Acceleration of wound healing using electrical fields: time for a stimulating discussion

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Abstract

Chronic wounds have a significant effect on patient morbidity and also result in high cost to both affected individuals and the healthcare system. Several engineering approaches have been devised to combat chronic cutaneous wounds; these can be broadly divided into chemical, biological and physical wound management. This review discusses the various approaches, with an emphasis on physical wound management, in particular the application of electric fields to accelerate wound healing.

Introduction

The ability to heal wounds caused by trauma, disease and environmental insults is one of the most fundamental defence mechanisms the body has against injury. However, there are circumstances where this physiological mechanism is impaired and wounds either heal very slowly or not at all. These types of wounds, referred to as chronic wounds, are characterised by tissue destruction and impaired blood circulation, which deprives the wound site of cells critical for wound healing such as macrophages, endothelial cells and fibroblasts. Chronic wounds occur when the normal wound healing process is disrupted; examples include pressure ulcers, ischaemic ulcers and venous ulcers [1].

Chronic wounds result in both significant patient morbidity and high cost to both affected individuals and the healthcare system. It has been estimated that in the United States alone, 5 million patients suffer from chronic non-healing wounds, costing the health system $20 billion annually; this is projected to grow a further 10% each year [2]. These costs cover diagnostic and surgical procedures of wounds, pharmaceuticals, wound closure devices, and hospital and physician charges.

Pressure ulcers from immobilised patients often require long periods of hospitalisation for treatment. In the USA, it is estimated that 1.5-3 million people require treatment for pressure ulcers; this figure contributes to an annual cost of approximately $5 billion [3]. Further, there are approximately 150 million sufferers of diabetes mellitus worldwide and, within this population, 15% will develop foot ulcers at least once in their lifetime due to vascular complications including vascular disease and peripheral neuropathy [4]. An estimated 25-50% of diabetic care costs are attributed to diabetic vascular complications, including approximately 85,000 lower limb amputations each year [5], accounting for more than 60% of all non-traumatic lower limb amputations [6].

Around 500,000-600,000 people in the USA suffer from venous ulcers [7]; a further 0.2-1% of the population in developed countries are affected by this ailment [8]. Treating these patients costs approximately $9685 per patient [9] and a total of $2.5-3 billion annually. Much more difficult to quantify are the indirect costs to the patients and the community through lost work time or even loss of employment or forced retirement, impaired quality of life due to social isolation, pain and discomfort, and impaired mobility. Estimates of these indirect costs suggest that, in developed counties alone, there is a loss of productivity of around 2 million working days [6].

Although these figures are mainly from the USA, its population comprises approximately 50% of the worldwide market for medical products and extrapolation of the data demonstrates the enormous impact of chronic wounds globally. There is clearly a necessity for further research and development in treating chronic wounds to improve the quality of life in people with predisposition to these wounds, as well as to reduce the economic burden to the healthcare system worldwide.

Throughout the years, there have been numerous treatments devised to combat chronic wounds. However, debridement and 'moist' wound healing are still commonly practised treatments used today. The importance of appropriate management of the wound bed is recognised as an essential part of the treatment approach to chronic wounds [10]. Failure

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of many novel treatments to dramatically impact on wound healing rates clearly suggests that better understanding of wound physiology at the cellular and molecular level is required to develop design criteria for new wound treatments. Novel approaches to managing and treating chronic wounds are continually being proposed, encompassing chemical, biological or physical treatments, or even combinations of these different approaches [11]. Table 1 summarises examples of these approaches to wound healing.

The objective of this review is to define the three major approaches outlined above and to assess the current status of new approaches to wound treatment, with a focus on the potential for electrical fields to enhance wound healing.

Chemical wound management

Chemical wound management refers to the use of new synthetic materials and medicated dressings to treat chronic wounds. This includes pharmaceutical approaches to combating infection as well as focusing on balancing cellular metabolic abnormalities implicated in chronic wounds.

The selection of wound dressing type depends on the underlying wound aetiology. Medicated hydrogel wound dressings have been proposed to not only treat infections but also provide a moist environment to enhance epithelialisation and aid in the wound healing process. Unlike saline-soaked gauze, hydrogels not only prevent the wound from drying but, used in combination with medication, can also assist in the controlled release of active agents. This approach can prolong treatment times in a local manner and ultimately may lead to increased patient compliance.

For example, since impaired nitric oxide (NO) metabolism has been implicated in delayed wound healing, a NO releasing hydrogel dressing has been proposed to achieve both moist wound healing and a pharmacological effect [26]. NO hydrogels increased extracellular matrix (ECM) production *in vitro* and improved the tissue quality of wounds in a mouse wound healing impaired model. However, the rate of wound closure was not changed [12].

Other studies have shown an acceleration of wound contraction and healing in a mouse model could be achieved with chitosan hydrogels which are proposed to be inherently antibacterial [13]. Hydrogels, however, have a disadvantage in that they are not appropriate for wounds with high exudate levels and thus are only able to be used in a proportion of chronic wounds.

Biological wound management

Wound healing requires the production of many soluble, bioactive factors to promote the wound healing process. Biological wound management involves the application onto

Table 1. Summary of major current approaches to wound healing, with specific examples and links to referenced papers.

Chemical	
Wound dressings [11]	Absorbent 'textile'
	Low-adhesives
	Vapour permeable films
	Hydrocolloids
	Hydrogels
	Alginates
	Foams
	Beads
Pharmaceutical	NO releasing hydrogels [12]
	Antibacterial hydrogels [13]
Biological	
Growth factors	rhPDGF (Regranex®) [14-17]
	Vitronectin:IGF complexes [18]
Engineered skin	CellSpray® and ReCell® [19]
Matrix-based products [11]	Integra® Dermal Regeneration Template
	BioMend®
	InFuse®
	Alloderm® and Cymetra®
	Seprapack® and Sepramesh®
	Innocoll
	SkinTemp® and Medfil®
	CosmoDerm™ and CosmoPlast™
	OP-1™
Cell-based products [11]	Isolagen
	Carticel®
Cell-matrix composite products [11]	Apligraf®
	Dermagraft®
	Transcyte®
	OrCel®
	Epicel®
Physical	
Hyperbaric oxygen [20]	
Whirlpool/pulses lavage [21, 22]	
Low energy laser [23, 24]	He-Ne
Ultrasound [25]	Low intensity (0.3-1 W/cm^2)
	High intensity (1-1.5 W/cm^2)
Electrical stimulation (see Tables 2-4).	Direct current
	Sinusoidal AC
	Pulsed AC (biphasic)
	Pulsed AC (monophasic)

the wound of selected biological agents in the cytokines and growth factor families to help facilitate wound healing [14-18]. Biological approaches may also include treatment of the wound area via tissue engineering techniques such as application of engineered skin equivalents [27] or combinations of growth factor application and skin grafts or isolated epithelial cells [19, 28].

Platelet-derived growth factor (PDGF), a key cytokine involved in the wound healing process, is released by platelets during blood clotting, one of the earliest cellular responses to an injury. PDGF stimulates fibroblast proliferation during the earlier phases of wound healing and is also involved in the latter phases of wound healing by promoting collagenase production from fibroblasts for wound remodelling. Although PDGF is initially released by platelets, it is also released by macrophages, vascular endothelial cells, fibroblasts and epithelial cells, and vascular smooth muscle cells also release it during the latter stages of wound healing [29].

Topical application of recombinant human PDGF (rhPDGF) has been used in the past to treat patients with persistent non-healing wounds where other conventional methods such as moist hydrogel dressings, topical application of antibiotics, wound debridements, and even other forms of physical therapies have proven to be unsuccessful [14]. Hom & Manivel [15] reported a case study in which a patient with a 12 year old chronic wound due to radiation therapy had received topical application of rhPDGF when conventional methods of wound management had failed. It was found that, after a 6 month course of rhPDGF treatment, sufficient granulation tissue developed to allow split-thickness skin graft to cover the remaining exposed tissue to take place. A follow up 1 year post rhPDGF treatment showed the wound area had completely re-epithelialised. Other studies have reported rhPDGF can increase the incidence of complete wound closure of neuropathic diabetic ulcers and decrease the time to achieve complete wound closure [14, 16]. Recombinant PDGF was also effective in the treatment of chronic full thickness pressure ulcers [17].

Another promising novel approach to wound management using bioactive factors is the use of vitronectin (VN) complexed with insulin-like growth factor (IGF). A recent study demonstrated that VN:IGF complexes can enhance keratinocyte migration and stimulate cell proliferation *in vitro* [18]. The study further reported positive wound healing outcomes using topical VN:IGF complexes in an *in vivo* porcine burn model. These findings include faster wound re-epithelialisation, with wounds tending to be less thick and with overall wound appearance better than the control-treated group.

In recent years, another form of biological treatment using maggot debridement therapy has re-emerged. Such therapies employ the use of the larvae of *Phaenicia sericata* for wound debridement, disinfection and enhanced healing. Studies have suggested that maggot debridement therapy is more effective and efficient compared to conventional wound debridement [30]. Furthermore, maggot therapy has been associated with controlling the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in wounds [31]. In recent studies, maggot larvae excretions/secretions from *Lucilia sericata* have been shown to promote human dermal neonatal fibroblast migration and is implicated in the healing of chronic wounds [32].

The versatile hydrogel is not only used to deliver chemical agents to the wound site but can also be used to deliver biological agents that promote wound healing. One such biological agent is the family of glycosaminoglycans (GAG). Glycosaminoglycans are polysaccharides found in the ECM of all vertebrates and are required for the maintenance of normal cellular functions. Kirker *et al.* [33] studied the wound healing properties of GAG hydrogel films on a rat model. It was found that after 5 days of treatment, a significant increase in wound re-epithelialisation was observed on wounds treated with GAG hydrogel; however, no differences in inflammatory response or wound contraction were observed. Also, after 10 days of treatment, wounds treated with GAG hydrogel contained more fibro-vascular tissue. It was hypothesised that GAG hydrogel provided a moist environment to facilitate assembly of other ECM components to promote cellular migration across the wound.

Delivery of growth factors for wound care management have largely been disappointing in both animal models and clinical trials, possibly due to the complex nature of wound healing *in vivo*. Wound healing involves a multitude of physiological and cellular interactions working in synergy. Treatment of chronic wounds with one growth factor alone may not be enough to help restore the body's balance of growth factors and cellular metabolic activities that is required for progression of chronic wounds to heal.

Physical wound management

Physical modalities used to treat chronic wounds include hyperbaric oxygen, whirlpool/pulsed lavage, low energy laser, ultrasound and electrical stimulation. These techniques are reviewed briefly, with electrical stimulation being examined in more detail. Hyperbaric oxygen utilises the principle that chronic wounds are often caused by tissue hypoxia due to poor circulation. Treatment with hyperbaric oxygen involves patients breathing 100% oxygen at pressures often two to three times greater than atmospheric pressure. This type of therapy may assist in wound healing by enhancing keratinocyte migration and accelerated epidermal maturation [20]. Although a promising therapeutic approach, as suggested in a recent critical review [34], access to the required equipment and infrastructure is currently limited.

Whirlpool or pulsed lavage, otherwise known as hydrotherapy, is used as a form of wound debridement and is achieved by the whirling and agitation of water in contact with the wound. It is also proposed that this process can dilute bacterial load found on wounds [21]. However, a recent review on wound cleansing techniques and its effectiveness on healing rates of pressure ulcers have found that there is still a lack of evidence to support the use of whirlpool [22]. Low energy laser treatment, also known as 'cold' laser, is the application of low-energy, low-power laser that may stimulate the physiological processes of wound healing. This is hypothesised to be via reducing inflammation and increasing collagen fibres [23], endothelial cell migration, proliferation and NO excretion [24].

Another physical therapy uses ultrasound, applying sound waves at frequencies greater than 20kHz (above human hearing range) at intensities of 1-1.5W/cm^2 to improve scar/wound outcome during the latter stage of wound healing. Ultrasound at lower intensities (0.3-1W/cm^2) has been shown to enhance collagen synthesis and tensile strength, angiogenesis and cellular recruitment [25] and may be a promising approach to wound healing, although little robust evidence is available [34].

Electrical stimulation is the application of an external electrical stimulus to promote and accelerate wound healing. Much of the literature has cited positive wound healing outcomes after an external stimulus is applied; however, there is little consensus on the most effective stimulation parameters. These positive wound healing outcomes extend to bone healing [35, 36] and in recent years have also been considered for application to nerve repair based on positive observations both *in vivo* and *in vitro* [37-39]. Of the physical modalities used to enhance wound healing, electrical stimulation with a focus on cutaneous (soft-tissue) healing will be discussed in detail.

Electrical stimulation

Historically, electricity was used over 300 years ago to treat cutaneous wounds when it was found that charged gold leaf prevented smallpox scars. During the 1960s several researchers reported on the positive healing effects of electrical stimulation on cutaneous wound healing both in animal and human wound models. These effects included faster wound healing and re-epithelialisation rates, and stronger scar tissues [40-42]. Since these studies, there has been a plethora of studies involving a variety of electrical parameters and experimental models.

It has been proposed that an external electrical stimulus mimics the human body's endogenous bioelectric systems that promote wound healing. Barker *et al.* [43] showed that, in normal uninjured human skin, a difference of electrical potentials can be detected and measured ranging between 10-60mV depending on the location of electrodes on the body surface. When the skin layers have been interrupted due to wounding, studies have shown that the injured tissue has a higher potential compared with the surrounding intact skin [44]. It has also been found that the transepithelial potential is low at the wound, increases with the distance from the wound and normalises to a value similar to the un-wounded skin at a distance of a few millimetres [43]. Thus it has been suggested that the endogenous electrical phenomena observed in wounds are not just side effects but instead play an active role in healing [45].

It has been well established that moisture can aid wound healing. Re-epithelialisation, in particular, can be up to 40% faster compared to air-exposed wounds [46]. This may be due to the bioelectric current flow being impeded on dry wounds and, conversely, moist wounds may provide pathways for the endogenous bioelectric current to flow more readily across the wound.

Based on the observed endogenous electrical properties, it has been hypothesised that external application of electrical current can be employed to assist in the healing of chronic wounds. There have been many studies on the effects of direct current (DC) and alternating current (sinusoidal or pulsed) in wound healing models in both humans and animals [47-54], or on cells involved in wound healing [55-57]. Pulsed current can be either monophasic or biphasic. Biphasic current includes a forward phase and a reverse phase and is either charge balanced (net zero DC current) or charge unbalanced (net DC current offset). Some examples of these various waveforms are shown in Figure 1. For the purpose of this review, sinusoidal alternating current is referred to as AC and other forms of AC such as pulsed will be specified accordingly.

DC stimulation regime typically involves the application of a current that is 1 second in duration or longer. Currents that are unidirectional but less than 1 second in duration are classified as alternating current (either sinusoidal or pulsed). Employment of DC in experimental models *in vivo* and *in vitro* can produce undesirable electrothermal, electrochemical as well as the desired electrophysical effects. An electrothermal effect is the process in which heat is released due to an electric current passing through a conductor and is governed by Joule's law. This effect can be minimised by reducing the current and treatment time, thus avoiding thermal damage. Electrochemical effects describe the chemical reaction that takes place between the electrodes and stimulation bath/tissues which may result in pH changes or release of electrode by-products which can cause chemical burns, blisters or be highly toxic to cells and tissues.

Depending on the waveform parameters, alternating current and biphasic stimulation can potentially eliminate the electrothermal and electrochemical effects observed in DC stimulation, thus reducing the undesirable side effects

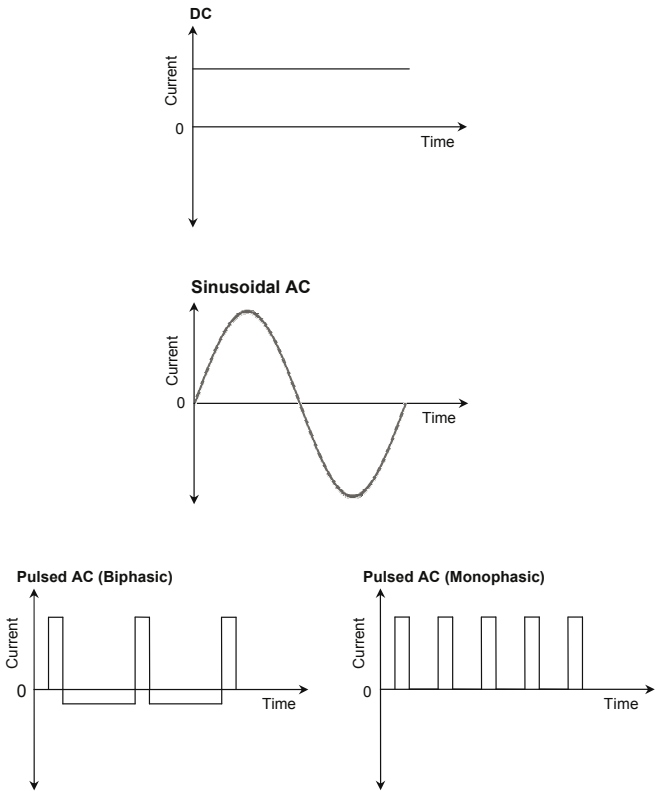

Figure 1. Illustration of various waveforms such as direct current (DC), sinusoidal alternating current waveform with net zero DC offset (AC), and alternating pulsed current (pulsed AC) in the form of biphasic current or monophasic current.

of electrical stimulation. An example of how waveform parameters may affect cytotoxcity is shown in Figure 2 [58]. Viability of a non-adherent lymphoblastic cell line KG1a was assessed after stimulation with AC waveform of amplitude 8mA (peak to peak) for 3 hours via pure platinum round wire electrodes (0.5 mm diameter). The frequencies of AC waves used were 10Hz, 60Hz and 200kHz. Controls were cells not exposed to electrodes or current and sham treatments were cells exposed to electrodes but were disconnected from the stimulator. Using cell staining techniques, it was found that AC stimulation using a low frequency (10Hz) affected KG1a viability 0 hours post stimulation and also at 30 hours post stimulation, indicating cell growth is reduced after low frequency AC stimulation in manner somewhat similar to DC stimulation. In contrast, KG1a cells stimulated at frequencies 60Hz and 200kHz showed no difference in cell viability. Correlating with viability data, cell growth was also not affected by AC stimulation at 60Hz and 200kHz [58].

Alternating current and pulsed current, in particular biphasic stimulation on wound healing models both *in vivo* and *in vitro,* have been studied less extensively compared to DC stimulation studies. This may be due to the more 'complex' nature of the waveforms compared to DC, thus introducing more variables such as waveform shape and frequencies that may impact on the cellular response to electrical stimulation.

Electrical stimulation in vivo

The literature reflects that, on the whole, *in vivo* studies of electrical stimulation in chronic wounds were performed before significant *in vitro* mechanistic studies were conducted. As early as 1969, *in vivo* clinical studies were taking place. These were followed by animal studies, mainly focused on the effects of electrical stimulation on bone healing [35, 36, 59, 60]. Studies on cutaneous wound healing in animals became more prevalent during the 1990s. A summary of *in vivo* studies, both on animals and humans using various stimulation regimes, are shown in Tables 2 & 3.

A wide variety of animal models have been used in studies to date. Animal type, electrical stimulation parameters and electrodes, and also outcome measures, are all highly variable between studies. While the majority of studies suggest some improvements in wound healing, the mechanisms for this are not clear. In terms of AC stimulation in animal wound healing models, both positive and negative outcomes have been reported. Studies of AC stimulation in the form of sinusoidal AC waveforms on rat skin incisions with wounds being electrically stimulated (1V, 100µA, 100Hz) 15 minutes per day showed an increase in collagen at the wound site compared to un-stimulated controls. Wound strength was comparable to control groups [61]. Wound strength is achieved by crosslinking of collagen III fibres into collagen I bundles during maturation/remodelling phase of wound healing. Although electrical stimulation in this study was found to promote collagen synthesis, the duration of this study did not allow wounds to reach this phase of wound healing.

Also using a rat skin model, Leffmann *et al.* [54] studied cutaneous wound healing using microamperage stimulation in the form of monophasic pulsed current. The electrical stimulation regime was 2 hour daily treatments of 100µA at 0.3Hz for 14 days on six rats, and control groups involved six rats receiving no stimulation. Analysis of wound size measurements and histological observations of fibroblast occurrence indicated that there was no significant differences between electrically treated and control groups at the end of the 14 day period.

Similar to animal studies, variable wound healing outcomes have been observed for electrical stimulation on human wounds. In general, electrical stimulation appears to increase wound healing rates and reduced wound size was observed in electrically treated wounds [40, 45, 47]. However, several of these studies lacked sufficient control groups [42, 49, 65, 66]. Furthermore, it is difficult to compare the data reported from these clinical studies due to the wide variety of outcome measures being reported. It is possible that the mixed results reported by

Table 2. Summary of electrical stimulation effects in animal models.

Method of study	Electrode type & spacing	Electrical parameters	Outcome measures and results
Wistar rats [61]	DC: steel sutures used as electrodes placed on the skin on either side and 1cm from the incision AC: lead plates (1cm^2) placed on both sides 1.5cm from the incision	DC 1V, 20µA (constant current or voltage unknown) Time: 60 min daily (duration not given) AC: Sinusoidal 1V, 100µA (peak), 300Hz Time: 15 min daily (duration not given)	Collagen content: significant increase for DC and AC stimulation groups Tensile strength: no significant difference for DC and AC stimulation groups
Handford mini-pigs (pressure sore model) [75] Gp 1: Denervated controls Gp 2: Denervated AC-stimulated skin Gp 3: Denervated DC-stimulated skin	Commercially available 3cm Encore Plus, UniPatch (conductive silver electrode patches) Electrodes placed 1cm distal (cathode) and proximal (anode) from wound periphery	DC 0.7mA (constant current) Maximum current density 30-200µA/cm^2 Time: 2h per day, 5 days a week for 30 days AC: Biphasic current (charge balanced) Current density below contraction levels (1189±219µA/cm^2) 40Hz, 300µs pulse width, 4s duty cycle (4s on, 4s off) Time: 2h per day, 5 days a week for 30 days	Healing time: unstimulated controls took longer to heal compared to DC and AC stimulation groups. Wound morphology: increased vascularity in DC and AC groups. No differences between DC and AC Wound strength: DC and AC stimulated skin samples perpendicular to current flow showed no change/ reduction in strength compared to normal skin Wound elasticity: stiffness of skin samples located parallel to current flow (DC and AC) was reduced by 50% compared to normal skin. However, stiffness was comparable to unstimulated denervated skin
Sprague-Dawley rats [54]	Carbonised rubber electrodes (0.41x0.41cm) Negative electrode placed directly on wound Positive electrode placed on shaved area contralateral to wound	AC: monophasic pulsed current 100µA, 0.3Hz at 50% duty cycle (3s on, 3s off) Time: 2h daily, 14 days	Wound size: no change Histological observations: Epithelial thickness: no significant difference Fibroblast count: no significant difference Blood vessel count: no significant difference
Yucatan mini pigs [63] Surgically induced partial-thickness, full-thickness and incisional wounds	4x13cm electrodes – maybe provided by commercial stimulator (Myomatic 1 microamperage pulsed galvanic stimulator) Placed on wounds for 7 consecutive days	AC: monophasic pulsed current Charge density: 5.8x10^{-6} C/cm^2 100µA, 60V, 0.1Hz, 3s duty cycle (3s on, 3s off) Time: 1h daily, 5 days	Wound size: no significant difference Visual appearance: no significant difference Tensile strength: no significant difference Collagen density: no significant difference Hydroxyproline deposition: no significant difference
Handford minipigs (pressure ulcer healing) [64]	Electrode material not specified Electrodes placed distally and proximally to the wound periphery	DC Current flow from proximal to distal electrode at constant amplitude of 0.6mA AC: biphasic pulsed current (charge balanced) 7-10mA, 40Hz, 300µs pulse width, 4s duty cycle (4s on, 4s off) Time: 2h daily, 5 days/week for up to 30 days	Wound reduction rate: DC and AC stimulated wounds showed accelerated wound closure Gross wound observations: wound contraction more rapid for ES groups compared to controls Histological observations, soluble protein concentrations, wound infections and mechanical properties: ES had minimal or no effect on all these parameters

these authors are due to the study method employed and inconsistency in electrode placement, electrode material, electrical stimulation parameters, and how data were analysed.

Jercinovic *et al.* [47] studied the effects of biphasic current stimulation on pressure ulcers, showing significant differences in stimulated and un-stimulated controls groups can be observed depending on how data were fitted into the author's wound healing model. It is worth noting that, on the whole, the study found healing rates of stimulated groups tended to be faster compared to un-stimulated controls. Although these studies have not all resulted in positive wound healing outcomes, and methodological flaws in clinical trials such as lack of control subjects do exist, the literature does appear to indicate that electrical stimulation appears to positively affect wound healing. What has been lacking in the studies is an understanding of the mechanism for hypothesised

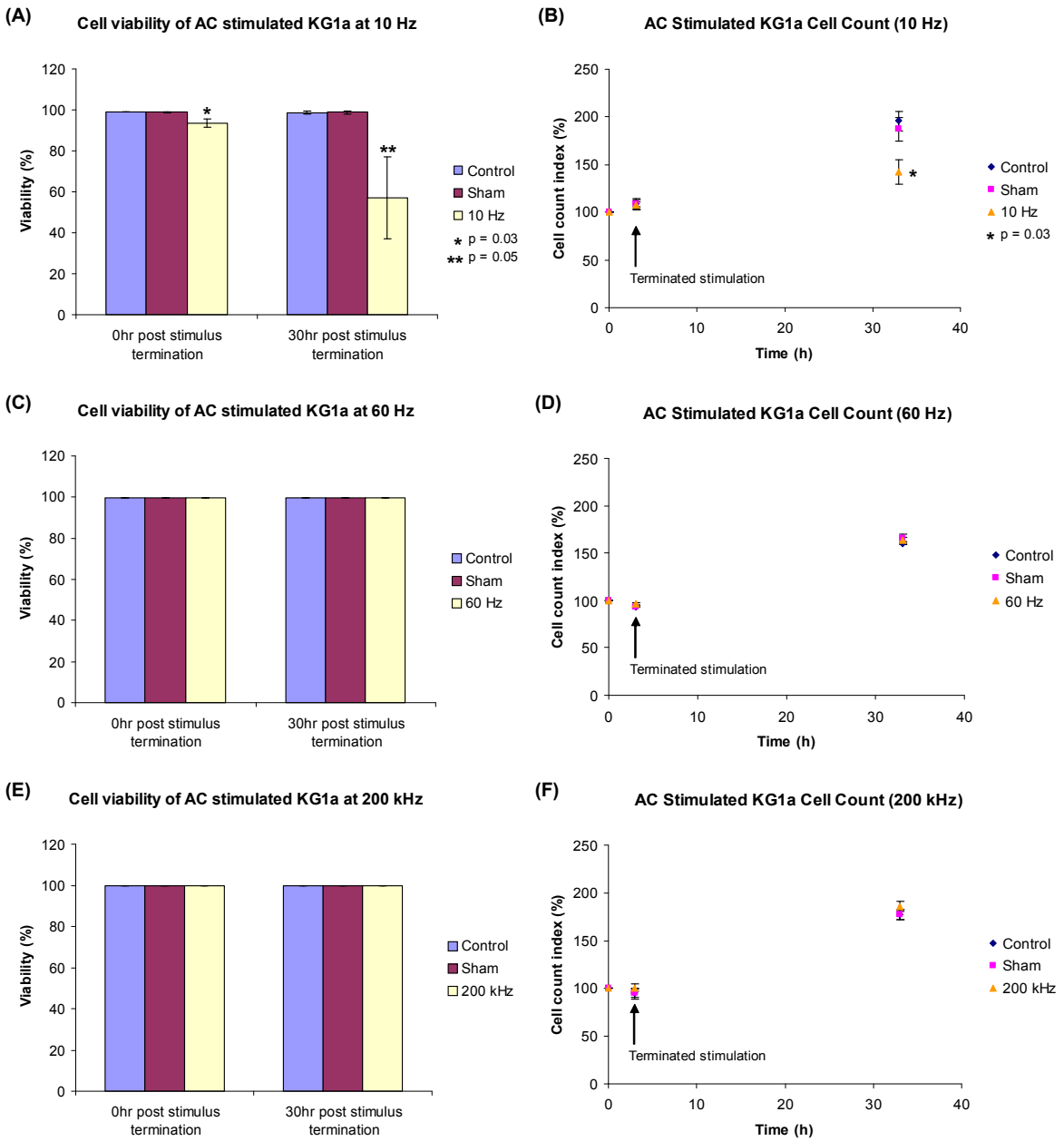

Figure 2. Viability and cell count of lymphoblastic KG1a cells exposed to AC stimulation (8mA peak to peak) for 3 hours via platinum round wire electrodes. Frequency of AC waveform was 10Hz (A-B), 60Hz (C-D) and 200kHz (E-F). Control groups were cells not exposed to electrodes and sham controls were cells exposed to electrodes but not connected to stimulator. Data were collected at two time points (0 hours post stimulation and 30 hours post stimulation). Data represent mean ± SEM. Students' t-test comparing treatment group and sham/control groups were conducted to obtain p-values [58].

Table 3. Summary of clinical trials involving electrical stimulation (ES) on wound healing.

Method of study	Electrode type & spacing	Electrical parameters	Outcome measures and results
67 patients (83 ischaemic ulcers) Contralateral wounds comparable in size and aetiology (8 of 67 patients); one ulcer received treatment and the others used as controls Total of 75 out of 83 ischaemic skin ulcers received electrotherapy [42]	2x2" copper mesh electrode –ve electrode on top of wound +ve electrode 15cm proximal to lesion (polarity of 'treatment' electrode switched after each growth plateau phase is observed)	DC 600μA constant current Time: 2h on, 4h off (repeated 3 times daily) until lesion completely healed	Number of fully healed wounds: 34 lesions (40%) healed completely 41 remaining lesions showed improvement ranging from 0-97% Wound infections: ES reduced the number of pathogens located on lesions Although there were lack of controls, the results revealed all but one lesion responded well to ES
30 patients with chronic dermal ischaemic ulcers Control group matched by age, diagnosis, wound aetiology, location and size The number of controls equalled number of experimental patients [40]	Stainless steel mesh electrodes and also flexible carbon material used as electrodes –ve electrode placed on wound +ve electrode placed 15-25cm proximal to wound (electrode polarities reversed after 3 days until the wound healed or a plateau in healing was observed)	DC 300-500μA for innervated tissues 500-700μA for denervated skin Current densities: 0.03-0.11mA/cm^2 Time: 2h twice daily, 5 days/week for 5 weeks	Healing rate: 1.5 to 2.5 times faster for stimulation groups compared to controls Wound strength: stronger scar tissue by palpation of closed wound and original wound margins for stimulated group Wound infections: less bacterial colonies observed for both stimulation groups
73 patients (109 pressure ulcers) 31 patients (control) 42 patients (ES) 20 patients (cross-over)* ** After 4 weeks of conventional treatment experienced ES treatment* [47]	50 or 75mm diameter self-adhering electrodes (Pals Plus Platinum) Placed on healthy skin 3cm from edge of ulcer	AC: Asymmetric biphasic current (charge balanced) Up to 35mA (varied for each patient) for minimal muscle contraction 40Hz, 250μs pulse width, 4s duty cycle (4s on, 4s off) Time: 2h daily, 5 days/week for 4 weeks	Healing rates: overall, faster healing rates were observed for ES groups 19 patients from cross-over group had increased healing rates after the treatment was switched to the same ES regime as ES groups
50 patients with spinal cord injuries having pressure sores: 16 patients assigned as Gp 1 17 patients assigned as Gp 2 17 patients assigned as Gp 3 [45]	Conducting rubber electrodes Gp 1: +ve electrode placed on top of wound 4 electrodes attached around the skin of wound representing a ring-shaped –ve electrode Gp 2: 2 electrodes placed on skin at ulcer edge across the wound; one +ve, one –ve Gp 3: Same placement as Gp 2 with electrodes connected to stimulators but power source disconnected	Gp 1: DC 0.6mA current Time: 2h daily (duration NS) Gp 2: DC 0.6mA current Time: 2h daily (duration NS) Gp 3: no current delivered	Average relative healing rates: Gp 1: 7.8%/day* Gp 2: 4.8%/day Gp 3: 4.2%/day ** statistically significant difference compared to unstimulated controls (Gp 3)* No significant difference in average relative healing rates between Gp 2 and Gp 3
23 patients with chronic wounds (wounds from all patients were electrically stimulated) Wounds were present for an average of 18.5 months prior to ES [65]	Conductive silicone electrodes placed above and below the wound	DC: constant current microamperage Current: 3μA for first cycle; 400nA for subsequent 8 cycles of treatment Cycle time: 23min; polarity reversed after 11.5min Treatment time: 3.5h daily, 5 days/week	Healing time: 34.8% achieved complete healing after an average of 45.6h of treatment 39.1% achieved ≥50% healing after an average of 39.7h of treatment

improvements in wound healing. Extensive *in vitro* studies have been reported in the literature in an attempt to address this knowledge gap.

Electrical stimulation in vitro

Since early reports of enhanced wound healing *in vivo*, many *in vitro* studies attempting to demonstrate the mechanism of action of electrical stimulation have been reported. A summary of the effects of electrical stimulation using various stimulation regimes on wound healing models *in vitro* is shown in Table 4. Similar to *in vivo* studies, the parameters used in these *in vitro* studies are highly variable. On the whole, it appears that cells are affected in different ways by a variety of electrical stimulation regimes. Such effects include physical changes such as alteration of cell shape and directional migration and biochemical changes such as growth factor production and protein and DNA synthesis.

Many of the *in vitro* studies initially examined cell migration behaviour. Migration studies of DC stimulation of cells suggested that stimulated cells display directional migration towards either the anode or cathode, depending on cell type. Erickson & Nuccitelli [67] observed embryonic quail fibroblast migration towards the cathode for DC field strengths of 1-10mV/mm. This effect was also observed in skin-derived keratinocytes [71]. Cell migration towards the anode was observed when DC fields of 75-100mV/mm were applied to human umbilical vein endothelial cells (HUVEC) [70]. Interestingly, human monocyte-derived macrophages were observed to migrate in a zigzag manner perpendicular to an applied AC field when a stimulus of 2mV/mm (peak to peak) at a frequency of 1Hz was applied [56]. All of the above studies used silver/silver chloride electrodes.

Studies examining other aspects of cell behaviour during electrical stimulation of cells *in vitro* have also been conducted. DC-stimulated HUVEC have been shown to elongate and reorientate in a direction perpendicular to the applied field. Furthermore, these cells had an increased production of vascular endothelial growth factor (VEGF), indicating electrical stimulation may promote angiogenesis to assist wound healing [70]. Asymmetric biphasic current has been implicated in development of differentiated cell morphology of keratinocytes [55]. Biphasic current, in the form of high voltage pulses, has also been associated with increases in collagen and DNA synthesis, and with cell migration [68, 72].

As outlined above, cell migration is a key event in normal wound healing that involves migration of an appropriately timed sequence of specific cell types to the site of injury. This migration sequence is essential for localised synthesis of soluble proteins such as chemotactic cytokines and structural proteins such as collagen. Since electrically directed cell migration *in vitro* may be translated to promotion of cell migration to the wounded tissue *in vivo*, this is often a focus for mechanistic studies.

Mechanisms of accelerated wound healing by electrical stimulation

A gap exists in the understanding of how electrical stimulation may bring about positive effects and, until the mechanisms are better understood, selecting appropriate stimulation regimes for *in vivo* use will remain an empirical exercise. *In vitro* studies on some of the proposed mechanisms used by cells in response to an electrical stimulus have suggested that electrical stimulation may affect migration and proliferation of the cells involved via expression of integrin receptors, up-regulation of protein kinase A, epidermal and vascular endothelial growth factors and expression of their respective receptors.

It has been suggested that directional cellular migration is mediated through ß2 integrin receptors when it was found that antibody blocking of ß2 integrin receptors on macrophages caused changes in cell movement. In addition, blocking ß1 integrin receptors did not impede electric field induced migration [73]. Both protein kinase A (PKA) and protein kinase C (PKC) have a pivotal role in intracellular signalling pathways [74] and the regulation of many cellular functions is achieved by phosphorylation of proteins, enzymes and receptors. Observations of human keratinocytes treated with a PKA inhibitor showed a markedly decreased directional motility when electrically stimulated, whereas inhibition of PKC did not significantly affect directional migration of these cells [69]. Combined, these studies suggest involvement of ß2 integrin receptors and the PKA intracellular signalling pathway during directed migration induced by DC stimulation.

Growth factors such as epidermal growth factor (EGF) and insulin have been reported to be involved in human keratinocyte migration. It was found that absence of these growth factors reduced cell migration rate during DC stimulation, but directional migration was still maintained [75]. Also, when EGF receptor was blocked with low concentrations of a protein tyrosine kinase inhibitor specific to EGF receptors, directional migration of keratinocytes was inhibited and at higher concentrations; migration rate was also decreased [76]. The same study also found EGF receptor aggregated on cathode face of cells after being stimulated and, when treated with an EGF receptor inhibitor, EGF receptor localisation ceased.

Few studies have examined the role of ECM in migration of electrically stimulated cells, in particular cells involved in wound healing. Studies have been conducted on the migratory effects of electrically stimulated cells seeded on collagen substrate [69, 75-77]. However, there is a lack of studies on the effects of electrical stimulation on cell migration on various ECM substrates. This is a significant gap in

Table 4. Summary of electrical stimulation (ES) on wound healing models in vitro.

Method of study	Electrode type & spacing	Electrical parameters	Outcome measures and results
Embryonic quail fibroblasts [67]	Ag/AgCl wire immersed in saline-filled wells that were connected to the chamber wells by 10cm long 2% agar bridges 45x50mm chamber	DC Field strength: 1-600mV/mm (constant voltage) Time: 90min	Direction of migration Migration towards cathode when field strength was about 150mV/mm Movement rapidly reversed when field is reversed Average migration speed: 0.8-0.9µm/min
Human fibroblasts IMR-90 cell line [68]	Stainless steel Flat, rectangular (2.2x1.5cm) Spaced approx 7.5cm apart	AC: High voltage pulsed current 0-300V, 60-120Hz, 100µs pulse width (twin-spike) Time: 20min	Maximal protein synthesis: 50V (100Hz) Maximal DNA synthesis: 75V (100Hz) ** Inhibition of protein and DNA synthesis at intensities greater than 250V*
Epidermal explants (keratinocyte culture) [55]	Platinum electrodes (50x2x0.5mm) Electrodes placed 60mm apart	AC: Asymmetric biphasic current (charge balanced) 20mA, 0.25ms pulse width, 40Hz frequency, 4s duty cycle (4s on, 4s off) Time: 40min/day, 11 days	Cell growth: ES suppressed of cell growth Cell morphology: ES had affected cell differentiation (based on histology) More epidermal layers were formed around stimulated explants
Human macrophage Human blood monocyte isolation ◊ monocyte/macrophage transformation [56]	Ag-AgCl electrodes in agar bridge Agar bridges: 8-10cm long (filled with Steinberg's saline gelled by 2% agar) Ag-AgCl electrodes, and electrode baths contain Steinberg's saline Chamber geometry: 60x10x0.2mm	AC: Sinusoidal 2mV/mm (peak to peak), 10Hz Time: 60-90min Migrating macrophages recorded in real time at 10min intervals	Migration: ES macrophages migrated in a zigzag manner approximately perpendicular to the electric field vector with increased migration rate compared to unstimulated controls and is ß2 integrin dependent but not ß1 Cell morphology: stimulated cells had elongated shape, membrane ruffles, and a reorganisation of microfilaments from peripheral ring-like structures to focal podosome structures
Human keratinocytes (grown on collagen) [69]	Ag-AgCl wire immersed in saline-filled wells that were connected to the chamber wells by 10cm long 2% agar bridges 45x50mm stimulation chamber	DC: physiologic electric field Voltage: 100mV/mm Current <0.6mA (minimise joule heating) Time: 1h	Direction of cell migration: ES cells migrated towards the cathode. Random migration was observed for unstimulated control cells Involvement of PKA: PKA inhibitor showed a markedly decreased directional motility when electrically stimulated, and inhibition of PKC did not significantly affect directional migration of these cells
HUVEC cell line [70]	Ag-AgCl wire immersed in Steinberg's solution that were connected to the chamber wells by 15cm long agar salt bridges 22x10x0.2mm stimulation chamber	DC: physiologic electrical field Voltage: 100-300mV/mm Time: up to 72h	Cell alignment: Stimulated cell axis perpendicular to electrical field and is both time and voltage dependent Cell elongation and alignment required VEGF receptor activation

the knowledge in this area, particularly given that the composition of ECM varies throughout the stages of wound healing. For example, fibrin is the predominant component of the ECM during the initial phases of wound healing, followed by collagen III during the intermediate (proliferative) phase of wound healing. Collagen III is cross-linked during the latter (maturation/remodelling) phase of wound healing, thus giving rise to an ECM comprised mainly of collagen I. Further mechanistic studies on aspects relating to the effects of electrical stimulation on cell behaviour, in particular the impact of ECM on migration under external electrical stimulation, would be of great interest.

Conclusion

Although electrical stimulation for accelerating wound healing has been studied over several decades, there remain many questions on both the underlying mechanisms and the specific waveforms and timing of stimulation that should be applied for optimal effect. It is clear that by increasing understanding of the cellular mechanisms, new approaches to therapy can be devised. However, better designed studies with appropriate controls and consistency of outcome measures are essential.

The complex nature of the wound healing process *in vivo* has also impeded a general understanding of the mechanisms of electrical and wound healing as it is very difficult to replicate wound healing conditions *in vitro*. The many studies that support the positive effect of electrical stimulation in the acceleration of wound healing suggest that further research is justified in this field. Potential applications include not only the frequently reported chronic cutaneous wound therapies, but also encompass others such as bone healing and neural repair and regeneration.

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