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Title: An Obstructive Chronic Pancreatitis Model Established Through Electrocoagulation

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **YES**

2.3.1, 2.3.2, 2.3.3, 2.4.1, 2.4.2, 2.5.1, 2.6.1, 2.6.2, 2.6.3, 2.7.2, 2.8.1, 2.8.2, 2.9.1, 2.9.2, 2.10.1, 2.10.2, 2.11.1, 2.11.2

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

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **10/13/2024**

When you are ready to submit your video files, please contact our China Location Producer, Yuan Yue.

Current Protocol Length
Number of Steps: 20
Number of Shots: 40

Introduction

1.1. **Haojie Huang:** There are many methods for building animal models of chronic pancreatitis, including intraperitoneal injection of caerulein and ligation of pancreatic duct, which have their limitations **[1]**.

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

What research gap are you addressing with your protocol?

1.2. **Haojie Huang:** Our team recently developed a new electrocoagulation model of chronic pancreatitis in mice, which has the advantage that it does not require long injection drugs or difficult methods to master, and it can easily establish the chronic pancreatitis model **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.10.1 and 2.10.2*

What new scientific questions have your results paved the way for?

1.3. **Zhenghui Yang:** The use of electrocoagulation to induce chronic pancreatitis in mice provides a more convenient and experimental model, offering a promising avenue for future research in this field **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.5.1 and LAB MEDIA: Figure 4*

Ethics Title Card

This research has been approved by the Naval Medical University's Committee

Protocol

2. Induction of Obstructive Chronic Pancreatitis in Mice via Electrocoagulation

Demonstrator: Zhenghui Yang

Protocol

2.1. To begin, place the anesthetized mouse on a heating pad to maintain body temperature **[1-TXT]**. Using a trimmer, shave the hair between the chest and lower abdomen **[2]**. Spray 75% alcohol evenly on the shaved area to prepare for the operation **[3]**.

2.1.1. WIDE: Establishing shot of talent placing the anesthetized mouse on the heating pad. **TXT: Anesthesia: 200 µL of Tribromoethanol/10 g of body weight**

2.1.2. Talent shaving the hair using a trimmer between the chest and lower abdomen.

2.1.3. Talent spraying 75% alcohol on the shaved area.

2.2. Fix the mouse to the surgical plate using surgical tape **[1]**.

2.2.1. Talent securing the mouse on the surgical plate using surgical tape.

2.3. Next, use scissors to make a 3-millimeter-long incision in the central abdomen **[1]**, followed by a 1.5 millimeter incision between the upper abdomen and the xiphoid process **[2]**. Use an abdominal expander to locate the area of the pancreas for electrocoagulation **[3]**.

2.3.1. Making a 3-millimeter-long incision in the central abdomen.

2.3.2. Making a 1.5-millimeter incision between the upper abdomen and the xiphoid process.

2.3.3. Locating the pancreas using an abdominal expander.

2.4. Using a sterile cotton swab, identify the duodenum from the rear of the left upper abdomen **[1]** and turn it to visualize the bile duct connecting the liver **[2]**.

2.4.1. Identifying the duodenum with a sterile cotton swab.

2.4.2. Turning the duodenum to reveal the bile duct.

Tracing Surgical Site

2.5. Temporarily occlude the proximal common bile duct using a microvascular clip to prevent retrograde infusion into the liver **[1]**.

2.5.1. Placing a microvascular clip to occlude the proximal common bile duct.

2.6. Connect the 0.25 by 0.35-millimeter polyethylene pipe to a 0.25-millimeter needle **[1]** and pierce the ampulla surrounding area, aiming for the large duodenal papilla **[2]**. Insert the tube into the papilla and pass it halfway into the bile duct, then stop the entry **[3]**.

2.6.1. Connecting the polyethylene pipe to a needle.

2.6.2. Inserting the needle into the ampulla area, targeting the duodenal papilla.

2.6.3. Inserting the tube halfway into the bile duct and stopping the entry.

2.7. Start the infusion pump **[1]** and infuse 0.2% methylene blue solution until the pancreatic duct becomes blue **[2-TXT]**.

2.7.1. Talent starting the infusion pump.

2.7.2. Shot of pancreatic duct being infused with methylene blue. **TXT: Infuse at 50 µL/10 g for 2 min at 50 µL/min**

2.8. After infusion, pull the polyethylene tube out **[1]** and remove the microvascular clip **[2]**.

2.8.1. Removing the polyethylene tube from the bile duct.

2.8.2. Removing the microvascular clip.

Electrocoagulation

2.9. Use sterile cotton swabs to fix the pancreas **[1]** and direct the electrocoagulation knife to the blue-colored area **[2-TXT]**.

2.9.1. Stabilizing the pancreas with cotton swabs.

2.9.2. Positioning the electrocoagulation knife at the blue-stained portion of the pancreas. **TXT: Electrocoagulation site: middle lower 2/3 between the common bile duct and the superior pancreaticoduodenal artery**

2.10. After setting the operating parameters of electrocoagulation, use pure copper as the electrode material and apply treatment to the pancreas for 2 to 3 seconds while avoiding blood vessels **[1-TXT]**. Continue electrocoagulation until the methylene blue-stained region changes color to yellow or brown **[2]**.

2.10.1. Applying electrocoagulation to the pancreas, carefully avoiding nearby blood vessels. **TXT: Electrocoagulation parameters: Voltage 220 V and Temperature 300 °C**

2.10.2. The blue color of the methylene blue-stained area changes to yellow or brown

2.11. When the blue staining solution is no longer visible in the pancreas, stop the electrocoagulation **[1]**. Close the abdomen using 4-0 *(four-oh)* nonabsorbent monofilament polypropylene sutures **[2-TXT]**.

2.11.1. Observing the area to ensure the blue staining is fully gone and stopping the electrocoagulation.

2.11.2. Suturing the abdomen with 4-0 nonabsorbent monofilament polypropylene sutures. **TXT: Do not perform electrocoagulation on the sham group mice**

2.12. After the surgery, place the mouse in a polyethylene box lined with bedding material **[1-TXT]**.

2.12.1. Talent placing the mouse in boxes with bedding, food, and water provided. **TXT: Provide food and water *ad libitum***

3. Serum Biochemical Analysis and Histopathological Examination of Pancreatic Tissue Post-Electrocoagulation in Mice

Demonstrator: Zhenghui Yang

Protocol

3.1. After electrocoagulation of the pancreatic duct, anesthetize the mouse and place it on an operating platform **[1-TXT]**.

3.1.1. Talent placing the electrocoagulated anesthetized mouse on an operating platform. **TXT: Anesthesia: 200 µL of Tribromoethanol/10 g of body weight**

3.2. To collect blood through the orbital plexus, gently hold the skin on the back to create a slight protrusion of the eyeball **[1]**. Then, place the end of a capillary tube in the corner of the eye and gently insert it under the eyeball at an angle of 30 to 45 degrees **[2]**. Rotate the capillary until blood starts to flow **[3]**.

3.2.1. Talent gently holding the mouse's back skin to slightly protrude the eyeball.

3.2.2. Talent positioning the capillary in the corner of the eye and inserting it under the eyeball at the correct angle.

3.2.3. Shot of the capillary being rotated to initiate blood flow.

3.3. After blood collection, close the eyelids by applying gentle pressure with gauze **[1]**.

3.3.1. Talent applying gauze to close the eyelids.

3.4. Centrifuge the blood at 1,200 *g* for 15 minutes **[1]** and collect the supernatant in a new tube **[2]**.

3.4.1. Talent placing the tube in a centrifuge.

3.4.2. Talent removing the serum supernatant and transferring it to a new tube.

3.5. Measure amylase, bilirubin, and hyaluronic acid using commercially available kits, following the manufacturer's recommendations **[1-TXT]**.

3.5.1. Talent preparing samples for amylase, bilirubin, and hyaluronic acid estimation with corresponding kits placed on a working platform visible in the frame. **TXT: Perform analysis on 7th, 14th, 21st, and 28th days of electrocoagulation**

3.6. Next, place the euthanized mouse on the operating table set at 4 degrees Celsius **[1-TXT]**. Using scissors and forceps, make a “V” incision in the abdominal wall to expose the abdominal cavity **[2]**. Remove the pancreas and divide it into three parts for staining **[3]**.

3.6.1. Talent placing the euthanized mouse on the cold operating table. **TXT: Euthanize the mouse by cervical dislocation**

3.6.2. Talent making a "V" incision in the abdominal wall with scissors and forceps.

3.6.3. Talent removing the pancreas and dividing it into three parts.

3.7. Fix the pancreas in 4% polyformaldehyde solution **[1]**. Then, embed the fixed pancreas into paraffin for sectioning **[2]**.

3.7.1. Talent placing the pancreatic tissue into 4% polyformaldehyde solution.

3.7.2. Talent embedding the fixed pancreas into paraffin blocks.

3.8. Cut the paraffin blocks into 0.5 millimeter-thick sections **[1]** and stain them with hematoxylin and eosin, as well as with Masson’s stain for immunohistochemical analysis **[2]**. Observe the stained pancreatic tissue under a light microscope **[3]**.

3.8.1. Shot of paraffin blocks being cut into 0.5 mm sections.

3.8.2. Talent staining the paraffin blocks with H & E and Masson’s stain.

3.8.3. Talent at the microscope, observing stained pancreatic sections.

Results

4. Results

4.1. Hematoxylin-eosin staining showed scattered pancreatic acinar cells and evidence of chronic pancreatitis in the electrocoagulation group on day 14 **[1]**. On day 28, signs of tissue recovery and reversal of pathology in the pancreatic acinar cells were observed **[2]**.

4.1.1. LAB MEDIA: Figure 2 *Video Editor: Highlight D14 image*

4.1.2. LAB MEDIA: Figure 2 *Video Editor: Highlight D28 image*

4.2. Masson staining revealed significantly more fibrosis in the electrocoagulation group **[1]** compared to the sham group **[2]**.

4.2.1. LAB MEDIA: Figure 3 *Video Editor: Highlight the image from the electrocoagulation group of Masson row*

4.2.2. LAB MEDIA: Figure 3 *Video Editor: Highlight the image from Sham group of Masson row*

4.3. Immunohistochemical staining showed elevated alpha-1 type 1 collagen and alpha-smooth muscle actin levels in the electrocoagulation group, indicating a higher degree of chronic pancreatitis **[1]**.

4.3.1. LAB MEDIA: Figure 3 *Video Editor: Highlight the images from the electrocoagulation group of COL-1 and SMA rows*

4.4. Hyaluronic acid levels increased in the electrocoagulation group, peaking around day 21 **[1]**, while remaining stable in the sham group **[2]**. The bilirubin levels rose significantly by day 14 in the electrocoagulation group, showing a peak around day 21 **[3]**, whereas the sham group showed no changes **[4]**.

4.4.1. LAB MEDIA: Figure 4A *Video editor: Highlight the 3rd point from the electrocoagulation (square markers) line*

4.4.2. LAB MEDIA: Figure 4A *Video editor: Highlight the sham group (circle markers) line*

4.4.3. LAB MEDIA: Figure 4B *Video editor: Highlight the 2nd and 3rd point from the electrocoagulation (square markers) line*

4.4.4. LAB MEDIA Figure 4B *Video editor: Highlight the sham group (circle markers) line*

4.5. Amylase levels in the electrocoagulation group spiked on day 14, followed by a decline by day 28 **[1]**, with the sham group showing little variation **[2]**.

4.5.1. LAB MEDIA: Figure 4C *Video editor: Highlight the 2nd,3rd, and 4th point from the electrocoagulation (square markers) line*

4.5.2. LAB MEDIA Figure 4C *Video editor: Highlight the sham group (circle markers) line*