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ELECTRICAL SIGNALING IN CONTROL OF OCULAR CELL BEHAVIORS

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Abstract

Epithelia of the cornea, lens and retina contain a vast array of ion channels and pumps. Together they produce a polarized flow of ions in and out of cells, as well as across the epithelia. These naturally occurring ion fluxes are essential to the hydration and metabolism of the ocular tissues, especially for the avascular cornea and lens. The directional transport of ions generates electric fields and currents in those tissues. Applied electric fields affect migration, division and proliferation of ocular cells which are important in homeostasis and healing of the ocular tissues. Abnormalities in any of those aspects may underlie many ocular diseases, for example chronic corneal ulcers, posterior capsule opacity after cataract surgery, and retinopathies. Electric field-inducing cellular responses, termed electrical signaling here, therefore may be an unexpected yet powerful mechanism in regulating ocular cell behavior. Both endogenous electric fields and applied electric fields could be exploited to regulate ocular cells. We aim to briefly describe the physiology of the naturally occurring electrical activities in the corneal, lens, and retinal epithelia, to provide experimental evidence of the effects of electric fields on ocular cell behaviors, and to suggest possible clinical implications.

Keywords

ion flux; electric field; cell migration; cell proliferation; wound healing; electrotaxis/galvanotaxis

1. INTRODUCTION

The eye is an electrically active organ, displaying many different types of electrical properties. The best-studied electrical activity in the eye is found in the retina, where incoming light excites the rod and cone photoreceptors, resulting in an electrical signal which propagates through the optic nerve and travels to the visual cortex where it is converted by the brain into an image. The corneal nerves may be excited by many types of

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stimuli. These are fast-acting electrical pulses like the action potentials of the nervous system.

The eye also has an electrical feature that is much less-well studied - slow-acting, long-lasting, steady, direct current (dc) electric fields (EFs). These include the transcorneal potential difference (TCPD), ionic currents at the lens, the transepithelial potential difference (TEP) of the retinal pigment epithelium (RPE) and the EFs/currents associated with injury (wound EFs/injury currents). In the cornea, ionic transport is required to transport water from the stroma to the tear film. The lens drives a unique current flow through itself that creates an internal microcirculation (Mathias et al., 2007). In the RPE ionic transport is required to move water from the subretinal space into the blood, and transport nutrients in the opposite direction, while also maintaining an optimal ionic composition in the subretinal space, required for photoreceptor excitation (Wimmers et al., 2007).

Accumulating experimental evidence suggests that this latter type of EF may provide an important signal that contributes to the control of cell behavior. The cells of the ocular tissues are exposed to EFs in their natural setting, and together with the finding that many cellular behaviors are affected by applied EFs of physiological magnitude, the role of EFs as a novel cell signaling mechanism is strongly suggested. We will use “electrical signaling” here specifically to describe this type of endogenous signaling mechanism and for signaling that may be incited by externally applied electric fields. Electrical signaling may represent a novel therapeutic strategy in ocular tissue repair and regeneration. Indeed, electrical stimulation is being tested clinically for the treatment of bone fracture, wound healing, and spinal cord injury.

This review aims to focus on 1) how the endogenous electrical activities are generated - the ionic transport mechanisms in the cornea, lens, and RPE; 2) the responses of ocular cells to applied EFs; 3) potential intracellular signaling mechanisms; and 4) clinical implications. For general and relevant background on electrical control of development, wound healing, and regeneration, please refer to the following elegant reviews (Funk et al., 2009; Levin, 2007; McCaig et al., 2005; McCaig et al., 2009; Mycielska and Djamgoz, 2004; Nuccitelli, 2003a, b; Ojingwa and Isseroff, 2003; Robinson and Messerli, 2003; Stewart et al., 2007).

2. BRIEF BACKGROUND IN ELECTRICITY AND ELECTROPHYSIOLOGY

Electric current is the movement or flow of electrically charged particles, while an EF is an influence produced by an electric charge on other charges in its vicinity. In a biological context, ions (e.g. Na^+ , K^+ , Cl^- , Ca^{2+} , etc.) are the charged particles whose flux constitutes electric currents. In biological tissue, which is a resistive as well as conductive medium, EFs form due to the presence of charged particles and the flow of electric currents. The EFs produced basically follow Ohm's law which states that voltage equals current multiplied by resistance: $V = I \times R$, where V is the voltage (measured in volts: V), I is the current (measured in amps: A), and R is the resistance (measured in ohms: Ω), although in biological tissue the situation is far more complex.

The resistivity is a measure of how strongly a material opposes the flow of electric current. Biological fluids typically have a resistivity value of $\sim 100 \Omega\text{-cm}$. The resistivity of tear and aqueous humor is $\sim 70 \Omega\text{-cm}$ and that for corneal stroma is $\sim 100 \Omega\text{-cm}$. Endothelium and epithelium have much higher resistivity values of $\sim 1,500$ and $250,000 \Omega\text{-cm}$ respectively (Gabriel et al., 1996; Guimera et al., 2010; Klyce, 1972; Lim and Fischbarg, 1981). When there is a voltage difference between any two points in conductive media current flows. In biological tissues or solutions, the EF and electric currents are therefore inseparable. The relationship between the current density and the EF is $E = J \times \rho$, where ρ is the static

resistivity (measured in ohm-metres, $\Omega\cdot\text{m}$), E is the magnitude of the electric field (measured in volts per metre, V/m), and J is the magnitude of the current density (measured in amperes per square metre, A/m^2). Most often in literature, the resistance value is used to quantify electrical resistance across a membrane structure, such as the cornea, epithelial and endothelial layers. The resistance across a membrane structure with defined area is expressed as the resistance times the areas, and presented as $\Omega\cdot\text{square centimeter}$. For example, the resistance of rabbit corneal endothelium was measured to be $\sim 70\ \Omega\cdot\text{cm}^2$ (Lim and Fischbarg, 1981).

Live cells maintain electrical potentials of varying magnitude across their plasma membranes (Fig. 1A). This is achieved by the active, polarized transport of ions by pumps and transporters, together with the selective permeability of channels in the cell membrane. Excitable cells such as neurons and muscle fibers have large membrane potentials ($\sim 70\ \text{mV}$), a voltage difference negative inside relative to outside the cells. Epithelial cells in cornea, lens, and retina also have significant membrane potentials. However, the membrane potential of these non-excitable cells is smaller ($\sim 30\ \text{mV}$) (Lichey et al., 1974; Mergler and Pleyer, 2007; Rae, 1979; Wiederholt and Koch, 1979).

Analogous to the cell membrane potential, epithelial cells form a “membrane” that collectively transports ions across the whole epithelial layer. At the same time, the epithelium maintains tight junctions between the cells preventing free diffusion of ions. The energy consuming transport of ions coupled with the epithelial barrier establish a steady, long-lasting TEP, similar to the membrane potential in cells (Fig. 1B). In the cornea, the TEP contributes to the generation of the TCPD. In the lens, large and consistent currents flow out from the equator and enter the anterior and posterior poles. In the retina, the RPE also generates and maintains a TEP.

EFs and current are vectors and it is this directional quality that makes EFs a candidate spatial organizer that is capable of imposing directional movement on molecules, cells, and tissues. The facts that ion channels and pumps are often localized to specific cells or parts of cells, and that the local resistance of cells or tissues may vary due to spatial variation in cell packing or tight junction density, means that EFs could be regulated spatially and temporally (McCaig et al., 2005).

3. ELECTRIC FIELDS IN THE CORNEA

Electrical potential differences (PDs) exist between the three layers of the cornea: the epithelium, stroma, and endothelium. Transport of ions and segregation of the ions in different tissue compartments generates the PD or EFs. We will firstly describe some ion transport mechanisms, then the electric potentials in the normal cornea and at corneal wounds, followed by experimental results on how corneal cells may be affected by such EFs.

3.1. Transport of ions across the cornea

The human corneal epithelium is about $50\ \mu\text{m}$ thick consisting of 5–7 layers of continuously renewing cells. The epithelial cells are joined by tight junctions and function as a semi-permeable, highly-electrically resistant membrane (Schermer et al., 1986; Tuli, 2005). The stroma makes up $\sim 90\%$ of the total corneal thickness and is primarily composed of collagen fibrils which are oriented in a parallel manner and organized in lamellae (Maurice, 1957). The endothelium consists of a monolayer of hexagonal cells (Konomi et al., 2005).

To remain transparent, corneal water balance/hydration level (deturgescence) must be regulated within a specific physiological range (Donn et al., 1959; Klyce, 1975, 1977; Klyce

and Crosson, 1985; Klyce and Wong, 1977; Maurice, 1951, 1967, 1972; Reinach et al., 2008b). Transport of ions coupled with water transport regulate the osmolarity balance between the corneal stroma, the anterior chamber, and the tear fluid, thus regulating deturgescence (Edelhauser, 2006). The corneal endothelium is responsible for ~ 90% of the fluid transport of the rabbit cornea. However, ion transport activity in the corneal epithelium also plays a significant role in maintaining proper hydration. Most of the following description will be on the corneal epithelium, because it maintains a far greater PD and generates significant wound EFs. We emphasize those aspects more relevant to generation of electric currents/EFs in corneal tissues. For more comprehensive reviews on ion transport and hydration of the cornea, please refer to these in-depth reviews and papers (Bonanno, 2011; Candia and Alvarez, 2008; Dikstein and Maurice, 1972a, b; Fischbarg and Lim, 1974; Fischbarg and Maurice, 2004; Hodson, 1974; Hodson and Miller, 1976; Klyce, 1977; Klyce and Wong, 1977; Mergler and Pleyer, 2007; Riley et al., 1995).

3.1.1. Expression of ion channels and transporters in the cornea—Corneal epithelial cells (CECs) and endothelial cells express a wide spectrum of ion channels, transporters and pumps (Fig. 2). These include K^+ , Na^+ , and Cl^- channels; transient receptor potential (TRP) channels; sodium proton exchangers (NHE); K^+/Cl^- co-transporters (KCC); Cl^-/HCO_3^- exchangers (CBE); Na^+/Ca^{2+} exchangers (SCE); $Na^+/K^+/Cl^-$ co-transporters (NKCC); Na^+/K^+ -ATPase; and Ca^{2+}/Mg^{2+} -ATPase. The functional expression of these diverse ion transporters and channels has been demonstrated by genetic, molecular, biochemical, immunostaining, and electrophysiological studies. These channels and transporters play important roles in regulating hydration levels of the cells, as well as the cornea as a whole (Reinach et al., 2008b).

Na^+/K^+ -ATPase is responsible for maintaining ionic gradients between the extracellular and intracellular spaces, required for cellular homeostasis and for the transepithelial movement of water and organic solutes in response to the differential osmotic gradients (Tsuji et al., 1992). Na^+/K^+ -ATPase is a multigene family of proteins with three α isoforms (α_1 , α_2 , α_3), three β isoforms (β_1 , β_2 , β_3) (Gick et al., 1993; Sweadner, 1989), and sometimes a γ subunit (Geering, 2006). The α subunit hydrolyzes ATP to ADP and in doing so shifts three Na^+ ions out of the cell and two K^+ ions into the cell (Delamere and Tamiya, 2004), while the β and γ subunits appear to have modulatory roles (Mathias et al., 2007). The α_1 subunit was found to be expressed predominantly in corneal endothelial cells and along the basolateral surfaces of basal epithelial cells. The α_2 and α_3 subunits distributed homogeneously in the epithelial layer. Corneas affected by Fuch's' dystrophy, a corneal adematous disease, show reduced epithelial immunostaining for Na^+/K^+ -ATPase subunits. Other alterations in epithelial Na^+/K^+ -ATPase point to the importance of epithelial changes in the development of corneal adematous diseases (Ljubimov et al., 2002).

Two isoforms of the $Na^+/K^+/Cl^-$ co-transporter (NKCC1 and NKCC2) are expressed in corneal epithelial and endothelial cells (Bildin et al., 2001a). Na^+/K^+ -ATPase and NKCC are suggested to be the major driving force for apical to basal transport of Na^+ and basal to tear transport of Cl^- (Reinach et al., 2008b).

Cl^- channels are expressed in both corneal epithelium and endothelium. Messenger RNA coding CIC Cl^- channels (CIC-2, -3, -5, -6, -7), cystic fibrosis transmembrane conductance regulator (CFTR), and Ca^{2+} -activated Cl^- channel-1 (CLCA-1) are positive in CECs (Fig. 3A). Some protein expression has also been confirmed (Fig. 3B), and Cl^- currents are present in human CECs (Fig. 4) (Cao et al., 2010). CIC-2 and CIC-3 immunolabeling confirmed the presence of these proteins in corneal epithelium (Davies et al., 2004). CFTR and CLCA-1 are also expressed in human CECs (Al-Nakkash et al., 2004; Al-Nakkash and Reinach, 2001; Connon et al., 2006; Itoh et al., 2000). Significantly for directional ion

transport, channels (e.g. CIC-2 and a CLCA) are distributed asymmetrically at the apical and basal layer of the corneal epithelium, respectively (Cao et al., 2010; Connon et al., 2006; Davies et al., 2004). CFTR plays a significant role in regulating the TCPD, as CFTR null mice fail to respond to perfusion of low Cl^- solution (Levin et al., 2006).

K^+ channel activities may be mediated by cholinergic receptor activation, Ca^{2+} level, arachidonic acid, and some fatty acids (Farrugia and Rae, 1992; Bockman et al., 1998; Takahira et al., 2001). K^+ channel activity modulates adrenoceptor stimulation of net Cl^- transport in CECs (Wolosin and Candia, 1987). A specific type of K^+ channel (Kv3.4) is expressed widely in CECs and is activated by ultraviolet irradiation (Lu, 2006). The hyperactivation of Kv3.4 results in rapid intracellular K^+ loss and cell volume shrinkage followed by apoptosis.

CECs express L-type Ca^{2+} channels and other Ca^{2+} influx pathways (Reinach et al., 2008a; Rich and Rae, 1995). Plasma membrane Ca^{2+} -ATPase is expressed in human and rabbit corneal epithelium. During wound healing, Ca^{2+} -ATPase changed expression distribution. More Ca^{2+} -ATPase was expressed at the basal side of the epithelial cells migrating into the wound bed (Talarico, 2010).

Immunostaining showed the presence of one of the typical epithelial Na^+ channels (ENaC) in the CECs, but another study showed inconsistent results (Mirshahi et al., 1999; Rauz et al., 2003).

Some channels and transporters have unique spatial localization. In contrast to the basolateral distribution of the α_1 subunit of Na^+/K^+ -ATPase, some Cl^- channels showed mostly apical distribution, and importantly CLC-6 and CFTR had a gradient of high expression toward the apical side (Fig. 5). The spatial difference in Na^+/K^+ -ATPase and Cl^- channels suggests directional transport of cations and anions. This is consistent with the measurements of ion transport, demonstrating directional transport of different ions described above (Candia and Alvarez, 2008; Fischbarg and Maurice, 2004; Klyce and Wong, 1977).

3.1.2. Ion transport in and across the epithelium—Baso-lateral distribution of Na^+/K^+ -ATPase suggests there are functionally inward (tear to basal) fluxes of positive charges making the basal side electrically positive. This is because, when activated, each transport cycle of Na^+/K^+ -ATPase pumps two K^+ ions into the cell and three Na^+ ions out of the cell (Jorgensen et al., 2003).

Active Cl^- transport is a secondary active process, because Cl^- uptake from the stroma into the epithelial layer is dependent upon basolateral membrane-coordinated Na^+/K^+ -ATPase and NKCC activity. Pump coupling to NKCC function is a result of Na^+/K^+ -ATPase establishing a bath-to-cell inwardly directed Na^+ gradient, which provides the chemical driving force for NKCC-mediated uphill intracellular Cl^- accumulation. Na^+/K^+ -ATPase activity has a variable load-dependent Na^+/K^+ stoichiometry (Candia et al., 1984; Reinach et al., 2008a).

The specific ionic fluxes that account for ion transport in corneal epithelia vary among species. In amphibians, it is essentially Cl^- flux (Zadunaisky and Lande, 1971). An inward flow of Na^+ ions has been observed in rabbit and frog cornea (Candia and Askew, 1968; Klyce, 1975; Reid et al., 2005; Van der Heyden et al., 1975), while Cl^- is actively transported outward from the aqueous humor, across the stroma and epithelium, into the tear film (Klyce and Wong, 1977; Reid et al., 2005; Zadunaisky, 1966). In mammals, this mechanism is accounted for by approximately equal contributions by inwardly directed net

Na^+ transport toward the stroma and outwardly directed active Cl^- transport into the tears (Klyce, 1975).

Transport of electrolytes is coupled with the transport of water between the stroma and tear fluid. Corneal epithelium transports Na^+ from the tear side to the basal side, and Cl^- in the opposite direction (Candia, 2004; Fischbarg et al., 2006; Klyce and Crosson, 1985; Levin and Verkman, 2005; Yang et al., 2001). Cl^- is actively transported cellularly. The active transport of Cl^- to the tear side creates a negative potential on this side. To maintain electroneutrality of solutions, the negative potential generated at the tear side draws Na^+ to move passively through the paracellular space to the tears to produce a net transport of NaCl . This would create a net osmotic gradient. Together with fluxes of other ions, e.g. HCO_3^- , the net transport generates osmotic gradients that drive water transport, with Cl^- secretion providing a primary driving force for water transport across the corneal epithelium (Fischbarg, 1997).

Aquaporins (AQPs) facilitate water transport in response to small osmotic gradients produced by salt pumping. The corneal epithelia of mouse, rat, and human express water-selective AQPs, AQP5 and AQP3. (Hamann et al., 1998; Levin and Verkman, 2004; Levin, 2005; Patil et al., 1997). The functional significance of various AQPs in ocular tissues has been confirmed in AQP-deficient mice. AQP deletion reduces transepithelial water permeability and hence the amount of fluid absorbed or secreted (Verkman et al., 2008). Altered AQP expression may play a role in various human corneal diseases associated with fluid imbalance (Levin and Verkman, 2006; Levin, 2005; Rabinowitz et al., 2005).

3.1.3. Regulation of the ion transport—Neuronal and non-neuronal mechanisms may regulate the functioning of ion transporters. The regulatory mechanisms appear to have interspecies differences. Adrenergic, serotonergic, and cholinergic receptors contribute to the control of ion transport activity (Klyce et al., 1982; Pesin and Candia, 1982a, b; Zadunaisky et al., 1973). The intracellular Cl^- accumulated through Cl^- channels egresses into the tear film (Fig. 2). These receptors activate several signaling pathways that control the activity of Cl^- channels in the corneal epithelium. Adrenergic signaling pathways elicit increases in fluid secretion into the tears through transient cytosolic increases in cAMP and intracellular Ca^{2+} . In addition to neuronal control, the active transport is regulated through many mechanisms, including those that are very important for wound healing, e.g. epidermal growth factor (EGF) increases NKCC activity in rat CECs and the increase in its activity lasts for more than an hour (Yang et al., 2000). Within the CIC family, Cl^- channels may be regulated by the cAMP-activated transmembrane conductance regulator (CFTR), Ca^{2+} -activated Cl^- channels (CaCC) - CICA2, and volume-regulated anion channels (Reinach et al., 2008a).

Growth factors, cytokines, ATP, mechanical forces, and UV irradiation are all known to induce ion fluxes in CECs (Lu, 2006; Reinach et al., 2008a). For example, growth factor receptor stimulation induced the activation of other types of K^+ channels through induction of CEC proliferation processes (Roderick et al., 2003). Therefore, selective K^+ channel activation via diverse receptor-activated events is essential for determining whether this activation induces cell death or promotes cell proliferation.

3.1.4. Ion transport across the endothelial layer—The corneal endothelium transports water and electrolytes between the stroma and anterior aqueous chamber. The corneal endothelium provides a pathway for nutrient uptake and waste removal via simple and facilitated diffusion, and secondary active transport mechanisms such as facilitated glucose transporter, glut-1 (Bildin et al., 2001b), lactate/ H^+ , and lactate/ Na^+ co-transporters (Giasson and Bonanno, 1994). The endothelium also secretes fluid via ionic transport in

order to counterbalance the continuous leak of fluid into the stroma (Bonanno, 2003). The “pump-leak” mechanism maintains corneal deturgescence and transparency by ensuring no net fluid transport occurs, as active secretion out of the cornea counterbalances the leak of fluid into the cornea (Maurice, 1972).

3.2. Electric potentials in the cornea

The polarized transport of ions, selective permeability to different ions, and electrical resistance between different compartments of tissues establish electric potential gradients in the cornea. Electrical PDs across the whole cornea (TCPDs) have been demonstrated with *in vitro* and *in vivo* measurements in many different animals. This was first described over half a century ago (Friedman and Kupfer, 1960; Ito et al., 2000; Klyce, 1972; Modrell and Potts, 1959; Potts and Modrell, 1957; van der Bijl et al., 2004). In rabbit, mouse, and frog corneas, TCPD measurements range from 15–33 mV, with the tear side negative (Klyce, 1972; Levin et al., 2006).

The corneal epithelium is electrogenic, which is to say that it is capable of producing its own electrical activity. As a collective layer, the epithelium produces a uniform and consistent TEP (Candia et al., 1968; Chiang et al., 1992; Fischer et al., 1972; Foulds and Barker, 1983; Lindemann, 1968). Detailed measurements demonstrated that the TEP contributes to over 90% of the TCPD. In various species, measurements of the corneal TEP have been found to range from ~20 to 40 mV (basal side positive compared to the tear side) (Fig. 6) (Akaike and Kiyohara, 1977; Candia et al., 1968; Chiang et al., 1992; Fischer et al., 1972; Klyce, 1972; Klyce and Crosson, 1985; Lindemann, 1968; Maurice, 1967). In isolated rabbit eyes, the TEP was identical in polarity with almost the same magnitude, to the simultaneously measured TCPD (Klyce, 1972).

In rabbit corneal epithelium, Klyce showed three iso-potential regions: beneath the superficial layer, the region between squamous and wing cells, and the transition region between wing and basal cells (Fig. 6). These three iso-potential regions correspond to the three resistance regions, with the first superficial layer having the highest electrical resistance (Fig. 6C).

Corneal endothelium is extremely leaky with the electrical resistance could be as low as only 25 $\Omega\text{-cm}^2$, due to its discontinuous tight junctions (Bonanno, 2003). The corneal transendothelial potential is only 0.5–1 mV, ~30–60 times smaller than the corneal TEP (Fischbarg and Lim, 1974; Hodson, 1977; Hodson and Miller, 1976). The transendothelial potential is dependent on the activity of $\text{Na}^+/\text{K}^+\text{-ATPase}$, which like most epithelial cells is located on the basolateral membrane of corneal endothelial cells, (Guggenheim and Hodson, 1994), and HCO_3^- (Hodson, 1974; Hodson and Miller, 1976), but to a lesser extent on the activity of Cl^- transporters (Barfort and Maurice, 1974; Fischbarg and Lim, 1974; Hodson, 1971).

Corneal transparency depends on regulation of the hydration of the corneal stroma. The stromal swelling pressure drives water into the cornea, while endothelial and epithelial layers counterbalance the pressure by their capacity to transport ions and water. Ion fluxes from the stromal to anterior chamber side, and to the tear film through channels and pumps, are responsible for the counterbalancing force. The identity and location of all of the components of this transport system are being gradually elucidated (Bonanno, 2003; Candia and Alvarez, 2008; Fischbarg and Maurice, 2004). In individual cells, the pumps and channels respond to osmotic challenge and regulate cell volume (Fig. 2) (Reinach et al., 2008a). The epithelium, with multiple layers and many cells as a functional unit contributes to regulation of stromal hydration through activation of asymmetrically distributed $\text{Na}^+/\text{K}^+\text{-ATPase}$, and other ion channels (Fig. 5, 7) (Cao et al., 2010; Ljubimov et al., 2002).

3.3. Electric fields at corneal wounds

While the role of ionic transport in maintaining corneal hydration has been the focus of most previous investigations, some less-recognized research suggests another potentially important role for ionic transport - in generating wound EFs. Recent experiments have produced strong evidence that the EFs thus generated may have an important role in corneal epithelial wound healing (Chiang et al., 1992; Reid et al., 2007; Reid et al., 2005; Zhao, 2009; Zhao et al., 2006). We will present a detailed account of wound-related EFs and cellular effects.

Injury disrupts the continuity of the epithelium, breaking down the tight junctions between cells that help maintain the PD, thus creating a short-circuit at the wound, allowing ions - Na^+ , Cl^- , K^+ etc. to flow into the wound freely from surrounding tissue (Fig. 7). Because ions can flow freely at the wound site, the PD here drops to 0 mV. The PD is maintained however, in the surrounding intact epithelium. The more positive potential underneath the intact epithelium relative to the wound drives electric currents (by convention, the flow of positive charge) into the wound, resulting in endogenous EFs pointing to the wound center (red arrow in Fig. 7B). The electrical differential between wounded and intact areas is known as the “wound EF”. Many different techniques have confirmed very consistently the existence of wound EFs (Chiang et al., 1992; Reid et al., 2007; Reid et al., 2005; Zhao, 2009; Zhao et al., 2006).

3.3.1. Magnitude of electric fields at corneal wounds—Chiang and co-workers, using micro-glass electrodes, measured a lateral potential drop along the outer surface of the cornea at the edge of wounds made in the corneal epithelium of the isolated bovine eye. Microglass electrodes measured lateral corneal wound EFs of ~42 mV/mm in the first 0.25 mm from the wound edge on the surface of the eye (Chiang et al., 1992). Because of the limitation of the spatial resolution of the measuring technique, the authors stated that “the 40 mV/mm average value reported here for the lateral EFs measured on the surface of the cornea at the edge of wounds made in the epithelium is almost certainly a minimum one”. They believe measurements with higher spatial resolution made closer to the edge of the wound would likely yield even higher values. Indeed, this value is smaller than those measured at skin wounds using other independent techniques such as the bioelectric field Imager. In mouse and human skin, 177 mV/mm has been measured with the bioelectric field imager (Nuccitelli et al., 2008).

Another different technique, the vibrating probe, is an ultra-sensitive micro-probe used to measure electric current non-invasively. This technique has permitted measurement of growth-, development-, and wound-related electric currents in a wide variety of organisms (Jaffe and Nuccitelli, 1974). Using the vibrating probe, we measured outward currents of up to $10 \mu\text{A}/\text{cm}^2$ in corneal wounds from rat, bovine, and human (Fig. 8) (Reid et al., 2010; Reid et al., 2007; Reid et al., 2005). In human corneal wounds, currents of $0.41 \mu\text{A}/\text{cm}^2$ were measured. The significantly weaker wound currents in human corneas compared to those in fresh rat and bovine corneas is likely due to the extended storage of the corneas.

3.3.2. Polarity of electric fields at corneal wounds—It may first appear that the corneal wound site being positive (anode) is in conflict with the idea of the field guiding cells into the wounds, because epithelial cells *in vitro* migrate toward the negative cathode (please see section 3.4. Corneal cells respond to electric fields). Direct measurements of the lateral fields at corneal wounds showed that the wound was relatively positive (Chiang et al., 1992). It should be noted that the microglass electrodes Chiang et al. used measured the surface EFs in the tear film. This is the return path of the currents (flow of positive charge) (blue arrow in Fig. 7B) driven by the positive TEP below the epithelial layers, out of the

wound margin. The bioelectric field imager and vibrating probe also measure EFs and currents on the surface of the wound and the outside vicinity of the wound.

However, what is more relevant is the lateral fields immediately below the upper epithelial layer, where the tight junctions exist and across which the TEP is established (Klyce, 1972; Wolosin, 1988). Immediately below the migrating surface epithelia, cells are tightly packed, with little extracellular space. The electric currents, by the laws of physics, must form a complete circuit. The current flowing away from the wound on the outer surface of the epithelium (blue dashed arrow in Fig. 7B) must flow through the epithelium back to the basal side to complete the circuit. The ion channels and pumps in the cells at the wound edge thus keep transporting ions (black arrows in Fig. 7B). This transport is likely to have an active component in an effort to maintain the TEP, like a battery, to keep the current flowing in the direction of the wound in the healing epithelium (red arrow in Fig. 7B). This is the direction in which active cell migration occurs. Tissue resistance may be substantial and may establish larger lateral fields with the wound being negative (red arrow in Fig. 7B). Thus, the most superficial layer of epithelium may “see” the wound as an anode, whereas the lower surface would see a cathode at the wound.

A new technique, the microneedle array, has demonstrated such voltage gradients in the epidermis of human skin with the field vector pointing toward the wound. The microneedle arrays were placed on skin with the electrode tips piercing and recording in-depth below the skin surface so that they lie below the stratum corneum, but above the basement membrane. The results confirmed the existence of a variation in skin potential that is maximum near the wound, and a resulting transverse EF directed toward the wound (more negative potential approaching the wound). The field strength at the wound edge is ~40 mV/mm (Mukerjee et al., 2006). This recording confirmed the generation of a lateral EF in human skin with the field direction pointing toward the wound in epidermis. This measurement is consistent with Nuccitelli's estimation based on measurements with the bioelectric field imager - about 40 mV/mm within the epidermis with the negative pole of the field at the wound site (Nuccitelli et al., 2008).

Thus, the EF is orientated in the same direction that CECs migrate and proliferate to heal the wound. Wounding establishes a steady, laterally-oriented EF with the cathode at the wound centre. The current runs laterally under the basal surfaces of the epithelial cells and returns laterally within the tear film across the apical surface. The TEP in the surrounding intact epithelium ‘pushes’ ions out of the wound, producing a large and rapidly increasing electric current (Reid et al., 2005). This ion flow generates EFs in the surrounding tissues, orientated toward the wound site (Fig. 7).

Mammalian skin also maintains a TEP, and the first person to measure injury currents was Emil Du-Bois Reymond who, using a galvanometer, noted a current exiting a wound on his finger (Du Bois-Reymond, 1843; Du Bois-Reymond, 1860). When the skin is cut, a wound-induced EF arises for the same reasons as outlined for corneal epithelium, with the current returning under the cornified layer. Importantly, wound-induced EFs persist until the migrating epithelial cells at the leading edges re-seal the wound and re-establish a high electrical resistance across the tissue (Danjo and Gipson, 1998; Hudspeth, 1982; Keese et al., 2004).

3.3.3. Wound electric fields are actively regulated—EFs have been described as a leakage at the wound site, i.e. a passive process. However, corneal wound EFs contain an actively-regulated component. This is supported by the following experimental evidence. Firstly, the time course of wound electric currents showed a gradual increase after wounding. It takes 60–90 min for the currents to reach a peak after wounding rat cornea

(Reid et al., 2005). If it is a pure leakage, the currents should reach the peak immediately or very soon after the epithelial breakdown. Secondly, drug treatment with aminophylline and other drugs which enhance Cl^- transport significantly enhanced the electric currents, while inhibition of ion transportation significantly reduced the currents (Reid et al., 2010; Reid et al., 2007; Reid et al., 2005). Our recent experiments showed that wounding induced changes in the expression level and distribution of Na^+/K^+ ATPase and some Cl^- channels (Cao et al., unpublished results).

Electric currents at human skin wounds also have a dynamic time course. At accidentally amputated finger tips, the currents reached a peak 6–8 days after amputation in children who regenerated the tip (Illingworth and Barker, 1980). The regulation of the current size appeared to coincide with the expression level of Na^+/K^+ ATPase at skin wounds, which gradually increased until day 7 and diminished at day 9 (Dube et al., 2010). The wound electrical signaling thus, may be actively regulated, through expression and activity of the pumps and channels. It could also be regulated by modification of tight junctions.

3.3.4. Ionic components of the electric current at corneal wounds—At corneal wounds, ions such as Na^+ , K^+ , Ca^{2+} , Cl^- and Mg^{2+} among others may contribute to the overall net electric current. Using ion-substituted artificial tear solution and vibrating probe techniques, we found that Cl^- and Na^+ are the major components of electric currents in rat corneal wounds. Na^+ appeared to be the major component of ionic transport in the resting (non-wounded) rat cornea and of the wound center leakage current, whereas Cl^- was a more important component of the endogenous electrical current at the wound edges (Fig. 9).

Ion-selective probes allow direct measurement of the contribution of each specific ion to the wound electric current. At rat cornea wounds, Ca^{2+} efflux increased steadily, whereas K^+ showed an initial large efflux which rapidly decreased. A significant observation was a persistent large influx of Cl^- , which had a time course similar to the net wound electric currents (Fig. 10) (Vieira et al., 2011).

Therefore, the outward electric currents occurring naturally at rat corneal wounds are carried mainly by a large influx of Cl^- ions, and in part by effluxes of Na^+ , Ca^{2+} and K^+ ions. Ca^{2+} and Cl^- fluxes appear to be mainly actively regulated, while K^+ flux appears to be largely due to leakage. The dynamic changes of electric currents and specific ion fluxes after wounding suggest that electrical signaling is an active response to injury.

3.4. Corneal cells respond to electric fields

Corneal cells migrate and proliferate in the presence of endogenous EFs, especially when the cornea is wounded. Applied EFs have been used to mimic the endogenous EFs to test their effects on cultured CECs *in vitro*. We will not discuss the methods and techniques used to apply EFs to cultured cells and tissues, because they have been described in detail previously (Song et al., 2007; Tai et al., 2009). Experimental evidence suggests that endogenous wound EFs provide a powerful signal for corneal epithelial wound healing.

3.4.1. Directional migration of corneal epithelial cells in electric fields—

Cultured CECs respond to applied EFs of physiological magnitude by migrating toward the cathode (Fig. 11A). Soong et al. demonstrated that EFs induced directional migration (galvanotaxis or electrotaxis) of CECs in the cathodal direction, with a threshold of 4 V/cm and a latency of 20 min (Soong et al., 1990a). Subsequent investigation by other groups demonstrated a much smaller threshold (<100 mV/mm) and response time within 10 min after onset of the EF (Farboud et al., 2000; Zhao et al., 1996b).

Monolayers and sheets of epithelial cells also migrate directionally in an applied EF (Zhao et al., 1996a) (Fig. 11B). An EF as low as 12.5 mV/mm is able to direct the migration of bovine CECs in culture (Zhao, 2009; Zhao et al., 2006). This collective migration has not been shown in chemotaxis responses. Compared to dissociated cells, the monolayer or sheets of cells migrate more directionally in an EF of much lower voltage (Zhao, 2009; Zhao et al., 1996a, b). Cells coupled by gap junctions react as a single unit to an applied EF and it has been suggested that gap junction coupling may increase cellular sensitivity to weak EFs as the perturbations induced by an applied EF are greater for groups of cells that are electrically coupled than for single cells (Cooper and Keller, 1984). This is pertinent as the *in vivo* healing of corneal epithelial wounds is accomplished by *en mass* directional growth of epithelium into the wound. *In vivo* wound healing is led by closely associated groups of cells rather than isolated single cells (Imanishi et al., 2000; Lu et al., 2001; Suzuki et al., 2003).

Increasing field strength induces a stronger electrotaxis/galvanotaxis response in bovine as well as human CECs (Zhao et al., 1997). CECs from rat, bovine, and human respond to an EF as low as 25 mV/mm (0.5 mV across a cell of 20 μ m diameter), well within the physiological range (Farboud et al., 2000; Zhao et al., 1996a, b; Zhao et al., 1997).

3.4.2. Electric fields provide an overriding guidance cue for corneal epithelial cells—Multiple guidance cues exist at the wound to guide the cells to proliferate and migrate directionally into the wound. Generally accepted guidance cues include injury stimulation, contact inhibition release, free edge, chemotaxis, and mechanical forces. Injury disrupts the plasma membranes of the cells at the wound margin which induces Ca^{2+} entry and K^{+} release. Wounded cells and tissue may release chemical substances (growth factors, cytokines, ATP) that attract cells into the wound by chemotaxis (directional cell migration in response to chemical gradients). Contact inhibition release or free edge, and simply the availability of space may also provide cells with directional signals (Block et al., 2010; Zhao et al., 2006) (Fig. 12A).

We compared the above directional cues with applied EFs. Surprisingly we found that EFs of physiological strength guide CECs, overriding other co-existing directional signals. When applied EFs of physiological strength (as small as 25 mV/mm) are applied against those well accepted directional guidance cues, CECs migrate in the direction of the EFs and ignore the other cues (Zhao, 2009; Zhao et al., 2006) (Fig. 12B).

Stratified multi-layered epithelial cells in a healing wound also respond to an applied EF. The stratified CECs also move away from the wound or into the wound following an applied EF and ignore other directional cues (Fig. 13). No other known directional signals, either biochemical or biophysical, have such an overriding guidance effect.

3.4.3. Electric fields guide other cellular behaviors—CECs also elongate and reorientate so that their long axis comes to lie perpendicular to the EF vector (Soong et al., 1990a; Zhao et al., 1996b; Zhao et al., 1997) (see Fig. 11A, 1 and 6 hours). Non-dividing CECs normally established a long axis perpendicular to the EF vector and when they came to divide they did so with a cleavage plane perpendicular to the EF vector. In a group of eighteen dividing cells almost all aligned themselves with the division axis parallel to the EF and the cleavage plane perpendicular, suggesting that the mitotic spindle had aligned parallel to the EF vector (Zhao et al., 1999b).

Cell proliferation rate and division orientation correlate with the endogenous EFs at corneal wounds. The endogenous EFs at corneal wounds were manipulated using drugs with different pharmacological actions but sharing the common feature of affecting ion transport.

The wound-induced EFs controlled the orientation of cell division; most epithelial cells divided with a cleavage plane parallel to the wound edge and perpendicular to the EF vector. Increasing or decreasing the EF pharmacologically, respectively increased or decreased the extent of oriented cell division. An endogenous EF also enhanced the frequency of cell division (Song et al., 2002).

Nerve sprouting in the healing epithelium also appears to be regulated by endogenous EFs. In a rat *in vivo* corneal model, similar pharmacological manipulation was used to enhance or inhibit the endogenous EF. The nerve sprouting, the direction of nerve growth, and the rate of epithelial wound healing correlated significantly with the wound-induced EFs (Song et al., 2004).

3.4.4. Electric fields control corneal epithelial wound healing—To achieve better wound healing *in vivo* is one ultimate goal of understanding the electric signaling at corneal wounds. Several attempts have been made with promising results. While experiments from different groups described above provide compelling and consistent evidence for a strong effect of EFs in guiding migration of CECs, *in vivo* experimental results are inconsistent and confusing.

Because the wound EFs are produced by ion transport, it is possible to pharmacologically manipulate the EFs. We used different pharmacological agents to stimulate or inhibit the TCPD and wound EFs. Increasing or decreasing wound EFs sped up or slowed down corneal epithelial wound healing, respectively (Reid et al., 2005; Song et al., 2004; Song et al., 2002; Zhao et al., 2006). For example, using AgNO₃ which enhances epithelial ion transport and increases the TCPD (Klyce and Marshall, 1982), significantly increased the wound electric currents (Fig. 14A). Furosemide inhibits Cl⁻ secretion and reduces the TCPD (Candia et al., 1986; Nagel and Carrasquer, 1989). Furosemide significantly reduced electric currents in rat corneal wounds. Application of these two, and several other similar drugs, consistently increased or decreased wound electric currents. Application of eye drops containing those drugs to rat corneal wounds *in vivo* sped up or slowed down wound healing, consistent with the effects on the wound electric currents (Fig. 14) (Reid et al., 2005; Song et al., 2004; Song et al., 2002; Zhao et al., 2006).

Sta Iglesia and Vanable used microglass electrodes to apply EFs to corneal wounds on bovine eyeball cultures (Sta Iglesia and Vanable, 1998). They found that the strength of the EFs in the vicinity of circular lesions in the bovine cornea influenced the rate of re-epithelialization. They treated the wounds with Na⁺-depleted Hanks' solution, or with the application of dc EFs. Both treatments increased the field strength and sped up wound healing significantly. When they reversed the field's polarity, a similar pattern was also observed. Those results appear to suggest that the presence of the fields, regardless of polarity, is important for corneal wound healing. The authors suggested two possibilities. The first is that the fields promote epithelialization by some means other than guiding cell migration. The second is that populations of cells take part in the wound healing – one migrates toward the cathode, and the other migrates toward the anode.

A more recent study using *Pax6*^{+/-} mice yielded some contradictory results. *Pax6*^{+/-} mice have an expression dose-dependent phenotype (Dora et al., 2008). One study reported that *Pax6*^{+/-} mice tended to heal corneal epithelial wound faster (Ramaesh et al., 2006). Other reports found that *Pax6*^{+/-} mice healed corneal epithelial wounds significantly slower (Dora et al., 2008; Leiper et al., 2006). Surprisingly, in some *Pax6*^{+/-} mice, the endogenous electric currents at corneal wounds flowed in the opposite direction, inward, albeit only at about one-10th of the magnitude. Wound healing in those corneas was not different from that in wild type controls (Kucerova et al., 2011).

The very consistent directional effects of EFs on the migration of CECs *in vitro*, significant effects of direct application of EFs at bovine corneal wounds, and the complexity of the *Pax6*^{+/-} mouse model call for further and more definitive experiments. Sta Iglesia and Vavable argue for the necessity of the presence of EFs, while in *Pax6*^{+/-} mice, significantly reduced EFs or even reversed polarity did not affect healing. Both the directional and non-directional processes, such as activation of multiple signaling pathways will need to be investigated.

3.4.5. Electric fields guide migration of corneal fibroblasts and endothelial cells—An externally applied dc EF guides the movement of cultured rabbit corneal stromal fibroblasts. The cells became spindle shaped, underwent galvanotropism by aligning their long axes perpendicular to the field, and migrated in the anodal (upfield) direction. The field strength for stromal cell migration appeared to be significantly higher (~6 V/cm) (Soong et al., 1990a; Soong et al., 1990b).

Cultured rabbit corneal endothelial cells responded to steady EFs (2–6 V/cm) by elongating their somata perpendicular to the field vector and by migrating toward the anode. During these directional movements, pseudopodia and ruffled membranes formed preferentially on the anodal side of the cells, while they retracted on the cathodal side (Chang et al., 1996).

3.5. Possible clinical intervention

Can the above information on corneal wound EFs contribute to the promotion of corneal wound healing? One possible approach we have explored is to modulate the ion transport, and therefore also the wound EFs at corneal wounds. Using donor human corneas, we have shown that electric current at human corneal wounds can be significantly increased with aminophylline or Cl⁻-free solution (Reid et al., 2010). Because corneal wound currents may regulate cell migration, cell division and nerve sprouting at wounds, pharmacological modulation of the endogenous wound EFs may offer a practical approach for chronic corneal wounds and related conditions. Substituting certain ions in the bathing solution also has significant effects on the electrical properties of the cornea. Eye drops could be developed with defined ionic components to facilitate corneal wound healing. This approach offers bioelectric stimulation without electrodes and can be readily tested in patients.

A significant piece of information lacking in this field is whether chronic or non-healing corneal wounds have defective electric signaling. The *Pax*^{+/-} mouse model did show abnormal electric currents at corneal wounds, but due to the complicated healing response, the relationship between the endogenous EFs and wound healing remain inconclusive. Elucidation of wound EFs at chronic corneal wounds in diabetes and other conditions will significantly strengthen our understanding of this biophysical factor.

Direct application of EFs to the cornea is possible using a contact lens with built-in circuitry. Recent advances in micro-fabrication have produced an integrated amperometric contact lens (Cong and Pan, 2008; Yao et al., 2011). Similar contact lenses with integral circuitry may harvest and deliver electric currents to the cornea.

4. ELECTRIC CURRENTS AT THE LENS

The ocular lens has a unique electric current flow which has been suggested to provide a circulatory mechanism to facilitate metabolisms in this avascular organ. In addition, the electric currents may have other effects on lens cells, such as regulating migration, proliferation, and differentiation. However, the experimental evidence is scarce and we would like to summarize the limited information to suggest this mostly under-explored role.

In order to focus light onto the retina, the lens must be crystal clear (Mathias et al., 1997). The absence of light-scattering elements requires an absence of blood vessels which would normally bring nutrients to cells and remove waste products. The lens consists of two types of cells - long fiber cells make up the bulk of the lens, while a monolayer of epithelial cells covers the anterior surface of the fibers (McAvoy and Chamberlain, 1989). The lens epithelial cells (LECs) proliferate, migrate, orientate, elongate, and differentiate into fiber cells at the equator. New fiber cells are continuously added throughout life, with the interior cells being the oldest, and they must remain viable for the entire lifetime of the organism (Mathias et al., 2007; Mathias et al., 1997; McAvoy and Chamberlain, 1989).

4.1. Electric currents form a unique pattern around the lens

It is now known that the lens is not inert like nail or hair. Instead, it is a living, dynamic organ that carries out a host of biochemical and physiological processes necessary for homeostasis and possibly for regeneration. The lens has a unique pattern of electric currents, which have been proposed to serve as an alternative circulatory system.

The vibrating probe technique revealed these electric currents. Large electric currents ($\sim 26 \mu\text{A}/\text{cm}^2$ in frog and rat lens) flow inward at the anterior and posterior poles, and outward at the equator (Robinson and Patterson, 1982; Wind et al., 1988b) (Fig. 15A). Experimental results on expression patterns of membrane ion pumps and channels across the lens, cell electrophysiological parameters, and measurements of ion currents at the lens, all support such an electrical circulation (Mathias and Rae, 2004; Mathias et al., 1997). The large outward electric currents at the equator, and inward currents at the anterior and posterior poles, have been confirmed by many labs, ranging from $\sim 20\text{--}40 \mu\text{A}/\text{cm}^2$ (Candia and Zamudio, 2002; Mathias et al., 2007; Mathias and Rae, 1985a, b; Mathias et al., 1997; Parmelee, 1986; Parmelee et al., 1985; Patterson, 1988; Robinson and Patterson, 1982; Wind et al., 1988a) (Fig. 15).

The physiological role of the ionic circulation has been hypothesized to be a micro-internal circulatory system. The lens "circulation" is likely accompanied by fluid flow which will bring nutrients and remove waste (Mathias et al., 2007; Mathias et al., 1997). The ionic circulation in this way will compensate for the lack of vasculature (Mathias et al., 2007).

Studies using the vibrating probe and Ussing-like chambers showed that Na^+ and K^+ fluxes are the major contributors to the circulating electric currents (Candia and Zamudio, 2002; Wind et al., 1988b). K^+ efflux plus the Na^+/K^+ -ATPase-generated current comprise the currents at the equatorial surface and an area anterior to it. Passive influx of Na^+ occurs at both the anterior polar region and posterior lens surface. Inhibition of Na^+/K^+ -ATPase with ouabain significantly reduced this ion flow (Wind et al., 1988a). The lens currents can be regulated. Beta-adrenergic agonists increased the equatorial current of the frog lens by a mechanism that is mediated by cAMP (Walsh and Patterson, 1993), while Ca^{2+} level and oxidants in the bathing solution also regulated the lens currents (Walsh and Patterson, 1989, 1991).

Detailed mechanisms of lens current generation and the physiological role of the current as an internal circulatory system have been reviewed in detail (Beebe and Truscott, 2010; Donaldson et al., 2010; Mathias et al., 2007; Mathias et al., 1997). Here we will mainly focus on the electrical properties of the ionic circulation, and its possible effects on migration, proliferation, and homeostasis of lens cells.

4.2. Ion pumps and channels in the lens

LECs and fiber cells have drastically different Na^+/K^+ -ATPase activities. Na^+/K^+ -ATPase activity is found predominantly in the LECs and surface fiber cells, although Western blot

analysis determined that LECs and fiber cells express a similar amount of Na^+/K^+ -ATPase protein (Delamere and Dean, 1993). However, the older, more internal fiber cells have very little Na^+/K^+ -ATPase activity (Baghieri and Garner, 1992; Candia and Alvarez, 2008; Candia et al., 1971; Neville et al., 1978; Palva and Palkama, 1976; Delamere and Tamiya, 2004).

The Na^+/K^+ -ATPase activities in LECs are spatially distributed. The subcellular distribution of Na^+/K^+ -ATPase in LECs is on the basolateral side. Therefore, Na^+/K^+ -ATPase activity drives net current from the apical side to the basal side. The equatorial regions of the lens have the highest activities, while cells in the anterior epithelium have a low activity. Using an Ussing-like chamber, and by local application of the Na^+/K^+ -ATPase inhibitor ouabain, Candia and Zamudio mapped the surface distribution of the Na^+/K^+ -ATPase current (I_p , $\mu\text{A}/\text{cm}^2$). They concluded that I_p was too small to be detected at either pole but reached a maximum of $10 \mu\text{A}/\text{cm}^2$ at the equator (Candia and Zamudio, 2002). Whole-cell patch-clamp studies provided similar results (Gao et al., 2000; Tamiya et al., 2003). The asymmetrical distribution of Na^+/K^+ -ATPase activity in the epithelium and fiber cells (and the equatorial and anterior epithelium) may contribute to the ionic currents and therefore the electrical currents that flow out at the equator and in at the poles.

A high K^+ conductance has been found in LECs (Mathias et al., 2007; Mathias and Rae, 1985a; Mathias et al., 1985). K^+ that is transported into the lens via the Na^+/K^+ -ATPase, therefore mostly leaks out of the same cells (predominantly anterior epithelial cells). The lens expresses at least three major K^+ channel types: inward rectifiers, large-conductance Ca^{2+} -activated channels, and delayed rectifiers (Mathias et al., 1997). Lens cells have a small Na^+ permeability but this leads to a significant total influx of Na^+ due to the large number of fiber cells (Mathias et al., 2007). Low resistance gap junctions connect all fiber cells so when Na^+ enters them it flows from cell to cell through these gap junctions until it reaches the surface epithelial cells and is then transported out of the lens by Na^+/K^+ -ATPase (Fig. 15B). In this model of circulating Na^+ current, extracellular current flows inward from the surface of the lens to the center, while intracellular current flows outward from the center to the surface. As well as Na^+/K^+ -ATPase and fiber cell gap junctions, this model also requires the presence of fiber cell Na^+ channels. Cl^- plays a significant role in lens cell volume regulation but not in lens circulation under normal conditions (Mathias et al., 2007).

4.3. Electric currents in cataract

A link has been found between abnormal elevation of lens Na^+ and the opacification of the lens cortex that occurs in age-related human cataract (Delamere and Tamiya, 2004). The transparent human lens contains some 17 mM Na^+ and 130 mM K^+ (data as mmol/kg lens water) (Patmore and Duncan, 1980). The levels of Na^+ and K^+ in the lens are established by the action of Na^+/K^+ -ATPase which exports Na^+ from the lens and imports K^+ inward to balance ion leakage in the opposite direction. However, in human age-related cortical cataracts and human diabetic cataracts, lens Na^+ is abnormally elevated (often higher than 100 mmol/kg lens water) and the K^+ is abnormally low (often below 80 mmol/kg lens water) (Davies et al., 1984; Duncan and Bushell, 1975). As a result of the osmotic disturbance caused by the ionic changes, water accumulates in lens cells. Eventually this leads to cell lysis and the appearance of fluid droplets that scatter light and impair transparency (Delamere and Tamiya, 2004).

4.4. Electric currents following lens capsule disruption

Wounding the lens by removal of part of the anterior capsule induces significant changes in the electric current pattern of the lens. Normal lenses have a small inward current across the anterior surface ($0.22\text{--}0.38 \mu\text{A}/\text{cm}^2$) but removal of part of the anterior capsule and the lens

nucleus resulted in larger outward currents ($1.8\text{--}2.6\ \mu\text{A}/\text{cm}^2$). A small cut $\sim 1\text{ mm}$ in length significantly reduced outward equatorial currents to 56% of their original size and removing a larger part of the anterior capsule $\sim 1\text{ mm}$ in diameter reduced equatorial currents to almost $0\ \mu\text{A}/\text{cm}^2$ ($0.4 \pm 0.56\ \mu\text{A}/\text{cm}^2$) (Lois et al., 2010; Wang et al., 2003c) (Fig. 16). The ionic components of these currents have not been determined. It is likely that multiple ions originally transported by the equatorial LECs make up the currents.

The large leakage or short circuitry due to disruption of the lens capsule and epithelium is likely to significantly reduce the PD across the lens epithelium. As discussed below (section **4.5.2. Applied Electric Fields Regulate Proliferation of Lens Epithelial Cells**) the presence of an EF inhibited cell proliferation. One may argue that release of this inhibition mechanism may contribute to the aberrant proliferation after cataract surgery, which results in posterior capsule opacification (PCO).

4.5. Responses of lens cells to electric fields

LECs proliferate and migrate in the presence of constant ionic currents and accompanying EFs. The PD across the lens capsule measured $\sim -79\text{ mV}$ and -64 mV in frog and rat lens, respectively (Wind et al., 1988a). The thickness of the epithelium is $\sim 5\ \mu\text{m}$. Theoretically, the voltage drop may reach $50\text{ mV}/5\ \mu\text{m}$, $\sim 10,000\text{ mV}/\text{mm}$ across the epithelium layer in resting status. This may have some effects on lens cell migration and proliferation as discussed in the following sections.

4.5.1. Applied electric fields direct migration and orientation of lens epithelial cells—LECs proliferate at the equator to give rise to new lens fiber cells and migration is initiated at the equator where cells encounter increasing gradients of growth factors from the ciliary body and fibroblast growth factor (FGF) from the vitreous body. The cells first divide and then migrate and initiate differentiation. Migration continues until the fiber cell contacts the anterior tip of a symmetrically located migrating fiber cell and the two cells join, detach from the basement membrane (lens capsule), and form a lens suture (Sue Menko, 2002).

Cultures of bovine and human LECs respond to applied EFs with directional migration and increased migratory rate, elongation, reorientation of the long axis of the cell, and an increase in the flattening size of individual cells (Fig. 17). Primary human LECs and primary bovine LECs, and a transformed human cell line all showed directed cell migration in a voltage-dependent manner, with a response threshold between $100\text{--}150\text{ mV}/\text{mm}$ and $25\text{--}50\text{ mV}/\text{mm}$, respectively. Small sheets of LECs also migrated directionally. Reversing the polarity reversed the direction of migration of all three cell types (Wang et al., 2000) (Fig. 17). Primary human LECs migrated cathodally with a response threshold less than $100\text{ mV}/\text{mm}$. Interestingly, LECs from different regions of the lens (where they will experience different EFs) moved in different directions (Wang et al., 2003b).

The migration of LECs from peripheral and central regions showed an intriguing phenomenon; in an EF of $150\text{--}250\text{ mV}/\text{mm}$, both central and peripheral LECs migrated anodally, however, in a low EF of $50\text{ mV}/\text{mm}$ peripheral LECs migrated cathodally, while central LECs showed no response (Wang et al., 2000; Wang et al., 2003b). This is intriguing as it shows that cells derived from the same line can show different responses to an EF depending on 1) their location relative to the EF vector, 2) slight differences in their anatomical location and morphology, and 3) EF strength, suggesting that a mechanism more complex than a simple directional cue is in play. This happens to have parallels with the *in vivo* situation. LECs which are generated in the peripheral (equatorial) regions may migrate in one of two directions *in vivo*. Either they migrate toward the equator, elongate, and trans-differentiate, to become lens fiber cells, or very few cells may migrate in the opposite

direction, toward the front of the lens, to compensate for ongoing apoptosis of LECs, as the epithelium turns over (Wang et al., 2003b).

LECs migrated at more than double the migration rate (compared to no EF control) in an EF of 50 mV/mm or higher (Wang et al., 2000). LECs also re-orientated to align perpendicular to the applied EFs. In control cultures without an applied EF, the orientation of the long axis of each LEC was distributed randomly. In an applied EF however, LECs re-oriented to lie perpendicular to the field vector (Wang et al., 2000). We sometimes observed that in an EF, LECs expanded and flattened (Fig. 17). These responses mimic the alignment, expansion of cell surface area, and flattening of LECs *in vivo*.

4.5.2. Applied electric fields regulate proliferation of lens epithelial cells—

LECs have a much slower proliferation rate than CECs. Applied EFs significantly inhibited proliferation of LECs over 24–48 hours. The simple presence of an EF of 200 mV/mm significantly reduced the growth rate by 50, 75, and 85% at 24, 48, and 72 hours, respectively, after onset of the field (Wang et al., 2003a). The reduction in cell numbers was due to inhibition of cell cycle progression, not apoptosis. Flow cytometry and Western blotting suggested that this occurred due to the EF affecting levels of important cell cycle control enzymes. The G1 specific cyclin, cyclin E, was suppressed whilst the cyclin-cdk complex inhibitor p27^{kip1} was enhanced, thus restricting G1 to S phase transition (Wang et al., 2003a).

4.6. Possible clinical implications

There are endogenous EFs and electric currents *in vivo*, and applied EFs regulate migration, orientation, and proliferation of LECs. It may not be too far-fetched to propose that the ionic currents in the lens might contribute to controlling the cellular behaviors of LECs *in vivo*. The EF-regulated cell behavior may have significant implications in the normal lens, in the etiology of PCO, and in lens regeneration. The EFs may regulate cell proliferation, migration, elongation, and flattening. These are important cellular behaviors required for LEC maturation into proper fiber cells. Therefore, dis-regulated endogenous EFs may contribute to PCO.

Electrical signaling may regulate behavior of LECs in PCO. The mainstay treatment for cataract is phacoemulsification, which involves removal of most of the anterior lens capsule, anterior lens epithelium, disaggregated fibers, and surrounding cortex, leaving only the posterior capsule, lens bow (germinal area), and anterior capsule-lens epithelium periphery in place. An artificial lens is implanted as common belief dictates that human lens regeneration does not occur (Lois et al., 2010). However, this treatment is associated with a high incidence of PCO. PCO occurs due to inappropriate proliferation and migration of LECs, which normally line the residual anterior and equatorial areas of the lens, onto the intact posterior capsule, where they compromise vision (Apple et al., 1992). Phacoemulsification is expected to significantly alter the endogenous current (Fig. 16). The normal electric control of migration and proliferation may become aberrant, and contribute to the PCO. It is not known whether the EFs regulate differentiation of LECs. Future research in this direction may provide some insights.

The electrical signal may be a critical component in lens regeneration. Following cataract extraction a mini lens or “Soemmerring’s ring” develops in the majority of cases (Apple et al., 1992). The Soemmerring’s ring is formed by the gradual build up of lens-like material at the equator where the anterior lens capsule and its underlying epithelium, lens bow, and posterior lens capsule are present (Lois et al., 2003). We hypothesized an important role for the anterior lens capsule-anterior lens epithelium (the parts removed during cataract

surgery), and proposed that preservation of the whole capsule with its attached anterior lens epithelium may allow for complete regeneration of the lens (Lois et al., 2010).

Cocteau and d'Etoille were the first to observe regeneration of mammalian lens material when the lens capsule and lens epithelium were left intact (Cocteau, 1824). Similar observations were made in rabbits (Gwon et al., 1989) and in mice (Call et al., 2004). Removal of the anterior lens capsule-anterior lens epithelium disrupts the inward currents at the anterior lens surface (Wang et al., 2005) and these electrical signals control the cellular events that lead to lens formation, i.e. elongation, migration, division, and cell cycle progression (Wang et al., 2003b). We studied the electrical events occurring following a modified method of lens extraction which left the anterior lens capsule-anterior lens epithelium in place to determine whether anterior capsule-anterior lens epithelium removal disrupts the endogenous electrical signals and whether the absence of these leads to de-regulated LEC migration and proliferation, and PCO (Lois et al., 2010).

When the anterior capsule was preserved, lens regeneration occurred. Despite the initial changes in EFs, by eight weeks (coinciding with the full development of the regenerated lens) inward currents had returned and resembled those seen in the controls, while outward currents remained at the site of capsulotomy (Lois et al., 2010). The interaction between biochemical signals is critical and should not be ignored in this model. Maintenance of the lens capsule structure to some extent by not removing the anterior flap may generate a biochemical/biophysical interaction which is optimal for lens regeneration. Research has demonstrated the importance of FGF in regulation of lens cell proliferation, differentiation, and migration. The interaction of those biochemical cues and electrical signaling could be a fertile research field.

5. ELECTRIC POTENTIAL IN THE RETINA L PIGMENT EPITHELIUM

The retina has many types of electric activities. Our focus here will be on the steady TEP generated by the RPE and their possible patho-physiological significance. The actual effects *in vivo* are likely to be very sophisticated. The RPE is a monolayer of cuboidal epithelial cells which lies in close association with the rod and cone photoreceptors. The RPE forms the outer blood-retinal barrier and regulates the environment of the outer retina. The RPE functions as three types of cell: epithelium, macrophage, and glia (Steinberg, 1985). The basolateral membrane faces Bruch's membrane which separates the RPE from the fenestrated endothelium of the choriocapillaris (Strauss, 2005).

5.1. Ion transport at the retinal pigment epithelium

The RPE transports ions, water, and metabolites between the subretinal space and the blood and this is important in the prevention of edema (Hamann, 2002; Marmor, 1999). The RPE also transports nutrients such as glucose, retinol, and fatty acids from the blood to the photoreceptors (Baehr et al., 2003; Besch et al., 2003; Thompson and Gal, 2003). Another important function of the RPE is the maintenance of photoreceptor excitability, through the voltage-dependent ion conductance of the apical membrane which enables stabilization of the ionic composition of the subretinal space (Steinberg et al., 1983). Please refer to other comprehensive reviews on ion channels in RPE (Levin and Verkman, 2006; Mergler and Pleyer, 2007; Sparrow et al., 2010; Wimmers et al., 2007).

The RPE has a very different Na^+/K^+ -ATPase distribution from other epithelial cells, including CECs and LECs (Gundersen et al., 1991; Rizzolo, 1999). The RPE contains high levels of Na^+/K^+ -ATPase on the apical side rather than on the basolateral side as in other epithelia (Miller et al., 1978). Expression levels of cadherin and rearrangement of the

cytoskeleton in RPE cells appear to be responsible for the assembly of Na^+/K^+ -ATPase with opposite polarity to other epithelial cells (Burke et al., 2000).

The RPE expresses voltage- and ligand-gated K^+ , Cl^- , and Ca^{2+} -conducting channels. K^+ and Cl^- channels are involved in transepithelial ion transport and volume regulation. Voltage-gated Ca^{2+} channels act as regulators of secretory activity, and ligand-gated cation channels contribute to RPE function by providing driving forces for ion transport, or by influencing intracellular Ca^{2+} homeostasis. The apical membrane contains a relatively large K^+ conductance (Quinn and Miller, 1992) and changes in intracellular K^+ in the RPE alters the current produced by Na^+/K^+ -ATPase on the apical membrane, consistent with the finding that intracellular K^+ is a competitive inhibitor of Na^+/K^+ -ATPase (Oakley et al., 1978).

5.2. Transepithelial potential in the retinal pigment epithelium

Similar to the TEP in the corneal and lens epithelia, a TEP is present in the RPE (Griff et al., 1985; Hughes et al., 1988; Maminishkis et al., 2006; Miller et al., 1978; Oakley et al., 1978; Quinn et al., 2001; Scharschmidt et al., 1988). In contrast to the corneal epithelium and lens epithelium in which the TEPs are positive at the basement membrane side relative to the apical side, in RPE the basal potential is negative relative to the apical potential. In adult human RPE the apical membrane has been shown to have a mean resting cell membrane potential of -49 ± 7 mV, a TEP of -1.9 ± 0.6 mV, and a resistance of $-79 \pm 48 \Omega\text{-cm}^2$ (Quinn and Miller, 1992). The magnitude of the TEP compared to that in the corneal and lens epithelia is much smaller. The field strength across the RPE therefore, is likely to be 1.9 mV over 5 μm , ~ 400 mV/mm. This is one-50th of that in lens epithelium and smaller than that in corneal epithelium.

The TEP of the RPE is regulated by Na^+/K^+ -ATPase inhibition, K^+ concentration, HCO_3^- and taurine, which is necessary for normal vision. Acute exposure of the apical, but not basal membrane of the RPE to taurine reduced the normally apically positive TEP in bullfrog RPE. With continued taurine exposure, the initial reduction in TEP was sometimes followed by a recovery of the TEP toward baseline. This recovery was abolished by strophanthidin or ouabain, indicating an important role of the apical Na^+/K^+ -ATPase. Increasing extracellular K^+ hyperpolarized the apical membrane and increased the TEP. Taurine reversibly doubled these responses, but did not change total epithelial resistance or the ratio of apical-to-basal membrane resistance, and ouabain abolished these responses. Collectively, these findings indicate the presence of an electrogenic $\text{Na}^+/\text{taurine}$ co-transport mechanism in the apical membrane of the bullfrog RPE (Gallemore et al., 1997; Hillenkamp et al., 2006; McBee et al., 2001). They also provide direct evidence that taurine produces a Na^+ -dependent increase in electrogenic pumping by the apical Na^+/K^+ -ATPase (Scharschmidt et al., 1988). Collectively, these ion channels determine the physiology of the RPE (Wimmers et al., 2007; Wollmann et al., 2006).

We have measured the resting electric currents at bovine retinal epithelium *in vitro*. Consistent with the distribution of Na^+/K^+ -ATPase, the intact RPE presents small outward currents (flow of positive charge from basal to apical). Upon wounding which disrupts the RPE barrier, a large inward (apical to basal) current was detected (Reid et al., unpublished results).

5.3. Retinal cells respond to applied electric fields

Much higher EFs (600–1000 mV/mm) are required to guide migration of human RPE (hRPE) cells, as compared to most cell types studied (Han et al., 2009b; Sulik et al., 1992). Cell edges facing the anode retracted their extensions, while cathodal edges and longitudinal

ends developed lamellipodia and ruffled membranes (Sulik et al., 1992). EF exposure also induced a cathodal accumulation of F-actin and $\beta 1$ integrin in hRPE cells (Han et al., 2009b). The directional migration was directed toward the cathode.

We investigated the responses of hRPE cells to applied EFs of various strengths, under different conditions. Primary hRPE cells migrated toward the anode at all EF strengths of 50 mV/mm or stronger. ARPE19 cells showed significant anodal migration at 300 mV/mm. Sheets of hRPE cells also migrate in an EF. Cell clusters appeared to migrate significantly faster than single cells (Gamboa et al., 2010) (Fig. 18).

At all EF strengths of 100 mV/mm or stronger, primary hRPE cells migrated faster than controls. Translocation rate was voltage-dependent, with faster rates at higher field strengths. Translocation rate was significantly reduced in the absence of serum or gelatin and was significantly greater in elongated cells as compared to round or amorphous cells (Gamboa et al., 2010). In “early” confluence primary hRPE cell monolayers, control wounds showed centripetal migration into the centre of the wound with no significant difference in rate between cells at the anodal or cathodal sides. Exposure to an EF of 300 mV/mm induced anodal migration at both sides of the wound and when exposed to an EF of 600 mV/mm, the hRPE cells showed elongated spindle shapes and aligned perpendicularly to the EF (Gamboa et al., 2010).

5.4. Physiological significance

Normal TEP appears to be essential for healthy RPE, but in addition to investigations of TEP in ion transportation, no research has been directed to test the role of TEP in various RPE functions. It is worthwhile to consider the TEP in RPE function and integrity, as malfunction or death of the RPE underlies many important diseases including age-related macular degeneration and diabetic retinopathy.

Disorders that result from malfunction of ion channels, either through gain of function, loss of function, or by dominant negative effects leading to a reduced number of channels in the cell membrane, are termed channelopathies (Wimmers et al., 2007). Degenerative diseases of the retina have been found to involve mainly Cl^- channel functions. Inactivation of the ClC-2 gene in the mouse results in a phenotype very similar to that of retinitis pigmentosa in man and the RPE in these mice shows no TEP (Bosl et al., 2001). This suggests the importance of transepithelial transport in the maintenance of photoreceptor health and function (Wimmers et al., 2007).

Mutations in VMD2 (the gene coding for bestrophin-1) cause an inherited form of macular degeneration with juvenile onset, known as Best’s vitelliform macular dystrophy (Cross and Bard, 1974; Godel et al., 1986; Marquardt et al., 1998; Petrukhin et al., 1998; Weingeist et al., 1982). Best’s disease is characterized by a reduction of the light-rise or light-peak in the patient’s electro-oculogram (Cross and Bard, 1974). The light-rise results from an increase in the basolateral membrane Cl^- conductance. Mutations in the VMD2 gene lead to a reduction in the basolateral membrane Cl^- conductance (Wimmers et al., 2007). The VMD2 gene product, bestrophin-1, appears to function as a Ca^{2+} -dependent Cl^- channel (Sun et al., 2002). Of 15 different mutant bestrophins investigated to date, all lead to a loss in Cl^- channel function with a dominant negative effect on the wild-type allele.

Exploiting bioelectric phenomena as a diagnostic and therapeutic tool is possible. Corneal epithelial wound healing can be stimulated with EFs. Healing of denuded areas in the RPE and restoring its function is desirable. Aberrant endogenous EFs could be a potential reason for defective healing. Applied EFs could be used for this purpose, stimulating and

accelerating proliferation, directional migration, and subsequently repopulation of RPE cells in RPE-denuded areas of Bruch's membrane.

6. SIGNALING MECHANISMS OF ELECTRIC FIELD-DIRECTED CELL BEHAVIORS

The most obvious effects of EFs on cells are the directional (vectorial) responses – cells migrate, orientate, elongate, divide, and spread in a directional manner. This has been the focus of many studies. There is likely another aspect – non-vectorial effects. These include activation of important intracellular signaling pathways that may affect diverse cellular behaviors, such as motility, proliferation, and apoptosis. The vectorial and non-vectorial effects are similar to the chemotactic and chemokinetic effects of growth factors and chemokines. Because of limited understanding of how the weak extracellular EFs induce intracellular responses, these effects are much less recognized and studied. Elucidating the mechanisms of EF transmission will shed more light on both the vectorial effects (electrotactic) and non-vectorial effects (electrokinetic). The following will focus on mechanisms that may underlie EF-induced directional cell migration.

6.1. Electric fields redistribute membrane receptors

6.1.1. Asymmetric distribution of growth factor receptors and membrane lipid

—An EF induces epidermal growth factor receptors (EGFRs) and membrane lipids to colocalize and redistribute cathodally in bovine CECs. Electrotaxis of CECs requires serum growth factors, and migration also depends on the substrate. Living and fixed CECs which had been exposed to an EF showed asymmetric distribution of EGFRs as early as 10 min after EF onset. Membrane lipid stained with DiD showed that redistribution of EGFR and DiD was virtually identical, with greater DiD and EGFR staining cathodally than anodally (Zhao et al., 1999a; Zhao et al., 2002). EGFR signaling is also required for galvanotaxis of keratinocyte and breast cancer cells (Fang et al., 1999). EF-enhanced directional migration correlates well with the expression level of EGFR/ErbB1 (Pu et al., 2007).

6.1.2. Asymmetric distribution of integrin receptors—In hRPE cells migrating toward the cathode, $\beta 1$ integrin distributed together with F-actin on the cathodal side (Han et al., 2009a). Integrins $\alpha 5$, and $\alpha 5\beta 1$ redistribute and aggregate differentially in fibroblasts cells that migrate directionally in an EF (Brown and Loew, 1994). Knockout of $\beta 4$ abrogated the electrotactic response of keratinocytes, which could be recovered by transfection and expression of $\beta 4$ integrin. EGF addition also recovered the electrotactic response of $\beta 4$ integrin null cells (Pullar et al., 2006). It is therefore likely that multiple signaling mechanisms underlie EF-induced cell migration.

6.1.3. Movement of membrane molecules - electrophoresis and electro-osmosis—Cell membranes are fluid in structure and permit movement of proteins and lipids in the plane of the membrane. The majority of proteins and lipids externally protruding from the plasma membrane are negatively charged, whereas lecithin and sphingomyelin have a positive charge, while cholesterol and certain glycolipids are neutral (Jaffe, 1977). Indeed, the surfaces of most cells bear a net negative charge (McLaughlin and Poo, 1981). Jaffe suggested that application of an EF parallel to the surface of a cell should redistribute charged macromolecules that are free to move laterally in the plasma membrane through the process of electrophoresis (Jaffe, 1977).

Con A receptors (Poo and Robinson, 1977), Ach receptors (Orida and Poo, 1978), and Fcε receptors (McCloskey et al., 1984) have been shown to be redistributed to the cathodal side by EFs, as have most other receptors studied, which is surprising considering that most

glycoproteins have a net negative charge at physiological pH (Robinson, 1985). These observations led to the suggestion of electro-osmosis - the imposition of an EF parallel to the surface of a cell should produce an electro-osmotic flow of fluid near the surface of the cell. The overall negative charge on the cell surface attracts mobile counterions (e.g. Na^+) to accumulate in an “aqueous diffuse double layer” adjacent to the surface of the cell. The movement of counterions induces a movement of fluid parallel to the surface of the cell. This electro-osmotic flow exerts a hydrodynamic force on the mobile macromolecules, ultimately causing even negatively charged macromolecules to accumulate at the negative side of the cell (McLaughlin and Poo, 1981). In support of this theory, pretreatment of embryonic muscle cells with neuraminidase, a treatment that removes negatively charged sialic acid residues, or with diI lipid which adds positive charges to the surface of the cell, both resulted in anodal accumulation of Con A receptors (McLaughlin and Poo, 1981).

The role of electrical charge on redistribution of cell membrane molecules was supported by a recent study using mammalian cell lines (3T3, HeLa, and CHO cells). Neuraminidase treatment removes sialic acids on the cell membrane, which reduces total cell surface charge, while cationic avidin treatment actually reverses the directional polarization of sialic acids. However, neither treatment altered directionality or speed of motility in a consistent manner. Polarization of the bulk of charged cell surface proteins may not be necessary nor sufficient to cause motility in an EF (Finkelstein et al., 2007).

6.2. Electric fields activate intracellular signaling pathways

Application of an EF to cells cultured in serum-free medium activates multiple intracellular signaling pathways, including phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and Src. Significantly, the activation is polarized toward the direction of cell migration (Zhao et al., 2006).

6.2.1. Electric fields induced asymmetric activation of PI3K/PTEN signaling—

PI3Ks are a family of important intracellular signaling molecules involved in many cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. PI3Ks phosphorylate the 3 position hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns). In many types of cells, PI3K signaling acts as a compass mechanism in chemotaxis (Firtel and Chung, 2000; Haugh et al., 2000; Iijima and Devreotes, 2002; Servant et al., 2000; Xu et al., 2003). Application of an EF to keratinocytes or neutrophils activated PI3K signaling pathways as indicated by increased phosphorylation of Akt (Fig. 19).

In neutrophil-like cells (DMSO-treated HL60 cells), we used a green fluorescence protein (PHAkt-GFP) to show polarized activation of PI3K pathways. When PI3K is activated, PtdIns(3,4,5) P_3 (PIP_3) is produced. PHAkt-GFP binds to PIP_3 revealing the location of PI3K activation. We found that PHAkt-GFP moved to the plasma membrane and was polarized to the leading edge of cells migrating in an EF. When the field polarity was reversed the PI3K activation was polarized to the new cathodal side, and directional migration of the cells toward the new cathode ensued (Fig. 20) (Zhao et al., 2006). Genetic experiments support an important role for PI3K in electrotaxis. When the gene *P110 γ* that codes for one catalytic subunit of a PI3K isoform (PI3K γ) was disrupted, activation of Akt, Src, P38, and ERK1/2 were all impaired in response to an EF. Electrotactic migration of keratinocytes and neutrophils was inhibited by the loss of PI3K γ (Zhao et al., 2006). Dermal fibroblasts from *p110 γ* (the PI3K gene) null mice showed significantly reduced directional migration (Guo et al., 2010).

6.2.2. Polarized activation of MAPK signaling—One important downstream signaling element of the EGFR, activated by EFs, is the MAPK - ERK1/2. Applied EFs activate ERK1/2 in CECs and LECs. Importantly, the activation appeared to be asymmetric. Marked cathodal asymmetry of dp-ERK was evident after 1.5 hours in an EF of 150 mV/mm, especially in the cathode-facing lamellipodia. In control cells ERK1/2 activation was largely perinuclear (Zhao et al., 2002).

In LECs it was shown that EF-induced activation of ERK1/2 required serum. ERK1/2 activation was enhanced in peripheral LECs at both 50 and 200 mV/mm, which stimulated migration in different directions. However, in central LECs which showed directional migration only at higher EF strengths, ERK1/2 activation was enhanced only at 200 mV/mm. U0126 (a MAPK inhibitor) prevented activation of ERK1/2 in LECs and inhibited EF-directed migration (Wang et al., 2003c).

U0126 inhibited wound healing in corneal and lens epithelial monolayer wounds with or without exposure to an EF. Wounding and application of an EF enhanced activation of ERK1/2 independently and U016 completely inhibited these activations. Immunocytochemical staining showed an asymmetric activation of ERK1/2 in EF-exposed wounds, with much weaker fluorescence in cathode-facing wounds (the LECs migrated anodally) (Wang et al., 2003c; Zhao et al., 1999a; Zhao et al., 2002).

Src tyrosine kinase activity is required for polarization in fibroblasts, and acts via the small GTPases Rac1 and Cdc42 (Timpson et al., 2001). The Src tyrosine kinase inhibitor, PP2, completely abolished Golgi polarization and changes in morphology. In an EF of 300 mV/mm, Src and Akt (the downstream effector of PI3K) were activated preferentially at the cells cathode-facing side (Pu and Zhao, 2005). In EF-exposed CECs and keratinocytes, Src activation was polarized in the direction of migration (Kucerova et al., 2010; Zhao et al., 2006).

6.2.3. Ca²⁺ signaling and galvanotaxis—While the majority of research found a significant role for Ca²⁺ in electrotaxis, some reported a non-essential role for Ca²⁺. Diverse stimuli increase intracellular Ca²⁺ which is associated with cytoskeleton remodeling. Myosin light chain kinase functions depend on Ca²⁺/calmodulin. Transient, graded increases in intracellular free Ca²⁺ are often seen in migrating cells and they correlate with the direction of movement (Cooper and Schliwa, 1985; Hong et al., 2000; Lee et al., 1999; Witke et al., 1993; Yamamoto et al., 1982; Zheng, 2000). Increasing Ca²⁺ levels have also been shown to be involved in the detachment of the uropod (Lee et al., 1999). Ca²⁺ release induces biased motility of growth cones (Hong et al., 2000; Zheng, 2000). Actin-binding proteins such as α -actinin and severin are sensitive to intracellular free Ca²⁺ (Witke et al., 1993; Yamamoto et al., 1982).

Ca²⁺ signaling therefore has long been proposed to play a role in the electrotactic response. Ca²⁺ has been shown to be required for electrotaxis in neural crest cells, mouse embryo fibroblasts, and fish and human keratocytes (Cooper and Schliwa, 1985; Onuma and Hui, 1985, 1988; Trollinger et al., 2002). Blockers of voltage-gated Ca²⁺ channels have been shown to inhibit galvanotaxis (Trollinger et al., 2002). However, except at very high voltages, physiological EFs which induce evident cell migration have not been shown to increase intracellular [Ca²⁺] (Brust-Mascher and Webb, 1998). In contrast, two strains of mouse fibroblasts exhibit electrotaxis independent of Ca²⁺ signaling (Brown and Loew, 1994). Several laboratories have tried to image the intracellular Ca²⁺ but no convincing data has been obtained. With faster imaging techniques, it may be possible to image in a more definitive way whether Ca²⁺ fluctuation is induced by physiological EFs.

6.3. Polarization of organelles

Polarized distribution of organelles (Golgi apparatus, microtubule organization center) contribute to directional cell migration. Asymmetry of the Golgi apparatus plays a role in the anterograde supply of membrane components to the cell leading edge for membrane protrusion (Bershadsky and Futerman, 1994; Nabi, 1999; Prigozhina and Waterman-Storer, 2004; Ridley et al., 2003). In an EF, Golgi apparatus and actin polarized cathodally in CHO cells (Pu and Zhao, 2005).

Golgi polarization correlates linearly with electrotaxis. Brefeldin A which induces dispersal of Golgi completely abolished EF-induced Golgi polarization and significantly inhibited EF-directed migration (Cao et al., 2011; Pu and Zhao, 2005). Detailed time-lapse imaging however, demonstrated a novel insight. While Golgi polarization and directional cell migration depend on each other, initiation of directional migration does not require Golgi polarization. Golgi then polarizes and facilitates and augments the directional migration (Pu and Zhao, 2005).

6.4. Shared and different mechanisms underlying galvanotaxis and chemotaxis

The last few sections suggest that chemotaxis and electrotaxis share many common molecules and features. There are however some differences between electrotaxis and chemotaxis.

In chemotaxis of *Dictyostelium* cells, G protein-coupled receptors (GPCRs) bind chemoattractants and regulate or bias pseudopod formation at the leading edge (Chung et al., 2001; Parent and Devreotes, 1999). Electrotaxis of *Dictyostelium* cells however, appears to be independent of GPCRs, while GPCR signaling is essential for chemotaxis of *Dictyostelium* cells. The G protein $G\alpha_2$ subunit and $G\beta\gamma$ complex couple together with receptors to transduce signals from chemoattractants. The $G\beta$ subunit is essential for chemotaxis to all chemoattractants (Jin et al., 1998; Wu et al., 1995). However, similar to $cAR1^-/cAR3^-$ and $G\alpha_2^-$ cells, $G\beta^-$ cells maintained significant directional migration in an EF although it was markedly suppressed (Zhao et al., 2002).

Membrane potential may play a different role in electrotaxis than chemotaxis. One of the immediate changes induced by extracellular EFs is a change in membrane potential. The anodal side of the plasma membrane becomes hyperpolarized (more polarized), while the cathodal side of the plasma membrane becomes depolarized (less polarized). It is difficult to precisely control the membrane potential in mammalian cells. We recently exploited the tolerance of *Dictyostelium* cells to changes in membrane potentials. We drastically changed the membrane potential by changing $[K^+]$, or pH in the bathing buffer. Electrotaxis was significantly inhibited while chemotaxis remained largely unchanged (Van Duijn et al., 1990; Gao et al., unpublished results).

It has been suggested that cells align perpendicular to an EF in order to minimize the voltage drop across themselves (Robinson, 1985). However, not all cell types align perpendicularly in an EF. CHO cells and differentiated *Dictyostelium* elongate parallel to an EF (Pu and Zhao, 2005; Zhao et al., 2004). The role of EF-induced changes in membrane potential in cellular responses is yet to be elucidated.

6.5. The sensor(s) remains mysterious

Although we have discovered some of the intracellular signaling pathways that are activated following EF exposure, and that alterations are made to membrane electric potential, membrane lipid distribution, and membrane receptor distribution, as well as intracellular distribution of many organelles such as the actin cytoskeleton and the Golgi apparatus, and

that certain genes (i.e. PTEN, Pax6) play a role, exactly how cells sense the EF and transduce it into an intracellular signal remains one of the most important and elusive questions. We expect significant efforts in the coming years on elucidation of the “sensors”/sensing mechanisms for small EFs.

7. CONCLUDING REMARKS

EFs resulting from ionic fluxes in ocular tissues appear to be an important regulator of many important aspects of ocular cell behavior. An important role for these EFs is likely to be in wound healing where naturally occurring wound EFs regulate cell migration, proliferation, and alignment of the cells. This biophysical signal may work together with other better-understood cues such as growth factors, other signals elicited by wounding, and the extracellular matrix, to modulate wound healing and regeneration. Further understanding of this aspect of electrically-controlled ocular cell behavior is likely to offer new insights into ocular tissue homeostasis and wound healing. It may also lead to development of tissue engineering techniques to repair damaged or diseased ocular tissues.

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REFERENCES

- Akaike N, Kiyohara T. Metabolic component in the epithelial intracellular potential of rabbit cornea. *Pflugers Arch.* 1977; 369:33–37. [PubMed: 560006]
- Al-Nakkash L, Iserovich P, Coca-Prados M, Yang H, Reinach PS. Functional and molecular characterization of a volume-activated chloride channel in rabbit corneal epithelial cells. *J Membr Biol.* 2004; 201:41–49. [PubMed: 15635811]
- Al-Nakkash L, Reinach PS. Activation of a CFTR-mediated chloride current in a rabbit corneal epithelial cell line. *Invest Ophthalmol Vis Sci.* 2001; 42:2364–2370. [PubMed: 11527951]
- Apple DJ, Solomon KD, Tetz MR, Assia EI, Holland EY, Legler UF, Tsai JC, Castaneda VE, Hoggatt JP, Kostick AM. Posterior capsule opacification. *Surv Ophthalmol.* 1992; 37:73–116. [PubMed: 1455302]
- Baehr W, Wu SM, Bird AC, Palczewski K. The retinoid cycle and retina disease. *Vision Res.* 2003; 43:2957–2958. [PubMed: 14611932]
- Baghieri S, Garner MH. Na,K-ATPase and phospholipid degradation in bovine and human lenses. *Curr Eye Res.* 1992; 11:459–467. [PubMed: 1318822]
- Barfort P, Maurice D. Electrical potential and fluid transport across the corneal endothelium. *Exp Eye Res.* 1974; 19:11–19. [PubMed: 4413199]
- Beebe DC, Truscott RJ. Counterpoint: The lens fluid circulation model—a critical appraisal. *Invest Ophthalmol Vis Sci.* 2010; 51:2306–2310. discussion 2310-2302. [PubMed: 20435605]
- Bershadsky AD, Futerman AH. Disruption of the Golgi apparatus by brefeldin A blocks cell polarization and inhibits directed cell migration. *Proc Natl Acad Sci U S A.* 1994; 91:5686–5689. [PubMed: 8202549]
- Besch D, Jagle H, Scholl HP, Seeliger MW, Zrenner E. Inherited multifocal RPE-diseases: mechanisms for local dysfunction in global retinoid cycle gene defects. *Vision Res.* 2003; 43:3095–3108. [PubMed: 14611947]
- Bildin VN, Iserovich P, Fischbarg J, Reinach PS. Differential expression of Na:K:2Cl cotransporter, glucose transporter 1, and aquaporin 1 in freshly isolated and cultured bovine corneal tissues. *Experimental biology and medicine.* 2001a; 226:919–926. [PubMed: 11682698]

- Bildin VN, Iserovich P, Fischbarg J, Reinach PS. Differential expression of Na:K:2Cl cotransporter, glucose transporter 1, and aquaporin 1 in freshly isolated and cultured bovine corneal tissues. *Exp Biol Med* (Maywood). 2001b; 226:919–926. [PubMed: 11682698]
- Block ER, Tolino MA, Lozano JS, Lathrop KL, Sullenberger RS, Mazie AR, Klarlund JK. Free edges in epithelial cell sheets stimulate epidermal growth factor receptor signaling. *Molecular biology of the cell*. 2010; 21:2172–2181. [PubMed: 20462956]
- Bonanno JA. Identity and regulation of ion transport mechanisms in the corneal endothelium. *Prog Retin Eye Res*. 2003; 22:69–94. [PubMed: 12597924]
- Bonanno JA. Molecular mechanisms underlying the corneal endothelial pump. *Experimental eye research*. 2011
- Bosl MR, Stein V, Hubner C, Zdebik AA, Jordt SE, Mukhopadhyay AK, Davidoff MS, Holstein AF, Jentsch TJ. Male germ cells and photoreceptors, both dependent on close cell-cell interactions, degenerate upon CIC-2 Cl(−) channel disruption. *EMBO J*. 2001; 20:1289–1299. [PubMed: 11250895]
- Brown MJ, Loew LM. Electric field-directed fibroblast locomotion involves cell surface molecular reorganization and is calcium independent. *The Journal of cell biology*. 1994; 127:117–128. [PubMed: 7929557]
- Brust-Mascher I, Webb WW. Calcium waves induced by large voltage pulses in fish keratocytes. *Biophys J*. 1998; 75:1669–1678. [PubMed: 9746509]
- Burke JM, Cao F, Irving PE. High levels of E-/P-cadherin: correlation with decreased apical polarity of Na/K ATPase in bovine RPE cells in situ. *Invest Ophthalmol Vis Sci*. 2000; 41:1945–1952. [PubMed: 10845621]
- Call MK, Grogg MW, Del Rio-Tsonis K, Tsonis PA. Lens regeneration in mice: implications in cataracts. *Exp Eye Res*. 2004; 78:297–299. [PubMed: 14729361]
- Candia OA. Electrolyte and fluid transport across corneal, conjunctival and lens epithelia. *Exp Eye Res*. 2004; 78:527–535. [PubMed: 15106931]
- Candia OA, Alvarez LJ. Fluid transport phenomena in ocular epithelia. *Prog Retin Eye Res*. 2008; 27:197–212. [PubMed: 18289913]
- Candia OA, Askew WA. Active sodium transport in the isolated bullfrog cornea. *Biochim Biophys Acta*. 1968; 163:262–265. [PubMed: 5686282]
- Candia OA, Bentley PJ, Mills CD. Short-circuit current and active Na transport across isolated lens of the toad. *Am J Physiol*. 1971; 220:558–564. [PubMed: 5540912]
- Candia OA, Grillone LR, Chu TC. Forskolin effects on frog and rabbit corneal epithelium ion transport. *The American journal of physiology*. 1986; 251:C448–C454. [PubMed: 3092677]
- Candia OA, Zadunaisky JA, Bajandas F. Electrical potential profile of the isolated frog cornea. *Invest Ophthalmol*. 1968; 7:405–415. [PubMed: 5663551]
- Candia OA, Zamudio AC. Regional distribution of the Na(+) and K(+) currents around the crystalline lens of rabbit. *Am J Physiol Cell Physiol*. 2002; 282:C252–C262. [PubMed: 11788336]
- Cao L, Pu J, Zhao M. GSK-3beta is essential for physiological electric field-directed Golgi polarization and optimal electrotaxis. *Cell Mol Life Sci*. 2011
- Cao L, Zhang XD, Liu X, Chen TY, Zhao M. Chloride channels and transporters in human corneal epithelium. *Exp Eye Res*. 2010; 90:771–779. [PubMed: 20346358]
- Chang PC, Sulik GI, Soong HK, Parkinson WC. Galvanotropic and galvanotactic responses of corneal endothelial cells. *J Formos Med Assoc*. 1996; 95:623–627. [PubMed: 8870433]
- Chiang M, Robinson KR, Venable JW Jr. Electrical fields in the vicinity of epithelial wounds in the isolated bovine eye. *Exp Eye Res*. 1992; 54:999–1003. [PubMed: 1521590]
- Chung CY, Funamoto S, Firtel RA. Signaling pathways controlling cell polarity and chemotaxis. *Trends Biochem Sci*. 2001; 26:557–566. [PubMed: 11551793]
- Cocteau, dE. Reproduction du cristalline. *J. Physiol. Exp. Pathol*. 1824; 1:30–44.
- Cong HL, Pan TR. Photopatternable conductive PDMS materials for microfabrication. *Adv Funct Mater*. 2008; 18:1912–1921.
- Connon C, Kawasaki S, Liles M, Koizumi N, Yamasaki K, Nakamura T, Quantock A, Kinoshita S. Gene expression and immunolocalisation of a calcium-activated chloride channel during the

- stratification of cultivated and developing corneal epithelium. *Cell and Tissue Research*. 2006; 323:177–182. [PubMed: 16158324]
- Cooper MS, Keller RE. Perpendicular orientation and directional migration of amphibian neural crest cells in dc electrical fields. *Proc Natl Acad Sci U S A*. 1984; 81:160–164. [PubMed: 6582473]
- Cooper MS, Schliwa M. Electrical and ionic controls of tissue cell locomotion in DC electric fields. *J Neurosci Res*. 1985; 13:223–244. [PubMed: 3973934]
- Cross HE, Bard L. Electro-oculography in Best's macular dystrophy. *Am J Ophthalmol*. 1974; 77:46–50. [PubMed: 4824173]
- Danjo Y, Gipson IK. Actin 'purse string' filaments are anchored by E-cadherin-mediated adherens junctions at the leading edge of the epithelial wound, providing coordinated cell movement. *J Cell Sci*. 1998; 111(Pt 22):3323–3332. [PubMed: 9788874]
- Davies N, Akhtar S, Turner HC, Candia OA, To CH, Guggenheim JA. Chloride channel gene expression in the rabbit cornea. *Molecular vision*. 2004; 10:1028–1037. [PubMed: 15635293]
- Davies PD, Duncan G, Pynsent PB, Arber DL, Lucas VA. Aqueous humour glucose concentration in cataract patients and its effect on the lens. *Exp Eye Res*. 1984; 39:605–609. [PubMed: 6519197]
- Delamere NA, Dean WL. Distribution of lens sodium-potassium-adenosine triphosphatase. *Invest Ophthalmol Vis Sci*. 1993; 34:2159–2163. [PubMed: 8389341]
- Delamere NA, Tamiya S. Expression, regulation and function of Na,K-ATPase in the lens. *Prog Retin Eye Res*. 2004; 23:593–615. [PubMed: 15388076]
- Dikstein S, Maurice DM. The active control of corneal hydration. *Isr J Med Sci*. 1972a; 8:1523–1528. [PubMed: 4647816]
- Dikstein S, Maurice DM. The metabolic basis to the fluid pump in the cornea. *J Physiol*. 1972b; 221:29–41. [PubMed: 4259586]
- Donaldson PJ, Musil LS, Mathias RT. Point: A critical appraisal of the lens circulation model--an experimental paradigm for understanding the maintenance of lens transparency? *Invest Ophthalmol Vis Sci*. 2010; 51:2303–2306. [PubMed: 20435604]
- Donn A, Maurice DM, Mills NL. Studies on the living cornea in vitro. II. The active transport of sodium across the epithelium. *Arch Ophthalmol*. 1959; 62:748–757. [PubMed: 13817537]
- Dora N, Ou J, Kucerova R, Parisi I, West JD, Collinson JM. PAX6 dosage effects on corneal development, growth, and wound healing. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2008; 237:1295–1306. [PubMed: 18386822]
- Du Bois-Reymond E. Vorläufiger Abriss einer Untersuchung über den sogenannten Froschstrom und die electomotorischen Fische. *Ann. Phy. U. Chem*. 1843; 58:1–30.
- Du Bois-Reymond, E. Untersuchungen über thierische Electricität, Zweiter Band, Zweite Abtheilung (Erste Lieferung). Berlin: Georg Reimer; 1860.
- Dube J, Rochette-Drouin O, Levesque P, Gauvin R, Roberge CJ, Auger FA, Goulet D, Bourdages M, Plante M, Germain L, Moulin VJ. Restoration of the transepithelial potential within tissue-engineered human skin in vitro and during the wound healing process in vivo. *Tissue Eng Part A*. 2010; 16:3055–3063. [PubMed: 20486795]
- Duncan G, Bushell AR. Ion analyses of human cataractous lenses. *Exp Eye Res*. 1975; 20:223–230. [PubMed: 1122997]
- Edelhauser HF. The balance between corneal transparency and edema: the Proctor Lecture. *Invest Ophthalmol Vis Sci*. 2006; 47:1754–1767. [PubMed: 16638979]
- Fang KS, Ionides E, Oster G, Nuccitelli R, Isseroff RR. Epidermal growth factor receptor relocalization and kinase activity are necessary for directional migration of keratinocytes in DC electric fields. *J Cell Sci*. 1999; 112(Pt 12):1967–1978. [PubMed: 10341215]
- Farboud B, Nuccitelli R, Schwab IR, Isseroff RR. DC electric fields induce rapid directional migration in cultured human corneal epithelial cells. *Exp Eye Res*. 2000; 70:667–673. [PubMed: 10870525]
- Finkelstein EI, Chao PH, Hung CT, Bulinski JC. Electric field-induced polarization of charged cell surface proteins does not determine the direction of galvanotaxis. *Cell Motil Cytoskeleton*. 2007; 64:833–846. [PubMed: 17685443]
- Firtel RA, Chung CY. The molecular genetics of chemotaxis: sensing and responding to chemoattractant gradients. *Bioessays*. 2000; 22:603–615. [PubMed: 10878573]

- Fischbarg J. Mechanism of fluid transport across corneal endothelium and other epithelial layers: a possible explanation based on cyclic cell volume regulatory changes. *Br J Ophthalmol.* 1997; 81:85–89. [PubMed: 9135416]
- Fischbarg J, Diecke FP, Iserovich P, Rubashkin A. The Role of the Tight Junction in Paracellular Fluid Transport across Corneal Endothelium. Electro-osmosis as a Driving Force. *J Membr Biol.* 2006; 210:117–130. [PubMed: 16868674]
- Fischbarg J, Lim JJ. Role of cations, anions and carbonic anhydrase in fluid transport across rabbit corneal endothelium. *J Physiol.* 1974; 241:647–675. [PubMed: 4215880]
- Fischbarg J, Maurice DM. An update on corneal hydration control. *Exp Eye Res.* 2004; 78:537–541. [PubMed: 15106932]
- Fischer F, Voigt G, Liegl O, Wiederholt M. Potential difference and short-circuit current in isolated human cornea. *Pflugers Arch.* 1972; 332 Suppl 332:R391.
- Foulds IS, Barker AT. Human skin battery potentials and their possible role in wound healing. *Br J Dermatol.* 1983; 109:515–522. [PubMed: 6639877]
- Friedman E, Kupfer C. Transcorneal potential in vivo. *Archives of ophthalmology.* 1960; 64:892–896. [PubMed: 13701996]
- Funk RH, Monsees T, Ozkucur N. Electromagnetic effects - From cell biology to medicine. *Prog Histochem Cytochem.* 2009; 43:177–264. [PubMed: 19167986]
- Gabriel S, Lau RW, Gabriel C. The dielectric properties of biological tissues: III. Parametric models for the dielectric spectrum of tissues. *Phys Med Biol.* 1996; 41:2271–2293. [PubMed: 8938026]
- Gallemore RP, Hughes BA, Miller SS. Retinal pigment epithelial transport mechanisms and their contributions to the electroretinogram. *Progress in Retinal and Eye Research.* 1997; 16:509–566.
- Gamboa OL, Pu J, Townend J, Forrester JV, Zhao M, McCaig C, Lois N. Electrical stimulation of retinal pigment epithelial cells. *Exp Eye Res.* 2010; 91:195–204. [PubMed: 20457155]
- Gao J, Sun X, Yatsula V, Wymore RS, Mathias RT. Isoform-specific function and distribution of Na/K pumps in the frog lens epithelium. *J Membr Biol.* 2000; 178:89–101. [PubMed: 11083898]
- Geering K. FXYD proteins: new regulators of Na-K-ATPase. *Am J Physiol Renal Physiol.* 2006; 290:F241–F250. [PubMed: 16403837]
- Giasson C, Bonanno JA. Facilitated transport of lactate by rabbit corneal endothelium. *Exp Eye Res.* 1994; 59:73–81. [PubMed: 7835399]
- Gick GG, Hatala MA, Chon D, Ismail-Beigi F. Na,K-ATPase in several tissues of the rat: tissue-specific expression of subunit mRNAs and enzyme activity. *J Membr Biol.* 1993; 131:229–236. [PubMed: 8388058]
- Godel V, Chaine G, Regenbogen L, Coscas G. Best's vitelliform macular dystrophy. *Acta Ophthalmol Suppl.* 1986; 175:1–31. [PubMed: 3006423]
- Griff ER, Shirao Y, Steinberg RH. Ba²⁺ unmasks K⁺ modulation of the Na⁺-K⁺ pump in the frog retinal pigment epithelium. *J Gen Physiol.* 1985; 86:853–876. [PubMed: 2416871]
- Guggenheim JA, Hodson SA. Localization of Na⁺/K⁺-ATPase in the bovine corneal endothelium. *Biochim Biophys Acta.* 1994; 1189:127–134. [PubMed: 8292616]
- Guimera A, Ivorra A, Gabriel G, Villa R. Non-invasive assessment of corneal endothelial permeability by means of electrical impedance measurements. *Med Eng Phys.* 2010; 32:1107–1115. [PubMed: 20832346]
- Gundersen D, Orlowski J, Rodriguez-Boulan E. Apical polarity of Na,K-ATPase in retinal pigment epithelium is linked to a reversal of the ankyrin-fodrin submembrane cytoskeleton. *The Journal of cell biology.* 1991; 112:863–872. [PubMed: 1847929]
- Guo A, Song B, Reid B, Gu Y, Forrester JV, Jahoda CA, Zhao M. Effects of physiological electric fields on migration of human dermal fibroblasts. *J Invest Dermatol.* 2010; 130:2320–2327. [PubMed: 20410911]
- Gwon A, Enomoto H, Horowitz J, Garner MH. Induction of de novo synthesis of crystalline lenses in aphakic rabbits. *Exp Eye Res.* 1989; 49:913–926. [PubMed: 2612588]
- Hamann S. Molecular mechanisms of water transport in the eye. *Int Rev Cytol.* 2002; 215:395–431. [PubMed: 11952236]

- Hamann S, Zeuthen T, La Cour M, Nagelhus EA, Ottersen OP, Agre P, Nielsen S. Aquaporins in complex tissues: distribution of aquaporins 1–5 in human and rat eye. *Am J Physiol.* 1998; 274:C1332–C1345. [PubMed: 9612221]
- Han J, Yan XL, Han QH, Li Y, Zhu J, Hui YN. Electric fields contribute to directed migration of human retinal pigment epithelial cells via interaction between F-actin and beta1 integrin. *Current eye research.* 2009a; 34:438–446. [PubMed: 19899978]
- Han J, Yan XL, Han QH, Li Y, Zhu J, Hui YN. Electric fields contribute to directed migration of human retinal pigment epithelial cells via interaction between F-actin and beta1 integrin. *Curr Eye Res.* 2009b; 34:438–446. [PubMed: 19899978]
- Haugh JM, Codazzi F, Teruel M, Meyer T. Spatial sensing in fibroblasts mediated by 3' phosphoinositides. *The Journal of cell biology.* 2000; 151:1269–1280. [PubMed: 11121441]
- Hillenkamp J, Hussain AA, Jackson TL, Cunningham JR, Marshall J. Effect of taurine and apical potassium concentration on electrophysiologic parameters of bovine retinal pigment epithelium. *Experimental eye research.* 2006; 82:258–264. [PubMed: 16102750]
- Hodson S. Evidence for a bicarbonate-dependent sodium pump in corneal endothelium. *Exp Eye Res.* 1971; 11:20–29. [PubMed: 5130518]
- Hodson S. The regulation of corneal hydration by a salt pump requiring the presence of sodium and bicarbonate ions. *J Physiol.* 1974; 236:271–302. [PubMed: 16992435]
- Hodson S. The endothelial pump of the cornea. *Invest Ophthalmol Vis Sci.* 1977; 16:589–591. [PubMed: 873720]
- Hodson S, Miller F. The bicarbonate ion pump in the endothelium which regulates the hydration of rabbit cornea. *J Physiol.* 1976; 263:563–577. [PubMed: 828203]
- Hong K, Nishiyama M, Henley J, Tessier-Lavigne M, Poo M. Calcium signalling in the guidance of nerve growth by netrin-1. *Nature.* 2000; 403:93–98. [PubMed: 10638760]
- Hudspeth AJ. The recovery of local transepithelial resistance following single-cell lesions. *Exp Cell Res.* 1982; 138:331–342. [PubMed: 7075690]
- Hughes BA, Miller SS, Joseph DP, Edelman JL. cAMP stimulates the Na⁺-K⁺ pump in frog retinal pigment epithelium. *Am J Physiol.* 1988; 254:C84–C98. [PubMed: 2447798]
- Iijima M, Devreotes P. Tumor suppressor PTEN mediates sensing of chemoattractant gradients. *Cell.* 2002; 109:599–610. [PubMed: 12062103]
- Illingworth CM, Barker AT. Measurement of electrical currents emerging during the regeneration of amputated finger tips in children. *Clinical Physics and Physiological Measurement.* 1980; 1:87.
- Imanishi J, Kamiyama K, Iguchi I, Kita M, Sotozono C, Kinoshita S. Growth factors: importance in wound healing and maintenance of transparency of the cornea. *Prog Retin Eye Res.* 2000; 19:113–129. [PubMed: 10614683]
- Ito Y, Cai H, Koizumi Y, Hori R, Terao M, Kimura T, Takagi S, Tomohiro M. Effects of lipid composition on the transcorneal penetration of liposomes containing disulfiram, a potential anti-cataract agent, in the rabbit. *Biol Pharm Bull.* 2000; 23:327–333. [PubMed: 10726888]
- Itoh R, Kawamoto S, Miyamoto Y, Kinoshita S, Okubo K. Isolation and characterization of a Ca(2+)-activated chloride channel from human corneal epithelium. *Curr Eye Res.* 2000; 21:918–925. [PubMed: 11262615]
- Jaffe LF. Electrophoresis along cell membranes. *Nature.* 1977; 265:600–602. [PubMed: 859558]
- Jaffe LF, Nuccitelli R. An ultrasensitive vibrating probe for measuring steady extracellular currents. *The Journal of cell biology.* 1974; 63:614–628. [PubMed: 4421919]
- Jin T, Amzel M, Devreotes PN, Wu L. Selection of gbeta subunits with point mutations that fail to activate specific signaling pathways in vivo: dissecting cellular responses mediated by a heterotrimeric G protein in Dictyostelium discoideum. *Mol Biol Cell.* 1998; 9:2949–2961. [PubMed: 9763454]
- Jorgensen PL, Hakansson KO, Karlsh SJ. Structure and mechanism of Na,K-ATPase: functional sites and their interactions. *Annu Rev Physiol.* 2003; 65:817–849. [PubMed: 12524462]
- Keese CR, Wegener J, Walker SR, Giaever I. Electrical wound-healing assay for cells in vitro. *Proc Natl Acad Sci U S A.* 2004; 101:1554–1559. [PubMed: 14747654]

- Klyce SD. Electrical profiles in the corneal epithelium. *J Physiol.* 1972; 226:407–429. [PubMed: 4538944]
- Klyce SD. Transport of Na, Cl, and water by the rabbit corneal epithelium at resting potential. *Am J Physiol.* 1975; 228:1446–1452. [PubMed: 165732]
- Klyce SD. Enhancing fluid secretion by the corneal epithelium. *Invest Ophthalmol Vis Sci.* 1977; 16:968–973. [PubMed: 908650]
- Klyce SD, Crosson CE. Transport processes across the rabbit corneal epithelium: a review. *Curr Eye Res.* 1985; 4:323–331. [PubMed: 3893897]
- Klyce SD, Marshall WS. Effects of Ag⁺ on ion transport by the corneal epithelium of the rabbit. *The Journal of membrane biology.* 1982; 66:133–144. [PubMed: 7077648]
- Klyce SD, Palkama KA, Harkonen M, Marshall WS, Huhtaniitty S, Mann KP, Neufeld AH. Neural serotonin stimulates chloride transport in the rabbit corneal epithelium. *Invest Ophthalmol Vis Sci.* 1982; 23:181–192. [PubMed: 6178711]
- Klyce SD, Wong RK. Site and mode of adrenaline action on chloride transport across the rabbit corneal epithelium. *J Physiol.* 1977; 266:777–799. [PubMed: 864618]
- Konomi K, Zhu C, Harris D, Joyce NC. Comparison of the proliferative capacity of human corneal endothelial cells from the central and peripheral areas. *Invest Ophthalmol Vis Sci.* 2005; 46:4086–4091. [PubMed: 16249484]
- Kucerova R, Walczysko P, Reid B, Ou J, Leiper LJ, Rajnicek AM, McCaig CD, Zhao M, Collinson JM. The role of electrical signals in murine corneal wound re-epithelialisations. *J Cell Physiol.* 2010
- Kucerova R, Walczysko P, Reid B, Ou J, Leiper LJ, Rajnicek AM, McCaig CD, Zhao M, Collinson JM. The role of electrical signals in murine corneal wound re-epithelialization. *J Cell Physiol.* 2011; 226:1544–1553. [PubMed: 20945376]
- Lee J, Ishihara A, Oxford G, Johnson B, Jacobson K. Regulation of cell movement is mediated by stretch-activated calcium channels. *Nature.* 1999; 400:382–386. [PubMed: 10432119]
- Leiper LJ, Walczysko P, Kucerova R, Ou J, Shanley LJ, Lawson D, Forrester JV, McCaig CD, Zhao M, Collinson JM. The roles of calcium signaling and ERK1/2 phosphorylation in a Pax6^{+/–} mouse model of epithelial wound-healing delay. *BMC Biol.* 2006; 4:27. [PubMed: 16914058]
- Levin M. Large-scale biophysics: ion flows and regeneration. *Trends in cell biology.* 2007; 17:261–270. [PubMed: 17498955]
- Levin MH, Kim JK, Hu J, Verkman AS. Potential difference measurements of ocular surface Na⁺ absorption analyzed using an electrokinetic model. *Invest Ophthalmol Vis Sci.* 2006; 47:306–316. [PubMed: 16384978]
- Levin MH, Verkman AS. Aquaporin-dependent water permeation at the mouse ocular surface: in vivo microfluorimetric measurements in cornea and conjunctiva. *Invest Ophthalmol Vis Sci.* 2004; 45:4423–4432. [PubMed: 15557451]
- Levin MH, Verkman AS. CFTR-regulated chloride transport at the ocular surface in living mice measured by potential differences. *Invest Ophthalmol Vis Sci.* 2005; 46:1428–1434. [PubMed: 15790911]
- Levin MH, Verkman AS. Aquaporins and CFTR in ocular epithelial fluid transport. *J Membr Biol.* 2006; 210:105–115. [PubMed: 16868675]
- Levin V. Delayed corneal epithelial wound healing in aquaporin-3-null mice. *Invest. Ophthalmol. Vis. Sci.* 2005; 45 E-Abstract 3618.
- Lichey HJ, Fischer F, Wiederholt M. Intracellular potentials in the isolated human cornea. *Pflugers Archiv : European journal of physiology.* 1974; 346:351–360. [PubMed: 4856395]
- Lim JJ, Fischbarg J. Electrical properties of rabbit corneal endothelium as determined from impedance measurements. *Biophysical journal.* 1981; 36:677–695. [PubMed: 7326329]
- Lindemann B. Resting potential of isolated beef cornea. *Exp Eye Res.* 1968; 7:62–69. [PubMed: 5640367]
- Ljubimov AV, Atilano SR, Garner MH, Maguen E, Nesburn AB, Kenney MC. Extracellular matrix and Na⁺,K⁺-ATPase in human corneas following cataract surgery: comparison with bullous keratopathy and Fuchs' dystrophy corneas. *Cornea.* 2002; 21:74–80. [PubMed: 11805512]

- Lois N, Dawson R, McKinnon AD, Forrester JV. A new model of posterior capsule opacification in rodents. *Invest Ophthalmol Vis Sci*. 2003; 44:3450–3457. [PubMed: 12882794]
- Lois N, Reid B, Song B, Zhao M, Forrester J, McCaig C. Electric currents and lens regeneration in the rat. *Exp Eye Res*. 2010; 90:316–323. [PubMed: 19931246]
- Lu L. Stress-induced corneal epithelial apoptosis mediated by K⁺ channel activation. *Progress in retinal and eye research*. 2006; 25:515–538. [PubMed: 16962363]
- Lu L, Reinach PS, Kao WW. Corneal epithelial wound healing. *Exp Biol Med (Maywood)*. 2001; 226:653–664. [PubMed: 11444101]
- Maminishkis A, Chen S, Jalickee S, Banzon T, Shi G, Wang FE, Ehalt T, Hammer JA, Miller SS. Confluent monolayers of cultured human fetal retinal pigment epithelium exhibit morphology and physiology of native tissue. *Invest Ophthalmol Vis Sci*. 2006; 47:3612–3624. [PubMed: 16877436]
- Marmor MF. Mechanisms of fluid accumulation in retinal edema. *Doc Ophthalmol*. 1999; 97:239–249. [PubMed: 10896337]
- Marquardt A, Stohr H, Passmore LA, Kramer F, Rivera A, Weber BH. Mutations in a novel gene, VMD2, encoding a protein of unknown properties cause juvenile-onset vitelliform macular dystrophy (Best's disease). *Hum Mol Genet*. 1998; 7:1517–1525. [PubMed: 9700209]
- Mathias RT, Kistler J, Donaldson P. The lens circulation. *J Membr Biol*. 2007; 216:1–16. [PubMed: 17568975]
- Mathias RT, Rae JL. Steady state voltages in the frog lens. *Curr Eye Res*. 1985a; 4:421–430. [PubMed: 4017633]
- Mathias RT, Rae JL. Transport properties of the lens. *Am J Physiol*. 1985b; 249:C181–C190. [PubMed: 2994483]
- Mathias RT, Rae JL. The lens: local transport and global transparency. *Experimental eye research*. 2004; 78:689–698. [PubMed: 15106948]
- Mathias RT, Rae JL, Baldo GJ. Physiological properties of the normal lens. *Physiol Rev*. 1997; 77:21–50. [PubMed: 9016299]
- Mathias RT, Rae JL, Ebihara L, McCarthy RT. The localization of transport properties in the frog lens. *Biophys J*. 1985; 48:423–434. [PubMed: 3876116]
- Maurice DM. The permeability to sodium ions of the living rabbit's cornea. *J Physiol*. 1951; 112:367–391. [PubMed: 14825218]
- Maurice DM. The structure and transparency of the cornea. *J Physiol*. 1957; 136:263–286. [PubMed: 13429485]
- Maurice DM. Epithelial potential of the cornea. *Exp Eye Res*. 1967; 6:138–140. [PubMed: 6024067]
- Maurice DM. The location of the fluid pump in the cornea. *J Physiol*. 1972; 221:43–54. [PubMed: 5016991]
- McAvoy JW, Chamberlain CG. Fibroblast growth factor (FGF) induces different responses in lens epithelial cells depending on its concentration. *Development*. 1989; 107:221–228. [PubMed: 2632221]
- McBee JK, Palczewski K, Baehr W, Pepperberg DR. Confronting complexity: the interlink of phototransduction and retinoid metabolism in the vertebrate retina. *Progress in retinal and eye research*. 2001; 20:469–529. [PubMed: 11390257]
- McCaig CD, Rajnicek AM, Song B, Zhao M. Controlling cell behavior electrically: current views and future potential. *Physiol Rev*. 2005; 85:943–978. [PubMed: 15987799]
- McCaig CD, Song B, Rajnicek AM. Electrical dimensions in cell science. *Journal of cell science*. 2009; 122:4267–4276. [PubMed: 19923270]
- McCloskey MA, Liu ZY, Poo MM. Lateral electromigration and diffusion of Fc epsilon receptors on rat basophilic leukemia cells: effects of IgE binding. *The Journal of cell biology*. 1984; 99:778–787. [PubMed: 6236227]
- McLaughlin S, Poo MM. The role of electro-osmosis in the electric-field-induced movement of charged macromolecules on the surfaces of cells. *Biophys J*. 1981; 34:85–93. [PubMed: 6894257]

- Mergler S, Pleyer U. The human corneal endothelium: New in sights into electrophysiology and ion channels. *Progress in Retinal and Eye Research*. 2007; 26:359–378. [PubMed: 17446115]
- Miller SS, Steinberg RH, Oakley B 2nd. The electrogenic sodium pump of the frog retinal pigment epithelium. *J Membr Biol*. 1978; 44:259–279. [PubMed: 313450]
- Mirshahi M, Nicolas C, Mirshahi S, Golestaneh N, d'Hermies F, Agarwal MK. Immunochemical analysis of the sodium channel in rodent and human eye. *Experimental eye research*. 1999; 69:21–32. [PubMed: 10375446]
- Modrell RW, Potts AM. The influence of medium composition, pH and temperature on the transcorneal potential. *American journal of ophthalmology*. 1959; 48:834–842. [PubMed: 14423155]
- Mukerjee EV, Isseroff RR, Nuccitelli R, Collins SD, Smith RL. Microneedle array for measuring wound generated electric fields. *Conf Proc IEEE Eng Med Biol Soc*. 2006; 1:4326–4328. [PubMed: 17947077]
- Mycielska ME, Djamgoz MB. Cellular mechanisms of direct-current electric field effects: galvanotaxis and metastatic disease. *J Cell Sci*. 2004; 117:1631–1639. [PubMed: 15075225]
- Nabi IR. The polarization of the motile cell. *J Cell Sci*. 1999; 112(Pt 12):1803–1811. [PubMed: 10341200]
- Nagel W, Carrasquer G. Effect of loop diuretics on bullfrog cornea epithelium. *The American journal of physiology*. 1989; 256:C750–C755. [PubMed: 2784943]
- Neville MC, Paterson CA, Hamilton PM. Evidence for two sodium pumps in the crystalline lens of the rabbit eye. *Exp Eye Res*. 1978; 27:637–648. [PubMed: 216566]
- Nuccitelli R. Endogenous electric fields in embryos during development, regeneration and wound healing. *Radiat Prot Dosimetry*. 2003a; 106:375–383. [PubMed: 14690282]
- Nuccitelli R. A role for endogenous electric fields in wound healing. *Curr Top Dev Biol*. 2003b; 58:1–26. [PubMed: 14711011]
- Nuccitelli R, Nuccitelli P, Ramlatchan S, Sanger R, Smith PJ. Imaging the electric field associated with mouse and human skin wounds. *Wound Repair Regen*. 2008; 16:432–441. [PubMed: 18471262]
- Oakley B 2nd, Miller SS, Steinberg RH. Effect of intracellular potassium upon the electrogenic pump of frog retinal pigment epithelium. *J Membr Biol*. 1978; 44:281–307. [PubMed: 313451]
- Ojingwa JC, Isseroff RR. Electrical stimulation of wound healing. *J Invest Dermatol*. 2003; 121:1–12. [PubMed: 12839557]
- Onuma EK, Hui SW. A calcium requirement for electric field-induced cell shape changes and preferential orientation. *Cell Calcium*. 1985; 6:281–292. [PubMed: 3926320]
- Onuma EK, Hui SW. Electric field-directed cell shape changes, displacement, and cytoskeletal reorganization are calcium dependent. *The Journal of cell biology*. 1988; 106:2067–2075. [PubMed: 3133378]
- Orida N, Poo MM. Electrophoretic movement and localisation of acetylcholine receptors in the embryonic muscle cell membrane. *Nature*. 1978; 275:31–35. [PubMed: 683340]
- Palva M, Palkama A. Electronmicroscopical, histochemical and biochemical findings on the Na-K-ATPase activity in the epithelium of the rat lens. *Exp Eye Res*. 1976; 22:229–236. [PubMed: 131692]
- Parent CA, Devreotes PN. A cell's sense of direction. *Science*. 1999; 284:765–770. [PubMed: 10221901]
- Parmelee JT. Measurement of steady currents around the frog lens. *Exp Eye Res*. 1986; 42:433–441. [PubMed: 3487463]
- Parmelee JT, Robinson KR, Patterson JW. Effects of calcium on the steady outward currents at the equator of the rat lens. *Invest Ophthalmol Vis Sci*. 1985; 26:1343–1348. [PubMed: 4044162]
- Patil RV, Saito I, Yang X, Wax MB. Expression of aquaporins in the rat ocular tissue. *Exp Eye Res*. 1997; 64:203–209. [PubMed: 9176054]
- Patmore L, Duncan G. Voltage-dependent potassium channels in the amphibian lens membranes: evidence from radiotracer and electrical conductance measurements. *Exp Eye Res*. 1980; 31:637–650. [PubMed: 6260521]

- Patterson JW. Characterization of the equatorial current of the lens. *Ophthalmic Res.* 1988; 20:139–142. [PubMed: 3263600]
- Pesin SR, Candia OA. Acetylcholine concentration and its role in ionic transport by the corneal epithelium. *Invest Ophthalmol Vis Sci.* 1982a; 22:651–659. [PubMed: 6978868]
- Pesin SR, Candia OA. Na⁺ and Cl[−] fluxes, and effects of pharmacological agents on the short-circuit current of the isolated rabbit iris-ciliary body. *Curr Eye Res.* 1982b; 2:815–827. [PubMed: 6197237]
- Petrukhin K, Koisti MJ, Bakall B, Li W, Xie G, Marknell T, Sandgren O, Forsman K, Holmgren G, Andreasson S, Vujic M, Bergen AA, McGarty-Dugan V, Figueroa D, Austin CP, Metzker ML, Caskey CT, Wadelius C. Identification of the gene responsible for Best macular dystrophy. *Nat Genet.* 1998; 19:241–247. [PubMed: 9662395]
- Poo M, Robinson KR. Electrophoresis of concanavalin A receptors along embryonic muscle cell membrane. *Nature.* 1977; 265:602–605. [PubMed: 859559]
- Potts AM, Modrell RW. The transcorneal potential. *American journal of ophthalmology.* 1957; 44:284–290. [PubMed: 13469992]
- Prigozhina NL, Waterman-Storer CM. Protein kinase D-mediated anterograde membrane trafficking is required for fibroblast motility. *Curr Biol.* 2004; 14:88–98. [PubMed: 14738729]
- Pu J, McCaig CD, Cao L, Zhao ZQ, Segall JE, Zhao M. EGF receptor signalling is essential for electric-field-directed migration of breast cancer cells. *Journal of Cell Science.* 2007; 120:3395–3403. [PubMed: 17881501]
- Pu J, Zhao M. Golgi polarization in a strong electric field. *J Cell Sci.* 2005; 118:1117–1128. [PubMed: 15728257]
- Pullar CE, Baier BS, Kariya Y, Russell AJ, Horst BA, Marinkovich MP, Isseroff RR. beta4 integrin and epidermal growth factor coordinately regulate electric field-mediated directional migration via Rac1. *Mol Biol Cell.* 2006; 17:4925–4935. [PubMed: 16914518]
- Quinn RH, Miller SS. Ion transport mechanisms in native human retinal pigment epithelium. *Invest Ophthalmol Vis Sci.* 1992; 33:3513–3527. [PubMed: 1334477]
- Quinn RH, Quong JN, Miller SS. Adrenergic receptor activated ion transport in human fetal retinal pigment epithelium. *Invest Ophthalmol Vis Sci.* 2001; 42:255–264. [PubMed: 11133877]
- Rabinowitz YS, Dong L, Wistow G. Gene expression profile studies of human keratoconus cornea for NEIBank: a novel cornea-expressed gene and the absence of transcripts for aquaporin 5. *Invest Ophthalmol Vis Sci.* 2005; 46:1239–1246. [PubMed: 15790884]
- Rae JL. The electrophysiology of the crystalline lens. *Curr Top Eye Res.* 1979; 1:37–90. [PubMed: 233657]
- Ramaesh T, Ramaesh K, Leask R, Springbett A, Riley SC, Dhillon B, West JD. Increased apoptosis and abnormal wound-healing responses in the heterozygous Pax6^{+/-} mouse cornea. *Investigative ophthalmology & visual science.* 2006; 47:1911–1917. [PubMed: 16638998]
- Rauz S, Walker EA, Murray PI, Stewart PM. Expression and distribution of the serum and glucocorticoid regulated kinase and the epithelial sodium channel subunits in the human cornea. *Experimental eye research.* 2003; 77:101–108. [PubMed: 12823993]
- Reid B, Graue-Hernandez E, Mannis M, Zhao M. Modulating Endogenous Electric Currents in Human Corneal Wounds—A Novel Approach of Bioelectric Stimulation Without Electrodes. *Cornea.* 2010 in press.
- Reid B, Nuccitelli R, Zhao M. Non-invasive measurement of bioelectric currents with a vibrating probe. *Nat Protoc.* 2007; 2:661–669. [PubMed: 17406628]
- Reid B, Song B, McCaig CD, Zhao M. Wound healing in rat cornea: the role of electric currents. *Faseb J.* 2005; 19:379–386. [PubMed: 15746181]
- Reinach, PS.; Capó-Aponte, JE.; Mergler, S.; Pokorny, KS. Roles of Corneal Epithelial Ion Transport Mechanisms in Mediating Responses to Cytokines and Osmotic Stress. Totowa, NJ: Humana Press; 2008a.
- Reinach PS, Capó-Aponte JE, Mergler S, Pokorny KS. Roles of Corneal Epithelial Ion Transport Mechanisms in Mediating Responses to Cytokines and Osmotic Stress, Ocular Transporters. *Ophthalmic Diseases And Drug Delivery.* 2008b:17–46.

- Rich A, Rae JL. Calcium entry in rabbit corneal epithelial cells: evidence for a nonvoltage dependent pathway. *The Journal of membrane biology*. 1995; 144:177–184. [PubMed: 7541085]
- Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR. Cell migration: integrating signals from front to back. *Science*. 2003; 302:1704–1709. [PubMed: 14657486]
- Riley MV, Winkler BS, Czajkowski CA, Peters MI. The roles of bicarbonate and CO₂ in transendothelial fluid movement and control of corneal thickness. *Invest Ophthalmol Vis Sci*. 1995; 36:103–112. [PubMed: 7822137]
- Rizzolo LJ. Polarization of the Na⁺, K⁺-ATPase in epithelia derived from the neuroepithelium. *Int Rev Cytol*. 1999; 185:195–235. [PubMed: 9750268]
- Robinson KR. The responses of cells to electrical fields: a review. *The Journal of cell biology*. 1985; 101:2023–2027. [PubMed: 3905820]
- Robinson KR, Messerli MA. Left/right, up/down: the role of endogenous electrical fields as directional signals in development, repair and invasion. *Bioessays*. 2003; 25:759–766. [PubMed: 12879446]
- Robinson KR, Patterson JW. Localization of steady currents in the lens. *Curr Eye Res*. 1982; 2:843–847. [PubMed: 7187641]
- Roderick C, Reinach PS, Wang L, Lu L. Modulation of rabbit corneal epithelial cell proliferation by growth factor-regulated K⁺ channel activity. *J Membr Biol*. 2003; 196:41–50. [PubMed: 14724755]
- Scharschmidt BF, Griff ER, Steinberg RH. Effect of taurine on the isolated retinal pigment epithelium of the frog: electrophysiologic evidence for stimulation of an apical, electrogenic Na⁺-K⁺ pump. *J Membr Biol*. 1988; 106:71–81. [PubMed: 3265731]
- Schermer A, Galvin S, Sun TT. Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. *The Journal of cell biology*. 1986; 103:49–62. [PubMed: 2424919]
- Servant G, Weiner OD, Herzmark P, Balla T, Sedat JW, Bourne HR. Polarization of chemoattractant receptor signaling during neutrophil chemotaxis. *Science*. 2000; 287:1037–1040. [PubMed: 10669415]
- Song B, Gu Y, Pu J, Reid B, Zhao Z, Zhao M. Application of direct current electric fields to cells and tissues in vitro and modulation of wound electric field in vivo. *Nat Protoc*. 2007; 2:1479–1489. [PubMed: 17545984]
- Song B, Zhao M, Forrester J, McCaig C. Nerve regeneration and wound healing are stimulated and directed by an endogenous electrical field in vivo. *J Cell Sci*. 2004; 117:4681–4690. [PubMed: 15371524]
- Song B, Zhao M, Forrester JV, McCaig CD. Electrical cues regulate the orientation and frequency of cell division and the rate of wound healing in vivo. *Proc Natl Acad Sci U S A*. 2002; 99:13577–13582. [PubMed: 12368473]
- Soong HK, Parkinson WC, Bafna S, Sulik GL, Huang SC. Movements of cultured corneal epithelial cells and stromal fibroblasts in electric fields. *Invest Ophthalmol Vis Sci*. 1990a; 31:2278–2282. [PubMed: 2242993]
- Soong HK, Parkinson WC, Sulik GL, Bafna S. Effects of electric fields on cytoskeleton of corneal stromal fibroblasts. *Current eye research*. 1990b; 9:893–901. [PubMed: 2123147]
- Sparrow JR, Hicks D, Hamel CP. The retinal pigment epithelium in health and disease. *Curr Mol Med*. 2010; 10:802–823. [PubMed: 21091424]
- Sta Iglesia DD, Venable JW Jr. Endogenous lateral electric fields around bovine corneal lesions are necessary for and can enhance normal rates of wound healing. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 1998; 6:531–542.
- Steinberg RH. Interactions between the retinal pigment epithelium and the neural retina. *Doc Ophthalmol*. 1985; 60:327–346. [PubMed: 3905312]
- Steinberg RH, Linsenmeier RA, Griff ER. Three light-evoked responses of the retinal pigment epithelium. *Vision Res*. 1983; 23:1315–1323. [PubMed: 6606894]

- Stewart S, Rojas-Munoz A, Izipisua Belmonte JC. Bioelectricity and epimorphic regeneration. *BioEssays : news and reviews in molecular, cellular and developmental biology*. 2007; 29:1133–1137.
- Strauss O. The retinal pigment epithelium in visual function. *Physiol Rev*. 2005; 85:845–881. [PubMed: 15987797]
- Sue Menko A. Lens epithelial cell differentiation. *Exp Eye Res*. 2002; 75:485–490. [PubMed: 12457861]
- Sulik GL, Soong HK, Chang PC, Parkinson WC, Elnor SG, Elnor VM. Effects of steady electric fields on human retinal pigment epithelial cell orientation and migration in culture. *Acta Ophthalmol (Copenh)*. 1992; 70:115–122. [PubMed: 1557964]
- Sun H, Tsunenari T, Yau KW, Nathans J. The vitelliform macular dystrophy protein defines a new family of chloride channels. *Proc Natl Acad Sci U S A*. 2002; 99:4008–4013. [PubMed: 11904445]
- Suzuki K, Saito J, Yanai R, Yamada N, Chikama T, Seki K, Nishida T. Cell-matrix and cell-cell interactions during corneal epithelial wound healing. *Prog Retin Eye Res*. 2003; 22:113–133. [PubMed: 12604055]
- Sweadner KJ. Isozymes of the Na⁺/K⁺-ATPase. *Biochim Biophys Acta*. 1989; 988:185–220. [PubMed: 2541792]
- Tai G, Reid B, Cao L, Zhao M. Electrotaxis and wound healing: experimental methods to study electric fields as a directional signal for cell migration. *Methods Mol Biol*. 2009; 571:77–97. [PubMed: 19763960]
- Talarico EF Jr. Plasma membrane calcium-ATPase isoform four distribution changes during corneal epithelial wound healing. *Molecular vision*. 2010; 16:2259–2272. [PubMed: 21139678]
- Tamiya S, Dean WL, Paterson CA, Delamere NA. Regional distribution of Na,K-ATPase activity in porcine lens epithelium. *Invest Ophthalmol Vis Sci*. 2003; 44:4395–4399. [PubMed: 14507885]
- Thompson DA, Gal A. Vitamin A metabolism in the retinal pigment epithelium: genes, mutations, and diseases. *Prog Retin Eye Res*. 2003; 22:683–703. [PubMed: 12892646]
- Timpson P, Jones GE, Frame MC, Brunton VG. Coordination of cell polarization and migration by the Rho family GTPases requires Src tyrosine kinase activity. *Curr Biol*. 2001; 11:1836–1846. [PubMed: 11728306]
- Trollinger DR, Isseroff RR, Nuccitelli R. Calcium channel blockers inhibit galvanotaxis in human keratinocytes. *J Cell Physiol*. 2002; 193:1–9. [PubMed: 12209874]
- Tsuji T, Lin LR, Murata Y, Reddy VN. Immunohistochemical localization of Na,K-ATPase in the in situ lens, cultured human lens epithelium and lentoid. *Exp Eye Res*. 1992; 55:469–478. [PubMed: 1330668]
- Tuli, G.; Schultz. *Modulation of Corneal Wound Healing*. Krachmer, JH.; M, M.; Holland, EJ., editors. Elsevier Mosby; 2005. p. 133-150.
- van der Bijl P, van Eyk AD, Seifart HI, Meyer D. In vitro transcorneal penetration of metronidazole and its potential use as adjunct therapy in Acanthamoeba keratitis. *Cornea*. 2004; 23:386–389. [PubMed: 15097135]
- Van der Heyden C, Weekers JF, Schoffeniels E. Sodium and chloride transport across the isolated rabbit cornea. *Exp Eye Res*. 1975; 20:89–96. [PubMed: 1193193]
- Van Duijn B, Vogelzang SA, Ypey DL, Van der Molen LG, Van Haastert PJ. Normal chemotaxis in Dictyostelium discoideum cells with a depolarized plasma membrane potential. *J Cell Sci*. 1990; 95(Pt 1):177–183. [PubMed: 2161858]
- Verkman AS, Ruiz-Ederra J, Levin MH. Functions of aquaporins in the eye. *Prog Retin Eye Res*. 2008; 27:420–433. [PubMed: 18501660]
- Vieira AC, Reid B, Cao L, Mannis MJ, Schwab IR, Zhao M. Ionic components of electric current at rat corneal wounds. *PLoS One*. 2011; 6:e17411. [PubMed: 21364900]
- Walsh S, Patterson JW. EGTA modulates equatorial currents in frog lenses. *Experimental eye research*. 1989; 49:705–715. [PubMed: 2512177]
- Walsh S, Patterson JW. Effects of oxidants on lens transport. *Investigative ophthalmology & visual science*. 1991; 32:1648–1658. [PubMed: 1707864]

- Walsh SP, Patterson JW. Topical norepinephrine, isoproterenol, terbutaline, forskolin and 8-bromo cyclic AMP increase lens equatorial current. *Experimental eye research*. 1993; 56:267–273. [PubMed: 8472783]
- Wang E, Reid B, Lois N, Forrester JV, McCaig CD, Zhao M. Electrical inhibition of lens epithelial cell proliferation: an additional factor in secondary cataract? *FASEB J*. 2005; 19:842–844. [PubMed: 15764648]
- Wang E, Yin Y, Zhao M, Forrester JV, McCaig CD. Physiological electric fields control the G1/S phase cell cycle checkpoint to inhibit endothelial cell proliferation. *Faseb J*. 2003a; 17:458–460. [PubMed: 12551844]
- Wang E, Zhao M, Forrester JV, CD MC. Re-orientation and faster, directed migration of lens epithelial cells in a physiological electric field. *Exp Eye Res*. 2000; 71:91–98. [PubMed: 10880279]
- Wang E, Zhao M, Forrester JV, McCaig CD. Bi-directional migration of lens epithelial cells in a physiological electrical field. *Exp Eye Res*. 2003b; 76:29–37. [PubMed: 12589773]
- Wang E, Zhao M, Forrester JV, McCaig CD. Electric fields and MAP kinase signaling can regulate early wound healing in lens epithelium. *Invest Ophthalmol Vis Sci*. 2003c; 44:244–249. [PubMed: 12506081]
- Weingeist TA, Kobrin JL, Watzke RC. Histopathology of Best's macular dystrophy. *Arch Ophthalmol*. 1982; 100:1108–1114. [PubMed: 7092654]
- Wiederholt M, Koch M. Effect of intraocular irrigating solutions on intracellular membrane potentials and swelling rate of isolated human and rabbit cornea. *Investigative ophthalmology & visual science*. 1979; 18:313–317. [PubMed: 422335]
- Wimmers S, Karl MO, Strauss O. Ion channels in the RPE. *Prog Retin Eye Res*. 2007; 26:263–301. [PubMed: 17258931]
- Wind BE, Walsh S, Patterson JW. Effect of ouabain on lens equatorial currents. *Invest Ophthalmol Vis Sci*. 1988a; 29:1753–1755. [PubMed: 3182208]
- Wind BE, Walsh S, Patterson JW. Equatorial potassium currents in lenses. *Exp Eye Res*. 1988b; 46:117–130. [PubMed: 3258245]
- Witke W, Hofmann A, Koppel B, Schleicher M, Noegel AA. The Ca(2+)-binding domains in non-muscle type alpha-actinin: biochemical and genetic analysis. *The Journal of cell biology*. 1993; 121:599–606. [PubMed: 8486739]
- Wollmann G, Lenzner S, Berger W, Rosenthal R, Karl MO, Strauss O. Voltage-dependent ion channels in the mouse RPE: comparison with Norrie disease mice. *Vision Res*. 2006; 46:688–698. [PubMed: 16289664]
- Wolosin JM. Regeneration of resistance and ion transport in rabbit corneal epithelium after induced surface cell exfoliation. *The Journal of membrane biology*. 1988; 104:45–55. [PubMed: 3184177]
- Wu L, Valkema R, Van Haastert PJ, Devreotes PN. The G protein beta subunit is essential for multiple responses to chemoattractants in Dictyostelium. *The Journal of cell biology*. 1995; 129:1667–1675. [PubMed: 7790362]
- Xu J, Wang F, Van Keymeulen A, Herzmark P, Straight A, Kelly K, Takuwa Y, Sugimoto N, Mitchison T, Bourne HR. Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell*. 2003; 114:201–214. [PubMed: 12887922]
- Yamamoto K, Pardee JD, Reidler J, Stryer L, Spudich JA. Mechanism of interaction of Dictyostelium severin with actin filaments. *The Journal of cell biology*. 1982; 95:711–719. [PubMed: 6897549]
- Yang H, Reinach PS, Koniarek JP, Wang Z, Iserovich P, Fischbarg J. Fluid transport by cultured corneal epithelial cell layers. *Br J Ophthalmol*. 2000; 84:199–204. [PubMed: 10655198]
- Yang H, Wang Z, Miyamoto Y, Reinach PS. Cell signaling pathways mediating epidermal growth factor stimulation of Na:K:2Cl cotransport activity in rabbit corneal epithelial cells. *J Membr Biol*. 2001; 183:93–101. [PubMed: 11562791]
- Yao H, Shum AJ, Cowan M, Lahdesmaki I, Parviz BA. A contact lens with embedded sensor for monitoring tear glucose level. *Biosens Bioelectron*. 2011; 26:3290–3296. [PubMed: 21257302]
- Zadunaisky JA. Active transport of chloride in frog cornea. *Am J Physiol*. 1966; 211:506–512. [PubMed: 5921111]

- Zadunaisky JA, Lande MA. Active chloride transport and control of corneal transparency. *Am J Physiol.* 1971; 221:1837–1844. [PubMed: 5124331]
- Zadunaisky JA, Lande MA, Chalfie M, Neufeld AH. Ion pumps in the cornea and their stimulation by epinephrine and cyclic-AMP. *Exp Eye Res.* 1973; 15:577–584. [PubMed: 4351365]
- Zhao M. Electrical fields in wound healing-An overriding signal that directs cell migration. *Seminars in cell & developmental biology.* 2009; 20:674–682. [PubMed: 19146969]
- Zhao M, Agius-Fernandez A, Forrester JV, McCaig CD. Directed migration of corneal epithelial sheets in physiological electric fields. *Invest Ophthalmol Vis Sci.* 1996a; 37:2548–2558. [PubMed: 8977469]
- Zhao M, Agius-Fernandez A, Forrester JV, McCaig CD. Orientation and directed migration of cultured corneal epithelial cells in small electric fields are serum dependent. *J Cell Sci.* 1996b; 109(Pt 6):1405–1414. [PubMed: 8799828]
- Zhao M, Bai H, Wang E, Forrester JV, McCaig CD. Electrical stimulation directly induces pre-angiogenic responses in vascular endothelial cells by signaling through VEGF receptors. *J Cell Sci.* 2004; 117:397–405. [PubMed: 14679307]
- Zhao M, Dick A, Forrester JV, McCaig CD. Electric field-directed cell motility involves up-regulated expression and asymmetric redistribution of the epidermal growth factor receptors and is enhanced by fibronectin and laminin. *Mol Biol Cell.* 1999a; 10:1259–1276. [PubMed: 10198071]
- Zhao M, Forrester JV, McCaig CD. A small, physiological electric field orients cell division. *Proc Natl Acad Sci U S A.* 1999b; 96:4942–4946. [PubMed: 10220398]
- Zhao M, McCaig CD, Agius-Fernandez A, Forrester JV, Araki-Sasaki K. Human corneal epithelial cells reorient and migrate cathodally in a small applied electric field. *Curr Eye Res.* 1997; 16:973–984. [PubMed: 9330848]
- Zhao M, Pu J, Forrester JV, McCaig CD. Membrane lipids, EGF receptors, and intracellular signals colocalize and are polarized in epithelial cells moving directionally in a physiological electric field. *FASEB J.* 2002; 16:857–859. [PubMed: 11967227]
- Zhao M, Song B, Pu J, Wada T, Reid B, Tai G, Wang F, Guo A, Walczysko P, Gu Y, Sasaki T, Suzuki A, Forrester JV, Bourne HR, Devreotes PN, McCaig CD, Penninger JM. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. *Nature.* 2006; 442:457–460. [PubMed: 16871217]
- Zheng JQ. Turning of nerve growth cones induced by localized increases in intracellular calcium ions. *Nature.* 2000; 403:89–93. [PubMed: 10638759]

A Membrane potential

B Trans-epithelial potential

Figure 1. Membrane potential and transepithelial potential

A. Live cells maintain a resting electric potential across the cell membrane, inside negative.

B. An epithelial layer maintains a trans-epithelial potential (TEP) across itself.

Figure 2. A schematic diagram showing ion transporting molecules (ion channels, pumps and transporters) in a corneal epithelial cell. 1,2,3,4,5

Sodium, potassium and chloride channels, respectively; **6.** TRP channels; **7.** SCE - sodium/calcium exchangers; **8.** Sodium/organic co-transporters; **9.** Magnesium/calcium ATPase; **10.** NKCC - sodium/potassium/chloride co-transporters; **11.** Sodium/potassium ATPase; **12.** NHE - sodium/proton exchangers; **13.** Potassium/proton antiporters; **14.** Potassium/chloride co-transporters; **15.** Chloride/bicarbonate exchangers. After Levin et al. (Invest. Ophthalmol. Vis. Sci. 2006;47:306–316).

Figure 3. mRNAs and proteins of chloride channels in human corneal epithelial cells

A. RT-PCR products for members of the CLC family. Transformed human corneal epithelial cell samples (tHCE) were used. DNA ladder is on the left. Tissue specific markers are on the right. **B.** Protein expression. Reprinted with permission from: Cao et al. (Exp. Eye Res. 2010;90(6):771–779).

Figure 4. Chloride currents recorded in human corneal epithelial cells (HCE)

Whole-cell chloride currents recorded from primary cultured HCE (A) and transformed HCE (B). The currents were recorded in bath solutions containing 140 mM chloride (left) or 30 mM chloride (middle). The current-voltage relationships are shown on the right. The zero currents in the recordings are indicated by the dotted lines. Reprinted with permission from: Cao et al. (Exp. Eye Res. 2010;90(6):771–779).

CLC family members in human corneal epithelium

Figure 5. Immunofluorescence staining for CLC family members in human corneal epithelium
In red, CLC family members CLC-2, CLC-3, CLC-4, CLC-6 and CFTR show distinct distribution in human corneal epithelium. Tubulin and DAPI (in blue; labeling nuclei) staining were used as controls. **A.** CLC-2 is found at both the apical (top) and basal layers of corneal epithelium. **B, C.** CLC-3 and CLC-4 are expressed at the apical layer only. **D, E.** CLC-6 and CFTR are distributed preferentially at the apical layer with expression levels gradually decreasing toward the basal layer. Tear side is orientated toward the top and basal to the bottom. Reprinted with permission from: Cao et al. (Exp. Eye Res. 2010;90(6):771–779).

Figure 6. Electric potential in rabbit cornea

A. Schematic illustration of the cornea showing the epithelium (left), a portion of the stroma (st), and the endothelium (e). On the basis of a dye study, the following regions in the electrical profiles were identified: α , the outer membrane of the squamous cell; β , the inner membrane of the basal (b) cells; sw , the region between squamous (s) and wing (w) cells; and wb , the transition region between wing and basal cells. **B.** Average potential profile for the epithelium. The extracellular gradient in potential from the sub-epithelial stroma to the aqueous humour was less than 1 mV. **C.** Average resistance profile for the epithelium. The stroma and endothelium contributed only a few percent to total corneal resistance in these preparations. (Reprinted with permission from: Klyce. (J. Physiol. 1972;226(2):407–429).

Figure 7. Electric potential difference across corneal epithelium, and the generation of wound electric fields

A. An electric potential difference, positive at the basal side relative to the tear side, is generated and maintained by net directional electrogenic movement of ions (mainly sodium and chloride) through pumps and channels. **B.** Wounding which breaks the corneal epithelial barrier (left) leads to the generation of endogenous wound electric currents/fields. Active ion transportation which continues in the intact epithelium (black arrows) underlies the endogenous wound electric field (red solid arrow pointing towards the wound). The return path of the currents (flow of positive charge) is represented by the blue dashed arrow.

Figure 8. Measurement of endogenous wound electric currents in cornea

A. Photograph (side view) of mouse cornea wound. White arrows indicate wound edges. Red dots show measuring positions (a–k). Probe is in measuring position (f). Scale bar = 500 μm . **B.** Graph showing electric current profile across wound. Current is highest at wound edges, positions (c) and (i). **C.** Wound current vs. time. Rat cornea wound current increases dramatically after wounding and is maintained at high levels for several hours. A,B. Reprinted with permission from: Reid et al. (Nat. Protoc. 2007;2;661–669). C. Reprinted with permission from: Reid et al. (FASEB J. 2005;19:379–386).

Figure 9. Sodium and chloride fluxes are major components of endogenous electric currents and fields

A. The small outward currents in normal intact rat cornea were significantly reduced (actually reversed to become inward) in sodium-free solution (* $P < 0.02$). **B.** Corneal wounds had larger currents which were significantly increased in chloride-free solution (** $P < 0.001$). Sodium-free solution significantly reduced wound currents (# $P < 0.03$). Reprinted with permission from: Reid et al. (FASEB J. 2005;19:379–386).

Figure 10. Relative contributions of ions to wound current

A. Wound Timelapse. Overlaying wound electric current data (red) with compiled ion flux data (flux of all ions combined; blue) shows that electric current increase after wounding is due to an increasing ion flux (mainly chloride). **B.** Combined wound ion flux. Chloride is the major ion contributing to the normal (control) wound current. Enhancement of wound current by aminophylline (Amin.) is mostly due to stimulation of chloride flux, but also by reversal of sodium flux (inward to outward). Reprinted with permission from: Vieira et al. (PLoS One. 2011;6(2):e17411. doi:10.1371/journal.pone.0017411).

Figure 11. Applied electric fields guide migration of corneal epithelial cells

A. An isolated corneal epithelial cell responded to an applied electric field by migrating toward the cathode (0–1 h). Reversing the field (6 and 8 h) reversed the cell migration direction (red arrows). **B.** Corneal epithelial cell sheets migrate toward the cathode. Pseudo-color images show cathodally-directed migration. EF = 150 mV/mm. Arrow indicates the field vector. A. Reprinted with permission from: Zhao et al. (J. Cell Sci. 1996;109(6):1405–1414). B. Adapted with permission from: Zhao et al. (Invest. Ophthalmol. Vis. Sci. 1996;37:2548–2558).

Figure 12. Wound electric fields may serve as a predominant guidance cue for cell migration into the wound

A. Generally accepted guidance cues for directing epithelial cell migration into the wound include injury stimulation, chemotaxis/haptotaxis, contact inhibition release, wound void, population pressure, and mechanical forces. Endogenous electric fields oriented into the wound (red arrow) have been proposed to be one of the major guidance cues. **B, C.** An electric field directs migration of corneal epithelial cells in a monolayer model of wound healing. The applied electric field (150 mV/mm) can even make the wound open (0–96 min). Reprinted with permission from: Zhao et al. (Nature. 2006;442(7101):457–60). See online movie: <http://www.nature.com/nature/journal/v442/n7101/extref/nature04925-s2.mov>

Figure 13. Electric fields direct the migration of stratified epithelia in wound healing

A. Stratified corneal epithelial cells move into the wound under control conditions without an applied electric field. **B,C.** An applied electric field directs the migration of stratified corneal epithelial cells to move away from the wound (**B**) or into the wound (**C**) when the polarity of the applied electric field is reversed. Dotted lines represent the wound edge at the start and dashed lines show the wound edge after the indicated period of time. Arrowheads indicate wound edges and the direction of cell migration. EF in B,C = 150 mV/mm. Reprinted with permission from: Zhao et al. (Nature. 2006;442(7101):457–60). See online movie: <http://www.nature.com/nature/journal/v442/n7101/extref/nature04925-s5.mov>

Figure 14. Endogenous wound electric currents modulate corneal epithelial wound healing *in vivo*

A. Treatment with silver nitrate (AgNO₃) which enhances epithelial ion transport increased the endogenous electric current at the wound edge. In contrast, furosemide (furo) which inhibits ion transport resulted in a marked decrease in the endogenous electric current at the wound edge (**P<0.05). **B.** These treatments in turn increased or decreased wound healing rates of rat cornea *in vivo*. Wounds were labeled with fluorescein. For clarity, the wound edge is marked with dots. **C.** There is a strong correlation between wound healing rate (y-axis) and endogenous wound electric current (x-axis). Data are mean ± s.e.m. from 3–12 independent eyes. Adapted with permission from: Reid et al. (FASEB J. 2005;19:379–386).

Figure 15. Lens circulation

A. Pattern of electrical current flow around the lens. Currents exit at the equator and enter at both poles. **B.** Factors responsible for generating lens currents. The transmembrane sodium gradient, which is generated by sodium/potassium pumps, pulls sodium across fiber cell membranes from the extracellular to the intracellular space. Thus, inward radial current is extracellular (1), whereas outward radial current is intracellular (2). Sodium/potassium pump current density, IP ($1A/cm^2$), is highest at the equatorial surface, where the pumps are concentrated to transport the circulating flux of sodium out of the lens. Reprinted with permission from: Mathias et al. (J. Membr. Biol. 2007;216(1):1–16).

Figure 16. Cutting the lens capsule induces significant changes in lens electric current

A. Cutting the anterior capsule (left) as in cataract surgery significantly reduced outward equatorial currents (right). **B.** Cutting the lens capsule also induced a large inward current at the cut. A. Adapted with permission from: Wang et al. (FASEB J. 2005;19(7):842–844). B. Reprinted with permission from: Reid et al. (Nat. Protoc. 2007;2(3):661–669).

Figure 17. Applied EFs direct migration, expansion, orientation and elongation of primary human lens epithelial cells (PHLECs)

Perpendicular orientation and cathodal migration of PHLECs in an EF of 250 mV/mm; polarity shown. **A.** No EF (0 hr). **B.** EF for 5 h, cathode at left. Cells aligned perpendicular to the EF and migrated cathodally with lamellipodia facing the cathode. **C.** EF reversed for 5 h. LECs and their lamellipodia moved toward the new cathode. Reprinted with permission from: Wang et al. (Exp. Eye Res. 2000;71(1):91–98).

Figure 18. An applied EF directs migration, orientation and elongation of retinal pigment epithelium (RPE) cells

Human RPE cells migrate toward the anode (left) while also elongating and reorientating the long axis of their cell body. EF = 600 mV/mm.

Figure 19. An applied EF activates intracellular signaling pathways

Phosphorylation of Akt (Ser 473) and ERK in primary cultures of mouse keratinocytes in serum-free medium was stimulated in an EF of 150 mV/mm. Adapted with permission from: Zhao et al. (Nature. 2006;442(7101):457–60).

Figure 20. An EF activates PI3 kinase signaling asymmetrically in a migrating cell

Dynamic distribution of PHAkt-GFP, as a probe to detect PIP_3 production, was observed to concentrate at the leading edge of HL-60 cells. When polarity of the EF was reversed (170 s), PHAkt-GFP redistributed towards the new leading edge (arrowheads). Arrows show cell migration direction. Reprinted with permission from: Zhao et al. (Nature. 2006;442(7101): 457–60). See online movie:

<http://www.nature.com/nature/journal/v442/n7101/extref/nature04925-s6.mov>