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Title: Microfluidic Chip for Axonal Injury Models Construction and Enabling Multi-Omics Analysis

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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? **Yes**

   SCOPE: 6.3.1-6.3.3
   Videographer: Please film these shots with a SCOPE kit as backup

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

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 26
Number of Shots: 56

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Bing Zhou:** We have developed large-scale microfluidic chips to enable multi-omics analysis, with the aim of elucidating the metabolic mechanisms of neurons following axonal injury.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What technologies are currently used to advance research in your field?

1.2. **Qianwen Zhnag:** We mainly utilize microfluidic technology, metabolic flux analysis and transcriptomics to advance the research on the metabolic mechanisms of neurons during axonal injury and regeneration.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What significant findings have you established in your field?

1.3. **Ruixuan Liu:** We found that there are metabolic differences among cortical neurons at different developmental stages and that young neurons undergo metabolic remodeling after axonal injury.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

1.4. **Jiaxin Sun:** The core advantage of our protocol lies in the large-scale microfluidic platform design and operational standards, which enable accurate multi-omics analysis of millions of cells.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Ethics Committee at Beihang University

Protocol

2. Fabrication and Sterilization of Polydimethylsiloxane (PDMS) Microfluidic Devices

Demonstrator: Jiaxin Sun

2.1. To begin, transfer the PDMS base and curing agent into a centrifuge tube **[1-TXT]**. Place the centrifuge tube containing the mixture into a centrifugal stirrer mixer **[2]**.

2.1.1. WIDE: Talent transferring weighed out PDMS base and curing agent into a centrifuge tube. **TXT: PDMS: Curing agent: 10:1**

2.1.2. Talent placing the centrifuge tube into a centrifugal stirrer mixer.

2.2. Centrifuge at 2000 *g* for 4 minutes twice, to mix and to degas **[1]**. Pour 13 to 15 grams of the degassed PDMS into the microfluidic mold, ensuring the bottom of the mold is completely covered **[2]**.

2.2.1. Talent inputting centrifugation parameters.

2.2.2. Talent pouring the degassed PDMS into the mold and evenly covering the bottom surface.

2.3. Now place the mold in a vacuum desiccator **[1]**. Then use a vacuum pump to evacuate air for 5 to 10 minutes to thoroughly remove bubbles from the PDMS **[2]**.

2.3.1. Talent placing the mold into a vacuum desiccator.

2.3.2. Talent activating the vacuum pump to evacuate air.

2.4. Using a rubber bulb, gently tap the surface of the PDMS to break any remaining surface bubbles **[1]**. Transfer the mold to a convection oven and bake at 80 degrees Celsius for 100 minutes to cure the polydimethylsiloxane **[2]**.

2.4.1. Talent using a rubber bulb to tap on the surface of the mold.

2.4.2. Talent placing the mold into a convection oven and setting it to 80 degrees Celsius.

2.5. Once the PDMS is fully cured, gently slide the tip of a scalpel under the edge of the PDMS to carefully lift and separate it from the mold **[1]**. Using a biopsy punch with a diameter of 2.0 to 2.5 millimeters, create holes in the PDMS for culture medium infusion and cell loading **[2]**.

2.5.1. Shot of a scalpel tip being inserted under the edge of the cured polydimethylsiloxane and lifting it out of the mold.

2.5.2. Talent using a biopsy punch to create inlet and outlet holes in the

polydimethylsiloxane.

2.6. Remove any surface impurities from the PDMS using adhesive tape **[1]**, then place it in a clean glass dish and wrap it with aluminum foil for storage **[2]**.

2.6.1. Talent dabbing the PDMS surface with adhesive tape to remove dust.

2.6.2. Talent placing the cleaned PDMS in a glass dish and wrapping it in aluminum foil.

2.7. Prior to the experiment, autoclave the microfluidic device at 121 degrees Celsius and 101 kilopascals for 5 minutes to ensure sterility **[1]**.

2.7.1. Talent placing the wrapped device in the autoclave and setting the sterilization conditions.

3. Coating Coverslips with Poly-D-Lysine for Microfluidic Cell Culture

Demonstrator: Ruixuan Liu

3.1. Place a coverslip intended for conventional microfluidic devices in a 35-millimeter culture dish **[1]**. Add 2 milliliters of 0.1 milligram per milliliter poly-D-lysine solution to the dish **[2]**. Incubate the dish at 37 degrees Celsius for 6 hours or overnight **[3]**.

3.1.1. Talent placing the coverslip in the center of the 35 millimeter culture dish.

3.1.2. Talent pipetting 2 milliliters of poly-D-lysine solution into the dish.

3.1.3. Talent placing the dish into a 37 degrees Celsius incubator.

3.2. After incubation, carefully retrieve the poly-D-lysine solution for reuse or proper disposal **[1]**. Now add 2 milliliters of sterile ultrapure water to the dish **[2].** Rotate it 50 times clockwise, followed by 50 times counterclockwise **[3]**.

3.2.1. Talent removing the dish from the incubator and aspirating the poly-D-lysine solution.

3.2.2. Talent pipetting 2 mL of sterile ultrapure water into the dish.

3.2.3. Talent rotating the dish back and forth with sterile water.

3.3. Aspirate the water using a vacuum pump connected to a sterile pipette tip, collecting waste in a liquid waste container **[1-TXT]**. After all washes are complete, allow the coverslips to air-dry naturally in a biosafety cabinet before use **[2]**.

3.3.1. Talent aspirating the water from the dish into a waste container using a pipette connected to a vacuum pump. **TXT: Repeat wash for total of 3 washes**

3.3.2. Talent placing washed coverslips in a biosafety cabinet for air drying.

4. *In Vitro* Culture of Cortical Neurons in Conventional or Large-Scale Microfluidic Devices

Demonstrator: Ruixuan Liu

4.1. Using sterile fine-tip forceps, place the autoclaved microfluidic chip at the center of the glass surface with microchannels facing downward **[1]**. Gently press the chip onto the glass surface using a 200-microliter pipette tip to ensure complete adhesion **[2]**.

4.1.1. Talent using fine-tip forceps to position the chip at the center of the coverslip.

4.1.2. Talent pressing down on the chip using a 200 microliter pipette tip.

4.2. Next, mark the left chamber with a dot using a permanent marker to designate the soma compartment **[1]**. Aspirate 10 microliters of neuronal suspension with a 10-microliter pipette **[2]**. Then slowly dispense the suspension into the loading port above the marked soma compartment **[3]**.

4.2.1. Talent marking the left chamber of the microfluidic chip with a dot.

4.2.2. Talent aspirating neuronal suspension using a pipette.

4.2.3. Talent dispensing cell suspension into the soma port.

4.3. Confirm proper fluid flow into the lower reservoir of the left chamber **[1]**. Transfer the culture dish to a humidified incubator set at 37 degrees Celsius and 5 percent carbon dioxide for 20 minutes **[2]**.

4.3.1. Talent visually inspecting the flow of fluid into the reservoir.

4.3.2. Talent placing the dish into a humidified incubator.

4.4. After incubation, place the dish under a 20X microscope to confirm media perfusion from the soma compartment to the axonal compartment through the microchannels **[1]**.

4.4.1. SCOPE: 4.4.1.mp4 00:00-00:25

4.5. Now, pipette 150 microliters of neuronal basal medium to the upper port of the axonal compartment **[1]**. Similarly, add 150 microliters of complete neuronal medium to the upper port of the soma compartment **[2]**.

4.5.1. LAB MEDIA: 4.5.1&4.5.2.mp4 00:04-00:13

4.5.2. LAB MEDIA: 4.5.1&4.5.2.mp4 00:14-00:20

4.6. Next, pour 20 milliliters of ultrapure water into a 150-millimeter culture dish to create a humidity chamber **[1]**. Place the 35-millimeter culture dish inside the large dish **[2]**. Then transfer the entire culture system to the incubator and maintain for the required duration **[3]**.

4.6.1. Talent pouring water into a large dish.

4.6.2. Talent placing the smaller culture dish inside the larger humidity chamber.

4.6.3. Talent transferring the entire setup into the incubator.

4.7. Use a 10-centimeter wide Petri dish for placement of the large-scale microfluidic device **[1]**. Choose either full-channel or interval-channel seeding based on

experimental needs, as each method requires different axonal injury protocols **[2]**. After seeding is completed, add 5 milliliters of complete neuron culture medium to the Petri dish to maintain neuron growth **[3]**.

4.7.1. Talent placing the large-scale microfluidic device in a 10 centimeter dish.

4.7.2. Talent performing either full-channel or interval-channel seeding.

4.7.3. Talent pipetting neuron culture medium into the large Petri dish.

5. Establishing an *In Vitro* Axonal Injury Model in Conventional Microfluidic Devices

Demonstrator: Ruixuan Liu

5.1. Transfer an appropriate amount of neuronal basal medium into a new 15-milliliter centrifuge tube for later use **[1]**. Place a microfluidic device containing cortical neurons on a clean bench **[2].**

5.1.1. Talent pipetting neuronal basal medium into a clean centrifuge tube.

5.1.2. Talent removing the cultured device and placing it on a clean workbench.

5.2. Using lint-free paper, dry the bottom of the 35-millimeter culture dish **[1]**. Then use a 200-microliter pipette to aspirate the medium from the two right-side holes of the microfluidic device **[2]**.

5.2.1. Talent drying the base of the dish with lint-free paper.

5.2.2. Talent aspirating medium from both right-side holes.

5.3. Next, connect a vacuum pump tubing to a sterile, filter-free 200 microliter pipette tip **[1]**. Turn on the vacuum pump at a suction rate of 60 liters per minute **[2]** and aim the tip at the connection point between the lower hole of the axon terminal chamber and the chamber to aspirate the old medium, causing axon breakage due to negative pressure **[3]**.

5.3.1. Talent connecting vacuum tubing to a pipette tip.

5.3.2. Shot of pump being turned on.

5.3.3. Talent aspirating fluid from the axon chamber.

5.4. Replace the pipette tip **[1]** and draw 150 microliters of neuronal basal medium, slowly adding it through the lower hole of the right chamber **[2-TXT]**.

5.4.1. Shot of the pipette tip being replaced.

5.4.2. Talent slowly adding fresh medium via a new tip. **TXT: Use the two right-side holes alternately to add liquid to one and aspirate from the other immediately**

5.5. After the performing addition and aspiration 4 times, replace with fresh complete neuronal medium **[1]**. Then aspirate the old medium from the cell body side hole **[2]** and add fresh complete neuronal medium containing drugs if needed **[3]**.

5.5.1. Talent adding fresh complete medium to complete the process.

5.5.2. Talent aspirating old medium from the soma side.

5.5.3. Talent adding drug-containing medium to the soma chamber.

5.6. Place the microfluidic device along with the culture dish in a 150-millimeter culture dish and continue culturing for the required time such as 24 hours **[1]**.

5.6.1. Talent placing the culture dish inside the larger dish for incubation.

6. Large-Scale Microfluidic Device Axonal Injury

Demonstrator: Ruixuan Liu

6.1. For manual axonal injury, seed neurons into all chambers of the large-scale microfluidic device **[1]**. Incubate the device at 37 degrees Celsius in a humidified incubator with 5 percent carbon dioxide for three days **[2]**.

6.1.1. Talent pipetting neurons into each chamber of the device.

6.1.2. Talent placing the seeded device into a humidified incubator.

6.2. On day 7 *in vitro*, remove the culture dish containing the microfluidic device from the incubator and place it on a sterile stage **[1]**. Prepare a microscope with 20X magnification and sterilize the work area using 75 percent ethanol **[2]**.

6.2.1. Talent moving the culture dish onto a sterile platform.

6.2.2. Talent wiping down the workspace and microscope with ethanol.

6.3. Hold a 10-microliter sterile filtered pipette tip and identify axon bundles in the original microchannels under microscope guidance **[1]**. Perform longitudinal scratches along each axon bundle using the pipette tip **[2]**. Observe the axon breakage under the microscope in real time **[3]**.
Videographer: Please film these shots with a SCOPE kit as backup

6.3.1. SCOPE: View of axon bundles inside microchannels under 20 times magnification.

6.3.2. SCOPE: Talent using pipette tip to scratch along axons.

6.3.3. SCOPE: Real-time visualization of broken axons after scratching.

Results

7. Results

7.1. The microchannels of a standard microfluidic device permit the growth of axons but not soma **[1]**, as confirmed by βIII *(beta-three)* -tubulin staining at 7 days *in vitro* **[2]**.

7.1.1. LAB MEDIA: Figure 2 *Video editor: Please highlight image A*

7.1.2. LAB MEDIA: Figure 2B *Video editor: Please highlight image B*

7.2. Neuronal density showed no significant difference following axonal injury, indicating no observable neuronal death **[1]**.

7.2.1. LAB MEDIA: Figure 2 *Video editor: Please highlight image C and emphasize the bar graph*

7.3. A large-scale microfluidic device with a 3-dimensional expandable design was developed for multi-omics analyses **[1]**. Both unpunched and perforated PDMS replicas adhered securely to 10-centimeter dishes or PC boards **[2]**.

7.3.1. LAB MEDIA: Figure 3A–C. *Video editor: Please show images A to C sequentially*

7.3.2. LAB MEDIA: Figure 3D,E.

7.4. Axonal damage induced by pipette tip scratching led to clearly severed axons with disrupted morphology **[1]**, in contrast to intact axons prior to injury **[2]**.

7.4.1. LAB MEDIA: Figure 4

7.4.2. LAB MEDIA: Figure 4 *Video editor: Please highlight images A and C*

7.5. Axonal regeneration was observed at both 6 hours and 24 hours following injury, while uninjured axons remained stable **[1]**.

7.5.1. LAB MEDIA: Figure 5 *Video editor: Please highlight images B when VO says "6 hours", C when VO says "24 hours" and A when Vo says "uninjured axons remained stable"*

7.6. Neuronal protein concentration rose significantly from 1420.4 micrograms per milliliter at baseline **[1]** to 1748.9 per milliliter at 6 hours **[2]**, and 1823.7 micrograms per milliliter at 24 hours post-injury **[3]**.

7.6.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the first bar labeled "(–)Ax"*

7.6.2. LAB MEDIA: Figure 6B. *Video editor: Highlight the second bar labeled "(+)Ax6h"*

7.6.3. LAB MEDIA: Figure 6B. *Video editor: Highlight the third bar labeled "(+)Ax24h"*

7.7. RNA yields from control and axon-injured groups were nearly identical **[1]**. RNA sequencing revealed 595 injury-specific genes and 609 control-specific genes, with

17,471 genes shared between groups **[2]**.

7.7.1. LAB MEDIA: Figure 7A.

7.7.2. LAB MEDIA: Figure 7B. *Video editor: Emphasize the non-overlapping blue and red regions of the Venn diagram, then the overlapping central region.*

7.8. KEGG pathway analysis identified significant upregulation of oxidative phosphorylation, reactive oxygen species response, mitophagy, and TCA cycle pathways post-injury **[1]**.

7.8.1. LAB MEDIA: Figure 7C.

Pronunciation Guide:

1. **Microfluidic**

- Pronunciation link: https://www.howtopronounce.com/microfluidic How To Pronounce
- IPA: /ˌmaɪkroʊfləˈwɪdɪk/
- Phonetic Spelling: my-kroh-fluh-WID-ik

2. **Polydimethylsiloxane** (often abbreviated PDMS)

- Pronunciation link: https://www.howtopronounce.com/polydimethylsiloxane How To Pronounce
- IPA: /ˌpɑliːˌdaɪˈmɛθəlˌsɪlɒkˌseɪn/
- Phonetic Spelling: pah-lee-dye-METH-ul-sil-ox-ayn

3. **Axonal**

- Pronunciation link: https://www.merriam-webster.com/dictionary/axonal (if available) — I didn't find a specific link in the search results for "axonal."
- IPA: /ˈæk.sə.nəl/
- Phonetic Spelling: AK-suh-nuhl

4. **Omics** (in "multi-omics")

- Pronunciation link: you might use Merriam-Webster / general dictionaries (standard word) — not in the snippet results.
- IPA: /ˈoʊ-mɪks/
- Phonetic Spelling: OH-miks

5. **Metabolic flux analysis**

- "Metabolic"
  - Pronunciation link: https://www.merriam-webster.com/dictionary/metabolic
  - IPA: /ˌmɛtəˈbɒlɪk/ (US: /ˌmɛtəˈbɑlɪk/)
  - Phonetic Spelling: meh-tuh-BOL-ik
- "Flux"
  - IPA: /flʌks/
  - Phonetic Spelling: flucks
- "Analysis"
  - Pronunciation link: https://www.merriam-webster.com/dictionary/analysis
  - IPA: /əˈnæləsɪs/
  - Phonetic Spelling: uh-NAL-uh-sis

6. **Transcriptomics**

- Pronunciation link: not found in current snippet; likely "transcriptome" + "-ics"
- IPA: /trænsˌkrɪpˈtɒmɪks/ (US: /trænsˌkrɪpˈtoʊmɪks/)
- Phonetic Spelling: trans-crip-TOM-iks

7. **Cortical neurons**

- "Cortical"
  - Pronunciation link: Merriam-Webster (if available) – not in snippet
  - IPA: /ˈkɔːrtɪkəl/ (US: /ˈkɔːrtɪkəl/ or /ˈkɔːrtɪkəl/)
  - Phonetic Spelling: KOR-ti-kul
- "Neurons"
  - Pronunciation link: Merriam-Webster (standard)

- IPA: /ˈnʊərɒnz/ (US: /ˈnʊrɑnz/)
- Phonetic Spelling: NYOO-rons

8. **βIII (beta-three) -tubulin**

- "Beta-three tubulin"
  - "Beta" /ˈbeɪtə/ (BAY-tuh)
  - "Tubulin" /ˈtjuːbjʊlɪn/ (US sometimes /ˈtjuːblɪn/) – "TOO-byoo-lin" or "TOO-blin" depending on accent

9. **Reactive oxygen species**

- "Reactive"
  - IPA: /riˈæktɪv/
  - Phonetic Spelling: ree-AK-tiv
- "Oxygen" /ˈɒksɪdʒən/ (US: /ˈɑːksɪdʒən/) – OK-si-jen
- "Species" /ˈspiːʃiːz/ – SPEE-sheez

10. **Mitophagy**

- Pronunciation link: likely in scientific dictionaries; not found in snippet
- IPA: /ˌmaɪtəˈfɑːdʒi/ (US: /ˌmaɪtoʊˈfɑːdʒi/)
- Phonetic Spelling: my-toh-FAH-jee

11. **Oxidative phosphorylation**

- "Oxidative" /ˈɑːksɪdeɪtɪv/ (US: /ˈɑksɪdeɪtɪv/) – oks-ih-DAY-tiv
- "Phosphorylation" /ˌfɑsfoʊrəˈleɪʃən/ – FOS-foh-ruh-LAY-shun

12. **TCA cycle** (Tricarboxylic Acid cycle)

- "Tricarboxylic" /ˌtraɪkɑːrˈbɒksɪlɪk/ (US variant)
- Phonetic Spelling: try-car-BOK-si-lik
- "Cycle" /ˈsaɪkəl/ – SY-kul