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Title: Electric-Field-Induced Neural Precursor Cell Differentiation in Microfluidic Devices

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? **27.22 meters**

Current Protocol Length

Number of Steps: 21
Number of Shots: 39

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. This study presents a protocol for the differentiation of neural precursor cells solely induced by direct current pulse stimulation in a microfluidic system.

1.1.1. *4.3.1.*

1.2. This technique provides a beneficial approach for reducing the experimental setup size, sample volume, and reagent volume. Furthermore, the MOE chip is suitable for confocal microscopy observations.

1.2.1. *4.4.4.*

OPTIONAL:

1.3. The simple direct current pulse treatment can control mouse neural precursor cells' fate and could be used to develop therapeutic strategies for nervous system disorders.

1.3.1. *5.1.1, Figure 7.*

Protocol

2. Design and fabrication of the multichannel, optically transparent, electrotactic chip and coating poly-L-lysine on the substrate in the cell culture regions

2.1. To begin, draw patterns for individual PMMA layers and the double-sided tape using appropriate software **[1-TXT]**.

2.1.1. Talent drawing patterns using AutoCAD software. **TEXT: PMMA- polymethyl methacrylate**

2.2. Switch on the carbon dioxide laser scriber **[1]** and connect it to a personal computer **[2]**. Open the designed pattern file using the software **[3]**.

2.2.1. Talent switching on the laser scriber.

2.2.2. Talent connecting the laser scriber to the computer.

2.2.3. Talent opening the desired file in the software.

2.3. Place the PMMA sheets or double-sided tape on the platform of the laser scriber **[1]**, then focus the laser onto the surface of the PMMA sheets or the double-sided tape using the auto-focus tool **[2]**.

2.3.1. Talent placing the PMMA sheets or the tape on the platform of laser scriber.

2.3.2. Talent focusing the laser on the surface of the tape and sheets.

2.4. Select the laser scriber as the printer, then use the laser scriber to start the direct ablation on the PMMA sheet or double-sided tape **[1]**.

2.4.1. Talent selecting the laser scriber as printer.

2.5. Remove the protective film from the PMMA sheets **[1]**, and clean the surface using nitrogen gas **[2]**.

2.5.1. Talent removing the protective film from the PMMA sheets.

2.5.2. Talent cleaning the surface of the film using nitrogen gas.

2.6. For bonding together multiple layers of PMMA sheets, stack three pieces of 1-millimeter PMMA sheets **[1]** and bond them under a pressure of 5 kilograms per

square centimeter in a thermal bonder for 30 minutes at 110 degrees Celsius to form the flow or electrical stimulation channel assembly **[2]**.

2.6.1. Talent stacking the layers of PMMA sheets.

2.6.2. Talent bonding the layers in thermal bonder.

2.7. Adhere 12 pieces of adaptors to the individual openings in Layer 1 of the MOE chip assembly with fast-acting cyanoacrylate glue **[1-TXT]**.

2.7.1. Talent adhering 12 pieces of adaptors with glue. **TEXT: MOE- multichannel, optically transparent, electrotactic**

2.8. Disinfect the 1-millimeter PMMA substrates, the double-sided tape, and the 3-millimeter optical grade PMMA using ultraviolet irradiation for 30 minutes before assembling the chip **[1]**.

2.8.1. Talent disinfecting the substrates, tape, and PMMA using UV.

2.9. Adhere the 1-millimeter PMMA substrates on the 3-millimeter optical grade PMMA with the double-sided tape to complete the PMMA assembly **[1]**. Adhere the cleaned cover glass to the PMMA assembly with the double-sided tape **[2]**. *Videographer: This step is important!*

2.9.1. Talent adhering the substrates on PMMA sheets with tape.

2.9.2. Talent adhering the cover glass to the PMMA assembly.

3. Coating poly-L-lysine on the substrate in the cell culture regions and preparation of the salt bridge network

3.1. Seal the openings of the agar bridge adaptors with white finger-tight plugs **[1]**. Connect the flat-bottom connector to the MOE chip assembly via the medium inlet and outlet adaptors **[2-TXT]**, then connect the cone-Luer adaptor to the 3-way stopcocks **[3]**.

3.1.1. Talent sealing the openings of the agar bridge adaptors with white finger tight plugs.

3.1.2. Talent connecting the connector to MOE chip assembly. **TEXT: MOE-multichannel, optically transparent, electrotactic**

3.1.3. Talent connecting the cone-Luer adaptor to the stopcocks.

3.2. Add 2 milliliters of 0.01% PLL solution using a 3-milliliter syringe that connects to the 3-way stopcock of the medium inlet **[1-TXT]** and connect an empty 3-milliliter syringe to the 3-way stopcock of the medium outlet **[2]**.

3.2.1. Talent adding PLL solution to the 3-way stopcock. **TEXT: PLL- poly-L-lysine**

3.2.2. Talent connecting empty syringe to the 3-way stopcock.

3.3. Fill the cell culture regions with the PLL solution **[1]** and slowly pump the coating solution back and forth **[2]**. Close the two 3-way stopcocks to seal the solution inside the culture regions **[3]**. *Videographer: This step is important!*

3.3.1. Talent filling the PLL solution in cell culture regions.

3.3.2. Talent pumping the coating solution back and forth.

3.3.3. Talent closing both the 3-way stopcocks.

3.4. Incubate the MOE chip at 37 degrees Celsius overnight in an incubator filled with 5% carbon dioxide atmosphere **[1]**.

3.4.1. Talent incubating the MOE chip in the incubator.

3.5. Open the two 3-way stopcocks **[1]** and flush away bubbles in the channels by manually pumping the coating solution back and forth in the channel using the two syringes **[2]**.

3.5.1. Talent opening the two 3-way stopcocks.

3.5.2. Talent pumping the coating solution in the channel.

3.6. Draw 3 milliliters of complete medium into a 3-milliliter syringe that connects to the 3-way stopcock of the medium inlet **[1]** and add it to replace the coating solution in the cell culture regions **[2]**.

3.6.1. Talent drawing complete medium into syringe.

3.6.2. Talent adding the complete medium in cell culture regions to replace the coating solution.

3.7. Replace the white finger-tight plug with the Luer adaptor **[1]** and inject 3% hot agarose to fill the adaptor **[2]**. *Videographer: This step is difficult and important!*

3.7.1. Talent replacing the tight plug with Luer adaptor.

3.7.2. Talent injecting agarose in the adaptor.

3.8. Connect the Luer lock syringe to the Luer adaptor **[1]** and inject 3% hot agarose through the black rubber bung to fill the Luer lock syringe. Allow 10 to 20 minutes for the agarose to cool down and solidify **[2]**. *Videographer: This step is important!*

3.8.1. Talent connecting Luer lock syringe to the adaptor.

3.8.2. Talent injecting hot agarose to the Luer lock syringe.

4. Setup of the microfluidic system for DC pulse stimulation

4.1. Install the cell-seeded MOE chip onto the transparent ITO heater that is fastened on a programmable X-Y-Z motorized stage maintained at 37 degrees Celsius **[1-TXT]**.

4.1.1. Talent installing the MOE chip onto ITO heater. **TEXT: ITO-Indium-Tin-Oxide**

4.2. Infuse the mNPCs by manual pumping into the MOE chip via the medium outlet **[1-TXT]**, then incubate the cell-seeded MOE chip on the ITO heater for 4 hours **[2]**. *Videographer: This step is important!*

4.2.1. Talent infusing the mNPCs into the MOE chip. **TEXT: mNPCs- mouse neural stem and progenitor cells**

4.2.2. Talent incubating the MOE chip on ITO heater.

4.3. Use electrical wires to connect an EF multiplexer to the MOE chip via the electrodes on the chip **[1-TXT]**. Connect an EF multiplexer and a function generator to an amplifier to output square-wave DC pulses **[2-TXT]**.

4.3.1. Talent connecting EF multiplexer to MOE chip. **TEXT: EF-electric fields**

4.3.2. Talent connecting an EF multiplexer and a function generator to an amplifier. **TEXT: 300 mV/mm magnitude, 100 Hz frequency at 50% duty cycles (50% time-on and 50% time-off)**

4.4. Remove all the flat bottom connector and adaptors on the MOE chip **[1]** and fill the adaptors with PBS **[2]**, then seal the adaptor openings with parafilm **[3]**. After immunostaining, observe the cells using a confocal fluorescence microscope **[4]**. *Videographer: This step is important!*

4.4.1. Talent removes all of the Flat bottom connector and Luer adaptor on MOE chip.

4.4.2. Talent fills the PBS into the adaptors.

4.4.3. Talent sealing the openings of adaptors with parafilm.

4.4.4. Talent observing the cells under fluorescence microscope.

Results

5. Differentiation of the mNPC cells in the control group and in the DC pulse stimulation group at DIV 3, 7, and 14

5.1. The DC pulse stimulation resulted in simultaneous mNPCs differentiating into neurons, astrocytes, and oligodendrocytes in stem cell maintenance medium **[1]**.

5.1.1. LAB MEDIA: Figure 7

5.2. The percentages of neurons, astrocytes, and oligodendrocytes in the control group **[1]** and in the stimulation group are shown here **[2]**.

5.2.1. LAB MEDIA: Figure 7A-7C.

5.2.2. LAB MEDIA: Figure 7D-7F.

5.3. After the DC pulse treatment, the mNPCs expressed significantly high numbers of neurons at DIV 7 **[1]**. Astrocytes at DIV 3 **[2]** and oligodendrocytes at DIV 7 and DIV 14 were present at relatively higher levels in the stimulation groups than in the control group **[3]**.

5.3.1. LAB MEDIA: Figure 7B and 7E. *Video editor focus on the yellow region in pie chart.*

5.3.2. LAB MEDIA: Figure 7A and 7D. *Video editor focus on the grey region in pie chart.*

5.3.3. LAB MEDIA: Figure 7B, 7C, 7E and 7F. *Video editor focus on the blue region in pie chart.*

Conclusion

6. Conclusion Interview Statements

6.1. Neurospheres formed by 30 to 40 cells are preferred for initiating mNPC differentiation. Overgrowth of mNPCs will impair cell survival during the differentiation process.

6.1.1. *4.2.1.*