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Title: Injection of Hydrogel Biomaterial Scaffolds to The Brain After Stroke

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **No, S. Thomas Carmichael will provide video.**

Protocol Length

Number of Shots: **49**

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. **Tatiana Segura**: The reproducible injection of hydrogel scaffolds into a rodent ischemic stroke model allows for the study of how materials and other delivered therapeutics affect brain repair, regeneration, and behavioral improvement.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested b-roll: 3.8.1.*
- 1.2. **Tatiana Segura**: The main advantages of using the photothrombotic stroke model to study brain-material interactions are the reproducible location and size, which allow injection of materials into the stroke core without imaging.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera
- 1.3. **S. Thomas Carmichael**: The phototrombotic stroke model is designed to be a relevant model of ischemic stroke in a region of the brain, the motor cortex, that is commonly affected by stroke in humans and to provide a relevant behavioral read out for stroke, which is motor control of the limb so as to evaluate a candidate therapeutic like a biopolymer hydrogel.
 - 1.3.1. Added: INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **NOTE: Authors recorded this on their own, it's uploaded to AWS**

Ethics Title Card

- 1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Duke University.

Protocol

2. Photothrombotic Stroke Model

NOTE: Authors mentioned that some shots were merged, please check videographer's log (I didn't receive any videographer notes for this one)

- 2.1. Confirm a lack of response to toe pinch in an anesthetized mouse [1-TXT], then weigh the mouse to determine how much Rose Bengal dye to inject [2] and secure it to a stereotaxic device equipped with a laser [3-TXT]. *Videographer: This step is difficult!*
 - 2.1.1. WIDE: Talent pinching toe *Videographer: More Talent than mouse in shot*
TEXT: Anesthesia: 4% -> 1.5% isoflurane
 - 2.1.2. Talent placing mouse onto balance *Videographer: More Talent than mouse in shot*
 - 2.1.3. Talent securing mouse to device *Videographer: More Talent than mouse in shot* **TEXT: See text for device and laser preparation details**
- 2.2. After shaving and disinfecting the surgical area, use forceps to tent the skin between the ears and eyes [1] and insert a blade into the raised skin to make a lateral, 1 to 2-centimeter midline incision in the skin [2].
 - 2.2.1. Skin being tented
 - 2.2.2. Incision being made
- 2.3. Separate the skin [1] and press the forceps lightly onto the parietal bones to locate the bregma [2].
 - 2.3.1. Skin being separated
 - 2.3.2. Bones being pressed
- 2.4. Use a surgical marker to label the bregma with a dot [1] and, wearing laser safety goggles, move the laser over the skull of the mouse [2], fixing the stereotaxic device at a 90-degree angle [3]. *Videographer: This step is important!*

- 2.4.1. Bregma being marked
- 2.4.2. Laser being moved over skull
- 2.4.3. Device being fixed at 90° angle
- 2.5. Select the 10-milliwatt pre-set **[1]** and turn on the laser **[2]**.
 - 2.5.1. Talent selecting pre-set
 - 2.5.2. Talent turning on laser
- 2.6. Adjust the x- and y-axis until the laser is directly over the bregma **[1]**.
 - 2.6.1. Talent adjusting axes
- 2.7. Reset the x and y on the digital display console **[1]** and move the laser 1.8 millimeters left of the bregma **[2]**.
 - 2.7.1. Talent resetting x and y
 - 2.7.2. Talent moving laser
- 2.8. Bring the laser as close to the skull as possible without letting the laser touch the skull **[1]** and move the laser 2.2 millimeters left of the bregma to ensure that it can move without any obstructions **[2]**.
 - 2.8.1. Laser being moved closed to skull
 - 2.8.2. Laser being moved left of bregma
- 2.9. When the laser is in position, turn it off **[1]** and use a disposable 29-gauge needle to intraperitoneally inject the appropriate volume of Rose Bengal dye **[2]**.
 - 2.9.1. Talent turning off laser
 - 2.9.2. Dye being injected
- 2.10. Immediately start a timer to allow the dye to circulate systemically for 7 minutes **[1]**.

2.10.1. Talent starting timer

2.11. While the dye is circulating, select the pre-set for 40 milliwatts without turning on the laser **[1]**.

2.11.1. Talent selecting pre-set

2.12. When the timer goes off, turn on the laser **[1]** and set the timer for 10 minutes **[2]**.

2.12.1. Talent, wearing goggles, turning on laser

2.12.2. Talent setting timer

2.13. When the timer goes off again, move the laser to 2.2 millimeters left of the bregma **[1]** and set another 10-minute timer **[2]**.

2.13.1. Laser being moved

2.13.2. Talent setting timer

2.14. When the second 10-minute timer has gone off, change the laser back to 10 milliwatts **[1]** and move it to 2 millimeters left of the bregma **[2]**.

2.14.1. Talent changing laser to 10 mW

2.14.2. Laser being moved

2.15. Mark this spot with the surgical marker **[1]** and turn off the laser **[2]**.

2.15.1. Spot being marked

2.15.2. Talent turning off laser

2.16. Lift the laser **[1]** and unlock it from the 90-degree angle so it can be moved away from the mouse **[2]**.

2.16.1. Talent lifting laser *Videographer: Can combine 3.16.1. and 3.16.2. as appropriate*

2.16.2. Talent unlocking laser *Videographer: Can combine 3.16.1. and 3.16.2. as appropriate*

2.17. Use cotton to apply sterile saline to the surgical area as necessary [1] and drill the 2-millimeter mark in small bursts perpendicular to the surface of the skull [2-TXT].
Videographer: This step is important!

2.17.1. Saline being applied

2.17.2. Mark being drilled **TEXT: Remove excess blood with cotton**

2.18. Place a small wipe next to the mouse [1] and use the forceps to pull the skin close together and upward [2].

2.18.1. Wipe being placed

2.18.2. Skin being pulled

2.19. Dab any large drops of surgical glue at the end of the tip on the wipe [1] and sparingly apply surgical glue to the skin [2].

2.19.1. Glue being dabbed

2.19.2. Glue being applied

2.20. Hold skin together with forceps for 10 seconds [1], then continue to apply glue until no additional openings are visible in the skin [2].

2.20.1. Skin being held

2.20.2. Glue being applied

3. Hydrogel Injection

3.1. For hydrogel injection into the stroke region, attach injection pumps to the stereotaxic device [1] and set the flow rate to 1 microliter per minute and the volume to 4 microliters [2].

3.1.1. WIDE: Talent attaching pump(s) to device

- 3.1.2. Talent setting flow rate and/or volume
- 3.2. Reopen the incision over the brain [1] and use saline-soaked cotton to clean any debris from the skull [2]. A white-yellow circle of tissue and the burr hole from the drill will be visible [3-TXT].
 - 3.2.1. Incision being opened
 - 3.2.2. Tissue/debris being wiped
 - 3.2.3. Shot of tissue/burr hole **TEXT: If no dead tissue visible, no stroke occurred**
- 3.3. If the burr hole is not centered above the stroke, re-drill to center it [1] and lock the injection pump at 90 degrees over the mouse [2].
 - 3.3.1. Talent attaching bit to drill
 - 3.3.2. Pump being locked over mouse
- 3.4. Clean the glass syringe with sterile saline [1] and remove the needle [2].
 - 3.4.1. Syringe being cleaned
 - 3.4.2. Syringe being disassembled
- 3.5. Use a 25-microliter positive displacement pipette to backload the syringe with 10 microliters of hydrogel [1] and use the plunger to push the gel all the way to the front of the syringe [2].
 - 3.5.1. Talent loading syringe with hydrogel
 - 3.5.2. Gel being pushed to front of syringe
- 3.6. Reassemble the syringe [1] and continue pushing until the gel visibly exudes from the needle [2].
 - 3.6.1. Needle being attached to syringe
 - 3.6.2. Gel being pushed

3.7. Load the syringe onto the pump **[1-TXT]** and move the pump in the x and y directions to orient the syringe over the burr hole **[2]**.

3.7.1. Talent loading syringe **TEXT: See text for syringe position adjustment details**

3.7.2. Syringe being oriented

3.8. Move the pump in the z direction until the needle touches the top of the hole **[1]** and reset the z on the digital display console **[2]**. *Videographer: This step is important!*

3.8.1. Needle being moved to burr hole

3.8.2. Z being reset

3.9. Move the syringe 0.75 millimeters in the z direction **[1-TXT]** and press **Start** on the pump console to inject 4-6 microliters of the hydrogel at a 1 microliter per minute flow rate **[2-TXT]**. *Videographer: This step is difficult and important!*

3.9.1. Syringe being moved **TEXT: If syringe resistance or bending, re-drill hole**

3.9.2. Start being pressed **TEXT: Optional: Use stereomicroscope for injection**

3.10. When all of the hydrogel has been delivered, set a 5-minute timer to allow the gel to begin crosslinking **[1]**.

3.10.1. Talent setting timer, with stereotaxis device visible in frame as possible
Videographer: More Talent than mouse in shot

3.11. After 5 minutes, turn the z knob to slowly pull up the syringe **[1]**. When the needle is far enough away from the skull, unlock and remove the pump **[2]**.

3.11.1. Knob being turned/needle being lifted

3.11.2. Talent unlocking/removing pump

3.12. Then close the skin over the skull **[1]** and place the mouse in a clean cage with monitoring until it has recovered from the anesthesia **[2]**.

3.12.1. Suture/glue being placed

3.12.2. Talent placing mouse into cage *Videographer: More Talent than mouse in shot*

4. Sample Collection

4.1. At the appropriate experimental endpoint, use blunt thin forceps **[1]** to carefully remove pieces of the skull to expose the brain **[2-TXT]**, using small surgical scissors to cut away the bone over the stroke site as necessary **[3]**. *Videographer: This step is difficult and important!*

4.1.1. WIDE: Talent selecting forceps

4.1.2. Skull pieces being removed **TEXT: Euthanasia: anesthesia + decapitation**

4.1.3. Skull pieces being cut

4.2. After harvesting, place the brain in 10-15 milliliters of 4% paraformaldehyde overnight for 16-24 hours **[1]** before placing the tissue into 30% sucrose **[2]**.

4.2.1. Talent placing brain in PFA, with PFA container visible in frame

4.2.2. Brain being placed into sucrose, with sucrose container visible in frame

4.3. The brain will be ready for cryosection when it sinks to the bottom of the tube **[1-TXT]**.

4.3.1. Shot of brain at bottom of tube **TEXT: i.e., 2-3 d**

Results

5. Results: Representative Hydrogel Biomaterial Scaffold Injection and Analysis

- 5.1. Five days after stroke induction [1], the infarct can be visualized during surgery [2] and by TTC (T-T-C) and immunohistochemically stained slides imaging [3-TXT].
 - 5.1.1. LAB MEDIA: Figures 1B and 2B
 - 5.1.2. LAB MEDIA: Figures 1B and 2B *Video Editor: please emphasize tissue within dashed circle in Figure 1B*
 - 5.1.3. LAB MEDIA: Figures 1B and 2B *Video Editor: please emphasize Figure 2B* **TEXT: TTC: 2,3,5-triphenyltetrazolium chloride**
- 5.2. An increase in the laser diameter with a 2x lens [1] leads to a visual increase in the stroke lesion that spans over 2.5 millimeters [2] versus a single 1-millimeter section using the laser only [3].
 - 5.2.1. LAB MEDIA: Figure 2C
 - 5.2.2. LAB MEDIA: Figure 2C *Video Editor: please emphasize Stroke 2x lens images*
 - 5.2.3. LAB MEDIA: Figure 2C *Video Editor: please emphasize Stroke no lens images*
- 5.3. Hyaluronic acid-based hydrogel microparticle injection into the stroke lesion five days after stroke [1] results in the formation of microannealed particles with a porous scaffold that facilitates cell migration into the stroke core [2].
 - 5.3.1. LAB MEDIA: Figure 4B
 - 5.3.2. LAB MEDIA: Figure 4B *Video Editor: please emphasize Hydrogel images*
- 5.4. Hyaluronic hydrogel injections that match the modulus of the cortex [1] lead to a significant decrease in the reactive astrocytes within the peri-infarct [2] as well as inside the microannealed particle gel [3].
 - 5.4.1. LAB MEDIA: Figures 5C-5E *Video Editor: please emphasize HA-MAP images in Figure 5C*
 - 5.4.2. LAB MEDIA: Figures 5C-5E *Video Editor: please emphasize HA-MAP (infarct) data cluster in Figures 5D and 5E*
 - 5.4.3. LAB MEDIA: Figures 5C-5E *Video Editor: please emphasize HA-MAP data cluster in Figures 5D and 5E*
- 5.5. Furthermore, microglia that were also within the hydrogel express pro-repair M2 markers [1], suggesting that microannealed particle hydrogel can reduce astrocyte

reactivity **[2]** and promote a more anti-inflammatory environment with pro-recovery astrocytes and microglia in just two days following injection **[3]**.

- 5.5.1. LAB MEDIA: Figures 5F-5H *Video Editor: please emphasize bottom row merged images*
- 5.5.2. LAB MEDIA: Figures 5F-5H *Video Editor: please add/emphasize asterisks in Figure 5H*
- 5.5.3. LAB MEDIA: Figures 5F-5H

Conclusion

6. Conclusion Interview Statements

6.1. **Tatiana Segura**: The scaffolds-tissue can be retrieved and used for molecular, cellular or tissue level analyses such as scRNA-seq, flowcytometry, or clarity. Also, behavioral analysis can be performed to assess behavioral improvement.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested b-roll: 4.2.2.*

6.2. **S. Thomas Carmichael**: this approach delivers a candidate therapeutic, a biopolymer hydrogel, into the stroke cavity, which is a potential cavity in stroke and area of dead tissue that can accept a transplant. Additionally, this cavity is adjacent to the area of the brain that undergoes the most plasticity after stroke, the peri-infarct tissue. So a candidate therapeutic like a biopolymer hydrogel can deliver cells or small molecules to the part of the brain that is really the target for recovery in stroke.

6.2.1. Added: INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **NOTE: Authors recorded this on their own, it's uploaded to AWS**

