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# Electrical stimulation: a novel therapeutic strategy to heal biological wounds

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Electrical stimulation (ES) has emerged as a powerful therapeutic modality for enhancing biological wound healing. This non-invasive technique utilizes low-level electrical currents to promote tissue regeneration and expedite the wound healing process. ES has been shown to accelerate wound closure, reduce inflammation, enhance angiogenesis, and modulate cell migration and proliferation through various mechanisms. The principle goal of wound management is the rapid recovery of the anatomical continuity of the skin, to prevent infections from the external environment and maintain homeostasis conditions inside. ES at the wound site is a compelling strategy for skin wound repair. Several ES applications are described in medical literature like AC, DC, and PC to improve cutaneous perfusion and accelerate wound healing. This review aimed to evaluate the primary factors and provides an overview of the potential benefits and mechanisms of ES in wound healing, and its ability to stimulate cellular responses, promote tissue regeneration, and improve overall healing outcomes. We also shed light on the application of ES which holds excellent promise as an adjunct therapy for various types of wounds, including chronic wounds, diabetic ulcers, and surgical incisions.



## 1. Introduction

Skin comprises cells that form a protective layer (epidermis and dermis) and their secretions. To create a semipermeable barrier against various microorganisms, the epidermis and dermis cells along with their secretions are required. Wounds are formed when the skin's barrier function is compromised by surgery, burns, unintentional injury, skin disorders, microbial infection, or metabolic dysfunction to repair and restore tissue functioning after injury, so wound healing is a highly complex biological process.[1–3] Following an injury, the skin undergoes a series of interconnected molecular and cellular processes to heal effectively.[4,5] Initially, hemostasis stops blood flow at the wound site, followed by an inflammatory phase that enhances vascular permeability, facilitating the influx of essential nutrients, enzymes, and immune cells such as lymphocytes, neutrophils, and macrophages. As the wound progresses into the proliferative phase, fibroblasts migrate to the wound bed in response to growth factors, secreting a temporary extracellular matrix (ECM) that acts as a scaffold for cellular growth.[6] Concurrently, endothelial cells experience rapid growth, and angiogenesis occurs in the granulation tissue to support the high metabolic demands. During the final remodeling phase, the vascular network regresses, and extensive replacement and remodeling of the ECM and collagen take place. In cases of chronic wounds, the inflammatory phase may persist for weeks to months, hindering progression into the proliferative and remodeling stages, ultimately leading to chronicity.[7] Chronic wounds prone to slow healing include venous leg ulcers, pressure ulcers, and diabetic foot ulcers, often requiring more than a year to heal completely.[8]

ES stands as a non-pharmacological, non-invasive physical stimulus.[9] The array of biological effects attributed to ES is extensive. At the molecular level, it can facilitate the movement of both charged and uncharged biomolecules through biological membranes, employing mechanisms such as electrophoresis and electroosmosis. ES can interact with several physical components at the cellular level, including the cytoskeleton, membrane-bound proteins, intracellular organelles, and ion channels.[10] These interactions affect cellular activities and functions such as migration, contraction, orientation, and

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proliferation.[11] ES has been used in various biological and clinical applications due to its direct effects on cells and biomolecules. Electrical signals are being used in regenerative medicine to aid stem cell differentiation, tissue regeneration, cell proliferation, maturation and the remodelling of artificial tissue constructs.[10] This encompasses direct current (DC), alternating current (AC), pulsed current (PC), and pulsed electromagnetic fields (PEMF). ES has also been identified as a regulator of wound healing, influencing the migration of epidermal stem cells (EpSCs) within skin cells.[12] The process of skin wound healing is intricately orchestrated, involving inflammation, granulation tissue formation, matrix remodelling, and re-epithelialization. Enhancing wound healing necessitates the development of a wound dressing serving as a barrier to foster wound healing process and promote the regeneration of skin tissue. Consequently, there is a significant desire for a novel therapeutic approach capable of facilitating the regenerative activities of skin cells, actively contributing to skin wound healing, and promoting tissue regeneration. By stimulating cell proliferation, creating less condensed collagen fibrils, and changing macrophage responses, ES can shift the damage response from healing/scarring to regeneration.[13] Also, some research shows that the triboelectric nanogenerator (TENG)-generated ACES of 50 A has been proven to boost fibroblast cell growth.[14]

Numerous wound care dressings of current time are passive in nature and cannot to respond to changes in the wound environment actively. These passive dressings, in certain instances, may release substances such as anti-inflammatory drugs, antibiotics, antibacterial compounds, angiogenic factors, and absorb excessive exudate.[15] More sophisticated dressings of this kind passively release biological elements and compounds to support the process of tissue healing.[16–18] A notable drawback of existing wound care products is their incapacity to furnish information regarding the status of the wound bed and its healing progression. As a result, patients need to undergo frequent screenings to evaluate the healing process and be examined for potential infections. The heightened frequency of visits, essential for continuous monitoring, not only contributes to the overall treatment cost but also places added strain on medical facilities. Moreover, frequent trips to medical centers pose a significant challenge, particularly for patients residing in remote areas. Another critical limitation associated with passive wound care products lies in their inability to discern variations among different stages of wound healing. Throughout the healing process, the rates of physiological processes differ, leading to variations in the concentration of necessary factors and drugs over time. Additionally, challenges arise in the proper use of antibiotics, as incorrect and prophylactic usage may contribute to the development of antibiotic-resistant bacteria.[19,20]

In this review, our objective is to: (1) discuss the fundamental mechanisms of tissue and cellular response to ES (2) review on dominant factors like voltage and morphology of healing to either study or utilize ES (3) review the clinical evidence on the efficacy of ES for wound healing (4) discuss the critical needs and gaps on using AC, DC and PC in healing therapy. In our review, the term "electrical stimulation" encompasses a wide-ranging definition in (Table 1). It signifies the physiological stimulation of cellular and tissue activities achieved by applying an electrical field or current. Additionally, it includes the physical aspect of "stimulation", wherein faster molecular transports through biological membranes are facilitated. Further research and clinical studies are warranted to optimize stimulation parameters, determine optimal treatment protocols, and enhance our understanding of the underlying cellular and molecular mechanisms involved in ES-mediated wound healing. The integration of electrical stimulation into clinical practice has the potential to revolutionize wound care and improve patient outcomes.

## 2. Current scenario of wound healing in market

Wound-healing technology is a big business venture offering more than $15 billion for wound-closed goods and a further $12 billion on the skin-scar prevention market. Approximately 2–4.5 million individuals in the United States are believed to be impacted by chronic wounds, resulting in an economic burden of $25 billion per year on the U.S. economy, as shown in (Fig. 1). This burden is on the rise due to escalating healthcare costs, an aging population, and an increased prevalence of comorbidities, such as diabetes.[17,27,28] Intelligent systems, sensing, response, or reporting devices, or a combination of them, can solve many of the problems related to wound healing, especially for chronic wounds. Skin wounds, prevalent in accidental injuries or surgical procedures, compromise skin integrity and heighten the risk of infection. Recent research indicates a close correlation between wound repair and the electric field of the wound and skin. Wound healing unfolds through a sequence of carefully orchestrated biological events. Several local and systemic factors can hinder the successful progression of wound healing, including chronic inflammation, poor perfusion, elevated local pressure, inadequate nutrition and infection.[29] Vascularization of the wound bed is essential for ensuring proper oxygenation and delivery of nutrients to the healing tissue.[30] In recent times, ES therapy has emerged as a potential method to accelerate wound healing (as shown in Table 1), complemented by progress in the development of flexible electronic devices aimed at enhancing recovery speed.[31] Nevertheless, stubborn non-healing wounds, like diabetic foot ulcers and pathological scars, persist as notable medical issues, presenting both physiological and psychological hurdles for patients. The process of wound contraction, a fundamental physiological mechanism, assumes a critical role in the context of delayed healing.[32] Chronic wounds, characterized by a hypoxic environment and an abundance of proteins conducive to bacterial growth, are particularly prone to bacterial infections.[33] While various pathogens commonly inhabit chronic wounds, their presence doesn't necessarily indicate wound infection. Some studies suggest that low levels of bacteria may contribute to promoting wound healing. Bacteria have been identified as producers of proteolytic enzymes that aid in

Table 1 Attributes of frequently employed waveforms for ES therapy.[21]

| Type of ES | Characteristics | Reference |
|---|---|---|
| Direct current (DC) | Continuous flow of electric charge in a monophasic waveform (in one direction) involves delivering currents ranging from 20–200 μA at a low voltage | 22 |
| Alternating current (AC) | Biphasic waveform, characterized by two symmetrical electrical pulses alternating one after the other, typically utilizes voltages between 50–150 V, depending on tissue hydration | 22 and 23 |
| Pulsed current (PC) | Intermittent flow of charged particles with gaps in current flow can exhibit either a monophasic or biphasic waveform. Currents ranging from 1.2–1.5 mA can be supplied to the tissue at high voltage | 24 and 25 |
| Degenerate wave (DW) | A specific type of waveform employed in certain biofeedback devices involves a constant current of 0.3 mA, delivering an electric field of 10 mV $mm^{-1}$ between the electrodes | 26 |

digestion of debris and stimulate protease release from neutrophils. However, beyond a critical colonization threshold, infection occurs, leading to impaired wound healing.[30]

## 3. Biology of wound-healing

Our skin is specialized in connecting with the outside and offers a range of critical homeostatic tasks, from thermostability regulation to the detection of environmental stimuli. The skin works primarily as a defense barrier that prevents desiccation and harm to interior tissues mechanically, chemically, thermally, and phonetically. This defense includes an advanced immune barrier reaction to defend against pathogenic infection and promote commensal microbes through an ingeniously tailored host–microbiota axis.[34–39] The skin has also developed efficient and fast mechanisms to seal violations in a process known collectively as the wound healing response. Wound cure comprises several stages initiated by biochemical pathways both intra- and intercellular which harmoniously coordinate to restore tissue homeostasis and integrity. There are additional cellular elements, such as cascades of inflammatory and coagulatory processes. Several cells are engaged as immune components, such as keratinocyte, fibroblasts, and endothelial cells, macrophages, neutrophils, monocytes, and lymphocytes.[40,41] Healing also involves various cell groups, the extra cells, and soluble mediators, such as growth factors and cytokines. Although the healing process is ongoing, it may be split arbitrarily into four phases: (i) coagulation and hemostasis; (ii) inflammation; (iii) proliferation; and (iv) scar-type wound remodeling, resulting in architectural and physiological recovery after damage (Fig. 2). These steps are detailed in the following sections.

### 3.1 Coagulation and hemostasis

The injured blood vessels contracted immediately after injury, and a blood clot develops, avoiding vascular damage and exsanguination.[41] When they meet the vascular subendothelial matrix, platelets are activated, which play an essential role in hemostasis and coagulation. Platelet receptors (*e.g.*, glycoprotein VI) interact with (ECM) proteins that foster adhesion to the blood vessel wall (*e.g.*, collagen, Willebrand-factor, and fibronectin). Thrombin stimulates activation of the platelet, producing structural changes and releasing alpha and dense pellets containing the coagulation-enhancing bioactive chemicals. Fibrin, fibronectin, vitronectin, thrombospondin and insoluble clot (eschar) are mainly used for plugging the wound and preventing bleeding. Thrombospondin also fulfils a variety of secondary tasks, including protection of arriving immune cells against bacterial invasion, act as a cytokine reservoir and growth factor which regulates the behaviour of early repair cells.[43]

In recruiting immune cells to the site of injury, platelets are essential, either by directly trapping immune cells or by releasing a chemokine secretome after degranulation. The secretome platelet also includes development factors, including fibroblasts and keratinocytes, that encourage resident skin cells.[44] Platelets have a major role in the early suppression of bacterial inflammations as the most numerous cell type during early healing. They express many amazing receptors (TLRs).[45] The coagulation process is disrupted, avoiding excessive thrombosis once sufficient coagulation has occurred. The aggregate platelets here are blocked by prostacyclin, antithrombin III-inhibited thrombin, activated protein C coagulation factors V and VII. At the same time, smooth muscle cells and endothelial cells repair the damaged artery wall and multiply in response to the released PDGF.[46,47] Endothelial parents are also recruited in support, as there is limited proliferative potential of regenerative endothelial cells.

### 3.2 Inflammation

The principal defence against pathogenic wound invasion has evolved from innate inflammation. The initiation of this immune response is prompted by signals of injury, including damage-associated molecular patterns (DAMPs) produced from bacterial components by necrotic cells and tissues, along with molecular patterns associated with disease (PAMPs). Through the binding pattern recognition receptors, these PAMPs and DAMPs enable resident immune cells, like mast cells, Langerhans cells, T-cells, and macrophages, to generate inflammatory pathways downstream.[48] After releasing chemokines, and pro-

 

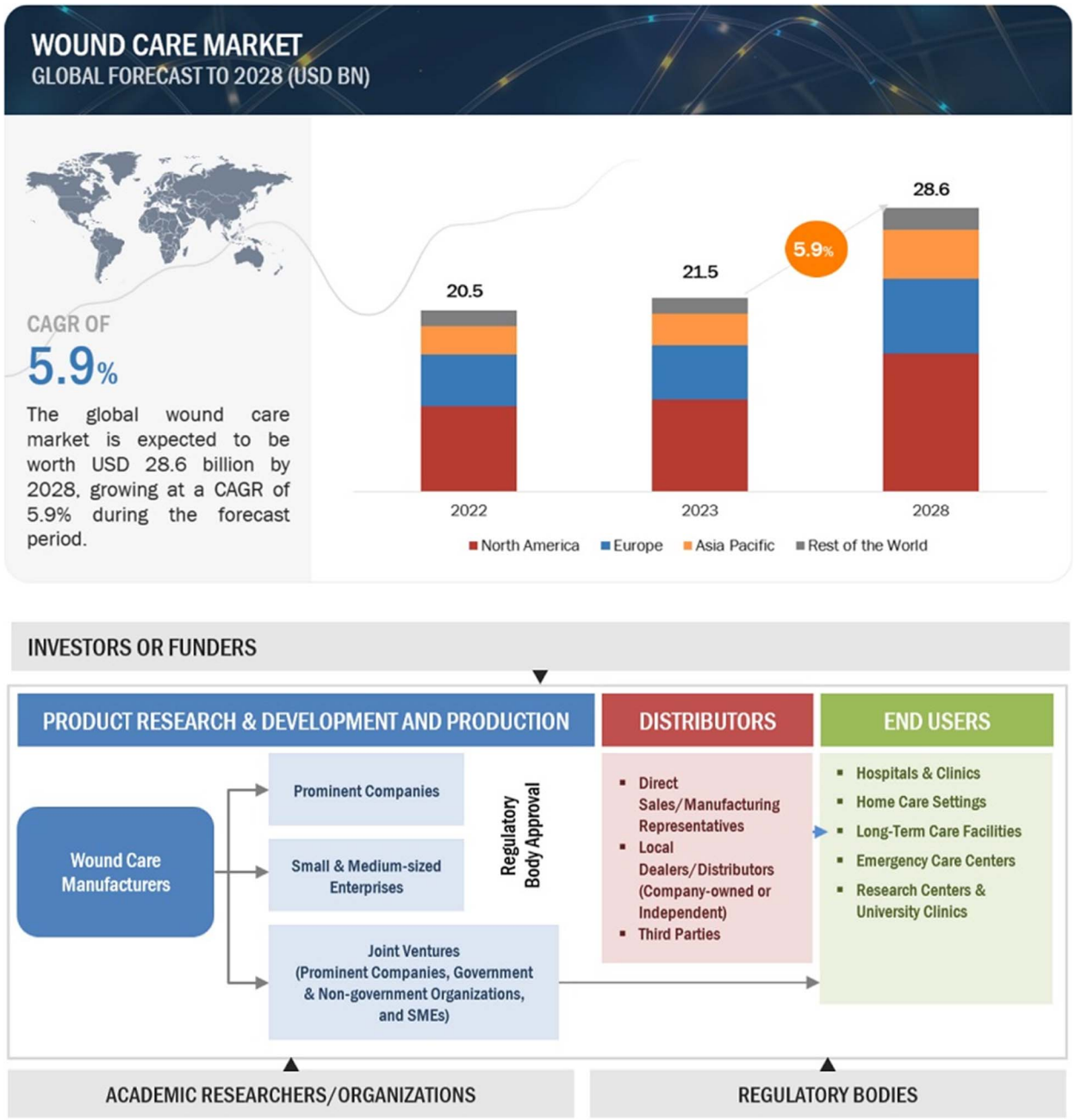


Fig. 1 Wound care market strategies for the future economic.[17,27,28]

inflammatory cytokines, leukocytes circulate to the injury site. Pro-inflammatory chemicals are also an incentive to vasodilate, which enhances neutrophil and monocyte adherence and diapedesis together with the production of endothelial cell-access molecules, such as selectins.[49,50] Indeed, selectins have been clearly demonstrated in immune cell recruitment with the impairment of immune cell infiltrations and wound healing by genetic and pharmacological blockades of E and P-selectin.

### 3.3 Proliferation

The proliferation healing phase is characterized by significant activation in wound closure, matrix deposition, and angiogenesis involving endothelial cells, macrophages, fibroblasts, and keratinocytes. Keratinocytes undergo activation triggered by alterations in mechanical tension and electrical gradients, along with exposure to hydrogen peroxide, pathogens, growth factors, and cytokines, often beginning as early as 12 hours after the occurrence of damage. This stimulation induces keratinocytes on the wound edge to undergo an incisive and migratory phenotypic partial epithelial–mesenchymal transition.[51,52] A shift from top-to-bottom polarity to the front to rear polarity occurs, facilitating the migration of leading keratinocytes in a process known as re-epithelialization. These keratinocytes move laterally across the wound to reconstruct the

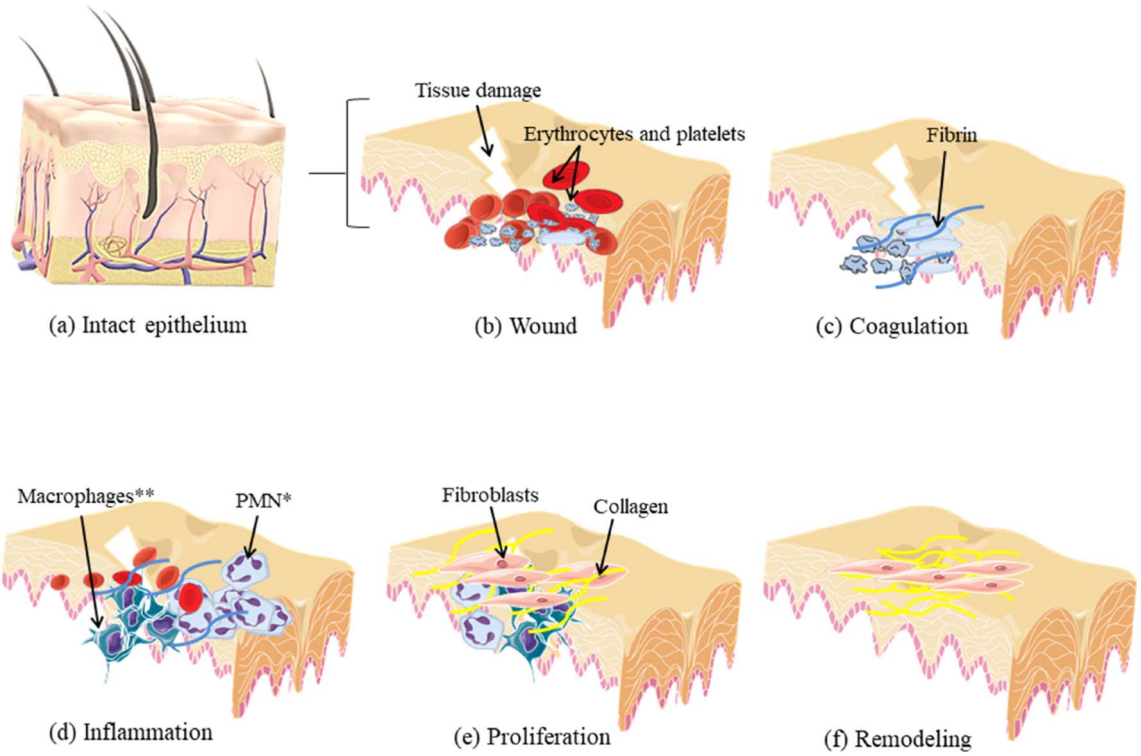


Fig. 2 Restoration of architecture and physiology following damage. The phases of biological wound healing process in (a–d) following the wound, there is an inflammatory phase that includes blood component leakage (which is followed by platelet aggregation, blood clotting, and inflammation); (e) keratinocytes, fibroblasts, and endothelial cells proliferate; (f) and after that granular tissue remodelling and re-epithelialization is the final stage. Reproduced from ref. 42 with permission from MDPI, copyright 2022.

epidermal layer. With PCK$\alpha$-mediated adjustments in demos stickiness and EF-medical modifications in adherence joints, keratinocytes modulate their cell adhesion, enabling them to replace order by the migrating epithelial sheet. Keratinocytes release the matrix metalloproteinase (MMPs) in the neo-epidermis to help them move and develop new ECM proteins to rebuild their membrane basement.[53,54]

### 3.4 Matrix remodelling

The ECM is restructured to cover the full injury from the first deposition of a fibrin clot to the creation of a mature collagen-rich scar of type I several years later. Initial fibrin clot replacement is done by proteoglycan, fibronectin, and hyaluronan, suggesting that the fibroblasts are the main type of cell responsible for wound ECM remodelling and later repair of mature collagen fibrils.[52,55] Proteoglycan helps build mature collagen fibrils that are joined together and act as a cell migration conduit. Approximately 80 percent of the collagen type I is present in non-injured adult skin. Granulation tissue, by contrast, consists primarily of the embryo-associated collagen type III (about 30%). With healing, collagen type III will be replaced by collagen kind I, and the tensile strength of the growing scar will be improved directly. The integrity and architecture of the ECM scar are never entirely unwounded in the skin. Collagen fibrils in scar dermis assume massive, parallel bundles and basket-weaved orientation. Wound scar tissue, therefore, only provides up to 80% of pre-wounding strength in post-injury.[56]

### 3.5 Microbiology of chronic wounds

On a cellular level, slow-healing "chronic" wounds (existing for more than four weeks) are characterized by extended inflammation, disorganized or insufficient ECM deposition, reduced neovascularization, and delayed reepithelialisation, as shown in Fig. 3.

Chronic wounds in the extremities often coincide with conditions such as venous valve insufficiency, arterial disease, or vasculitis, all of which significantly affect both the healing

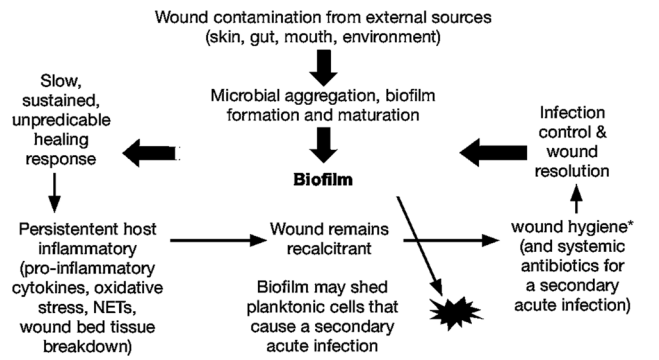


Fig. 3 Advancement and resolution of infections in chronic wounds.[57]

process and vulnerability to infections. These infections in chronic wounds are responsible for roughly 85% of all non-traumatic lower-limb amputations and contribute to 7–8% of mortality among patients with spinal cord injuries. Their concentration, species composition, and the host's response determine microbes' role in infection. Low concentrations of germs often found on the skin or other regions of the body, such as, streptococci, *Pseudomonas*, coliform bacteria and staphylococci, contaminate and even colonize wounds. This is regarded as typical and isn't thought to be an infection or a barrier to healing. Indeed, wound colonization by these common microorganisms has been found to aid healing in some cases. Inflammation (warmth, erythema, induration, soreness), purulent discharges, and, for chronic wounds, foul odor, delayed healing, necrosis, and discolored or friable granulation tissue, are all clinical indicators of the change from colonization to infection. Infection by some aggressive microbe strains, on the other hand, can occur at virtually any dose. Clinical microbiology has concentrated on establishing the quantities and identities of microorganisms to forecast and battle wound infections.[15,58–60]

Extensive research has been conducted to assess the impact of microorganisms on chronic wounds, employing various methods to discern their potential involvement in non-healing. Challenges arise due to the diverse approaches in specimen collection and microbiological analysis, along with variations in patient demographics, ulcer etiology, and infection status, making comparisons between studies challenging. Additionally, clinical studies often have limitations in scope and rely on assumptions regarding the relative pathogenicity of microorganisms. Anaerobic organisms, in addition to aerobes, are regularly found in wounds with varying degrees of success. *Peptostreptococcus* species, along with pigmented and non-pigmented *Prevotella*/*Porphyromonas* species, emerged as the predominant isolates found in both infected and non-infected leg ulcers. Existing literature on the subject suggests that the microbial composition of chronic wounds remains relatively stable over time. Hansson *et al.* proposed the microflora of chronic wounds to be a highly stable entity, noting that 90% of ulcers monitored for four months, or until healing occurred, exhibited the presence of at least one resident organism identified in all monthly swabs.[61] Nevertheless, there is a lack of definitive studies on bacterial succession in chronic wounds, the impact of antibiotics on this succession, or the relationships between bacterial lineages and the healing process.[58,62,63]

### 3.6 Factors affecting wound healing

Numerous environmental and personal factors influence the process of wound healing. The efficacy of therapy may be limited by factors such as microbial infections, ischemia,[64] or the patient's ageing, which weakens the body's natural ability to regenerate.[65] Broadly speaking, two primary categories of agents influence wound healing: systemic factors, which pertain to the overall health condition of the patient, and local factors, which directly affect the characteristics of the wound itself. Local agents encompass infections, venous sufficiency, and oxygenation levels. At the same time, the second category includes age, gender, stress, ischemia, chronic diseases, levels of sex hormones, dietary habits, underlying comorbidities, and addictions such as alcoholism. Other authors make distinctions among subcategories. The divide influenced by the Beyene group's work[66] is depicted in the diagram in Fig. 4. Usually, multiple variables contribute to the increasing impairment of wound healing at the same time. Because of this, getting rid of some of them can greatly enhance the standard of care. Numerous methods are used in modern medicine to lessen the impact of adverse systemic and local variables.[68]

On the other hand, the impact of poor nutrition, which impedes the body's capacity to mend wounds, can be readily neutralised using straightforward techniques. This if the proper wound care is used, the chance of bacterial infection can be reduced. However, several factors that impact wound healing continue to pose a substantial therapeutic challenge. In particular, the agents associated with the diseases of civilization provide challenges because it is not easy to reverse the years of neglect that have damaged the organism's general state.[69,70] An increasing number of writers emphasise the role that nutrition plays in the proper healing of wounds. Malnutrition and vitamin deficiencies can hinder the body's capacity to repair wounds. Because of this, modern therapy is moving towards a more holistic approach and involves the use of medications that support both the body's overall functioning and localised effects. For instance, because the metabolic demand for arginine increases during acute stress, supplementing with it as an adjuvant treatment for wound care can yield excellent effects. Because it affects the immune system and promotes wound healing, arginine is essential during times of damage and increased growth. In a similar vein, oral glutamine supplementation has been shown to raise the amount of mature collagen and decrease the propensity for wounds to rupture.[71]

However, as noted by Arnold and Barbul, maintaining the proper blood glucose level is essential for the treatment of diabetic patients.[72] And last, it is also impossible to ignore the role that protein dosages play in wound healing. Malnutrition deficient in protein and calories lowers antibody levels, phagocytic activity, T-cell function, and wound tensile strength. The body is unable to prevent infection of the wound as a result. The list of factors that affect healing is considerably longer. To help with the process, adequate amounts of micronutrients, vitamins, or fatty acids must be present. Furthermore, deficiencies in specific components may impact the therapeutic pathways. The Hayman group research effectively demonstrated the supportive role that appropriate nutrition played during the therapy.[73] The clinical investigation conducted by the team revealed that the healing of the pressure ulcer was enhanced using high-energy supplements that were enhanced by nutrition. Physiological stress is the next component influencing the healing of wounds. Research has indicated that stress impedes the normal healing process at the site of damage by greatly delaying it. Moreover, it may lead to psychologically ill behaviours (ranging from worry and depression to negative habits like drug and alcohol misuse) that can be harmful on their own to the healing process of wounds. Growing older is a known risk

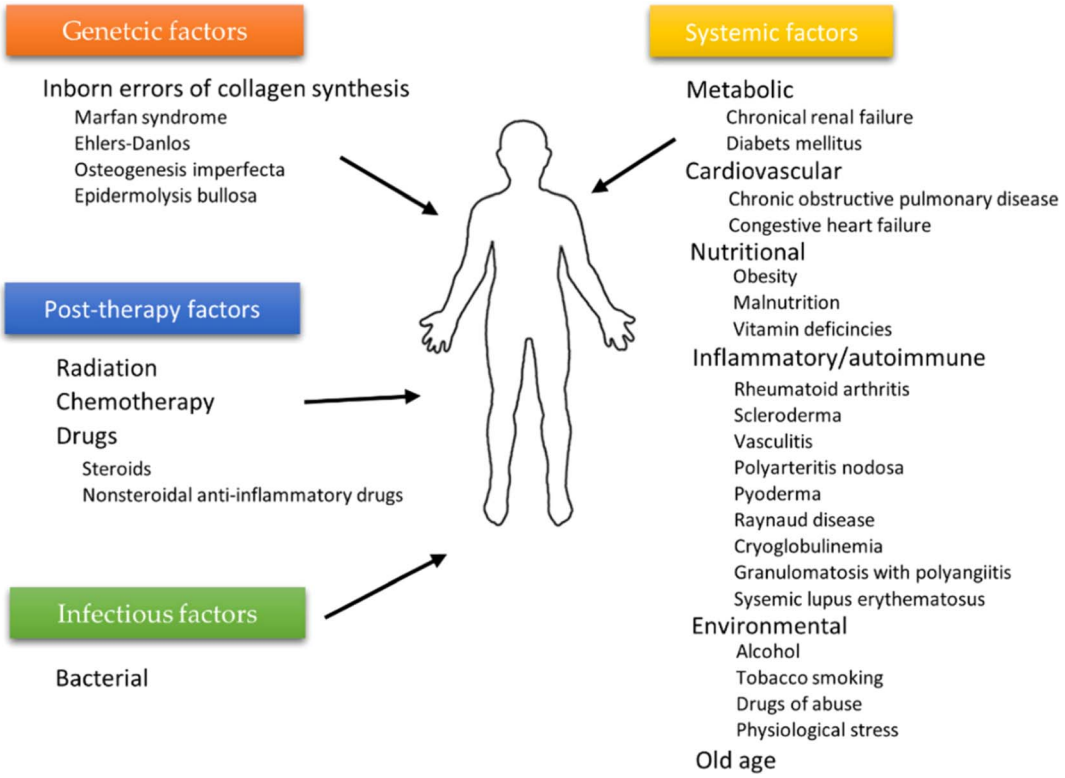


Fig. 4 Various factors affect the wound healing process, reproduced from ref. 67 with permission from MDPI, copyright 2022.

factor that lowers the efficacy of wound healing. This is a factor that needs to be carefully studied, especially because the number of people over 60 is growing worldwide.[74] Significant advancements in molecular and cellular biology during the past few decades have expanded our understanding of how ageing affects cell function. Numerous studies indicate that the primary cause of senescent cells' malfunction is their compromised ability to respond to stress. Additionally, a few treatments to lessen age-related deterioration in wound healing have been tried.[75] Growing older is a known risk factor that lowers the efficacy of wound healing.[75] This is a factor that needs to be carefully studied, mainly because the number of people over 60 is growing worldwide. Significant advancements in molecular and cellular biology during the past few decades have expanded our understanding of how ageing affects cell function. Numerous studies indicate that the primary cause of senescent cells' malfunction is their compromised ability to respond to stress. Additionally, several treatments to lessen age-related deterioration in wound healing have been tried.[76]

### 3.7 Stinctions between the processes of wound healing and regeneration

Skin grafts are required to replace surface deficits that cannot be rectified by simple wound margin approximation. Grafts are intrusive procedures that might result in significant consequences for the patient; as a result, they are only used when there are no other options. The problem of overcoming the current hurdles in wound care can be solved by using novel management strategies. Regenerative medicine is a relatively young field of medicine that aims to improve the regeneration process through a multidisciplinary approach that focuses on both problem solving and correcting defects in the physiological process of wound healing. Regeneration (Fig. 5) is a physiological mechanism in less phylogenetically evolved creatures; many larval and adult animals may regenerate significant portions of their body plan following transection or amputation; regrettably, this occurs only during the initial part of intrauterine life in humans.[78–80]

Research in regenerative medicine provides diverse approaches to accelerate and enhance wound healing. Utilizing growth factors, stem cells, and nanomaterials offer direct or indirect stimulations of wound healing by modifying the wound environment. This integrative approach paves the way for new possibilities in tissue regeneration in the future. Collaboration is essential for connecting clinicians with scientific engineering skills along with commercial organizations and guiding new technologies to an effective and safe application.[81] All the criteria offered by other professions can be assessed separately and then aggregated and clinically evaluated to combine patient demands with accessible technologies. Safety is a top issue in clinical practice, and best-fit risk analysis must consider diseases and therapies offered by regenerative medicine. The prompt clinical availability of these treatments is critical, particularly in the case of acute damage or injuries that jeopardise a patient's life. The financial aspect is also significant; high-quality results are required to justify the price of new

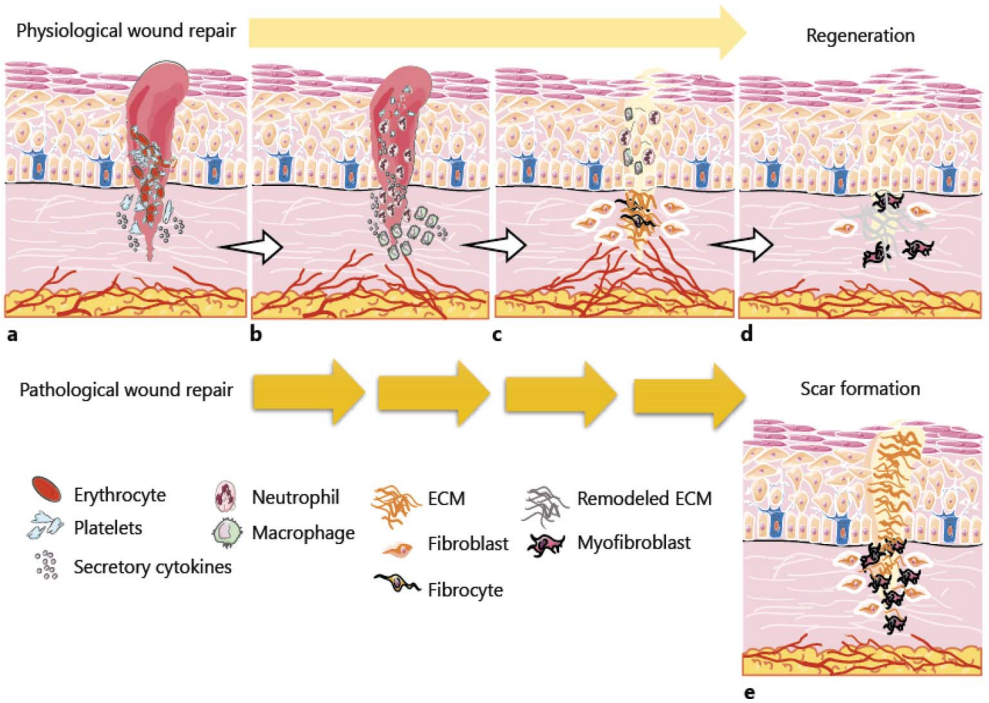


Fig. 5 The process of skin wound healing, also known as reparative or regenerative response. Following foetal tissue damage and gestational traumas, the skin regenerates through a four-stage physiological wound healing process. (a) During the hemostasis phase, platelets aid in the blood clot's creation and the cytokine release that triggers the recruitment of inflammatory cells. (b) During the inflammatory phase, pathogens and injured cells are phagocytosed by neutrophils and macrophages that have been activated. Additionally, macrophages help the shift from inflammation to proliferation, whereas neutrophils release cytokines that heighten inflammatory responses. (c) The vascular network is repaired, the provisional matrix is replaced by granular tissue, and re-epithelialization results in a covered wound surface during the proliferative phase. (d) Collagen type III fibres are replaced with collagen type I fibres during the remodelling process, which sees stage fibroblasts develop into myofibroblasts. (e) Any anomalies that cause accelerated cell responses or delayed repair drive wounds towards pathological or reparative healing, which is what happens to adult humans and results in the creation of cutaneous scars, reproduced from ref. 77 with permission from Karger Publishers, copyright 2022.

technology. Finally, due to skin's structural and functional complexity, there is yet no complete solution for skin regeneration.

## 4. ES in wound healing

The human body is known to have an endogenous bioelectric system, which generates natural electrochemical impulses in several parts of the body, including the heart, brain, muscles, and bones. The endogenous electric potential of our skin, sometimes known as the "skin battery", also has an impact on the healing process of wounds.[82] There aren't any free electrons in physiological solution to move the current. It is, therefore, transported by charged ions. Electrical potentials are produced by asymmetric ionic fluxes throughout the tissues (Fig. 6A). A transepithelial electric potential known as the "skin battery" is produced when ions pass through the epidermis' $Na^+/K^+$ ATPase pumps. 20 skin injury generates current of injury, which is necessary for proper wound healing (Fig. 6B). The skin battery is shorted out by this electrical leak, which is a persistent lateral electrical potential. The major ions in this electrical current are $Cl^-$, $K^+$, $Na^+$, and $Ca^{2+}$. An electrical potential with the negative pole at the centre of the wound and the positive pole at its edge is created by the damage current, which is detectable within 2–3 mm of the wound and ranges from roughly 10 to 60 mV 8.20, and draws cells to the site of damage. In a damp environment, the current is maintained; when a wound dries out, it stops. It was discovered in 1983 that ionic flux, injury current, and healing rate are all related. Rats' injured corneal epithelium showed increased $Cl^-$ and $Na^+$ influx with $AgNO_3$, which significantly increased the current of injury and improved wound healing. On the other hand, the current of injury was reduced considerably in rat corneal wounds treated with furosemide (a substance that blocks $Cl^-$ efflux), leading to impaired corneal wounds. An inherent electrical potential of 10–60 mV occurs between the epidermal and sub-epidermal layers of healthy skin. The aforementioned phenomenon is predominantly ascribed to the movement of ions *via* ion channels and the periodic depolarization and repolarization of cells. Around a wound, this *trans*-epithelial voltage, or TEP, rises significantly.[84]

In the given illustration (Fig. 7) it is shown how an injury disrupts the epithelium which causes a short-circuit to the TEP, which in turn propels positive electrical flow in the direction of the lesion. Clinical tests have revealed that the voltage difference between the wound site and the intact skin ranges between

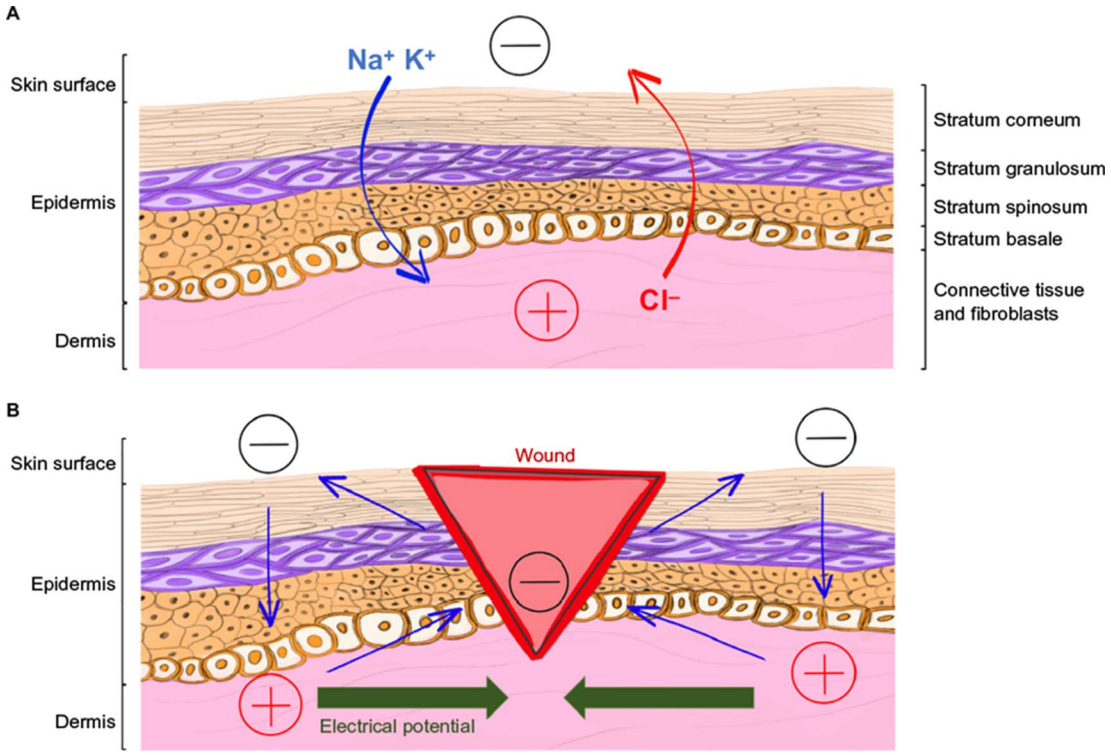


Fig. 6 Endogenous cutaneous bioelectric current both prior to and during damage. Through the ionic flow of $Na^+$, $K^+$, and $Cl^-$, the unbroken skin layers of the epidermis and dermis (A) generate a polarity with positive (+) and negative (−) poles, maintaining the skin battery across the body. A wound (B) causes current to flow out of it (blue), creating an endogenous electrical potential (green) with the positive pole (+) distant from the wound and the negative pole (−) in the centre of the wound, reproduced from ref. 83 with permission from Dove Medical Press, copyright 2017.

100 and 150 mV $mm^{-1}$.[86] Injuries cause an electric current to flow through them. These endogenous electric fields are essential for wound healing because they cause endogenous currents, which in turn serve as a stimulus for cellular migration and aid in the repair of wounds. It's also important to note that the average healing rate is thought to drop by 25% in the absence of this current. The investigation of ES as a means of accelerating wound healing for a range of applications is driven by this phenomena.[87]

Miniaturization of wound healing equipment into a wearable patch is poised to broaden the application of ES for wound healing in medical contexts, akin to the recent advancements in wearable electronics that are increasingly becoming pervasive in our daily lives. A lightweight patch adhering to the skin, without impeding the natural movement of the body, offers greater practicality compared to traditional wired electrical devices. Previous *in vitro* studies revealed that keratinocyte migration speeds vary (the predominant cells in the epidermis, the outermost layer of skin).[88] With increasing externally applied direct current voltage, the number of fibroblasts (the most common cells in the dermis, a skin layer beneath the epidermis) increases. In principle, the current density is

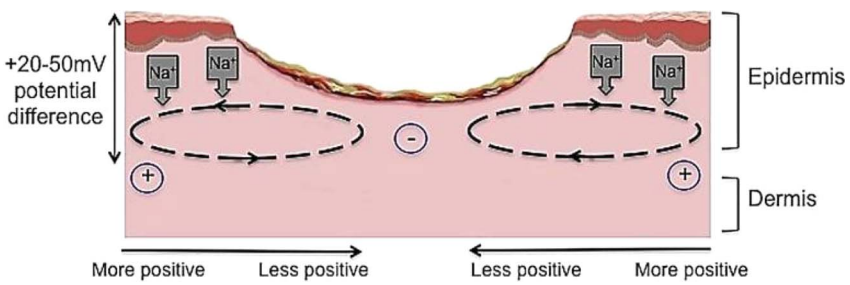


Fig. 7 It is believed that the injury's existing state has a significant role in the start of repair. Human skin that is not injured has a transcutaneous current potential of 20–50 mV and an endogenous electrical potential. This is produced when sodium ions pass through the epidermis' $Na^+/K^+$ ATPase pumps. Epithelial disruption is the mechanism that generates the current of damage. A lateral electrical field is produced by a current passing through the wound pathway after skin damage, reproduced from ref. 85 with permission from MDPI, copyright 2014.

expected to have a positive correlation with the applied voltage, although some studies in this context have utilized an electric field rather than current density as a parameter.

To effectively utilize a bioelectric plaster for wound healing, two crucial factors must be considered. Firstly, the bioelectric plaster must be biologically safe, as it comes into direct contact with both the skin and the wound. Secondly, it must have the capacity to sustain an electric current for a minimum duration ranging from a few hours to days, until the wound achieves closure.[72,89] Consequently, the research findings provide valuable insights into the design principles for the bioelectric plaster, highlighting the importance of generating a continuous electric current on the skin to optimize cell migration. Even the TENG is a good candidate for generating electric pulses because it's cheap, easy to make, wearable, and flexible, among other things. Bioabsorbable TENG (BN-TENG) based on natural materials was reported by Jiang *et al.* for regulating cardiomyocyte beating.[90,91] Tissue repair and cell regulation may be aided by electric stimulation. Self-powered cardiomyocyte stimulation can be used to repair abnormal cardiomyocytes, which could be a future solution for some heart diseases. The ES systems use bridge rectifier, PDMS-packed IDT electrodes, and BN-TENG. The cell migrated towards the middle of the scratch after it was scratched. The cells in all the electrical groups moved towards the centre of the scratch, and the scratch was nearly recovered after 24 hours. This control group had much fewer cells migrate to the scratch's margin.

In comparison to the DC group, both the AC and the iTENG sources exhibited superior cell migration.[92,93] Long *et al.* demonstrated an electrical wound healing bandage based on wearable TENG.[94] TENG and the dressing electrodes were the two parts of the bandage. The TENG works by sliding the PTFE layer back and forth against the copper layer. In the absence of an electric field, the substantial wound heals radially. Tissue staining results indicated that the electric field-treated skin exhibited complete wound healing, while the control skin lacked epithelialization over the wound area. Self-powered wound healing bandages and devices hold significant promise for expedited wound healing without the associated risk of cosmetic concerns. Hu *et al.* demonstrated the efficacy of the rotary triboelectric nanogenerator (RD-TENG) in promoting fibroblast proliferation and enhancing the migration of L929 cells in 2019, as explained in the relevant section.[14] The RD-TENG-stimulated cells moved 67.1% faster than the control group. The expression of migration-related genes was also investigated. In the RD-TENG stimulated cells, gene expression was higher. TENG's potential for tissue formation and remodelling was demonstrated in this study.

### 4.1 Types of ES

Fracture repair, pain control, and wound healing are all possible with the use of ES. These several electrical applications include DC, AC, HVPC (high voltage pulsed current), and DC with reduced intensity (LIDC). A pulsed electromagnetic field (PEMF) is the most used treatment for non-unions, and transcutaneous electrical nerve stimulation (TENS) is the most frequently used treatment for pain control. FREMS (frequency rhythmic electrical modulation systems) is a sort of transcutaneous electrotherapy that employs ES that changes the amount of stimulation (pulse, frequency, duration, and voltage), all while varying continuously. Even though many distinct methods of ES appear in the literature, all of them seem to produce favourable benefits (Fig. 8).[95–97]

**4.1.1 PEMF.** PEMF therapy stands as a safe and non-invasive method to enhance health, acting as a mimicry of the Earth's natural electromagnetic frequencies. Essentially functioning as a recharger of the body's electrical battery, it offers several notable benefits, including increased energy and circulation, reduced muscular spasms, improved sleep, accelerated healing of bone fractures, and diminished pain and inflammation. In the words of Bryant A. Meyers, "the body is self-regulating, self-regenerating, and self-healing", and PEMF therapy serves as a means to provide the necessary energy for these inherent processes.[98]

**4.1.2 TENS.** Transcutaneous electrical nerve stimulation (TENS) is a therapeutic technique that employs low voltage electrical current to relieve pain. A TENS unit comprises a battery-operated device that administers electrical impulses *via* electrodes placed on the skin's surface. These electrodes are positioned either directly over or near nerves responsible for localized pain or at specific trigger points. Two theories explain the mechanism of TENS. According to one theory, the electric current activates nerve cells, impeding the transmission of pain signals and modifying pain perception. Alternatively, another theory suggests that nerve stimulation boosts the production of endorphins, the body's natural pain-relieving chemicals, thereby diminishing the sensation of pain.[99,100] According to one study a particular waveform was made up of a pattern of five pulses that were 10 ms apart, 0.1 to 0.2 ms long, and repeated at a rate of 2 Hz. The pulses ranged between 25 and 50 mA in current. Their study encompassed 19 patients, all with 5.2 $cm^2$ leprous ulcers lasting at least two months. Within 12 weeks, all 19 patients achieved complete recovery. The absence of controls and the limited sample size indicate that this study cannot be considered conclusive, even though it does highlight the potential of TENS stimulation as a wound treatment.[101]

A similar waveform was employed in a second trial done the same year to treat ischemic skin flaps brought on by reconstructive surgery for breast cancer. 24 patients participated in this trial by Lundeberg *et al.*, 10 of whom served as controls and received a sham procedure. The treatment group's stimulation level was adjusted to three times the threshold at which tingling sensations were perceived. The study's findings were also encouraging; compared to the control group, which saw an 80% incidence of necrosis, ES generally increased blood flow and had no such events.[102]

**4.1.3 HVPC.** In the 1940s, American scientists at Bell Laboratories introduced high-volt pulsed current (HVPC), which is characterized by twin-peak, monophasic pulses featuring very short durations (less than 200 ms) and operating at voltages ranging from 150 to 500 volts. The primary goal of administering HVPC is to accelerate the healing process of cutaneous wounds. Additionally, it finds applications in

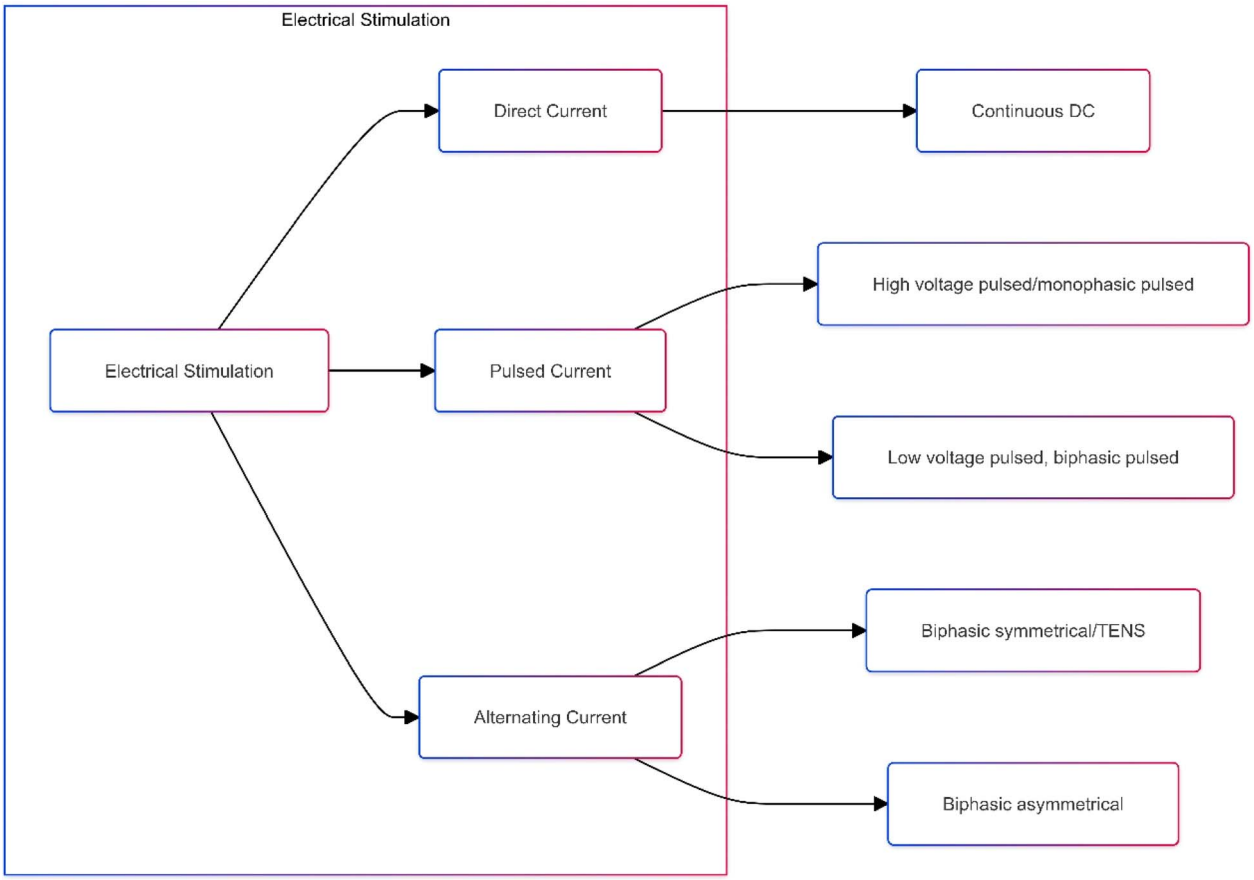


Fig. 8 Schematic diagram of different types of ES.

reducing muscle spasms, muscle re-education, pain modulation, and preventing edema. HVPC harnesses a potent electromagnetic force exceeding 150 volts (up to a maximum of 500 volts). The "galvanotaxis effect" induced by HVPC prompts polarized cells to migrate towards either the cathode or the anode. This phenomenon is thought to stimulate the migration of platelets, lymphocytes, macrophages, and neutrophils, thus initiating the "inflammatory phase" of the tissue healing response. Subsequent fibroblast migration to the wound site enhances the synthesis of new tissue during the "proliferation phase" of the healing response. In the final "remodeling and maturation phase" of the healing response, the migration of epidermal cells and keratinocytes proves beneficial. Reports indicate promising outcomes with HVPC treatment for pressure ulcers. For instance, pressure ulcers treated with HVPC for 45 minutes per day, five times a week, healed completely in 7.3 weeks, whereas untreated ulcers experienced a 29% increase in size. Other studies reported an 80% reduction in pressure ulcers treated with HVPC (220 V; 100 Hz) for 1 hour per day for 20 days, compared to a 52% reduction in a control group.[103,104]

**4.1.4 Pulsed current.** Pulsed current (PC) refers to the transient flow of electrons or ions in a unidirectional or bidirectional manner, characterized by short pulses followed by longer periods of no current flow. Each pulse is treated as a distinct electrical event within a series or train of pulses, separated by intervals of time.

Pulsed current has the potential to solve some of the problems associated with DC stimulation. According to a study, pulsed current stimulation typically takes the form of a square wave with pulses lasting less than 1 ms, or less than 5% of the overall duration (resulting in a duty cycle of less than 5%). This design prevents it from being a polar signal and also mitigates discomfort and the accumulation of acid/alkaline substances that can occur with continuous current.[105] The initial experiments on pulsed current stimulation utilized the Dermapulse and Vara/Pulse® wound healing stimulators, manufactured by Staodynamics Inc. Both of these devices can be programmed to deliver a square wave with a 35 mA amplitude and a frequency of 64 or 128 Hz. The duty cycle at 60 Hz was 0.84%, and at 128 Hz it was 1.68%. In both studies, stimulation was administered twice daily for four weeks in 30 minutes sessions. Initially, the electrode placed over the wound had a negative polarity, and the frequency was set to 128 Hz until necrotic tissue was removed from the wound. Following this, the polarity was changed every three days. Once the wound ceased descending to the muscle level, the frequency was reduced to 64 Hz, and the polarity was switched daily. In both experiments, the recovery rate of the stimulation group was twice as high as that of the control group.[106]

Jünger *et al.* conducted a follow-up study on the Dermapulse® device between 1997 and 2006 utilising a similar procedure to the earlier trials, with the exception that the stimulation was cycled periodically between 7 days of negative

 

and 3 days of favourable results. Transcutaneous oxygen partial pressure, a gauge of a tissue's capillary density, wound size, oxygen level, reported pain, and others were all significantly improved by stimulation after four months compared to controls.[107] Badmos and Adegoke tested an electrostimulation device in 2001 that delivered pulsed current at a frequency of 30 Hz and a duty cycle of one third. They discovered that the area of the ulcers in their therapy group shrank by 22.2%, compared to just 2.6% in the control group. This does suggest that stimulation promoted faster healing, but as there were only three patients in each group and there was a wide range of patient characteristics, the result cannot be regarded as statistically significant.[22]

Another study that used stimulation that might possibly be regarded as pulsed current was Baker *et al.*'s 1996 publication. Three various stimulation waveforms were evaluated in this instance. The first had a short, intense positive pulse followed by a weaker, longer negative pulse, so that the total charge transferred in either direction was equal. The second one mirrored the first, with the only difference being that the negative phase had an equal duration as the positive one but was comparatively weaker. The resulting waveform maintained the same shape as the initial waveform but had a reduced overall amplitude (4 mA for the positive pulse). The amplitudes of the first two waveforms were individually adjusted for each patient just below the motor threshold, which is the level of stimulation that triggers muscle activity. There were no statistically significant differences in the healing rates among the three distinct stimulation groups and the control group. However, when examining only the patients who fully recovered during therapy, the balanced waveform demonstrated a slight improvement compared to the unbalanced waveform and notably outperformed (with a significance level of $p < 0.05$) both the lower amplitude and control groups. Nonetheless, the significance of this outcome is constrained not only by the complex procedure needed to achieve a significant result but also by the electrode placement, which diverged from the more typical configuration of positioning one electrode on intact skin and the other directly on the wound. Consequently, this study does not offer further insights into the ramifications of charge entering or leaving the wound.[108]

Several features define PC, including waveform, amplitude, duration, and frequency. PC waveforms may take on either a monophasic or biphasic configuration.

*4.1.4.1 Monophasic pulsed current.* A monophasic pulse is defined by a brief movement of electrons or ions away from the isoelectric line, followed by a return to the zero line after a finite duration, typically around 1 millisecond. In the clinical wound-healing literature, two documented monophasic PC waveforms are notable. These include the rectangular or square waveform of low-voltage MPC (microcurrent pulsed current) and the twin-peaked waveform of high-voltage, monophasic PC (HVPC). MPC pulses are consistently shorter than 1 ms in duration. Importantly, MPC does not impact the pH of the environment and does not cause harm to the skin or tissues.[109,110]

*4.1.4.2 Biphasic pulsed currents.* The biphasic PC waveform comprises two phases and is bidirectional. In the initial phase, electrons or ions move away from the isoelectric line, then revert to baseline after a specific duration. Subsequently, the second phase involves movement in the opposite direction, swiftly returning to baseline afterward (Fig. 9). The biphasic waveform may exhibit asymmetry or symmetry about the isoelectric line. In symmetric biphasic waveforms, phase changes are electrically equivalent or balanced, lacking polarity, which is deemed undesirable. Both electrically balanced and unbalanced asymmetric biphasic waves are feasible. Within the clinical wound-healing literature, documented biphasic waveforms include symmetrical (charge-balanced) and asymmetrical (charge-unbalanced) variations. Investigations have shown positive treatment effects when employing an asymmetrical, charge unbalanced (polarized) waveform.[89,112,113]

**4.1.5 Low-intensity direct current (LIDS).** Low-intensity direct current (LIDC) is the name given to the first active electrostimulation method that has been studied. It typically involves currents of less than 1 mA that are either continuously given or pulsed at a low frequency for at least one second. Wolcott *et al.* conducted the initial investigation into LIDC in 1969.[114] Here, a continuous DC current of 400–800 A was used to treat patients with ischemic skin ulcers. Three times a day, two-hours bouts of current were applied. The positive electrode was 15 cm away from the site and the electrode on the wound itself was negative for the first three days. After switching the electrodes, the positive electrode was left on the wound to promote healing. It was implied that a plateau occurred when the wound size stopped shrinking, although specific standards were not stated. Positive stimulation enhanced healing but also fostered bacterial development, while negative stimulation seemed to exert an antimicrobial effect but hindered wound healing. This is the cause for the polarity shift.

The combination of LIDC stimulation with bandages soaked in povidone-iodine or saline solution was explored by Katelaris *et al.* in 1987.[115] They applied a cathode over the wound while using a stimulator that delivered 20 A of current. The researchers could not find any evidence that ES accelerated the healing of venous ulcers in this instance. In fact, it was discovered that povidone-iodine and ES dramatically retarded recovery. The negatively charged iodine molecules would be repelled by the negative electrode above the wound, which would allow them to enter the tissue more deeply. Here, their mild toxicity to human cells would hinder the development of new tissue. Additionally, there are potential limitations in this study, such as the limited number of patients, the small sample size, and the relatively low current, thus it is not certain that LIDC is useless in general. It does, however, demonstrate the need for caution when selecting the materials used in conjunction with electrostimulation therapy to prevent negative side effects.

The same year, Amin and Fakhri looked into how LIDC affected chronic burn injuries. Two times each week, 25 mA of electricity was delivered for 10 minutes on either side of the wound as their treatment. Re-epithelialization, or the renewal of skin, started in all but one of their 20 patients within the day three, and the lesion was fully healed within the third month. Additionally, after receiving electrostimulation therapy,

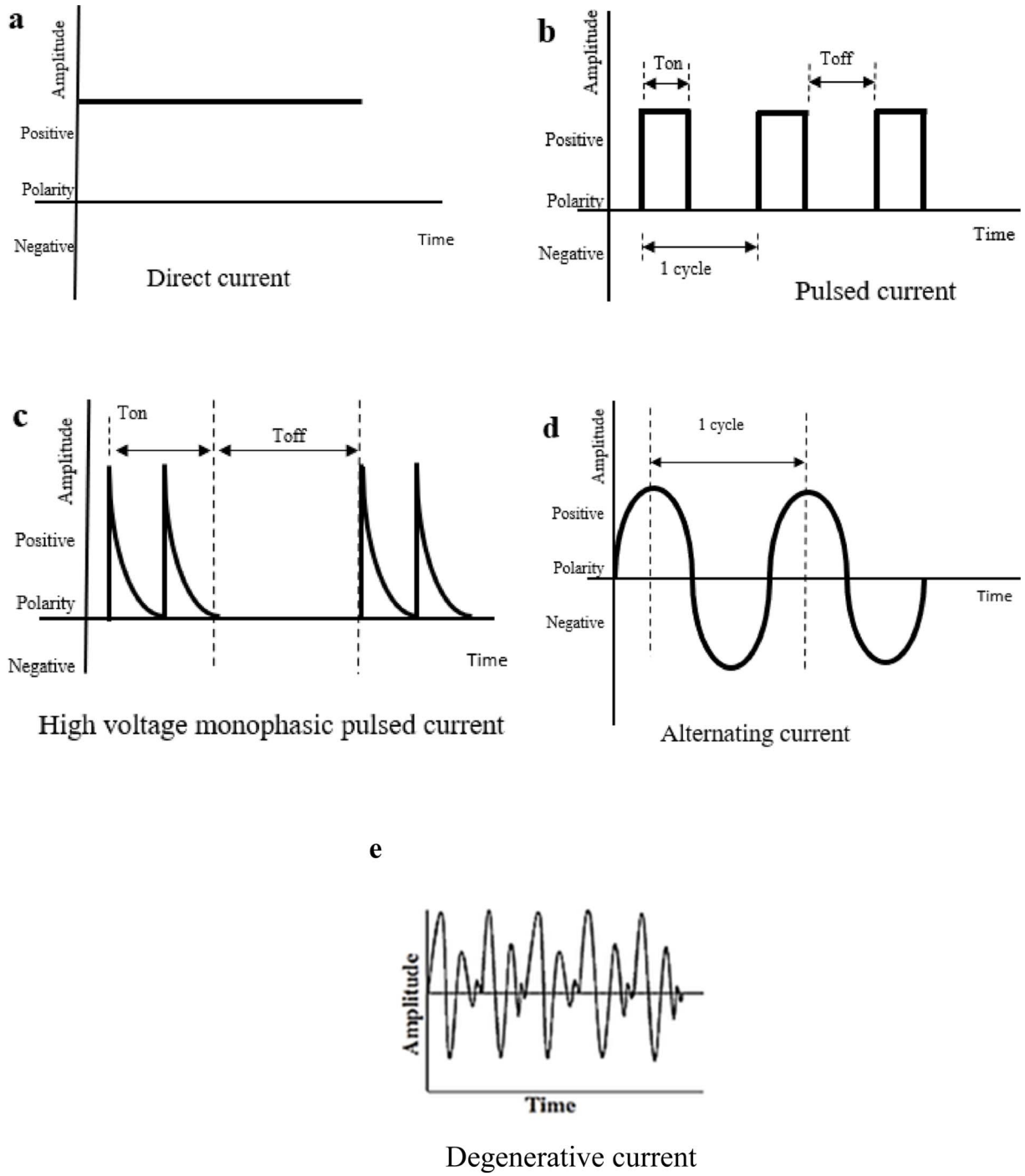


Fig. 9 The characteristics of waveform in DC (a), PC (b), HVMPC (c), AC (d) and (e) degenerate wave form with formulas on basic measurements displayed, reproduced from ref. 111 with permission from MDPI, copyright 2021.

a number of individuals for whom skin transplants had previously been attempted experienced success.[116]

Despite numerous studies suggesting its effectiveness in promoting wound healing and its ability to closely mimic the natural current of injury, the LIDC (low-intensity direct current) is rarely utilized in modern settings. This reluctance is attributed to the potential drawbacks of prolonged exposure to DC currents, even at levels below 1 mA. Specifically, the cathode draws positive hydrogen ions ($H^+$), leading to the production of acid, while the anode attracts negative hydroxide ions ($OH^-$), resulting in alkalis. Consequently, this can create an alkaline environment beneath the anode and an acidic environment beneath the cathode. Such conditions may irritate the skin and create an unfavorable environment for the cellular processes necessary for wound healing.[117]

**4.1.6 FREMS.** FREMS, a groundbreaking electrotherapy, represents a recent innovation in the field. Distinguishing itself from traditional electrotherapy systems, FREMS automatically applies sequences of modulated electrical stimuli with varying pulse frequencies and durations. The concept behind FREMS is based on the idea that delivering a series of sub-threshold electrical stimuli through the skin near a motor nerve *via* a non-invasive device would generate composite motor action potentials in excitable tissues. Unlike conventional electrotherapies that rely on single low-intensity, short-duration shocks, which struggle to penetrate the skin's dielectric barrier and stimulate underlying neuronal, muscular, and circulatory tissues, FREMS achieves this effect through specific sequences of weak impulses. These impulses feature rapid fluctuations in pulse frequency and duration, gradually recruiting membrane potentials in the stimulated tissues.[77,81] A FREMS signal is made up of a number of brief pulses that occur at a rapidly varying frequency. Musculoskeletal discomfort was the initial indication for its use in 2004. Since then, FREMS has been the subject of extensive research, much of it centred on its potential to alleviate nerve damage, or diabetic neuropathy brought on by diabetes, a task at which it appears to have promise. The use of pulses with changing frequencies is justified by the notion that these fluctuations "probably permit a modulation of peripheral and central systems".[118] Studies investigating the efficacy of FREMS stimulation on chronic leg ulcers have been conducted by Janković and Bini.[119] Results indicated that topical treatment alone did not significantly reduce the size of 7 ulcers or alleviate pain to the same extent as topical treatment combined with FREMS stimulation. Similarly, another researcher observed that wounds treated with FREMS experienced a significant reduction in size at 15 and 30 days into the treatment period compared to the control group ($p < 0.05$). However, this significance did not persist beyond day 60.[119] When FREMS were tested just on venous ulcers, separate from the effects of diabetes, the third study likewise indicated promising benefits. In the survey conducted by Santamato *et al.*, the 10 patients in the treatment group exhibited wound area reductions approximately six times greater than those of the 10 patients in the control group after 15 days of therapy and 30 days of follow-up. Additionally, patients undergoing treatment reported significantly lower levels of pain compared to the control group.[120] The conclusive study by Magnoni *et al.* encompassed a total of 30 treated patients and 30 controls, all of whom had chronic ulcers of various types.[121]. Additionally, this study found that the therapy group experienced noticeably improved outcomes in terms of both reported pain and wound size. Nevertheless, none of these studies incorporated a sham procedure, leading to the patients being aware of their group assignments. Consequently, discerning whether the observed benefits in wound healing were due to the treatment or the placebo effect remains challenging.[122]

**4.1.7 Wireless micro current stimulation.** The clinically positive effects of Wireless Micro Current Stimulation (WMCS), a cutting-edge technique that is an alternative to electrode-based ES and is one of the non-invasive or non-contact therapy modalities of ES, have recently been demonstrated by us and others.[123] In the case of WMCS, akin to the electrode-based ES approach, it harnesses the conducting capability of charged air gas. This relies on the property of nitrogen ($N_2$) and/or oxygen ($O_2$) molecules to either accept or donate electrons, facilitating the distribution of currents and voltages within the tissue.[124] According to one study, WMCS can be a useful technique for treating diabetic-related wounds, chronic wounds that are difficult to cure, firework burns,[124] and Martorell's ulcer.[125] The positive therapeutic outcomes observed in both of these interconnected yet distinct methodologies are primarily attributed to the tissue potentials and currents generated, whether by electrode-based ES or WMCS.[126] Through the restoration of the natural current of injury and reactivating the body's physiological tissue regeneration processes, WMCS promotes wound healing of pressure ulcers. Notably, WMCS inhibits granulocyte aggregation to limit inflammatory reactions, then promotes myofibroblast activity and collagen fibre synthesis. For wounds with a variety of etiopathologies that are persistent and non-healing, WMCS provides a special therapy option. By replicating and reinstating the natural electrical current that has been disturbed in wounded skin, WMCS in particular is thought to promote wound healing.[127,128]

**4.1.8 Biphasic waveform.** Utilizing a biphasic waveform, where the delivered charge in each direction is equal, represents an entirely different form of stimulation. While there are certain benefits to this, chief among them being that there is no pH change, several of the mechanisms covered in section III are directional and hence need a polar signal to work. Biphasic stimulation research has not been extensively pursued as a result. The exclusions are listed in detail below. Jercinovic *et al.* released the initial investigation into biphasic stimulation in 1994. They employed a waveform known as functional electrical stimulation (FES), which consists of trains of pulses that, as they approach zero, slightly overshoot and then gradually exponentially decay back up, maintaining a balanced overall charge transfer. While 48 patients served as controls and received only conventional treatment, 61 spinal cord injury patients with pressure ulcers were treated using this waveform. They discovered that electrostimulation-treated wounds healed 1.5 to 2 times more quickly.[129]

Ibrahim *et al.* explored the impacts of biphasic stimulation on individuals with severe partial thickness burns in 2019. They compared the results of ES with negative pressure wound therapy. This method employs suction to eliminate excess fluids from the wound while simultaneously enhancing blood flow and tissue regeneration. 1 Hz, 300 A square waves were used for the ES In terms of reducing wound size (1.6 and 1.3 times, respectively) and bacterial colony count (both resulting in a slight decrease compared to the nearly two times increase observed in controls), both ES and negative pressure outperformed the standard wound care control significantly. Patients who received ES had a marginally superior reduction in wound size, whereas those who received negative pressure had fewer bacterial colonies. It's feasible that combining negative pressure and ES could improve performance on both metrics, but this wasn't tested.[122]

### 4.2 Direction of current

ES can be classified into two forms based on the directionality of the current (or voltage): bidirectional current (or voltage) and unidirectional current (or voltage). Direct current (DC) and unidirectional pulse current (PC) are both components of the unidirectional current. The unidirectional current is defined by the unidirectional flow of charged particles, indicating that its polarity is continuous (unbalanced). The unidirectional current can imitate the endogenous electric field thanks to this property. Hence, the cathode is connected to the wound's centre and the anode of the ES device is always fixed on the normal skin surrounding the wound (Fig. 10). However, if the uneven current stimulates the wound repeatedly over an extended period of time, it will cause heat effects and skin damage.[130]

The current with reverse polarity is bidirectional. Near the electrode, the charged particles will exhibit alternate radial ranges when bidirectional current stimulates the wound. Typically, the two electrodes of the ES device are positioned on the normal skin, on either side of the wound (Fig. 10B). Applying this current to the wound might significantly lessen or perhaps completely prevent the heat effect.[131]

### 4.3 Electricity affecting the infection

Several studies suggest bacterial load and infection significantly contribute to chronic wounds and delayed healing. Acute and chronic wounds can be linked to bacterial colonization of >105 organisms per gram of tissue, and the likelihood of infection and delayed wound healing is increased.[132–134] The study showed that the healing rate was negatively correlated with log CFU. A 44% delay occurred in wound healing for every log CFU order. In 1983, doctors Halbert and Rohr tested 83 amputated limbs for bacterial growth and found that those who healed more slowly had higher levels of germs in their leg ulcers. Colonized ulcers were more prolonged to present, were larger at presentation, and had a longer duration to healing than non-colonized ulcers.[135,136] The ability of ES to help lower bacterial load and clinical illnesses is certainly noteworthy.

HVPC exhibited bacteriostatic properties after being applied to bacteria after 2 hours at 250 V or greater on *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, according to the research conducted by Kincaid *et al.*[137] Studies have found numerous different types of ES to have inhibitory effects on the development of multiple types of bacteria.[137–139] ES has bacteriostatic and antibacterial effects on the wound environment, which may lead to improved wound closure. However, we could not locate any clinical studies that indicate infection or adverse events in the RCTs.

## 5. Mechanism of ES in wound healing

Numerous studies have supported the use of ES therapy in conjunction with conventional wound care. Electrical current delivery *via* electrodes applied to the skin, either directly or near the wound, is known as electrode stimulation.[140] It has been demonstrated that ES has positive effects on the various stages of cutaneous wound healing in both chronic (Fig. 11) and acute (Fig. 12) wounds.[141] According to some research, ES can boost perfusion, lessen infection, enhance cellular immunity, and quicken the healing of cutaneous wounds. Human skin that is not injured has a transcutaneous current potential of 10–60 mV and an endogenous electrical potential. This is produced when sodium ions pass through the epidermis' $Na^+/K^+$ ATPase pumps. As a result, it is believed that the injury's current state is essential for starting repair.[142]

### 5.1 Mechanisms of cellular response to ES

ES may activate diverse intracellular signalling pathways. This can change the intracellular microenvironment, affecting cell proliferation, differentiation, and migration.[143] During a potential difference established between the surface electrodes, ion movements occur. In the context of electrical terminals, the anode signifies the positive terminal, while the cathode signifies the negative terminal. This configuration results in the positive terminal attracting negative ions and repelling positive ions towards the negative terminal. This procedure causes a current to pass from the anode to the cathode, charging nerve trunks with electricity.[144] Nerve cells and muscle fibres typically maintain a resting membrane potential of around −70 to −90 mV relative to the extracellular fluid. A change in this membrane potential close to the outer cell membrane brought

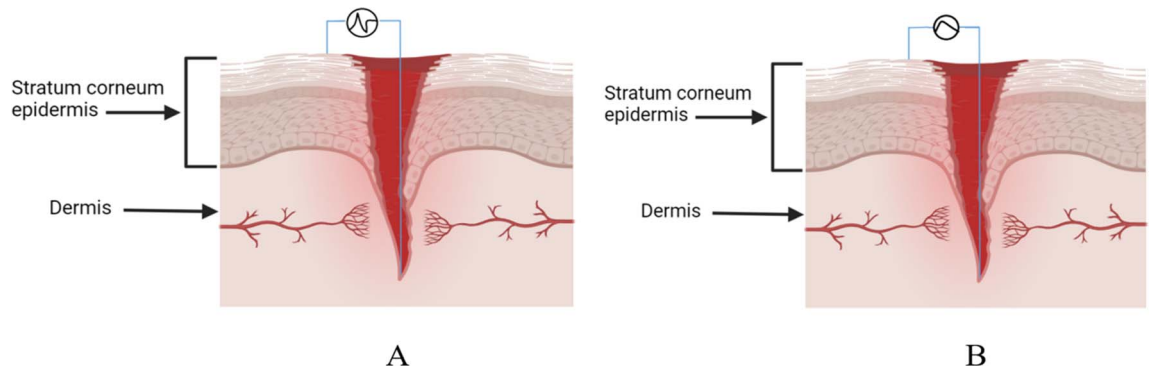


Fig. 10 (A) The positive electrode is often placed on the normal skin and the negative electrode is placed in the wound when the ES waveforms are unidirectional current. (B) Both electrodes are typically on normal skin when the ES waveforms are bidirectional current.

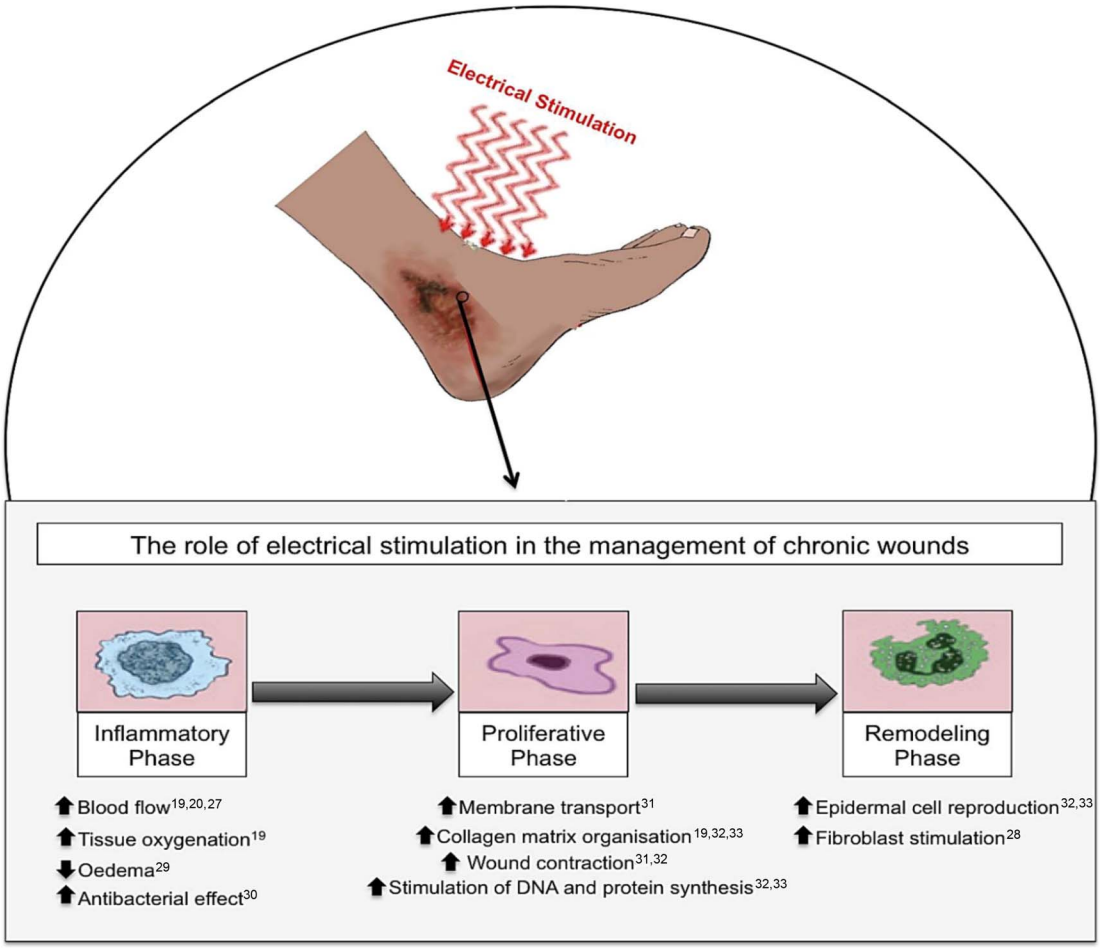


Fig. 11 It has been demonstrated that ES, in the form of PC, DC, and AC, improves cutaneous wound healing in chronic wounds. Applying ES to a chronic wound provides positive effects on the wound during its three stages of healing: the phases of inflammation, proliferation, and remodelling. Phase of inflammation: ES reduces oedema, boosts fibroblasts, improves blood flow, and oxygenates tissues while also having a stronger antimicrobial impact. Proliferative phase: ES promotes wound contraction, collagen matrix organisation, membrane transport, and the creation of proteins and DNA. Remodelling phase: ES stimulates fibroblasts, promotes migration and proliferation of epidermal cells, and improves wound healing, reproduced from ref. 85 with permission from MDPI, copyright 2014.

on by ES causes induced action potentials and contractions in the muscles.[145] Higher stimulation frequencies (20–50 Hz) are required for tetanic muscular contractions as opposed to voluntary contractions, which happen at firing rates of 4–12 Hz.[146] ES of nerve trunks activates both motor and sensory nerves, causing direct muscle contractions and reflex-induced muscle responses. Sensory stimuli may aid neuroplasticity in the central nervous system. Due to distance and nerve fibre thickness, muscle belly stimulation attracts fibres close to the electrodes, resulting in random activation orders. In contrast, nerve trunk stimulation causes the entire muscle to become uniformly active. Repeated use of muscle belly stimulation can lead to localized fatigue.[147]

In order to promote the development of tissues, directed cell migration, and wound healing, regeneration, and accordance are essential in regenerative medicine. G-protein coupled receptors, cell polarisation, voltage-gated ion channels, integrins, and endogenous electric fields are some of the mechanisms involved in these activities.[148] Due to its capacity to stimulate specific cell signalling pathways close to the cathode or anode, ES draws more attention as a physical technique. This results in cell movement and alignment. The development of tissue engineering and regenerative medicine applications is greatly enhanced by the ability to affect cell behaviour.

### 5.2 Alignment of cells in response to ES

Numerous strategies exist that enable the induction of cell movement and alignment by environmental variables, such as the addition of bioactive substances or the provision of unique surface patterns.[149] However, studies have demonstrated that physical approaches are superior to all other ways for directing cell migration and alignment. Essentially, when an electrical current is generated from the anode to the cathode, it sends an electric charge into the nerve trunk.[150]

When ES is used on the nerve trunk, it triggers both the motor nerves, which convey signals from the central nervous system to the muscles and the sensory nerves, which transmit sensory information from the body to the CNS.[150] Activating

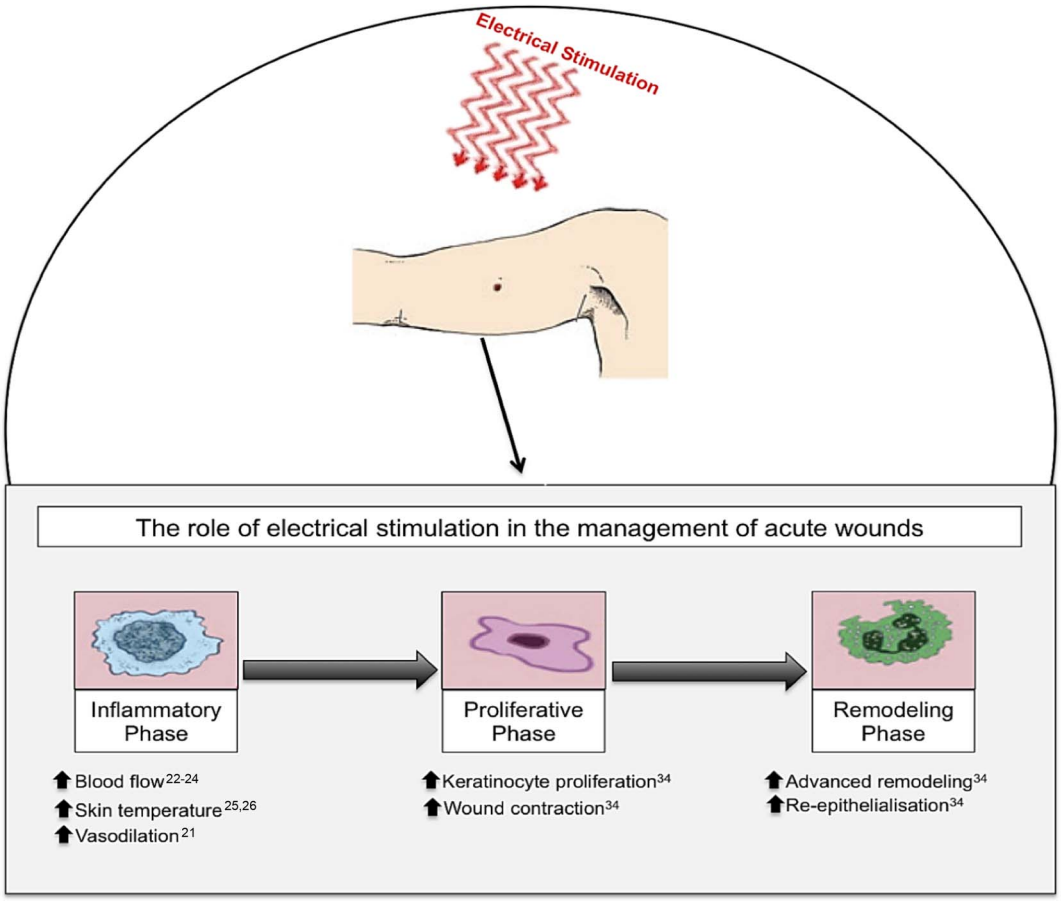


Fig. 12 It has been demonstrated that ES, in the forms of direct current (DC), pulsed current (PC), and biofeedback ES, is helpful for cutaneous wound healing in acute wounds. The application of ES to an acute wound has positive effects on the wound during each of the three stages of wound healing: inflammation, proliferation, and remodelling. Phase of inflammation: ES raises skin temperature, blood flow, and vasodilation. Phase of proliferation: ES promotes wound contraction and keratinocyte proliferation. Remodelling phase: ES promotes re-epithelialization and moves the remodelling face forward, improving wound healing, reproduced from ref. 85 with permission from MDPI, copyright 2014.

motor nerves directly causes muscle contractions in the muscles they connect to, while the activation of sensory nerves can indirectly lead to muscle contractions by triggering spinal reflexes. Additionally, sensory stimulation, involving these reflex pathways is believed to be helpful in promoting changes in the central nervous system, a concept known as neuroplasticity. When ES is applied to the muscle itself, it depends on factors such as the distance between the electrodes and the nerve endings and the thickness of the nerve fibres.[151]

Stimulating the muscle directly in the belly region activates specific muscle fibres in close proximity to the electrodes,[152] whereas stimulating the nerve trunk results in a more uniform activation of muscle fibres across the entire muscle mass. Furthermore, muscle belly stimulation, because it targets localized muscle fibres, can lead to muscle fatigue when repeatedly applied. When electrically stimulating muscles and nerves, electrical signals typically travel along the motor axon in the direction of the muscle (orthodromically), causing muscle contractions.[153] However, these impulses can also travel in the opposite direction, away from the muscles towards the central nervous system (antidromically). It is only possible with ES that this bidirectional transmission takes place; it does not happen when muscles are activated voluntarily.[150] ES is frequently thought to have a side effect known as antidromic activation along the motor neurons. It is additionally proposed that this type of antidromic propagation can contribute to neuroplasticity when ES occurs.

Directed cell migration and alignment play a pivotal role in wound healing, tissue regeneration, and tissue formation, making them crucial in the field of regenerative medicine.[143] The capacity to control and regulate these processes is of immense value. The underlying mechanisms responsible for these effects include factors such as, integrins, G-protein-coupled receptors, cell polarization, voltage-gated ion channels, and the presence of natural electric fields. Among various physical methods, ES has garnered significant attention due to its ability to activate specific signalling pathways in cells located near the cathode or anode, leading to the induction of cell migration and alignment.[154]

### 5.3 Influence of ES on cellular alignment

There are various approaches for prompting cell alignment and migration through external stimuli, including the introduction

 

of bioactive agents and the provision of distinct surface patterns. Research has demonstrated that among these methods, physical techniques like ES outperform all others in steering cell migration and alignment.[155–157]

ES significantly influences cell alignment, effectively reorienting random cells towards a specific alignment. The direction of this alignment gradually shifts in response to changes in the direction of the applied ES. Certain cell types, such as endothelial progenitor cells, adipose-derived stromal cells, bone marrow stromal cells, cardiac adipose tissue-derived progenitor cells, and vascular endothelial cells, align themselves perpendicular to the electric field vectors to minimize the field gradient across their structures. Conversely, cells like ventricular myocytes, PC-12 cells, myoblasts, osteoblasts, and cardiomyocytes align parallel to the field vectors due to ES-induced cytoskeletal rearrangements.[157] Typically, the intensity of ES remains below 10 V $cm^{-1}$, and as this intensity increases, cells exhibit better alignment; however, this improvement in alignment is often accompanied by a relative decrease in cell activity.[158–160]

### 5.4 Mechanism of ES for enhancing cell migration

The precise electrotaxis mechanism remains elusive. It likely involves several factors, including the cell's internal environment, ion channels, membrane receptors, transport proteins, and the interaction of signal pathways like TGFβ1/ERK/NF-κB and Wnt/GSK3β.[161–163] The significance of epidermal growth factor receptors (EGFR) in ES-induced cell migration has garnered substantial attention from scientists.[159,163] Through a feedback loop involving EGFR, ES controls the synthesis of cytokines and growth factors that are produced later. EGFR is expressed asymmetrically in front and behind cells, activating signalling pathways that encourage cell migration. Blocking EGFR with erlotinib, significantly reduces the electrotaxis of neural precursor cells (NPCs).[164]

Multiple signalling pathways, including the MAPK-ERK1/2 and PI3K/Akt pathways, are activated when the EGFR is active. Typically, MAPK-ERK1/2 takes part in signal transduction from outside sources in a variety of signalling pathways. Cell migration is aided by MEK phosphorylation, which activates the downstream proteins ERK1 and ERK2. The MAPK pathway appears to be involved in the electrotaxis of mesenchymal stem cells (mASCs), as inhibiting the pathway reduced migration.[165] Additionally, it has been noted that static monophasic ES can control epithelial cell migration and proliferation by triggering the ERK1/2 subunit of the MAPK signalling cascade.[166]

The PI3K/Akt signaling pathway plays a pivotal and extensively studied role in cellular responses to ES.[167] ES induces a notable upsurge in the expression of downstream protein PIP3 and the phosphorylation of Akt, leading to the uneven distribution of PIP3 and cytoskeletal proteins towards the cathode. Meng *et al.* demonstrated that the cathode-directed migration of NPCs under ES necessitates the activation of the PI3K pathway.[168] Pharmacological or genetic inhibition of PI3K/Akt disrupts electrotaxis, underscoring its crucial role. Conversely, ES boosts Akt phosphorylation and PIP3 fluorescence, underscoring the pivotal involvement of the PI3K/Akt pathway in ES induced directed NPC migration. Additionally, genetic disruption of PTEN, a phosphatase that inhibits PI3K signaling, augments ES induced ERK and Akt phosphorylation, resulting in significantly enhanced cell directionality.[169]

Further the signalling pathways, ion channels like voltage-gated calcium channels play a pivotal role in cell response during ES.[170] ES induces ion flow through channels and transporters, causing intracellular molecular polarization and cytoskeletal changes, ultimately directing cell migration.[171] Calcium influx leads to ongoing cathodal cell migration, and preventing calcium channels or intracellular calcium-related components reduces or completely blocks ES effects.[160]

Apart from its effects on migration, ES also has a significant impact on proliferation and the regulation of differentiation processes.[172] One of the main problems in regenerative medicine is making up for lost cells from illness or damage.[173] For example, following significant cell loss, various tissues often develop fibrous collagen scars, such as in the heart, creating an ischemic environment and restricting oxygen supply. The challenge of getting enough cells for transplantation is a significant obstacle in these situations.[174] As a result, there has been much interest in the ability of seed cells to multiply and differentiate into other cell types.

### 5.5 Impact of ES on cell proliferation

Cell proliferation can be boosted by effective ES, which is usually applied at levels below 1 V $cm^{-1}$ and within a particular range.[175,176] Higher ES levels in this intensity range result in faster proliferation rates. Preosteoblasts, osteoblasts, unconstrained somatic human stem cells, human umbilical vein endothelial cells, neural stem cells, and human dermal fibroblasts all show a 0.2 to 1.5 fold increase in proliferation while retaining their regular cellular metabolic activity and phenotype.[177] Additionally, high-intensity ES that is administered for a very brief period (less than 1 ms) and above 100 V $cm^{-1}$ encourages cell multiplication, however extremely high intensities might cause cell death.

### 5.6 Effect of directional changes on accelerated cell migration

A cell or a group of cells can migrate in a direction dependent on environmental stimuli through a process known as directional cell migration. Various studies have demonstrated that when exposed to chemotactic signals, cells align themselves in the direction of migration in laboratory settings and living organisms.[178] Interestingly, when cells are cultivated *in vitro*, they can show polarisation and travel in a specific direction even without exogenous chemoattractant.[179] When a wound is healing, fibroblasts migrate to the wound site in accordance with the orientation of collagen, which is an essential sort of cell migration.[180] It's vital to remember that a reduction in cell motility or unchecked cell migration might cause physiological abnormalities and hamper wound healing. The key to managing wounds effectively is controlling cell migration. Wound dressings are now the most widely used technique for

wound treatment.[180] However, traditional wound dressings like gauze, rubber, and foam focus more on controlling leakage than changing the ways in which cells usually behave. This strategy can result in an unexpected inflammatory response or a more passive healing process. As a result, there is a great need to develop novel approaches to wound healing that actively direct cell migration and quicken the healing of skin wounds.

The successful healing of skin wounds relies on the migration of three key skin cell types: keratinocytes, fibroblasts, and endothelial cells.[181] These migrations are crucial for fundamental wound healing processes like re-epithelialization, fibroplasia, and neovascularization. The combination of piezoelectric and triboelectric effects stands out in the realm of multi-energy harvesting devices. Their structural and operational similarities enable seamless integration, resulting in more straightforward, more efficient devices with complementary attributes. This synergy enhances the feasibility and performance of multi-energy-harvesting technologies. The inherent in materials, the piezoelectric effect converts mechanical deformation into electricity, which is vital in body tissues like skin for healing. Although there is evidence of some charge transfer within the human body, some behaviours, such as fast potential growth, make charge penetration simpler and may even occur below breakdown voltage. This happens when a voltage is applied quickly, causing a spike in the capacitive current.

## 6. Morphology characteristics of ES devices

Electrode manufacturing is a pivotal initial phase in the production of batteries, focusing on crafting the cathode and anode, which are fundamental components of a battery. This complex procedure involves numerous critical processes, each of which helps to produce effective, high-performance electrodes.[182] The mixing process, which entails precisely measuring and blending the necessary raw ingredients for the cathode and anode, is where it starts. This involves mixing solvents and active ingredients to create slurries, which are crucial to the composition of batteries.

Following that, these slurries are applied to copper and aluminium foils during the "coating process", which is carried out by a trained coater. The thinly coated electrodes are then dried in furnaces that are above 100 °C in temperature. The electrodes are then compressed during the "roll-pressing process" in a calendar, reducing their thickness and increasing energy density. The flattened electrodes are accurately cut into the required proportions during the "slitting and notching" stage. The last step is notching, which entails cutting a V-shaped notch and connecting cathode (+) and anode (−) tabs. Batteries, the backbone of several contemporary technologies and applications, rely heavily on the quality and efficiency of the precise electrode production process.[83,183]

### 6.1 Dimensional overview

Cell migration plays a vital role in various physiological and growth phases, including organ formation, immune response, and wound healing.[184,185] Variations in the topography of the (ECM) are a crucial ECM-dependent component that govern cell migration in multiple dimensions. Abnormal cell motility can aid in the spread of cancer and other illnesses. Fibroblast migration is necessary for both healthy wound healing and the harmful matrix deposition seen in conditions such as fibrosis. These mesenchymal cells, which are found throughout the human body, are in charge of secreting and maintaining the extracellular matrix. They may look alike, yet they have different patterns of gene expression that are associated with their developmental origins and positions along axes of development. In a conventional two-dimensional migration model, the ECM is supplied to cells as a flat sheet of globular molecules, resulting in a flattened cell shape with apical/basal polarity and most of the contractile apparatus attached to the two-dimensional surface.

The human body is full of fibroblasts, which are essential for wound healing and tissue maintenance because they can move through the local tissue environment and perform tasks like remodelling, degradation, and repair of the matrix. Fibroblasts are usually cultivated on 2D surfaces, such as plastic or glass, that have been modified to encourage cell and protein adhesion in conventional cell culture techniques. This configuration places artificial limitations on cell movement by limiting it to a flat 2D plane, even if it is useful for a variety of microscopic imaging techniques, enabling the observation and monitoring of cell migration in response to chemotactic gradients or other stimuli.[126]

Researchers have studied fibroblast behaviour in reconstituted ECM hydrogels which are usually derived from purified type I collagen in great detail in order to better understand fibroblast behaviour in three-dimensional (3D) settings that more closely resemble physiological conditions.[186] Collagen I can be extracted using acid or pepsin from tissues high in collagen, such as tendons, and then kept in solution. Collagen naturally goes through a process called fibrillogenesis when exposed to physiological pH and temperature. This process involves the formation of linear fibers from collagen monomers, resulting in a hydrogel with a loose network of collagen fibers interspersed with fluid. Various factors, such as temperature, pH, ionic strength, ion stoichiometry, and monomer concentration used during gelation, can influence the microstructure of this collagen gel.[187] Fibroblasts can be incorporated into the hydrogels either after the gel has formed or during the gelation process, enabling their embedding within the 3D fibrillar network. This approach allows researchers to investigate cell interactions within such model matrices.

The migration of fibroblasts on 2D surfaces is substantially different from their movement within 3D fibrous matrix. Fibroblasts can migrate more quickly and unidirectionally in 3D settings than on comparatively flat 2D surfaces. It is interesting to note that these 3D migratory properties can be reproduced by lowering the cell-substrate interactions from 2D to 1D by altering 2D surfaces to restrict adhesion and migration to straight, narrow lines.[12,47,188] In a similar vein, the introduction of micro topographical cues on intricate 2D surfaces can change migratory behaviours to resemble 3D matrix behaviour. These

results indicate that, in contrast to typical 2D migration properties, fibroblasts may be able to migrate in 1D along the matrix fibres while moving through weakly organised 3D fibrillar matrices.

The debate regarding whether cells migrating within 3D matrices completely forego the formation of focal adhesions, crucial for 2D migration, remains contentious. While there is conflicting evidence regarding the clustering of adhesion proteins such as paxillin in 3D matrix migration, a more intriguing observation is that in 3D environments, the depletion of adhesion proteins like talin, p130Cas, or genetic vinculin deficiency can yield opposing effects on migration speed and persistence. In contrast to 2D migration, 3D migration demonstrates a significant correlation between cell speed and the growth rate of pseudopodial protrusions.[14,160] Additionally, fibroblasts engaged in 3D migration lack the polarity essential for 2D migration, such as polarized activation of Rac, Cdc42, and phosphatidylinositol-3,4,5-trisphosphate. Notably, physiological 3D collagen fosters the formation of a newly identified adhesion structure known as a linear invadosome.[12]

This structure is comparable to conventional invadosomes found in 2D experimental culture methods and facilitates matrix invasion. These structures might be essential for directing specific proteolytic activity to promote migration across densely packed fibrillar matrices. Although some research points to differences between 2D and 3D cell migration, both scenarios are governed by conserved principles.[189] To mimic observations in 2D systems, 3D collagen matrices, for instance, can be designed with spatial gradients in mechanical characteristics and ligand density. This will cause fibroblasts to redistribute over time to locations with higher stiffness and ligand density.

Notably, physiological 3D collagen stimulates the formation of a linear invadosome, a newly discovered adhesion structure.[165] This structure promotes matrix invasion and is similar to traditional invadosomes used in 2D experimental culture techniques. These structures may be crucial for controlling proteolytic activity to facilitate migration through densely packed fibrillar matrices. While some studies suggest that 2D and 3D cell migration differ from one another, conserved principles apply to both situations. For example, 3D collagen matrices can be created with spatial gradients in ligand density and mechanical properties to match observations in 2D systems. Fibroblasts will eventually shift as a result to areas with greater stiffness and ligand density. This suggests that both 2D and 3D matrices are subject to some basic physical and mechanosensitive features of migration.[189]

The decision of the source material for 3D collagen matrices collagen extracted with acid or pepsin has significant effects on our comprehension of cell movement in fibrillar 3D environments.[190] Nonhelical telopeptides at the ends of collagen molecules are removed by pepsin extraction, which affects fibrillogenesis and the creation of cross-links. While pepsin-extracted collagen matrices can be infiltrated using broad-spectrum matrix metalloprotease inhibitors, telopeptide-intact (acid-extracted) 3D collagen matrices require matrix metalloprotease (MMP)-14. To provide appropriate pathways for invasion, increased matrix density requires matrix deformation or proteolytic breakdown. It has been established that local proteolysis is necessary for 3D invasion and migration. This further supports the significance of proteolysis in navigating thick, cross-linked fibrillar matrices.

Although matrices made from collagen that has been acid-extracted still require local proteolytic activity, they have drawbacks. Although the distribution of fibrillar proteins in these reconstituted matrices is homogeneous, they do not have the molecular and topological complexity of intact tissues. Cells in complex matrices can move along (ECM) bundles that are aligned or pre-existing routes; this is a characteristic that is not usually seen in homogeneous regenerated ECMs.[184] Over time, fibroblasts do reorganise collagen fibrils in ECM hydrogels into oriented bundles that resemble some features of intact tissue. However, this cell-mediated reorganisation modifies the mechanical properties of the matrix, also the local ligand density. And the matrix architecture in a spatially heterogeneous way, negating the initial benefit of controlled conditions.[7,29,95,184]

### 6.2 Positive and negative current impact on wound

The epidermis generates an electrical voltage referred to as the transepithelial potential (TEP) or the "skin battery". In humans, the average voltage gradient of the TEP between the outermost skin layer (stratum corneum) and the basal side ranges from 10 mV to 60 mV, depending on the measured region.[191,192] The TEP is a result of the active transport of $Na^+$ to the basal side and $Cl^-$ out apically.[193] Tight junctions connecting epithelial cells act as the primary electrical resistance barrier, with directional ion transport and this barrier collectively establishing the TEP. Similar TEPs are observed in various epithelial tissues such as the cornea, gastrointestinal duct, urinary duct, and respiratory duct. In the corneal epithelium, the TEP is maintained around 40 mV through asymmetric ion pumping.[188] The TEP forms the foundation for generating endogenous electric fields after tissue injury. Wounds, such as those breaking the skin's epidermal layer, naturally generate endogenous direct current electric fields (EFs) *in vivo*. These EFs involve a TEP due to charge accumulations on and inside the epidermal surface. Uneven distribution of ion channels like $Na^+$, $K^+$, and $Na^+/K^+$ ATPase in the skin's mucosal surface establishes a transepithelial potential difference (TEPD).[194] When the epidermis is injured, an electrical leak occurs. The wound's resistance is lower than that of intact skin, resulting in an endogenous EF due to ion movement between the epidermis and dermis.

## 7. Factors affecting wound healing

The wound-healing process consists of various highly integrated and overlapping phases like inflammation, reduction of oedema, migration and proliferation of cells, tissue remodeling or resolution and scar formation. These steps and the biochemical results they produce must take place in the correct order, at the right time, and with the right amount of intensity.

Numerous immune cells, including macrophages, mast cells, lymphocytes, mast cells, and neutrophils, are involved in the immunological response. These cells can absorb pathogen and cell debris, produce a number of cytokines and growth factors at the wound, and increase the signal sent by the wound during the coagulation phase. The presence of electrical fields causes reactions in lymphocytes, mast cells, neutrophils, and macrophages.[195]

### 7.1 Recruitment of immunocytes

Accelerating the recruitment of cytokines and immunocytes during the early stages of inflammation can help facilitate wound healing. The recruitment process should be accelerated during the entire inflammatory phase to hasten wound healing. Studies have demonstrated that endogenous ES can cause neutrophils, lymphocytes, and macrophages to move to the site. According to Brandon *et al.*,[186] voltage-gated potassium (KV) channels play a major role in mediating the electric response of macrophages, including their recruitment to the wound. Francis *et al.* discovered that DC electric fields could regulate T lymphocyte migration towards the cathode *in vitro* and *in vivo.*[196] Wang *et al.*'s important discovery that ES affects the activation of signalling mediators during the inflammatory phase is significant.[197] To monitor immune cells and chemicals, they inserted a polyvinyl alcohol (PVA) sponge under the skin and utilized PC ES to attract neutrophils to it. The phosphorylation of extracellular signal-regulated kinase (ERK) may be associated with the recruitment of neutrophils.[18,186]

### 7.2 Resolution of inflammation

The length of the inflammatory response will have a significant impact on how quickly a wound heals. ES can reduce the quantity of immune cells and cytokines during the late stages of inflammation, which may play a part in the inflammation's resolution. ES of a PC has been shown the decrease quantity of mast cells. ES has been shown to lower the amount of polymorphonuclear leukocytes (PNL) and mast cells in the wound, according to various studies.[198,199] According to their study, the ES group's period of inflammation was shorter than that of the control group. Seren *et al.* found that transcutaneous electrical nerve stimulation (TENS) dramatically reduced the expression levels of pro-inflammatory cytokines in the skin, interleukin-6 (IL-6), interleukin-1 beta (IL-1), and including tumour necrosis factor (TNF). According to the research, TENS can accelerate recovery by reducing inflammation.[183]

### 7.3 Antibacterial activity on inflammation

The inflammatory cells are unable to eradicate the bacteria if their concentration reaches a particular point. The wound will already be infected at this point. The leading cause of slow wound healing is persistent wound infection.[200] Although bacterial infections can be treated with antibiotics, prolonged use of these drugs may make it difficult to control chronic wounds and increase antibiotic resistance.[201] The influence of exogenous ES on *In Vivo* Antibacterial Properties was initially explored by Wolcott *et al.*[202] They employed negative polarity DC to address chronic wounds containing *Proteus* and *Pseudomonas* species. The outcome revealed that, within a few days, the chronic wound became free of pathogens. In a rabbit wound, Rowley *et al.* discovered that negative polarity DC had an antimicrobial impact on *Pseudomonas aeruginosa*.[203] According to Bolton *et al.*, DC ES would have an antibacterial impact at the anode but not the cathode. They discovered a good association between the duration and current density of ES and the antibacterial effect. It's possible that ES alone won't produce the best antibacterial result. To enhance the antibacterial action, several researchers have added antibacterial compounds based on ES.[204]

For instance, Chu *et al.* discovered that under DC ES, the antibacterial activity of the silver nylon dressing was increased.[205] These trials produced encouraging findings and demonstrated the importance of the antibacterial treatment with ES. There are some indications of an indirect antibacterial impact. However, the mechanism of direct ES's antibacterial activity is still unclear.[83] The antibacterial indirect effects of the ES can be realised by attracting neutrophils and macrophages. The bacteria in chronic wounds, on the other hand, have been shown to form highly antibiotic-resistant polymicrobial biofilms instead of existing in a free condition. It has been demonstrated that ES can lessen bacterial adhesion, which may diminish the likelihood of biofilm development.[206] The question of whether ES can injure human cells given their fragility still has to be resolved. However, because skin capacitance exists, the extracellular environment will be comparatively constant at the proper current intensity to guarantee the security of the cell's living environment. The quick alteration of the living environment (such as the change in pH) makes it possible to efficiently kill germs that are adhering to the skin.[207] All these studies on the antibacterial effects of ES on wounds consistently indicate that ES can hinder the growth of microorganisms, subsequently contributing to a slower wound healing process. With minimal detrimental effects on cells, these investigations collectively suggest that ES can indirectly facilitate wound healing by reducing the number of pathogens present or by decreasing the motility of the wound.[208]

### 7.4 Oedema

Oedema is induced by increased blood pressure in the vessels, leading to a substantial reduction in local blood flow and tissue oxygenation for the patient. In other words, oedema can slow the healing process. Researchers discovered that ES helped lessen oedema in animal models as early as the 1980s. According to Taylor *et al.*, cathodic ES therapy can lessen oedema.[23] Young *et al.* noted a significant reduction in edema levels in both the lesion and surrounding tissues during the treatment of venous leg ulcers (VLUs) using the same pulsed direct current ES (Fig. 13).[25]

### 7.5 Impact on tissue blood flow (TBF)

Wound healing has been aided using ES to boost tissue blood flow. The increased blood flow aids the distribution of

nutrients. Zeinab Khalil *et al.* discovered that low-frequency ES can enhance the vascular reactivity of old rats by treating wounds with non-invasive TENS technology.[210] Additionally, scientists discovered that ageing rats' vascular responses around sensory neurons can be stimulated to speed up wound healing when subjected to low-frequency ES.[211] According to multiple studies, the skin blood flow (BF) in chronic wounds significantly increased, and the rate of wound healing saw a 60% improvement when the lesion was electrically stimulated for four weeks in a warm environment (32 °C). Another study suggests that increased vasodilation, leading to enhanced blood flow, may contribute to the wounds' improved responsiveness to ES. These findings collectively indicate that ES therapy plays a role in enhancing tissue blood flow and expediting the process of wound healing.[212]

### 7.6 Impact of cell migration and proliferation

During the proliferation phase, new granulation tissue and epithelium predominantly form. Studies have revealed the involvement of epithelial cells, fibroblasts, and endothelial cells—known to be responsive to electrical signals—in this phase. These cells migrate in response to the endogenous electric field generated by damaged tissue. Electrical signals regulate cell-directed migration through pathways such as phosphatidylinositol-3-OH kinase-g (PI3K) and phosphatase and tensin homolog (PTEN), as reported by Zhao *et al.*[213] The electric field's capacity to provide directional cues for cells and override other directional cues like chemokine gradients and pressure gradients in cell migration is a critical aspect of ES's ability to accelerate wound healing, a function that other forms of treatment cannot substitute. Numerous studies have demonstrated that exogenous ES promotes cell proliferation and accelerates wound healing.[214]

### 7.7 Scar formation

The remodelling phase of the healing process is the one that causes the most worry in therapeutic care. A significant amount of collagen is deposited during this stage, which is directly related to scarring. Scarring has an impact on the patient's look as well as perhaps on their everyday activities. When

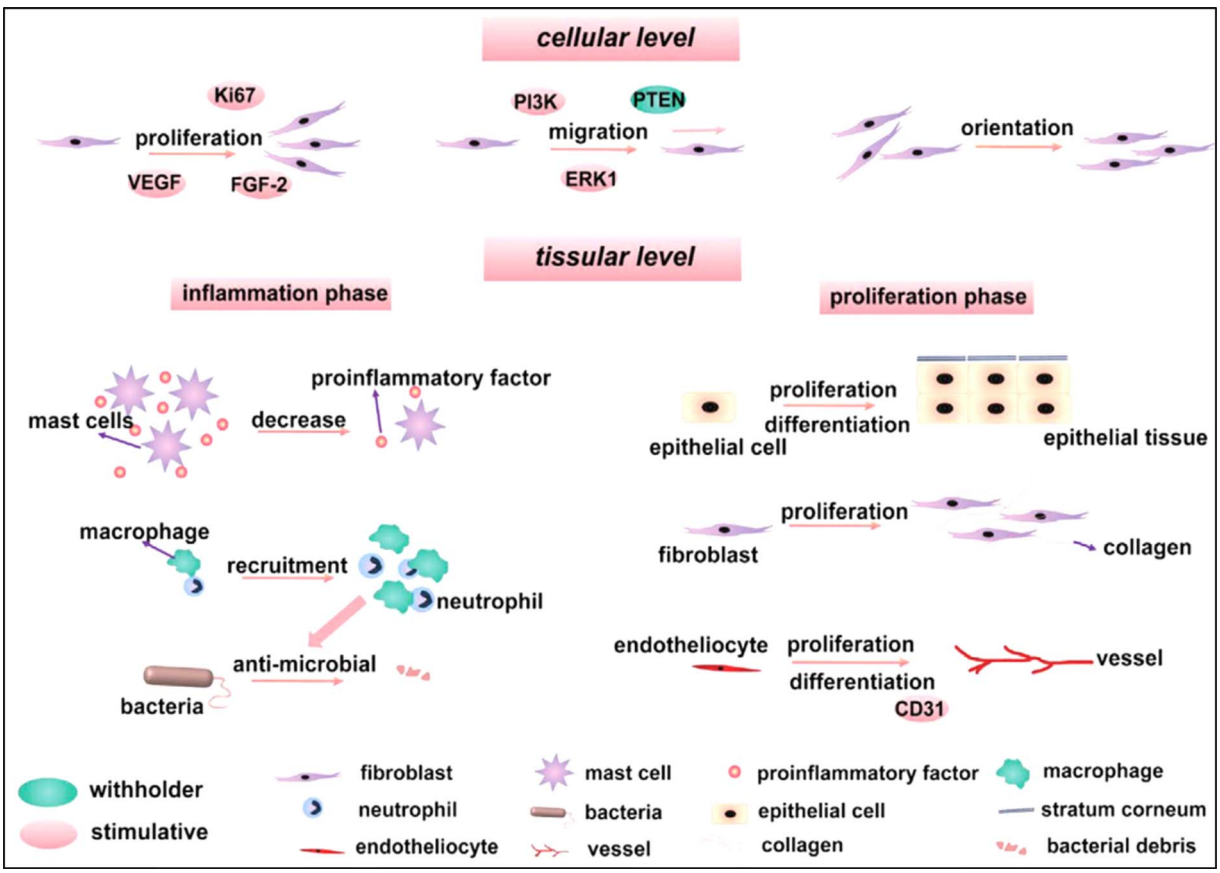


Fig. 13 The cellular and tissular effects of ES on wounds. ES has the potential to speed up cell proliferation at the cellular level by upregulating the production of VEGF, FGF-2, and Ki67. Additionally, ES may facilitate cell migration by upregulating PTEN expression and encouraging the production of PI3K and ERK1. Furthermore, the orientation of cells may also be guided by ES. ES primarily benefits the phases of proliferation and inflammation at the tissular level. ES may attract neutrophils and macrophages during the inflammatory phase and aid in the reduction of inflammation by lowering the quantity of proinflammatory factors and mast cells. Additionally, it may have antimicrobial properties due to the recruitment of immune cells. ES can speed up the proliferation and differentiation of endotheliocytes, epithelial cells, and fibroblasts, during the proliferation phase. The expression of CD31 may have an impact on endotheliocyte differentiation, reproduced from ref. 209 with permission from John Wiley and Sons, copyright 2021.

investigating the impact of ES on wound healing, very few researchers have taken into account the influence of ES on scars.[215]

Weiss *et al.* investigated the connection between ES and scarring for the first time.[216] According to Kambic *et al.*, AC and DC can influence scar toughness in addition to helping in wound healing.[217] Low-voltage pulsed current (LVPC) ES was utilised by Habiba *et al.* to investigate a putative wound-healing mechanism in diabetic mice. They discovered a positive correlation between the intensity of ES and the collagen deposition in deep scars. The findings suggested that ES might have an impact on scars' tensile strength.[105]

## 8. Advantages of ES in wound healing

Since they have become more popular over the past few years, the majority of electrotherapy devices can now produce low-intensity currents (LIC). When a wound is sustained, the body creates an injury current that aids in healing. However, this current may progressively decrease, occasionally leading to slowed or incomplete wound healing. Thus, by maintaining the LIC throughout the various stages of healing, healing may be sped up by administering the same LIC externally. Because LIC resembles the currents naturally produced by the human body when it is injured, other scientists have noted that the effectiveness of LIC in promoting wound healing is significant. Despite its extremely low amplitude, it is reasonable to wonder whether this specific type of currents may exhibit a favorable range of amplitudes for enhancing wound healing.[218] There are two types of low-intensity current: low-intensity direct current (LIDC) and low-intensity pulsed direct current (LIPDC).

According to recent studies, LIDC between 200 and 800 A is useful for boosting and speeding up wound healing. It is important to note that no blood or serous exudate was found in any trial, proving that low-intensity ES is suitable for an intensity range of 200 to 800 A.[202] Research utilizing low-intensity pulsed direct currents (LIPDCs) has indicated that an intensity range of 300 to 600 A can effectively promote the healing of stage II and III pressure ulcers that were not adequately treated with standard compression therapy. Therefore, it indicates that an intensity range of 300 to 630 A is the preferred intensity for treating these particular lesions.[219] Regarding methodological concerns, locating research specifically employing low-intensity current (LIC) for wound healing proved challenging, necessitating sophisticated search techniques. This challenge may be attributed to the literature's lack of differentiation between LIC and other currents with intensities exceeding 1 mA, commonly grouped under the term “electrical stimulation”. In each study, the control or sham-treatment group received standard wound care, ensuring that treatment was not withheld, aligning with fundamental medical ethics principles. This must be emphasised. Because the control group received conventional care, the pace of healing was accelerated in respect to standard care rather than no care at all. This fact demonstrates that LIC cannot be used alone but can be used in conjunction with conventional wound care, as suggested by recent study.[218]

No firm conclusion or generalisation could be made regarding the impact of LIC on wound healing. There is agreement among researchers only in terms of intensity. Every other factor varies between experiments. Due to the lack of trials for each type of lesion, the effectiveness on a particular form of ulcer could not be determined. The LIC generators used in the investigations have been retired, although this has no impact because all publications sufficiently give parameters and technical details. Another consideration is that different outcome measures and criteria have been employed in studies, making it difficult to compare the results and draw conclusions to some extent. Despite this, the encouraging findings suggest that LICs may help to stimulate and speed up wound healing. The criteria above precluded findings on the effectiveness and scope of LICs in promoting and speeding wound healing.[220]

The Fenzian system, employing degenerate waves (DW) as its waveform, has been utilized to address acute cutaneous wound healing and the manifestations of abnormal skin scarring. Research on ES has predominantly focused on pressure ulcers, venous ulcers, vascular ulcers, and diabetic foot wounds.[26] The heterogeneity in outcome assessments, ES type, and dosage of the medication across trials is one of the difficulties in evaluating these data. The majority of the studies were of limited scale, and several had scant follow-up and short treatment durations. Furthermore, the primary endpoint in several of the studies was not complete wound healing (*i.e.*, complete wound closure).[221] Alterations in wound area were commonly employed instead of complete wound closure due to the abbreviated duration of the studies. Examining the impact of ES on acute wounds is essential as standardizing chronic wounds proves challenging. Human controlled trials examining the function of ES in acute cutaneous wounds were lacking. It has been demonstrated that biofeedback ES is a useful technique for improving cutaneous wound healing. In a controlled investigation, a noticeably enhanced blood flow was observed on day 14. It is still challenging to determine which stages of wound healing this gadget would be most effective for based on the results so far. It is significant to note that not all ES treatments and delivery methods have an impact on every stage of wound healing (Fig. 14).[222]

Electroconductive scaffolds hold immense potential for biomedical applications, particularly in tissue engineering and regenerative medicine.[223] These scaffolds, often made from materials like conductive polymers, carbon-based materials, or metallic nanoparticles, can provide electrical stimulation to cells, enhancing cell proliferation, differentiation, and overall tissue regeneration. However, one significant limitation of electroconductive scaffolds is the risk of tissue death due to hyperstimulation.[224] This hyperstimulation can lead to various adverse effects, including apoptosis, necrosis, and impaired tissue function. To address this issue, researchers have explored several chemical processes and materials, including the incorporation of insulating polymers, to mitigate the risks associated with electroconductive scaffolds. One of the primary strategies to prevent hyperstimulation-induced tissue death is the incorporation of insulating polymers. These polymers, such as poly(lactic-*co*-glycolic acid) (PLGA), polycaprolactone (PCL), and

 

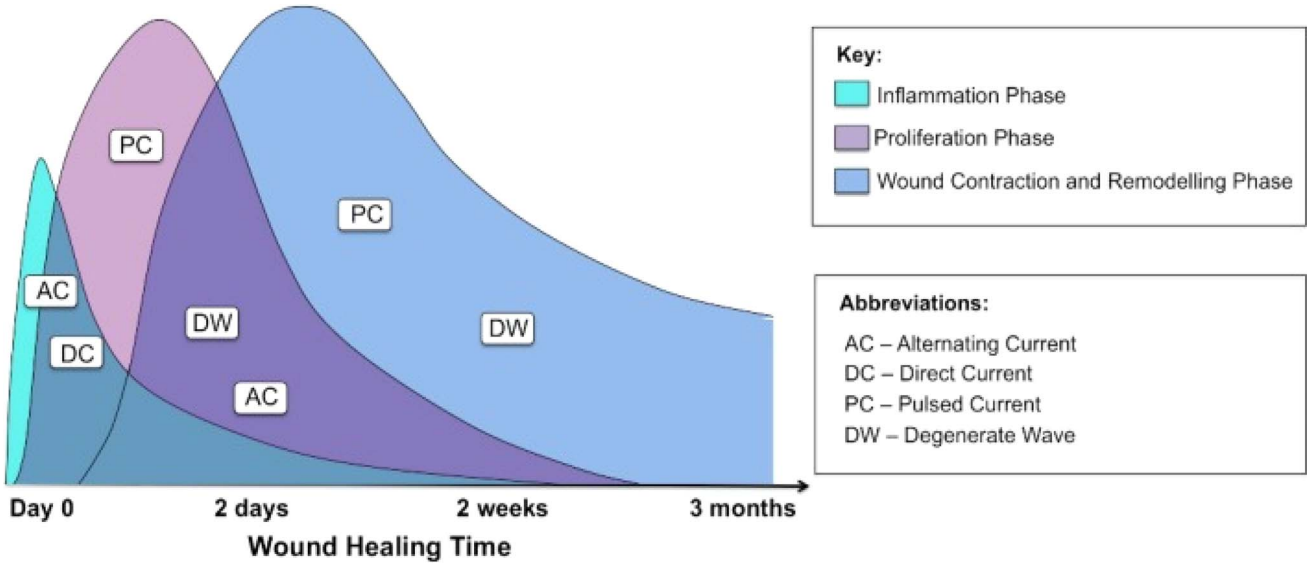


Fig. 14 Visual depiction illustrating the three stages of acute cutaneous wound healing and the suitability of various ES waveforms for each phase: inflammatory, proliferative, and remodelling, reproduced from ref. 85 with permission from MDPI, copyright 2014.

polyethylene glycol (PEG), are non-conductive and can be used to coat or blend with conductive materials. By modulating the electrical conductivity of the scaffold, these polymers help to control the intensity and distribution of electrical signals, reducing the risk of hyperstimulation. For instance, PLGA has been widely used as a biodegradable insulating polymer that can be combined with conductive materials to create scaffolds with controlled conductivity and degradation rates, promoting tissue regeneration while minimizing adverse effects.

## 9. Future prospects of ES

The three elements of tissue engineering scaffold, growth facto, and cells have grown in prominence in regenerative medicine during the past few decades. In skin tissue engineering, the creation of an electroconductive scaffold that can produce ES is finding more applications. The future prospects of ES for healing biological wounds are highly promising, driven by advancements in biomedical engineering and a deeper understanding of bioelectricity's role in tissue repair. ES can significantly accelerate the migration and proliferation of essential cells involved in wound healing, such as fibroblasts, keratinocytes, and endothelial cells, leading to faster wound closure and tissue regeneration. Additionally, ES promotes angiogenesis, which is crucial for supplying nutrients and oxygen to healing tissues, thereby enhancing the overall healing process (Fig. 15).[24,225–228]

One of the most exciting developments is the integration of ES into wearable medical devices, such as smart bandages. These devices can deliver controlled electrical currents to wounds, potentially improving healing rates while allowing patients to maintain mobility and perform daily activities. Future ES systems could be programmable to deliver specific electrical patterns tailored to different stages of wound healing or responsive to real-time changes in the wound environment, optimizing therapeutic outcomes for various wound types.[229–231]

Combining ES with other therapies, such as growth factors, stem cell therapy, or pharmacological agents, presents another promising avenue. ES can improve the integration and effectiveness of transplanted stem cells or enhance the delivery and action of therapeutic agents at the wound site, offering a synergistic approach to wound care. Ongoing research aims to understand better the mechanisms by which ES influences cellular and molecular pathways in wound healing. Insights into these processes will enable the optimization of ES parameters, such as voltage, frequency, and duration, to maximize therapeutic benefits.[20,187,232–234]

Personalized medicine is another frontier where ES could make a significant impact. Advances in personalized medicine allow for the customization of ES treatments based on individual patient characteristics, such as genetic background, wound type, and healing capacity. This customization could lead to more effective and efficient wound-healing solutions tailored to each patient's needs.[235,236]

The path to broader clinical adoption of ES will involve continued clinical trials to demonstrate safety and efficacy in various wound types and patient populations. Achieving regulatory approvals for new ES devices and protocols will be crucial for their integration into standard wound care practices. As evidence supporting the benefits of ES accumulates, it is likely to become an integral part of standard wound care, particularly for chronic and hard-to-heal wounds. This integration will require training for healthcare providers and the development of guidelines and protocols to ensure the safe and effective use of ES in clinical settings.[237–240]

In summary, the future of electrical stimulation in wound healing holds great potential, driven by technological innovations, enhanced mechanistic understanding, and integration

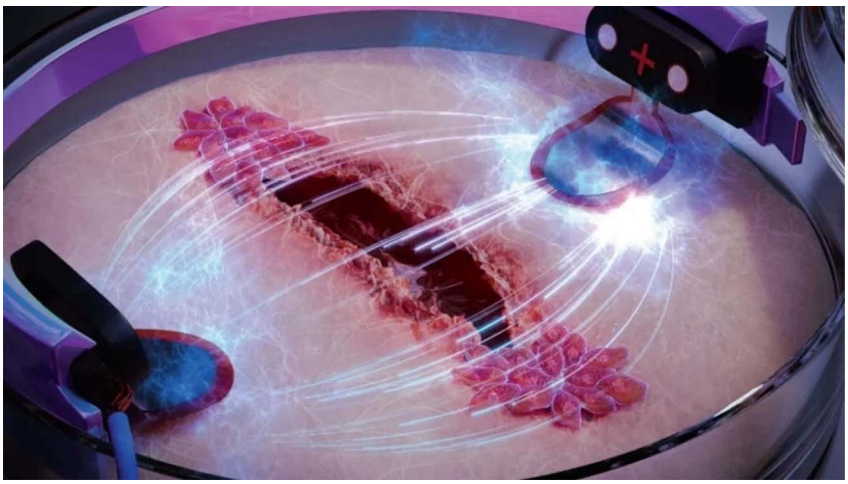

Fig. 15 Future prospects of treating wounds using ES, image credit: Chalmers University of Technology.

into multidisciplinary treatment approaches. These advancements promise to improve patient outcomes and transform the management of wounds in clinical settings.

Future studies should explore the controlled delivery of drugs and other beneficial agents, employing more sophisticated *ex vivo* models or animal studies to offer more robust support for potential human trials.

## 10. Conclusion

The recovery of chronic wounds remains a persistent challenge for healthcare professionals, presenting a significant public health concern. Natural endogenous electrical potentials that our bodies generate around an injury are believed to speed up the healing process by directing cells to the location of damage. Exogenous ES mimics physiological processes and has demonstrated effectiveness in expediting wound healing. Research shows that ES can reduce inflammation, regulate bacterial growth, enhance wound blood perfusion, promote fibroblast migration, induce angiogenesis, and stimulate keratinocyte activity. This therapeutic approach has shown promise in addressing both acute and chronic wounds. Specifically, wounds treated with ES, particularly pulsed current, have significantly reduced in size, particularly chronic wounds. We thoroughly discussed the fundamental biological process of wound healing in accordance to morphology and regeneration process. The study delves into the roles of HVPC, LIDC, PEMF, TENS, FREMS, and DC–AC in further reinforcing the contribution of ES to the enhancement of cutaneous wound repair, supported by measurable objective criteria and histological analysis. There is compelling evidence, both invasive and non-invasive, indicating that ES treatments lead to increased angiogenesis. Moreover, this therapeutic approach holds promise for addressing delayed chronic wounds. Furthermore, self-sustaining ES, a wearable wound-healing device that offers intriguing new possibilities for the management of wound care, is a result of recent advancements in nanogenerator technology.

## Ethics approval and consent to participate

No animals have been harmed and no ethics have been violated.

## Data availability

Data will be available upon request.

## Author contributions

Conceptualization by S. P.; writing—original draft preparation S. P., A. G., A. P., D. S., R. M., S. R. and S. M.; all authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

The authors declare that they have no conflict of interest.

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