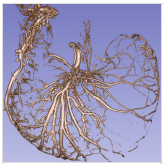

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Micro-CT imaging of rat stomach vasculature

Deborah Jaffey[1], Logan Chesney[1], Terry Powley[1]

[1]Purdue University

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SPARC
Tech. support email: info@neuinfo.org

Deborah Jaffey

ABSTRACT

This protocol describes how to prepare a rat stomach for micro-CT imaging of the vasculature. A lead-containing silicone-based curable material is injected into the heart at perfusion and flows through the vasculature and cures in place. Subsequent microCT imaging then provides a 3D description of the stomach vasculature.

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KEYWORDS

micro-CT, vasculature, Microfil, tracing

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MATERIALS TEXT

MATERIALS

Ketamine **Patterson**

Veterinary Catalog #07-803-6637

Xylazine **Akorn Animal**

Health Catalog #NDC: 59399-110-20 Step 3

Heparin **Henry Schein Animal**

Health Catalog #049130

Microfil MV-122 **Flow Tech**

Inc Catalog #MV-122 Step 5

Microfil MV-Diluent **Flow Tech**

Inc Catalog #MV-Diluent

ABSTRACT

This protocol describes how to prepare a rat stomach for micro-CT imaging of the vasculature. A lead-containing silicone-based curable material is injected into the heart at perfusion and flows through the vasculature and cures in place. Subsequent microCT imaging then provides a 3D description of the stomach vasculature.

Set-up

1 Feed the rat per the details given in protocol "Micro-CT imaging of iodine-stained rat stomach".

2 Heat one liter of saline (either sterile or prepared with 1L ultrapure water + 8.7 g NaCl) to between 37 °C to 42 °C.

3 Weigh the rat to determine the appropriate dose of ketamine + xylazine cocktail. Prepared cocktail consists of 10 mL of 100 mg/mL ketamine and 1 mL of 100 mg/mL xylazine for a total volume of 11 mL. Accepted dose is 2-3 mL/kg body weight, depending on the fed/fasting state of the animal.

Ketamine **Patterson**

Veterinary Catalog #07-803-6637

Xylazine **Akorn Animal**

Health Catalog #NDC: 59399-110-20

4 Prepare two syringes: one with a 23G needle to be used for intraperitoneal injection of ketamine + xylazine, and one with a 26G needle for intracardiac injection of heparin. 500 USP units of heparin are used for each rat.

5 Mix the Microfil compound and diluent in an appropriate (at least 40 mL) container, preferably sealable and disposable (avoid using glass, as it is difficult to clean). The specific Microfil compound used is MV-122, due to its high concentration of radiopaque lead chromate.

Microfil MV-122 **Flow Tech**

Inc Catalog #MV-122

The preferred ratio of compound to diluent was determined (through trial and error) to be 3 mL of compound to 5 mL of diluent. To ensure thorough vascular fill, you must use a total volume (while maintaining the 3:5 ratio) that exceeds the estimated volume of blood in the rat. Gently shake or stir the solution to mix, but take care not to mix ao vigorously that bubbles are produced. **DO NOT ADD THE CURING AGENT AT THIS POINT.** If the ratio of compound to diluent is too high then penetration of the vasculature is poor; if the ratio of compound to diluent is too low then the material

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will tend to leak out of the stomach.

6 Prepare the peristalsis pump (Masterflex series) with appropriate input and output tubing to accommodate an 18G blunted needle attached to a 1 mL syringe. Set the pump to 25 mL/min or an equivalent flow rate.

Perfusion

7 Administer the appropriate dose of ketamine/xylazine intraperitoneally.

8 Expose the thoracic cavity of the rat. Take care not to expose the abdomen so that it can be kept at or as close to 37 °C as possible for the rest of the procedure.

9 Turn the pump on to begin the flow of saline through an 18G blunted needle. Inject the heparin into the left ventricle of the rat. Immediately after, thread the 18G needle carefully through the left ventricle and into the aorta without piercing the interventricular septum. If the septum is pierced, the procedure may still continue, but the vascular fill may not be optimal due to partial redirection to the pulmonary circuit. Cut the right atrium and partially sever the inferior vena cava (IVC). Keep in mind that the IVC will need to be clamped later.

10 Carefully insert an infant feeding tube into the stomach through the esophagus and leave in place through the perfusion to ensure that the lower esophageal sphincter remains open. For a 300 g male Sprague-Dawley rat, the distance from mouth opening to the LES is about 125 mm.

11 Make sure throughout the procedure to administer warm saline in and around the abdominal cavity of the rat at least once per minute to minimize cooling. Experience has shown that if the stomach cools too much little penetration of the forestomach vasculature occurs

12 After perfusion of around 200 mL saline, add the curing agent to the Microfil solution (assuming 12 mL of MV-122 compound and 20 mL of diluent), and immediately mix gently (avoid bubbles) but thoroughly for 1 to 2 minutes to achieve homogeneity. Start a 20-minute timer upon the addition of the curing agent, by which point significant polymerization will have occurred.

13 After 250 mL of saline has been perfused, stop the pump and move the input end of the tubing into the Microfil solution. Secure the tubing into the solution container. Lower the pump rate down to 2 mL/min, and start the pump. It will likely take a couple of minutes to travel through the tubing before it reaches the rat, but filling of the aorta and coronary arteries should be visible almost immediately upon reaching the needle.While making sure to keep administering saline to the abdominal cavity, keep the pump running until the end of the 20 minute timer or until the Microfil solution is depleted, whichever comes first. Stop the pump, clamp both the perfusion line and the IVC (distal to the cut), and wait at least an additional 70 minutes before dissection to allow the Microfil compound to adequately cure.

Dissection

14 Open the abdomen and dissect out the stomach, leaving about a centimeter each of esophagus and duodenum. Make sure not to remove the gastroepiploic artery along the greater curvature of the corpus/antrum, as it can serve as a useful axis for orientation during scanning.

15 Immediately place the stomach in a solution of 4% paraformaldehyde in 0.01M PBS. Allow at least 24 hours of fixation time before scanning.

16 After the tissue is fixed the stomach can be flushed. This is not required but can give better results if subsequent iodine staining is planned. To flush the stomach, hold the stomach under the surface of a container of saline and gently massage the stomach to push food out through the pylorus (which should be open due to the presence of the feeding

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tube during perfusion). If the contents just consist of Dietgel as intended, this should not be too difficult. Allow the stomach to refill with saline and repeat to remove the contents of the stomach.

Scanning Preparation

17 Prior to scanning, make a thick solution of gelatin in which the stomach can be suspended while scanning. The preferred concentration is 4% to 5% of type B gelatin in ultrapure water (40 g/L to 50 g/L). This concentration of gelatin is rigid enough to hold the stomach into place during scanning, but soft enough to allow the stomach to be manipulated into the appropriate orientation without the exertion of excess force on the tissue. The advantage of supporting the stomach in gelatin is that the stomach does not then rest on a rigid surface to which it will conform, hence distorting the shape of the stomach.

18 The gelatin solution should be made a day ahead of scanning to allow it to thoroughly cool. If you experience blurred scans when using gelatin, the gelatin can be refrigerated before scanning and between scans to avoid softening. An appropriate vessel for holding the stomach and gelatin is a 4-ounce, circular, transparent, polypropylene (PP) container with a sealable top.

19 An hour or so before scanning, the stomach needs to be filled full with saline and tied off with thread to ensure consistent stomach dimensions.

19.1 Tie off the esophagus with a bow of cotton thread, leaving preferably at least 1 cm of esophagus between the LES and the bow.

19.2 Attach a 5 Fr feeding tube to a 10 or 20 mL syringe filled with PBS, and thread the tube through the duodenum, through the pylorus, and into the stomach, being careful not to damage or distort the stomach.

19.3 Hold the duodenum (with tubing) skyward allowing liquids and gas to escape the stomach, and slowly (so as to not force the tubing off the needle) plunge the syringe to inflate the stomach. Continue to do this until bubbles no longer emerge to the surface. Slowly remove the tube while still plunging until the distal end of the tube is at the junction of your index finger and thumb, at the open end of the duodenum—still ensure that no bubbles are present at this point, or you will have to reinsert the tube and repeat the saline injection.

19.4 Using a small pair of hemostats, clamp the duodenum directly below where you are holding it, making sure not to clamp the end of the tube.

19.5 Tie a bow about 2-3 mm proximal to the clamp and then remove the clamp. The stomach should now be fully inflated with saline. The purpose of this step is to ensure that the stomach has repeatable dimensions and is not partially collapsed or and doesn't contain air.

Scanning

20 The instrument used for microCT scanning is

Quantum GX2
MicroCT imaging system
Perkin Elmer **CLS149276**

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21 Warm up the scanner. If possible, set the warmup voltage to 55 kV and current to 144 µA.

22 Under a ventilated hood, remove the stomach from 4% PF solution and rinse with 10X PBS, saline, or a similar buffer. Partially fill a 4 oz. PP container ¾ with gelatin. Using a smooth tool and/or gloved fingers, place the stomach into the container and manipulate it into the appropriate scanning orientation. Completely submerge the stomach so that it does not dry out. The standard orientation is ventral side up, esophagus facing the back of the scanner, and pylorus facing left. Make sure that the stomach is lying as flat as possible in the horizontal plane.

23 Open the door, place the stomach onto the bore between the beam and detector, and close the door. The Quantum GX2 will allow for fine adjustments to position (forward/backward, left/right, up/down) that can be accessed in Live Mode. For the highest pixel/voxel resolution, it is necessary to reduce the field of view (FOV) to as small as possible while still capturing the entire stomach. Using Live Mode allows the user to preview the scan by rotating the beam/detector position around the stomach prior to actual scanning, so it is useful in determining the smallest possible field of view. With the exception of particularly large stomachs, most rat stomachs can fit within a 45 mm field of view (which yields 90 µm voxel resolution), but some may require a 60 mm FOV, or in extreme cases, a 72 mm FOV.

24 Set the scanning parameters to High Resolution, with at least a 14 minute scan time.

25 Use Live Mode to view the orientation of the stomach, and use buttons located on the front of the scanner to alter translational position. It may also be necessary to remove the stomach and carefully manipulate it to achieve the correct position and alignment. After this is done, start the scan.

26 When scan is complete, transfer stomach to a PP container filled with saline/PBS and rinse off all the gelatin.

27 Once back in the lab, remove tie-off threads and return all stomachs to 4% PF and restore at 4 °C.

28 Once it has been confirmed that a good microCT scan of the vasculature has been obtained, the stomach as a whole can be imaged by soaking in an I_2 / KI solution (see this protocol "Micro-CT imaging of iodine-stained rat stomach") starting from step 18 of that protocol. If only light iodine staining of the complete stomach is wanted so that the vasculature remains visible, then reduce the soak time and/or soak the stomach in PBS following the iodine soak to partially leach out the iodine.

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