it by polymerase chain reaction (PCR) [62]. The 16S rRNA gene is ideal as it is present in all bacteria and it contains both conserved and hyper-variable regions, which allows binding sites for PCR primers and taxonomic classification [63-66]. This provides a more robust approach in terms of identification and relative quantity of bacteria present compared to culture based techniques [67-71]. Mycobiome identification involves DNA sequencing based on the fungal 18S rDNA and internal transcribed spacer (ITS). As with 16S, 18S rDNA has conserved and variable regions, which allow species identification. ITS allow fungal identification at the species or subspecies level [72].

Initial culture independent methods employed Sanger sequencing which has been the standard method used to sequence the human genome [73]. The advantage of Sanger sequencing is that it allows sequencing the full length of the 16S rRNA or the 18S gene. However, it has proven to be expensive and time consuming with low throughput to completely explore the diversity of the human microbiome. Next generation sequencing platforms provide more precise identification of the microbiota and cost effectiveness. These platform families include 454 [74], Illumina [75], SOLiD, Ion Torrent [76] and PacBio [65].

The first step for all microbiological diagnostics is sampling and the quality of the report depends on the quality of this step. Sampling is a significant dilemma and potential cause for bias also in microbiome research. Sampling types used range from superficial skin swabs, curettage, debridement and biopsies. Superficial skin swabs may not catch the full diversity of bacteria found in deeper layers [67].

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Curettage and debridement includes mostly non-viable tissue and will give an inaccurate picture of the more superficial microbiota. Skin biopsies would seem to provide the optimum representation of the skin microbiome. However, studies have shown comparable level of wound microbial burden between the non-invasive and invasive sampling techniques [67,77], which is promising as biopsies are not always feasible and can be traumatic to the patient.

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The culture independent approaches described above are not without limitations. They are not able to distinguish between live and dead organisms, they can be costly and time consuming and although they provide a thorough taxonomic classification of microbial species present they provide no information on function. Shotgun metagenomic sequencing allows examination of both the taxonomy and functionality of the microbiome [78,79]. It involves a minimal need for DNA amplification, which provides greater accuracy in determining the relative proportions of each microbe. It has provided a deep insight into the functional role of microbes in the gut [80]. However the small amount of DNA provided by skin sampling and the lack of reference genome sequences limits its current use in skin microbiome studies.

Cutaneous wound microbiome

Next generation sequencing has enabled characterisation of the microbiome based on three dimensions which are important in understanding the role of microbes on wound outcomes: total microbial load, microbial diversity, and presence of pathogenic organisms [81]. Over 60 different bacterial genera have been identified to

be present in chronic wounds (table 1) [69,82-84]. No studies as of yet have characterised the cutaneous wound mycobiome or virome.

The four dominant phylotypes identified in chronic wounds are *Staphylococcus, Corynebacterium, Clostridiales and Pseudomonas* [85] (figure 1). In a recent large study of over 2000 wounds, Wolcott et al reported a high proportion of *Staphylococcus species* in wounds of differing aetiologies [86]. In line with this, Han et al found varying amounts of *Staphylococci* in almost all wounds they studied [87]. Dowd et al identified *Peptoniphilus, Enterobacter, Stenotrophomonas, Finegoldia*, and *Serratia spp.* as common findings in pooled diabetic foot, venous leg and pressure wound samples [68]. A more recent study by the same group focused on the bacterial microbiome of diabetic foot wounds and *Corynebacterium* spp. was found to be the most prevalent bacterial genus although this was not present in all samples [50]. They also showed that anaerobes including *Bacteroides, Peptoniphilus, Fingoldia, Anaerococcus*, and *Peptostreptococcus spp*. are present in diabetic foot wounds. This has been confirmed in a number of other studies since [69,86]. Price et al identified a mean of 10 different bacterial families present in chronic wounds with anaerobic *Clostridiales family XI* amongst the most prevalent bacteria [88].

Due to methodological limitations the bacterial load and diversity was underestimated in some of the earlier studies [50,68]. In a more recent study by Gontcharova et al, the skin microbiome was shown to be significantly more diverse compared to wounded skin [89]. However, they also identified anaerobic bacteria, *Corynebacterium* and *Staphylococcus* the most prevalent species in wounds.

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Gardner et al studied neuropathic diabetic foot wounds and noted that the wound microbiome profile was dependent on ulcer depth and duration [90]. Deeper wounds and those of a longer duration had a greater microbial diversity and a higher relative abundance of anaerobic bacteria and gram-negative *Proteobacteria* than those of shorter duration whereas shallower wounds contained a greater abundance of *Staphylococci* [90]. Tuttle et al showed a higher bacterial abundance and diversity in chronic wounds that failed to heal in 6 months compared to similar aetiological wounds that healed completely [91]. They also found that the proportion of *Actinomycetales* was increased in wounds that had not healed, and that of *Pseudomonadaceae* was increased in wounds that healed.

Horton et al identified an association between certain skin microbiomes and skin abscess formation [92]. They showed that the peri-abscess skin sample microbiome was similar to that of the contralateral skin samples but ~~different~~ differed from control patients. This highlights the potential role of the individual's skin microbiome in determining risk of developing skin disorders.

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Antimicrobial effects of electrical stimulation

Mechanism of action of electrical stimulation

ES has a bacteriostatic and bactericidal effect. However, the exact mechanism still remains unknown with both direct and indirect mechanisms suggested (figure 2). Liu et al proposed ES may directly disrupt the integrity of the bacterial membrane [93]. The exact mechanism of bacterial membrane disruption is unknown; however, one suggestion is that ES produced electrons repeatedly exciting the bacterial cell membranes cause eventual leakage of cellular constituents [94]. There are many indirect mechanisms, which have been proposed which include ~~production of substances secondary to electrolysis,~~ pH changes, temperature variations and ~~galvanotaxis~~electrotaxis. ~~ES leads to the production of gas and toxic substances such as hydrogen peroxide and chlorine secondary to electrolysis, which may explain its indirect antibacterial effect [94-97]. However, it seems unlikely to be a predominant factor in bacterial inhibition as ES without the production of these substances still demonstrates antimicrobial effects [98] and neutralisation of these bi-products would be expected in human wounds [95].~~ The acidity and alkalinity is pole dependent, with low pH at the anode and high pH at the cathode [95,96]. The ES modality also seems to be important with significant alterations in pH noted with DC compared to HVMPC [95]. Although pH changes secondary to ES seem to effect bacterial growth, their transient nature makes it unlikely to be the major mechanism of bacterial inhibition [97]. Temperature variations have been found to be minimal during the application of ES and this seems unlikely to affect bacterial viability [97]. ~~Galvanotaxis~~ Electrotaxis occurs secondary to ES [98] and we agree with Asadi and Torkaman [11] that this migratory flow of cells may include attraction of antimicrobial

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cells to the wound, which may explain the indirect antimicrobial activity of ES. One of the key processes of biofilm formation includes attachment and adherence which have been shown to be reduced with ES [99]. More recent findings suggest ES may affect gene expression with repression of quorum sensing genes identified following the application of ES [100].The above evidence proposes both direct and indirect antimicrobial mechanisms of ES with direct mechanisms appearing more predominant [7,11].

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Planktonic

In vitro

Various ES modalities including direct current (DC), ~~low intensity direct current (LIDC),~~ alternating current (AC), high voltage monophasic pulsed current (HVMPC) ~~and~~, low voltage monophasic pulsed current (LVMPC) and low voltage biphasic pulsed current (LVBPC) have been studied *in vitro* with respect to their antimicrobial effects on planktonic bacteria (~~figure 3;~~ table 2). These have varied in protocols with regards to current type, intensity, duration and polarity. The majority of studies involved initial growth of the microorganisms in various broths followed by spread of the suspension into specifically prepared agar media-filled (solidified agarose, nutrient, Mueller-Hinton and eosin methylene blue) petri plates containing the electrodes for ES. There have been a few exceptions where bacteria were grown on cotton patches or specially designed Teflon coupons.

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Barranco et al investigated the effect of DC delivered at different intensities in a petri dish (0.4, 4, 40 or 400 µA) using four different types of electrodes (silver, gold, platinum and stainless steel) on *S. aureus* [101]. Overall findings suggested the greater the intensity of current, the greater the antibacterial effect. However, the increased current intensity was associated with gas formation, electrode corrosion and significant pH shifts. The use of silver positive polarity electrode was far superior at lower currents and at this intensity had negligible detrimental effects [101]. The benefits of silver electrode use were supported by Spadaro et al who showed a significant bacteriostatic effect on four bacterial species when used as the anode at low current intensity [102]. The beneficial antimicrobial effects of silver are well documented [103] and electrically stimulated release of silver ions has been shown to significantly improve this effect [103-105].

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A weak DC of 100 µA delivered via a silver anode electrode had a bactericidal effect on gram negative bacteria and a bacteriostatic effect on gram positive micro-organisms [94]. On the contrary the same group had earlier found the opposite [106]. Liu et al found even weaker DC (10 µA) had a bactericidal effect at the cathode on *S. epidermidis* and *S. aureus* [93]. The effects of ES on *Candida albicans* were investigated by Karba et al [107]. They applied ~~LI~~DC at 0.2-1 mA for between 2 to 18 hours using either the electrodes directly immersed in the culture medium or over agar bridges. They found that the inhibitory action of the ES was proportional to the magnitude and application time.

Rowley was the first to investigate the antibacterial effects of AC and DC [108]. He found growth rates of *Escherichia coli* were affected very little or not at all by AC while a significant bacteriostatic effect occurred with DC. Interestingly Petrofsky et al investigated the effects of AC and DC ES on *S. aureus, E. coli* and *P. aeruginosa* and showed AC had a positive antibacterial effect over DC stimulation against *P. aeruginosa* [109]. They applied AC at 5 and 20 mA for 30 minutes and showed a significant growth reduction of *P. aeruginosa*. DC stimulation at 100 µA over the same time period had no bacteriostatic effect. In contrast Maadi et al who found AC had no inhibitory effect on growth of *P. aeruginosa* [110].

Daeschlein et al investigated the inhibitory effect of LVMPC on common gram positive and negative pathogens of chronic wounds [111]. They found a significant decrease in all microorganisms irrespective of polarity when compared to controls. However they did find, as did Barranco et al [101], that positive polarity has a higher antibacterial effect than negative polarity [111].

Merriman et al investigated the antimicrobial effect of four different types of ES stimulation current [95]. They found application of microampere direct current and HVMPC had a time and polarity independent effect on bacterial growth. Whereas low voltage monophasic and biphasic pulsed current had no antibacterial effect. Guffey and Asmussen found a difference between the antimicrobial effects of HVMPC and DC [112]. They applied HVMPC at 50 to 800 mA and 100 pulses per second at a maximum 160 V for 30 minutes and showed it had no effect on the levels of *S. aureus*, while DC applied at 1, 5 and 10 mA inhibited its growth. In contrast Kincaid

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and Lavoie showed HVMPC (150-350 V, 1-4 hour duration and 120 pulses per second) had an inhibitory effect against *S. aureus, E. coli* and *P. aeruginosa,* which was dose and exposure duration dependent [113]. However they used a higher voltage intensity and longer duration compared to Guffey and Asmussen [112]. Similarly Szuminsky et al found HVMPC applied at 500 V for 30 minutes at 120 pulses per second had bactericidal effects against *S. aureus, E. coli, Klebsiella sp.* and *P. aeruginosa* at both polarities [97]. These observations suggest the effect of HVMPC may be more dependent on intensity rather than treatment duration. It is important to note that extrapolation of these findings to human subjects needs to be considered cautiously as these parameters of ES delivery would not be clinically possible. Gomes et al were the first to investigate the effect of fixed diphasic – Bernard current on bacterial inhibition [114]. Cultures of *S. aureus*, *P. aeruginosa* and *E. coli* were stimulated with either fixed diphasic – Bernard current at 3, 6 and 9 mA for 15 and 30 minutes, or HVMPC at 32, 64 and 95 V for 30 and 60 minutes. There was a decrease in bacterial counts for the two current types, but the most effective reduction was in fixed diphasic – Bernard where there was no pole dependent effect. However, the different currents, the different application times and stimulation intensities produced results indicating a time dependent or a voltage dependent response.

In vivo

There is limited data on the antimicrobial effects of ES on planktonic bacteria *in vivo* than *in vitro* (table 3). ES must be charge-balanced in order to be safe for tissue health. Wolcott et al, were the first to investigate this *in vivo* [115]. They applied

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negative polarity ~~LI~~DC to chronic human wounds colonised with *Pseudomonas* and *Proteus* species and observed pathogen free wounds within several days. Rowley et al found that application of negative polarity DC at 1 mA for 3 days to rabbit wounds infected with *P. aeruginosa* had a bacteriostatic effect [116]. Bolton et al noted DC stimulation of intact human skin at varying current intensities ranging from 0-100 μA led to bactericidal effects at the anode with no effect noted at the negative electrode [96]. They found that the longer the duration of stimulation and the higher the current density the greater the degree of antimicrobial effect. The antimicrobial effect of electrically stimulated silver has been investigated only once *in vivo*. Chu et al showed an enhancing antimicrobial effect of DC stimulated silver nylon dressings [117].

Biofilm

In vitro

Only two studies have assessed the effect of an electric wound dressing on biofilm [100,118] (table 4). The electric dressing utilised was Procellera™ (Vomaris Innovations, Inc) which is a single layer dressing consisting of a matrix of alternating silver and zinc that are held in position on a polyester substrate. The dressing system is unlike the other modalities described and is activated in the presence of a conductive fluid and produces a DC voltage of 0.5-0.9 V. The electric field produced is topographical, uncontrollable and potentially provides an unbalanced ion flux. Banerjee et al successfully showed biofilm disruption in *P. aeruginosa* biofilm within 24 hours of dressing application with the use of imaging and gene expression studies [100]. Kim et al applied the dressing to a variety of antibiotic sensitive and

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multi-drug resistant bacterial strains and found a bactericidal effect, except in the case of *Enterococcus* species, where a bacteriostatic effect was noted [118].

Ten studies have investigated the bioelectric effect, which is the phenomenon whereby ES increases the efficacy of antibiotics. All except the study by Caubet et al [119] who utilised both DC and an alternating radio frequency electric current, assessed the bioelectric effect of DC [120-128]. Bacterial biofilms of multiple species were assessed including *E. coli* [119], *P. aeruginosa* [120,121,123,124,127], *S. epidermidis* [121,122,125], *S. aureus* [121,128], *Streptococcus gordonii* [126] and *Klebsiella Pneumoniae* [127]. The majority of studies found an enhancing effect of ES on the efficacy of antibiotics against the biofilms [119,120,122,123,126-128]. However, Del Pozo et al found varying degrees of antibiotic enhancement by ES across different bacteria and antibiotics and concluded that the bioelectric effect is not generalizable across all microorganisms and antimicrobial agents [121]. Jass et al found the ES enhancement was antibiotic dependent [124] and Sandvik et al found no additional benefit to biofilm disruption with the addition of antibiotics to ES biofilms [125]. The relationship between current intensity, biofilm disruption and the bioelectric effect has also been investigated. Del Pozo et al found a direct relationship between current intensity and antimicrobial effect, however, this direct relationship was not evident with the addition of various antibiotic agents [121]. On the contrary, Haddad et al found a greater electrical current intensity increased the effectiveness of Vancomycin against *S. epidermidis* biofilm, although they only investigated one bacterial species and one antibiotic [122]. Supporting Del Pozo et al, both Jass et al [123] and Sandvik et al [125] found no benefit of increased current intensity on the enhancement of the bioelectric effect.

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The materials on which the biofilms are formed vary widely between the studies. Metals have been commonly used such as titanium [122,129-131], steel [120,132,133] and platinum [134] to resemble prosthetic models. Other materials used include a dialysis membrane [123,124], Teflon [121,135,136], polyvinyl chloride urinary catheter model [137], carbon fabric conductive scaffold [138], polycarbonate [100,125-127], glass [119] and a composite consisting of synthetic hydroxyapatite and zinc oxide [139].

In vivo

The investigation into the effect of ES on biofilms is sparse. Only 6 *in vivo* studies have assessed the effect of ES on biofilms with the majority showing positive outcomes (table 5). Although none of the studies to date have directly investigated the effect of ES on cutaneous wound bacterial biofilms, the outcomes presented below are promising and potentially translatable.

A study in goats assessing the effect of 100 µA of DC ES applied to metal pins inoculated with *S. epidermidis* inserted into the tibia showed a marked reduction in clinical signs of infection compared to controls. However, sample numbers were relatively low and there were no techniques used to quantify the biofilm [140]. A similar study was conducted by Del Pozo et al where they applied a higher DC current (200 µA) to *S. epidermidis* inoculated stainless steel implanted into the tibia of rabbits [141]. They had a second treatment group which received intravenous doxycycline as well as a third control group for comparison. Bacterial load quantification showed a significant reduction in counts for both ES and doxycycline

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treated rabbits compared to controls and also found ES treatment was significantly more efficacious than antibiotic treatment [141]. Post inoculation incubation time is an important factor in biofilm development and in the latter study [141] treatment did not commence until after 4 weeks post inoculation allowing time for the biofilm to develop, however, there was no imaging available to confirm biofilm formation. Conversely, Paryavi et al showed no benefit of DC ES in reducing bacterial load compared to control in an instrumented rabbit model [142]. However, they did investigate a different microbe (*S. aureus*) and used a lower intensity current (60 μA).

Using a capacitive coupling ES device, Gilotra et al evaluated the bioelectric effect in a rabbit spine infection model [143]. Rabbits with spinal rods implanted were infected with *S. aureus* following a single dose of intravenous antibiotics. After 7 days of ES, instrumentation related infection was significantly reduced compared to controls (36% vs. 81%), although there was no difference in soft tissue infection burden and total bacterial load [143]. Ehrensberger et al found application of cathodic voltage-controlled ES at 1.8 V to titanium for 1 hour implanted into a rodent model with preformed methicillin-resistant *S. aureus* biofilm led to a significant reduction in bacterial counts both on the implant and the surrounding bone tissue [131]. Using a similar rodent model, the same group found ES enhanced the activity of vancomycin in reducing the implant bacterial burden but had no benefit in reducing bone bacterial burden compared to antibiotic treatment alone [144].

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Microbiome

In vitro

There are no studies to date investigating the effect of ES on the microbiome *in vitro*.

In vivo

There are no studies to date investigating the effect of ES on the microbiome *in vivo*.

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Future perspective

Cutaneous wound infections play a pivotal role in delayed wound healing. The growing need for non-pharmacological management of such infections is growing with the increasing rate of anti-microbial resistance. ES has the potential to bridge this current predicament.

The majority of studies presented above utilise ES in the *in vitro* environment in both planktonic and biofilm bacterial states. Although studies on planktonic bacteria are helpful in establishing potential ES mechanism of action, their translatability to biofilm and clinical use is very limited. Also the varying use of different ES modalities and parameters also provide no consensus yet as to the best ES device for bacterial management. The use of ES in inhibiting bacterial biofilms is far more clinically relevant. Although there is a good body of *in vitro* studies developing, only a couple of studies have assessed the impact of a clinically useable electric dressing [100,118]. Both these studies have shown promising results with regards to biofilm disruption. However, the ES Procellera^{TM} delivers to the wound is not controllable or quantifiable. Also, as with planktonic bacterial models, the varying use of different ES parameters and study protocols makes concluding which is the best form of ES in the management of biofilms currently difficult.

Although *in vivo* studies on planktonic bacteria are useful and have shown positive outcomes, we feel the role of ES is more clinically suited to the management of chronic highly resistant wound biofilms. There are only a handful of *in vivo* studies assessing the impact of ES on bacterial biofilms. These studies have shown promising results, however, the majority of studies were assessing the effect of ES

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on biofilm formed on metallic implants. Nnone of the studies were directly assessing the effect of ES on cutaneous wound biofilms and with the delicate nature of human cutaneous wounds, these findings are unlikely to be directly translatable..

The use of ES in the management of cutaneous wound biofilms may not be clinically successful when used in isolation. This possibility makes the bioelectric effect of significant interest. Both *in vitro* and *in vivo* studies have shown in the majority of cases an enhancing effect of ES on antibiotic efficacy against biofilms. However, again only a limited number of bacterial species and antibiotic agents have been investigated and all in non-cutaneous wound models.

The majority of studies to date have assessed the antimicrobial efficacy of ES against single species biofilms and easily culturable species. However, microbes reside in chronic wounds in polymicrobial biofilms and culture independent techniques have allowed the identification of a wider microbial community residing in chronic wounds. No studies to date have assessed the impact of ES on the microbiome profile. Next generation sequencing techniques are providing a greater insight not only into the diversity of microbes that reside in cutaneous wounds but also the functional potential of the microbial community. There is currently a need to investigate the effect of ES on the microbiome to provide a deeper insight into its role in the management of infections and wound healing.

ES has the potential to be an effective antimicrobial tool specifically against microbes that may delay cutaneous wound healing either used in isolation or as an adjunct to antibiotics and we agree with Korzendorfer and Hettrick that current evidence is

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limited and lacking and relevant clinical studies are needed [145]. In order to investigate this, revolutionary studies over the next decade are needed focusing on key specific areas. Firstly, standardised protocols are necessary to develop robust evidence supporting the use of ES in cutaneous wound management. Secondly, clinically relevant biofilm models are necessary which would have more translatability to clinical practice. Finally, the use of next generation sequencing techniques to identify how ES modifies the wound microbiome to enhance wound healing is a vital and currently unexplored area.

There is currently a disconnect between cutaneous wound infections, biofilms, microbiome and ES. Future studies combining the fields of ES, biofilm and microbiome research are necessary and an exciting prospect to fully elucidate the use of ES in the management of cutaneous wounds.

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Executive summary

Introduction

- Chronic cutaneous wounds have a major impact on patients' quality of life and mobility and present a huge burden on the healthcare system.
- Due to major challenges in current management of cutaneous wound infections, new non-pharmacological approaches are urgently required.
- ES could promote cutaneous wound healing by imitating the natural electrical current that occurs in wounds.

Cutaneous wound biofilms and skin microbiome

- Bacteria in chronic wounds reside as highly antibiotic resistant polymicrobial biofilms, which are fundamental in impairing cutaneous wound healing.
- Culture based methods are frequently used to isolate, identify, and study skin microbes. The sensitivity of these methods is limited whereby data using them underestimate the diversity of bacteria present.
- Culture independent sequencing techniques have allowed comprehensive identification of the cutaneous wound microbiome.

Antimicrobial effects of electrical stimulation

- The majority of studies have investigated with positive outcomes the use of ES *in vitro* in regards to both planktonic and biofilm bacterial states.
- There is a lack of high quality *in vivo* data specifically related to cutaneous wound infection.
- There are currently no studies investigating the ability of ES to alter the microbiome both *in vitro* and *in vivo*.

Future perspective

- Current evidence suggests ES is a successful anti-microbial treatment.

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- However, there is currently a disconnect between cutaneous wound infections, biofilms, microbiome and ES.
- Future studies combining the fields of ES, biofilm and microbiome research are necessary and an exciting prospect to fully elucidate the use of ES in the management of cutaneous wounds.

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Table 1. Studies using next generation sequencing technology to analyse microbiome of chronic wounds

Reference	Wound type and location	Sampling method	Microbial identification methods	Principle findings
[50]	Diabetic	Debridement	bTEFAP	Most prevalent bacterial genus was *Corynebacterium spp.*
[68]	Diabetic Pressure Venous	Debridement	Culure methods PRAPS FRACS PRADS	Each wound type revealed marked differences in bacterial populations
[69]	Diabetic Post-surgical Pressure Venous	Debridement	Culture methods Pyrosequencing (454 Roche)	145 genera identified with molecular methods compared to only 14 identified by culture techniques
[82]	Venous	Debridement	bTEFAP and bTEFAP titanium	Predominant organisms include *Bacteroidales*, various anaerobes, *Staphylococcus*, *Corynebacterium* and *Serratia*
[83]	Pressure	Debridement	bTEFAP	Polymicrobial in nature with no single bacterium exclusively colonising the wounds

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[84]	Neuropathic Other Post-surgical Pressure Venous	Curettage	Pyrosequencing (Roche 454)	58 bacterial families and 91 bacterial genera characterised
[85]	Arterial Diabetic Scalp Venous	Curettage Surface swabbing	Culture methods MegaBACE 1000 DNA sequencer ABI3730 DNA sequencer	Individual wounds contained 4-22 phylotypes Different diversity and dominance information given between molecular and culture methods
[86]	Diabetic Post-surgical Pressure Venous	Debridement	Pyrosequencing (454 Roche)	High proportion of *Staphylococcus* and *Pseudomonas spp.* High prevalence of anaerobic bacteria and bacteria traditionally considered commensal
[87]	Arterial Diabetic Other Pressure Traumatic Venous	Biopsy Curettage	Culture methods Pyrosequencing (454 Roche)	Pyrosequencing revealed increased bacterial diversity with an average of 17 genera in each wound

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[88]	Diabetic Post-surgical Pressure Venous	Curettage	Culture methods Pyrosequencing (454 Roche)	Gene analyses revealed approximately 4 fold more bacterial families in wounds than estimated by culture
[89]	Diabetic Normal skin	Debridement Surface swabbing	bTEFAP	Intact skin significantly more diverse than wounds Wounds show heightened levels of anaerobic bacteria
[90]	Diabetic	Surface swabbing	Culture methods Pyrosequencing (454 Roche)	Wound depth positively correlated with abundance of anaerobic bacteria, and negatively correlated with abundance of *Staphylococcus*
[91]	Venous	Debridement	Culture methods Ibis T5000 universal biosensor bTEFAP and bTEFAP titanium	Significantly higher bacterial abundance and diversity in wounds that had not healed *Actinomycetales* was increased in wounds that had not healed *Pseudomonadaceae* was increased in wounds that had healed

bTEFAP - bacterial tag encoded FLX amplicon pyrosequencing; PRAPS - partial ribosomal amplification and pyrosequencing; FRACS - full ribosomal amplification, cloning and Sanger sequencing; PRADS – partial ribosomal amplification, density gradient gel electrophoresis and Sanger sequencing.

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Table 2. Planktonic *in vitro* studies

Reference	ES	Pathogens	Treatment Parameters	Principale findings
[93]	DC	*S. epidermidis* *S. aureus*	CI: 10-100 µA Voltage: 9 V Duration: 16 h P: anode and cathode ET: carbon	Antimicrobial effect at low CI around cathode
[101]	DC	*S. aureus*	CI: 0.4-400 µA Voltage: 0.4-9.3 V Duration: 24-48 h P: anode ET: silver, gold, platinum and stainless steel	CI directly proportional to bacteriostatic effect

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[95]	DC HVPC LVMPC LVBPC HVMPC	*S. aureus*	CI: 500 µA (DC); 30 mA (LVMPC + LVBPC); NS/A (HVMPC) Voltage: 0-102 V (DC); NS/A (LVMPC + LVBPC); 250 V (HVMPC) Duration: 1 h/d for 3 d P: aAnode and cathode ET: stainless steel PW: 120 µs (LVMPC + LVBPC); NS/A spaced 70 µs apart (HVMPC) IPI: 7.69 ms (LVMPC); 7.47 ms (LVBPC); 9.93 ms (HVMPC) PF: 128 pps (LVMPC + LVBPC); 100 pps (HVMPC)	Antibacterial effect with DC and HVMPC irrespective of polarity
[97]	HVMPC	*S. aureus* *E. coli* *Klebsiella* *P. aeruginosa*	CI: NS/A Voltage: 500 V Duration: 30 m Voltage: 500 V P: anode and cathode ET: stainless steel PW: 7 µs spaced 70 µs apart IPI: 8-9 ms PF: 120 pps	Antibacterial effects irrespective of polarity

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[104]	DC	*S. aureus* *E. coli* *P. vulgaris* *P. aeruginosa*	CI: 0.4-400 μA Voltage: 0.086 - ≥ 9.5 V Duration: 24 h P: anode and cathode ET: silver, platinum, stainless steel, gold and copper	Antibacterial effect at all ET at high CI Only silver electrode used as anode had bacteriostatic effect at low CI
[94]	DC	Gram +ve Gram -ve	CI: 100 μA Voltage: NS/A Duration: NS/A P: aAnode and cathode ET: silver	Bacteriostatic effect on gram +ve Bactericidal effect on gram -ve
[106]	DC	*S aureus* *P aeruginosa*	CI: 26-800 μA Voltage: NS/A Duration: NS/A P: aAnode ET: silver	Bacteriostatic effect on P. aeruginosa Bactericidal effect on S. aureus
[107]	DC	*C. albicans*	CI: 200-1000 μA Voltage: NS/A Duration: 12-18 h P: cathode ET: platinum-iridium alloy	Antimicrobial effect proportional to CI and application time

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[108]	DC AC	*E. coli*	CI: 1-140 mA (DC); 15-30 mA (AC) Voltage: NS/A Duration: NS/A P: cathode ET: platinum PW: NS/A (AC) PF: NS/A (AC)	DC showed bacteriostatic effect AC showed no effect
[109]	DC AC	*S. aureus* *E. coli* *P. aeruginosa*	CI: 100 µA (DC); 5-20 mA (AC) Duration: 30 m Voltage: NS/A P: NS/A ET: carbonised rubber PW: 250 µs (AC) PF: 30 pps (AC)	AC reduced growth of P. aeruginosa
[110]	DC AC	*P. aeruginosa*	CI: NS/A Voltage: 1.5-10 V Duration: up to 19 h P: aanode and cathode ET: Stainless steel PW: 20 ms PF: 50 pps	DC inhibited growth AC had no effect

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[111]	LVMPC	*S. aureus* *S. epidermidis* *E. faecium* *E. coli* *P. aeruginosa* *Klebsiella*	CI: 42 mA Voltage: NS/A Duration: 30 m P: anode and cathode ET: hydrogel PW: 140 μs IPI: NS/A PF: 128 pps	Antibacterial effect on all microorganisms irrespective of polarity
[112]	DC HVMPC	*S. aureus*	CI: 1-10 mA (DC); 50-800 mA (HVMPC) Voltage: NS/A (DC); <160 V (HVMPC) Duration: 30 m P: anode and cathode ET: NS/A PW: NS/A IPI: NS/A PF: NS/A	DC had antibacterial effect at all CI HVMPC had no antibacterial effect
[113]	HVMPC	*S. aureus* *E. coli* *P. aeruginosa*	CI: NS/A Voltage: 150-300 V Duration: 1-4 h P: anode and cathode ET: stainless steel PW: NS/A spaced 55 μs apart IPI: NS/A PF: 120 pps	Antibacterial effect against all organisms

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[114]	FDBC HVMPC	*S. aureus* *P. aeruginosa* *E. coli*	CI: 3-9 mA (FDBC); NS/A (HVMPC) Voltage: 2.72-6.97 V[2] (FDBC); 32-95 V (HVMPC) Duration: 0-30 minutes (FDBC); 0-1 h (HVMPC) P: a~~A~~node and cathode ET: stainless steel PW: 10 ms (FDBC); 15 µs (HVMPC) IPI: NS/A PF: 100 pps	FDBC had a more effective reduction in bacterial counts irrespective of polarity

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ES – electrical stimulation; DC – direct current; AC – alternating current; ~~LVPC – low voltage pulsed current;~~ HVMPC – high voltage monophasic pulsed current; LVMPC – low voltage monophasic pulsed current; LVBPC – low voltage biphasic pulsed current; FDBC - fixed diphasic Bernard current; CI – current intensity; P – polarity; ET – electrode type; A – ampere; V~~v~~ – volt; PW – pulse width; IPI – inter pulse interval; PF – pulse frequency; pps – pulses per second; NS/A – not stated/available; ~~.~~

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Table 3. Planktonic *in vivo* studies

Reference	ES / model	Pathogens	Treatment Parameters	Principale findings
[96]	DC Human (n=28)	*S. epidermidis*	CI: 10-100 μA Voltage: 6-9 V Duration: 4 or 18 h P: anode and cathode ET: carbon and aluminum	Bactericidal effect at anode No effect at cathode
[115]	DC Human (n=75)	*P. aeruginosa* *Proteus*	CI: 200-800 μA Voltage: NS/A Duration: 6 h/d for 0.8-15.4 weeks P: anode and cathode ET: NS/A	Cathodal treated wounds pathogen free in few days
[116]	DC Rabbit (n=140)	*P. aeruginosa*	CI: 1 mA Voltage: NS/A Duration: 72 h P: cathode ET: copper	Bacteriostatic effect
[117]	DC Rat (n=360)	*P. aeruginosa*	CI: 0.4-40 μA Voltage: NS/A Duration: 5 d P: anode and cathode ET: silver	Anodal enhancing antimicrobial effect

ES – electrical stimulation; DC – direct current; CI – current intensity; P – polarity; ET – electrode type; A – ampere; V – volts; NS/A – not stated/available.

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Table 4. Biofilm *in vitro* studies

Reference	ES / model	Pathogens	Treatment Parameters	Principale findings
[125]	DC Antibiotics Polycarbonate	*S. epidermidis*	CI: 2-5 mA Voltage: NS/A Duration: 24 h P: anode and cathode ET: platinum ~~Antibiotics~~	Addition of ES to antibiotic treated biofilms had no significant benefit in reducing biofilm viability
[121]	DC Antibiotics Teflon	*P. aeruginosa* *S. aureus* *S. epidermidis*	CI: 0-2000 mA Voltage: NS/A Duration: 24 h P: anode and cathode ET: stainless steel and graphite ~~Antibiotics~~	Bioelectric effect was not observed for all bacteria and antibiotic combinations
[100]	Procellera™ Polycarbonate	*P. aeruginosa*	CI: 2-10 µA Voltage: 0.3-0.9 V Duration: 24 h P: anode ET: silver and zinc	Markedly disrupted biofilm integrity using SEM Reduced biofilm thickness and number of live bacteria Repressed quorum sensing genes

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[118]	Procellera™ Textile materials	*A. baumannii* *A. calcoaceticus* *E. faecalis* *E. coli* *K. pneumoniae* *P. aeruginosa* *S.aureus* *S.aureus (MRSA)* *S. epidermidis* *E. Faecium* *E. raffinosus*	CI: 2-10 μA Voltage: 0.3-0.9 V Duration: 24-48 h P: anode ET: silver and zinc	Procellera™ had a bactericidal effect against all study organisms except Enterococcus species where a bacteriostatic effect was noted
[119]	DC + RF Antibiotics Glass	*E. coli*	CI: 200 mA + 20 V (DC); 150 mA + 10 MHz (RF) ~~Antibiotics~~ Voltage: ~ 20 V (DC) Duration: 24 h P: NS/A (DC) ET: stainless steel PF: 10000000 pps (RF)	Synergistic effect between ES and antibiotics DC produces a more pronounced bioelectric effect than RF
[120]	DC Antibiotics Steel	*P. aeruginosa*	CI: 50 mA Voltage: 10V ~~Antibiotics~~ Duration: 24-48 h P: anode and cathode ET: stainless steel	Combined ES and antibiotic treatment was more successful in reducing bacterial count compared to either treatment used in isolation

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[122]	DC Antibiotics Titanium	*S. epidermidis*	CI: 22-333 μA Voltage: 4 V Antibiotics Duration: 24 h P: anode and cathode ET: stainless steel	Bioelectric effect was enhanced with higher current intensity
[123]	NS/A Dialysis membrane	*P. aeruginosa*	CI: 0-20 mA/cm^2 Antibiotics Voltage: NS/A Duration: 12 h P: NS/A ET: NS/A	ES alone had no effect on biofilm formation Addition of ES to antibiotic treatment enhanced anti-microbial activity of antibiotic
[124]	NS/A Antibiotics Dialysis membrane	*P. aeruginosa*	CI: 0-9 mA/cm^2 Antibiotics Voltage: NS/A Duration: 12 h P: NS/A ET: NS/A	Addition of ES to antibiotic treatment did not enhance anti-microbial activity of all antibiotics used
[126]	DC Antibiotics polycarbonate	*S. gordonii*	CI: 2 mA Antibiotics Voltage: 1-8 V Duration: 24 h P: NS/A ET:stainless steel	ES enhanced the antibiotic efficacy against biofilm formation

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[127]	DC Antibiotics polycarbonate	*P. aeruginosa* *K. pneumoniae*	CI: 0-10 mA ~~Antibiotics~~ Voltage: 4-7 V @ 1 mA Duration: 24 h P: anode and cathode ET: platinum	Bioelectric effect against biofilm formation noted
[128]	DC Antibiotics Polypyrrole/chitosan	*S. aureus*	CI: 200 μA ~~Antibiotics~~ Voltage: NS/A Duration: 4 h P: anode and cathode ET: copper	ES significantly enhanced antibiotic killing efficacy of biofilm
[129]	DC Titanium	*S. aureus*	CI: 3645 A Voltage: 20 V Duration: 6 m P: anode and cathode ET: platinum and titanium	ES coupled with anodised nanotubular titanium led to a significant decrease in biofilm formation compared to controls
[130]	LVBPC Titanium	*P. aeruginosa*	CI: NS/A Voltage: 0.5-5 V Duration: 6 d P: anode and cathode ET: titanium PW: NS/A IPI: 0-0.5 s PF: 200-10000 pps~~Hz~~	Greatest effect of ES against biofilm formation (50% reduction) was seen with ES at 5 V and 200 Hz

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[131]	CVCES Titanium	*S. aureus* *(MRSA)*	CI: NS/A Voltage: 1.8 V Duration: 1 h P: cathode ET: silver and graphite ~~1.8 V~~	92% reduction in bacterial count with the use of CVCES compared to control
[132]	DC + BC Steel	*S. epidermidis*	CI: 60-100 μA (DC); 60 100 μA ~~+ 1Hz~~ (BC) Voltage: NS/A (DC + BC) Duration: 400 m P: anode and cathode ET: stainless steel and indium tin oxide PF: 1 pps	Detachment of biofilm from steel is higher with the use of DC compared to BC
[133]	BC Steel	*S. epidermidis*	CI: 15-100 μA Voltage: 1.5–1.7 V ~~0.1-2 Hz~~ Duration: 150 m P: anode and cathode ET: stainless steel and indium tin oxide PF: 0.1-2 pps	Percentage of bacterial detachment from steel was frequency dependent

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[134]	DC Platinum	*K. pneumoniae* *P. fluorescens* *P. aeruginosa*	CI: 50 µA Voltage: 1.3 V Duration: NS/A P: anode and cathode ET: platinum~~0.016-20 Hz~~	Biofilm viability was polarity dependent with anodic polarity reducing biofilm thickness by 26%
[135]	DC Teflon	*S. epidermidis*	CI: 2000 mA Voltage: NS/A Duration: 4-24 h P: anode and cathode ET: stainless steel and graphite	Reduced bacterial count with ES application compared to controls at 4 and 24 hours
[136]	DC Teflon	*S. epidermidis* *P. aeruginosa* *E. coli* *P. acnes* *C. albicans*	CI: 200-500 µA Voltage: NS/A Duration: 4-48 h P: anode and cathode ET: platinum	ES effect on species is time dependent ES was not effective in reducing *P. acnes* biofilm
[137]	DC Polyvinyl chloride urinary catheter model	*S. epidermidis* *E. coli* *P. aeruginosa* *C. parapsilosis*	CI: 0-500 µA Voltage: NS/A Duration: 24 h – 4 d P: anode and cathode ET: platinum	Biofilm reduction was time and current intensity dependent
[138]	DC Carbon fabric conductive scaffold	*A. baumannii*	CI: NS/A Voltage: 400-800 mV Duration: 24 h P: cathode ET: carbon and silver	80% decrease in biofilm surface coverage within 24 hours

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[139]	DC HA-ZnO	*S. aureus* *(MRSA)*	CI: 0 A Voltage: 1 V/cm Duration: 6-24 h P: anode and cathode ET: stainless steel	Time dependent decrease in bacterial viability and biofilm formation

ES – electrical stimulation; V – volts; SEM – scanning electron microscopy; DC – direct current; HAZno – Hydroxyapatite zinc oxide; RF – radio frequency; A – ampere; Hz – hertz; CVCES – Cathodic voltage controlled electrical stimulation; NS/A – not stated/availablespecified; PC – pulsed current; BC – block current; MRSA – methicillin resistant *staphylococcus aureus*. LVBPC – low voltage biphasic pulsed current; CI – current intensity; P – polarity; ET – electrode type; PW – pulse width; IPI – inter pulse interval; PF – pulse frequency; pps – pulses per second.

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Table 5. Biofilm *in vivo* studies

Reference	ES / model	Pathogens	Treatment Parameters	Principalle findings
[131]	CVCES Rat (n=16)	*S. aureus (MRSA)*	CI: NS/A Voltage: 1.8 V Duration: 1 h Polarity: cathode ET: silver and platinum	ES significantly reduced bacterial load by 87% in the bone and 98% in the titanium implant
[140]	DC Goat (n=9)	*S. epidermidis*	100 µACI: 100 µA Voltage: 9 V Duration: 24 h/d for 21 d Polarity: anode and cathode ET: stainless steel and platinum	Implant infection rate reduced in ES treated animals compared to controls (11% vs. 89%)
[141]	DC Antibiotics Rabbit (n=39)	*S. epidermidis*	200 mA 6VCI: 200 µA Voltage: 6 V Duration: Treatment initiated 4 weeks after inoculation and administered for 21 d Polarity: anode and cathode ET: stainless steel	Bacterial load significantly reduced in ES and antibiotic only groups compared to controls ES more efficacious than antibiotic treatment

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[142]	DC Rabbit (n=17)	*S. aureus* *(MRSA)*	~~60 µA~~CI: 60 µA Voltage: NS/A Duration: 24 h/d for 7 d Polarity: cathode ET: stainless steel	No significant reduction in biofilm observed with ES compared to controls
[143]	AC Rabbit (n=30)	*S. aureus*	CI: 6 mA (max 14 mA) Antibiotics Voltage: NS/A Duration: 24 h/d for 7 d ET: - hydrogel PW: NS/A PF: NS/A	Overall bacterial burden was not reduced with ES
[144]	CVCES Antibiotics Rat (n=20)	*S. aureus* *(MRSA)*	~~1.8 V~~ ~~Antibiotics~~CI: NS/A Voltage: 1.8 V Duration: treatment initiated 1 week after inoculation and administered for 1 h either on d7 or on d7 + d21 Polarity: cathode ET: titanium	ES had a significant bioelectric effect on implant bacterial burden ES had no significant bioelectric effect on bone bacterial burden

ES – electrical stimulation; DC – direct current; A – ampere; V – volts; CVCES – Cathodic voltage controlled electrical stimulation; AC – alternating current; MRSA – methicillin resistant *staphylococcus aureus*. CI – current intensity; P – polarity; ET – electrode type; PW – pulse width; PF – pulse frequency; pps – pulses per second; NS/A – not stated/available;

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Figure Legend

Figure 1. Common bacteria identified in chronic wounds by sequencing methods. The commonest bacteria based on phylum, family, genus or species identified from 13 studies assessing the microbiome of chronic wounds using sequencing techniques.

Figure 2. Proposed antimicrobial mechanism of action of electrical stimulation. Figure highlights the direct (bacterial membrane disruption and inhibit bacterial proliferation) and indirect (pH~~ and~~, temperature alterations~~, production of toxic substances~~ and ~~galvanotaxis~~electrotaxis) antimicrobial effects of electrical stimulation.

~~**Figure 3.** Electrical stimulation waveforms.~~

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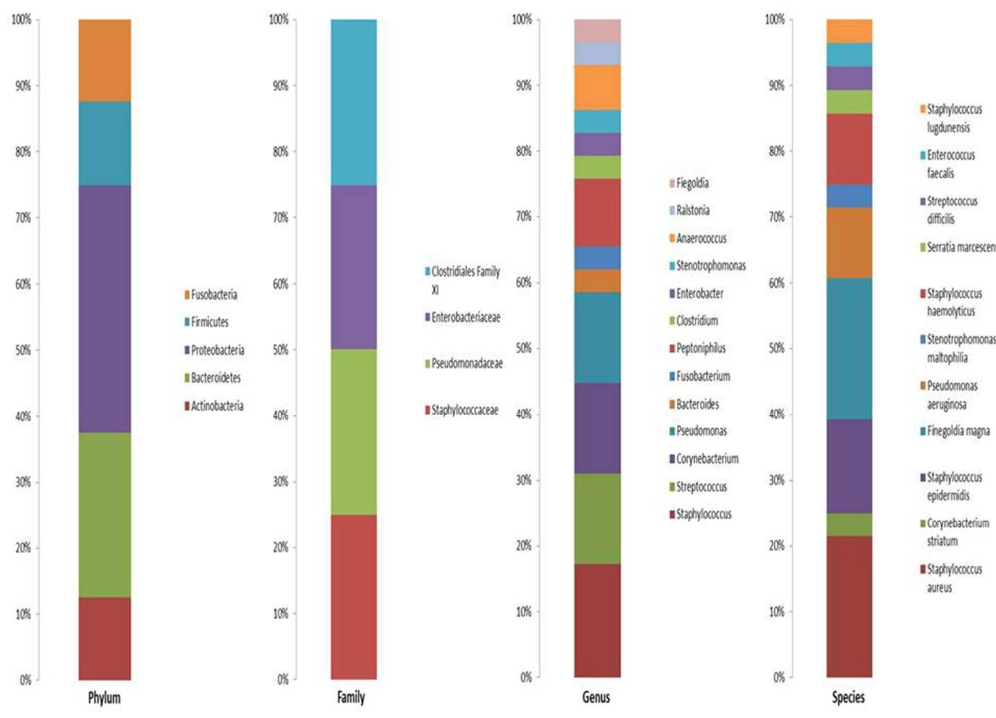

Figure 1. Common bacteria identified in chronic wounds by sequencing methods. The commonest bacteria based on phylum, family, genus or species identified from 13 studies assessing the microbiome of chronic wounds using sequencing techniques.

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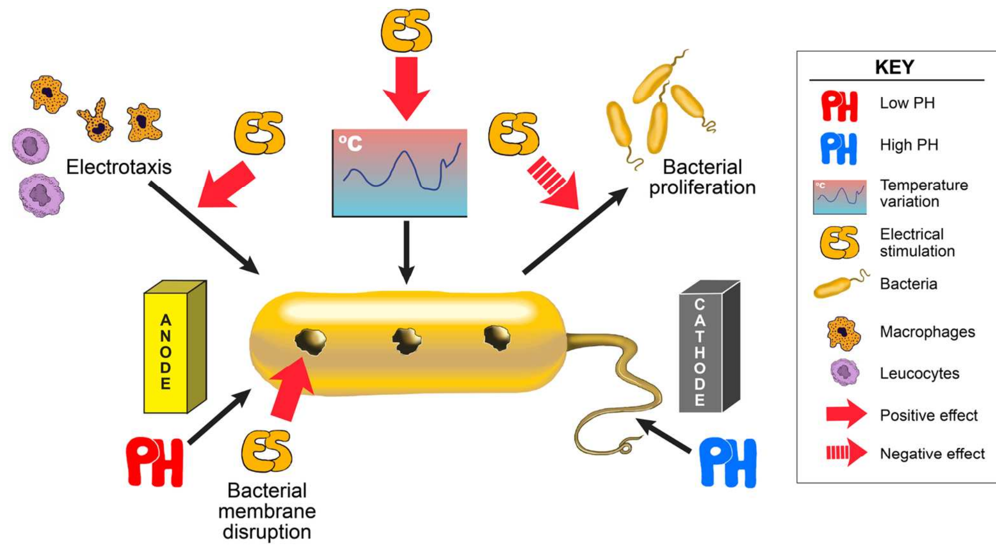

Figure 2. Proposed antimicrobial mechanism of action of electrical stimulation. Figure highlights the direct (bacterial membrane disruption and inhibit bacterial proliferation) and indirect (pH, temperature alterations and electrotaxis) antimicrobial effects of electrical stimulation.

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