A Role for Endogenous Electric Fields in Wound Healing

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ABSTRACT

This review focuses on the experimental evidence supporting a role for endogenous electric fields in wound healing in vertebrates. Most wounds involve the disruption of epithelial layers composing the skin or surrounding organs in the body. These epithelia generate a steady voltage across themselves that will drive an injury current out of the wounded region, generating a lateral electric field that has been measured in four different cases to be 40-200 mV/mm. Many epithelial cells including human keratinocytes have the ability to detect electric fields of this magnitude and respond with directed migration. Their response typically requires Ca^{2+} influx, the presence of specific growth factors and intracellular kinase activity. Protein kinase C is required by neural crest cells and cAMP-dependent protein kinase is used in keratinocytes while Mitogen-activated protein kinase is required by corneal epithelial cells. Several recent experiments support a role for electric fields in the stimulation of wound healing in the developing frog neurula, adult newt skin and adult mammalian cornea. Some experiments indicate that when the electric field is removed the wound healing rate is 25% slower. In addition, nearly every clinical trial using electric fields to stimulate healing in mammalian wounds reports a significant increase in the rate of healing from 13 to 50%. However, these trials have utilized many different field strengths and polarities so much work is needed to optimize this approach for the treatment of mammalian wounds.

INTRODUCTION

Most wounds involve the disruption of the epithelial layers composing the skin or surrounding organs in the body. They pose a serious threat to the well being of the organism by allowing both the invasion of microorganisms and the leakage of internal body fluids. Therefore, wound healing is one of the most important regenerative processes that most organisms exhibit. It usually involves the migration and proliferation of epidermal cells to reseal the epidermal layer. A recent review described the process, “The initial inflammatory response leads to the influx of macrophages and neutrophils, which release cytokines, growth factors, and nitric oxide, and induce nearby keratinocytes to migrate across the wounded epithelium (Hackam and Ford 2002). While cytokines and growth factors can indeed stimulate keratinocyte migration, a much earlier stimulus of directed migration that is triggered by wounding is not even mentioned by those authors. I am referring to the electrical field generated by the flow of current out of the wound. As covered in detail below, this wound field is generated by the transepithelial

potential driving current out of the low resistance pathway at the wound and comes into being immediately upon wounding. This wound field-stimulated migration is known as galvanotaxis and has been studied extensively in human keratinocytes and mammalian corneal epithelial cells. Here I will review the evidence for the involvement of endogenous electric fields in vertebrate wound healing.

There are earlier reviews of the role of electric fields in regeneration and wound healing that may be of interest to the reader (Borgens 1982;Jaffe and Vanable 1984;Nuccitelli 1984;Robinson 1985;Nuccitelli 1988;Vanable, Jr. 1989;Borgens, et al. 1989;McCaig and Zhao 1997;McCaig, et al. 2002;Cho 2002) and due to the availability of this earlier work, I will concentrate mainly on more recent studies conducted for the most part during the past 10 years.

Most biologists learn early in their studies that all cells generate a voltage across their plasma membranes called the membrane potential. This voltage is used in many different ways, including aiding the transport of molecules across the membrane through coupled transport, rapid signaling to the entire cell that an event has occurred somewhere on its surface, and for relaying signals long distances as in the case of neurons. When we shift our attention from the single cell to the multicellular domain, we find a striking parallel. Just as the cell is surrounded by a plasma membrane, all of our organs are bounded by an outer epithelium and indeed the largest organ in our bodies, our skin, is a multi-layered epithelium. However, In contrast to the extensive coverage given to the electrical properties of the plasma membrane, the electrical properties of epithelia are usually hardly mentioned in the textbooks. Yet these properties are very important for organ function. The epithelium is to the organ as the plasma membrane is to the cell. Just as the plasma membrane forms a boundary that controls what goes in and out of the cell, the bounding epithelium determines what goes in and out of the organ that it encapsulates. The cells composing each epithelial layer are coupled with gap junctions so that they can be thought of electrically to be one continuous layer, much as we think of the plasma membrane. Analogous to the plasma membrane, all epithelia generate a voltage across this epithelial layer that has many different uses. The polarity of this voltage is usually inside positive, which is opposite in sign to the plasma membrane potential that is generally inside negative. How is this transepithelial potential (TEP) generated?

The TEP is due for the most part to the polarized distribution of ion channels in the epithelial cells. Most Na^+ channels are localized to the apical membrane and most K^+ channels are found in the basolateral membranes. Since the Na^+/K^+ ATPase maintains high internal K^+ and low internal Na^+, this localization of channels leads to Na^+ influx across the apical membrane and K^+ efflux across the basolateral membrane. This flow of positive charge into the apical end and out of the basal end of the epithelial cells constitutes a transepithelial ion flow that must complete the current loop by flowing back through a paracellular route to the apical side of the epithelium (fig. 1). The transepithelial potential will be proportional to the resistance of this paracellular pathway but typical values for this TEP range from 15 to 60 mV, inside positive in our bodies.

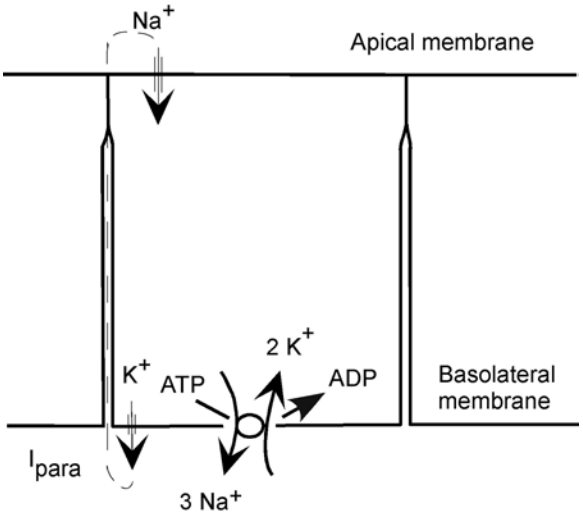

Figure 1. Diagram of a typical epithelial cell in a monolayer with Na^+ channels localized on the apical plasma membrane and K^+ channels localized on the basolateral membranes along with the Na^+/K^+-ATPase. This asymmetric distribution of ion channels generates a transcellular flow of positive current that must flow back between the cells through the paracellular pathway (I_{para}). This current flow generates a transepithelial potential that is positive on the basolateral side of the monolayer

It is this TEP that is the driving force for most endogenous electric fields in embryos and adults. This voltage across epithelia will drive current out of regions of low resistance where there has been a break in the epithelium (wounds) or where tight junction resistance is low such as along the primitive streak (Jaffe and Stern 1979;Winkel and Nuccitelli 1989) or the posterior intestinal portal (Hotary and Robinson 1990) in chick or mouse embryos or at the forming limb bud in amphibian and chick embryos (Robinson 1983;Borgens, et al. 1983;Altizer, et al. 2001). This "leakage current" will in turn generate a lateral electric field along its path that will be proportional to the resistivity in that region. This electric field results from Ohm's Law in a conductive medium, $E=J\rho$, where J is current density and ρ is the local resistivity. Typical values for such fields will be discussed next.

ENDOGENOUS ELECTRIC FIELDS MEASURED NEAR WOUNDS

The earliest measurements of the electrical phenomena associated with wounds did not measure the electric field itself, but rather the current flowing out of the wound. DuBois-Reymond (1843) used a unique galvanometer that he built with more than two miles of wire and measured about 1 μA flowing out of a cut in one of his fingers. This was confirmed in 1849 and 1910 by other investigators and the history of these measurements is presented in a scholarly review by Vanable (1991). More modern techniques have also been used to study this wound current. The "leakage current" that is driven out of epithelia in low resistance regions has been measured using the vibrating probe technique (Jaffe and Nuccitelli 1974) in several systems. One of the earliest such measurements was a current as large as 100 $\mu A/cm^2$ leaving the stumps of regenerating newt limbs (Borgens, et al. 1977). Similar measurements have also been made on fingertip amputation currents in humans (Illingworth and Barker 1980) where up to 30 $\mu A/cm^2$ was detected leaving the accidentally amputated stump for about three weeks. These currents will certainly generate electric fields just beneath the epidermis that will be proportional to the resistivity encountered in the tissue. The range of human tissue resistivity spans 200 to 1000 ohm-cm (Faes, et al. 1999) so these currents would be expected to generate an electric field within the tissue of about 10-100 mV/mm.

However, since this tissue resistivity can vary substantially as a function of cell density and tissue anatomy, it is always more reliable to measure these fields

directly in the tissue rather than estimating them based on the transembryonic current density. This has been accomplished in four different wound types in skin and cornea. The classic approach to these measurements is to use KCl-filled glass microelectrodes to penetrate the outer epithelium and measure the voltage just beneath it in several positions along a line leading away from the wound. However, for skin measurements, another approach is more common. That is to measure the potential gradient just beneath the stratum corneum on the surface of the epidermis either with surface electrodes or by other means. The field generated by the current flowing between the upper surface of the epidermis and the stratum corneum is often larger than that generated below the epidermis due to the higher resistivity of that upper region. The range of field strengths measured in the four cases in the literature is surprisingly small, between 40 and 200 mV/mm (Table 1). The field direction is a function of position. Beneath the epidermis the field polarity has the negative pole at the wound center and above the epidermis the wound current is flowing in the opposite direction so that the positive pole is at the wound (fig. 2).

These wound fields have some useful properties for signaling. First, they appear immediately upon wounding since the TEP is continuously present to drive current out of any low-resistance region as soon as it is formed. Second, the lateral electric field illustrated in fig. 2 that is generated by the wound current will persist until the resistance increases as the wound heals. Thus we have a signal that is immediate and persistent. These are ideal properties for a physiological signal to stimulate wound

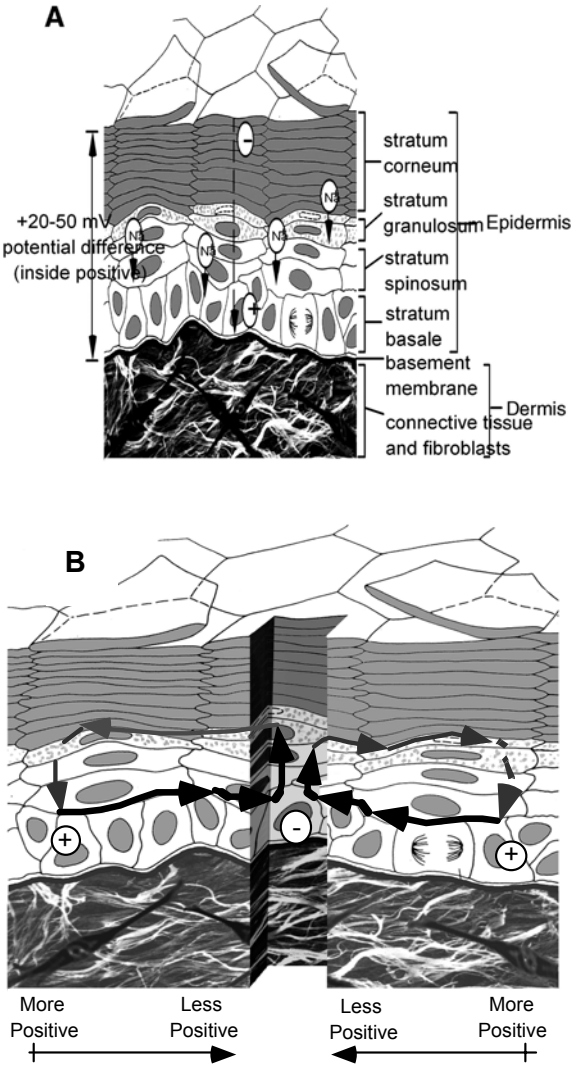

Figure 2. Generation of skin wound electric fields. **A.** Unbroken skin maintains a "skin battery" or transepithelial potential, generated by the apical influx of Na^+ and basolateral efflux of K^+. **B.** When wounded, the potential drives current flow through the newly formed low resistance pathway generating an electric field whose negative vector points toward the wound center at the lower portion of the epidermis and away from the wound on the upper portion below the stratum corneum

healing. If the epithelial cells forming the epidermis were able to detect such electric fields, they would be able to initiate wound healing immediately upon wounding. This is in fact the case, as I will discuss in the next section.

EPITHELIAL CELLS EXHIBITING GALVANOTAXIS

The phenomenon of galvanotaxis was first observed over one hundred years ago in leukocytes (Dineur 1891). Since then more than 15 cell types have been found to exhibit this ability to detect external electric fields (table 2) and to migrate along field lines (Robinson 1985;Nuccitelli 1988) but not all cells exhibit this behavior(Grahn, et al. 2003)(Sillman, et al., 2003). It is not clear why some of those cells such as amoebae and slime molds have developed this capability since they may not encounter electric fields in their native environment. However, epithelial cells will all be exposed to electric fields generated by leakage currents driven by the TEP and it is not at all surprising that these cells have evolved the ability to sense and respond to small electric fields.

Embryonic cells

Embryonic cells that are known to migrate long distances within embryos were among the first to be found to exhibit galvanotaxis. Neural crest cells from both avians and amphibians (Nuccitelli and Erickson 1983;Stump and Robinson 1983b;Cooper and Keller 1984) as well as fibroblasts from avians, amphibians and mammals (Luther, et al. 1983;Erickson and Nuccitelli 1984;Yang, et al. 1984) respond to fields as low as 10 mV/mm by exhibiting enhanced migratory activity in the direction of the negative pole. As the field is increased, the degree of directed migration increases, reaching a maximal directed orientation of 0.8 on a scale of 0 to 1 at 100 mV/mm. At this field strength, approximately 98% of the cells actively migrate toward the negative pole.

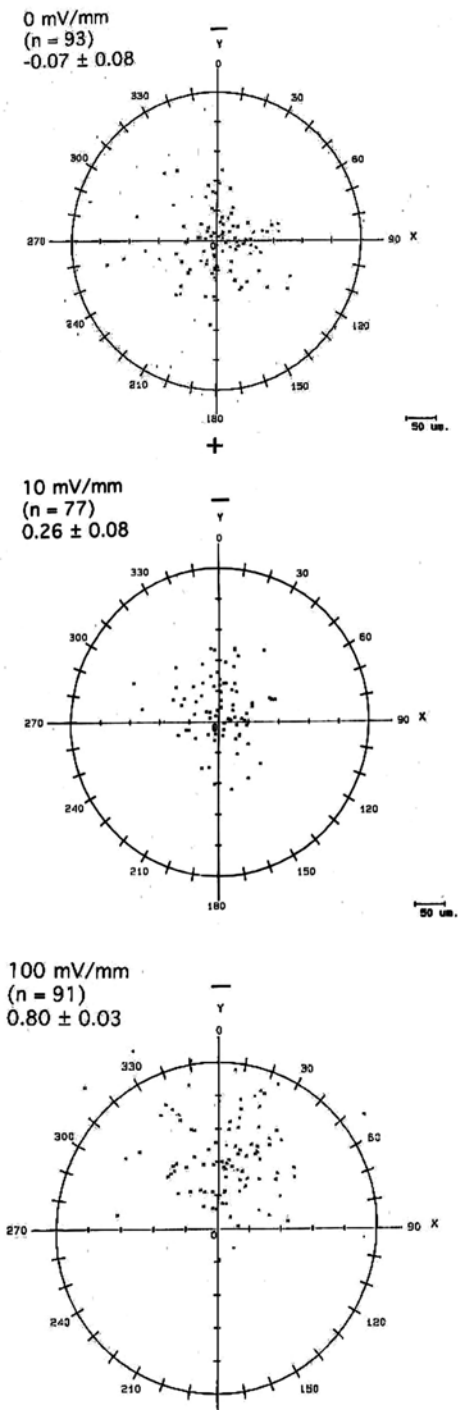

Figure 3. Galvanotaxis of human keratinocytes. Every point represents the position of a single cell after 2 h in the indicated field strength after starting at the origin. **Top:** No field control exhibits average cosine φ = -0.07. **Middle:** 10 mV/mm exhibits significant migration toward the negative pole. **Bottom:** 100 mV/mm stimulates significant galvanotaxis in 98% of cells. (with permission of Nishimura, et al., 1996)

Epidermal cells

Epidermal cells from amphibians, fish and human skin also respond very strongly to physiological electric fields. Fish scale keratocytes are very fast movers and migrate toward the cathode in an imposed field (Cooper and Schliwa 1986a;Cooper and Schliwa 1986b). Neither the spontaneous locomotion nor the electrically guided motility were found to be microtubule dependent. The lamellipodial extension and locomotion of keratocytes are reversibly inhibited by a variety of calcium channel antagonists, whereas their motility is unaffected by hyperpolarizing and depolarizing (low and high K+) media.

Rivkah Isseroff's group at UC Davis has collaborated with me to study primary human keratinocytes in culture. These cells migrate well on glass coverslips coated with collagen and their motility is highly sensitive to imposed dc electric fields. In fields as low as 10 mV/mm, more cells migrate toward the cathode than toward the anode (fig. 3). The optimal field strength for this response is 100 mV/mm at which 98% of the cells migrate toward the cathode, exhibiting and average cosine of 0.8 on a scale of 0 to 1.

They exhibit a response half-time of about 10 minutes and their directed response requires Ca^{2+} influx (Fang, et al. 1998;Trollinger, et al. 2002b) and epidermal growth factor (EGF) receptor phosphorylation (Fang, et al. 1999). Inhibitor studies implicate a role for PKA in galvanotaxis but not PKC or myosin light chain kinase (Pullar, et al. 2001). The presence of a growth factor (EFG) is required for galvanotaxis in these cells as well as the corneal epithelial cell system described next.

Corneal epithelial cells

Corneal epithelial cells from bovine, rabbit and human sources have been extensively investigated (Table 2). Many of these studies have been conducted *in vitro* and a few were done *in situ*. In every case, these cells migrate toward the negative pole of the imposed field and responded to fields on the order of 100 mV/mm. There has not been much work using lower field strengths so the threshold field is not accurately known. However, the endogenous field near the bovine cornea wound of 42 mV/mm (Chiang, et al. 1992) would suggest that these corneal epithelial cells should be able to respond to fields of this magnitude. Some very interesting results have emerged recently from *in situ* studies on the rat cornea system that will be discussed below.

Lens epithelial cells

McCaig's group has also studied the galvanotaxis of lens epithelial cells from central and peripheral regions (Wang, et al. 2003a;Wang, et al. 2003b). Field-directed cell migration required serum, or growth factors. Cells cultured in serum-free medium are blinded to the electric field but their ability to respond was restored partially by the addition of basic fibroblast growth factor. The direction of cell migration depended on both the field strength and the origin of the lens epithelial cells. Both central and peripheral lens epithelial cells moved anodally at 150-250 mV/mm, but surprisingly the peripheral cells migrated in the opposite direction in 50mV/mm. This bi-directional migration depending on field strength is not very common and could prove to be quite useful for studies of the mechanism used by these cells to detect the field. The only previous reports of a bi-directional response involved the polarization of

algal eggs in which some batches exhibited the opposite polarization response to the same field (Peng and Jaffe 1976) and some exhibited a bipolar response as a function of field strength. Neuronal growth can exhibit a bi-directional response to the same field if the substrate charge is modified (Rajnicek, et al. 1998).

HOW DO MOTILE CELLS SENSE AND RESPOND TO THE IMPOSED ELECTRIC FIELD?

This question has been the focus of most studies of galvanotaxis over the past few years. Two clues to the mechanism seem to be widely applicable: 1) Ca^{2+} influx appears to be required for many of the cells and they are unable to detect the field when Ca^{2+} influx is blocked by removing extracellular Ca^{2+} or by adding Ca^{2+} channel blockers (Cooper and Schliwa 1986a;Nuccitelli and Smart 1989; Nuccitelli and Smart 1991;Fang, et al. 1998;Trollinger, et al. 2002a); 2) Growth factors are required along with protein kinase activity. It was found early on that specific growth factors in the culture media such as EGF and FGF were absolutely required for galvanotaxis of human keratinocytes (Fang, et al. 1998), corneal epithelial cells (Zhao, et al. 1996) and lens epithelial cells (Wang, et al. 2003b). Subsequent work indicated that EGF receptor phosphorylation was required and inhibitors of EGF receptor phosphorylation would make human keratinocytes blind to the imposed electric field (fig. 4) while reducing their migration rate only slightly. The phosphorylated receptor is rapidly localized to the cathodal side of these cells (Fang, et al. 1999;Zhao, et al. 2002). With significant asymmetry being detected within five minutes of field application. The two mechanisms that have been proposed to accomplish this

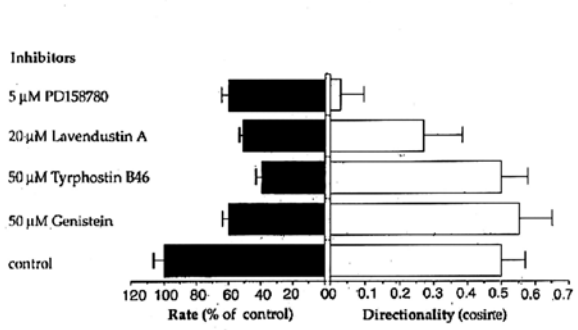

Figure 4. Response of human keratinocytes to a 100 mV/mm electric field in the presence of various tyrosine kinase inhibitors. PD158780 is the most specific inhibitor of EGFR phosphorylation and completely blinds the cells to the field whole only inhibiting their rate of movement by 40%.

localization are localized secretion and lateral electrophoresis (Jaffe 1977) discussed in detail below. However, more experimental work is needed to determine the exact mechanism generating this receptor asymmetry.

Receptor phosphorylation requires protein kinase activity and several kinases are known to be involved (table 3). Protein kinase C was the first found to be needed for the galvanotaxis response in neural crest cells (Nuccitelli, et al. 1993), but it does not appear to be required for the response of human keratinocytes (Pullar, et al. 2001) where the activity of cAMP-dependent protein kinase (PKA) appears to be more critical. Corneal epithelial cells utilize the mitogen-activated protein (MAP) kinase, ERK1/2 (Zhao, et al. 2002) as do lens epithelial cells (Wang, et al. 2003b). In the corneal cells, a cathodal accumulation of lipids, EGF receptors and ERK1/2 induces but does not depend on a cathodal redistribution of F-actin. Inhibitors of MAP kinase signaling inhibit the directed cell migration and cathodal asymmetry of ERK and F-actin. In lens epithelial cells they observed an asymmetric activation of ERK, with much weaker activity in cathode-facing

wounds than in those facing the anode. This correlates well with the wound healing response in which anode-facing wounds healed faster than cathode-facing ones (Wang, et al. 2003b).

Given these clues, there have been several speculations regarding the mechanism by which cells can sense the presence of external electrical fields. The most direct of these is **via the redistribution of lipid or protein molecules in the plasma membrane (Jaffe, 1977)**. Electrical fields can exert force on charged molecules to move them directly by electrophoresis or indirectly via electro-osmosis. Electrophoresis is the movement of a charged molecule in an electric field that is a function of its net charge and molecular weight, whereas electro-osmosis refers to the fluid flow through the medium that results from the movement of the waters of hydration that surround all charges. The bulk water movement of electro-osmosis can actually "drag" membrane lipids or proteins in the direction opposite to that in which electrophoresis would move them. Most membrane proteins and lipids have a net negative charge and would be electrophoresed towards the positive pole of an electric field. However, many cells exhibit the opposite protein redistribution toward the positive pole! How does this happen? Each of the negative charges on the membrane proteins and lipids must be counter-balanced by a positive charge in solution and each of those charges is surrounded by waters of hydration. The external electric field will move the positive counter-charges towards the negative pole and the waters of hydration will move with the positive charges. This causes a bulk water movement toward the negative pole that can actually drag membrane proteins along with it, even if those proteins have a net negative charge. The strength of electro-osmosis will be highly dependent on the net surface charge and reducing this charge by removing sugar groups for example can actually change the direction of movement of membrane proteins (McLaughlin and Poo 1981). Once the redistribution of proteins and lipids occurs, asymmetry is established and the redistributed proteins can influence cell motility via the signaling cascades discussed above.

The second speculation regarding the mechanism by which cells could sense the field involves localized changes in the membrane potential. All cells generate a voltage across their plasma membrane that is about 70 mV inside negative in animal cells and often much larger in plant cells. When placed in an electrical field, the voltage across the plasma membrane will be modified most in regions that are perpendicular to the field lines. The ends of the cell that face the two poles of the field will experience the largest effect. The voltage drop across the cell is determined by the field strength multiplied by the length of the cell along the field lines. For a 100 μm long cell in a 100 mV/mm field, this voltage drop would be 10 mV. Since current passing through a cell will encounter the bulk of the resistance at the plasma membrane and very little across the cytoplasm, approximately half of this voltage drop will occur across each membrane facing the poles. That means that the plasma membrane at the end of the cell facing the positive pole will have 5 mV more voltage across it and the membrane facing the negative pole will have 5 mV less across it. The cytoplasm will also have a small voltage across it typically in the microvolt range due to its much

lower resistivity. So the question becomes can this 7% change in the membrane potential in a localized region influence cell motility? Voltage-gated ion channels typically open upon a depolarization of about 10-20 mV so this small change is not likely to trigger channel opening. However, it will certainly bias the force driving ions through any open channels. Positive ions will experience a larger force driving them into the cell at the membrane region facing the positive pole of the field and a slightly lower force driving influx at the end of the cell facing the negative pole. This could contribute to the generation of an intracellular ion concentration asymmetry that might influence the direction of cell motility.

The obvious candidate ion to consider is Ca^{2+} since many cells require Ca^{2+} influx for galvanotaxis. Ca^{2+} will be driven into the cell by both the negative membrane potential and the concentration gradient since extracellular $[Ca^{2+}]$ is typically 10,000 times greater than intracellular $[Ca^{2+}]$. The best way to determine the total driving force on Ca^{2+} is to calculate it's equilibrium potential from this concentration gradient. The equilibrium potential is the voltage at which as much Ca^{2+} would be forced out per unit time by the voltage as would leak in per unit time due to the high concentration outside. It is given by the Nernst equation and for most mammalian cells with $[Ca^{2+}]_o$ of 1.2 mM and $[Ca^{2+}]_I$ of 10^{-4} mM, this equilibrium potential would be 114 mV. Since most cells have a resting potential near -70 mV, Ca^{2+} is 184 mV away from this equilibrium. A 5 mV change in the membrane potential will only increase the total driving force by 3.8% at the positive pole and reduce it by 3.8% at the negative pole. However, over time, the consequent asymmetry in flux could result in an intracellular ion concentration gradient that might influence cell motility.

CAN IMPOSED ELECTRIC FIELDS BE USED TO STIMULATE WOUND HEALING?

Now that we know that endogenous electric fields in the range of 40-200 mV/mm are naturally present near wounds and that skin cells respond to fields of this magnitude with directed motility, the possibility arises that the electric field may be playing a role in the stimulation of wound healing. There have only been a few well-controlled experiments designed to test this hypothesis to date along with many more clinical attempts that have been less satisfactory. I will briefly review these here.

Robinson's group carried out the first controlled experiment designed to investigate the role of the electric field in wound healing utilizing a very simple system, the neurula stage frog embryo (Stump and Robinson 1986;Rajnicek, et al. 1988). Transected frog embryos will heal completely within 7 hours in a pond water medium with a rapid purse string-like contraction requiring microfilaments but not Na^+. This is followed by a slower phase that is blocked by either Na^+-free medium, or the addition of amiloride, benzamil or ouabain, drugs that inhibit Na^+ flux through the epithelium as indicated by a rapid reduction in the transepithelial potential. This indicates that the slow phase of wound healing requires the endogenous Na^+-carried electric current and certainly supports a role for the electric field in wound healing.

The second well-controlled experiment to test the role of electric fields in wound

healing was conducted on the newt by Vanable's group (Chiang, et al. 1991). They made a small skin wound in one hindlimb digit on both the right and left foot of *Notophthalamus viridescens* and monitored the healing rate while changing the lateral electric field near the wounds by passing current through one digit, across the body and out the contralateral digit (fig. 5). The amount

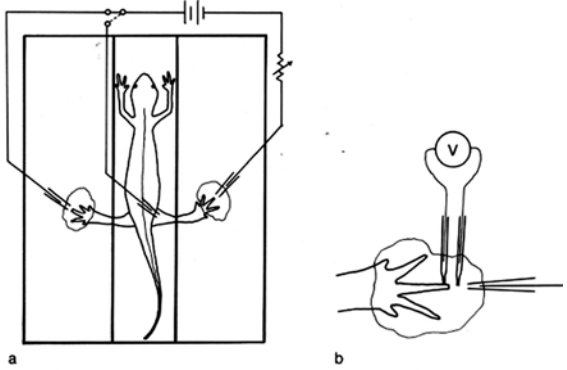

Figure 5. Diagram of experiment designed to modify electric field within newt digit during wound healing. a. The newt was placed in a three-compartment chamber with only the feet immersed in pond water. b. View of one current-supplying electrode and the two microelectrodes used for voltage measurements (Reproduced with permission from Chiang, et al., 1991)

of current passed was adjusted so that the lateral field of one wound was zero while the contralateral wound had an enhanced field. They observed that the wounds with the enhanced field healed more rapidly than the wounds with the zero field. When digits on one side were treated with 30 μM benzamil in an artificial pond water so that their wound fields were reduced to approximately zero, and the contralateral wounds were kept in artificial pond water without benzamil so that they had normal wound fields, there was significantly less epithelization of the benzamil-treated wounds than of the control wounds. This effect on wound healing was reversed by adding currents that restored the normal wound fields, but not by adding currents that reversed the wound fields to the opposite polarity. When currents were added to reverse the wound fields on one side of the animal, leaving the contralateral wounds free of added currents, the wounds with the reversed fields healed more poorly than the contralateral wounds with normal fields. These results are consistent with the hypothesis that the intrinsic lateral electric fields in the vicinity of wounds promote epithelization of these wounds. These experiments are the most elegant ones to date on this question and the overall conclusion is that in the absence of a lateral electric field, the rate of wound healing is reduced by about 25% (fig. 6).

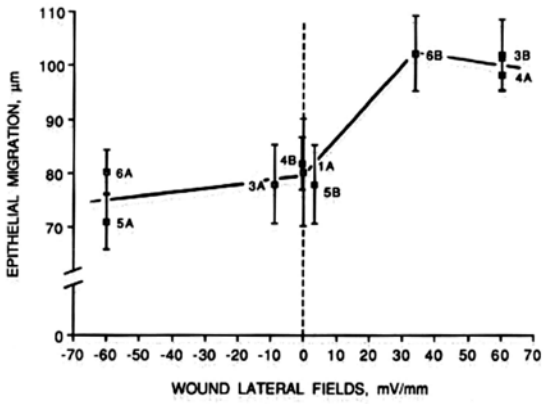

Figure 6. Epithelization after 4 h of healing vs lateral field. When the lateral electric field is reduced to 0 the rate of healing is reduced by about 25%. (Reproduced with permission of Chiang, et al., 1991)

Vanable's group went on to ask if applying a larger electric field could increase the rate of wound healing. The endogenous lateral field near a wound is typically 40 mV/mm. Augmenting this to 80 and 100 mV/m reduced the rate of healing so they concluded that the newt epithelialization rate was nearly maximal at the normal field strength (Sta Iglesia, et al. 1996).

Vanable's group has also contributed the third well controlled experiment to determine the involvement of electric fields in wound healing (Sta Iglesia and Vanable, Jr. 1998). Here they used

bovine corneal lesions with a 1.5 mm circular wound. A decrease in the field strength by submersion of the lesions or by treating the lesions with the Na+-channel blocker, benzamil, significantly retarded healing. An increase in the field strength of lesions treated with Na+-depleted Hanks' solution, by the addition of direct current, increased epithelization. Epithelization was fastest in wounds with field strengths raised to -80 mV/mm, more than twice the normal field strength present in wounds maintained in Hanks' solution alone. Epithelization decreased, however, when the field strengths were increased to -120 mV/mm. A similar pattern was also observed when the field's polarity was reversed. By manipulating and monitoring the field strengths, they demonstrated for the first time that increased wound field strengths enhance corneal wound epithelization, and that field strengths with reversed polarity also enhance this epithelization.

The final well-controlled study was also conducted on a cornea preparation *in situ*. McCaig's group used the rat cornea to study wound healing in response to a similar circular wound (Song, et al. 2002a) .They manipulated the endogenous lateral electric field near the wound by using drugs with differing actions (fig. 7). They also found that the rate of wound closure was highly sensitive to the field strength. .

In addition to influencing the rate of wound closure, the wound-induced field influenced the orientation of cell division. Most epithelial cells divided with a cleavage plane parallel to the wound edge and perpendicular to the field vector. Increasing or decreasing the field pharmacologically, respectively increased or decreased the extent of oriented cell division. In addition, cells

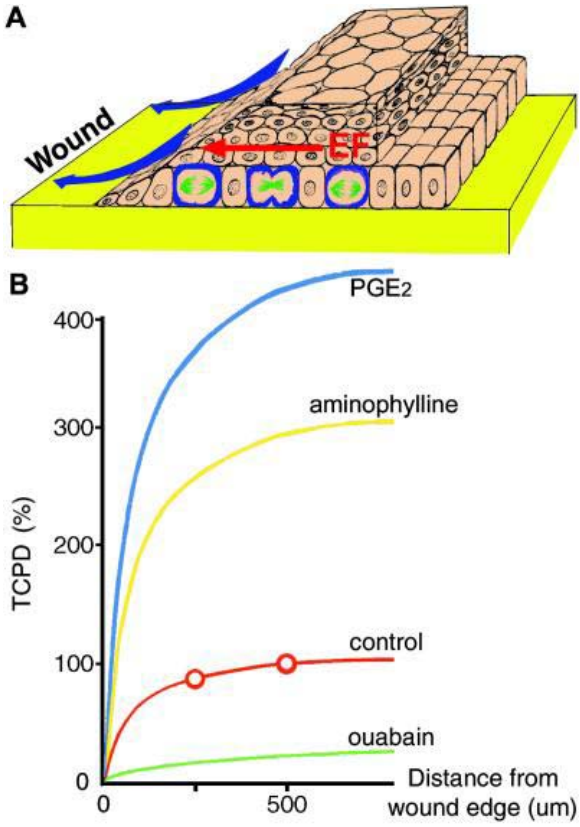

Figure 7. The transcorneal potential can be manipulated with different drugs and its effect on wound healing, cell division and nerve sprout orientation determined. (reprinted with permission from Song, et al., 2002a)

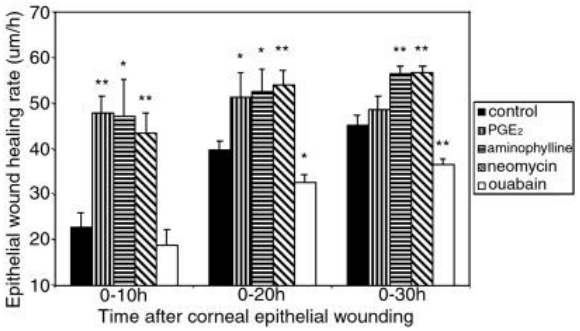

Figure 8. Corneal epithelial wound healing rate is influenced by the electric field. The rate is greatest when PGE_2 or aminophylline are present and these trigger the largest transcorneal potential as seen in fig. 7. Ouabain reduces this potential and generates the lowest epithelial wound healing rate. (Reprinted with permission from Song, et al., 2002)

closest to the wound edge, where the field was highest, were oriented most strongly by the field. The frequency of cell division was also enhanced by the

endogenous electric field. Because the endogenous field also regulated the wound-healing rate (fig. 8), it may act as one control of the interplay between cell migration and cell division during healing.

One very important additional observation made by McCaig's group was the effect of these endogenous fields on nearby nerve growth (Song, et al. 2002b). The endogenous electric field near the wound has a very strong orienting effect on the direction of sensory nerve sprouting and growth. Between 16 and 20 hours after wounding a large number of nerve sprouts project directly towards the cut wound edge in a whole-mount rat cornea (fig. 9). Reducing the wound field with ouabain randomizes nerve fiber orientation, suggesting that the electric field is the main orienting influence for these nerve sprouts. It has been known for decades that nerve growth can be oriented by imposed electric fields (Sisken and Smith 1975;Jaffe and Poo 1979), but this is one of only a few well documented examples in which a naturally occurring electric field has been found to exert a strong influence over neuronal growth. In addition to nerve growth, the axis of cell division was also strongly influenced by this endogenous electric field. Treatments that enhanced the field resulted in a greater degree of mitotic spindle alignment perpendicular to the field lines (fig. 9).

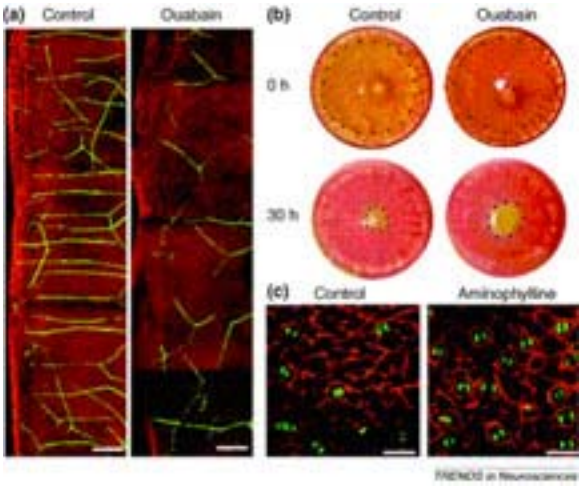

Figure 9. Observations made near a wound in rat cornea. A. Neuron outgrowths are strongly aligned by the endogenous electric field and exhibit a more random orientation when the field is reduced by ouabain addition. b. The rate of corneal wound healing is reduced in the presence of ouabain. c. The frequency and orientation of division planes is influenced by the field strength. Aminophylline increases the transcorneal potential and stimulates an increase in the rate of cell division and orientation of the axis of division perpendicular to the field. (reprinted from McCaig, et al., 2002)

CLINICAL TRIALS USING ELECTRIC FIELDS TO STIMULATE WOUND HEALING

While these well-controlled experiments are fairly recent, there are a large number of earlier clinical attempts to improve wound healing with electrical stimulation. There is much interest in finding improved methods for wound management and in the past five years alone, there are more than 8 reviews of the clinical trials using electrical stimulation (Fleischli and Laughlin 1997;Lampe 1998;Devine 1998;Braddock, et al. 1999;Bogie, et al. 2000;Evans, et al. 2001;Cullum, et al. 2001;Akai and Hayashi 2002). All of these reviews conclude that electro-therapy usually improves the rate of wound healing by 13 to 50% and sometimes stimulates the healing of chronic wounds for which no other conventional therapy had been successful. The most extensive review of the clinical literature remains Vanable's book chapter (Vanable, Jr. 1989) in which he describes in detail the three negative reports in the literature (Carey and Lepley, Jr. 1962;Wu, et al. 1967;Steckel, et al. 1984) followed by 11 positive reports grouped by methodology. Some investigators placed the cathode in the wound for the duration of the experiment (Assimacopoulos 1968b;

Assimacopoulos 1968a;Konikoff 1976), some used the anode at the wound throughout (Alvarez, et al. 1983) and others alternate polarity with the cathode in the wound first followed by the anode (Wolcott, et al. 1969;Carley and Wainapel 1985;Rowley, et al. 1974b;Rowley, et al. 1974a;Wheeler, et al. 1971;Gault and Gatens, Jr. 1976;Page and Gault 1975).

The three negative reports are not convincing. Carey and Lepley (1962) studied a small number of rabbit back skin wounds using very high current densities of around 400 $\mu A/cm^2$ and observed much necrosis at the anode. Wu, et al. (1967) ignored skin healing and Steckel et al. (1984) studied horse skin wounds in which those with electrodes had purulent inflammation.

The most popular approach for stimulating wound healing in humans uses both polarities of imposed fields sequentially, with the cathode in the wound first, followed by the anode. The rationale for using the cathode first is that this renders the wound free from infection (Rowley 1972;Rowley, et al. 1974a). There are reports of significant stimulation of healing in over 300 ulcers with this approach but the control group was always a small fraction of the size of the experimental group. Nevertheless, these studies indicate that electric fields promote the healing of chronic wounds. It is also clear that much work is needed to determine the optimal protocol for electric field application since so many different treatments have been used by these investigators. This will require further studies on model systems such as the mammalian cornea and skin as well as much more work applying fields to human skin wounds.

SUMMARY

1. Our skin is a polarized, multilayered epithelium that generates a transepithelial potential of 20-50 mV across itself. Wounds in this organ generate a low resistance pathway through which current will flow. This flow of current from all regions around the wound generates a lateral electric field that points toward the wound from every direction around it. The magnitude of this lateral field ranges between 40-200 mV/mm in mammalian wounds but there are still no reliable measurements of the lateral electric field near human skin wounds.

2. These wound fields have useful properties for signaling because they appear immediately upon wounding and persist until the wound heals.

3. Many cells have the ability to detect electric fields of this magnitude and 13 of these are discussed here. Most of these cells migrate toward the cathode of an imposed electric field with the optimal response occurring in fields on the order of 100 mV/mm.

4. The mechanisms used by these cells to sense the electric field often require Ca^{2+} influx, the presence of growth factors and protein kinase activity. PKC is required by neural crest cells and PKA is used in keratinocytes while MAP kinase is required by corneal epithelial cells.

5. The immediate target of these fields is likely to be the plasma membrane. Due to its presence at the outer boundary of the cell and its large resistance, it will have the most interaction the electric field. The field could act to redistribute charged lipid and protein molecules within the plasma membrane or modify the membrane

potential at the ends of the cell facing the poles of the electric field.

6. Several well-controlled experiments support a role for electric fields in the stimulation of wound healing in frog neurula, newt skin, and mammalian cornea. The endogenous field near a wound in the rat cornea influences the rate of wound healing, the orientation of cell division and the orientation of nerve sprouting.

7. Several clinical trials have reported that the healing of mammalian wounds can be promoted by electric fields. However, many different field strengths and polarities have been used so much work is needed to optimize this approach for the treatment of mammalian wounds. Most importantly, the lateral electric field near human skin wounds must be measured to guide the informed design of fields to stimulate wound healing.

Table 1. Endogenous electric fields measured near wounds

Species	Tissue	Wound type	E Field (mV/mm)	Reference
Bovine	Cornea	cut	42	(Chiang, et al. 1992;Sta Iglesia and Vanable, Jr. 1998)
Notophthalmus viridescens	digit	Digit tip amputation	40	(Chiang, et al. 1989;Iglesia, et al. 1996;McGinnis and Vanable, Jr. 1986)
Notophthalmus viridescens	Limb stump	Amputation	7-50	(McGinnis and Vanable, Jr. 1986)
Guinea pig	Skin	Small cut	100-200	(Barker, et al. 1982)

Table 2. Vertebrate cells exhibiting galvanotaxis

Cell Type	Response Direction/threshold (mV/mm)	Reference
Neural crest cells		
Quail	Cathode/10	(Nuccitelli, et al. 1993;Nuccitelli and Erickson 1983)
Xenopus	Cathode/10	(Stump and Robinson 1983a)
Ambystoma	Cathode/nd*	(Cooper and Keller 1984)
Fibroblasts		
Quail somite	Cathode/10	(Nuccitelli and Erickson 1983;Erickson and Nuccitelli 1984)
Mouse C3H/10T1/2	Cathode/nd*	(Yang, et al. 1984)
Mouse NIH 3T3 and SV101	Cathode/nd*	(Brown and Loew 1994)
Cornea		
Rat epithelial	Cathode/nd*	(Song, et al. 2002a)
Rabbit epithelial	Cathode/400	(Soong, et al. 1990a)
Rabbit endothelial	Anode/200	(Chang, et al. 1996)
Rabbit stromal fibroblast	Anode/600	(Soong, et al. 1990b)
Bovine	Cathode/100	(Zhao, et al. 1996)
Human	Cathode/100	(Farboud, et al. 2000)
Lens	Cathode/50	(Wang, et al. 2003a)
Bovine	Anode/150-200	
Retina	Cathode/600	(Sulik, et al. 1992)
Human pigment epithelial		
Vascular endothelium	Cathode/100	(Li and Kolega 2002)
Bovine		
Human granulocyte	Anode/100	(Rapp, et al. 1988)
Human leukocyte	Both Anodel and Cathode/nd*	(Dineur 1891;Fukushima, et al. 1953)
Human macrophage	Anode/nd*	(Orida and Feldman 1982;Cho, et al. 2002)
Rabbit osteoclasts	Anode/nd*	(Ferrier, et al. 1986)
Rabbit osteoblasts	Cathode/nd*	(Ferrier, et al. 1986)
Bovine chondrocyte	Cathode/80	(Chao, et al. 2000)
Rat prostate cancer cell line. MAT-LyLu	Cathode/10	(Djamgoz, et al. 2001)
Epidermal cells		
Xenopus embryo	Cathode/nd*	(Luther, et al. 1983)
Fish scale	Cathode/50	(Cooper and Schliwa 1986b)
Human skin	Cathode/10	(Nishimura, et al. 1996)

*not determined

Table 3. Signal transduction molecules involved in galvanotaxis

Species	Tissue	Required Molecule	Reference
Quail	Neural crest	PKC, PKA	(Nuccitelli, et al. 1993)
Bovine	Lens	TGF-α, βFGF, ERK1/2, MAPK	(Wang, et al. 2003b;Wang, et al. 2003a)
Bovine	Cornea	HGF, EGF, EGFR, MAPK	(Zhao, et al. 1996;Zhao, et al. 2002;McBain, et al. 2003)
Human	Skin	EGFR, EGF, PKA, Ca^{2+}	(Pullar, et al. 2001;Fang, et al. 1999;Fang, et al. 1998;Trollinger, et al. 2002b)

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