

Video Article

Electric Field-controlled Directed Migration of Neural Progenitor Cells in 2D and 3D Environments

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Abstract

Endogenous electric fields (EFs) occur naturally *in vivo* and play a critical role during tissue/organ development and regeneration, including that of the central nervous system^{1,2}. These endogenous EFs are generated by cellular regulation of ionic transport combined with the electrical resistance of cells and tissues. It has been reported that applied EF treatment can promote functional repair of spinal cord injuries in animals and humans^{3,4}. In particular, EF-directed cell migration has been demonstrated in a wide variety of cell types^{5,6}, including neural progenitor cells (NPCs)^{7,8}. Application of direct current (DC) EFs is not a commonly available technique in most laboratories. We have described detailed protocols for the application of DC EFs to cell and tissue cultures previously^{5,11}. Here we present a video demonstration of standard methods based on a calculated field strength to set up 2D and 3D environments for NPCs, and to investigate cellular responses to EF stimulation in both single cell growth conditions in 2D, and the organotypic spinal cord slice in 3D. The spinal cord slice is an ideal recipient tissue for studying NPC *ex vivo* behaviours, post-transplantation, because the cytoarchitectonic tissue organization is well preserved within these cultures^{9,10}. Additionally, this *ex vivo* model also allows procedures that are not technically feasible to track cells *in vivo* using time-lapse recording at the single cell level. It is critically essential to evaluate cell behaviours in not only a 2D environment, but also in a 3D organotypic condition which mimicks the *in vivo* environment. This system will allow high-resolution imaging using cover glass-based dishes in tissue or organ culture with 3D tracking of single cell migration *in vitro* and *ex vivo* and can be an intermediate step before moving onto *in vivo* paradigms.

Video Link

The video component of this article can be found at <https://www.jove.com/video/3453/>

Protocol

1. Neural progenitor cell isolation

1. Dissect whole brains from E14-16 mice and place in cold DMEM/F12 basal medium. Remove all meninges under an anatomical microscope and transfer brains into a 35 mm Petri dish.
2. Use fine forceps to mechanically dissociate brains into tissue fragments and transfer them to a 15 ml tube, then centrifuge samples at 800 rpm for 3 min to remove debris.
3. Add DMEM/F12 containing bFGF and EGF and triturate with a 1 ml pipette.
4. Pass the cell suspension through a cell strainer to obtain a single cell suspension.
5. Plate cells into flasks at 2.5×10^4 cells/ml, then perform a full medium change every 3 days and passage the cells every 6 days.
6. After at least 5 passages, digest neurospheres to single cells using trypsin and EDTA and grow on poly-D-lysine/laminin-coated electrotactic chambers (prepared as described below in 2). Use the growing medium containing N2-supplement, bFGF and EGF at all times to maintain properties of NPCs.

2. Preparation of the electrotactic chamber

1. Prepare 22 x 11 mm strips of glass by dividing autoclaved 22 x 22 mm no.1 thickness coverslips in half using a diamond pen.
2. Create a free-standing glass well of interior dimensions 22 x 10 mm by gluing together four vertically standing 22 x 11 mm strips with high-vacuum silicone grease. Allow the wells to completely dry and harden overnight.

3. The following day, attach two 22 x 11 mm glass strips, parallel to each other leaving a gap of 10 mm, to the base of a 100 mm culture dish using silicone grease. Seal off the region between these strips by placing a 22 x 22 mm cover slip at each end, attached to the bottom of the dish with grease on three sides, but not that closest to the centre.
4. Place the glass well prepared in step 2.2 onto the cover slips so that the interior walls create a confined space for seeding the cells onto the bottom of the plate. Water-proof all joints with silicone grease. Coat this confined region sequentially with poly-D-lysine then laminin: add 1 ml poly-D-lysine into the chamber and leave for 5 min to allow the poly-D-lysine to bind to the bottom of the plate; wash the chamber with sterile PBS twice, then dilute laminin in sterile PBS to yield 20 µg/ml and use to cover the bottom of the plate. Leave at room temperature over night.
5. The following day, harvest cells and prepare 1 ml of suspension containing 1×10^4 cells. Remove laminin from the glass well, allowing it to air dry completely, and replace with 1 ml of cell suspension. Place the dish in the incubator at 37° C for a minimum of 4 hours to allow for attachment.
6. Once the cells are sufficiently confluent remove all medium from the chamber. Carefully remove the glass well. Form a roof over the cells by carefully attaching, with silicone grease, an autoclaved 22 x 22 mm glass coverslip bridging between the two 22 x 11 mm strips. Cover the cells with a few drops of medium to avoid drying out.
7. Form an isolated medium reservoir at each end of the chamber by creating two watertight silicone grease barriers that run from one edge of the dish to the other, over the chamber roof. Fill the chamber with fresh medium, ensuring a flow-through from one medium reservoir to the other. Return the dish to the incubator for 12 hours to allow for cell recovery.

3. Application of an electric field to the electrotactic chamber

1. Prepare a lid to cover the dish by drilling two holes, one positioned over each reservoir of the migration chamber.
2. Replace all medium in the chamber with culture medium containing 25 mM HEPES buffer and transfer the dish to the temperature-controlled imaging system. Set up the experimental parameters for time-lapse and multi-position recording. Align the chamber so that the cathode and anode are on the left and right respectively, to ensure that the EF vector runs horizontally as viewed down the microscope and recorded in the imaging system.
3. Fill two beakers with Steinberg's solution (58 mM NaCl, 0.67 mM KCl, 0.44 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1.3 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 4.6 mM Trizma base, pH 7.4). Connect a separate beaker to each medium reservoir using pre-prepared glass bridges (glass tubes ~13 cm long and ~3 mm in diameter, and bent into a U-shape by heating in a Bunsen flame), filled with 2 % (w/v) Steinberg's-agar solution, passing through the holes in the lid. Complete the electric circuit by placing Ag/AgCl electrodes connected to a DC power supply into each beaker of Steinberg's solution.
4. Set the voltage dial on the power supply to 0 and switch on. Measure the voltage across the electrotactic chamber while turning up the voltage dial, using a voltage meter, and adjust to suit the experimental requirements.
5. Start time-lapse recording. Perform a medium change and readjust the voltage, as required, every hour. Fresh medium, drugs, or chemical agents may be added to the reservoirs as required. When perform medium change, two options can be considered as below:
 1. Option No.1 - To pause the time-lapse recording temporarily, carefully remove glass bridges from chamber to avoid perturbing cover lid, use a sterilized Pasteur pipette gently replace all medium with fresh medium and put the glass bridges back, then resume the recording.
 2. Option No.2 - Alternatively, custom made cover lid with 4 holes (two positioned over each reservoir of the migration chamber) can also be used for medium change purpose. Two for glass bridges connection, the other two for changing medium. Option 2 allows continuous recording without interference during medium change.

4. Preparation of the organotypic spinal cord slice

1. Dissect lumbar spinal cords of 2 week old C57 BL/6 mice.
2. Slice spinal cords into 500 µm thick sections with a Mcllwain tissue chopper.
3. Separate slices under an anatomical microscope and select slices with intact sagittal/axial spinal cord structure.
4. Plate slices in a 35 mm Petri dish containing 30 µl Matrigel and place them as close to the centre as possible and maintain them in a 5 % CO_2 incubator at 37 °C for at least 30 min until the Matrigel proteins self-assemble to produce a thin film that covers the surface of the spinal cord slice. It is very important to make sure that Matrigel has been assembled completely, incubate the Petri dish at 37 °C for another 30 min if necessary.
5. Add 4-6 ml DMEM/F-12 medium containing 25 mM HEPES buffer and 15-20% fetal calf serum very gently so as to avoid medium flow directly onto the slice. Ensure that the slice is not entirely submerged in medium, leaving the surface of explants well exposed to the air. Change medium twice weekly.

5. Injection of Hoechst 33342 labelled NPCs into the organotypic spinal cord slice

1. Prepare the NPC suspension at 1×10^6 cells/µl.
2. Pre-incubate the cell suspension in medium with 5 µM Hoechst 33342 for 30 min.
3. Use capillary glass tube to microinject 2 µl of suspension into the spinal cord slice slowly under a microscope. Make sure capillary glass tube passes through the matrigel (pink part under microscope) and reach to the inside of spinal cord slice (grey tissue under microscope), remain inside the spinal cord slice for at least 30 seconds to avoid cell suspension run over. Put the petri dish containing spinal cord slice into incubator (37°C 5% CO_2) and leave it over night.
4. Next day, apply an EF of 500 mV/mm to the spinal cord slice containing Hoechst 33342-labelled NPCs in the electrotactic chamber (using the methods described in 3).

6. Representative Results

When NPCs were exposed to a range of physiological EFs they showed highly directed cell migration towards the cathode (Figure 1). The same observation was also made at a single cell level on the organotypic spinal cord slice *ex vivo* model, a 3D environment mimicking *in vivo* conditions (Figure 2).

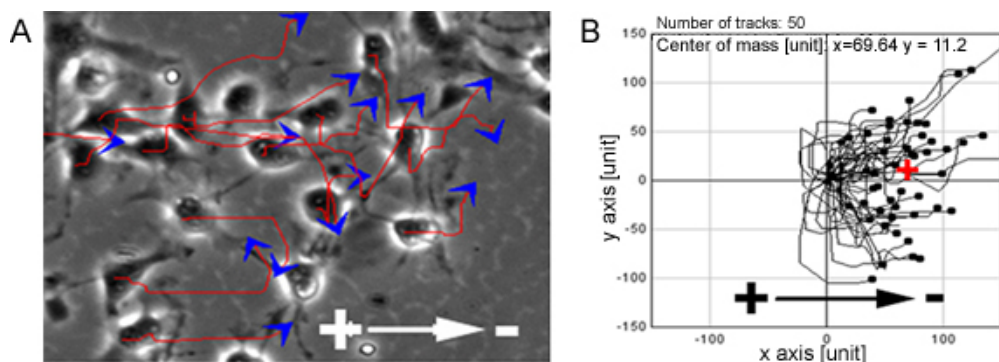


Figure 1. NPCs show directed migration in EFs. PCs showed highly directed migration towards the cathode when exposed to EFs, red lines and blue arrows represent trajectories and direction of cell movement (A). B shows migration paths of NPCs. Bar: 50 μ m.

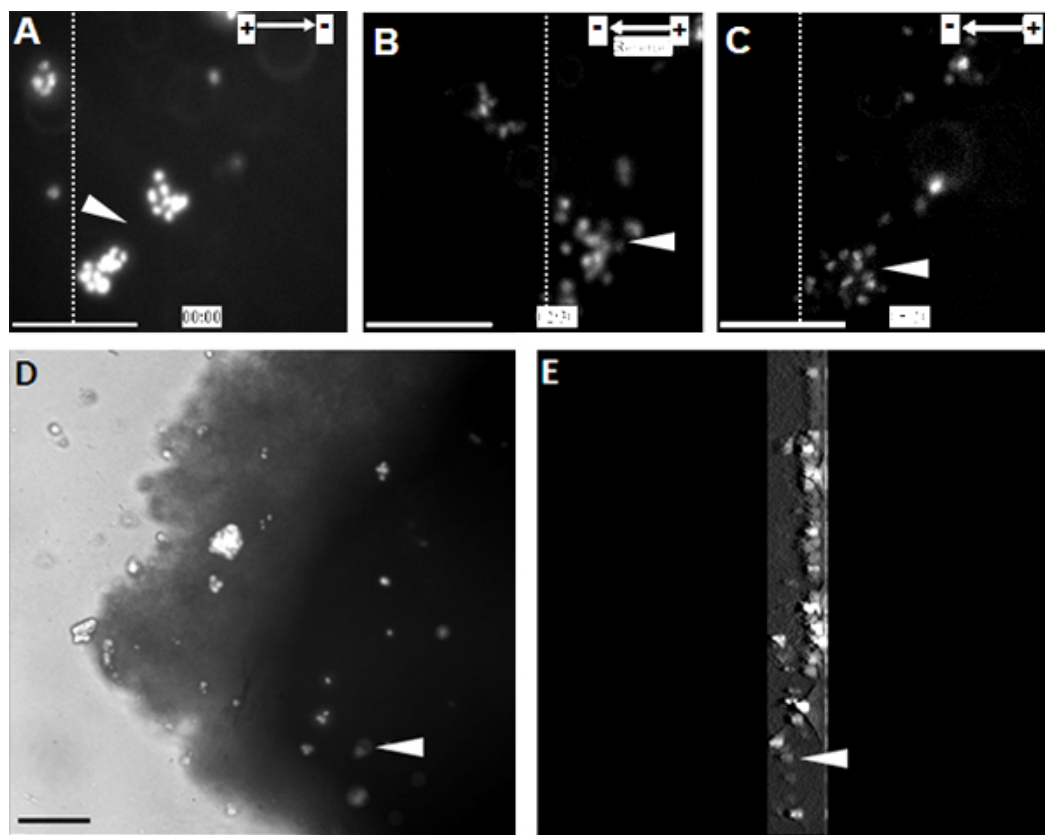


Figure 2. Transplanted NPCs show directed migration towards the cathode in the organotypic spinal cord slice. (A) NPCs labeled with Hoechst 33342 were transplanted into the organotypic spinal cord slice at the starting point of the EF treatment. NPCs migrated directionally towards the cathode for 2.5 hours, at which point the EF polarity was reversed (B). Altering EF polarity triggered a sharp reversal of electrotaxis towards the new cathode (C). (D) Image of transplanted NPCs within the spinal cord slice at the end of the time-lapse recording. (E) A 3D reconstruction of transplanted NPCs within the spinal cord slice. 3D scanning sections were 300 μ m in thickness, starting from the middle and ending at the bottom of the slice. Dotted lines indicate the relative positions of the same population of transplanted cells at the beginning, reversal, and end points of the EF treatment (A - C, respectively). Arrow heads indicate the same population of Hoechst 33342 labelled NPCs. Bar: 50 μ m.

Discussion

The protocols we use are based on previous studies^{5,11}. Using these methods, stable culture and electric current conditions can be maintained while applying an EF via agar bridges, Steinberg's solution, and Ag/AgCl electrodes, to cells or slices cultured in custom-designed electrotactic

chambers of standardised and precise dimensions. The depth of chambers can be adjusted to accommodate for different sample thicknesses¹¹, and in the case of cells, chamber size can be modified to accommodate however many cells are required (e.g. Western blot analysis requires many cells). We have modified and developed these methods to allow procedures that are not technically feasible to track cells *in vivo* using time-lapse recording at a single cell level. The majority of the previous research on cellular responses to EFs, has been conducted in 2D environments. The *in vivo* study of the electrotactic response of NPCs at a single cell level would provide pivotal evidence to substantiate any physiological relevance. However, this is technically very difficult to achieve with current technology, especially when trying to obtain real-time cell migration recordings. We have taken further steps to establish an *ex vivo* organotypic spinal cord slice culture model, closely mimicking the *in vivo* environment, demonstrating that cells still possess an electrotactic response in a 3D environment, as shown in Figure 2. This method could also be suitable for observing the effects of EFs on a wide variety of cell types within an organotypic 3D environment, such as wound healing in epithelial tissue, angiogenesis in the aortic ring or chick CAM, and possibly genetic effects in the chick embryo.

Disclosures

We have nothing to disclose.

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