The Spark of Life: The Role of Electric Fields in Regulating Cell Behaviour Using the Eye as a Model System

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Introduction

There have been many instances where clinical observation and insight have generated concepts and theories regarding physiological mechanisms which, when tested experimentally, have produced deeper understanding of how cells function and how development is regulated. For many years, it has been recognised that exogenous, and naturally occurring endogenous, electric fields exert some unexplained influence over how cells behave and interact with one another, at both the level of individual cells and the whole organism (for historical review, see Piccolino [1]). Recently, observations on the patterns made when corneal epithelial wounds (abrasions) heal over time, led Dua et al. [2, 3] to re-iterate the notion that electromagnetic fields, possibly related to the natural dipolar organisation of the eye, might have a bearing on how cells migrate to close the wound. In particular, in certain forms of clinical pathology such as hurricane keratopathy, the corneal epithelial cells adopt a migratory whorling pattern which can be reproduced in vitro by proliferating corneal epithelial cells in response to a low magnetic field (fig. 1) [2, 3]. This raised questions not only about whether electric fields might influence corneal epithelial cell migration but if so, how this effect might be achieved at the cellular level.

Electrotaxis or galvanotaxis has been a recognised phenomenon for over 100 years [reviewed in 4]. Previous
work by a number of groups over several years has shown that exogenously applied electric fields induce directed migration of a range of cell types and that neurites in particular showed a marked polarisation of growth cone guidance towards the cathode (fig. 2) [5]. Much further work has shown that these effects have a general applicability to many aspects of cell physiology, and developmental biology as well as influences on pathological processes such as wound healing, angiogenesis, and tumour growth and metastases, and the field has recently been extensively reviewed [6].

Mysterious Electricity: How Is This Physical Phenomenon Integrated into Cell Behaviour?

Electricity is a physical phenomenon that centres upon the behaviour of charged particles: it is a form of energy created by the movement of electrons, positrons, ions and other subatomic particles, all of which are the stuff of molecules and cells. As a result, the work done and the forces generated by this energy or by these moving particles can be described in simple mathematical equations, e.g. work, energy and force are described thus:

\[ F = q_1 \cdot q_2 / 4 \pi \varepsilon_0 \chi^2 \]

where \( F \) is the force, and \( q \) refers to the amount of charge on the particles. \( q \) is measured in coulombs (C). \( \chi \) is the distance between the charged particles, \( \varepsilon_0 \) is a correction factor relating to the ‘permitivity’ or conductivity of the medium, and \( K \) is the dielectric constant with a value of 1 when the electricity is flowing in a vacuum.

An electric field (E) is the ratio of the electrical force (F) to the size of the charge (q). E is a vector and is measured in volts.
The electrical potential, or potential difference (PD), is the difference in voltage which occurs from one side of an electric field to the other. The electric current (I) is the flow of charge across the field and is expressed thus:

\[ I = \frac{dq}{dt} \text{ (Cs}^{-1}) \text{ (amps)} \]

The electrical resistance is the ratio of the voltage difference between two points in the field and the current across the field.

These values have real meaning in biological systems; for instance the PD across the corneal epithelium (trans-epithelial potential difference, TEPD) is generated by the segregation of ions, mainly Na\(^+\) and Cl\(^-\), between the tear fluid and the corneal stroma and can be directly measured (it measures around 40–50 mV, inside positive) (fig. 3) [7]. Thus the epithelial layers of surfaces behave like batteries in which the TEPD is sustained by ionic pumps and channels working against the resistance of tight junctions (fig. 4).

When the epithelial layer is breached, the PD at the site of the wound falls to zero and current flows outwards from the wound edge creating an electric field with possible effects on the now activated cells, which are engaging in migratory and proliferative responses (fig. 5).

**Modelling the Effects of Electric Fields on Cell Behaviour in vitro**

Cell biologists have long studied the behaviour of living cells in vitro using various methods to evaluate motility, migration, adhesion and proliferation, most commonly on 2D protein-coated glass or plastic substrates. Early studies have shown that various cell types can respond to electric fields (EF) in vitro by migrating usually towards the cathode, some of the earliest being those of Ludloff (1895) (cited by Ogawa et. al. [8]) and Verworn (1896) (cited by Erickson and Nuccitelli [4]). Difficulties have arisen in early studies due to the instability of the electric charge under the influence of thermal currents, electrode deposits, and other disturbances in the fluid phase system, but these technical difficulties were over-
come in the 1950s by using agar salt bridges which 'damp' the system and permit a stable current flow (fig. 6) [9]. Modern microscopy techniques incorporate the cell culture system and electric current flow chamber, mounted in a heated chamber on the viewing system of an inverted microscope, thus permitting time lapse imaging, image capture and analysis, and various other customised modifications [10–12].

**Effects of EF on Epithelial Cells**

Initial studies using corneal epithelial cells in vitro showed that cell migration occurred directionally towards the cathode, that the effect was dependent on the strength of the electric field and was reversible by reversing the current flow, that the field force was within the range of endogenous EF, that migration was not only directional but that the rate of migration varied with the field strength, but importantly movement was minimal in the absence of serum (fig. 7) [10–12]. In addition, directedness was enhanced significantly on appropriate matrices such as fibronectin and laminin [13]. Since serum represented a major source of growth factors known to be involved in epithelial chemotaxis such as EGF and HGF, this implicated ligand-growth factor interactions in the electrotactic response. Indeed this proved to be the case since polarisation of EGF receptors on corneal epithelial cells could be induced cathodally when the cells were migrating in serum-rich medium in an electric field. Interestingly, actin cytoskeletal elements and EGF receptors interdigitated in their insertions into the cell membrane during this EF-induced response. These effects were also not artefacts of the procedures used to evaluate the cells by confocal microscopy since binding of fluorescently labelled EGF protein could be observed in a polarised distribution on the cathodal side of live unfixed cells in an EF [11, 13, 14]. The observed effects of EF on EGF receptors were duplicated with other receptor types such as FGF and TGFβ receptors which were distributed with their ligands cathodally.

**EF also Regulate Spindle Orientation during Cytokinesis**

During studies on cell migration lasting for prolonged periods it was observed that some cells underwent cytokinesis and cell division. More importantly, dividing nu-
**Fig. 7.** Corneal epithelial cells migrate to the cathode.

**Fig. 8.** The axis of cell division orients orthogonal to the EF.
clei appeared to orient themselves along the vector of the EF (fig. 8) with the axis of cell division orthogonal to the field. This was demonstrated quantitatively by showing that the vast majority of cells oriented their cytokinetic machinery within 60–120° of the EF vector and most of the dividing cells displayed a similar orthogonal polarisation. As with cell migration, these effects were associated with similar distribution of TGFβ receptor II distribution to the polar ends of the mitotic spindle and vectorial actin polarisation in the dividing cell. This novel observation led to speculation that this cellular response to small physiological EF could explain some aspects of the known effects of EF in determining cell fate during development and differentiation [6, 15].

**Small Physiological EF Direct Cell Movement during Wound Closure**

The above studies on single cells, particularly those demonstrating effects on cell division, suggested that EF might regulate the behaviour of groups of cells for instance cell sheets during embryonic development or wound healing. In order to study this, in vitro models of wound closure were developed using epithelial cell sheets and EF were applied at different times after generating a ‘wound’ in the culture system. As predicted, the cell sheet migrated cathodally as an intact monolayer of cells; remarkably, however, the ‘wound’ in the culture system could be opened or closed merely by reversing the current vector (fig. 9) providing the strongest demonstration so far of the effect of EF on cell behaviour [16]. During these studies, an in vitro organ culture model of corneal wound closure was developed which showed the integrated ‘tumbling’ behaviour of the different epithelial cell layers in 3D and providing new insight into how initial single cell epithelial coverage of the denuded surface of the fresh wound was followed by the cell crowding effect of neighbouring cells to fill in the gap [17].

**Endogenous EF Regulate Epithelial Cell Behaviour and Re-Innervation in Healing Wounds in vivo**

The above in vitro studies naturally led to attempts to demonstrate similar effects in vivo. The cornea provides an ideal model for direct visualisation of healing wounds since the size of the denuded area of corneal stroma can be evaluated using fluorescent dyes [18]. However, for assessment of the effects of EF, current methods do not permit exogenous application of electrodes to generate sustained EF, necessary for modulation of a healing corneal wound over several hours. The effects of EF on this model, however, can be evaluated indirectly. Since the PD across the epithelium is generated by channels and pumps maintaining an ionic concentration difference between the tear fluid and the corneal stroma, disruption of ion transport by pharmacological intervention can alter the magnitude of the PD.
positively or negatively, by a significant amount; and independently of any other (primary) effects of each individual agent. Using a concentric corneal abrasion technique, the rate of wound closure was observed to be significantly faster when agents were used which increased the transcorneal PD than when they reduced this potential (fig. 10). Interestingly, as an aside, one of the drugs (silver nitrate) which promoted wound healing and increased the TCPD has been used historically as a clinical pharmacological agent to treat ocular surface inflammation.

In addition to promoting wound closure, the later re-innervation of the closed wound was also seen to be affected differentially by EF-modifying agents. When corneal epithelial wounds are induced, neurite termini are disrupted, and repopulation of the wound with sprouting neurite extensions takes approximately 24 h. Interestingly, in regular linear wounds, neurite extensions align themselves perpendicular to the wound edge. This effect appears to be under the control of the wound-induced EF since agents which increase the TCPD induce faster, oriented re-innervation of the wound compared to untreated controls while agents which decrease the TCPD have the opposite effect [19].

EF-modulating agents also had remarkable effects on proliferating epithelial cells in the healing corneal wound in vivo: both the number of proliferating cells close to the wound edge and their orientation (axis of polarisation of the mitotic spindle) parallel to the wound edge were increased as the TCPD increased and decreased correspondingly when the TCPD was decreased, confirming the results of the in vitro studies [20].

These data demonstrated that small endogenous physiological EF not only affected cell migration but had significantly wider effects on cell behaviour by increasing proliferation rates, by directing orientation of nerve sprouts and probably have significant influence on tissue differentiation and organogenesis by ordering the alignment and orientation of dividing cells. This effect probably underpins the well-recorded effects of EF on processes such as neural plate migration and dorso-ventral positioning during development [for review, see 6].

![Fig. 10. Modulation of transcorneal potential difference (TCPD) and effect on closure of corneal epithelial wound. a Series of photographs of corneal abrasions treated with various agents which alter the TCPD (taken from [16]). b Correlation between area of wound and change in TCPD [16].](image)

**Do All Cells Migrate in Response to EF and Do They All Migrate to the Cathode?**

Almost all cell types tested in vitro respond to the application of a small electric field; this includes paramecium (one of the first cells tested [9]), amoebae, various bacterial cells as well as a wide range of mammalian cells. However, some cells do not show an electrotactic (i.e. directional) response to EF and this includes melanocytes. Melanocytes have an intrinsic random motility but in marked contrast to keratinocytes, they fail to show EF-induced directional migration [21].

This failure of some cell types to respond to EF suggests some degree of specificity in the response, rather
than a non-specific general response to a physical insult. Specificity in responsiveness is also suggested by alternate responsiveness, i.e. while most cells respond to small EF by migrating cathodally, some cells, particularly endothelial cells, migrate anodally (table 1). Furthermore, certain cell types are dually responsive such as lens epithelial cells. Previous studies have shown that the lens body generates a low level electric current flowing outwards from the lens equator and returning at the anterior and posterior poles. Strikingly, this endogenous current flow is reflected in the behaviour of cells in vitro: cells cultured from the anterior epithelium in vitro migrate cathodally while those from the region of the lens equator migrate anodally [22]. Recent studies by our group have shown that lens regeneration can be promoted if the anterior lens capsular wound and its associated epithelial layer is allowed to heal after lens removal [23, and Lois et al., in preparation]. This effect also correlates with restoration of the endogenous TEPD.

In addition to preferred directionality for some cell types, certain cell types can switch their directionality from cathodal to anodal depending on the strength of the applied EF. The physiological basis for the overall differential responsiveness of cells to EF remains unknown but may be reflected in the variability in cell surface proteins/receptors and their associated level of charged residues (see later).

### Role of EF in Tissue Differentiation/Morphogenesis

Several studies have implicated physiological endogenous EF in tissue morphogenesis during development [reviewed in 24, 25]. Similar effects are likely during tissue and organ repair in vivo. An in vitro correlate of such cell behaviour is angiogenesis in vitro. When endothelial cells are cultured in vitro, particularly in 3D culture systems, they progress through a series of stages: initially there is adhesion, migration and proliferation, followed by some level of apoptosis/inhibition of proliferation leading to formation of ‘angiogenic’ tubes, which develop branching and cell-fusing behaviour.

Endothelial cells cultured in vitro in the presence of EF proliferate more slowly, an effect which is mediated by inhibition of cyclin E and upregulation of p27kip1. In addition, they align themselves orthogonal to the EF, a behavioural response which is dependent on VEGF release [26–28]. Current studies in our group are now aimed at determining whether EF modify in vitro tube formation.

| Table 1. Direction of cell migration in response to EF |
|----------------|----------------|
| **Anode**       | **Cathode**    |
| Human granulocytes | Corneal epithelium |
| Corneal endothelium | Aortic endothelium |
| HUVECs           | Human RPE cells |
| Metastatic breast epithelial cells | Amphibian neural crest |
| Lens epithelium | Fibroblasts |
| Lens epithelium | Fish epidermis |
| Metastatic prostate cells | Lens epithelium |

1 Direction depends on source of cells and strength of EF.

### What Are the Molecular Mechanisms of Electrotaxis?

From the above it is clear that EF regulate several biological processes including cell migration, division, differentiation, secretion, intracellular signalling events, cell alignment and positioning with effects on diverse processes such as wound repair, development, angiogenesis, tissue differentiation, tumour metastasis and many other processes.

Precisely how EF modify cell behaviour and function is not clear. Considering one aspect of cell behaviour alone, namely cell migration, several receptor-mediated in vitro phenomena are already well-recognised processes, including chemotaxis [29] and haptotaxis [30]. Several theories on how electrotaxis is induced have been proposed. For instance, since serum and growth factors, as well as an appropriate substrate, are required in order to generate a significant electrotactic response, a trivial explanation could be that the movement of cells in an EF is simply another form of electrophoresis, as occurs with protein electrophoresis. However, this explanation is unlikely for two reasons: first, the predominant charge on most cells is negative, yet the majority of cells migrate towards the cathode, and in fact some cells reverse their polarity simply with a different strength of EF, as discussed above; in addition, almost all cells migrate by crawling on a surface while proteins migrating electrophoretically do so in solution.

Electrophoresis has been suggested as an indirect mechanism by acting on ligand-receptor interactions, in an analogous mechanism to spatial receptor-ligand interactions driving chemotactic responses [29]. However, most peptide ligands are negatively charged and during electrophoresis would migrate towards the anode, while
their cognate receptors translocate in the membrane to the cathode under the influence of an electric field.

Recently, mobilisation of intracellular calcium stores has been proposed as a mechanism for directed migration in response to an electric field [31, 32] and calcium-channel blockers have been shown to inhibit galvanotaxis [33]. A possible role for calcium has certain attractions. Cells migrate in extracellular fluid which contains calcium ions and in a polarised EF, the concentration of both intracellular and extracellular Ca$$^{++}$$ ions would tend to be greater towards the anodal side of the cell; at the onset of an externally applied electric field the distribution of anodally distributed Ca$$^{++}$$ ions would instantly be perturbed; using fluorescent dyes this might be demonstrable in vitro as a ‘calcium wave’ and current experiments by other groups are directed towards testing this hypothesis [32]. Ca$$^{++}$$ ions are extensively involved in many cell-signalling processes and cytoskeletal perturbations, which also occur during cell migration and activation, and these effects may reflect how EF modify intracellular signalling processes and cytoskeletal perturbations, which also occur during cell migration and activation, and these effects may reflect how EF modify intracellular events to initiate change in behaviour. According to Djamgoz and colleagues [32], passive accumulation of Ca$$^{++}$$ ions at the anodal side of the cell in an EF is sufficient to induce contraction of the cytoskeleton and propel the cell towards the cathode.

However, Ca$$^{++}$$ waves in themselves do not explain the molecular mechanism of EF-induced cellular changes. Clearly, some direct effect of electricity, and more specifically the flow of electric charge or current, must play a part in how cell behaviour is altered. Cells are composed of a protein-rich aqueous gel-like substance and are separated from a similarly protein-rich aqueous micro-environment by a lipid bilayer or cell membrane, which is also organised into discrete domains, themselves crowded with transmembrane proteins connecting the intracellular and extracellular spaces. Throughout this complex molecular arrangement, water molecules are dispersed and contribute extensively to the overall conformation and interactions of the many cellular components. For instance the role of ‘internal’ water molecules in stabilising intramolecular bonds in proteins is well recognised [34].

The arrangement of external water molecules has also generated considerable interest. Water is itself a charged molecule and exists as a dipole [35, 36], and some have suggested that application of an EF to cells in an aqueous environment generates forces acting on the surface of the cell through a form of hydrodynamic drag, termed electro-osmosis. This effect can occur because of the partitioning of the larger Na$$^{+}$$ ions externally and smaller K$$^{+}$$ ions internally, through various membrane channels and metabolic pumps. External sodium thus attracts to it a larger aqueous shell of water molecules than occurs internally and thus creates a stronger external dragging force in the presence of an electric field [37]. At a purely physical level, it has been calculated that ‘hydrodynamic friction’ is more relevant at low or moderately charged surfaces compared to the higher direct ‘electrofriction’ which occurs on highly charged surfaces [38]. To some degree, EF can exert variable forces on the cell depending on the nature of the charge on cell surface proteins and their interactions with Na$$^{+}$$ ions and their aqueous shells.

This notion has been modified and taken further by the concept of ‘structured water’ [39–41]. According to this paradigm, external water (i.e. water surrounding cells, proteins and other molecules in solution) exists in two general states: bulk water and surface or interfacial water. Bulk water exists in a form similar to the ion-associated aqueous shells described above due to the larger intermolecular distances of small molecules in solution. However, as water molecules come into contact with the surface of a protein, they become ordered in such a way that their positive charges line up along the negative surface charges of the protein, with further layers of water molecules organising themselves in alternating orientation around the protein molecule (fig. 11, 12). When several polymeric proteins are packed in solution, the structured water molecules allow an overall supramolecular structure with increasing intramolecular forces, leading to the formation of a polymeric gel.

A view of the cell membrane as a lipid bilayer with discrete domains containing a few suspended surface proteins and receptors has changed with the realisation that, in fact, the cell membrane is actually packed with protein molecules and contains few protein-free regions (fig. 13). Accordingly, cell surface proteins will associate extensively with water molecules both intra- and extracellularly. At the cell surface, ‘structured water’ molecules may actually determine the partitioning of ions by preventing intracellular movement of the larger Na$$^{+}$$ ions, and thus maintaining a transmembrane concentration differential (fig. 14). Intracellularly, cytoskeletal proteins, particularly at the cell cortex, will be packed together with ‘structured’ water molecules in a gel formation held together with Ca$$^{++}$$ ions. When the cell is exposed to an EF, there is likely to be an instantaneous disruption of the arrangement of structured water molecules due to the effect of the current flow on the dipolar water molecules. This affects not only the external surface but also the cortical gel,
Fig. 11. \( \text{H}_2\text{O} \) exists as a dipole and may adopt a structured network of water dipoles around the polymer (interfacial water).

Fig. 12. A polymer water gel: biopolymers with the network of ‘structured water’ constitute a gel, e.g. extracellular matrix and cytoplasm of cells.

Fig. 13. The cell membrane (lipid bilayer) is ‘crowded’ with many large trans-membrane proteins of various sizes, shape and charge.

Fig. 14. An associated cytoplasmic cortical gel, also with its network of structured water (plus \( \text{Ca}^{++} \) ions).

Fig. 15. Application of a DC EF disrupts the layer of structured water.

Fig. 16. Entry of \( \text{Na}^{+} \) ions and escape of \( \text{K}^{+} \) ions occurs when layer of structured/bound water is disrupted.
which loses its gel structure to become more of a sol, and releases large amounts of trapped Ca$^{++}$ ions (thus leading to the calcium wave). Disruption of extracellular structured water allows rapid influx of Na$^+$ ions with concomitant release of intracellular K$^+$ ions (fig. 15, 16).

Loss of gel structure (gel-sol phase transition) in the cortical cytoplasm leads to lamellipodial protrusion with migration of intramembranous charged proteins which accumulate at the leading edge of the cell (fig. 17). Meanwhile, electrophoretic effects of the EF on soluble ligand (e.g. EGF, TGF$a$, HGF [14, 16]) in the extracellular fluid will induce ligand movement towards the anode, thus increasing the stochastic interactions with the receptors accumulating at the cathodal pole of the migrating cell, moving in the opposite direction (fig. 18). Thus the multiple effects of a DC physiological endogenously gener-
ated EF may be mediated by disruption of the cell-associated network of structured water, leading to gel-sol phase transition and focal exchange of ions, involved in transcellular potential differences.

The possibility that such phenomena as receptor-ligand interactions and cell-signalling mechanisms underpin EF-induced process such as cell migration and wound healing has been largely corroborated by recent evidence showing the central role of PI3kγ/PTEN regulation of the effects of EF as they have been shown to underpin chemotaxis [16]. Although the above concepts relating to structured water are not revealed in these processes, they offer a general view of how such events may be mediated. There is considerable controversy over whether ‘structured water’ as described above actually exists [34] although the concept of interfacial water, previously described as ‘bound water’, has been mooted extensively for many years. However, in the context of the mechanism of electrostatic responses, the concept of structured water can now be formally tested.

**Clinical Applicability**

The central role of endogenous, physiological EF in cell behaviour may be of considerable clinical relevance. Effects on the developing embryo and fetus are self-evident but more directly, the use of electro-stimulation or inhibition (as the case may be) may assist in the healing and repair of wounds and damaged tissues such as in the eye, CNS, skin, bone and many other sites. Even the possibility of inducing regeneration in tissues such as neural tissue and lens can be entertained. Applicability in the field of therapy/prevention of tumour metastases can be envisaged. From both a fundamental physiological investigative standpoint and a clinical practical use, electricity may offer marvellous insights into the function and behaviour of living tissues.

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**References**


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