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Title: A Rat Carotid Artery Pressure-Controlled Segmental Balloon Injury with Periadventitial Therapeutic Application

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Y, LEICA DUAL HEAD MZ6 MICROSCOPE**

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)? **N**

Script Length

Number of shots: **41**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Edward Bahnson**: The pressure-controlled segmental balloon injury is an experimental procedure that mimics the balloon angioplasty that is performed in human patients with symptomatic atherosclerotic disease **[1]**.

REQUIRED:

1.2. **Nick Buglak**: This restenosis model is performed in a similar manner to balloon angioplasty in the clinical setting, allowing a defined area of vascular injury and complete re-endothelization within 2 weeks of injury to be achieved **[1]**.

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of North Carolina – Chapel Hill.

Protocol

2. Common Carotid Artery (CCA) Identification

2.1. After confirming a lack of response to pedal reflex in an anesthetized adult rat **[1-TXT]**, place the rat under a dissecting microscope **[2]** and use a scalpel to make a straight, 1.5-2-centimeter, superficial, longitudinal skin incision along the neckline between the jaw bones **[3]**.

2.1.1. WIDE: Talent pinching toe *Videographer: More Talent than rat in shot* **TEXT: Anesthesia: 5 -> 1.5% isoflurane**
2.1.2. Talent adjusting microscope over rat *Videographer: More Talent than rat in shot*
2.1.3. Incision being made

2.2. Make a second incision through the connective tissue under the skin until the muscle layer is exposed **[1]** and displace the salivary glands underneath the skin to access the muscle tissue **[2]**.

2.2.1. SCOPE: Incision being made *Videographer: Important step*
2.2.2. SCOPE: Glands being displaced *Videographer: Important step*

2.3. Insert closed scissor tips between the muscle layer and connective tissue **[1]** and gently open the scissors while pulling the skin upward to bluntly separate the connective tissue from the muscle **[2]**.

2.3.1. SCOPE: Tips being inserted
2.3.2. SCOPE: Tips being opened/skin being pulled

2.4. Dissect the sternohyoid and sternomastoid muscles longitudinally along the left side of the trachea **[1]** until the omohyoid muscle, which runs perpendicular to the two superficial muscles, is observed **[2]**.

2.4.1. SCOPE: Muscles being dissected *Videographer: Important step; Video Editor: please emphasize muscles when mentioned if necessary/possible*
2.4.2. SCOPE: Shot of omohyoid muscle *Videographer: Important step; Video Editor: please emphasize muscle when mentioned if necessary/possible*

2.5. Use forceps to gently create a window separating the perpendicular omohyoid muscle from the longitudinal sternohyoid muscle running over the trachea **[1-TXT]** and reach the forceps under the omohyoid muscle **[2]** to separate the sternohyoid and sternomastoid muscles to expose the common carotid artery **[3]**.

2.5.1. SCOPE: Window being created **TEXT: Caution: Do not damage trachea**
2.5.2. SCOPE: Forceps being inserted
2.5.3. SCOPE: Muscles being cut/CCA being exposed

3. CCA Dissection

3.1. To isolate the common carotid artery, dissect the artery near the bifurcation **[1]** until the internal and external carotid arteries are exposed **[2]**.

3.1.1. WIDE: Talent dissecting artery *Videographer: More Talent than rat in shot*
3.1.2. SCOPE: Arteries being dissected/exposed

3.2. Using pre-cut Prolene sutures, ligate the superior thyroid and external carotid arteries near their respective bifurcations **[1]**, leaving the majority of the suture to one side of the knot and grabbing each suture with a curved hemostat **[2]**.

3.2.1. SCOPE: Arteries being ligated
3.2.2. SCOPE: Shot of suture on one side, then sutures being grabbed

3.3. Finish dissecting around the internal carotid artery **[1]** and reach the forceps under and around the artery **[2]**.

3.3.1. SCOPE: Artery being dissected
3.3.2. SCOPE: Forceps being reached under and around

3.4. Use a non-crushing vascular clamp to carefully clamp the occipital artery with the internal carotid artery to achieve distal control without kinking the vessels **[1]**.

3.4.1. SCOPE: Clamp being placed *Videographer: Important/difficult step*

3.5. Dissect the common carotid artery proximal to the bifurcation, taking care to separate the vagus nerve from the artery **[1]**, and reach forceps under and around the common carotid artery **[2]**.

3.5.1. SCOPE: Artery being dissected
3.5.2. SCOPE: Forceps being reached under and around

3.6. Then use a non-crushing vascular clamp to achieve proximal control, placing the clamp at least 5 millimeters from the bifurcation **[1]**.

3.6.1. SCOPE: Clamp being placed

4. Carotid Artery Pressure-Controlled Segmental Balloon Injury

4.1. To induce the balloon injury, maneuver the curved hemostats **[1]**, holding each ligated artery branch, to expose the bifurcation between the external carotid artery and superior branch **[2]**.

4.1.1. WIDE: Talent manipulating hemostats *Videographer: More Talent than rat in shot*
4.1.2. SCOPE: Branches being held with hemostats

4.2. Gently dissect the tissue at the bifurcation to clear the arteriotomy site as much as possible **[1]** and use microdissection scissors to make an arteriotomy incision between the external carotid artery and the superior branch **[2]**.

4.2.1. SCOPE: Tissue being dissected *Videographer: Important/difficult step*
4.2.2. SCOPE: Incision being made *Videographer: Important/difficult step*

4.3. **Nick Buglak**: Take care to clear the site prior to inserting the microscissors and to ensure that one blade of the microscissors is fully inserted into the artery before making the incision **[1]**.

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

4.4. Use a cotton swab to push all of the blood out of the common carotid artery **[1]** and to clean up the arteriotomy site **[2]**.

4.4.1. SCOPE: Blood being flushed
4.4.2. SCOPE: Site being cleaned

4.5. Insert the uninflated balloon catheter through the arteriotomy by lifting the arteriotomy with forceps **[1]** and advance the balloon into the artery until the proximal end of the balloon is past the bifurcation. If the balloon does not easily advance into the common carotid **[2]**, reposition the angle of the balloon before trying again **[3]**.

4.5.1. SCOPE: Balloon being inserted *Videographer: Important step*
4.5.2. SCOPE: Balloon being advanced unsuccessfully (attempt 1). NOTE: the balloon would not go past the bifurcation causing the clamp on the internal carotid artery to move. This emphasizes the importance of not having a kink in the common carotid artery which Talent speaks about in concluding statement 6.1.
4.5.3. SCOPE: Balloon being advanced successfully, after readjusting the angle of insertion (attempt 2) *Videographer: Important step*

4.6. Tape the catheter to the anesthesia nose cone so the balloon does not slip out of the artery during the inflation **[1]** and slowly fill the balloon to 5 atmospheres of pressure **[2]**.

4.6.1. SCOPE: Catheter being taped
4.6.2. SCOPE: Balloon being inflated

4.7. Leave the balloon in the artery for 5 minutes to induce the arterial injury **[1-TXT]** before deflating and gently removing the balloon through the arteriotomy **[2]**.

4.7.1. Talent setting timer, with setup but minimal rat in frame **TEXT: Ensure pressure remains constant for entire 5 min**
4.7.2. SCOPE: Balloon being deflated and/or removed

4.8. Gently squeeze the clamp to flush out the artery **[1]** and ligate the external carotid artery proximal to the arteriotomy **[2]**.

4.8.1. SCOPE: Clamp being squeezed/artery being flushed
4.8.2. SCOPE: ECA being ligated

4.9. Remove the clamps from the common and internal carotid arteries to restore blood flow **[1-TXT]** and apply 50 microliters of therapeutic gel periadventitially along the left and right sides of injured artery **[2]**.

4.9.1. SCOPE: Clamp(s) being removed *Videographer: Important step;* **TEXT: Confirm no visible bleeding around arteriotomy**
4.9.2. SCOPE: Gel being applied *Videographer: Important step;*

4.10. Then use interrupted 4-0 or 6-0 vicryl sutures to close the connective tissue **[1]** and close the skin with a running 4-0 nylon suture **[2]**.

4.10.1. Connective tissue suture being placed NOTE:We took shots under the SCOPE and of the Talent for both suturing steps in case the SCOPE shots are more informative
4.10.2. Skin suture(s) being placed

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.

2.2., 2.4., 3.4., 4.2., 4.5., 4.9.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
3.4. placing the clamp around the internal carotid artery can be difficult. If the clamp is placed too close to the common carotid artery then this will cause a kink in the common carotid artery and not allow the balloon to pass through easily even if all other steps are performed properly.
4.2. making the arteriotomy is without a doubt the most difficult step to ensure the model is performed correctly. Clearing the arteriotomy site as much as possible prior to actually creating the arteriotomy is critical. After making the arteriotomy, inserting forceps through the arteriotomy to ensure they are indeed inside of the artery further verifies that the step was performed correctly.

Results

5. Results: Representative Hematoxylin & Eosin (H&E) and Light Sheet Fluorescence Microscopy Imaging

5.1. Here representative images **[1]** of H&E-stained arterial cross-sections of healthy **[2]**, injured **[3]**, and treated arteries are shown **[4]**.

5.1.1. LAB MEDIA: Figure 2A
5.1.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize Figure 2A and magnified image*
5.1.3. LAB MEDIA: Figure 2A *Video Editor: please emphasize Figure 2B and magnified image*
5.1.4. LAB MEDIA: Figure 2A *Video Editor: please emphasize Figure 2C*

5.2. Using ImageJ, the perimeter of the neointima **[1]**, as well as the internal **[2]** and external elastic lamina can be traced to quantify their respective areas **[3]**.

5.2.1. LAB MEDIA: Figure 2E *Video Editor: please emphasize black dotted line*
5.2.2. LAB MEDIA: Figure 2E *Video Editor: please emphasize yellow dotted line*
5.2.3. LAB MEDIA: Figure 2E *Video Editor: please emphasize blue dotted line*

5.3. Light sheet fluorescence microscopy can also be used to visualize the entire region of injury along the length of the artery **[1]**.

5.3.1. LAB MEDIA: Figures 4A and 4B *Video Editor: please add/emphasize arrowheads in Figure 4B image*

5.4. The images can then be rendered using software for quantifying the intima-media ratio **[1]**.

5.4.1. LAB MEDIA: Figures 4A and 4B *Video Editor: please add Figure 4C*

Conclusion

6. Conclusion Interview Statements

6.1. **Nick Buglak**: Be sure to place the internal carotid clamp so as not to kink the common carotid and to completely clear the arteriotomy site before inserting the microdissection scissors **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *(3.4., 4.2, 4.5.2, 4.5.3)*

6.2. **Nick Buglak**: Many therapeutic approaches can be used at the end of the surgery to attempt to mitigate the pathological response that occurs inside the carotid artery **[1]**.

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