Video Article

Electrically Conductive Scaffold to Modulate and Deliver Stem Cells

Byeongtaek Oh[1], Alexa Levinson[1], Vivek Lam[1], Shang Song[1], Paul George[1,2]

[1]Department of Neurology and Neurological Sciences, Stanford University School of Medicine

[2]Stanford Stroke Center and Stanford University School of Medicine

Correspondence to: Paul George at pgeorge1@stanford.edu

URL: https://www.jove.com/video/57367
DOI: doi:10.3791/57367

Keywords: Bioengineering, Issue 134, Pyrrole, Electrical Stimulation, Neural Progenitor Cell, Conductive Polymer, Stroke, Stem Cell

Date Published: 4/13/2018

Citation: Oh, B., Levinson, A., Lam, V., Song, S., George, P. Electrically Conductive Scaffold to Modulate and Deliver Stem Cells. *J. Vis. Exp.* (134), e57367, doi:10.3791/57367 (2018).

Abstract

Stem cell therapy has emerged as an exciting stroke therapeutic, but the optimal delivery method remains unclear. While the technique of microinjection has been used for decades to deliver stem cells in stroke models, this technique is limited by the lack of ability to manipulate the stem cells prior to injection. This paper details a method of using an electrically conductive polymer scaffold for stem cell delivery. Electrical stimulation of stem cells using a conductive polymer scaffold alters the stem cell's genes involved in cell survival, inflammatory response, and synaptic remodeling. After electrical preconditioning, the stem cells on the scaffold are transplanted intracranially in a distal middle cerebral artery occlusion rat model. This protocol describes a powerful technique to manipulate stem cells via a conductive polymer scaffold and creates a new tool to further develop stem cell-based therapy.

Video Link

The video component of this article can be found at https://www.jove.com/video/57367/

Introduction

Stroke is the second leading cause of death in the world and the fifth leading cause of death in the United States. Despite these high death rates, treatments for stroke recovery currently remain a challenge with no viable medical options currently available[1]. There are currently about 300 clinical trials dealing with ischemic strokes, of which only 40 utilize stem cells. Previous studies have shown that stem cell therapies have a beneficial effect on stroke repair[2,3]. Paracrine factors such as brain-derived neurotrophic factor (BDNF) and thrombospondin-1 (THBS-1) released from transplanted human neural progenitor cells (hNPCs) have shown improved functional recovery through mechanisms associated with an increase in synapse formation, angiogenesis, dendritic branching and new axonal projections, as well as modulating the immune system[4,5,6]. However, the optimal delivery methods of the stem cells remain elusive.

Successful stem cell delivery to the brain remains a challenge. Currently, injectable hydrogel and polymeric scaffolding systems have been introduced to deliver stem cells. These delivery methods protect stem cells during transplantation as well as offer protection from the harsh post-stroke environment including the host's inflammatory response and hypoxic conditions[7,8,9,10]. However, the most commonly used materials are inert, which limits the use of continuous modulation (*i.e.*, electrical stimulation) of the cells[11]. Electrical stimulation is a cue that influences differentiation, ion channel density, and neurite outgrowth of stem cells[12]. As compared to inert polymers, conductive polymers can carry a current allowing for electrical stimulation and manipulation of stem cells[2]. However, the precise mechanism by which electrical stimulation modulates neurotrophic factor release (*i.e.,* BDNF and THBS-1) is still not fully explored.

In this protocol, we describe the steps to construct a cell culture system consisting of a conductive polymer scaffold, polypyrrole (PPy), that allows for *in vitro* electrical stimulation. Because of the manner in which the cell culture system is fabricated, subsequent implantation of the stem cell-seeded scaffold onto the peri-infarct cortex is possible. For this system, we electrically precondition stem cells on the scaffold for a short time period prior to implantation. Following electrical stimulation, the conductive polymer scaffold carrying the cells is successfully implanted intracranially using a minimally invasive method.

Protocol

All stem cell and animal procedures were approved by Stanford's Stem Cell Research Oversight committee and by Stanford University's Administrative Panel on Laboratory Animal Care (SCRO-616 and APLAC-31909).

1. Etching of ITO Glass

1. Prepare the indium tin oxide (ITO)-covered glass slide by placing a masking agent over the conductive side of the glass.
   NOTE: Most commercial transparent tapes can be used as a masking agent.

1. Remove the excess mask using a blade, leaving only the desired shape of the final ITO design covered on the glass.

2. Combine 5% (v/v) of nitric acid and 45% (v/v) of HCl in a glass beaker inside a fume hood to prepare the etching solution.
    1. Use a hot plate and a thermometer to ensure that the temperature of etching solution is maintained at 70 °C.

3. Once the etching solution reaches 70 °C, place the masked-ITO glass slide into the solution for 2 minutes to remove the excess ITO layer.
NOTE: Tape masking protects the ITO layer from the exposure to acid solution.
4. Remove the slide from the solution and rinse it with deionized (DI) H_2O.
5. Carefully remove the mask and measure resistance using a multimeter to ensure that the remaining ITO is intact. Readouts of the desired etched area should be approximately 0 Ω.

2. Preparation of Pyrrole Solution

1. In a 1000 mL glass bottle, dissolve 70 g of sodium dodecylbenzenesulfonate (NaDBS) in 600 mL of DI H_2O.
2. Once the NaDBS is dissolved, add 14 mL of 0.2 M pyrrole and 386 mL of DI H_2O to the solution.
3. Cover the solution with aluminum foil and store at 4 °C.

3. Electroplating of Polypyrrole on ITO glass

1. Warm the pyrrole solution to room temperature until NaDBS is fully redissolved.
NOTE: This takes about 15 to 20 minutes.
2. Pour 25 mL of the pyrrole solution into a beaker.
3. Connect the etched ITO glass to a multimeter and submerge the slide into the pyrrole solution.
    1. Ensure that the side with 0 Ω resistance is facing towards the platinum mesh reference electrode.

4. Apply an electrical current to initiate electroplating of PPy on the ITO surface using a multimeter (current controlled at 3 mA/cm^2 for 4 hours).
5. Remove the ITO glass from the multimeter and wash electroplated-PPy in DI H_2O to remove residual surfactant.
6. Gently detach the electroplated-PPy plate from the ITO glass using a razor blade.
    1. Store the PPy plate at room temperature.

4. Preparation of Polydimethylsiloxane (PDMS)

1. Using a spatula and a weigh dish, mix 18 g of base agent with 2 g of curing agent and pour the mixture into two 10 cm x 8 cm glass molds.
2. Remove the bubbles from the molds using a vacuum chamber for 15 - 20 minutes.
3. Place the molds in a 70 °C oven for 3 h.
4. Once cooled, use a flat spatula to remove the PDMS from the molds.

5. Fabrication of the *In Vitro* Electrical Stimulation Chamber

1. Autoclave the metal plates (WxL, 2.5 cm x 12.5 cm) and flow valves at 120 °C for 1 hour.
2. Using a chamber slide as a template, cut two pieces of PDMS.
NOTE: One piece of PDMS serves as the perimeter of the cell chamber with cutouts of two adjoining squares from within the cell chamber. The other PDMS piece is an outline of the chamber's perimeter.
    1. Cut out the inside cell chamber so that it is square shaped and matches the shape of the cell chamber. Ensure that the overall shape of both pieces of PDMS is rectangular and matches that of the chamber slide.

3. Layer the materials as following from bottom to top: (1) Metal plate, (2) PDMS (without cutouts), (3) PPy plate (perpendicular to PDMS), (4) PDMS (with cutouts), and (5) cell chamber.
4. Clamp the apparatus together once it is fully aligned.
5. Cut two 60 cm pieces of a metal wire and use silver paste to attach one wire to each end of the PPy plate that protrudes from the outside of the chamber. Ensure that the wires are long enough to extend from the apparatus to the outside of an incubator.
6. Once the silver paste is cured, reinforce and seal the wire connection with epoxy.
    1. Record the resistance using a multimeter to make sure that the applied field is the same in each chamber.
    2. For implantation, remove the wires; unclamp the cell chambers and PDMS and then them separate from the conductive scaffold. The dimension of the implanted scaffolds is approximately 1 mm x 3 mm x 0.25 mm.

6. Plating human Neural Progenitor Cells (hNPCs) on PPy

1. Sterilize the assembled chambers under UV light for 2 hours.
    1. After 1 hour, rotate the assembled chambers 90°.

2. After sterilization, coat the surface of PPy at the bottom of the chamber with 800 µL of a coating substrate and allow the substrate to solidify on the PPy plate in an incubator at 37 °C at 5% CO_2 for 1 h.
3. After 1 hour, remove the supernatant from chambers and gently rinse with 1 mL of DPBS +Ca +Mg per well.
NOTE: Avoid washing the surface of PPy forcefully as this will cause the detachment of the coating substrate.
4. Using fresh cell media, mechanically dissociate cultured cells for use with the chambers from a tissue culture plate with gentle pipetting.

NOTE: Cells should be 90 - 100% confluent upon dissociation.
5. Collect hNPC pellets using centrifugation at 8000 x g for 5 minutes.
6. Using a hemocytometer, count and resuspend the cells at a volume of 100,000 cells/cm^2.
7. Plate 100,000 cells into each chamber well (100,000 cells/cm^2 in 1 mL of media).
8. Return chambers to incubator at 37 °C at 5% CO_2.

7. Electrical Stimulation of hNPCs

1. One day after seeding the cells on the chambers, use a waveform generator to apply electrical stimulation to the cells.
2. Prior to stimulation, remove old media from each chamber well and replenish with 800 µL of fresh media.
3. After the media is changed, place the chambers back into the incubator, extend the wires out of the incubator, and attach them to a waveform generator
4. Apply the stimulation to the cells with a square wave signal at ±400 mV with 100 Hz for 1 h.
5. After the stimulation, remove the wires and incubate the chambers for another day in an incubator set at 37 °C with 5% CO_2.
6. Perform subsequent biological analysis including cell viability and quantitative real-time polymerase chain reaction (qRT-PCR) following the manufacturer's protocol.

8. *In Vivo* PPy Implantation

1. Perform distal middle cerebral artery (dMCA) occlusion stroke models on T cell-deficient adult male nude rats (NIH-RNU 230 ± 30 g) as described previously[2]. Perform implantation one week post-dMCA occlusion.
2. One day prior to surgery, give ampicillin (1 mg/mL) to the rats in their cage water.
3. Anesthetize the rat in an induction chamber using 0.5 - 3% mg/kg of isoflurane administered via inhalation.
4. Confirm anesthetization of the rat by a lack of toe pinch reflex response.
    1. While the animal is under anesthesia, continue to monitor its membrane color, breathing pattern, and rectal temperature every 15 minutes.
5. Once anesthetization is confirmed, apply artificial tears on eyes of rat to prevent dryness.
6. Ensure that aseptic technique is maintained during this procedure by using sterile gloves, and a sterile surgical drape[13]. Keep sterile surgical instruments within a sterile field.
7. While the rat is under anesthesia, drill a craniectomy above the left cortex. Open the dura.
    1. Remove the dura mater from the brain using a micro-thin surgical needle.
8. Implant the conductive scaffolds from *in vitro* system onto the rat cortex.
   NOTE: For our experiments, we implanted scaffolds from *in vitro* day 3 after hNPC plating.
    1. Place the implant primarily on the penumbral cortex medial to the stroke lesion.
9. After the scaffold is placed, place surgicel over the implant to prevent movement with skin closure.
10. Suture the wound shut and subcutaneously inject the rats with buprenorphine SR at a dosage of 1 mg/kg to manage the pain associated with stereotaxic surgery and dMCA occlusion.
11. Place the rat in a recovery cage as it regains consciousness.
    1. Never leave the animal unattended while it is unconscious.
    2. Do not place the animal with others until it has fully gained consciousness.
12. Monitor animals daily for signs of pain including body weight loss, decreased food/water intake, reduced grooming/unkempt coat, decreased/increased spontaneous activity, abnormal posture, dehydration/skin tenting, sunken eyes, hiding, self-mutilation, rapid breathing, open mouthed breathing, twitching, trembling, tremor, and vocalization.
    1. Monitor the animals daily until it appears that they are eating, drinking, grooming, and gaining weight; and then weekly after weight gain.
13. Early euthanization via CO_2 inhalation and a secondary confirmation of euthanization (as to ensure the animal will not revive due to normal oxygen supply post CO_2 inhalation) will occur to any animal that appears to be not eating, drinking and gaining weight after the initial surgical procedure; appears in pain; or appears unable to complete the behavioral tests due to a larger than expected motor cortex deficit.

Representative Results

The schematic shown in **Figure 1** represents the overall workflow of the electrical stimulation of hNPCs and potential downstream applications. A current limitation in stem cell therapy is that stem cells are exposed to a harsh post-transplantation environment including inflammation and ischemic conditions. These difficult conditions likely limit their therapeutic efficacy[14,15]. The use of a conductive scaffold to protect hNPCs from this environment may augment hNPCs therapeutic benefits through electrical preconditioning. The first step in this stem cell delivery technique is the development of a conductive scaffold using an electroplating approach[2,16]. We characterized the scaffolds biocompatibility and optimized electrical preconditioning characteristics with hNPCs. Controls were defined as unstimulated stem cells grown on a tissue culture plate.

Various voltages were evaluated to determine the safety of electrical stimulation and to maximize the preconditioning efficacy. To ensure that the applied field is same in each chamber, the resistivity of assembled chamber was measured using a multimeter (Resistances (Ohm, Ω) of chambers were approximately 10 kΩ, and resistivity≈3 Ωm). According to the commercial vendor, the hNPCs used have shown no significant cytotoxicity in normal culture systems. hNPCs with or without exposure to electrical stimulation were stained with cell viability assays (Live: green; Dead: red) (**Figure 2**). These results indicate that hNPCs were viable after the electrical stimulation (±400 mV, 100 Hz for 1 h). To validate the cell viability assay, we performed cell viability testing using a resazurin assay. The results also demonstrate no significant cytotoxicity of electrical stimulation on hNPCs.

Our previous *in vivo* data demonstrated that paracrine factors released from hNPCs (SD56, NPCs derived from embryonic stem cells) with an exposure to electrical stimulation improve the recovery after stroke[2]. To explore further candidate factors known to be important in stroke recovery that are released from hNPC (Aruna Biomedical, NPCs derived from embryonic stem cells), BDNF and THBS-1 were evaluated. These factors have been extensively studied due to their role in neuronal outgrowth and increase in cell-to-cell interactions[17,18]. To investigate the efficacy of electrical stimulation on transcriptome changes for BDNF and THBS-1, qRT-PCR was performed including BDNF and THBS-1 genes with GAPDH as a housekeeping gene. Approximately 1 µg of total RNA was reverse-transcribed into cDNA according to the manufacturer's protocol. Normalized fold-change ratios were calculated by ΔΔCt method comparing gene expression in hNPCs on PPy and hNPCs on PPy with an exposure to electrical stimulation. Statistical analysis showed significant upregulations in gene expression of BDNF and THBS-1 between groups that were electrically stimulation dependent ($p \leq 0.01$) (**Figure 3**). This data suggests that optimization is possible to maximize hNPC efficacy without significant cell death.

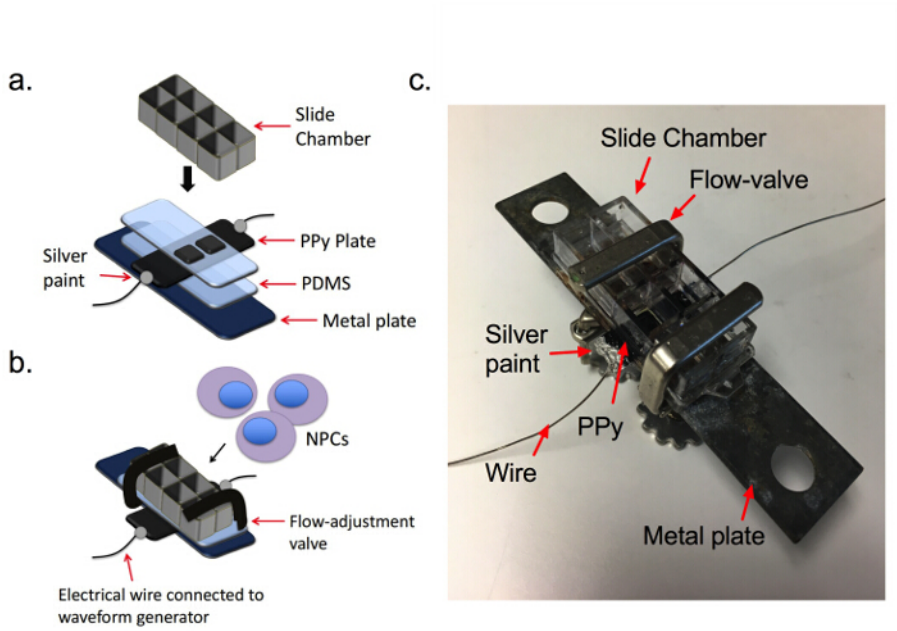

Figure 1. *In vitro* conductive polymer scaffold system for electrical stimulation. (A) A slide chamber is placed on top of PPy plate. **(B)** The schematic shows the fabrication of the *in vitro* electrical stimulation chamber with hNPCs plated on the surface of PPy. Flow valves are used to hold the slide chamber, PDMS, PPy, and metal plate together. **(C)** Image of the chamber. Please click here to view a larger version of this figure.

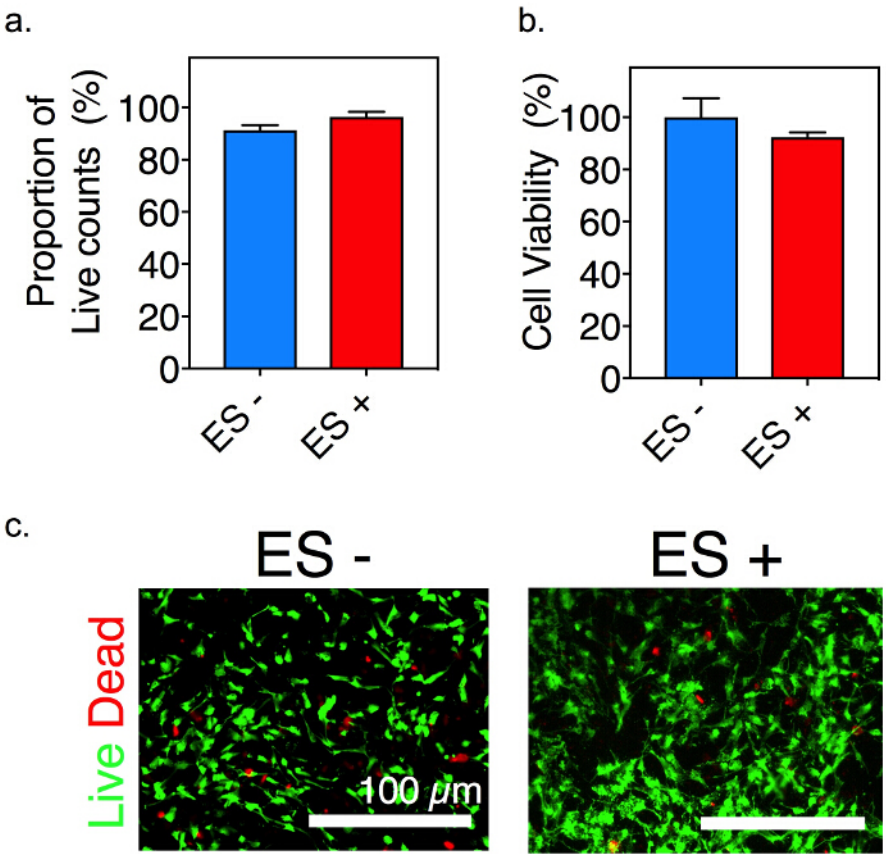

Figure 2. Cell viability assay in hNPCs with or without electrical stimulation. (A) Bar graph demonstrating live cell stained with Calcein-am. Data presented are mean ± S.D.; n = 4. **(B)** Cell viability assay using resazurin. Bar graph shows there was no cytotoxicity of electrical stimulation on hNPCs; n = 4. **(C)** Images of cell viability assay before and after electrical stimulation treatment. Green signal indicates live cells (Calcein-am), whereas red signal indicates dead cells (EtHD-1). ES indicates electrical stimulation. ES + or - indicates the presence or absence of electrical stimulation on cells. Please click here to view a larger version of this figure.

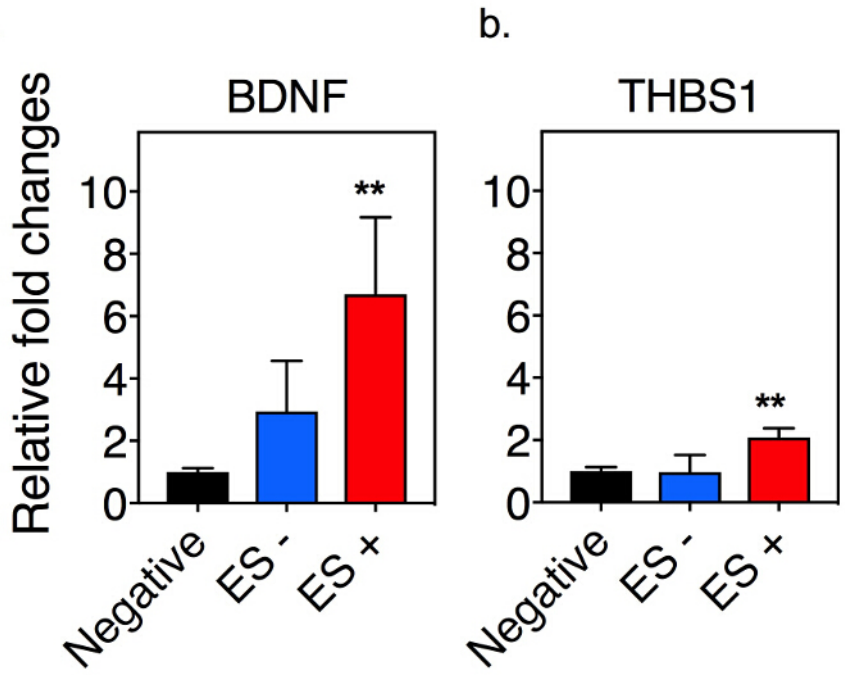

Figure 3. Gene expression changes with electrical stimulation. Bar graph demonstrating fold changes in gene expressions of BDNF **(A)** and THBS1 **(B)** in hNPCs (** indicates statistically significant between electrically preconditioned and all other groups, $p < 0.01$, error bars show S.D.; n = 4, one-way ANOVA). Negative indicates the control where cells were cultured on a regular tissue culture plate. ES + or - indicates the presence or absence of electrical stimulation on cells. Please click here to view a larger version of this figure.

Discussion

Growing evidence has demonstrated the promise of stem cells as a novel stroke therapy. This promise has resulted in a major effort to advance stem cell therapeutics to the bedside with at least 40 ongoing or completed clinical trials. Stroke pathology offers a unique neurological disorder that lends itself to stem cell therapy because after the acute insult, there is no neurodegenerative process preventing recovery. The exact mechanism of stem cell-enhanced stroke recovery remains unclear. Angiogenesis, synaptogenesis, and synaptic remodeling have all been shown to be important. When important molecules for synapse formation such as BDNF and THBS-1 are removed, stroke recovery is impaired[6,19]. hNPCs have improved functional recovery after stroke in numerous animal models[20,21]. The mechanisms of the hNPCs effect remains not fully understood, and the ideal delivery method for these cells is unknown. By developing a system that allows one to manipulate the important molecules released from hNPCs, one can help differentiate the integral recovery mechanisms and optimize stem cell therapy.

Successful cell delivery to the brain remains a challenge. Currently, a number of technologies have been developed using different biomaterial scaffolding systems to deliver stem cells to improve the survival and integration of stem cells[9,10]. However, due to the inert characteristics of these materials, it is difficult to modulate stem cells after their transplantation.

This article describes a method using a PPy scaffold to electrically manipulate the transcriptome of hNPCs as shown by the upregulation of BDNF and THBS-1 in our qRT-PCR data. Our previous study revealed that an increase in VEGFA expression on hNPCs after exposure to electrical stimulation improved functional recovery after stroke[2]. Here we demonstrate that additional neurotrophic factors such as BDNF and THBS-1 are also modulated after exposure to electrical stimulation in a different hNPC cell line, showing that our system can be used with numerous cell lines.

During the fabrication of the *in vitro* electrical stimulation chamber, it is critical that the metal wire is completely connected to PPy plate using silver paint and epoxy. If this connection is not sound, it causes varying electric fields even with the same parameters. Moreover, at times media may leak after the assembly of the scaffolding system. To resolve this issue, vacuum grease can be applied to seal the space between the PDMS and the PPy plate with care not to apply to the cell-seeding surface. One limitation due to the non-transparent nature of the PPy plate is that checking cells' confluency with standard light microscopy is limited.

The advantage of the method described in this protocol allows for modulation of hNPCs to help determine the mechanistic changes of the cells. Currently, electrical modulation has not been approached using conventional hydrogel or inert scaffolding systems. After electrical stimulation using the PPy scaffold, the hNPCs can be delivered without removal from the polymer surface to an *in vivo* model. Developing methods to further study disease models of stem cell applications allows us to better design translational applications. The electrical preconditioning approach, made possible by the conductive polymer system, can be applied to different cell types and allows for better understanding of the impact of electrical stimulation on a cell. The ability to optimize the cells *in vitro* and then without further manipulation (such as passaging or removal) allows for testing of these *in vitro* manipulations in *in vivo* disease states such as stroke. The ultimate aim is to utilize new techniques, such as the described conductive polymer scaffold, to advance stroke therapeutics and improve understanding of stroke recovery mechanisms.

Disclosures

The authors have no conflicts of interest to declare with this work.

Acknowledgements

We thank Dr. Kati Andreasson (Department of Neurology and Neurological Science, Stanford University) for use of the qRT-PCR machine. The work was supported by National Institutes of Health Grants K08NS098876 (to P.M.G.) and Stanford School of Medicine Dean's Postdoctoral Fellowship (to B.O.).

References

1. Moskowitz, M. A., Lo, E. H., & Iadecola, C. The Science of Stroke: Mechanisms in Search of Treatments. *Neuron.* **67** (2), 181-198 (2010).
2. George, P. M. *et al.* Electrical Preconditioning of Stem Cells with a Conductive Polymer Scaffold Enhances Stroke Recovery. *Biomaterials.* **142** 31-40 (2017).
3. Steinberg, G. K. *et al.* Clinical Outcomes of Transplanted Modified Bone Marrow-Derived Mesenchymal Stem Cells in Stroke: A Phase 1/2a Study. *Stroke.* **47** (7), 1817-1824 (2016).
4. George, P. M., & Steinberg, G. K. Novel Stroke Therapeutics: Unraveling Stroke Pathophysiology and Its Impact on Clinical Treatments. *Neuron.* **87** (2), 297-309 (2015).
5. Schabitz, W. R. *et al.* Intravenous Brain-Derived Neurotrophic Factor Enhances Poststroke Sensorimotor Recovery and Stimulates Neurogenesis. *Stroke.* **38** (7), 2165-2172 (2007).
6. Liauw, J. *et al.* Thrombospondins 1 and 2 Are Necessary For Synaptic Plasticity and Functional Recovery After Stroke. *J Cereb Blood Flow Metab.* **28** (10), 1722-1732 (2008).
7. Cantu, D. A., Hematti, P., & Kao, W. J. Cell Encapsulating Biomaterial Regulates Mesenchymal Stromal/Stem Cell Differentiation and Macrophage Immunophenotype. *Stem Cells Translational Medicine.* **1** (10), 740-749 (2012).
8. Chaudhuri, O. *et al.* Hydrogels with Tunable Stress Relaxation Regulate Stem Cell Fate and Activity. *Nat Mater.* **15** (3), 326-334 (2016).
9. Ballios, B. G. *et al.* A Hyaluronan-Based Injectable Hydrogel Improves the Survival and Integration of Stem Cell Progeny following Transplantation. *Stem Cell Reports.* **4** (6), 1031-1045 (2015).
10. Mothe, A. J., Tam, R. Y., Zahir, T., Tator, C. H., & Shoichet, M. S. Repair of the Injured Spinal Cord by Transplantation of Neural Stem Cells in a hyaluronan-based hydrogel. *Biomaterials.* **34** (15), 3775-3783 (2013).
11. Saini, M., Singh, Y., Arora, P., Arora, V., & Jain, K. Implant Biomaterials: A Comprehensive Review. *World J Clin Cases.* **3** (1), 52-57 (2015).
12. Huang, Y., Li, Y., Chen, J., Zhou, H., & Tan, S. Electrical Stimulation Elicits Neural Stem Cells Activation: New Perspectives in CNS Repair. *Front Hum Neurosci.* **9** (2015).
13. Pritchett-Corning, K. R., Mulder, G. B., Luo, Y., & White, W. J. Principles of Rodent Surgery for the New Surgeon. *J Vis Exp.* (47) (2011).
14. Abdelwahid, E. *et al.* Stem Cell Death and Survival in Heart Regeneration and Repair. *Apoptosis.* **21** (3), 252-268 (2016).
15. Wang, X. *et al.* The Clinical Status of Stem Cell Therapy for Ischemic Cardiomyopathy. *Stem Cells Int.* **2015** (2015).
16. George, P. M. *et al.* Fabrication and Biocompatibility of Polypyrrole Implants Suitable for Neural Prosthetics. *Biomaterials.* **26** (17), 3511-3519 (2005).
17. Aravamudan, B., Thompson, M., Pabelick, C., & Prakash, Y. S. Brain-Derived Neurotrophic Factor Induces Proliferation of Human Airway Smooth Muscle Cells. *J Cell Mol Med.* **16** (4), 812-823 (2012).
18. Lopes, N. *et al.* Thrombospondin 2 Regulates Cell Proliferation Induced by Rac1 Redox-Dependent Signaling. *Mol Cell Biol.* **23** (15), 5401-5408 (2003).
19. Ploughman, M. *et al.* Brain-Derived Neurotrophic Factor Contributes to Recovery of Skilled Reaching After Focal Ischemia in Rats. *Stroke.* **40** (4), 1490-1495 (2009).
20. Baker, E. W. *et al.* Induced Pluripotent Stem Cell-Derived Neural Stem Cell Therapy Enhances Recovery in an Ischemic Stroke Pig Model. *Sci Rep.* **7** (1), 10075 (2017).
21. Bacigaluppi, M. *et al.* Neural Stem Cell Transplantation Induces Stroke Recovery by Upregulating Glutamate Transporter GLT-1 in Astrocytes. *J Neurosci.* **36** (41), 10529-10544 (2016).

