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Title: Implantation of Human-Sized Coronary Stents into Rat Abdominal Aorta Using a Trans-Femoral Access

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **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)? **N**

Introduction

1. Introductory Interview Statements

Videographer NOTE: we forgot to record a separat headshot for Roberta during the interview and later on she had very strong mask-marks so we went through the interview-footage and selected the shot #7 with TC 00:06:02.21 as a suitable headshot

REQUIRED:

1.1. **Elisa A. Liehn**: This protocol uses an animal model to establish an accessible method for characterizing the effects of human coronary stents on vascular pathology **[1]**.

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

REQUIRED:

1.2. **Elisa A. Liehn**: The main advantages of our model are that is minimally invasive, highly reproducible, and easily performed, even by researchers with little operative experience **[1]**.

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

Introduction of Demonstrator on Camera

1.3. **Elisa A. Liehn**: Demonstrating the procedure will be Roberta Florescu, a physician from our laboratory **[1][2]**.

1.3.1. INTERVIEW: Author saying the above NOTE: Filmed in 2 locations to choose from

1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Ethics Title Card

1.4. Procedures involving animal subjects have been performed in accordance with the German animal welfare law (TSchG) and Directive 2010/63/EU pertaining to the

protection of animals used for scientific purposes and were approved by the Governmental Animal Care and Use Committee.

Protocol

2. Surgery Preparation

Videographer NOTE: everything filmed with animal #2 should be used in priority (animal #1 died in the middle of the procedure. We still filmed further, just as a backup, but the arteries aren't pulsating for example)

2.1. After confirming a lack of response to pedal reflex in an anesthetized adult rat **[1-TXT]**, place the rat on a heating pad in the supine position with the right hind limb fully extended and in line with the spine **[2]** and fix the upper and lower limbs with medical tape **[3]**.

2.1.1. WIDE: Talent pinching toe *Videographer: More Talent than rat in shot* **TEXT: Anesthesia: ketamine 100 mg/kg + xylazine 8 mg/kg -> 1.5% isoflurane**

2.1.2. Right hind limb being fully extended/aligned with spine

2.1.3. Limb(s) being taped

2.2. Apply ointment to the animal's eyes **[1]** and shave the fur from the groin and lower abdomen **[2]**.

2.2.1. ECU: Ointment being applied

2.2.2. Fur being shaved

2.3. Then sterilize the exposed skin with a povidone-iodine solution **[1]**.

2.3.1. Skin being wiped, with povidone-iodine container visible in frame

3. Stent Implantation

3.1. For implantation of the stent, make a 0.5-1-centimeter medial incision in the right groin to open the skin and underlying fascia **[1]** and bluntly dissect and probe the tissue until the pulsating left femoral artery is located **[2]**.

3.1.1. WIDE: Talent making incision *Videographer: More Talent than rat in shot*

3.1.2. Tissue being dissected/artery being located *Videographer: Important step*

3.2. Using very fine forceps, gently remove the connective tissue surrounding the artery **[1-TXT]** and carefully insert the top of the forceps under the vessel **[2]**.

3.2.1. Tissue being removed **TEXT: Caution: Do not damage femoral nerve or vein**

3.2.2. Forceps being inserted

3.3. Thread pieces of 4-0 silk suture under the distal and proximal parts of the artery to form slings **[1]** and clamp the ends of each of the two thread slings between the branches of a surgical clamp **[2]**.

3.3.1. Sutures being placed

3.3.2. Slings being clamped

3.4. Use the surgical clamps to control the artery **[1]** and gently stretch and lift the artery to temporarily interrupt the blood flow **[2]**.

3.4.1. Artery being controlled

3.4.2. Artery being lifted

3.5. Using sharp microscissors, quickly perform an arteriotomy in the middle of the femoral artery **[1]** and introduce a guidewire into the arteriotomy **[2]**.

3.5.1. Arteriotomy being performed *Videographer: Important step*

3.5.2. Guidewire being inserted *Videographer: Important step*

3.6. When the proximal thread sling is reached, move the surgical clamp to release the tension of the thread **[1]** and advance the guidewire toward the abdominal aorta **[2]**.

3.6.1. Clamp being moved

3.6.2. Guidewire being advanced

3.7. Introduce a 2.25- x 8-millimeter crimped and balloon-mounted coronary stent over the guide wire into the femoral artery **[1]**.

3.7.1. Stent being introduced *Videographer: Important step*

3.8. Advance the stent to the abdominal aorta **[1]** until it is just above the aortic bifurcation but below the renal arteries **[2]**.

3.8.1. Stent being advanced *Videographer: Difficult step*

3.8.2. Shot of stent in place *Videographer: Difficult step*

3.9. When the stent is in place, use an inflation syringe system to inflate the balloon catheter to its nominal pressure **[1]**.

3.9.1. Stent being inflated *Videographer: Important step*

3.10. After 15 seconds, deflate the balloon catheter **[1]** and maintain negative pressure according to the manufacturer's recommendations for the stent in use **[2]**.

3.10.1. Catheter being deflated *Videographer: Important step*

3.10.2. Negative pressure being maintained *Videographer: Important step*

3.11. Next, slowly withdraw the deflated catheter while leaving the stent in place **[1]**.

3.11.1. Catheter being withdrawn

3.12. Just before removing the guide wire, use the surgical clamp to create tension on the thread loop above the incision to interrupt the blood flow **[1]** and remove the guide wire **[2]**.

3.12.1. Tension being created

3.12.2. Catheter being removed

3.13. Directly ligate the vessel proximally **[1]** and tie the proximal and distal thread loops to ligate the femoral artery **[2]**.

3.13.1. Vessel being ligated

3.13.2. Thread loops being tied

3.14. Then confirm adequate hemostasis of the arteriotomy **[1-TXT]** and use 6-0 non-resorbable sutures to close the muscle overlying the artery and the skin incision **[2-TXT]**.

3.14.1. Hemostasis being confirmed **TEXT: Collateral arteries ensure limb perfusion**

3.14.2. Muscle being sutured **TEXT: See text for full post-operative care details**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.1., 3.5., 3.7., 3.9., 3.10.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.8., reserve rats will be available.

Results

4. Results: Representative Stent Implantation Outcome Evaluation

4.1. In this representative analysis **[1]**, human-sized coronary stents were successfully deployed with no sign of malapposition or vessel injury **[2]**.

4.1.1. LAB MEDIA: Table 1
4.1.2. LAB MEDIA: Table 1 *Video Editor: please emphasize first 4 rows of table*

4.2. Homozygous apolipoprotein E knockout rats **[1]** developed markedly elevated neointimal hyperplasia and in-stent restenosis **[2]** compared to wildtype rats **[3]**.

4.2.1. LAB MEDIA: Figure 2
4.2.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize NI region of third image in Figure 2B*
4.2.3. LAB MEDIA: Figure 2 *Video Editor: please emphasize NI region of third image in Figure 2A*

4.3. Although the apolipoprotein E knockout background renders animals more susceptible to atherosclerosis, no antecedent atherosclerotic plaques were observed in these mice **[1]**.

4.3.1. LAB MEDIA: Figure 2 *Video Editor: please emphasize Figure 2B images*

Conclusion

5. Conclusion Interview Statements

5.1. **Roberta Florescu**: Selecting the proper stent size for each animal and using rats weighing over 500 grams for the human coronary stent implantation are critical to the success of the procedure **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.8.) NOTE: Filmed in 2 locations to choose from

5.2. **Roberta Florescu**: Following this procedure, other clinical and pathology diagnostic tools can be used, such as optical coherence tomography or histological analysis **[1]**.

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