Recent Developments in Electrotaxis Assays

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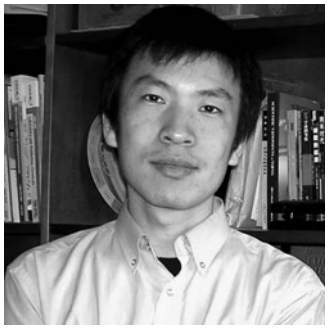
Significance: A wide range of cell types can migrate in response to physiological or externally applied direct current electric field (dcEF), a process termed electrotaxis. In particular, electrotaxis of epithelial cells to wound-generated dcEF for mediating wound healing is a well-accepted mechanism. In addition, various immune cells have been demonstrated to undergo electrotaxis, suggesting a link between electrotaxis and inflammatory responses in wound healing. Electrotaxis research will generate important insight into the electrical guiding mechanism for cell migration thereby providing the scientific basis to further develop clinical applications for wound care. Development of advanced electrotaxis assays will critically enable in-depth experimental electrotaxis studies *in vitro*.

Recent Advances: Recently, a number of new electrotaxis assays or new uses of previously developed assays for electrotaxis studies have been reported. These new developments provide improved solutions for experimental throughput, configuration of three-dimensional cell migration environments and coexisting guiding signals, measurements of collective electrotactic cell migration, and sorting electrotactic populations.

Critical Issues: These new developments face the challenge of playing a more important role to better understand the biological mechanisms underlying electrotaxis, in addition to making a stronger impact on relevant applications.

Future Directions: On one hand, specific electrotaxis assays should be further developed to improve its function and tested for a broader range of experimental conditions and electrotactic populations. On the other hand, joint efforts among electrotaxis researchers are needed to integrate the unique features of specific electrotaxis assays, allowing more advanced and efficient electrotaxis analyses to answer both basic science and clinical questions.



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Abbreviations and Acronyms

2D = two-dimensional
3D = three-dimensional
dcEF = direct current EF
ECM = extracellular matrix
EF = electric field
GFP = green fluorescent protein
hiPS = human induced pluripotent stem
PDMS = polydimethylsiloxane

SCOPE AND SIGNIFICANCE

Cell migration is mediated by complex interactions between cells and their environments. It plays an important role in physiological processes such as inflammation, wound healing, cancer metastasis, and neuronal guidance.[1–5] Among the diverse guiding mechanisms, electrotaxis or galvanotaxis—cell migration in response to direct current electric field (dcEF)—can direct the migration of various cell types such as epithelial cells,[6] endothelial cells,[7] cancer cells,[8] and immune cells.[9] Physiological dcEF produced at the wound and electrical attraction of epithelial cells for wound recovery is well known. Research toward better understanding the mechanisms of electrotaxis is an important and rapidly growing area with direct relevance to wound care. Sophisticated *in vitro* electrotaxis assays provide the important experimental platform for electrotaxis studies.

Dish-based assays are the current gold standard for *in vitro* electrotaxis

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measurements and have been widely adapted to study electrotaxis of various cell types.[5,8,10] In addition, microfluidic devices, which can better control electric field (EF) applications and the cell migration environments, have been increasingly developed and used for electrotaxis research.[11] Despite the successful use of these experimental tools, it is challenging to effectively address more advanced scientific questions and meet higher technical requirements for the rapidly growing electrotaxis research. For example, parallel experiments are required to compare cell migration in different dcEF, thus allowing more efficient electrotaxis analysis and minimal variations among devices. Furthermore, electrotaxis analysis in three-dimensional (3D) environments is required to better simulate the situation in wound tissues. It is also important to analyze cell migration in response to dcEF and other coexisting guiding signals, which occur in wound healing. In addition to single-cell electrotaxis, collective migratory behaviors of cell groups in response to dcEF, which is the migration mode of many relevant cell types in wound healing, need to be understood. Finally, it is of great interest to use electrotaxis for sorting applications. Research in these areas will require further development of electrotaxis assays to meet specific requirements.

Indeed, a number of new electrotaxis assays have been recently reported, providing improved experimental throughput and capabilities for configuring more complex and physiologically relevant cell migration environments, and for sorting electrotactic populations. In addition, several previously developed assays have been used for studying collective electrotactic cell migration. These technological developments provide advanced experimental tools for electrotaxis research. In this review, we highlight some of these new developments.

TRANSLATIONAL RELEVANCE

The new developments in electrotaxis assays reviewed in this article expand the capabilities of existing assays and thus allow for more advanced electrotaxis experiments. In addition to characterizing and functionally validating the developed assays, research enabled by these assays has shown promise in generating new insights of electrotaxis. Particularly, sophisticated *in vitro* electrotaxis assays better mimic the guiding environment in wound tissues and allow electrotaxis experiments with the extracellular matrix (ECM) and cell forms that are more relevant to the situations in wound healing. Research toward this direction will help us better understand the mechanisms underlying EF guided cell migration in wound healing.

CLINICAL RELEVANCE

Basic biomedical research provides the important foundation for developing new and better clinical applications. Despite extensive evidence for the role of electrotaxis in wound healing, it has not led to direct clinical success. A deep understanding of the electrical guiding mechanism for cell migration under physiological conditions must be achieved to develop effective clinical applications for wound healing. The new developments in electrotaxis assays reviewed in this article enable advanced *in vitro* electrotaxis studies toward several important directions.

DISCUSSION OF RECENT ADVANCES

Multifields assays

In most *in vitro* electrotaxis assays, only a single dcEF strength can be configured in each experiment and only one experiment can be done at a time. Therefore, observing cell responses in different conditions requires multiple experiments in a sequential manner. High-throughput electrotaxis assays are needed for parallel electrotaxis experiments. Lin *et al.* employed a transwell-based electrotaxis assay for high-throughput experimentation.[9] In this assay, a dcEF is generated in the transwell by affixing two platinum electrodes to the top and bottom wells. The percentage of cells migrated from the top well to the bottom well in response to dcEF is measured to determine electrotaxis. This assay is capable of performing up to 24 experiments in different conditions per transwell plate. Using this assay, electrotaxis of various lymphocyte subsets from human peripheral blood to the cathode of the applied dcEF was identified. However, the transwell assay requires a peripheral circuit to supply different dcEF in different wells, and the dcEF in transwells is not uniform. In addition, as an endpoint assay, it does not allow real-time cell migration monitoring. Huang *et al.* developed a microfluidic electrotaxis device, which can generate multiple dcEF in a single microchannel.[12] The device consists of a straight microchannel with three consecutive segments of different width. Thus, three different dcEF can be generated in different segments. Using this device, electrotaxis of different lung cancer cell lines was compared and correlated with their metastatic potential. However, different channel width result in different flow speed, which can potentially

complicate electrotaxis analysis due to flow-induced shear stress. To overcome this issue, Tsai *et al.* reported an improved microfluidic device, which can generate different dcEF with uniform flow speed (Fig. 1A).[13] In this device, the main channel is divided into four segments without varying the channel width and each segment is connected to the cathode via different channel paths. Thus, a different dcEF is generated in different segments with comparable internal flow speed. In addition to validating the developed device for testing electrotaxis of lung cancer cells, the device was further used to demonstrate and characterize cathode-directing electrotaxis of HSC-3 oral squamous cell carcinoma cells. Further development of microfluidic electrotaxis devices is expected to further increase the experimental throughput, and these devices can be applied to more effectively test electrotaxis of various cell types relevant to wound healing.

3D assays

Currently, most of the electrotaxis experiments investigate cell migration on a two-dimensional (2D) substrate. However, cells can migrate in both 2D and 3D tissue environments *in vivo,* and cell migration in 2D and 3D can be largely different. Compared with cell migration on a 2D substrate, cell migration in the 3D microenvironment requires the ability of cells to squeeze through the

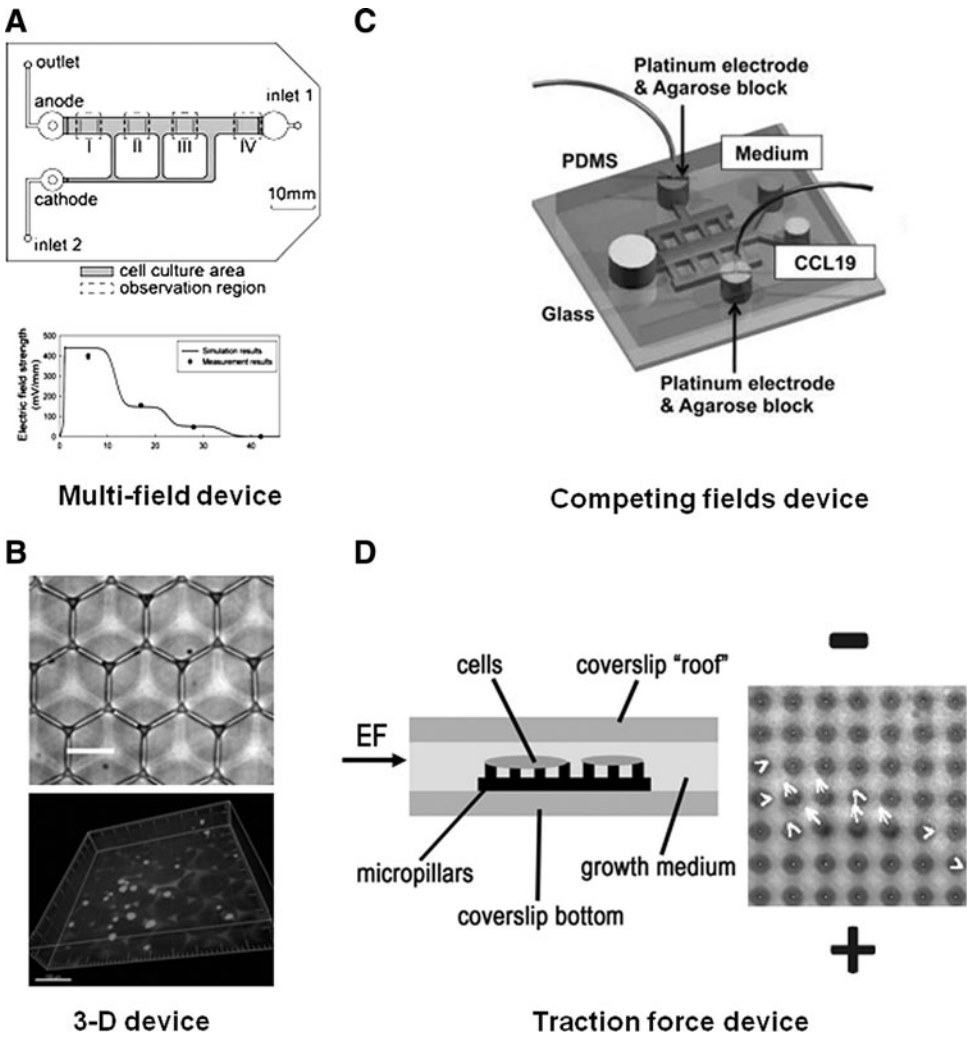

Figure 1. New developments in electrotaxis assays. **(A)** The microfluidic device for generating multiple dcEF with uniform flow. *Top*: The device design. *Bottom*: The simulated and measured dcEF in the device. Reprinted with permission from Tsai *et al.*[13] Copyright 2012, American Institute of Physics. **(B)** 3D electrotaxis assay. *Top*: The 3D scaffold (scale bar: 50 μm). *Bottom*: A reconstructed 3D fluorescent image of cells inside the 3D scaffold. Reprinted with permission from Sun *et al.*[15] Copyright 2012, American Institute of Physics. **(C)** The microfluidic device for generating controlled coexisting dcEF and chemical gradients. Reprinted with permission from Li *et al.*[19] Copyright 2012, American Institute of Physics. **(D)** The traction force assay. *Right*: The leading cell forces orient with dcEF in collective electrotaxis. Reprinted from Li et al.[27] with kind permission from Springer Science and Business Media. 3D, three-dimensional; dcEF, direct current electric field.

ECM. Therefore, it is important to develop biomimetic approaches to study the effect of dcEF on cell migration over 3D topology. Zhang *et al.* reported the use of the 3D Matrigel for studying electrotaxis of human induced pluripotent stem (hiPS) cells.[14] The cells were mixed with Matrigel followed by transference to the electrotactic chamber. The study showed that dcEF can stimulate and direct hiPS cell migration in 3D. Sun *et al.* developed a 3D scaffold to study the electrotactic response of lung cancer cells (Fig. 1B).[15] A polydimethylsiloxane (PDMS)-based microfluidic device was used to generate uniformly sized bubbles in gelatin by injecting a gelatin solution and nitrogen gas from two different inlets. The bubbles were then collected and the 3D scaffold was formed by chemical cross linking and degassing. The pore size is well controlled and uniform in the scaffold. Cells were easily seeded into the scaffolds using a pipette, and the scaffold was integrated into a polymethylmethacrylate-based electrotactic chamber for cell migration experiments. Using this device, different electrotactic cell migration was observed in 3D comparing to it on a gelatin-coated 2D substrate. Further optimization and development of 3D electrotaxis assays will enhance their use for studying electrotaxis of relevant cell types in wound healing in a more realistic ECM, which simulates wound tissues *in vivo*.

Coexisting fields assays

Diverse environmental signals such as chemical, electrical, and mechanical signals are presented to cells in a complex and coexisting manner to guide cell migration. Some examples include effective neutrophil navigation in coexisting chemical gradient arrays[16]; dendritic cell migration guided by coexisting soluble and surface-bound chemokine gradients.[17] In wound healing, a combination of dcEF, chemical gradients, and other cues provide coexisting guiding environments to direct the migration of relevant cell types at the wound. Interestingly, dcEF was suggested to override other guiding cues to direct cell migration in wound healing.[18] Improved knowledge of the competition between electrical and chemical guidance will help researchers better understand cell migration in electrochemical guiding environments and inspire new therapeutic applications by electrically manipulating cell migration. Li *et al.* reported a microfluidic device that can configure coexisting chemical gradients and dcEF in a controller manner (Fig. 1C).[19] In this device, laminar flow mixing was used to maintain chemical gradients in the main channel with minimized disturbance by the dcEF applied from the side wells. Using this device, it was shown that T cells migrate more strongly toward the cathode of dcEF in the presence of a competing chemokine gradient under specific experimental conditions. Thus, external dcEF applications may be used clinically to manipulate the migration of relevant cell types over coexisting tissue produced chemical guiding factors for mediating wound healing. More recently, Song *et al.* developed a microfluidic device to monitor cellular migration in response to dcEF and fluid shear stress in single, simultaneous, and sequential modes.[20] In this device, constant dcEF in the microchannel is maintained by a feedback control loop. The developed device was used to study fibroblast migration in response to costimulating dcEF and fluid shear stress. While wound produced dcEF is well known, fluid shear stress can also be induced by interstitial fluid loss in the connective tissue toward the wound. Separate dcEF or flow stimulations induce electrotactic cell migration or migration along the flow direction, respectively. Simultaneous dcEF and flow stimulations enhance directional cell migration. When dcEF and flow are applied sequentially, cell migration is affected by the applied stimulation as well as pre-existing stimulating conditions. Thus, electrotaxis of fibroblasts under flow conditions may benefit wound healing comparing to EF or fluid shear stress-based guidance alone. These studies showed promise for investigating cell migration in complex multifields guiding environments in wound healing and motivate further development of electrotaxis assays to permit more advanced studies.

Electrotactic sorting

Caenorhabditis elegans is a widely utilized model organism in biology research. Electrotaxis of *C. elegans* has been studied in direct current, alternating current, or pulse EF.[21–23] Research using *C. elegans* often requires sorting worms by their stages or separating normal worms and mutants. Besides various existing sorting methods, electrotaxis provides a simple and effective way to sort *C. elegans*. Manière *et al.* demonstrated separation of normal worms from mutants by their electrotactic motility in the electrophoresis agar gel.[24] The gel box is easy to fabricate and the method allows for sorting a large number of worms. Rezai *et al.* spatially separated different worms in a simple planar microchannel with a narrowed trap region based on the age-dependent or stage-dependent electrotactic responses of worms.[25] In addition, continuous sorting can be achieved in a

parallel manner. Han *et al.* proposed another sorting strategy based on the size-dependent motility and electrotaxis of worms in a microstructured channel.[26] This device features hexagonally arrayed microstructures. The study established the relationship between the worm size and the geometry of the environment, which was used to optimize the sorting performance. Integration of sorting and downstream analysis in an automated and high-throughput manner will lead to improved and broad future applications. Furthermore, the possibility of sorting cells based on electrotaxis should be explored. If successful, it will provide a method to identify and isolate cells with different electrotactic migration ability for both further basic electrotaxis research and tissue-engineering applications.

Electrotaxis assays for studying collective cell migration

In wound healing, large epithelial sheets migrate collectively as a group in defined directions and maintain tight cell–cell adhesion. Li *et al.* adapted the dish-based electrotaxis assay to study electrotaxis of epithelial sheets.[27] The study showed that cells in monolayer migrated more efficiently and directionally than isolated cells. Furthermore, E-cadherin-mediated cell–cell adhesion is essential for collective electrotactic migration. A force sensing assay with PDMS pillar arrays was integrated into the electrotaxis chamber to measure the traction force during cell migration (Fig. 1D). Using this assay, it is shown that the traction forces of the leading edge cells in an epithelial sheet orient with dcEF, leading collective electrotaxis. Further research toward better understanding electrotaxis of wound healing-related cell types in their physiological group format will provide important scientific basis for deriving effective therapeutic strategies for wound healing. Successful use of existing cell migration assays for studying collective electrotaxis will certainly motivate the development of more sophisticated assays to better suit the need of collective electrotaxis experiments.

SUMMARY AND FUTURE DIRECTIONS

In summary, some recent new developments and applications of electrotaxis assays were reviewed in this article. Although each assay focuses on addressing a specific issue, these studies show the growing technological development for advanced electrotaxis research. We believe such development will continue to grow in the future, which will further improve the functionalities of electrotaxis assays and enhance their utilizations.

The electrotaxis assays reviewed here were validated using specific electrotactic populations. There is room to further develop each electrotaxis assay to improve its performance and to test the assay with a broader range of experimental conditions and electrotactic populations. In addition, it will be beneficial to integrate the unique features of specific electrotaxis assays to allow more advanced electrotaxis analysis. For example, incorporating the designs of different coexisting field devices will allow investigation of cell migration in response to combinations of electrical, chemical, and mechanical guiding signals, which more closely mimic the physiological wound setting. Furthermore, incorporating the multifields and high-throughput designs to various electrotaxis assays (*e.g.*, 3D assays; coexisting field assays; collective electrotaxis assays) will significantly accelerate electrotaxis analysis in these assays and allow large-scale screening and quantifications.

Although electrotaxis has been mostly studied in cell cultures, it will be ultimately important to extend the study to realistic *in vivo* settings. On the other hand, *in vivo* electrotaxis studies will inevitably deal with issues such as less-controlled dcEF applications, complex guiding microenvironments, ECM and cell forms, as well as higher requirement for imaging. Biomemetics is a useful approach to simulate different aspects of *in vivo* situations. As reviewed in this article, progress toward this direction has been made toward studying electrotaxis in more physiologically relevant 3D ECM, multiple coexisting guiding fields, and of connected cell sheets. Direct visualization of green fluorescent protein (GFP)-tagged T lymphocyte migration guided by externally applied dcEF in the ear skin of a living GFP transgenic mouse using intravital confocal microscopy was also reported.[9] More advanced imaging techniques such as two-photon microscopy can be further used for *in vivo* or *ex vivo* electrotaxis studies in deeper tissues with an improved resolution. In addition, sophisticated experimental approaches need to be developed to control EF applications and its accompanying effects in tissues or complex ECM for electrotaxis studies. For example, sensitive probes and feedback controls are needed to monitor and maintain local EF for *in vivo* electrotaxis experiments. Furthermore, an accurately controlled fluidic perfusion system can help testing if dcEF can independently direct cell migration in *in vivo* settings or is complicated by other environmental factors. Taking together, electrotaxis studies to mimic *in vivo* situations or directly testing electrotaxis

in vivo will critically enable translation of basic research outcomes to clinical applications.

Although there is currently no clinical practice for wound healing by directly manipulating electrotaxis of relevant cells, electrical treatments for chronic wound with therapeutic benefits have been quite commonly used by medical practitioners such as physical therapists and acupuncturists. Progress has been made in treating human spinal cord injuries in a phase 1 trial by implanting an oscillating EF stimulator.[28] The potential electrotaxis-based therapeutic approach for wound healing may be beneficial to stimulate cells and tissues safely and more cost efficiently because the EF is applied at low magnitude using relatively simple electrical setups. On the other hand, it will be critical to optimize the applied EF in clinical applications for wound healing. A better understanding of EF guided cell migration will inspire the development of new EF-based treatments or other biophysical energies that can modulate physiological EF for wound care and other clinical applications.

TAKE-HOME MESSAGE

Technological advances

- The new electrotaxis assays allow parallel testing of cell migration to a range of dcEF strength.
- The new electrotaxis assays allow testing cell migration to dcEFs in 3D environments.
- The new electrotaxis assays allow testing how dcEFs and other guiding cues compete or collaborate to direct cell migration.
- The new electrotaxis assays allow sorting electrotactic populations.
- Electrotaxis assays have been successfully used for studying collective cell migration.

Basic science advances

- Research enabled by 3D electrotaxis assay showed that applied dcEF can stimulate and direct the migration of hiPS cells in a 3D Matrigel environment; lung cancer cell migration under dcEF is different in 2D and 3D environments.
- Research enabled by the coexisting field assay showed a stronger electrotactic attraction over chemical gradients for human T cell migration; dcEF and fluid shear stress have combined effects on fibroblast migration.
- Large epithelial sheets migrate more efficiently than isolated cells under dcEF; E-cadherin-mediated cell–cell adhesion as well as the forces of leading cells on the substratum play important roles in collective electrotaxis.

Clinical relevance

- The developed new assays will facilitate electrotaxis research with the potential to inspire new applications for wound care.
- The stronger electrotactic attraction over chemical gradients supports applying external dcEF to improve wound healing.
- Combinatorial stimulations by dcEF and fluid shear stress may clinically enhance wound healing.
- If electrotactic sorting of cells is possible, it will provide an effective method to identify and isolate relevant cell types with different electrotactic migration ability for tissue-engineering applications.
- Targeting electrotaxis of epithelial sheets may inspire new approaches to enhance epithelialization in nonhealing and chronic wounds.

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