

Skin-derived precursor cells undergo substrate-dependent galvanotaxis that can be modified by neighbouring cells

Stephanie N. Iwasa^{a,b}, Milos R. Popovic^{a,b}, Cindi M. Morshead^{a,b,c,*}

^a Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario M5S 3E1, Canada

^b Toronto Rehabilitation Institute - University Health Network, Toronto, Ontario M4G 3V9, Canada

^c Department of Surgery, University of Toronto, Toronto, Ontario M5S 3E1, Canada

ARTICLE INFO

Keywords:

Skin-derived precursors
Electric field
Cell migration
Galvanotaxis
Substrates
Co-cultures

ABSTRACT

Many cell types respond to electric fields (EFs) through cell migration, a process termed galvanotaxis. The galvanotactic response is critical for development and wound healing. Here we investigate whether skin-derived precursor cells (SKPs), which have the potential to differentiate into mesodermal and peripheral neural cell types, undergo directed migration in the presence of an EF. We found that EF application promotes SKP migration towards the anode. The migratory response is substrate-dependent as SKPs undergo directed migration on laminin and Matrigel, but not collagen. The majority of SKPs express the undifferentiated cell markers nestin, fibronectin and Sox2, after both EF application and in sister cultures with no EF application, suggesting that EFs do not promote cell differentiation. Co-cultures of SKPs and brain-derived neural precursor cells (NPCs), a population of cells that undergo rapid, cathode-directed migration, reveal that in the presence of NPCs an increased percentage of SKPs undergo galvanotaxis, providing evidence that cells can provide cues to modify the galvanotactic response. We propose that a better understanding of SKP migration in the presence of EFs may provide insight into improved strategies for wound repair.

1. Introduction

Endogenous EFs are important throughout development and in wound healing. Physiologically, EFs and electric potentials are created through the separation of ions. These EFs and electric potential patterns control an organism's anatomy and morphology (Levin et al., 2017; Levin and Martyniuk, 2018). EFs affect cellular processes such as migration, alignment, proliferation and differentiation (Thrivikraman et al., 2018). Galvanotaxis, which is the migration due to EFs, has been shown to override other migration cues (Funk, 2015; Zhao, 2009). Cells can sense EFs through the electrophoresis of charged membrane channels and the polarization of charged molecules triggering localized signalling pathways and cytoskeletal changes for cell migration (Allen et al., 2013; Huang et al., 2017; Nakajima et al., 2015). Further, the galvanotactic response is cell-type dependent. Cells are differentially electro-sensitive and migrate at different speeds and directions, *i.e.* towards the positive or negative EF terminal called the anode and cathode, respectively (Ozkucur et al., 2011; Sillman et al., 2003) and field strengths of 3 mV/mm to over 1000 mV/mm can elicit a

galvanotactic response depending on the cell type (Iwasa et al., 2017). One of the most well-studied stem cell types that undergo galvanotaxis are NPCs which demonstrate a robust, rapid and directed cathodal migration in response to EFs (Babona-Pilipos et al., 2011, 2015; Cao et al., 2013; Meng et al., 2011).

When an injury occurs, an EF is created which directs cell migration to the injury site. Understanding how EFs affect the migration of stem cells is important to understanding how wound repair occurs. In the skin, cell migratory responses to EFs are varied. Mature cell phenotypes such as human epidermal keratinocytes migrate towards the cathode in an EF (Nishimura et al., 1996), human dermal fibroblasts migrate towards the anode (Guo et al., 2010), and melanocytes have not been shown to undergo galvanotaxis (Grahm et al., 2003). With regard to dermal skin-derived stem cells, the galvanotactic response has not been examined. SKPs are found in the dermal papillae and are multipotent, self-renewing cells (Toma et al., 2001). These stem and progenitor cells give rise to adipocytes, dermal fibroblasts, skeletogenic cells, smooth muscle cells, Schwann cells and neuronal precursors (Biernaskie et al., 2009; Fernandes et al., 2008; Lavoie et al., 2009; McKenzie et al., 2006;

Abbreviations: SKPs, Skin-derived precursor cells; NPCs, Neural precursor cells; EFs, Electric fields

* Corresponding author at: Donnelly Centre, Room 1006, Department of Surgery, University of Toronto, Donnelly Centre 160 College St, Toronto, Ontario M5S 3E1, Canada.

E-mail addresses: stephanie.iwasa@mail.utoronto.ca (S.N. Iwasa), milos.popovic@utoronto.ca (M.R. Popovic), cindi.morshead@utoronto.ca (C.M. Morshead).

<https://doi.org/10.1016/j.scr.2018.07.019>

Received 2 February 2018; Received in revised form 13 June 2018; Accepted 18 July 2018

Available online 19 July 2018

1873-5061/ © 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Steinbach et al., 2011; Toma et al., 2001). These cells contribute to wound healing through migration and differentiation in response to injury. Indeed, SKPs migrate in response to biopsy punch wounds and their transplantation can promote wound repair in diabetic ulcers (Biernaskie et al., 2009; Ke et al., 2015). Further, factors that promote their proliferation *in vitro* can improve wound healing *in vivo* (Naska et al., 2016). Given the promising therapeutic potential of SKPs for enhancing skin repair, we sought to determine their responsiveness to EF application.

Herein, we examine the effects of EFs on SKP galvanotaxis. We demonstrate that SKPs undergo anodal galvanotaxis. SKP galvanotaxis is substrate-dependent and the presence of NPCs and their conditioned media modify their galvanotactic response.

2. Materials and methods

2.1. Ethics statement

All animal work was approved by the University of Toronto Animal Care Committee in accordance with institutional guidelines (protocol no. 20011515). All SKP dissections were performed on CD1 post-natal day 0 to 6 murine pups (Charles River). Neurosphere dissections were performed from adult C57/BL6 or transgenic mouse R26R-EYFP bred in house.

2.2. Cell culture

SKPs were obtained as previously described (Biernaskie et al., 2006; Toma et al., 2001). Briefly, back skin tissue from wild-type CD1 pups (postnatal day 0–6) was dissected free of other tissue, and was dissociated both mechanically and enzymatically with collagenase (1 mg/mL, Sigma-Aldrich). Cells were plated at most 50 cells/ μ L in T25 or T75 culture flasks (BD Falcon) in SKP media: (DMEM + Gluta-max:F12 + Glutamax, 3:1) with 1% penicillin/streptomycin (Invitrogen) supplemented with epidermal growth factor (EGF, 20 ng/mL; Sigma-Aldrich), basic fibroblast growth factor (bFGF, 40 ng/mL; Sigma-Aldrich) and 2% B27 supplement. SKPs were fed with the addition of 2–5 mL of fresh medium containing all growth factors and supplements every 2–5 days to replenish the entirety of the culture media. Every 7–21 days SKPs were passaged. As previously described, media and cells were transferred to new flasks to avoid SKP sphere contact with adherent cells. To passage, SKP-derived spheres were collected, mechanically and enzymatically dissociated into single cells and re-plated in SKP media. For all experiments, SKP spheres were used between passage numbers 1–3.

For co-culture experiments, NPCs were derived from a transgenic mouse which ubiquitously expressed yellow fluorescent protein. Neurospheres were isolated from adult mouse brains as previously described (Babona-Pilipos et al., 2011). Briefly, brains were removed and the periventricular regions surrounding the lateral ventricles were isolated. These were enzymatically and mechanically dissociated. The cells were plated in serum free media with EGF, (20 ng/mL; Sigma-Aldrich), bFGF, (10 ng/mL; Sigma-Aldrich) and heparin (2 μ g/mL, Sigma-Aldrich). The NPCs were passaged every 7 days and were used between passages 1–4 for the galvanotaxis assay.

For conditioned media (CM) collection, C57/BL6-derived neurosphere cells were plated at 40 cells/ μ L. The following day the flask was spun down at 1500 rpm and CM was collected and stored at -20°C or -80°C prior to use.

2.3. Galvanotaxis chambers and assay

Galvanotaxis chambers were constructed as described (Babona-Pilipos et al., 2011, 2012). Briefly, acid-treated glass slides were coated with poly-L-lysine (Sigma-Aldrich) plated with laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane (50 μ g/mL,

Sigma), collagen I rat tail (40 μ g/mL, Gibco) and 4% (v/v) Corning Matrigel Basement Membrane Matrix (BD Biosciences, Canada). Following coating, SKP spheres were triturated with a P200 and incubated for 17–22 h in the presence of EGF, bFGF and B27 at 37°C , 5% CO_2 in 100% humidity. Prior to being placed in the presence of the direct current EF, the chambers received a glass slide as a roof and were placed in the microscope for live-cell imaging. Silver wire (Alfa Aesar) chlorinated in bleach was used as electrodes and 1.5% agarose gel bridges were used to separate the toxic electrode-media interface from the cells.

For co-culture experiments, chamber construction was the same as above. Both SKP and NPC spheres were removed from their respective cultures, triturated and plated together on chambers coated with laminin. For CM experiments, on the day of chamber construction the CM was thawed and supplemented with EGF, bFGF and B27, and sterile filtered. The CM was added to the chambers and triturated SKP spheres were plated onto the galvanotaxis chamber and placed in the presence or absence of the EF.

2.4. Quantification of cell migration

Cells were live-imaged and tracked in the presence and absence of the EF. For SKP migration experiments, images were taken every minute for 150 min. At least 15 cells per experiment were tracked using AxioVision Cell Tracking Software and at least 3 chambers were run per experimental condition. For co-culture experiments, a microscope field of view, which included at least 15 cells that were non-fluorescent (SKPs) and 15 cells that were fluorescent (yellow fluorescent NPCs), was chosen. Images were taken every 5 or 10 min for 150 min. Cells were tracked with automatic tracking software or with the manual tracking option at 10 min intervals. X and Y-displacement shifts in the chamber itself were subtracted from the displacement of the tracked cells as appropriate in the following analyses.

For each individual cell the following parameters were calculated using the measurements as depicted in the diagram in Fig. 1A. The magnitude of the velocity ($|\text{velocity}|$) was calculated by dividing the cell displacement by the elapsed time. The directedness of the migration was calculated by dividing the cell displacement in the direction of the EF (x-displacement) by the cell displacement. This gives the cosine of the angle (θ) between the cell displacement vector and the EF vector. A directedness value of 1 is a cell migrating directly towards the cathode and a value of -1 is a cell migrating directly towards the anode. These values were averaged over the total number of cells tracked in each experimental condition.

Cell migration path graphs were plotted with each cell migration path starting at the origin (0,0) as seen in Fig. 1H–M and Fig. 3C and D. To quantify cell migration directional preference on laminin, NPCs or SKPs were considered to have migrated towards a terminal (cathode or anode), if its displacement towards a terminal was greater than the average of all cell displacements in the absence of an EF plus the standard error of the means for their respective cell type. Otherwise, the cell was considered undirected.

2.5. Immunocytochemistry

Immunocytochemistry was performed as previously described (Babona-Pilipos et al., 2011). Cells were plated overnight (17–22 h) in the absence of an EF. Cells were placed in the EF for 150 min and then fixed with 4% paraformaldehyde (Sigma) for 10–20 min at room temperature for 3 chambers. Control cells were plated overnight (17–24 h) in the absence of an EF for 3 chambers and then fixed. The cells were washed 3 times with PBS for 5 min and permeabilized with 0.3% Triton X-100 (Sigma) for 20 min at room temperature. The cells were exposed to 10% NGS (Jackson ImmunoResearch Laboratories) in PBS for 1 h at room temperature and then washed and incubated overnight in primary antibody cocktail at 4°C . The cells were then triple washed for 5 min

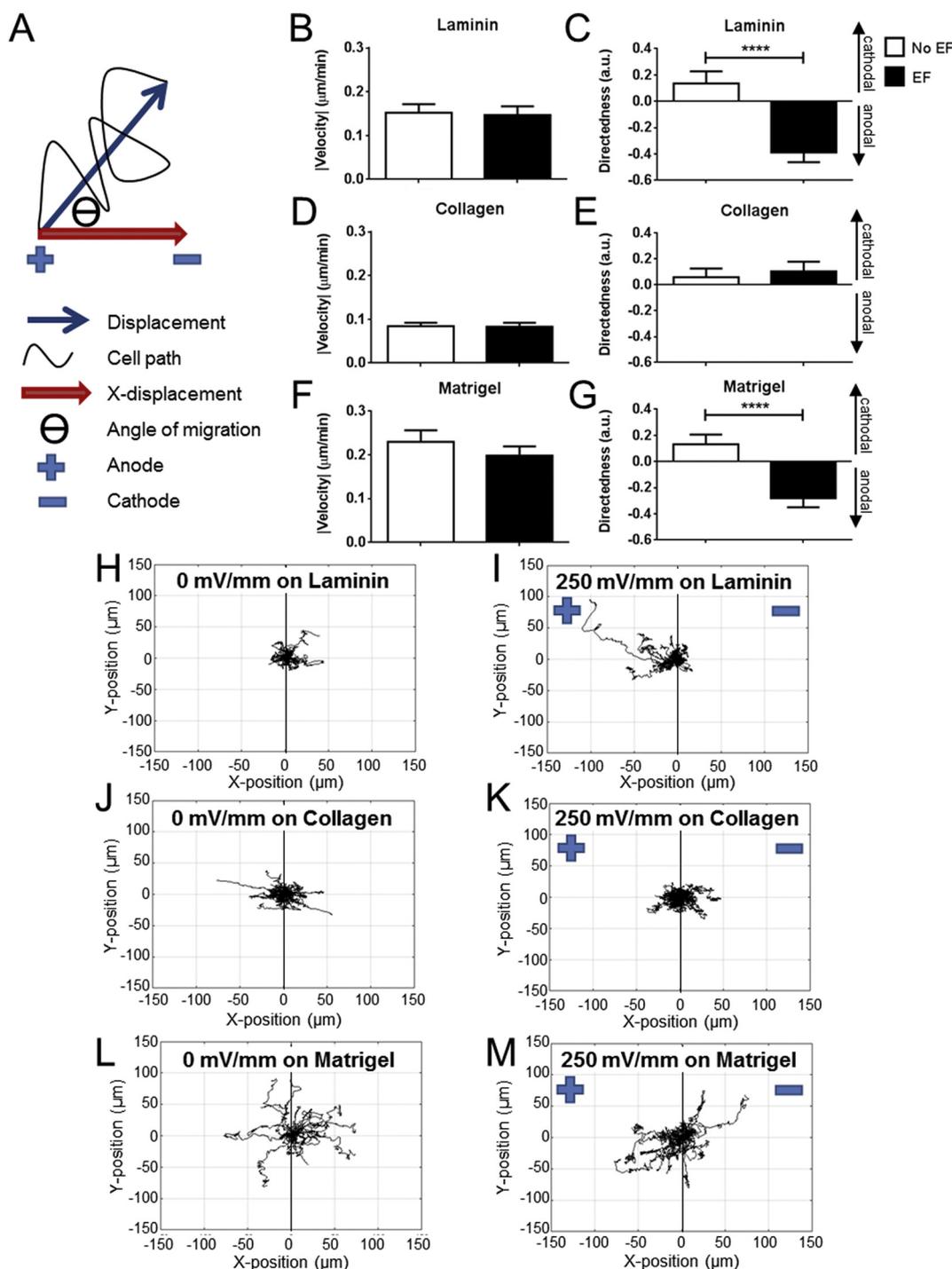


Fig. 1. SKPs migrate towards the anode in the presence of a direct current EF on laminin and Matrigel, but not on collagen. A) Diagram of an example cell path in black and the measured variables used to calculate the |velocity| and directedness of the cells. The average SKP |velocity| and directedness on B)-C) laminin, D)-E) collagen and F)-G) Matrigel was calculated in the presence and absence of a direct current EF over 3 experiments. H)-M) Representative tracked cell paths for one experiment of SKPs over 150 min. SKPs on laminin in the H) absence of an EF ($n = 26$) and I) in the presence of an EF ($n = 25$); on collagen in the J) absence of an EF ($n = 36$) and K) in the presence of an EF ($n = 39$); and on Matrigel in the L) absence of an EF ($n = 22$) and M) in the presence of an EF ($n = 21$). Anodes indicated by “+” and cathodes “-”. Data are represented as means \pm S.E.M., **** $p < 0.0001$.

each in PBS and incubated at room temperature for 1 h with a secondary antibody cocktail. The following primary antibodies were used in a cocktail: rabbit polyclonal anti-fibronectin (1:400, Sigma) and mouse monoclonal anti-nestin (1:400, Millipore). Rabbit polyclonal anti-Sox2 (1:200, Abcam) was used as a single antibody. The following secondary antibodies were incubated for 1 h at room temperature: goat-anti-rabbit conjugated with Alexafluor 488 (1:400, Invitrogen-Gibco)

and goat-anti-mouse conjugated with Alexafluor 568 (1:400, Invitrogen-Gibco). Cells were mounted with mounting media containing 4',6-diamidino-2-phenylindole (Vector Laboratories) for nuclear staining.

Images were taken and positive cells were counted in the region with a high density of plated cells and at 4 other locations: 1000 μm above, below and beside the initial imaging location. The percentage of

positive cells was recorded for each chamber and averaged over 3 chambers in the experimental condition.

2.6. Statistical analysis

Values are represented as means over all cells \pm standard error of means. Group means were compared using an unpaired *t*-test. Comparisons were done between percentages of cells showing a preference towards the anode, cathode and undirected pole using a Fisher's exact test. Comparisons of values to zero were done using a one-sample *t*-test.

3. Results

3.1. SKPs undergo anodal galvanotaxis

SKPs were plated in the presence of growth factors and placed in the presence of an EF for 150 min on two physiologically relevant substrates, laminin and collagen I which are present in the skin, as well as Matrigel which is an adhesive substrate containing a combination of laminin, collagen IV, TGF- β , and extracellular matrix proteins and growth factors found in mouse sarcoma. We utilized a field strength of 250 mV/mm, which induces NPC galvanotaxis (Babona-Pilipos et al., 2011). To characterize SKP migration we calculated the magnitude of the velocity, $|\text{velocity}|$, and directedness using the variables seen in Fig. 1A and described in Methods 2.4. Notably, in the absence of an EF the directedness was not significantly different from zero on any substrate examined (one sample *t*-test, (laminin) $p = 0.15$, (collagen) $p = 0.37$, (Matrigel) $p = 0.078$) (Fig. 1C,E,G). However, SKPs displayed directed migration towards the anode on laminin and Matrigel in the presence of an EF (Fig. 1C,G). On collagen, directedness was not significantly different in the presence or absence of the EF (unpaired *t*-test, $p = 0.65$) (Fig. 1E). These findings demonstrate that SKP galvanotaxis is substrate-dependent. Fig. 1H–M shows individual cell migration paths on laminin, collagen, and Matrigel substrates in the presence or absence of an EF.

With regard to SKP migration $|\text{velocity}|$, no significant difference was observed in the presence and absence of an EF on any substrate tested (Fig. 1B,D,F). No differences in the numbers of adherent cells on laminin, collagen and Matrigel were observed throughout the time lapse imaging. Although, cells continued to adhere to the substrates, cell morphology became less elongated and more rounded in the presence of an EF compared to no EF (Supplementary video 1–6 in the web version).

3.2. SKP electro-sensitivity

To test whether the weak galvanotactic migratory response of SKPs was due to suboptimal EF strength application, we chose to investigate EFs of 125 mV/mm and 500 mV/mm on laminin. At a lower field strength of 125 mV/mm, $|\text{velocity}|$ remained the same ($0.14 \pm 0.01 \mu\text{m}/\text{min}$) and migration was still directed towards the anode (-0.24 ± 0.06). Directedness at 125 mV/mm was not significantly different from that of 250 mV/mm EF. At a higher field strength of 500 mV/mm, cells died after 100 min of EF application. Thus we were unable to enhance the galvanotactic response of SKPs over a range of field strengths.

One of the most notable observations when analyzing SKP migration was that, unlike NPCs in the presence of an EF, only a subpopulation of SKPs displayed directed migration. In Fig. 2A, we analyzed the percentage of migrating cells. Approximately half of the 62 tracked cells were considered undirected in the presence or absence of an EF for both conditions (53% and 48%, no EF versus no EF condition, respectively). Hence, many SKPs were not responsive to the EF. However, more cells went towards the anode in the presence of an EF (42%) versus in the absence of an EF (18%). Of those directed cells, the proportion of cells

migrating to the anode and cathode was significantly different in the absence and presence of an EF (Fisher's exact test, $p = 0.0007$). This is very different from what is observed in NPCs where $> 98\%$ of NPCs undergo galvanotaxis towards the cathode under these same conditions (Babona-Pilipos et al., 2011). Together, these findings suggest that SKPs are generally less electro-sensitive than other precursor pools.

3.3. SKPs express nestin, fibronectin and Sox2 before and after EF application

SKPs have the potential to generate a variety of different cell types including neuronal, skeletogenic and dermal phenotypes (Biernaskie et al., 2009; Fernandes et al., 2006; Lavoie et al., 2009). We asked whether there was heterogeneity within the cells that might underlie the weak galvanotactic response as (1) different cell types display different galvanotactic responses in terms of their migration kinematics (Nakajima et al., 2015) and; (2) we have previously demonstrated that mature neural cell types do not undergo galvanotaxis (Babona-Pilipos et al., 2011). We sought to determine whether the time in culture, and in the presence of the EF, was inducing differentiation of SKP-derived cells which could ultimately account for the observed weak galvanotactic response. We performed immunocytochemistry on SKPs after EF application and in sister cultures with no EF application, ensuring the same time in culture prior to analysis. Antibodies used to evaluate markers of undifferentiated SKP cells include nestin, fibronectin and Sox2 (Biernaskie et al., 2009; Toma et al., 2001). There was a small, significant decrease in the percentage of cells expressing fibronectin (10%) and Sox2 (4%) at the end of the EF application however the vast majority of SKPs expressed undifferentiated cell markers in the presence or absence of the EF as shown in Fig. 2B. Representative images are found in Fig. 2C–D. The significant increase in anodally-directed cells (from 18 to 42% in the presence of the EF) cannot be accounted for by the cells that are expressing the undifferentiated markers.

3.4. SKPs and NPCs migrate in different directions when plated together in the presence of an EF

Our previous work demonstrated that NPCs are electro-sensitive cells that undergo cathodal galvanotaxis at a $|\text{velocity}|$ of $1.09 \pm 0.15 \mu\text{m}/\text{min}$ (Babona-Pilipos et al., 2011), which is approximately $5\text{--}7\times$ greater than what we observed with SKPs at 250 mV/mm. SKPs migrate much slower and in the opposite direction to NPCs plated on the same substrates (laminin, Matrigel) and in the same field strength (250 mV/mm). To ask whether cell-mediated factors may be influencing cell migratory behaviour in the EF, we performed co-culture experiments with SKPs and NPCs. NPCs expressing yellow fluorescent protein were co-cultured with SKPs from wild-type mice in the presence of growth factors. In co-cultures, NPCs migrated in the same rapid and cathodal-directed fashion as previously demonstrated when cultured alone (Babona-Pilipos et al., 2011) and SKPs continued to migrate towards the anode (Fig. 3A–B and supplementary video 7 in web version). The individual cell paths in the absence and presence of an EF are shown for NPCs (green) and SKPs (black) on the same graph to demonstrate their different migratory behaviours (Fig. 3C–D). Virtually all of the NPCs (95%) migrated towards the cathode (Fig. 3E), similar to what is observed in the presence of an EF when NPCs are plated alone (Babona-Pilipos et al., 2011, 2015). Notably, a smaller proportion of SKPs were undirected in the presence of an EF in NPC co-cultures (Fisher's exact test, $p = .01$) (Fig. 3E). These findings reveal that SKP migration is dependent on environmental factors that can be modified by NPCs. We next asked whether a secreted factor from NPCs could account for the change in SKP galvanotaxis. Conditioned media (CM) was collected from NPC cultures and placed on SKPs in the galvanotaxis chamber. Strikingly, there was a significant increase in the numbers of cathodally-directed migrating cells within the directed SKP population compared to SKPs alone (10% SKPs alone versus 42% for SKPs + CM)

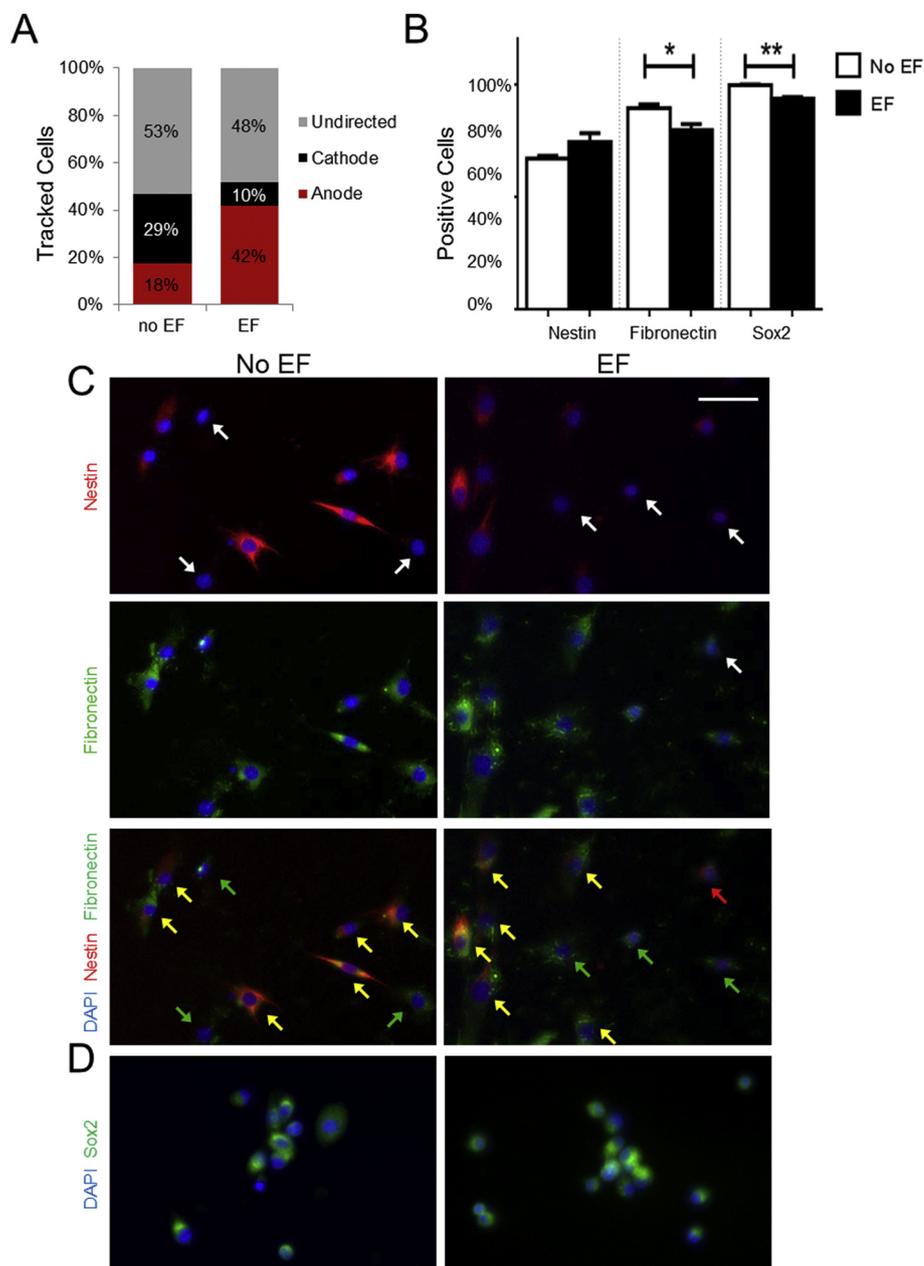


Fig. 2. Plated SKPs have a heterogeneous response to EFs and express nestin, fibronectin and Sox2. **A)** In 3 different chambers of cells plated on laminin, percentage of the cells that showed a directional preference ($n = 66$ cells, no EF) ($n = 62$ cells, EF) **B)** Percentages of cells that express nestin, fibronectin and Sox2 after being in the absence of an EF and in the presence of an EF. **C)** Representative images of plated chambers of SKPs that were in the absence and presence of an EF. White arrows indicate cells that are not expressing nestin and/or fibronectin. In merged images green arrows indicate cells expressing only fibronectin; red arrows indicate cells expressing only nestin and; yellow arrows indicate cells expressing nestin and fibronectin. **D)** Images of plated chambers of Sox2-positive SKPs that were in the absence and presence of an EF. Scale bar = 50 μm . Data are represented as means \pm S.E.M. * $p < 0.05$, ** $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Fisher's exact test, $p < 0.0001$) (Fig. 3E). These findings reveal that a factor in the NPC secretome is able to modify the galvanotactic response of SKPs.

4. Discussion

The migratory response of cells to EFs is important for directing cells to injury sites. Here we have demonstrated that SKPs undergo anodal galvanotaxis. Their response is heterogeneous unlike the rapid and robust cathodal migration of NPCs. Further, we demonstrate that SKP migration is influenced by the external environment as their migration is substrate-dependent and modified by the presence of NPCs.

The cellular mechanisms underlying differences in galvanotaxis between different cell populations is not well understood. However, the differentiation state of cells can influence cell migration through intracellular pH, membrane potential and ion channel expression, for example (Allen et al., 2013; Babona-Pilipos et al., 2011; Chang and Minc, 2014; Cui et al., 2017; Levin, 2014; Nakajima et al., 2015; Ozkucur et al., 2011). In our studies, we cannot exclude the possibility

that a subpopulation of multipotent SKPs were undergoing differentiation into a more mature phenotype within the culture conditions that could be affecting their galvanotactic response. However, even in an undifferentiated state, SKPs show differential migration patterns when transplanted *in vivo* (Biernaskie et al., 2009; Fernandes et al., 2004). In our experiments, cells were plated in conditions that maintain cells in an undifferentiated state and the vast majority of SKPs continued to express nestin, fibronectin and Sox2 demonstrating that differentiation is not the cause of the differential migration.

A role for external factors was seen as SKPs plated on different matrices underwent different galvanotactic responses. Laminin and collagen are components of the extracellular matrix of the dermis (Iorio et al., 2015; Meigel et al., 1977). Matrigel is a mixture predominantly of laminin and collagen IV. Interestingly, we found that the matrix regulated the directedness, but not [velocity], of SKPs. This dissociation between [velocity] and directedness suggests that the mechanisms underlying these distinct parameters of galvanotaxis are differentially regulated, comparable to what others have observed with human keratinocytes (Nishimura et al., 1996; Sheridan et al., 1996) and NPCs

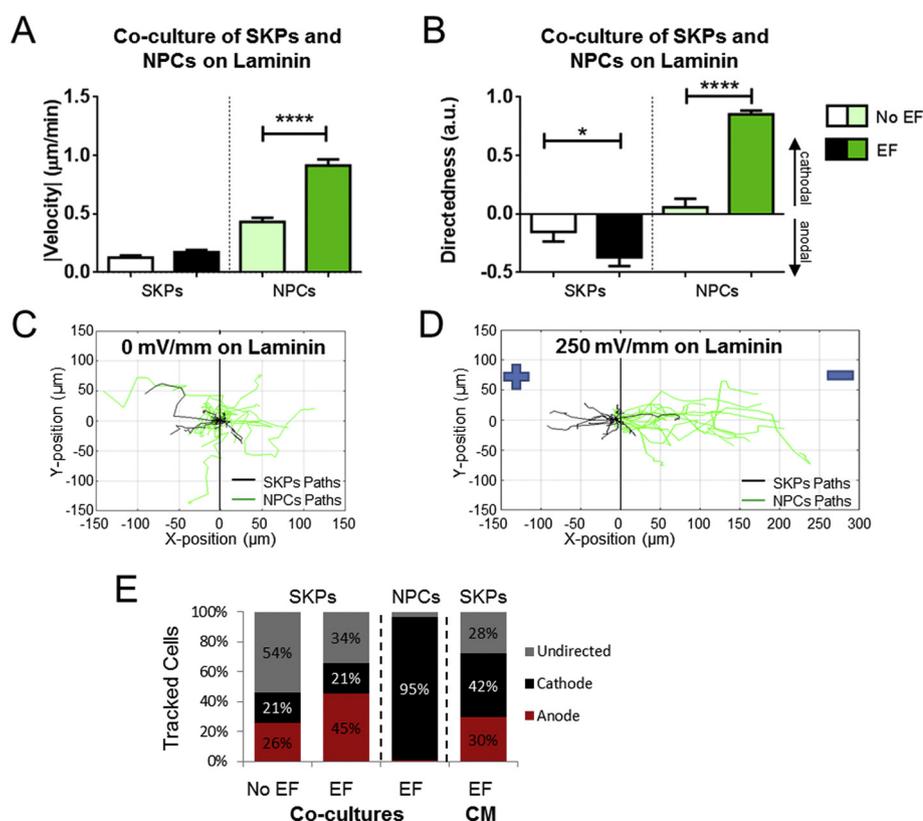


Fig. 3. SKPs and NPCs migrate in different directions when plated together in the presence of an EF. A) Average NPC and SKP |velocity| in the presence and absence of an EF over 5 experiments on laminin. B) Average SKP and NPC directedness in the presence of an EF over 5 experiments on laminin. C) Tracked cell paths of co-culture of SKPs and NPCs over 150 min in the absence of an EF: SKP paths ($n = 15$) and NPC paths ($n = 15$) D) the presence of an EF: SKP paths ($n = 20$) and NPC paths ($n = 15$). E) Cell migration directional preference in at least 3 different chambers of cells plated on laminin, in co-cultures of SKPs and NPCs ($n = 82, 97, 86$ SKPs with no EF, SKPs with EF, NPCs with EF) and in NPC conditioned media ($n = 205$, SKPs with EF). Data are represented as means \pm S.E.M. * $p < 0.05$, **** $p < 0.0001$.

(Babona-Pilipos et al., 2011, 2018). *In vivo*, SKPs are found in the dermal papillae and are exposed to different substrates in the skin such as collagens, laminin, fibronectin and other elastic fibers (Hsu et al., 2014). Transplanted SKPs migrate to hair follicle niches and also migrate to injury sites (Biernaskie et al., 2009). Having distinct migratory responses on different substrates can contribute to the cues necessary to direct SKP migration to desired final environments such as injury sites.

A number of pathways have been elucidated that mediate the EF-induced migration in response to environmental cues. These include: (1) focal adhesions to specific substrates, (2) matrix stiffness and/or morphology and (3) the surface charge of the substrate. Downstream effectors of adhesive interactions regulate the phosphatidylinositol 3-kinase (PI3K)-Akt signalling pathways, a pathway shown to be important in galvanotaxis (Yao et al., 2015). Concentrations of extracellular matrix molecules can affect EGF receptor expression which is also shown to play a role in galvanotaxis (Sheridan et al., 1996; Zhao et al., 1999). Differences in matrix stiffness or morphology are other potential players in establishing the galvanotactic response of cells. Indeed, brain tumor-initiating cells migrate towards the anode on poly-L-ornithine/laminin coated plates in a 2D environment but the same cells undergo cathodal migration in a collagen type I hyaluronic acid gel 3D environment (Huang et al., 2016). Surface charge has also been shown to influence EF-induced neurite growth and galvanotaxis of glial cells (Huang et al., 2017; Rajnicek et al., 1998). The mechanism of galvanotaxis in response to various environmental cues remains an important area for future investigation.

Interestingly, our co-culture experiments with NPCs and SKPs demonstrate that when placed in the same extracellular cues, SKPs and NPCs continue to respond differently, supporting the idea that cells use different signalling pathways when responding to EFs. Similar to when cultured alone, a large portion of the population of SKPs migrates towards the anode and virtually all NPCs migrate towards the cathode. However, in NPC co-cultures, a greater percentage of SKPs undergo directed migration. We demonstrated further that factors released from NPCs are affecting the directed migratory behaviour of SKPs. Factors

such as vascular endothelial growth factor, bFGF, nerve growth factor, glial cell-derived neurotrophic factor, insulin-like growth factor-1 and/or transforming growth factor β 2 are good candidates for modifying the galvanotactic response (Hawryluk et al., 2012; Ladewig et al., 2014). A comprehensive understanding of the interplay between EFs in different microenvironments and cells is critical for the development of tools to promote wound healing.

5. Conclusions

This study demonstrates that SKPs are able to undergo galvanotaxis and their migration is preferentially towards the anode. The fact that SKPs are less electro-sensitive in terms of migratory behaviour induced by the EF (directedness; |velocity|) and that the migration was substrate-dependent, plays an important role in how different cell types respond to tissue injury where EFs are generated. We have further demonstrated that external factors derived from neighbouring cells influence the migration, highlighting the complexity of the interplay between cells and their environment in response to electrical stimulation.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2018.07.019>.

Declarations of interest

None.

Funding

This research was funded by the Connaught Innovation Fund [grant number 502036] (CMM, MRP) and Ontario Institute of Regenerative Medicine [grant number 501670] (CMM, MRP). SNI was the recipient of the Barbara and Frank Milligan Award (University of Toronto) and the Wildcat Graduate Scholarship (University of Toronto). The funding sources had no involvement in the conduct of the research or

preparation of the article.

Acknowledgements

The authors would like to thank Sibel Naska, Smitha Paul and Neemat Mahmud for their suggestions on SKP culture. The authors would also like to thank Umalkhair Ahmed, Nancy Liu and Elana Sefton for their input in the manuscript preparation.

References

- Allen, G.M., Mogilner, A., Theriot, J.A., 2013. Electrophoresis of cellular membrane components creates the directional cue guiding keratocyte galvanotaxis. *Curr. Biol.* 23, 560–568. <https://doi.org/10.1016/j.cub.2013.02.047>.
- Babona-Pilipos, R., Droujinine, I.A., Popovic, M.R., Morshead, C.M., 2011. Adult subependymal neural precursors, but not differentiated cells, undergo rapid cathodal migration in the presence of direct current electric fields. *PLoS ONE* 6. <https://doi.org/10.1371/journal.pone.0023808>.
- Babona-Pilipos, R., Popovic, M.R., Morshead, C.M., 2012. A galvanotaxis assay for analysis of neural precursor cell migration kinetics in an externally applied direct current electric field. *J. Vis. Exp.* 1–8. <https://doi.org/10.3791/4193>.
- Babona-Pilipos, R., Pritchard-Oh, A., Popovic, M.R., Morshead, C.M., 2015. Biphasic monopolar electrical stimulation induces rapid and directed galvanotaxis in adult subependymal neural precursors. *Stem Cell Res Ther* 6, 67. <https://doi.org/10.1186/s13287-015-0049-6>.
- Babona-Pilipos, R., Liu, N., Pritchard-Oh, A., Mok, A., Badawi, D., Popovic, M.R., Morshead, C.M., 2018. Calcium influx differentially regulates migration velocity and directedness in response to electric field application. *Exp. Cell Res.* 368, 202–214. <https://doi.org/10.1016/j.yexcr.2018.04.031>.
- Biernaskie, J.A., McKenzie, I.A., Toma, J.G., Miller, F.D., 2006. Isolation of skin-derived precursors (SKPs) and differentiation and enrichment of their Schwann cell progeny. *Nat. Protoc.* 1, 2803–2812. <https://doi.org/10.1038/nprot.2006.422>.
- Biernaskie, J., Paris, M., Morozova, O., Fagan, B.M., Marra, M., Pevny, L., Miller, F.D., 2009. SKPs derive from hair follicle precursors and exhibit properties of adult dermal stem cells. *Cell Stem Cell* 5, 610–623. <https://doi.org/10.1016/j.stem.2009.10.019>.
- Cao, L., Wei, D., Reid, B., Zhao, S., Pu, J., Pan, T., Yamoah, E.N., Zhao, M., 2013. Endogenous electric currents might guide rostral migration of neuroblasts. *EMBO Rep.* 14, 184–190. <https://doi.org/10.1038/embor.2012.215>.
- Chang, F., Minc, N., 2014. Electrochemical control of cell and tissue polarity. *Annu. Rev. Cell Dev. Biol.* 30, 317–336. <https://doi.org/10.1146/annurev-cellbio-100913-013357>.
- Cui, M., Ge, H., Zhao, H., Zou, Y., Chen, Y., Feng, H., 2017. Electromagnetic fields for the regulation of neural stem cells. *Stem Cells Int.* 2017. <https://doi.org/10.1155/2017/9898439>.
- Fernandes, K.J.L., McKenzie, I.A., Mill, P., Smith, K.M., Akhavan, M., Barnabé-Heider, F., Biernaskie, J., Junek, A., Kobayashi, N.R., Toma, J.G., Kaplan, D.R., Labosky, P.A., Rafuse, V., Hui, C.-C., Miller, F.D., 2004. A dermal niche for multipotent adult skin-derived precursor cells. *Nat. Cell Biol.* 6, 1082–1093. <https://doi.org/10.1038/ncb1181>.
- Fernandes, K.J.L., Kobayashi, N.R., Gallagher, C.J., Barnabé-Heider, F., Aumont, A., Kaplan, D.R., Miller, F.D., 2006. Analysis of the neurogenic potential of multipotent skin-derived precursors. *Exp. Neurol.* 201, 32–48. <https://doi.org/10.1016/j.expneurol.2006.03.018>.
- Fernandes, K.J.L., Toma, J.G., Miller, F.D., 2008. Multipotent skin-derived precursors: adult neural crest-related precursors with therapeutic potential. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 363, 185–198. <https://doi.org/10.1098/rstb.2006.2020>.
- Funk, R.H.W., 2015. Endogenous electric fields as guiding cue for cell migration. *Front. Physiol.* 6, 1–8. <https://doi.org/10.3389/fphys.2015.00143>.
- Grahn, J.C., Reilly, D.A., Nuccitelli, R.L., Isseroff, R.R., 2003. Melanocytes do not migrate directionally in physiological DC electric fields. *Wound Repair Regen.* 11, 64–70. <https://doi.org/10.1046/j.1524-475X.2003.11110.x>.
- Guo, A., Song, B., Reid, B., Gu, Y., Forrester, J.V., Jahoda, C.A.B., Zhao, M., et al., 2010. Effects of physiological electric fields on migration of human dermal fibroblasts. *J. Invest. Dermatol.* 130, 2320–2327. <https://doi.org/10.1038/jid.2010.96>.
- Hawrylyuk, G.W.J., Mothe, A.J., Chamankhah, M., Wang, J., Tator, C., Fehlings, M.G., 2012. In vitro characterization of trophic factor expression in neural precursor cells. *Stem Cells Dev.* 21, 432–447. <https://doi.org/10.1089/scd.2011.0242>.
- Hsu, Y.-C., Li, L., Fuchs, E., 2014. Emerging interactions between skin stem cells and their niches. *Nat. Med.* 20, 847–856. <https://doi.org/10.1038/nm.3643>.
- Huang, Y.-J., Hoffmann, G., Wheeler, B., Schiapparelli, P., Quinones-Hinojosa, A., Searson, P., 2016. Cellular microenvironment modulates the galvanotaxis of brain tumor initiating cells. *Sci. Rep.* 6, 1–10. <https://doi.org/10.1038/srep21583>.
- Huang, Y.-J., Schiapparelli, P., Kozielski, K., Green, J., Lavell, E., Guerrero-Cazares, H., Quinones-Hinojosa, A., Searson, P., 2017. Electrophoresis of cell membrane heparan sulfate regulates galvanotaxis in glial cells. *J. Cell Sci.* 130, 203752. <https://doi.org/10.1242/jcs.203752>.
- Iorio, V., Troughton, L.D., Hamill, K.J., 2015. Laminins: roles and utility in wound repair. *Adv. wound care* 4, 250–263. <https://doi.org/10.1089/wound.2014.0533>.
- Iwasa, S.N., Babona-Pilipos, R., Morshead, C.M., 2017. Environmental Factors That Influence Stem Cell Migration: an “electric Field”. *Stem Cells Int.* 2017. <https://doi.org/10.1155/2017/4276927>.
- Ke, T., Yang, M., Mao, D., Zhu, M., Che, Y., Kong, D., Li, C., 2015. Co-transplantation of skin-derived precursors and collagen sponge facilitates diabetic wound healing by promoting local vascular regeneration. *Cell. Physiol. Biochem.* 37, 1725–1737. <https://doi.org/10.1159/000438537>.
- Ladewig, J., Koch, P., Brüstle, O., 2014. Auto-attraction of neural precursors and their neuronal progeny impairs neuronal migration. *Nat. Neurosci.* 17, 24–26. <https://doi.org/10.1038/nn.3583>.
- Lavoie, J.-F., Biernaskie, J.A., Chen, Y., Bagli, D., Alman, B., Kaplan, D.R., Miller, F.D., 2009. Skin-derived precursors differentiate into skeletogenic cell types and contribute to bone repair. *Stem Cells Dev.* 18, 893–906. <https://doi.org/10.1089/scd.2008.0260>.
- Levin, M., 2014. Molecular bioelectricity: how endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo. *Mol. Biol. Cell* 25, 3835–3850. <https://doi.org/10.1091/mbc.E13-12-0708>.
- Levin, M., Martyniuk, C.J., 2018. The bioelectric code: an ancient computational medium for dynamic control of growth and form. *Biosystems* 164, 76–93. <https://doi.org/10.1016/j.biosystems.2017.08.009>.
- Levin, M., Pezzullo, G., Finkelstein, J.M., 2017. Endogenous bioelectric signaling networks: exploiting voltage gradients for control of growth and form. *Annu. Rev. Biomed. Eng.* 19, 353–387. <https://doi.org/10.1146/annurev-bioeng-071114-040647>.
- McKenzie, I.A., Biernaskie, J., Toma, J.G., Midha, R., Miller, F.D., 2006. Skin-derived precursors generate myelinating Schwann cells for the injured and dysmyelinated nervous system. *J. Neurosci.* 26, 6651–6660. <https://doi.org/10.1523/JNEUROSCI.1007-06.2006>.
- Meigel, W.N., Gay, S., Weber, L., 1977. Dermal architecture and collagen type distribution. *Arch. Dermatol. Res.* 259, 1–10. <https://doi.org/10.1007/BF00562732>.
- Meng, X., Arocena, M., Penninger, J., Gage, F.H., Zhao, M., Song, B., 2011. PI3K mediated electrostatic of embryonic and adult neural progenitor cells in the presence of growth factors. *Exp. Neurol.* 227, 210–217. <https://doi.org/10.1016/j.expneurol.2010.11.002>.
- Nakajima, K., Zhu, K., Sun, Y.H., Hegyi, B., Zeng, Q., Murphy, C.J., Small, J.V., Chen-Izu, Y., Izumiya, Y., Penninger, J.M., Zhao, M., 2015. KCNJ15/Kir4.2 couples with polyamines to sense weak extracellular electric fields in galvanotaxis. *Nat. Commun.* 6, 8532. <https://doi.org/10.1038/ncomms9532>.
- Naska, S., Yuzwa, S.A., Johnston, A.P.W., Paul, S., Smith, K.M., Paris, M., Sefton, M.V., Datti, A., Miller, F.D., Kaplan, D.R., 2016. Identification of drugs that regulate dermal stem cells and enhance skin repair. *Stem Cell Rep.* 6, 74–84. <https://doi.org/10.1016/j.stemcr.2015.12.002>.
- Nishimura, K.Y., Isseroff, R.R., Nuccitelli, R., 1996. Human keratinocytes migrate to the negative pole in direct current electric fields comparable to those measured in mammalian wounds. *J. Cell Sci.* 109, 199–207. <https://doi.org/10.3389/fphys.2015.00143>. (Pt 1).
- Ozkucur, N., Perike, S., Sharma, P., Funk, R.H.W., 2011. Persistent directional cell migration requires ion transport proteins as direction sensors and membrane potential differences in order to maintain directedness. *BMC Cell Biol.* 12, 4. <https://doi.org/10.1186/1471-2121-12-4>.
- Rajnicek, A.M., Robinson, K.R., McCaig, C.D., 1998. The direction of neurite growth in a DC electric field depends on the substratum. *Mol. Biol. Cell* 9, 226A.
- Sheridan, D.M., Isseroff, R.R., Nuccitelli, R., 1996. Imposition of a physiological DC electric field alters the migratory response of human keratinocytes on extracellular matrix molecules. *J. Invest. Dermatol.* 106, 642–646. <https://doi.org/10.1111/1523-1747.ep12345456>.
- Sillman, A.L., Quang, D.M., Farboud, B., Fang, K.S., Nuccitelli, R., Isseroff, R.R., 2003. Human dermal fibroblasts do not exhibit directional migration on collagen I in direct-current electric fields of physiological strength. *Exp. Dermatol.* 12, 396–402. <https://doi.org/10.1034/j.1600-0625.2002.120406.x>.
- Steinbach, S.K., El-Mounayri, O., Dacosta, R.S., Frontini, M.J., Nong, Z., Maeda, A., Pickering, J.G., Miller, F.D., Husain, M., 2011. Directed differentiation of skin-derived precursors into functional vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* 31, 2938–2948. <https://doi.org/10.1161/ATVBAHA.111.232975>.
- Thrivikraman, G., Boda, S.K., Basu, B., 2018. Unraveling the mechanistic effects of electric field stimulation towards directing stem cell fate and function: a tissue engineering perspective. *Biomaterials* 150, 60–86. <https://doi.org/10.1016/j.biomaterials.2017.10.003>.
- Toma, J.G., Akhavan, M., Fernandes, K.J., Barnabé-Heider, F., Sadikot, A., Kaplan, D.R., Miller, F.D., 2001. Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat. Cell Biol.* 3, 778–784. <https://doi.org/10.1038/ncb0901-778>.
- Yao, L., Li, Y., Knapp, J., Smith, P., 2015. Exploration of molecular pathways mediating electric field-directed schwann cell migration by RNA-seq. *J. Cell. Physiol.* 230, 1515–1524. <https://doi.org/10.1002/jcp.24897>.
- Zhao, M., Dick, A., Forrester, J.V., McCaig, C.D., 1999. Electric field-directed cell motility involves up-regulated expression and asymmetric redistribution of the epidermal growth factor receptors and is enhanced by fibronectin and laminin. *Mol. Biol. Cell* 10, 1259–1276. <https://doi.org/10.1091/mbc.10.4.1259>.
- Zhao, M., 2009. Electrical fields in wound healing: an overriding signal that directs cell migration. *Semin. Cell Dev. Biol.* 20, 674–682.