### Title

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### Journal

Cold Spring Harbor Perspectives in Biology, 14(10)

### ISSN

1943-0264



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### Publication Date

2022-10-01

### DOI

10.1101/cshperspect.a041236

# Bioelectric Signaling: Role of Bioelectricity in Directional Cell Migration in Wound Healing

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In wound healing, individual cells' behaviors coordinate movement toward the wound center to restore small or large barrier defects. The migration of epithelial cells as a continuous sheet structure is one of the most important processes by which the skin barrier is restored. How such multicellular and tissue level movement is initiated upon injury, coordinated during healing, and stopped when wounds healed has been a research focus for decades. When skin is wounded, the compromised epithelial barrier generates endogenous electric fields (EFs), produced by ion channels and maintained by cell junctions. These EFs are present across wounds, with the cathodal pole at the wound center. Epithelial cells detect minute EFs and migrate directionally in response to electrical signals. It has long been postulated that the naturally occurring EFs facilitate wound healing by guiding cell migration. It is not until recently that experimental evidence has shown that large epithelial sheets of keratinocytes or corneal epithelial cells respond to applied EFs by collective directional migration. Although some of the mechanisms of the collective cell migration are similar to those used by isolated cells, there are unique mechanisms that govern the coordinated movement of the cohesive sheet. We will review the understanding of wound EFs and how epithelial cells and other cells important to wound healing respond to the electric signals individually as well as collectively. Mounting evidence suggests that wound bioelectrical signaling is an important mechanism in healing. Critical understanding and proper exploitation of this mechanism will be important for better wound healing and regeneration.

Q2 Electric fields (EFs) generated in physiological processes across the animal kingdom are one mechanism by which cells communicate with one another. Bioelectrical signaling plays an important part in processes at the cellular level such as stem cell differentiation and neuronal dendritic outgrowth (McMillen et al. 2021) and in integrated tissue processes such as embryogenesis, limb morphogenesis, and cancer (for review, see Chang and Minc 2014; Funk 2015; Levin 2021). In this review, we focus on the role of bioelectric signaling in the process of epithelial wound healing.

Editors: Xing Dai, Sabine Werner, Cheng-Ming Chuong, and Maksim Plikus
Additional Perspectives on Wound Healing: From Bench to Bedside available at www.cshperspectives.org


Advanced Online Article. Cite this article as *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a041236

A critical process in wound healing is epithelialization, in which keratinocytes or other types of epithelial cells migrate, proliferate, and differentiate to form continuous epidermis or epithelium (Fig. 1A; Martin 1997; Gurtner et al. 2008; Stappenbeck and Miyoshi 2009; Eming et al. 2014). Successful wound closure is defined by complete coverage of the wound bed by the continuous epidermis (i.e., epithelialization). In the absence of an intact epithelial barrier, a wound remains a portal for the entry of microorganisms and other pathogenic substances. An unepithelialized wound results in loss of local ionic, nutrient, and pH control, altering biochemical, biomechanical, and bioelectrical homeostasis. Together these, or some combination thereof, contribute to the clinical consequences known as chronic and nonhealing wounds that include diabetic foot ulcers, venous ulcers, and pressure ulcers, which constitute a major medical concern and financial burden to the healthcare system (Pastar et al. 2014; Olsson et al. 2019; Sen 2021). The incomplete understanding of the mechanisms for epithelialization in wound healing has stymied efforts to optimize this process.

Contrary to expectation, a defect in the proliferative capacity of keratinocytes appears not to be the major issue in impaired epithelialization. Rather, in most types of chronic wounds, counterintuitively, keratinocytes at the wound edge are hyperproliferative. In pressure ulcers, venous ulcers, and diabetic foot ulcers, the epidermis is characterized by hyperproliferation at the wound edge, attributed to *c-myc* activation and overexpression (Stojadinovic et al. 2005, 2008; Pastar et al. 2014). The hyperproliferative keratinocytes contribute to a thickening of the epidermis and cornified layer. Rather than a proliferative defect, activation of the β-catenin/c-MYC pathway(s) is suggested to contribute to impaired healing by inhibiting keratinocyte migration and altering their differentiation (Stojadinovic et al. 2005).

Control of migration of the keratinocytes thus may hold the key for successful wound epithelialization. Good epithelialization is characterized by keratinocyte migration to form a coherent sheet, growing/sliding into the wound to epithelialize the denuded wound bed (Fig. 1A; Zhao et al. 2003). A better understanding of the epithelialization process and of the bioelectrical signals that control it can ultimately lead to the development of effective therapeutic approaches to facilitate wound healing (Stojadinovic et al. 2005; Pastar et al. 2014). This perspective will focus on electrical signaling in wounds and provide an understanding of the intracellular signaling events that result in collective directional migration of epithelial sheets and provide support for positing that this may be one of the fundamental mechanisms promoting wound healing.

The migration of large epithelial sheets is poorly understood. Many important mechanisms and mediators have been demonstrated to contribute to epithelialization: injury stimulation, growth factors, cytokines, mechanical forces, denuded space available, and changes in wound bed stiffness (Pastar et al. 2014). Yet very few of them have been demonstrated to be able to mobilize and guide directional migration of cohesive epithelial sheets in experimental in vitro models. EFs can provide that signal. An endogenous EF is naturally generated when skin is wounded. The wound EF instantly comes into being when the epithelial barrier is compromised with the cathodal pole localized to the wound center. We demonstrated that EFs of physiological strength mobilize and guide migration not only of isolated keratinocytes but also of large epithelial sheets, as well as sheets of other types of cells that maintain intercellular junctions during migration (Zhao et al. 1996, 2006). More recently, these results have been confirmed demonstrating EF-guided collective migration of keratinocytes and other epithelial cells (Cohen et al. 2014; Zajdel et al. 2020, 2021; Shim et al. 2021).

We will first present experimental evidence demonstrating the electrical properties of the stratified epidermal epithelium and review the current understanding of how those electrical phenomena are generated in the epidermis. We then will review the experimental data that demonstrate how EFs serve as a powerful mechanism for mobilizing and guiding the migration of cells important for wound healing. Finally, we focus on the mechanisms underlying the collective migration of epidermal and corneal epithelial sheets

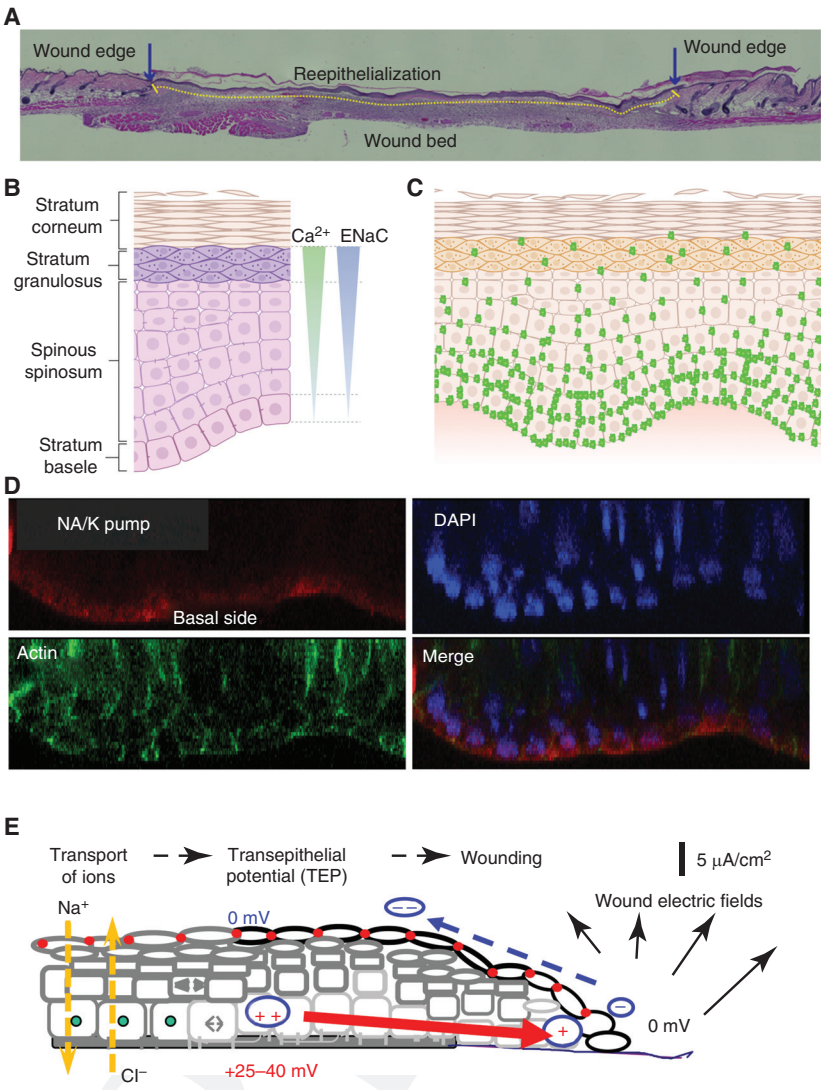


**Figure 1.** Gradients of ions and ion transporters, and endogenous electricity at a wound. (*A*) Fully reepithelialized mouse skin wound. C57BL/6J mouse was wounded on the dorsum skin with an 8-mm circular punch, and the wound tissue harvested on day 10 post-wounding. Arrows indicate original wound margin, marked by last hair follicle. The wound bed has totally reepithelialized (yellow dashed line). Although a functional epithelium has formed to provide a barrier for the wound, note the absence of regenerated hair follicles or other adnexa in the wound bed. (*B*) Schematic representation of epidermis architecture of newborn mouse. Continuous tight junctions were found mainly in the second layer of the stratum granulosum. The extracellular $Ca^{2+}$ gradient and the increased expression of sodium channel ENaC subunits in more differentiated cell layers are indicated. (*B*, Based on Guitard et al. 2004; redrawn by Chelsea Brown.) (*C*) Na/K pump is expressed higher in the basal layers than in apical layers. (*C*, Based on Dubé et al. 2010; redrawn by Chelsea Brown.) (*D*) Basal expression of Na/K pump in monolayer of Manin–Darby canine kidney (MDCK) cells. (*D*, Reprinted from Tran et al. 2013 under the terms of the Creative Commons Attribution License.) (*E*) Transport of ions establishes electrical potentials and produces wound electric currents. (*Left*) Expression and function of polarized ion channels and pumps transport ions (yellow arrows), which are separated by tight junctions (red dots), resulting in establishment of the transepithelial potential (TEP; 25–40 mV, basal side positive relative to the apical surface). Injury breaks down the electrical barrier, resulting in wound electric currents flow toward (red arrow) and out of (black arrows) wound. The currents return along the blue arrow, to reach adjacent normal epidermis, which like a battery keeps transport ions (yellow dotted arrows) to maintain current flow in the circuit. (*E*, Reprinted with modifications from Zhao 2009, with permission from Elsevier © 2009.)

in the process of wound healing and point out how the migration of other types of epithelial sheets can illustrate the shared mechanisms.

## EPIDERMIS IS A STRONG ELECTRICAL BARRIER

Epidermis, like other epithelia, has the responsibility of establishing a tissue barrier, separating the internal from the external environment. This barrier function is critical for maintaining internal fluid and electrolyte balance and preventing entry of infectious or toxic substances. The functions of epidermis include physical, chemical, immunological, and antimicrobial barrier roles (Niehues et al. 2018). The physical barrier consists of the cross-linked keratin cytoskeleton contributing to keratinocyte stiffness and resilience (Lulevich et al. 2010), the unique lipids generated in the different epidermal stratified layers (Feingold and Elias 2014a,b), and the biomechanical properties of the stratum corneum (Évora et al. 2021). The chemical barrier is attributed to unique cross-linked proteins in the stratum corneum aided by peptides and reactive oxygen species produced by the keratinocytes. The immune barrier is formed by the innate and adaptive immune systems, like Langerhans cells and T cells, and cytokine production by keratinocytes. Microbes residing on the skin surface are believed to form the commensal skin microbiome barrier to prevent pathogenic microbes and educate the innate immune system (Nakatsuji et al. 2021). Those barrier functions are paramount in skin health and diseases (Belkaid and Segre 2014; Brandner et al. 2015; Brettmann and de Guzman Strong 2018; Chen et al. 2018), including wound healing.

The skin, mainly the epidermis, prevents the free movement of electrolytes and ions, thus forming a strong electrical barrier. More than 99% of the body's resistance to electric current flow is at the skin (Fish and Geddes 2009). The electrical barrier is relatively easy to assess quantitatively, measuring either transepidermal/transepithelial electrical resistance (TEER) or skin electrical impedance. Those parameters provide reliable measures of the epidermal electrical barrier and have been used often to quantify skin barrier function, similar to the transepidermal water loss (TEWL) assay (Whelan 1950; Lewis and Basketter 1995; Abdayem et al. 2015). The TEER and TEWL both give a quantitative assessment of the epidermal barrier and can detect and quantify breaches of the skin barrier by proteases, cholera toxin, or mechanical stripping (Rinaldi et al. 2019). When injury to the skin or skin disease compromises the skin barrier, electrical resistance and electrical impedance change accordingly (Lodén et al. 1999; Shan et al. 2012; Bäsler et al. 2016; Rinaldi et al. 2021).

The electrical resistance of the skin, as an electric component, is measured in ohms (Ω). Dry skin may have a resistance measurement of >100,000 Ω because of the outer layer of dead cells in the stratum corneum and the contribution of the outermost viable layer of the stratum granulosum with its tight junctions (TJs) (Fish and Geddes 2009; Birgersson et al. 2013). When skin is fully hydrated, or immersed in water, the resistance is reduced to ∼1000 Ω, closer to that of monolayer cultures of other epithelia (Fish and Geddes 2009; Kong et al. 2011).

TJs are a complex of about 40 different transmembrane proteins embedded in the plasma membranes of adjacent cells, with extracellular domains joining one another, preventing the flow of molecules and ions through the space between adjacent cells. Keratinocytes, when differentiating from the basal to the superficial layers, demonstrate a gradient of TJ structural protein expression, which contributes to a tight physical barrier within the epithelium, particularly in the granular layer, preventing the free movement of ions and other electrolytes between different layers (Bazzoni and Dejana 2002; Furuse et al. 2002; Niessen 2007; Brandner et al. 2015; Yuki et al. 2016; Rübsam et al. 2017). Such an intercellular junctional difference corresponds to the keratin 10 gradients and other cell differentiation marker gradients (Pastar et al. 2014). The diffusion of small-molecule dyes across different layers of epidermis thus can be a good assessment of the skin barrier function and its recovery following barrier function breaches (Fig. 1B–D; Schmitz et al. 2015).

In stratified epithelium that migrates into a wound, as in skin and oral epithelium, occlud-

ing and claudin-1, two TJ component proteins, are present in migrating epithelial cells at the wound edge (Volksdorf et al. 2017; Shi et al. 2018; Leonardo et al. 2020). Injury results in increased claudin-1 localization to the superficial layers (Volksdorf et al. 2017). In corneal epithelium, higher-resolution imaging analysis revealed that another TJ protein, zonula occludens-1, is expressed right behind the very first cell at the wound leading edge (Danjo and Gipson 1998) (see Fig. 3, red dots between cells in the superficial layer). Because TJs form the high electrical resistance of the cohesive sheet, such an expression pattern ensures not only a continuous epithelial sheet and highly coordinated collective migration, but also focusing or funneling of the wound electric currents (wECs) and wound electric fields (wEFs) at the wound edge, because the currents are channeled through the relatively lower electrical resistance wound edge (Fig. 1E) (for details, please see below the subsection Electric Fields Naturally Occur at Wounds). Without such proper "electrical insulation" (i.e., expression level and localization of TJs), wEFs and wECs are expected to have an aberrant pattern, which, indeed, has been demonstrated in diabetic and Pax6 mutant corneas where TJ expression is aberrant (Leiper et al. 2006; Ou et al. 2008; Kucerova et al. 2011; Shen et al. 2016). Both the cornea and epidermis of diabetic animals have significantly lower levels and mislocated expression of TJs at their wounds (Volksdorf et al. 2017; Alfuraih et al. 2020). Importantly, TJ proteins in epidermis advancing in normally healing wounds are present predominantly in the superficial layers and the leading rows of keratinocytes, whereas the epidermis in chronic wounds loses this distinct feature: occludin and claudin-1 are absent in the superficial layers and at the leading tongue of epidermis migrating into the wound (Volksdorf et al. 2017; Shi et al. 2018; Leonardo et al. 2020).

## EPIDERMIS IS A BATTERY

Keratinocytes express abundant ion channels, pumps, and transporters (Olah et al. 2012). Activities of the channels and pumps not only affect the ionic homeostasis of cells, generating and maintaining the cell membrane potential of individual cells, but importantly also establish tissue level ionic gradients and electric potentials across epidermal layers. In addition to gradients of well-characterized differentiation markers, three categories of electrically relevant gradients have been experimentally demonstrated: ion channel and pumps, ions, and electric potential.

Ion channels and pumps are differentially expressed at various layers of the epidermis, in a polarized or gradient manner. Epithelial sodium channels (ENaC) and the atypical sodium channel Nax (scn7a) are most highly expressed in the granular layer and gradually decrease to the basal layer (Fig. 1B; Brouard et al. 1999; Guitard et al. 2004; Xu et al. 2015a; Adams 2016). Na/K pumps, however, demonstrate a reverse gradient, being most highly expressed in the basal layers and with reduced expression in more superficial layers of the tissue (Fig. 1C,D; Dubé et al. 2010; Moulin et al. 2012). This tissue level distribution is very similar to apical versus basal–lateral distribution of sodium channels and Na/K pumps exhibited by cultured monolayers of transporting epithelial cells (Fig. 1B-D; Krupinski and Beitel 2009; Enuka et al. 2012; Tran et al. 2013; Xu et al. 2015a). Other channels (e.g., transient receptor potential [TRP] channels V4 [TRPV4], aquaporin 3 and 5) also show very distinctive gradients in the epidermis (Blaydon and Kelsell 2014).

The epidermis also maintains distinct gradients of different ions, which are important for epidermal structure and function. A calcium gradient is maintained with the highest levels in the granular layer (Fig. 1B; Mauro et al. 1998; Elias et al. 2002; Leinonen et al. 2009; García et al. 2016; Streubel et al. 2018). Sodium and chloride have gradients with the highest concentration at the basal epidermal layer and in the dermis (von Zglinicki et al. 1993; Forslind et al. 1997; Mauro et al. 1998; Paweloszek et al. 2016; Tarnowska et al. 2020). The stratum corneum is acidic, with a pH of 4.1–5.8, whereas the basal layer of the epidermis has a pH value closer to 7.4. The acidification gradient has been attributed to filaggrin degradation, fatty acid content, sodium–hydrogen exchanger (NHE1) activation, and melanosome release (Proksch 2018).

When the skin barrier is disturbed, significant changes and even the total disappearance of gradients of calcium, chloride, and proton across the epidermis can occur (Mauro et al. 1998). Disturbance of the epidermal calcium gradients is seen in aging skin and is attributed to the noted impaired homeostasis of aged skin (Rinnerthaler et al. 2015).

Electrical potential gradients are present across the epidermis and other epithelia as a result of the active transport of ions that establish the ionic gradients (Ussing and Zerahn 1951). Transcellular transport across the cell membrane and paracellular transport of ions limited by TJs in the epithelial cells collectively establish unique electrical properties of the epithelium (Adams 2016). Ion channels and pumps translocate ions across the different layers of the epidermis, much like a battery or generator, generating ionic fluxes. Cell–cell junctions prevent the free movement of ions, like resistors. The combined results are the formation of ionic gradients and electrical potentials across the different layers in the epidermis, collectively forming the transepithelial potential (TEP) (Barker et al. 1982; Dubé et al. 2010; Kawai et al. 2011; Moulin et al. 2012). The TEPs have been demonstrated using various techniques in animal epithelia. Barker and colleagues used glass microelectrodes and silver chloride electrodes and demonstrated consistent potential differences of tens of millivolts across skin at various positions in guinea pigs and in human from scalp to sole (Illingworth and Barker 1980; Barker et al. 1982; Foulds and Barker 1983). The TEP normally measures ~20–40 mV across the epidermis that ranges from ~80–270 microns in thickness in humans (Illingworth and Barker 1980; Barker et al. 1982; Foulds and Barker 1983; Oltulu et al. 2018; Abe et al. 2019a, 2019b, 2021). The orientation of skin TEPs has the positive pole at the basal side of the epidermis relative to the negative pole at the skin surface (Fig. 1E).

Defective channels and skin barrier function are interrelated (Blaydon and Kelsell 2014). The functions of ion channels, gradients of ions, and the electric potential differences play critical roles in the differentiation of keratinocytes, epidermis stratification, and barrier establishment (Denda and Kumazawa 2002; Kumamoto et al. 2013). For example, the gain-of-function mutations in channels such as the aquaporin or transient receptor potential channels in human result in distinct epidermal phenotypes with abnormal differentiation and barrier function. The gain-of-function mutations in aquaporin 5 or TRP channels V3 result in keratoderma and hyperkeratotic plaques, respectively (Lin et al. 2012; Blaydon et al. 2013; Blaydon and Kelsell 2014).

Knockout of atypical sodium channel Nax results in deficient wound healing in a murine splinted excisional wound-healing model, in which healing is dependent on epithelialization, rather than wound contraction (Hou et al. 2021). The Na/K ATPase pump (Na/K pump) is required for the formation and maintenance of intercellular junctions and the development of epithelial tubes in mammals, zebrafish, *Drosophila*, and *Caenorhabditis elegans*. The association of the Na/K pump with cell junctions is proposed to enable vectorial transcellular ion transport and regulate the intraorganismal environment (Krupinski and Beitel 2009). Conversely, disrupting the barrier (e.g., by tape stripping) results in altered $Ca^{2+}$ ionic gradients (Ahn et al. 1999) and increased transepidermal sodium flux, presumed to be via changes in expression of the ENaCs (Xu et al. 2015b). These have physiological system consequences for the organism: disruption of the ionic and electrical gradients is shown to be closely correlated with skin injuries and diseases, such as atopic dermatitis, irritant contact dermatitis, ichthyosis, rosacea, and acne, in which the skin barrier functions, including the electrical barrier, are compromised (Proksch 2018).

## Electric Fields Naturally Occur at Wounds

The skin battery formed by activities of ion channels and pumps, together with electrical resistant cell–cell junctions, is maintained across the epithelium at the body surface. Injuries to the skin that result in fluid exudation (blood or tissue fluid) at the wound site short-circuit the TEP by providing an ionic path to connect the positive pole (the basal side) to the negative pole (surface) of the skin battery (Fig. 1E), thus generating an immediate wEF and concurrent wECs at the

breach. The wEF is established with the negative pole at the wound center; thus, wound electric currents flow into the wounds (red arrow in Fig. 1E). The conventional flow of positive charges is used to show the direction of the wEFs, and the wECs; both are reoriented to the wound center from all directions in the epidermis.

Numerous experiments over nearly two centuries from different laboratories have demonstrated the existence of the wEFs and wECs. To measure the wECs and wEFs, different techniques have been used, including galvanometer, glass microelectrodes, the vibrating probe, Ag/Cl microelectrodes, bioelectric imager, Kelvin probe, and microneedle arrays (Barker et al. 1982; Foulds and Barker 1983; Chiang et al. 1992; Mukerjee et al. 2006; Nuccitelli et al. 2008; Ahn et al. 2012; Gow et al. 2012). One of the founders of modern electrophysiology, Emil du Bois-Reymond, was the first one who measured electric currents flowing out of skin wounds (du Bois-Reymond 1843). Nuccitelli and Jaffe developed the vibrating probe for the measurement of wECs (Nuccitelli et al. 2008). Jaffe, Barker, Nuccitelli, Borgens, and our laboratories have used different techniques and demonstrated the coming into being and evolution of such wEFs at skin wounds, as well as in wounds of cornea, trachea epithelium, gut epithelium, ocular lens epithelium, and retinal pigment epithelium (Betz et al. 1980; Nuccitelli et al. 2003, 2008; McCaig et al. 2005; Mukerjee et al. 2006; Gamboa et al. 2010; Lois et al. 2010; Reid and Zhao 2011; Sun et al. 2019).

Biological tissues, tissue layers, groups of cells, interstitial fluid, and blood or other bathing solutions or culture media all have measurable electrical resistance. The current size ($I$), voltage ($V$), and resistance ($R$) at wounds and in the tissues follow Ohm's law, $V = I^* R$. We will describe the wound electrical signals as wECs and wEFs following the original experimental techniques and reports. Heterogeneity in complex tissue and cell resistance and electrical generation will be simplified in the following description.

The wound electric signals are immediate and persistent. The wEFs are produced instantaneously upon injury and persist until the epithelial barrier recovers (i.e., complete reepithelialization of the wound surface that prevents the short-circuiting of the epithelial battery at the wound). Illingworth and Barker measured electric currents flowing out of a fingertip wound following amputation and found that the currents persisted for weeks or even months and only disappeared after the fingertip wound had fully healed (Illingworth and Barker 1980). At simple skin incisional wounds, ear punch, and corneal wounds, wEFs persisted for hours, days, and weeks until presumably the barrier recovery is complete and the connection site that short-circuits the skin battery is terminated (Reid et al. 2007; Ferreira et al. 2016).

The wound electric signals are temporally regulated. When the resting skin battery is short-circuited, the wECs are instantaneously produced. However, the time to peak wEC values varies with the size of the initial wound. In small corneal or small skin incision wounds, peaks are reached in an hour or so (Reid et al. 2005, 2007; Zhao et al. 2006; Guo et al. 2010). At small incisional wounds in mouse and human skin, wEFs appear immediately following injury and continue to increase over a day or two, only to return to zero when the wound has completely healed (Nuccitelli et al. 2008; Guo et al. 2010). In large skin wounds (e.g., amputated fingertips), the wound currents reach their peak 4 d after amputation (Illingworth and Barker 1980). Interestingly, at amputated tadpole tail stumps, the wEFs and wECs demonstrate changing magnitude and even direction along a time course of 2–3 d, which correlates with local oxygen consumption and activities of sodium channels and can predict whether the tails will regenerate or not (Ferreira et al. 2016, 2018, 2020). The dynamic time course of wECs and wEFs strongly suggests that a significant component of the epithelial response to injury—namely, the generation and production and regulation of wound electric signals—is a key signal for healing, akin to other biochemical signals generated during the wound-healing process.

The wound electric signals are spatially controlled and converge at wound edge. Spatially, the wEFs are funneled toward the wound, with the strongest wEFs at the wound edge. Although the entire remaining epithelial tissue transports ions, only the TEP at the wound site is short-

circuited, forming a sink of electric currents. The TEP across all epithelia, and in this case of the epidermis, thus drives current to converge at the wound, building the strongest wEFs at the wound edge (Reid et al. 2005, 2007; Zhao et al. 2006; Ferreira et al. 2016). Glass microelectrode, vibrating probe, and microneedle measurements have demonstrated that >90% of the wEFs in both cornea and skin are focused within only a 1-mm range from the wound edge, and the rest (~10%) within the next 1–2 mm (Barker et al. 1982; Chiang et al. 1992; Reid et al. 2005; Mukerjee et al. 2006).

The physiological ranges of the wound electric signals are between a few to 150 mV/mm and a few to 4–20 uA/cm$^2$ depending on time and location of measurements. Those values are benchmarks when physiological EFs and ECs are discussed.

The wound electrical signaling requires activities of $Na^+$ channels, $Cl^-$ channels, and Na/K ATP pumps. Inhibition of Na channel with amiloride or benzamil, as well as inhibition of Na/K ATP pumps with ouabain, significantly reduce TEP and wECs and wEFs (Reid et al. 2005; Zhao et al. 2006; Nuccitelli et al. 2008; Tran et al. 2013). Of note, wEFs also decline with age, and wECs are significantly impaired in diabetic animals (Nuccitelli et al. 2011; Shen et al. 2016). These changes coincide with the disappearance of the epidermal $Ca^{2+}$ gradient in human skin (Streubel et al. 2018), which could contribute to faulty epidermal differentiation.

## EFs Guide Migration of Keratinocytes and Other Cells Important for Wound Healing

Directional migration of cells in an EF—namely, galvanotaxis/electrotaxis—in isolated keratinocytes, corneal epithelial cells, and many other types of cells has been reviewed (Robinson 1985; Wu and Lin 2014; Funk 2015; Feng and Kamp 2017). Experimental results using primary cultured keratinocytes and cell lines all suggest that very small EFs, as low as 8 mV/mm, guide directional migration of keratinocytes (Nishimura et al. 1996; Fang et al. 1999; Yang et al. 2013). Such a field strength is well within the physiological strength measured at the skin and other epithelial wounds (Chiang et al. 1992; Sheridan et al. 1996; Nuccitelli et al. 2003, 2008; Reid et al. 2005, 2007, 2011). Other types of cells that are important for wound healing, including dermal fibroblasts, neutrophils, melanocytes, macrophages, and endothelial cells, have been reported to respond to applied EFs, although significant variations in voltage and time required to induce direction migration are reported (Isseroff et al. 2001; Grahn et al. 2003; Sillman et al. 2003; Zhao et al. 2004; Guo et al. 2010; Wosik et al. 2018; Sun et al. 2019; Ammann and Slepian 2021).

Not only is the migratory speed of a keratinocyte a factor in moving it into the denuded area to resurface a wound but directing its migratory trajectory toward the wound is a critical function. Galvanotactic bioelectric signaling may be a key cue for this function. Numerous studies tracking the migratory paths of isolated skin keratinocytes or other epithelial cells have noted that when they are placed in an EF of physiological strength, they reorient and migrate toward the cathode (Cooper and Schliwa 1986; Nishimura et al. 1996). How the cell senses and responds to an EF with directed migration has been the subject of study for decades and is still not fully understood. SenGupta and colleagues have proposed four unifying principles that underlie any directional cell migratory response: (1) generation of the directional signal, (2) sensing of the signal, (3) transmission of the signal from the sensor to the cellular machinery that controls movement, and (4) conversion of signal to an asymmetrical force to move the cell directionally (SenGupta et al. 2021). For chemotactic stimuli, most of these principles have been identified. For galvanotaxis, identification of principle 2 remains a challenge. For the first principle, the generation of a signal, an endogenous EF at the site of the wound has been amply documented (Mukerjee et al. 2006). For principle 3, identification of the signal's transmission to the intracellular mechanical components that move the cell has also been documented. We have demonstrated leading-edge actin and myosin polymerization in epithelial cells exposed to EFs (Zhao et al. 2002; Sun et al. 2013). Numerous signaling pathways, similar to those called into play in chemotaxis, are also

polarized and activated at the leading edge in response to the application of the EF, including PI3 kinases/Pten, pSrc, and phosphorylation of glycogen synthase kinase 3β (GSK-3β), resulting in reorientation and polarization of the Golgi apparatus (Cao et al. 2011), membrane growth factor receptors, and integrins (Zhao et al. 2006; Zhao 2009; Kucerova et al. 2011; Zhu et al. 2019). Intracellular polyamines are also polarized by exposure to the EF and likely amplify signaling from the cell membrane $K^+$ channel Kir4.2 channel with which they are associated (Nakajima et al. 2015; Nakajima and Zhao 2016). The conversion of the signal to a force to propel the cell in a given direction, principle 4, also shares the common lamellipodial extension and protrusion mechanics that govern directional migration in response to a chemotactic gradient (Yang et al. 2013; Saltukoglu et al. 2015). It is principle 2, the identification of the most proximal sensor of the EF signal, that has remained elusive. The high resistance of the plasma membrane (Poo 1981) has led to the belief that sensing of the EF should occur on the cell membrane's outer surface. Indeed, electromigration of plasma membrane macromolecules or relocalization of outer membrane components in response to EF exposure has been noted for membrane lipids (Zhao et al. 2002), glycosylphosphatidylinositol-anchored membrane proteins (Sarkar et al. 2019), heparan sulfate and other sulfated glycosaminoglycans (Huang et al. 2017), epidermal growth factor (EGF) receptors (Zhao et al. 2002; Pu et al. 2007), galectin 3 (Liu et al. 2012), integrins (Sarkar et al. 2019; Zhu et al. 2019), purinergic receptors (Nakajima et al. 2019; Sarkar et al. 2019), and membrane lipid raft (Fig. 2; Zhao et al. 2002; Lin et al. 2017). Changes in the immediate cell membrane vicinity by alterations in extracellular pH or viscosity of the extracellular medium affect directionality of the EF response (Fang et al. 1999; Saltukoglu et al. 2015; Kobylkevich et al. 2018). A number of channels and receptors on the cell surface have also been implicated as proximal receptors with studies demonstrating their relocalization in the EF and loss of directionality in cells in which the receptors are inhibited or genetically ablated. For epithelial cells, the list includes one of the earliest recognized receptors to guide directional migration in EF, the EGF receptor (Fang et al. 1999; Zhao et al. 2002), and many others, including ENaC (Yang et al. 2013), the purinergic receptor (Saltukoglu et al. 2015), and the $\beta_2$ adrenergic receptor (Pullar and Isseroff 2005). Other cell types likely transmit the EF signal through other cell membrane receptors (e.g., in endothelial cells VEGF is critical to sensing the field [Zhao et al. 2004]). Yet, despite the identification of these membrane receptors as putative sensors, the mechanism by which the EF activates them remains unknown.

Experiments on in vivo galvanotaxis that are relevant to wound healing are limited, largely because of technical difficulties in application of EFs in a well-controlled manner in vivo and study cell migration. More amenable experimental models are needed. Using an ex vivo/ex ovo chick skin model, directional feather bud growth and the coordinated mesenchymal cell migration have been shown to be accompanied by dynamic changes of skin endogenous bioelectric currents (Li et al. 2018). Genetic and pharmacological manipulation in this model suggesting cell and tissue polarization mechanisms is regulated by calcium signaling, which probably coupled to changes in tissue level EFs, thus orchestrating coordinating cell movement and directional feather bud growth. Remarkably, application of EFs reorients the direction of feather bud globally (Jiang et al. 2021). Video microscopic tracing of green flourescent protein (GFP)-tagged immunocytes in the skin of mouse ears reveals that motile cutaneous T cells actively migrate toward the cathode of an applied direct current (DC) EF (Lin et al. 2008). These results suggest the importance of bioelectricity in collective cell behaviors in the skin in vivo, thus in wound healing (Li et al. 2018; Jiang et al. 2021). With significant development in bioelectronics and imaging techniques, better controlled application of EFs in vivo and the effects on cell migration are expected to produce some definitive results in the coming years.

## Physiological Electric Fields Guide Migration of Sheets of Epithelial Cells

Epithelialization is a collective behavior essential for wound healing. Collective cell migration is a

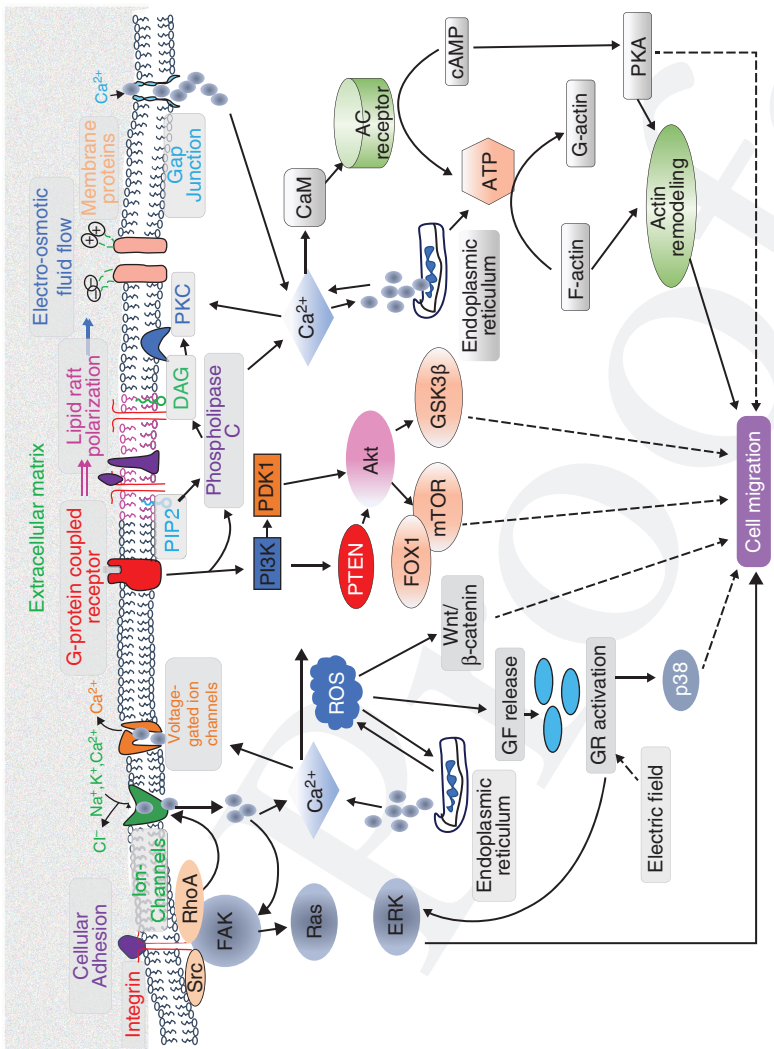


Figure 2. Putative signaling pathways involved in electrical signaling in galvanotaxis. An extracellular electric field may exert its effects on various cellular membrane components resulting in activation of downstream signaling pathways leading to directional cell migration. Cell membrane components implicated include integrins, ion channels, gap junctions, electrophoretic and electro-osmotic movement of charged molecules, lipid raft polarization, and membrane receptors (e.g., G-protein-coupled receptors, growth factor receptors). (ROS) Reactive oxygen species, (Src) intracellular protein-tyrosine kinase, (FAK) focal adhesion kinase, (Ras/ERK) extracellular signal regulated kinase pathway, (RhoA) ras homolog family member A, (GF) growth factor, (PIP2) phosphatidylinositol 4,5- bisphosphate, (DAG) diacylglycerol, (PKC) protein kinase C, (PI3K) phosphatidylinositol-3 kinase, (PDK1) 3-phosphoinositide-dependent protein kinase-1, (PTEN) phosphate and tensin homolog, (mTOR) mammalian target of rapamycin, (FOX1) forkhead box protein 1, (GSK3β) glycogen synthase kinase-3, (CaM) calmodulin, (AC) adenylyl cyclase, (PKA) protein kinase A, (cAMP) cyclic adenosine monophosphate. (Figure provided by Dr. Abijeet Mehta, Northwestern University.)

key feature of this process. Keratinocytes migrate as a cohesive sheet of epithelial tissue. However, other types of cells, such as neutrophils, macrophages, and fibroblasts, migrate into the wounds as individual cells without tight cell–cell contact and junctions. The majority of experiments to study the mechanisms by which cells sense and respond to EFs with directed migration or galvanotaxis have been performed on isolated cells in culture. That approach provides the advantage of being easy to analyze at the single-cell level.

To understand cohesive sheet movement, how exactly each keratinocyte's migration is related to its neighboring cells in the course of wound reepithelialization has been studied in detail in the past decades. Two models have been proposed to explain this movement in stratified epithelia, such as the skin and cornea: a "sliding" model and a "leapfrog" model (Donaldson and Mahan 1988; Stenn and Malhotra 1992). In the sliding model, the epithelium surrounding the wound migrates into the wound as a coherent sheet with little change of relative position between cells (Weiss 1961; Vaughan and Trinkaus 1966; Kuwabara et al. 1976; Brewitt 1979; Buck 1979; Radice 1980). In the leapfrog model, individual cells repeatedly crawl over each other (leapfrog) at the tip of the healing epithelium to access the wound bed (Krawczyk 1971; Winter 1972; Gibbins 1978; Ortonne et al. 1981; Garlick and Taichman 1994; Martin 1997). We used Hoffman microscopy to directly visualize individual cells in stratified corneal epithelium and imaged the migration patterns of cells in the different layers of the epithelium to reconstruct three-dimensional (3D) videos of individual cell movements. Video microscopy coupled with digital 3D reconstruction allowed direct visualization of the movement of individual cells in the epithelium and demonstrated that the stratified corneal epithelium resurfaces the wound area mainly using "sliding" movements. About 97% of cells during the healing process maintained their positions relative to their neighboring cells in $x$, $y$, $z$ directions ($x$ being the direction of migration into the wounds, $y$ the axis perpendicular to the wound edge, and $z$ the apical–basal axis). Only 3% of cells were seen to have demonstrated a small change in their position relative to neighbors (Zhao et al. 2003). Therefore, this study demonstrates that epithelial cells in a cohesive sheet migrate into the wound not only maintaining their tight intercellular junctions but also maintaining their original positions relative to neighboring cells.

Maintaining relative positions minimizes disruption of cell–cell junctions and promotes the coordination of cell migration as a cohesive tissue, rather than individual cells. The minimal disruption of intercellular junctions also confers the advantage of maintaining a functional barrier in the moving tissue as it migrates over the wound (Zhao et al. 2003). Two widely used models to study collective migration of epithelial cells that demonstrate the maintenance of good intercellular junctions are the scratch assay and barrier release assay (Liang et al. 2007; Das et al. 2015). In those models, cell membrane injury, biochemicals released from the newly generated free edge, contact inhibition release, and new cell-free space available for migrating cells provide strong stimuli for cells at the leading edge to polarize and guide collective migration into the in vitro wound and for a few individual cells to do so when their junctions to other cells are disrupted (Cao et al. 2011; Zhang et al. 2017). Our experiments demonstrated that EFs provide a powerful guidance mechanism that reinforces the mechanism previously identified in individual cells of membrane protrusion to result in cell migration of the sheet's leading-edge cells. The EFs also mobilize cells in the middle of cell sheets and, importantly, overrides guidance of the free edge by other coexisting directional migratory stimuli when they contradict the directional galvanotaxis guidance, in which case cells collectively retreat from the wounds, thus forming a trailing edge of the groups/sheets (Fig. 3; Zhao et al. 1996, 2006; Zhao 2009).

In the absence of applied EFs or other migratory stimuli, sheets of cultured keratinocyte and corneal epithelial cells do not show overall net migration of the cell sheets, other than migration of individual cells at the leading edge into the acellular space. Those leading-edge cells migrate into the space available, resulting in the expansion of cell sheets to cover larger areas through

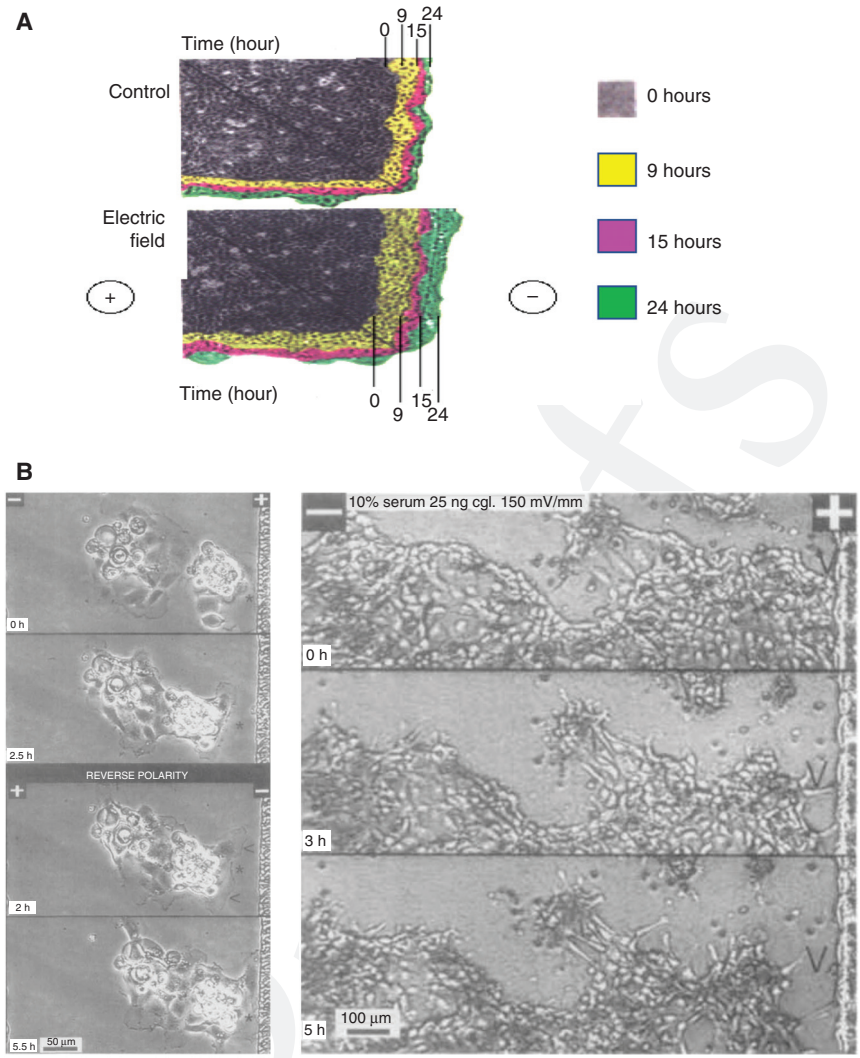


**Figure 3.** Collective galvanotaxis of sheets of keratinocyte (*A*) and corneal epithelial cells (*B*). (*A*) Electric fields enhance wound healing in vitro. Confluent cultures of neonatal human keratinocytes were "wounded" with a pipette tip. One culture dish was exposed to an applied DC electric field of 100 mV/mm (*lower* culture, labeled Electric field; + and – indicate anodal and cathodal poles of the applied field), and one culture was not exposed to an electric field (*upper* culture, labeled Control). Both cultures were returned to the incubator and maintained in tissue culture medium at 37°C. At 9, 15, and 24 h after the initial wounding, the cultures were removed from the incubator and the wounded edge digitally imaged. Images were overlain with respect to the time 0 edge and pseudo-colored for ease of visualization. Increased outgrowth from the cathodal-facing edge can be seen in the field-exposed culture. Collective migration in the direction of the EF is significantly greater than that of the wound edge that is perpendicular to the EF direction. (*B*) Groups and sheets of cultured primary corneal epithelial cells migrate directionally in an EF. A small group of cells (*left*) at 0 h initially adhered to the static reference scratch on the bottom of cell culture dish (to the *right* of the photos), then in an applied EF migrated collectively to the *left* joining another group of cells on the *left*. When the field polarity was reversed, the merged larger cell group migrated to the *right*. A much larger sheet of cells also moved to the *left* leaving the static scratch marker on the dish. Polarity, time, and scale are as indicated. These early images were taken using a film camera at the time when digital imaging was not available, thus the less-optimal resolution. Collective migration, however, is evident. Scale bars: (*left*) 50 um; (*right*) 100 um.

individual cells increasing their areas of coverage, whereas most cells in the middle of the cell sheets show very little motility. This is different from sheets of cultured MDCK cells, which provide an excellent model for collective migration because there is significant collective migration of small patches of cells in the middle of the cell sheets, as well as those at the leading edge (Sadati et al. 2013; Chepizhko et al. 2018). Surprisingly, in an EF, cells in the middle of the MDCK monolayer respond significantly better with higher migration speed and directionality, even better than the cells at the free edge (Cohen et al. 2014). This appears to be very different from what observed in wound healing and experiments with other types of cells (see below).

On the other hand, when exposed to EFs, sheets of keratinocytes and corneal epithelial cells show remarkable overall net migration of whole cell sheets (Zhao et al. 1996). Both the leading edge and trailing edge, as well as the cells across the whole sheet, migrate in the same direction; thus, the geometric center of the sheets migrates directionally (Fig. 3). Shortly after the onset of application of an EF (20–30 min), cells at the edge of the sheets facing the cathode begin to extend lamellipodia in the cathodal direction, becoming the leading edge. Conversely, the cells at the anode-facing edge begin to retract their membrane extensions and form the trailing edge. The cell sheets migrate toward the cathode persistently and steadily as a collective unit while exposed to the EF (Fig. 4). Sheets of corneal epithelial cells, keratinocytes, and fish keratocytes respond to EFs in a very similar way (i.e., overall collective galvanotaxis) (Cooper and Schliwa 1986; Zhao et al. 2006; Sun et al. 2016). During such migration, epithelial sheets of both keratinocytes and corneal epithelial cells maintain cell–cell junctions (Figs. 3–5; Zhao et al. 1996, 2006).

An EF as low as 50 mV/mm induces collective migration of whole sheets. The field strength is well within the physiological strength that was measured at corneal and skin wounds (Chiang et al. 1992; Reid et al. 2005, 2007; Nuccitelli et al. 2011).

Such collective galvanotaxis has subsequently been demonstrated in several other epithelial cell types (Li et al. 2012; Cohen et al. 2014; Zajdel et al. 2020, 2021; Shim et al. 2021). Elegant experimental designs and new engineering tools have better illustrated and confirmed the collective galvanotaxis of cohesive sheets, with some important differences noted in different cell types (Cohen et al. 2014; Zajdel et al. 2020, 2021; Shim et al. 2021). For example, in MDCK monolayers, collective galvanotaxis has been confirmed to depend on the size of the cell population and the existence of physical coupling between cells. However, unlike the corneal cell sheet response, the most active cell migration happens in the middle of the cell sheet, whereas the leading edge/free edge is insensitive (Cohen et al. 2014).

Cells at the leading edge of epithelial sheets lead. In corneal epithelial sheets, lamellipodia from the wound edge cells were much more prominent than those seen in dissociated cells (Zhao et al. 1996). Following reversal of the polarity of EFs and the cell membrane protrusion and retraction, directed movement of sheets was also reversed (Fig. 4A). The leading-edge cells of the sheets are charged with the role of exploring the environment. Some investigators believe that the wound edge cells integrate various signals (such as chemical, electrical, and/or mechanical cues) and then lead the whole sheet to migrate coordinately into a newly available space. This is seen in development, wound healing, and cancer metastasis (Jacinto et al. 2002). The role of the leading-edge cells has been shown in vivo, ex vivo, and in vitro for many types of epithelial cell sheet migration in development and wound healing. In these experiments the epithelial sheets move as a unit; the leading cells protrude, leading the movement, and the cells behind follow. The result is the overall movement of the epithelial cell sheets to the cathode, in the same direction as the endogenous wound EFs (Figs. 3 and 4; Zhao et al. 1996, 2006; McCaig et al. 2005). Epithelial cell sheets from cornea, trachea, and skin and fish keratocytes demonstrate more active migration of border cells, and upon stimulation those wound edge cells respond with significantly higher directionality and speed than the wound edge cells not in an EF (Cooper and Schliwa 1986; Kiehart et al. 2000; Zhao et al. 2006). The responses of the in vitro cultured cell sheets are very similar to wound healing after corneal

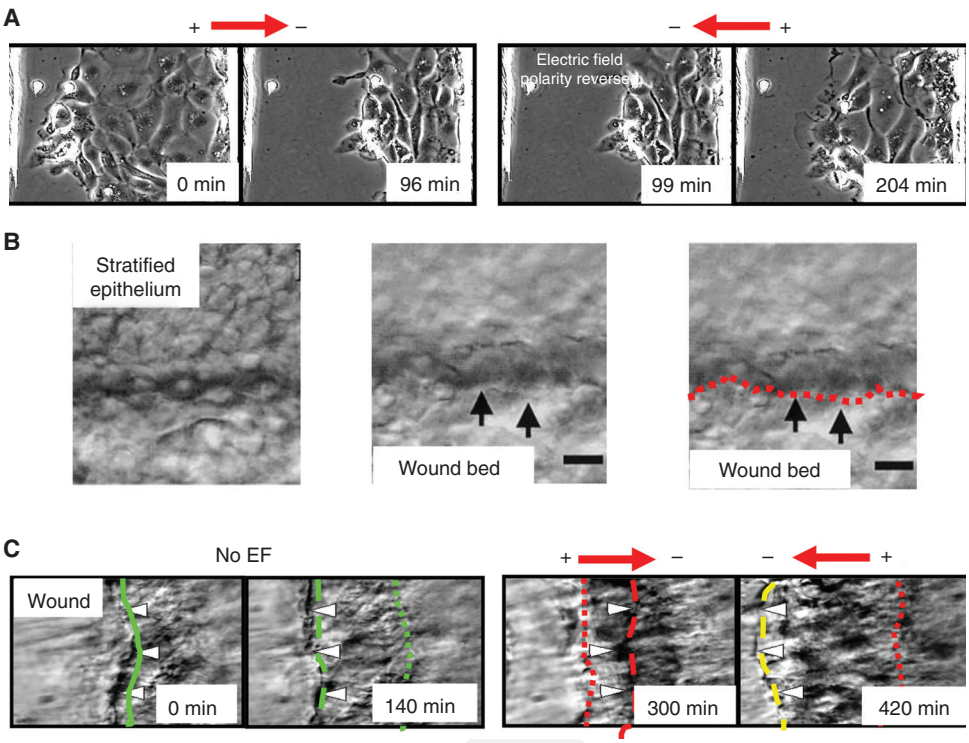


**Figure 4.** Electric fields as an overriding cue guide collective migration and leading-edge cells lead. (*A*) An EF guides collective migration of epithelial sheet overriding free edge and other co-existing guidance mechanisms. The cells follow guidance of EFs of physiological strength, ignoring direction of the space available to the *left* and contact inhibition to the *right*. When the EF points to the *right*, cells migrate to the *right* (*upper* panel); when the EF points to the *left*, cells migrate to the *left* (*lower* panel). The leading-edge cells, following EF direction, actively extending lamellipodia (red arrow). (*B*) Leading edge cells of collective migration in wound healing of an organ culture of cornea. Black arrows indicate lamellipodium like structure from the leading cells. Red dotted line in the low *right* indicates wound edge. (*C*) Collective galvanotaxis of stratified epithelium. 0–140 min: Stratified corneal epithelium migrates in situ in an ex vivo cornea culture to heal a wound (toward the *left*). 140–300 min: (*B*) An applied EF against default healing direction overrides coexisting guidance cues, epithelial cells migrate away from the wound. 300–420 min: (*C*) This wound-healing response is significantly enhanced by an electric field with the cathode at the wound. Colored lines indicate wound edge, arrowheads indicate movement of the wound edge in the indicated period of time. (*A*,*C*, Reprinted with modifications from Zhao et al. 2006, with permission from the authors and Nature Publishing Group © 2006. *B*, Reprinted with modifications from Zhao et al. 2003, with permission from John Wiley and Sons © 2003.)

epithelium injury in vivo and ex vivo (Chan et al. 1989; Gipson and Sugrue 1994). Our work has demonstrated that the leading and trailing edges of keratinocyte or MDCK cell sheets both respond to applied electrical cues, protrude and retract respectively, and migrate together and directionally as a coherent unit. Reversing the direction of the electrical cue induces complete reversal of migration direction of the cells in the leading edge, middle, and trailing edge of the cell sheet. Reversed migration of the whole sheet then follows (Li et al. 2012).

Collective galvanotaxis has better migration directionality and net directional speed when the group size increases (Zhao et al. 1996; Li et al. 2012). Cells in sheets moved more uniformly with similar directionality and speed than did dissociated cells. This first may appear to be counterintuitive, but from a system-wise approach, as in other collective biological behaviors, collectiv-

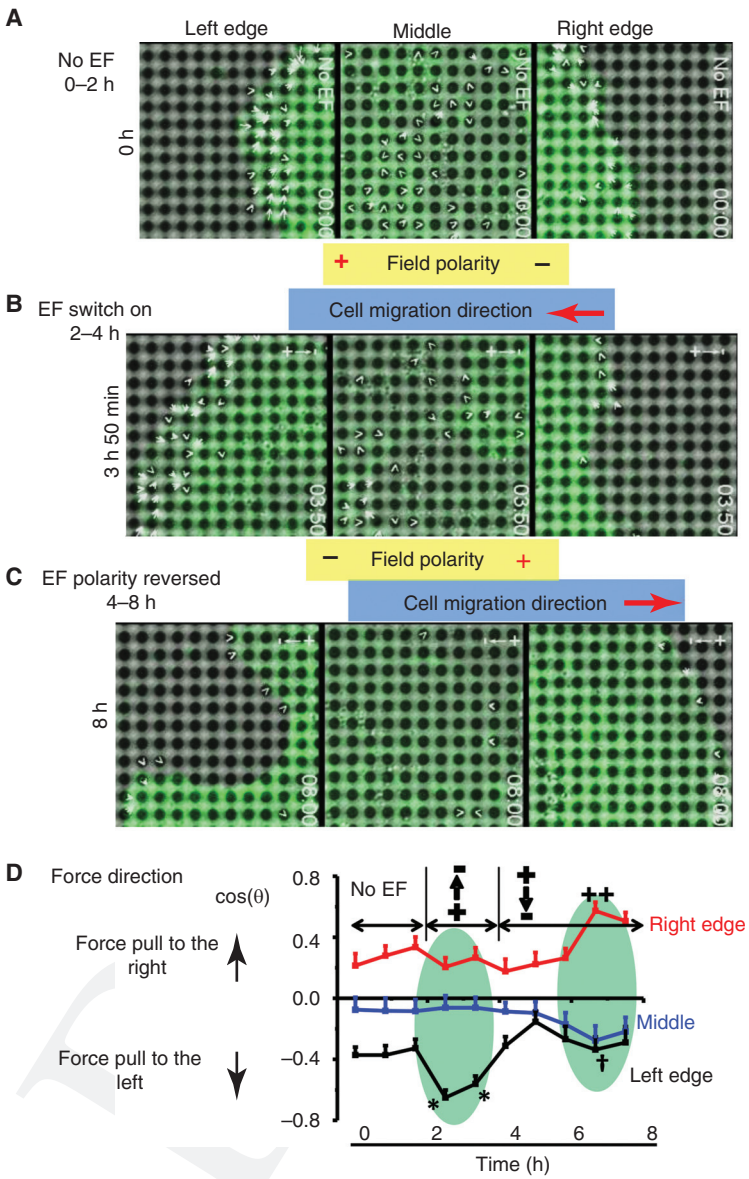


**Figure 5.** Leading-edge leads in collective galvanotaxis of sheets of MDCK II cells expressing green fluorescence protein (GFP) on traction force–sensing micropillars. The leading-edge cells actively migrate, reorientate, and increase traction forces to "lead" the cell sheet. (*A*) Migration of cells at edges and middle area of a cell sheet. Green area indicates GFP-expressing cells, black dots indicate the micropillars, and white arrowheads and their length indicate the direction and magnitude of traction forces. (*B*) In an electric field with anode on the *left*, cells on the *left* edge advance and lead; cells on the *right* edge retract. The sheet migrates to the *left*, to the anode. (*C*) When the field polarity is reversed, the anode is now on the *right*; cells at the *right* edge reverse migration direction and migrate to the *right*. Cells on the *left* edge retract. The sheet migrates to the *right*, to the anode. EF = 200 mV/mm. Polarity as shown. (*D*) Edge cells show stronger average traction force than those in the center of the sheet. Significant reorientation of the traction force is detected in the leading-edge cells (marked by the blue ovals). *: $P < 0.05$ when compared with that of same edge at 1 h; +: $P < 0.05$ when compared with that of the same edge at 3 h; ++: $P < 0.01$ when compared with that of the same edge at 1 or 3 h. The data are expressed as mean ± SEM. (*D*, Based on Li et al. 2012.)

ity reduces internal noise. The increased directional collective migration of grouped cells relative to isolated individual cells was demonstrated in analysis of collective chemotaxis of cultured neural crest cells (Theveneau et al. 2010). In a sheet, individual cells are guided not only by the EF, but also guided and constrained by the movements of neighboring cells, thus creating a more synchronized collective response. Dissociated cells often migrate in random trajectories. From the published galvanotaxis studies of almost all cell types in a culture where cells do not form cell sheets, a few dissociated cells even move in the direction opposite to that of most other cells in an EF of physiological strength, although the basis for this reversed migratory path is not known.

Corneal epithelial cells in sheets migrated in a more coordinated manner with a high degree in the similarity of migration directionality and speed than isolated cells. Displacement in the EF direction is more efficient (i.e., sheets of cells show higher speed in the field direction) than cells cultured under sparse conditions with no cell-to-cell connections (Zhao et al. 1996). This enhanced response of cells in a sheet to an EF as compared to individual cells is an important emerging behavioral characteristic. Cells collectively in a sheet have significantly increased sensitivity to weak physiological EFs. The most striking difference was found in MDCK cells, in which individual cells (not in contact with other cells) had a weak or zero galvanotaxis response to an applied field of 50–200 mV/mm, whereas when those cells aggregated into clusters or sheets, the galvanotactic response appears, and the response increases in proportion to the cell grouping size. Clusters of MDCK II cells with more than 20–50 cells and larger sheets show this directional migration, which was not observed in individual cells exposed to EFs of physiological strength 50–200 mV/mm (Li et al. 2012). The bigger the clusters or sheets, the more sensitive they are to weaker fields and the more readily they show collective galvanotaxis. MDCK II, MDCK I, rat kidney epithelial cells, monkey trachea epithelial cells, and corneal epithelial cells show this behavior (Li et al. 2012).

One potential mechanism for colony size–dependent responses is that gap junctions couple the electrical signal between cells, thereby propagating the detected electrical signal. For example, it has been hypothetically proposed that cells coupled by gap junctions will react as a single unit to an applied EF. Therefore, the hyperpolarization and depolarization of cell membranes in that unit increase proportionally by group size over uncoupled cells (Cooper 1984). In an EF of 20 mV/mm, a single cell 10 μm in diameter has a potential difference of 0.2 mV across its membrane. A group of electrically coupled cells 100 cells wide will collectively experience a 20-mV difference (Cooper 1984).

Another mechanism is mediated by mechanical coupling between cells. We demonstrated that the coupling through E-cadherin is responsible for the increased sensitivity and coordinated migration. In isolated cells, or when E-cadherin junctions were disrupted with antibodies, cell groups lost their mechanical coupling and with it their coordinated directional migration (Li et al. 2012). Mechanical coupling between cells is a dynamic material property; therefore, it appears to be one key factor in collective and coherent cell migration of the whole epithelial sheets. Indeed, our detailed analysis of traction forces in MDCK II cells demonstrated coordinated force generation in sheets that had overall migration. The leading cells in the direction of guidance cue reorientation of the direction of traction forces in the right direction and migrated together with the cells in the middle of the sheets as well as those at the trailing edge. Reversal of the direction of the galvanotaxis cue-induced significant decrease in the orientation of the traction forces in the cells now supposed to retract, whereas the cells at the new leading edge (facing the anode) showed significant coordination of traction forces and appeared to lead to migration of epithelial sheets, and the cells collectively migrated in the new direction (Fig. 5; Li et al. 2012).

## MOLECULAR AND CELLULAR MECHANISMS OF COLLECTIVE GALVANOTAXIS

Migration of epithelial sheets share signaling mechanisms with single cells that migrate in the EF. The PI3 K/Akt signaling pathway plays an essential role in galvanotaxis, which has been

demonstrated in many different types of cells using pharmacological and genetic approaches (Zhao et al. 2006). In primary cultures of keratinocytes, pharmacological blocking of PI3 K signaling or knockout of the catalytic subunit of PI3 kinase (p110γ) abolished PI3k/Akt signaling and galvanotaxis of both the single cells and cell sheets (Zhao et al. 2006). Collective galvanotaxis in stratified corneal epithelium is also significantly inhibited in p110γ knockout tissues (Zhao et al. 2006).

Migration of epithelial sheets requires maintenance of cell–cell junctions and E-cadherin expression. The physical constraint of neighboring cells and structural and functional coupling of cells via cell membrane molecules are some important changes that occur when single cells coalesce to form cell sheets. Some cell junction proteins have been investigated in collective galvanotaxis. The cadherins are a superfamily of adhesion molecules that play critical roles in cell recognition, tissue morphogenesis, and tumor suppression (Gumbiner 1996; Guillot and Lecuit 2013). E-cadherins are expressed in the epidermis and other epithelial tissues (Tinkle et al. 2004). E-cadherins play a critical role in collective galvanotaxis. In an MDCK monolayer, application of the E-cadherin antibody DECMA-1 abolished galvanotaxis, with cells losing their directionality, whereas their migration speed increased significantly (Li et al. 2012). This result was further confirmed using $Ca^{2+}$ free medium or other chemicals that disrupted cell–cell junctions in monolayer cultures (Li et al. 2012). In monolayers of mouse keratinocytes, cell–cell adhesion disruption with DECMA-1 also resulted in a decrease in migration directionality and increased cell migration speed (Shim et al. 2021). Those results from different epithelial cell types support conserved mechanisms of tissue-level response to bioelectrical signaling and may offer new tissue engineering options. It is worth noting that N-cadherin is required for collective chemotaxis in neural crest cells (Theveneau et al. 2010).

## TRANSLATION OF ELECTRICAL STIMULUS TO CLINICAL USE IN WOUND REPAIR

Despite the wealth of in vitro mechanistic support for the role of electric fields in orchestrating wound repair, there is a notable dearth of translation to clinical use. Indeed, no new devices utilizing ES have become approved for clinical use since this area was reviewed a decade ago (Isseroff and Dahle 2012). One battery-powered bandage device (Posifect) incorporated the concept of directional current flow, placing the anodal electrode at the periphery of the circular bandage, to direct cellular migration toward the center of the wound, where the cathodal electrode was embedded. However, this device, although available in the United Kingdom for a short while, is no longer available. The only commercially available, FDA-approved device delivering ES directly to a wound is Procellera, a woven bandage that has embedded microbatteries as an array of alternating zinc and silver microdots, creating multiple small EFs. This bandage lacks directionality in the array of the multiply generated EFs and is approved by the FDA for its antimicrobial function in the wound rather than for its ability to guide directional migration of cells within the wound.

With strong evidence supporting the electrical signaling as a fundamental mechanism in wound healing, one may wonder why what seems to be translatable into a simple therapy is still not readily available yet? Some of the intricacies that perhaps are limiting clinical translation include the spatial and temporal modulation of EFs in the wound: they are directionally localized and change over time, and any successful bandage device would have to be able to accommodate these changes in real time, essentially being a "smart bandage." Additional considerations include the different electrical stimuli required to directionally guide the migration and function of the various cell types, such as fibroblasts, macrophages, and endothelial cells, that contribute to healing. Another challenge is that the wounds, especially chronic wounds, are highly heterogenous in electric resistance or conductivity, which again makes the control of distribution of EFs or electric currents difficult. The interface of electron-electricity and the biological ionic-electricity is another hurdle. Electrical currents produced by conventional devices and biological electric currents are very different by the nature of the charge carriers. The electricity in those devices is carried by electrons, whereas that

in our body is carried by ions. Designing a device with onboard power may necessitate nonrechargeable batteries, which could complicate manufacture and delivery. The current state of research in the technology of EF delivering wound devices has been well-summarized in recent reviews (Cheah et al. 2021; Korupalli et al. 2021). Although this appears to be an active area of research investigation, interestingly, there are no current active registered clinical trials testing EF delivering bandages for wound repair. This appears to be an unmet translational need. Similar to other promising approaches to manage chronic wounds, electrical therapies will need solid understanding and investment to avoid a great hope turning to a hype. Some serious efforts are being made, including a DARPA initiative (https://researchfunding.duke.edu/bioelectronics-tissue-regeneration-betr; https://news.ucsc.edu/2020/02/wound-healing.html).

## CONCLUSION

Following significant advances of genomic and cellular understanding of wound healing, a major challenge is the control of coordinated growth and 3D recovery of the tissues that heal wounds. Successful healing relies on proper and timely epithelialization, with the primary goal to recover the barrier function, which requires epithelial cells to move as a cohesive sheet of tissue, maintaining cell–cell junctions. Experimental evidence suggests that signaling by the endogenous EFs generated at wound sites provides a powerful cue for cohesive cell sheet migration, which is thus a powerful mechanism for epithelialization in wound healing.

The bioelectrical signaling at wounds yet has more questions to be answered. What are the specific channels and pumps at wounds that generate the wEFs? This question will be better answered through the integration of bioelectric, molecular, and genetics approaches. In general, we believe that ion channels and pumps at the tissue level are the "generators," together with cell junctions as the "resistors" establishing a spatiotemporal electrical map to guide wound healing. Spatial and temporal expression, activation, and function of the "generators" and "resistors" at wounds will be one area for future investigation. How cells sense and respond to weak physiological strength EFs is another pressing question. Some screening approaches have been attempted and have identified some molecular mechanisms (Zhao et al. 2006; Gao et al. 2015; Nakajima et al. 2015; Nakajima and Zhao 2016). In chemotaxis, a very specific ligand-receptor signaling paradigm has been demonstrated from *Dictyostelium* cells, neutrophils, to migrating neurons. We suspect that electrical sensing is likely to be different from chemoattractant sensing. Technological advances in the following two areas will be critical: to map endogenous EFs in biological samples, and to establish an electronics–biology interface. Electrical measurements of action potentials and other high-frequency electrical signals are well-developed and widely used. Measurements of wound EFs, which are DC in nature, have not been easily adopted by the wider scientific community. For the electronics–biology interface, almost all electric power suppliers are electron currents, whereas the EFs and currents in our body are ionic. Good electronic–biological tissue interfaces to manipulate bioelectricity will be essential to research, translation, and future clinical application.

## ACKNOWLEDGMENTS

The research in the Zhao Laboratory and the Isseroff Laboratory at UC Davis are supported by a Defense Advanced Research Projects Agency (DARPA) grant (D20AC00003; Program Leader: Marco Rolando, University of California Santa Cruz), an Air Force Office of Scientific Research (AFOSR) Multidisciplinary Research Program of the University Research Initiative (MURI) grant (FA9550-16-1-0052; Program Leader: Wolfgang Losert, University of Maryland), and National Institutes of Health (NIH) grant 1R01EY019101. Work in the Zhao Laboratory is also supported by a National Eye Institute (NEI) Core Grant (P-30 EY012576; PI: Jack Werner), the Research to Prevent Blindness, Inc.

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Queries

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