Integrative Biology

CRITICAL REVIEW

View Article Online
View Journal | View Issue

Cite this: *Integr. Biol.*, 2014, 6, 817

Influence of electrotaxis on cell behaviour

Barbara Cortese,*[ab] Ilaria Elena Palamà,[a] Stefania D'Amone[a] and Giuseppe Gigli[acd]

Received 23rd June 2014,
Accepted 17th July 2014

DOI: 10.1039/c4ib00142g

www.rsc.org/ibiology

Understanding the mechanism of cell migration and interaction with the microenvironment is not only of critical significance to the function and biology of cells, but also has extreme relevance and impact on physiological processes and diseases such as morphogenesis, wound healing, neuron guidance, and cancer metastasis. External guidance factors such as topography and physical cues of the microenvironment promote directional migration and can target specific changes in cell motility and signalling mechanisms. Recent studies have shown that cells can directionally respond to applied electric fields (EFs), in both *in vitro* and *in vivo* settings, a phenomenon called electrotaxis. However, the exact cellular mechanisms for sensing electrical signals are still not fully well understood, and it is thus far unknown how cells recognize and respond to electric fields, although some studies have suggested that electromigration of some cell surface receptors and ion channels in cells could be involved. Applied electric fields may have a potential clinical role in guiding cell migration and present a more precise manageability to change the magnitude and direction of the electric field than most other guidance cues such as chemical cues. Here we present a review of recent studies used for studying electrotaxis to point out similarities, identify points of disagreement, and stimulate new directions for investigation. Insights into the mechanisms by which applied EFs direct cell migration, morphological change and development will enable current and future therapeutic applications to be optimized.

Insight, innovation, integration

Electrical cues can affect cellular behaviour (electrotaxis) and suggest a possible practical therapeutic strategy to activate signalling pathways that would contribute to control cell functionality. Here, we discuss the possible role of various regulatory mechanisms which occur under electrotaxis stimulation, along with their impact on cells. Our discussion on the role of extracellular electrical fields in controlling behaviour, cancer metastasis, neuron guidance and wound healing points out to identify similarities, aspects of disagreement of recent discoveries and stimulate new directions for investigation. Further fundamental investigations are still required to elucidate the bases of the responses to electrotaxis in different cells, and studies using improved experimental methodologies will help clarify the effect of electrically stimulated cells and medium conditions.

Introduction

The complex architecture of the microenvironment in which cells reside and interact exhibits a multifaceted milieu of physicochemical cues which play a critical role in various sets of cellular processes, in particular for the capacity of driving dynamic cellular phenomena.[1–6] The mechanisms underlying cell motility are extremely complex.[7] Considerable experimental research has contributed to increase the knowledge that provides insights into the cellular and physiological mechanisms by which cells respond to stimuli from their environment. Much is still being learned about the known sensitivity of cells to various physical and chemical stimuli, which cause a directional motion or "-taxis" (Fig. 1). Over the past few decades, multiple experimental and theoretical studies have reported on the response of living cells (orientation and migration) to the variation of gradients of soluble or surface attached chemicals (chemotaxis and haptotaxis, respectively),[8–11] topographical surface features (contact guidance),[12–14] light intensity (phototaxis),[15] extracellular tension (tensotaxis),[16] electrostatic potential (galvanotaxis),[17] gravitational potential (geotaxis),[18] or focused on the rigidity of the substrate (mechanotaxis or durotaxis),[19–21] or concurrent combination of several cues.[22] Nonetheless it is still not clear how this basic motility is coupled to the environmental cues and how specific stimuli may elicit

[a] *NNL, Institute of Nanoscience CNR, Via Arnesano, 73100 Lecce, Italy. E-mail: barbara.cortese@nano.cnr.it*
[b] *Department of Physics, University Sapienza, P. le A. Moro 5, Rome, I-00185, Italy*
[c] *Department of Mathematics and Physics, University of Salento, Via Monteroni, 73100 Lecce, Italy*
[d] *Center for Biomolecular Nanotechnologies (CNB) of Italian Institute of Technology (IIT), Via Barsanti, 73010 Arnesano (LE), Italy*

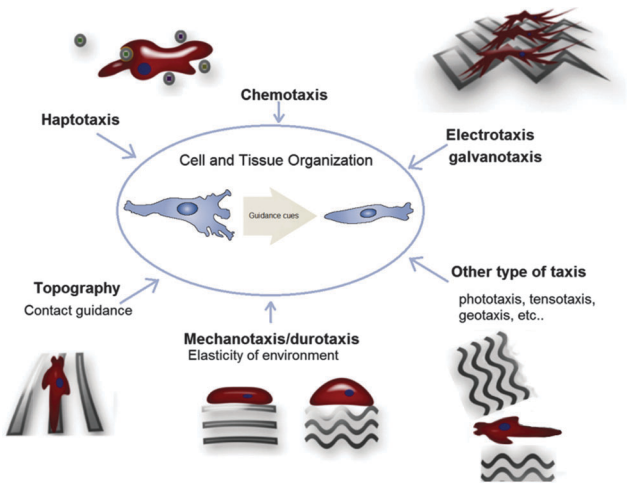

Fig. 1 Directional locomotion of cells is crucial in many processes, such as immune response and wound healing. The control of cell migration can be induced by means of various taxis (moves to/away from stimulus) as schematized.

certain responses. Also the inherent diversity of cells may include responses from background or collateral stimuli unintentionally created. Continuous efforts are invested to engineer substrates to isolate relevant stimuli which ideally may provide a pathway to obtain an absolute control over the stimulating signals to the cells. Among the above-mentioned gradients, chemotaxis may be the most well understood and is most commonly seen in *in vitro* experiments. Migration of cells through chemotaxis and haptotaxis can be controlled by modulating surface chemistry and surface adhesion proteins or substrate-bound chemoattractants of the extracellular scaffold, matrix or substrate. On the other hand, physical cues like topography can also provide important regulation of cell motility by providing contact guidance cues through geometrical constraints of the adhesion sites, inducing alignment or directional growth of cells.[12–14] Also, the rigidity of the surrounding ECM plays a role in regulating cell behaviour and distinctly influences cell migration. The tendency of cells to migrate from a soft to rigid matrix in the absence of any soluble biochemical stimuli (*e.g.* durotaxis) has been demonstrated in a large number of experimental observations, but is still poorly understood.[20,21] Significant research has now been conducted to observe the behaviours of cells in the presence of electric fields (EFs). Electrotaxis (or galvanotaxis) is the phenomenon by which cells migrate directionally in response to electric stimulation.[23] Increasing evidence has shown a strong influence of this phenomena on a number of basic processes, such as embryonic development,[24,25] directing nerve cell growth,[26,27] as well as pathophysiological conditions, as in wound healing,[28–30] angiogenesis and directing metastatic cancer cells.[31]

Taking advantage from the effects induced by electric field stimulation and being easy to control at the microsecond timeframe, electrotaxis is an ideal cue to modulate and contribute to cellular positioning in multiple physiologic settings. Moreover the magnitude and direction of the electric field can be more precisely and quickly changed than most other guidance cues such as chemical cues. Although electrotaxis may seem unlikely to occur in an *in vivo* situation, it is somewhat implicated in cell movements that occur throughout functional processes such as development, morphogenesis, and regeneration. For example, disruption of epithelial integrity leads to a spontaneous electric field oriented towards the wound (0.4–1.4 V cm^{-1}) and surrounding epithelial cells migrate directionally to cover the wounded tissue in a process that can be disrupted by interfering with the electric fields.[23,33]

The preferential direction of migration during electrotaxis varies among cell types and under different experimental conditions.[23,32] Several cell types change their initial direction of migration when an external electric field is applied and even revert their current migration direction when the electric field is reversed in polarity, supporting the fact that the electrical stimulus exerts higher control on cell migration than chemical guidance.[23,35] Mounting research supports the hypothesis that electrotactic cues seem to perform on the same downstream motility pathways as chemotaxis and general cell migration inducing reorientation of cell surface and signalling molecules.[34,35]

Table 1 summarizes the specifics of the wide array of studies of different cell types and organisms utilized and the use of different types of electric fields. Electrotaxis depends on the strength of the applied cue and on the presence of molecular activators. The related electric fields used are in the range of 0–15 V cm^{-1}.[36] A higher electric field strength can disrupt cell membranes (electroporation) and cause Joule heating damaging inevitably the membrane proteins.[37]

Although this field is still an emerging area of research, mounting evidence suggests that electrotactic sensing and the resulting migration or movement hold great potential in directing cell migration. It is however still a highly complex process, dependent on cell type, medium conditions, and diverse intracellular signalling cascades. Moreover, the molecules responsible for selecting the migration direction in electrotaxis have not been identified and the underlying molecular mechanisms governing these processes remain elusive. Extensive further research is needed to elucidate the physiological mechanisms for electrotaxis in different cell types and organisms. In this review, we discuss the possible role of various regulatory mechanisms which occur under electrotaxis stimulation, along with their impact on cells. We direct our discussion to the role of extracellular electrical fields and their roles in controlling behaviour, cancer metastasis, neuron guidance and wound healing. What becomes apparent from these studies is that external electrical cues produce emergent behaviours. Moreover, issues concerning the way in which these processes are controlled and coordinated, and their consequences on the cell shape and the types of cell movements, are among the many intriguing questions that still have to be answered to. This review details part of the current literature of electrotactic cues from both the hypothesized physical intracellular mechanisms that produce an electrotactic response and the cell motility perspectives. Recent advances in our understanding of electrotaxis, detailed in this review, can provide new insights essential

Table 1 A summary of various responses and mechanisms involved in different cells to various electric fields[a]

Cell type	V cm^{-1}	Time of exposure	Electric field	Direction	Mechanism involved observed	Ref.
Dermal fibroblasts	0.5–1	5 h	DC	Anode	PI3 kinase signalling	40
Schwann cells	0.03–1	2 h	DC	Anode	Voltage dependent	41
Breast cancer cells	0.5–4	1–3 h	DC	Anode	Induced polarization of epidermal growth factor receptor (EGFR)	104
Lung adenocarcinoma cell line	3	2 h	DC	Anode	Serum and EGFR independent	109
Prostate cancer cells, rat	0.1–4	6 h	DC	Cathode	Surface charge, VGSCs dependent	89
Granulocytes, human	10	10 min	DC	Bi-directional	Calcium dependent mechanism	81
Keratinocytes, human	1	1 h	DC	Cathode	Epidermal growth factor (EGFR) mechanism	60
C3H10T12 mouse embryo fibroblasts	1–10	30 min–2 h	DC	Cathode	Calcium dependent mechanism	72
Keratinocytes, human	2	1–3 h	DC	Cathode	Voltage gated sodium channel dependant mechanism	133
3T3 fibroblasts, rat	1–4	30 min	DC	Cathode	No influence of external calcium	69
Xenopus neurons	1	12 h	DC	Cathode	No influence of external calcium	70
Spinal neurite, amphibian	1–1.4	8–12 h	DC	Cathode	Substratum dependant and of surface charge density and adhesivity of the substratum	117
Osteogenic cell	10–15 and $\leq$5	5 h	DC	Calvarial osteoblasts migrated to the cathode. SaOS-2 cells migrated towards the anode.	Depolarization-activated calcium dependent mechanism	35
Dictyostelium cells	12	30 min	DC	Cathode	Membrane depolarisation	73
Keratocyte	0–10	1 h	DC	Cathode	Electrophoretic movement of cellular membrane components	50
MDCK (Madin–Darby canine kidney)	5	2 h	DC	Cathode	Leader cell dynamics	34
Human pancreatic carcinoma cells	100 ns, 30k	6 min	Nanosecond pulsed electric fields	NA	Stimulate an increase in reactive oxygen species (ROS), proportional to the number of pulses applied. The ROS increase is inhibited by blocking the increase in intracellular Ca^{2+}	83

[a] DC = direct current; AC = alternating current.

for establishing EF cell-based developmental models and for the generation of clinically relevant populations for cell therapy. Future studies will no doubt define specific signalling pathways for the guidance of cells and uncover the molecular mechanisms that regulate their directed migration.

Cellular behaviour and signalling of electrotaxis

As aforementioned, electrotactic behaviour has been observed in many cell types (Table 1). Motile cells exposed to an external direct current electric field will reorient and migrate along the direction of the cathodal or anodal electric potential. Cathodal electrotaxis behaviour is generally more commonly observed (*e.g.* bovine corneal epithelial cells, bovine aortic vascular endothelial cells, human retinal pigment epithelial cells, human keratinocytes, amphibian neural crest cells, fish epidermal cells and metastatic rat prostate cells).[21–52] However, a few studies including metastatic human breast cancer cells,[38] osteoclasts,[39] dermal fibroblasts,[40] and Schwann cells,[41] have reported an anodal behaviour. Induced polarized effects on ion transport, asymmetrical distribution on cellular membrane components[42] or redistribution of charged cell-surface molecules due to electrotaxis effect have also been reported.[37–39] These opposite responses indicate that the effects of EFs on cells are cell-type and species specific. Thus, cell electrotaxis needs to be established experimentally on individual basis.

Mechanism of intracellular motility pathways

In general, several theories and mechanisms on how EFs affect cells directly are debated.[46] Fig. 2 shows an overview of our present understanding of the mechanism underlying cell electrotactic behaviour. What is known is that electrical fields seem to be sensed through matching pathways involved in chemotaxis but do not operate simply by modulating the chemotactic systems of the cell.[47] To what extent though chemotaxis and electrotaxis principles or mechanisms are shared or overlap? There are situations *in vivo* where chemotaxis clearly coexist with endogenous EFs (*i.e.* cells engaged in wound healing migrate in the presence of a laterally oriented EF, which may be capable of inducing directed cell movement and a local chemical gradient). Apparently shared common intracellular signals are mediated through MAPK and PI3K signalling pathways.[48,49] Inhibition of PI3K as well as alternative signalling pathways such as vascular endothelial growth factor VEGF, extracellular signal-regulated kinases ERK and Rho/ROCK protein kinase was shown to disrupt the electrotactic response of cells.[23,49] Exposure of cells to electric fields induce rapid and sustained phosphorylation of extracellular-signal-regulated kinase (ERK), p38 mitogen-activated kinase (MAPK), Src and Akt on Ser 473. Phosphorylation of the Janus kinase JAK1 remained unchanged, indicating that electric currents activate

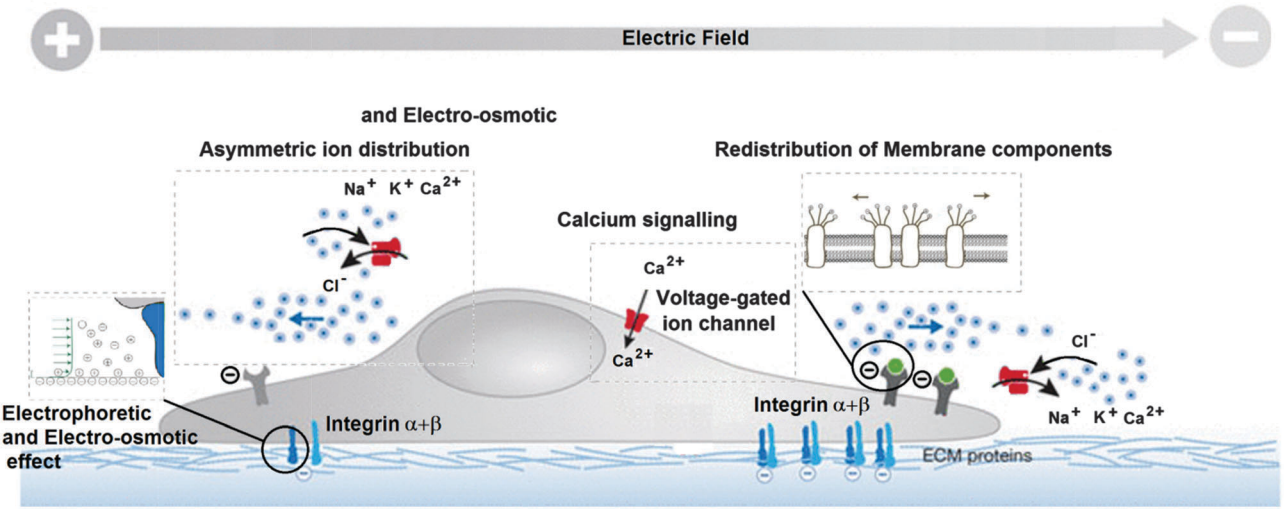

Fig. 2 Visual schematization of a hypothetical mechanism by which cells respond to electric fields.

only defined signalling pathways. The overall implication is that electric fields act downstream through chemotaxis sensors but possibly upstream of PI-3-kinase activation and polarization.[50] In a related study, Sun *et al.* investigated the intracellular mechanisms determining directionality in electrotaxis.[51] These authors proposed that cell motility can be modelled by a strong PI3K-dependent pathway that defines the front at the cathode and the myosin-dependent pathway that defines the back at the cathode which compete to determine the direction of migration in an electric field. Although it is not clear why the PI3K pathway dominates, these findings point to a model in which cells use actomyosin contractile networks as a compass to orient themselves in an electric field.

In chemotaxis the cell responds to a polarized gradient of ligand extracellularly by translocating the membrane and receptors in equal proportion to the leading edge, creating a greater area of highly folded membrane at the front. This induces activation of signalling molecules in a gradient from the leading to the trailing edge, and this determines directional migration. Similarly, an induced accumulation of membrane lipid and associated receptors cathodally/anodally in an EF would induce stronger signalling at the cathodal/anodal side, respectively (Fig. 2). This signalling pattern is analogous to the signalling pattern caused by a chemoattractant gradient.

It may be possible that electrophoresis of a chemoattractant is involved and that the pathways converge on the locomotory machinery of the cell. This strengthens the concept that molecular mechanisms canonical to chemotaxis but also the potential signal crosstalk between electrical and chemical migration cues are shared and exist in the spontaneously motile cells acting as an internal guiding system when provided with an external directional cue from an electric field. On the other hand the signal transduction pathways of chemotaxis and electrotaxis do not completely overlap. Inhibition of the phosphatase and tensin homolog (PTEN) signalling pathway has been shown to block the chemotactic response while improving the strength of electrotactic response.[23] Recently Li *et al.* provided evidence that the superoxide induced by direct current EF is a key element in mediating electrotaxis through the activation of ERKs and reorganization of the cytoskeleton.[52] Several more unknown pathways may be involved in electrotaxis, including sensing of the electric field, determining the direction to migrate, maintaining a persistent direction of migration, and the controlling of cell adhesion and migration speed. How does this affect the cell directionality? Directed cell movement requires two essential events: a localized cue and an asymmetrically activated intracellular signalling pathway. Assuming that the localized cue is provided by a gradient in voltage, this will create a spatial difference between a cell's leading edge compared to its trailing edge. The asymmetrically activated signalling pathways are provided by a high expression level of epidermal growth factor receptor EGFRs. EGFR kinase activation and autophosphorylation initiate intracellular signalling pathways which contribute to cell motility.

It therefore becomes increasingly clear that delineating the key mechanism by which cells respond to electric fields and identifying the "EF sensor" and the signalling proteins involved in the electrical directing may unravel the guiding role of the electric field.

Redistribution of charge of the membrane components

The search for a mechanism of electrotaxis also suggests that the redistribution of receptors, as a process of coupling electric fields to direct migration, may be possible. The immediate target of electric fields is likely to be the plasma membrane. The field could act to redistribute charged lipid and protein molecules within the plasma membrane or modify the membrane potential at the ends of the cell facing the poles of the electric field.[53] Biophysically speaking, the membrane potential is altered by the hyperpolarizing and depolarizing effect of the anode and cathode facing sides of the cell, respectively, which in turn can modify the movement of ions across the plasma membrane.[53] Asymmetrical distribution of cell membrane receptors, ion channels, receptor tyrosine

kinases, and integrins are thus driven in combination of an *in situ* electrophoretic effect (which is the lateral movement of charged components on the membrane driven by the EF) and an electro-osmotic effect, in which charged membrane components are swept by electro-osmotic flow (the flow across a material initiated by an applied electric current) generated by the EF.[28,30,38,54,55] Electrostatic and electro-osmotic forces will therefore apply mechanical force on the cell or on tension-sensitive cell-surface components triggering the initial signal of electrotactic movement of cells.[56–59] Chemically speaking, the activation of asymmetrically distributed membrane components (*e.g.* membrane receptors, ion channels, receptor tyrosine kinases and integrins) would lead to a polarized cellular signalling which conveys the directional cue.[28] It may be that the interaction between electrophoresis and electro-osmosis controls EF-directed migration, but little literature supports this theory. Very recently the role of the charge of membrane components as a crucial factor has been confirmed by Allen's group. Allen *et al.* hypothesised that electrophoretic redistribution of membrane components of motile cells in zebrafish keratocytes is the primary physical mechanism for motile cells to sense an electric field, ruling out directional sensing from electro-osmotic fluid flow.[50] Although electrotaxing cells reoriented in response to shear stress imposed by laminar fluid flow, the electro-osmotic fluid flow was not adequately strong to reorient electrotaxing cells. They argued that asymmetric transmembrane potential did not act as an electrotactic sensor and showed that directional motile response of keratocytes cells to the cathode in an electric field does not require extracellular sodium or potassium. They also showed that the motility of cells was insensitive to membrane potential as well as to perturbation of calcium, sodium, hydrogen, or chloride ion transport across the plasma membrane. The authors proposed that electric fields induced an asymmetric activation of intracellular signalling pathways, (including one dependent on PI3K) which in turn influenced cell motility. Keratinocyte experiments described by Fang *et al.* suggested that electrophoretic clustering of EGF receptors on the cathodal side of the plasma membrane was involved in regulating the direction of migration, but blocking with an inhibitor highly specific for EGFR prevented EGFR relocalization and abolished the directionality of keratinocytes migration, overriding the effect of the voltage gradient.[60]

Proper cell polarization is therefore a prerequisite for directional cell migration. The establishment of cell polarity suggests that there is a difference in molecular processes at the front and the rear of a migration cell. The receptors that transduce the EF differ according to the cell type. Still further experimental evidence is required to clarify the electro-migration ability of different cell types, the possible polarization/re-distribution of receptors on the cell surface by the electric field and their interplays may significantly allow the cell to sense the direction of the electric field and migrate directionally.

It is generally accepted the hypothesis that EF-induced membrane receptor polarization results in directional ligand sensing[44] or initiation of ligand-independent activation.[61,62] The signalling discrepancy initiates polarized intracellular signalling cascades leading to directed cell migration. Upon recognition of extracellular ligands, cell surface receptor tyrosine kinases (RTK) are activated and are key regulators of extracellular signalling. RTK activation has not been systematically examined, however studies showing various RTKS have been reported in the electrotaxis of different types of cells.

Therefore a more methodical and systematic approach should be carried out in the future to elucidate the cellular signalling network under the EF stimulation and identify the molecular mechanism for electrotaxis in different types of cells.

Calcium signalling role

Many studies are gradually converging on the role of intracellular calcium distribution, showing its importance in field-directed cell shape changes and movement.[28] Because $[Ca^{2+}]_i$ is known to regulate processes such as signal transduction, cytoskeletal reorganization, cell orientation and migration, and cell differentiation and proliferation, changes in $[Ca^{2+}]_i$ have been hypothesized to mediate the molecular and cellular effects induced by electric fields. Under the influence of externally applied electric fields, Ca^{2+} concentration increases significantly and is retained for the duration of exposure.[63] The role of calcium ions in the motility process leads to various possible calcium pathways into the cytoplasm voltage-gated calcium channels (VGCCs), Na^+/Ca^{2+} exchangers (NCX), plasma membrane Ca^{2+} ATP-ases (PMCAs), leakage channels, internal rearrangements by calcium ''waves,'' or eventually, calcium release (uptake) from internal stores.[46,64] Other intracellular signalling cascades reported in electrotaxis include PI3K, cAMP, PTEN, ERK1/2.[65–68] Voltage-gated Ca^{2+} channels play a critical role in the control of selective Ca^{2+} flow down their electrochemical gradient in response to a change in the membrane potential in various cells. To activate VGCCs, the electric field strength must be sufficiently large to induce a potential difference on the order of 100 mV. Furthermore, voltage-gated Ca^{2+} channels may open through action of the depolarizing potential on the cathodal side.

Increases in $[Ca^{2+}]_i$ can also be mediated by activation of stretch-activated cation channels (SACCs), which, upon opening, permit the influx of cations including Ca^{2+}.[69,70] $[Ca^{2+}]_i$ increases have also been proposed to mediate electric field-induced microfilament reorganization.

Some researchers purported that cells migrating toward the cathode have a cathode high $[Ca^{2+}]_i$ on the leading edge of the cell.[11,40,46,54] This side of the cell then contracts, thereby propelling the cell toward the cathode. This process continues until the voltage-gated Ca^{2+} channels near the cathodal side depolarise (open), thereby allowing Ca^{2+} influx.[38,71] Thus this causes some cell types to reorient towards the cathode, such as embryo fibroblasts,[72] human retinal pigment epithelial cells,[73] while other types towards the anode, like metastatic human breast cancer cells.[74] Using Ca^{2+} channel blockers (*e.g.* D-600, verapamil), Cooper and Nuccitelli reverted the electrotactic response influencing the directionality of migration with replacement of external Ca^{2+} by Mg^{2+}.[75,76]

Shanley and co-workers showed that in the electrotactic Dictyostelium cells, cAMP induced transient calcium flux whereas the $[Ca^{2+}]_i$ increase to dcEF was more sustained.[77] They also discussed the different characteristics of calcium response to direct current EF in electrotaxing cells showing a possible signalling divergence between chemotaxis and electrotaxis. Some possible factors such as the delayed calcium spikes upon dcEF stimulation and passive calcium influx were explained but the underlying mechanisms remain to be determined. Morita and his group showed that EGF pre-treatment of cells promotes calcium oscillations in response to glutamate, ATP, or thimerosal (which directly activates the inositol-1,4,5-triphosphate receptor) in astrocytes.[78,79] The epidermal growth factor (EGF) was also shown to directly induce transient $[Ca^{2+}]_i$ increase in breast cancer cells.[80] Interestingly, Franke *et al.* showed a bi-directional Ca^{2+}-dependent electrotactic response of granulocytes, although the phenomenon was not quite clear. They observed a movement towards the anode of the electric field in medium containing a higher Ca^{2+} content (2.5 mM), while in medium with a lower Ca^{2+} content (0.1 mM) the granulocytes moved towards the cathode.[81] In addition to Ca^{2+} ion channels also Na^+ and the associated downstream cellular signalling have been implicated to an electrotactic response.[38,73,77,81,82] The cellular response to nanosecond pulsed electric field (nsPEF) exposure was shown by Nuccitelli and his co-workers to stimulate an immediate increase in intracellular Ca^{2+} and an increase in reactive oxygen species (ROS) in human pancreatic carcinoma cells.[83] This ROS increase was proportional to the number of pulses applied and the increase was inhibited by blocking the increase in intracellular Ca^{2+} and by the presence of the anti-oxidant, as well as the presence of Ca^{2+} chelators in the intracellular and extracellular media. Thus the Ca^{2+} increase is probably a key step required for ROS generation. Onuma *et al.* showed that by reducing calcium influx suppressed electric field-induced cell-shape changes and preferential alignment of embryonic mouse fibroblasts.[84]

However despite the data confirming perturbations of intracellular calcium dynamics in cells exposed to EFs the importance of its role still remains debated. In fact, investigations reported by Borys suggested that internal Ca^{2+} signalling is not strictly required for EF-directed motility, and only when present it appears to act in a controlling role.[46] He also implied that the concentration difference in the Ca^{2+} ions between the cathodal and anodal sides of the cell may result from electrical repulsion of intracellular Ca^{2+} ions by Na^{2+} ions entering through the VGSCs on the cathodal side.[85] Brown followed by Palmer confirmed a Ca^{2+}-independent control of electrotaxis.[64,69] They reported that fibroblasts, in response to electric fields, showed a redistribution of the integrins which was a calcium-independent process, apparently without voltage-gated Ca^{2+} channels in the plasma membrane. They concluded that an alternative mechanism involving the lateral electro-migration of cell surface glycoproteins was implicated in cell-substratum adhesion. No voltage gated calcium influx and no extracellular calcium requirement was observed, showing that the fibroblasts exhibited cathode-directed migration regardless of the removal of extracellular calcium and the presence of EGTA. Therefore, which is the role of calcium in electrotaxis? Because calcium is a well-characterized signalling second messenger and is involved in a myriad of functions including secretion, neurotransmitter release, and muscle contraction, the modulation of calcium in cells or tissues could have other applications. Therefore an undisputed role for calcium in directional migration is yet to be confirmed. It may be that the earlier reports may not have been able to capture or analyse the Ca^{2+} transients at the leading edge. Thus, more careful time-lapse analysis of Ca^{2+} gradients should be performed on cells migrating in an applied EF.

Asymmetric membrane potential

Many studies also concord on the hypothesis of a gradient, electrically induced in the membrane potential, V_m, which can affect asymmetrically ion fluxes that could signal to the motile mechanism.[59,86–88] Membrane potential depolarization by EF has been shown to directly activate voltage-gated ion channels or voltage sensitive proteins.[88–90] Gao and his group showed that depolarization of the V_m, significantly inhibited electrotaxis. Moreover they observed that the electrotaxis of *Dictyostelium* cells was significantly altered by the pH of the bathing solution. Cells in solutions of a neutral pH (*i.e.* 6.5) had more negative V_ms and showed a major electrotactic response. Cells in basic or acidic solutions (pH 5.0 or pH 9.0) showed significantly reduced electrotaxis. What is more, they showed that increasing the extracellular $[K^+]$ significantly inhibited electrotaxis. They suggested that the reduced V_m might be responsible for Ca^{2+} signalling inhibition and thereby affect electrotaxis.[71]

Özkucu showed that a dcEF of 10 V cm^{-1} affected osteogenic cells by hyperpolarizing the membrane facing the anode by approximately 50 mV and depolarizing the cathodal side by the same amount.[35] They observed that increasing $[Ca^{2+}]_i$ can activate myosin light chain kinase, which in turn triggers actin-activated myosin ATPase, a major regulator of cell contraction. This suggests that regulation of intracellular Ca^{2+} levels is a key for dcEF induction of cell migration responses. Notably, directed motility and intracellular Ca^{2+} kinetics in osteoblast-like cells can be altered by dcEFs with different strength. In order to extend these ideas, research should necessary attempt to identify the exact cascades and which channel translating exogenous and endogenous electrical signals involved in the variety of intracellular responses by electric fields.

The effort to elucidate the different mechanism controlling the rate of cell motility from controlling directionality is complicated by the fact that the electrical properties of cells depend on the mode, strength, and duration of the applied electrotactic cue as well as the electrotactic chamber. Most of the experimental evidence is obtained from single chamber experimental systems which have scarcely changed in the past decades. Typically, *in vitro* systems adopt electrodes which couple current into a shallow channel containing cells.[26,43–45] The electrodes usually used to apply an electric field across a channel are agar bridges that are placed in an electrolyte for the purpose of preventing nonreversible cytotoxic reactions, such as hydrolysis, from occurring in the medium next to the

cells.[25,73] Although effective, this method produces only unidirectional fields, is time-consuming, and is difficult to standardize owing to assembly variability. Another important issue with this method is the gradual accumulation of cytotoxic species when reduction–oxidation reactions occur at the anode and cathode, so agar salt bridges are used to isolate the reactive by products from the cell culture. Microfluidic chambers hold great promises for better field shaping and control in order to analyse and compare multiple taxis and electrotaxis of cells. Most of these devices are fabricated from polydimethylsiloxane (PDMS, a transparent flexible polymer) or PMMA and provide excellent scalable environments for manipulation, immobilization and live imaging of cells.[91–94]

From the numerous studies, we conclude that a much wider range of possible signalling mechanisms must be examined to uncover better the mechanisms of electrotaxis.[89–91] More studies should look at the mechanism controlling the rate of cell motility and directionality. To address this issue, we need to observe more cell morphological rearrangements and intracellular calcium dynamics in response to EFs. It is also possible that different cells use different ionic mechanisms to influence the cytoskeleton and move within dcEFs. It may involve a stretch activated channel which may contribute to the local $[Ca^{2+}]_i$ increase due to the mechanical strain induced by the EF, and therefore cause a depolarization-activated calcium response. Still these are yet to be identified. Genetic and proteomics-based

Table 2 Synopsis of the mechanism involved, hypothesized to respond to electric fields

Electrotactic mechanism	Cell type	Outcome	Observation	Ref.
Membrane potential depolarization	Keratocyte, Dictyostelium cells	Activated voltage-gated ion channels or voltage sensitive proteins, as well as altering transmembrane ion fluxes as calcium ion channels and EGR	Hypothetically serves as a stimulus for cell locomotion	71
Activity of sodium channels	MAT-LyLu rat prostate cancer cells sodium–hydrogen transporter, NHE1, in WT-PSN broblasts, heK 293 cells, SaOS-2 and Calvarial osteoblasts	VGSCs are involved, Sodium–potassium adenosine–triphosphatase (NAKA) and sodium–hydrogen exchanger (NHE) act as directional sensors and Vmem as a regulatory cue	Activation mechanism still unknown	23, 53, 54, 71, 89 and 134
PI3K/Akt pathway, PTEN, myosin-dependent pathways and mitogen-activated protein kinases (MAPK)	Keratocyte, Dictyostelium cells, neural stem/progenitor cells, glioma cells	Fragments migrate to the anode, in the opposite direction of whole cells. Myosin II was essential for the direction sensing of fragments but not for parental cells, while PI3 kinase was essential for the direction sensing of whole cells but not for fragments	Competing signal transduction pathways, linked mechanistically to EGFs, superoxide regulates Akt and Erk1/2 activation	51, 52, 59 and 123
Directed collective motion	MDCK-II epithelial cells	Monolayers undergoing galvanotaxis obey Abercrombie's rule of contact inhibition.	Sheepdog collective response	34
Hyperpolarization of the anodal side and depolarization of the cathodal side of the cell	Keratocyte, Dictyostelium cells osteoblasts	May change the opening probability of voltage gated ion channels as well as create an asymmetric electro-motive force for ionic flow once ion channels are open	Inhibits Ca^{2+} signalling	50, 71 and 134
Electrophoretic redistribution of membrane components	Zebrafish keratocytes	The chemical polarization of the cellular membrane is transduced by intracellular signaling pathways.	Competing chemotactic signalling pathways may be the primary physical mechanism	50
Electrostatic and electro-osmotic forces at the plasma membrane	Mammalian cell lines (3T3, HeLa, and CHO cells), Keratocyte, Keratocyte, Fragments	Establishing a cathodal/anodal axis of polarity and re-orienting cells through hydrodynamic shear as seen with laminar fluid flow	Redistribution of charged cell-surface molecules, not involved in the directional sensing	51, 57 and 58
Calcium signalling	Influences keratinocytes C3H/10T1/2, broblasts Irrelevant to 3T3 broblasts and galvanotropism of Xenopus neurons	Higher Ca^{2+} rise occurs at the rear side of the migrating cells	Contradictory	28, 38 and 60–85
EGF growth factors	Keratinocytes, MDA-MB-231 breast cancer cells hippocampal cell line-derived NPCs	Depends on an induced asymmetry of EGF receptors and subsequently of F-actin. MAP kinase signaling pathway, which becomes activated asymmetrically, intracellular calcium ions increase possible involvement of the cell surface	Competing chemotactic signalling pathways	60, 106 and 125

approaches hold great promise to control and identify target signalling molecules/pathways.

In brief, we can conclude that EF involves a wide range of mechanisms which we have attempted to briefly summarize in Table 2. Of course much is still unknown, still experiments are underway to identify the mechanisms of sensing an EF. It has been shown that EFs may first induce a gradient in the membrane electric potential which in turn can affect asymmetrically ion fluxes that could signal to the motile machinery. Second, cells can be oriented by fluid flow, and an electro-osmotic flow may be generated near the charged plasma membrane, and influence the charge distribution. What has shown to have more dominance is the electrophoresis of charged membrane proteins which can drag the signalling proteins in the plasma membrane at one edge.

Following this conclusion, the electrophoretic force may be the triggering signal of electrotaxis of cells. The relationship between EGFR, calcium concentration, integrin distribution and microfilament reorganization should be placed as central questions for future studies. While either of these mechanisms could plausibly bias cell migration, it is important to ask first whether such interactions should strongly stimulate motile activity or is there is a separation between the physical mechanism of sensing an electric field and the following directional response. The issue is central to understanding why cells do not rapidly accommodate and ignore the electric field. To address why electric fields stimulate and persistently bias the motility of cells, it would be useful to examine how other cells respond to electrostatic cues.

Response of cancer cells to electrotactic cues

A large interest has been committed towards the effects of electrotaxis in the topic of cancer and its metastatic behaviour. Cell migration is a critical step in the process of metastatic propagation of tumour cells from the primary tumour to local and distant sites.[89] Migration of tumour cells is controlled by numerous factors, including chemoattractants, chemorepellents, extracellular matrix components and electric fields as purported by several reports.[87,95–104] Moreover highly metastatic cancer cells have shown a stronger response to an electric field than healthy or weakly metastatic cancer cells.[24,99] Biophysically, electric fields were shown to effectively redistribute charged mobile entities in the plane of the cell membrane. Voltage-gated sodium channels were reported to be involved in the electrotaxis of prostate cancer cells.[38] Also epidermal growth factor receptor (EGFR) signalling has been shown to be essential for EF-directed migration of breast cancer cells showing to polarize to the cathode-facing side in several cell types such as bovine corneal epithelial cells.[104,105] Pu and colleagues identified that the epidermal growth factor receptor (EGFR) pathway of A549 lung adenocarcinoma cells and breast cancer cells was affected by electrotaxis.[104] They showed that the cancer cells moved toward the anode of dcEF rather than to the cathode suggesting that electrotaxis of different cancer cells can be characteristically different in specific physiological environments and disease models. These experiments were confirmed by Wu *et al.*, who also showed the dcEF-induced polarization of the epidermal growth factor receptor (EGFR) and observed an increase of intracellular calcium ions in breast cancer cells.[106] They also hypothesized possible involvement of EGFR and calcium signalling in breast cancer cell electrotaxis. The electrotaxis of HeLa cells, cervical carcinoma cells, was shown to be dependent on a serine/threonine phosphatase and its substrate.[107] Huang's group has contributed to determine the involvement of electric fields in cancer metastasis using a microfluidic cell culture chip for long-term electrotaxis studies of human lung adenocarcinoma cell lines.[108] Their system consisted of an electrotactic chip with a single channel (single-field chip, SFC) or multi-channel (multi-field electrotactic chip, MFC) which provided uniform dcEF in the cell culture micro-chamber. Typically, the order of the electric field (EF) range was 75–375 mV mm^{-1} in the cell culture region of the channels. A positive correlation was observed between metastasis ability and electrotaxis response. The authors showed that the highly metastatic cells, the CL1–5 cells, showed stronger electrotactic response than the weakly metastatic cells, the CL1–0 cells. However, orientation of CL1–5 cells was not evident until 2 hour exposure in the EF. Recently Tsai showed contradicting results. They demonstrated that the lung adenocarcinoma cell line was serum and EGFR independent.[109] They suggested that blocking of EGFR signalling had no effect on the electrotaxis of the cells in spite of the high EGFR expression in the cells, and the electrotaxis of the lung cancer cells does not involve ligand-induced signalling of the EGFR pathway. Therefore, this suggests an involvement of different signalling pathways in electrotaxis interrelated to the different response time of the EF-induced directional migration and the cell body orientation of CL1–5 cells. This point of view was also confirmed in a recent work by Hammerick and coworkers.[110]

Furthermore Wang *et al.* used a microfluidic device to further demonstrate the biased growth of lung cancer cell filopodia towards the cathode of the external electric field as well as the polarized distribution of epidermal growth factor receptors (EGFRs) towards the cathodal side.[111] This may possibly be due a redistribution of the receptors on the cell surface induced by the electric field. For lung cancer cells with high migration and invasion abilities, they observed evident growth of filopodia biased on the side facing the cathode for 180–250 mV mm^{-1} EF strengths, which gradually decreased for higher EFs. Cells with weaker electrotaxis ability did not show evident cell responsivity and asymmetric filopodial growth and are not as evident as those of the highly invasive cells.

The speculation that electrical fields and chemical stimuli trigger the same intracellular kinase cascades was shown by Li *et al.* by monitoring the migration both *in vitro* in a microfluidic chamber and *in vivo* in mouse skin under the influence of a DC field.[14,112] They showed comparable motility and even higher orientation responses for activated T cells (in a 7 V cm^{-1} electric field) compared to an optimal T cell chemotaxis to a

100 nM CCL19 gradient which is similar to the fields observed in wound tissue. However, the authors were not able to exclude the possibility that electrically stimulated T cells produce chemo attractants which in turn can potentially form gradients in the microfluidic channel and thus induce chemotaxis.[47]

Because application of electric fields has proved to be a safe approach in clinical application, these studies hold promises for possible practical therapeutic strategies for cancer by activation of signalling pathways that would contribute to cancer cell angiogenesis.

Response of neurons to EFS

Electrical stimulation in the field of neurosciences is well established as a concept and is a powerful and broadly applicable therapeutic technology utilizing the voltage sensitivity of transmembrane ion channels and other fundamental processes of cellular electrical signalling.[113] The ability to direct the outgrowth of neuronal processes through the use of an extracellular electric field is renowned as galvanotropism. Recent research has shown therapeutic potential of EFs to promote nerve growth and axon regeneration under electrical stimulation or guiding long distance migration. But the guidance effect of EFs for cell migration and neurite growth has significant interspecies difference and has shown to be cell type dependent. For example, neurites from Xenopus neurons have been shown to respond to fields of less than 10 mV mm^{-1} growing toward the cathode.[114] Not only these neurons responded by orienting their neurites toward the cathode in an applied field, they also extended longer processes in the presence of a field (*i.e.* rat neurons grow perpendicular in an EF).[115] Whereas neurons from zebra fish do not respond to an 100 mV mm^{-1} applied EF *in vitro* at all.[116]

The direction of galvanotropism was shown to be influenced by both substratum charge and growth cone-to-substratum adhesivity. Rajnicek *et al.* showed that anodal galvanotropism was induced only by polylysine (positively charged) and not by laminin or Falcon plastic (both negatively charged).[117] However a reversal in surface charge was insufficient to reverse galvanotropism completely. This was associated to the possibility that substratum adhesivity and surface charge influenced the direction of growth cone turning. It is likely in fact that hypothetically an increased electrostatic attraction of the anodal or cathodal side of the growth cone leads to turning. Positive membrane proteins accumulate cathodally and negative membrane proteins accumulate anodally. However it may also be that that regardless of the substrate type, the cathode-facing regions of the growth cone may experience increased calcium influx *via* field-induced membrane depolarization or accumulation and activation of membrane proteins such as calcium channels or acetylcholine receptors. This in turn could stimulate cytoskeletal dynamics cathodally. Significant amount of research has shown a voltage dependent cathode-facing growth. Feng reported that small DC EFs, as low as 16 mV mm^{-1}, induced significant directional migration of neural human stem cells (NSCs) toward the cathode.[118] Migration directedness and the distance to the cathode increased with the increase of field strength. Reversal of the field polarity reversed migration of the cells, also showing that the electrotactic response was both time and voltage dependent. Recently, Li *et al.* investigated the role of DC EFs (of 50, 100 and 250 mV mm^{-1}) in directing the migration of mammalian neuronal stem/progenitor cells (NSPCs) showing directional response toward the cathode.[119] Their study uncovered the dose dependence role of EF as a directional guidance cue in controlling NSPC migration and revealed a novel signal transduction pathway in mediating electrotactic response (NMDAR/Racl/actin protein complex). Yao and co-workers demonstrated enhanced differentiation and perpendicular neural process growth of rat hippocampal-derived precursor neurons under stimulation of applied DC electrical fields in the range of 50–300 mV mm^{-1}.[120] At higher ranges (above 120 mV mm^{-1}), neurons exhibited a strong cathodal migration, which increased with the field strength. Moreover by switching the field polarity they showed a change in direction of neuron migration as well. Kim and his colleagues used microarray electrodes to direct neuronal migration toward the source of electrical current in primary hippocampal neuronal cell cultures.[121] By applying a wide range of parameters (15–60 μA, 16–496 μs and 5–220 Hz) they showed effective stimulation without inducing cell damage.[122] *In vitro* cathodal migration of NSPCs has also been observed in several other experiments using a similar stimulation setup. Arocena and co-workers applied EFs of 250 mV mm^{-2} on rat neural stem cells (NSCs), inducing neuronal migration.[49] They also showed enhanced migration speed with higher amplitudes.[123] Cao and colleagues found that high field strengths (>10 mV mm^{-1}) promoted clear and sustained directional migration towards the cathode. Furthermore, reversing the field direction with a high exogenous potential (50 mV mm^{-1}) caused cells to steer off course and move in the direction of the imposed field. They also implicated that the P2Y1 purinergic receptor, which is expressed specifically in migrating neuroblasts, was a mediator of the galvanotaxis.[124] Ariza showed first evidence of adult NPC differentiation affected in an EF *in vitro*. Treatment of NPCs with a 437 mV mm^{-1} direct current (DC) EF showed perpendicular alignment to the EF vector and a greater tendency to differentiate into neurons, but not into oligodendrocytes or astrocytes.[94]

Much is still to be understood about how developing neurons know which pathway to take. It has been suggested that EGFR polarization within the membrane leads to actin co-localization and polymerization, and these processes in turn trigger cathodal galvanotaxis. Meng *et al.* demonstrated that pharmacological and genetic inhibition of PI3K signaling significantly attenuated embryonic and hippocampal adult NPC migration.[123] Morshead's group demonstrated that EGF also plays a role in the galvanotaxis of SE-derived NPCs. In the presence of the EGFR inhibitor, undifferentiated NPCs experience significantly reduced migratory behaviour in the presence of a dcEF. However, they suggested that the mechanisms by which growth factors mediate galvanotaxis may vary between

hippocampal and subependyma-derived NPCs.[92] Asymmetric distribution of receptors might be decisive for electrically induced growth-cone guidance, whereas asymmetric epidermal growth factor (EGF) receptors and vascular endothelial growth factor (VEGF) receptors might transduce epithelial and endothelial responses to EFs.[48,60,105,125]

Calcium has been widely thought to play a controlling role in neurite outgrowth, but has shown inconclusive nature of evidence. The importance of calcium was in fact challenged by Palmer *et al.*, who showed that the effect of the electric field likely involved calcium and cAMP to coordinate the directional field response of growth cones but they concluded that neuronal galvanotropism was independent of the entry of external Ca^{2+} or of internal Ca^{2+} gradients.[70] However, it must be recognized that when Ca^{2+} is present, it may be the common second messenger used to transduce the EF signal. It has been suggested that the calcium channels may be redistributed *via* ''lateral electrophoresis'', leading to the formation of lateral cytoplasmic gradients of Ca^{2+}.[56,63] Another means by which applied electrical fields might affect Ca^{2+} distribution was presented by Bedlack *et al.* and Davenport and Kater, who found that N1E-115 mouse neuroblastoma cells and Helisoma neurons formed lateral Ca^{2+} gradients in response to large electric fields.[126,127]

Neurite outgrowth of neurons co-cultured with Schwann cells and electrical stimulation was examined by Koppes *et al.* in the presence of both biophysical and cellular cues. Following 8 h of electrical stimulation (0–100 mV mm^{-1}), a moderate electric field of 50 mV mm^{-1} resulted in significantly greater neurite outgrowth (114%) than both the unstimulated controls and all other field magnitudes tested (10, 100 mV mm^{-1}).[128] This electrically induced increase in neurite length may promote regrowth following injury. Furthermore, electrical stimulation at the injury site in conjunction with an aligned scaffold or prealigned glia may serve to both direct and promote robust axonal growth more efficiently. These results suggest that further investigation and application of EFs is warranted to elucidate the utility of EFs to control both morphologically and phenotypically behaviour of neurons. Despite the variety of *in vitro* research, still, only few present convincing *in vivo* evidence concerning electrotaxis and the mechanisms involved are still unclear.[30,129] Yet, to our knowledge, insufficient analysis of differentiation, alignment, proliferation, and viability *in vitro* within a continuous DC EF has to be investigated. Naturally, this also raises several interesting and open questions. Determining the outcome of migrating cells and behavioural measuring of functional recovery of lesions in an adequate approach may provide supportive information for the effect of electric fields. With progress, the use of EFs may be engineered to control differentiation and target injured sites by directing migration to replace cell loss. A broad range of work must be conducted to look at how electrical signals from the extracellular cues are translated into intracellular actions resulting in the electrotaxis effect but such combination may potentially develop into an indispensible therapeutic approach for brain treatment.

Wound healing

Accumulating experimental investigations suggest a much more important role than previously thought for electric signals among the many factors in directing cell migration in wound healing. The physiological EF with strength of tens to hundreds of mV per mm (mV mm^{-1}) originates from the difference in transepithelial potential (TEP), which is supposedly formed by the differential distribution of ion channels on polarized epithelial cells.[23,77] Cell membranes and epithelial tissues are semi-permeable barriers to ions and other charged species, resulting in charge separation and thus a natural electric potential across the layer. Immediately upon wounding the barrier is damaged and ions from either side will flow down its gradient, reducing the TEP.[130] This ionic current is suspected to act as a signal for cell growth towards the injury until the barrier is repaired and the electric potential is restored. The range of TEPs is generally of a few millivolts to tens of millivolts, corresponding to transcellular direct-current EFs of 50–500 mV mm^{-1}.[74,131,132]

The directional migration of cultured epithelial or corneal keratinocytes toward the cathode in an applied DC EF has been well investigated in the literature and it is fully recognized that electric signalling is a predominant signalling mechanism in guiding cell migration in wound healing.[23] Researchers have demonstrated control of cell migration, elongation, and polarization *in vivo* and *in vitro* when exposed to an EF of strength comparable to those within wounds (100 mV mm^{-1}). Application of EFs activates signalling molecules critical for wound healing in many types of exposed cells, including epidermal growth factor receptors (EGFRs), integrins, and phosphoinositide 3 (PI3) kinases and Pten (phosphatase and tensin homolog).[23,24,132] Most noticeably investigations showed that these signalling molecules are activated directionally, often towards the cathode-facing side of the cell.[47] Thus the EF provides a directional signal to guide migrating epithelial cells toward the wound centre.[60,105]

How though the wound EF mediates directional migration of epithelial cells is, as yet, not fully understood. Epidermal keratinocytes, in response to an EF, re-organize their lamellipodia facing the cathode, and migrate directionally *in vitro*. The mechanism for keratinocyte sensing of the EF is largely unknown, but a role for Ca^{2+} influx,[90] epidermal growth factor receptor (EGFR) phosphorylation,[59] and cAMP-dependent PKA has been reported.[65,68] Isseroff and his group proposed that an unidentified calcium channel was required for electrotaxis.[90] They showed that calcium channel blockers inhibited the directionality of keratinocytes, but the localization or the timing of the activation of the calcium channel remains unclear. Recently they proposed that the epithelial sodium channel, ENaC, also mediates electrotaxis.[133,134] Though further studies are still required to test if a calcium channel is downstream of ENaC or whether there are parallel pathways that control different cellular responses, the authors suggested that epithelial sodium channels act as a cell membrane EF mediating the protrusion process in response to an electric field.

EF-induced reorganization of charged surface receptors and of the cytoskeleton seems to be involved. Pullar and his co-workers demonstrated a crucial role for α6β4 integrin in sustaining directional migration in keratinocytes in response to an applied EF and demonstrate that the cooperative interaction of both EGF and β4 integrin is necessary to achieve the full biological response of EF-mediated directional migration.[65,68,130] They showed that knockout of β4 integrin abrogated the electrotaxis of keratinocytes in the absence of EGF, which can be recovered by transfection and expression of β4 integrin. Investigations carried out by Zhao *et al.* suggest that the tension between PTEN and PI3K is also relevant to electrotaxis in wound healing.[23] Using gradients of electric potential of the same magnitude as those observed in endogenous settings they showed that EFs are able to override other cues in directing cell migration, governing the movement of keratinocytes during wound repair, an observation that is consistent with previous findings. This might be particularly important in situations where different cues use overlapping signal transduction networks, perhaps leading one cue to prime or turn down the response to another cue. The authors also showed that EFs activated PI3 kinase/Akt signalling pathways in neutrophils and keratinocytes cultured in serum-free medium polarizing toward the site of the cathode in an EF. By reversing the polarity of the EF, they demonstrated that the PI3 kinase signalling was activated at the new cathode-facing side, as with the membrane protrusion, and a rearrangement of migration in the new direction was seen. These findings point to an essential role of the molecules PI3 kinase/Akt and PTEN which are responsible for directional guidance in chemotaxis, suggesting that electrotaxis and chemotaxis share common signalling pathways. However, the signal transduction pathways of chemotaxis and electrotaxis do not completely overlap because PTEN inhibition improves the strength of a cell.

To date though, analysis of single cell migration has been taken into consideration. Cells acting collectively may respond to the same signal differently compared to isolated cells. Cohen *et al.* showed surprising results by inducing directed collective cell motion.[34] The authors managed to manipulate cell migration of monolayers of cells without disrupting the structural integrity of the monolayer. Using shaped electric fields and precisely patterned epithelial monolayers, they demonstrated multiaxial control of migration trajectories within a single monolayer. However, these results evidenced the lack of physical coupling in many of the experiments reported. In fact, altering the perimeter-to-area ratio with a cell-based obstacle showed a pronounced effect on the controllability of induced cell behaviour showing insensitivity to galvanotactic cues. The authors evidenced that tissues are an active material suggesting that the physical coupling between cells and inter/intracellular feedback loops creates a collective decision network. Therefore electrotaxis has shown to be potentially useful clinically for wound healing applications. However the reported studies demonstrate great variability in the parameters of application leading to an inability to generate sufficient evidence to support any one standard therapeutic approach. A deeper understanding of EF-induced directional migration may improve our knowledge to control directional sensing and the signalling mechanisms required for specific biological processes. Hence further trials are required to fully elucidate the mechanism by which the protrusion processes are mediated in response to an EF.

Conclusions and future outlooks

In summary, we approach a way to understand how electrotactic forces and biochemical signalling events interact to guide cell movement also describing the recent developments in electrotaxis application. There remain many voids in our knowledge base.

Given that a static electric field induces reorientation of cell surface and signalling molecules, the application of this kind of stimulus appears to be more important for cell migration than chemical and hapotactic signals.[35] Undoubtedly, electric fields uniquely offer the possibility of long-range communication and control of directed motion of cells that can be transmitted rapidly and, with the appropriate circuitry, undiminished. Several challenges still remain for clinical applications. First of all, a quantitative analysis of the cell migration behaviour will be needed to determine thresholds, to fully uncover how electrical signals from the extracellular environment are translated into intracellular actions and determine the degree of dominance of one cue over another while assessing concurring or diverging mechanisms.[47] Electrotactic migration is not or in any case not completely mediated by chemotaxis and should be decoupled from this kind of stimulus. Indeed, it has been reported that external electric fields override other cell migration cues and therefore it may be important for practical cell recruitment applications such as wound healing and formation of new tissues.[32] When chemical gradients in an electric field were disrupted, cells responded just as well to the electric field. Chemotaxis cues only affect the cell polarisation direction by modulating the direction of the protrusions of the cell (pseudopods or lamellipods). On the other hand, electrotaxis affects cell direction and cell velocity due to electrostatic forces. Current literature has reported observable directional migration and long axis reorientation (electro-alignment). However, the number of different treatments of cells with various modalities such as growth factors, toxic agents and different mechanical and physical properties further increases the experimental conditions and poses a challenge for efficient determination of cell behaviour in a large number of situations. In fact separate reports have validated controversial data: most cell types respond to an electric field by migrating toward the cathode, although some (often similar) cell types respond by migrating to the anode. Systematic analysis of the intracellular signalling and cytoskeleton dynamics will be needed to recognize molecular mechanisms and identify important control elements of electrotaxis. As aforementioned, upon exposure of a cell to an EF, the cell membrane potentials change: the plasma membrane facing the cathode depolarises while the membrane facing the anode hyperpolarises.[28,38,71] In most studied cells, this is thought to depend on changes in Ca^{2+}.[38]

There is contradictory evidence that local calcium entry is involved in electrotactic responses; however, there are reasons for suspecting the involvement of calcium in this process. It appears that EFs would require endogenous growth factors to relay the directional information to the cytoskeletal players that produce cell migration and promote electrotactic signalling most likely by activating the PI3 kinase/Akt pathway. In fact inhibition of PI3 kinase disrupts the galvanotactic response of cells. It has been established that various cells can potentially respond to an exogenous EF, at least by means of passive Ca^{2+} influx, independent of sensing an external chemical gradient. Several important proteins and genes have been reported to be involved in the mechanisms that occur when cells sense an external electrical field. Reorganization of cell surface receptors or extracellular molecules to induce a gradient of either receptor or ligand, respectively, is likely an initial event, which activates cells asymmetrically and drives subsequent cytoskeletal reorganization and directed cell migration. This suggests that the redistribution of receptors in the membrane is essential for the electrotropic response. Moreover, while earlier studies have failed to identify any voltage gated expression, interpretation would merit further testing, using different tools to overcome the limitations of the techniques used until now (*i.e.* real time fluorescence imaging may now allow us to visualise spatially restricted changes within milliseconds of electric field application).

Second the design and fabrication of an environment which will allow physicochemical cues to control the single stimuli is missing. The current state of the art supports the hypothesis in which intracellular signalling pathways which are common in chemotaxis are used to transduce the electrotactic signal. A unified set of quantitative analysis might uncover governing principles compared to different systems. What's more, cell behaviour in a 3D environment can be significantly different from 2D cultures. Following the exposure to EFs, cell membranes undergo conformational alterations, which are not only directly involved in cell motility, but activate a variety of downstream intracellular signalling pathways leading to cell migration. Although varied strategies have been developed to stimulate electrically cells, most of them are still limited to a 2D approach. Advancements in microfabrication and materials science have provided tools to meet these challenges. In particular, microfluidics combined to control electric fields has shown a concurrent intracellular mechanism connecting electrotaxis and chemotaxis. 3D microfluidic cell culture systems can offer a biologically relevant model to conduct microscale cell-based research and applications. Practical application of electrical stimulation itself may need to be carefully considered and designed.

Finally, mathematical modelling and simulations could be important to understand the interplay of EFs, proton diffusion and cell shape changes essential for directional cell migration. In fact the great amount of knowledge regarding cell and tissue interactions with EF comes from experimental studies. Very few mathematical modelling have been used to obtain specific information of the cell–EF interaction. A physical model for cell motion was found to be valuable in controlling and planning cell motion during EF exposure.[135] However the influence of the EF in cell dynamics, especially cell migration, has not been numerically studied. They may aid to predict thresholds, assess molecular mechanisms and expect which cue will dominate over others. While the research is underway to uncover the basic theories and evidence that also the speed of migration and directedness of cell migration are enhanced and regulated by external electrical cues, there is still much to do in our understanding of how these processes are controlled within 3D surroundings and within a tissue. With further understanding of the molecular and cellular mechanisms, and development in techniques of application of EFs, electric stimulation may lead to effective and exciting therapies. Indeed, the tools to enable such a feat are emerging and will eventually lead to a molecular understanding of cell migration under physiological and pathological conditions. We are just beginning to uncover which molecular mechanisms are involved and how cells respond to EFs. In conclusion, further fundamental investigations are still required to elucidate the bases of the responses to EFs in different cells, and studies with improved experimental methodologies will help clarify the effect of electrically stimulated cells and medium conditions. Well-controlled trials and standardization of the device and protocols for electric stimulation are needed.

Notes and references

1 Y. Matsubayashi, M. Ebisuya, S. Honjoh and E. Nishida, *Curr. Biol.*, 2004, **14**, 731.
2 S. McDougall, J. Dallon, J. Sherratt and P. Maini, *Philos. Trans. R. Soc. London, Ser. A*, 2006, **364**, 1385.
3 R. Ayala, T. Shu and L. H. Tsai, *Cell*, 2007, **128**, 29.
4 M. E. Hatten, *Science*, 2002, **297**, 1660.
5 R. Keller, *Curr. Opin. Cell Biol.*, 2005, **17**, 533.
6 P. Friedl and K. Wolfs, *Nat. Rev. Cancer*, 2003, **3**, 362.
7 A. J. Ridley, *Science*, 2003, **302**, 1704.
8 S. B. Carter, *Nature*, 1965, **208**, 1183.
9 N. L. Jeon, H. Baskaran, S. K. W. Dertinger, G. M. Whitesides, L. Van de Water and M. Toner, *Nat. Biotechnol.*, 2002, **20**, 826.
10 L. Helmick, A. A. de Mayolo, Y. Zhang, C. M. Cheng, S. C. Watkins, C. Wu and P. R. LeDuc, *Nano Lett.*, 2008, **8**, 1303.
11 D. Bray, *Cell Movements: From Molecules to Motility*, Garland Publishing, New York, NJ, 2nd edn, 2001.
12 A. S. Curtis, B. Casey, J. O. Gallagher, D. Pasqui, M. A. Wood and C. D. Wilkinson, *Biophys. Chem.*, 2001, **94**, 275.
13 M. J. Dalby, N. Gadegaard, R. Tare, A. Andar, M. O. Riehle, P. Herzyk, C. D. Wilkinson and R. O. Oreffo, *Nat. Mater.*, 2007, **6**, 997.
14 B. Cortese, C. Piliego, I. Viola, S. D'Amone, R. Cingolani and G. Gigli, *Langmuir*, 2009, **25**, 7025.
15 J. Saranak and K. W. Foster, *Nature*, 1997, **387**, 465.
16 L. V. Beloussov, N. N. Louchinskaia and A. A. Stein, *Dev. Genes Evol.*, 2000, **210**, 92.
17 C. A. Erickson and R. Nuccitelli, *J. Cell Biol.*, 1984, **98**, 296.
18 C. F. Adams and A. J. Paul, *J. Crustacean Biol.*, 1999, **19**, 106.

19 C. M. Lo, H. B. Wang, M. Dembo and Y. L. Wang, *Biophys. J.*, 2000, **79**, 144.
20 B. Cortese, G. Gigli and M. Riehle, *Adv. Funct. Mater.*, 2009, **19**, 2961.
21 I. E. Palamà, S. D'Amone, A. M. Coluccia, M. Biasiucci and G. Gigli, *Integr. Biol.*, 2012, **4**, 228.
22 N. A. Hale, Y. Yang and P. Rajagopalan, *ACS Appl. Mater. Interfaces*, 2010, **2**, 2317.
23 M. Zhao, B. Song, J. Pu, T. Wada, B. Reid, G. Tai, F. Wang, A. Guo, P. Walczysko, Y. Gu, T. Sasaki, A. Suzuki, J. V. Forrester, H. R. Bourne, P. N. Devreotes, C. D. McCaig and J. M. Penninger, *Nature*, 2006, **442**, 457.
24 L. F. Jaffe and R. Nucitelli, *Annu. Rev. Biophys. Bioeng.*, 1977, **6**, 445.
25 C. D. McCaig, *J. Cell Sci.*, 1989, **93**, 723.
26 G. Yang, H. Long, J. Wu and H. Huang, *BMEI*, 2008, 548.
27 B. Song, Y. Gu, J. Pu, B. Reid, Z. Zhao and M. Zhao, *Nat. Protoc.*, 2007, **2**, 1479.
28 R. Nuccitelli, *Curr. Top. Dev. Biol.*, 2003, **58**, 1.
29 G. Tai, B. Reid, L. Cao and M. Zhao, *Methods Mol. Biol.*, 2009, **571**, 77.
30 M. A. Messerli and D. M. Graham, *Biol. Bull.*, 2011, **221**, 79.
31 X. Yan, J. Han, Z. Zhang, J. Wang, Q. Cheng, K. Gao, Y. Ni and Y. Wang, *Bioelectromagnetics*, 2009, **30**, 29.
32 M. Zhao, *Semin. Cell Dev. Biol.*, 2009, **20**, 674.
33 C. D. McCaig, A. M. Rajnicek, B. Song and M. Zhao, *Physiol. Rev.*, 2005, **85**, 943.
34 D. J. Cohen, W. J. Nelson and M. M. Maharbiz, *Nat. Mater.*, 2014, **13**, 409.
35 N. Özkucur, T. K. Monsees, S. Perike, H. Q. Do and R. H. W. Funk, *PLoS One*, 2009, **4**, e6131.
36 K. S. Cole, *Membranes, Ions and Impulses: A Chapter of Classical Biophysics*, Univ. of Calif. Press, Berkeley, 1968.
37 C. Polk and E. Postow, *Biological Effects of Electromagnetic Fields Handbooks, Manuals, Etc.*, CRC, Boca Raton, FL, 1995, p. 618.
38 M. E. Mycielska and M. B. A. Djamgoz, *J. Cell Sci.*, 2004, **117**, 1631.
39 J. Ferrier, S. M. Ross, J. Kanehisa and J. E. Aubin, *J. Cell. Physiol.*, 1986, **129**, 283.
40 A. Guo, B. Song, B. Reid, Y. Gu, J. V. Forrester, C. A. B. Jahoda and M. Zhao, *J. Invest. Dermatol.*, 2010, **130**, 2320–2327.
41 M. J. McKasson, L. Huang and K. R. Robinson, *Exp. Neurol.*, 2008, **211**, 585.
42 S. McLaughlin and M. M. Poo, *Biophys. J.*, 1981, **34**, 85.
43 F. Lin, F. Baldessari, C. C. Gyenge, T. Sato, R. D. Chambers, J. G. Santiago and E. C. Butcher, *J. Immunol.*, 2008, **181**, 2465.
44 C. D. McCaig, B. Song and A. M. Rajnicek, *J. Cell Sci.*, 2009, **122**, 4267.
45 P. G. Chao, R. Roy, R. L. Mauck, W. Liu, W. B. Valhmu and C. T. Hung, *J. Biomech. Eng.*, 2000, **122**, 261.
46 P. Borys, The role of passive calcium influx through the cell membrane in galvanotaxis, *Cell. Mol. Biol. Lett.*, 2013, **18**, 187.
47 K. Y. Nishimura, R. R. Isseroff and R. Nuccitelli, *J. Cell Sci.*, 1996, **109**, 199.
48 M. Zhao, J. Pu, J. V. Forrester and C. D. McCaig, *FASEB J.*, 2002, **16**, 857.
49 M. Arocena, M. Zhao, J. M. Collinson and B. Song, *J. Neurosci. Res.*, 2010, **88**, 3267.
50 G. M. Allen, A. Mogilner and J. A. Theriot, *Curr. Biol.*, 2013, **23**, 560.
51 Y. Sun, H. Do, J. Gao, R. Zhao, M. Zhao and A. Mogilner, *Curr. Biol.*, 2013, **23**, 569.
52 F. Li, T. Chen, S. Hu, J. Lin, R. Hu and H. Feng, *PLoS One*, 2013, **8**, e61195.
53 N. Özkucur, S. Perike, P. Sharma and R. H. W. Funk, *BMC Cell Biol.*, 2011, **12**, 4.
54 D. Gross, L. M. Loew and W. W. Webb, *Biophys. J.*, 1986, **50**, 339.
55 L. F. Jaffe, *Nature*, 1977, **265**, 600.
56 M. Poo, *Annu. Rev. Biophys. Bioeng.*, 1981, **10**, 245.
57 E. I. Finkelstein, P. H. G. Chao, C. T. Hung and J. C. Bulinski, *Cell Motil. Cytoskeleton*, 2007, **64**, 33.
58 L. Huang, P. Cormie, M. A. Messerli and K. R. Robinson, *J. Cell. Physiol.*, 2009, **219**, 162.
59 M. J. Sato, M. Ueda, H. Takagi, T. M. Watanabe and T. Yanagida, *BioSystems*, 2007, **88**, 261.
60 K. S. Fang, E. Ionides, G. Oster, R. Nuccitelli and R. R. Isseroff, *J. Cell Sci.*, 1999, **112**, 1967.
61 J. Stollberg and S. E. Fraser, *J. Neurosci.*, 1990, **10**, 247.
62 H. L. Zhang and H. B. Peng, *PLoS One*, 2011, **6**, e26805.
63 M. Poo and K. R. Robinson, *Nature*, 1977, **265**, 602.
64 G. R. Monteith, D. M. Andrew, H. M. Faddy and S. J. Roberts-Thomson, *Nat. Rev.*, 2007, **7**, 519.
65 C. E. Pullar and R. R. Isseroff, *J. Cell Sci.*, 2005, **118**, 2023.
66 E. Wang, M. Zhao, J. V. Forrester and C. D. McCaig, *Invest. Ophthalmol. Visual Sci.*, 2003, **44**, 244.
67 X. Meng, M. Arocena, J. Penninger, F. H. Gage, M. Zhao and B. Song, *Exp. Neurol.*, 2011, **227**, 210.
68 C. E. Pullar, R. R. Isseroff and R. Nuccitelli, *Cell Motil. Cytoskeleton*, 2001, **50**, 207.
69 M. J. Brown and L. M. Loew, *J. Cell Biol.*, 1994, **127**, 117.
70 A. M. Palmer, M. A. Messerli and K. R. Robinson, *J. Neurobiol.*, 2000, **45**, 30.
71 R. C. Gao, X.-D. Zhang, Y. H. Sun, Y. Kamimura, A. Mogilner, P. N. Devreotes and M. Zhao, *Eukaryotic Cell*, 2011, **10**, 1251.
72 E. Onuma and S. Hui, *Cell Calcium*, 1985, **6**, 281.
73 G. L. Sulik, H. K. Soong, P. C. Chang, W. C. Parkinson, S. G. Elner and V. M. Elner, *Acta Ophthalmol.*, 1992, **70**, 115.
74 S. P. Fraser, J. K. J. Diss, M. E. Mycielska, R. C. Coombes and M. B. A. Djamgoz, *Breast Cancer Res. Treat.*, 2002, **76**, S142.
75 M. S. Cooper and M. Schliwa, *J. Cell Biol.*, 1986, **102**, 1384.
76 R. Nuccitelli and T. Smart, *Biol. Bull.*, 1989, **176**, 130.
77 L. Shanley, P. Walczysko, M. Bain, D. MacEwan and M. Zhao, *J. Cell Sci.*, 2006, **119**, 4741.
78 M. Morita, C. Higuchi, T. Moto, N. Kozuka, J. Susuki, R. Itofusa, J. Yamashita and Y. Kudo, *J. Neurosci.*, 2003, **23**, 10944.
79 M. Morita and Y. Kudo, *Glia*, 2010, **58**, 1988.

80 S. Mishra and A. W. Hamburger, *Biochem. Biophys. Res. Commun.*, 1993, **191**, 1066.
81 K. Franke and H. Gruler, *Eur. Biophys. J.*, 1990, **18**, 334.
82 M. J. Sato, H. Kuwayama, W. N. van Egmond, A. L. Takayama, H. Takagi, P. J. van Haastert, T. Yanagida and M. Ueda, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 6667.
83 R. Nuccitelli, K. Lui, M. Kreis, B. Athos and P. Nuccitelli, *Biochem. Biophys. Res. Commun.*, 2013, **435**, 580.
84 E. K. Onuma and S.-W. Hui, *Cell Calcium*, 1985, **6**, 281.
85 P. Borys, *Eur. Biophys. J.*, 2012, **41**, 527.
86 N. Minc and F. Chang, *Curr. Biol.*, 2010, **20**, 710.
87 R. Morini and A. Becchetti, *Adv. Exp. Med. Biol.*, 2010, **674**, 95.
88 P. Uysal-Onganer and M. B. Djamgoz, *Mol. Cancer*, 2007, **6**, 76.
89 M. B. A. Djamgoz, M. Mycielska, Z. Madeja, S. Fraser and W. Korohoda, *J. Cell Sci.*, 2001, **114**, 2697.
90 D. R. Trollinger, R. R. Isseroff and R. Nuccitelli, *J. Cell. Physiol.*, 2002, **193**, 1.
91 S. Sakata, M. I. Hossain and Y. Okamura, *J. Physiol.*, 2011, **589**, 2687.
92 P. Rezai, A. Siddiqui, P. R. Selvaganapathy and B. P. Gupta, *Lab Chip*, 2010, **10**, 220.
93 M. Marotta, R. Bragós and A. M. Gómez-Foix, *BioTechniques*, 2004, **36**, 68.
94 C. A. Ariza, A. T. Fleury, C. J. Tormos, V. Petruk, S. Chawla, J. Oh, D. S. Sakaguchi and S. K. Mallapragada, *Stem Cell Rev. Rep.*, 2010, **6**, 585–600.
95 R. Babona-Pilipos, I. A. Droujinine, M. R. Popovic and C. i. M. Morshead, *PLoS One*, 2011, **6**, e23808.
96 J. E. Segall, S. Tyerech, L. Boselli, S. Masseling, J. Helft, A. Chan, J. Jones and J. Condeelis, *Clin. Exp. Metastasis*, 1996, **14**, 61.
97 M. D. Levine, L. A. Liotta and M. L. Stracke, *EXS*, 1995, **74**, 157.
98 R. Nuccitelli, *Adv. Cell Biol.*, 1988, **2**, 21.
99 C. Huang, J. Cheng, M. Yen and T. Young, *Biosens. Bioelectron.*, 2009, **24**, 3510.
100 X. Yan, J. Han, Z. Zhang, J. Wang, Q. Cheng, K. Gao, Y. Ni and Y. Wang, *Bioelectromagnetics*, 2009, **30**, 29.
101 M. Wartenberg, N. Wirtz, A. Grob, W. Niedermeier, J. Hescheler, S. C. Peters and H. Sauer, *Bioelectromagnetics*, 2008, **29**, 47.
102 I. A. Grimstad, *Exp. Cell Res.*, 1987, **173**, 515.
103 M. L. Stracke, S. A. Aznavoorian, M. E. Beckner, L. A. Liotta and E. Schiffmann, Cell motility, a principal requirement for metastasis, in *Cell Motility Factors*, ed. I. D. Goldberg, Birkhauser Verlag, Basel, 1991, pp. 147–162.
104 J. Pu, C. D. McCaig, L. Cao, Z. Zhao, J. E. Segall and M. Zhao, *J. Cell Sci.*, 2007, **120**, 3395.
105 M. Zhao, A. Dick, J. Forrester and C. McCaig, *Mol. Biol. Cell*, 1999, **10**, 1259.
106 D. Wu, X. Ma and F. Lin, *Cell Biochem. Biophys.*, 2013, **67**, 1115.
107 C. Martin-Granados, A. R. Prescott, N. Van Dessel, A. Van Eynde, M. Arocena, I. P. Klaska, J. Görnemann, M. Beullens, M. Bollen, J. V. Forrester and C. D. McCaig, *PLoS One*, 2012, **7**, e40769.
108 C.-W. Huang, H.-Y. Chen, M.-H. Yen, J. J. W. Chen, T.-H. Young and J.-Y. Cheng, *PLoS One*, 2011, **6**, e25928.
109 H.-F. Tsai, C.-W. Huang, H.-F. Chang, J. J. W. Chen, C.-H. Lee and J.-Y. Cheng, *PLoS One*, 2013, **8**, e73418.
110 K. E. Hammerick, M. T. Longaker and F. B. Prinz, *Biochem. Biophys. Res. Commun.*, 2010, **397**, 12.
111 C.-C. Wang, Y.-C. Kao, P.-Y. Chi, C.-W. Huang, J.-Y. Lin, C.-F. Chou, J.-Y. Cheng and C.-H. Lee, *Lab Chip*, 2011, **11**, 695.
112 J. Li, S. Nandagopal, D. Wu, S. F. Romanuik, K. Paul, D. J. Thomsonc and F. Lin, *Lab Chip*, 2011, **11**, 1298.
113 N. Patel and M. M. Poo, *J. Neurosci.*, 1982, **2**, 483.
114 L. Hinkle, C. D. McCaig and K. R. Robinson, *J. Physiol.*, 1981, **314**, 121.
115 A. M. Rajnicek, N. A. Gow and C. D. McCaig, *Exp. Physiol.*, 1992, **77**, 229.
116 P. Cormie and K. R. Robinson, *Neurosci. Lett.*, 2007, **411**, 128.
117 A. M. Rajnicek, K. R. Robinson and C. D. McCaig, *Dev. Biol.*, 1998, **203**, 412.
118 J.-F. Feng, J. Liu, X.-Z. Zhang, L. Zhang, J.-Y. Jiang, J. Nolta and M. Zhao, *Stem Cells*, 2012, **30**, 349.
119 L. Li, Y. H. El-Hayek, B. Liu, Y. Chen, E. Gomez, X. Wu, K. Ning, L. Li, N. Chang, L. Zhang, Z. Wang, X. Hu and Q. Wan, *Stem Cells*, 2008, **26**, 2193.
120 L. Yao, L. Shanley, C. McCaig and M. Zhao, *J. Cell. Physiol.*, 2008, **216**, 527.
121 S. H. Jeong, S. B. Jun, J. K. Song and S. J. Kim, *Med. Biol. Eng. Comput.*, 2009, **47**, 93.
122 S. S. Magavi, B. R. Leavitt and J. D. Macklis, *Nature*, 2000, **405**, 951.
123 X. Meng, M. Arocena, J. Penninger, F. H. Gage, M. Zhao and B. Song, *Exp. Neurol.*, 2011, **227**, 210.
124 L. Cao, D. Wei, B. Reid, S. Zhao, J. Pu, T. Pan, E. Yamoah and M. Zhao, *EMBO Rep.*, 2013, **14**, 184.
125 C. E. Pullar, B. S. Baier, Y. Kariya, A. J. Russell, B. A. Horst, M. P. Marinkovich and R. R. Isseroff, *Mol. Biol. Cell*, 2006, **17**, 4925.
126 R. S. Bedlack, M.-D. Wei and L. M. Loew, *Neuron*, 1992, **9**, 393.
127 R. Davenport and S. Kater, *Neuron*, 1992, **9**, 405.
128 A. N. Koppes, A. M. Seggio and D. M. Thompson, *J. Neural Eng.*, 2011, **8**, 046023.
129 D. Janigro, C. Perju, V. Fazio, K. Hallene, G. Dini, M. K. Agarwal and L. Cucullo, *BMC Cancer*, 2006, **6**, 72.
130 L. C. Kloth, *Int. J. Low Extrem. Wounds*, 2005, **4**, 23.
131 R. J. Fitzsimmons, S. L. Gordon, J. Kronberg, T. Ganey and A. A. Pilla, *J. Orthop. Res.*, 2008, **26**, 854.
132 A. Ramadan, M. Elsaidy and R. Zyada, *J. Wound Care*, 2008, **17**, 292.
133 H.-Y. Yang, R.-P. Charles, E. Hummler, D. L. Baines and R. R. Isseroff, *J. Cell Sci.*, 2013, **126**, 1942.
134 N. Özkucur, B. Song, S. Bola, L. Zhang, B. Reid, G. Fu, R. H. W. Funk and M. Zhao, *Cell. Mol. Life Sci.*, 2014, DOI: 10.1007/s00018-014-1632-1.
135 N. Ogawa, H. Okua, K. Hashimoto and M. Ishikawa, *J. Theor. Biol.*, 2006, **242**, 314.

