Dec 16, 2020

Micro-CT imaging of iodine-stained rat stomach

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1 Works for me dx.doi.org/10.17504/protocols.io.95ih84e

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ABSTRACT

This protocol describes how to prepare a rat stomach for micro-CT imaging to enable a 3D description of the organ. Iodine is used to stain the tissue post-perfusion. By varying the meal size and the delay time between feeding and perfusion, it is possible to determine how stomach volume changes, both overall and for stomach compartments. The DICOM file output can be used to generate a 3D scaffold of the stomach for modeling purposes.

DOI

dx.doi.org/10.17504/protocols.io.95ih84e

PROTOCOL CITATION

Deborah Jaffey, Terry Powley, Logan Chesney 2020. Micro-CT imaging of iodine-stained rat stomach. **protocols.io** https://dx.doi.org/10.17504/protocols.io.95ih84e

KEYWORDS

Micro-CT, rat, stomach, iodine

CREATED

Dec 04, 2019

LAST MODIFIED

Dec 16, 2020

PROTOCOL INTEGER ID

30602

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Animals

1

Sprague-Dawley

Rat **Envigo Catalog #RRID:RGD_70508**

(male, 228-330 g) were included in the study according to procedures approved by the Purdue Animal Care and Use Committee. Rats were housed individually in ventilated cages with elevated stainless steel wire floors during the controlled feeding part of the experiment to prevent the animals from accessing their feces and adding to stomach contents. The environment was maintained on a 12:12 h light-dark cycle (lights on at 6 am and lights off at 6 pm).

Training

2 Select 4 rats within the preferred weight distribution. Each animal is trained to consume a quantity of palatable Dietgel **ClearH20 Catalog #72-06-5022**

In the first 2 days, the animal is supplied with both regular rat chow and a half container of Dietgel (the Dietgel is put in a dish in the cage at 11AM) to accustom itself to the Dietgel.

In the following days, the animal is fasted for 12 hours (7PM to 7AM) and then is fed with the Dietgel only at 7AM. The animal is given 30 minutes to consume Dietgel; then regular meals are supplied to the animal afterwards regardless of whether it finishes the Dietgel or not.

After the diet training (~2 to 3 repetitions), each animal is able to naturally consume the Dietgel following overnight food restriction.

Feeding

3 On the day before the planned experiment, fast the rats for 12 hours overnight.

4 Weigh bowls on scale to obtain empty bowl weights.

5 Place half the contents of a container of Dietgel **ClearH20 Catalog #72-06-5022** in bowls and reweigh for filled bowl weights.

6 Place the filled bowls in the cages with the fasted rats and allow them to consume their fill of Dietgel. After 30 minutes or when they stop eating (whichever comes first), remove and reweigh the food bowls to determine the amount of Dietgel eaten by each rat, and record the time as t0. Do not disturb the animals while they are eating.

7 Select the two or three rats which have consumed the most Dietgel and assign the rats randomly to perfusion times from t0 and higher (leaving intervals of at least one hour between perfusions), and return the other rats to the general population.

Perfusion

8 Weigh rats to determine drug dose

9 IP inject a mixture of

Ketamine **Patterson Veterinary Catalog #07-803-6637**

and

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Xylazine **Akorn Animal Health Catalog #NDC: 59399-110-20**

(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. Use **2-3** mL/kg - too heavy a dose may result in premature cardiac arrest, leading to a poor perfusion).

10 Open thoracic cavity thoroughly to where heart, inferior vena cava (IVC), aorta can be easily seen and accessed, and administer 0.5 mL

Heparin **Henry Schein Animal Health Catalog #049130**

with a 26G needle into the right or left ventricle.

11 Prepare the peristalsis pump (Masterflex series) with appropriate input and output tubing. Start pump with 250 mL 40 °C saline at 25 mL/min, then thread a blunt 18G perfusion needle (w/tubing, attached to pump) through the apex of the left ventricle and into the aorta. Once secured in place, cut the right atrium and IVC to drain blood.

12 Try not to poke through to right atrium/ventricle on the way there. Clamp needle with hemostat if desired to disable backflow out of left ventricle. Do not go too far up the aorta or else flow may be reduced to the aortic arch and predominantly redirected to the brachiocephalic artery (check if the liver is clearing at a reasonable rate; if liver is not a tan color after ~45 seconds, then adjust needle position).

13 Thread one feeding tube (5 Fr or 8 Fr) through esophagus, making sure not to go too far to prevent stretching/damaging the stomach at greater curvature (feel slight resistance at LES) and keep tube in place during entire perfusion.

14 Cut small (feeding tube-sized) hole in duodenum 2-3 cm from pylorus. Thread feeding tube (5 Fr) through hole and into pylorus and keep tube in place during entire perfusion. Cover exposed areas with Kimwipes and spray frequently with saline to keep moist.

15 After 250 mL saline, switch to 500 mL of 4% paraformaldehyde in 0.01M PBS (also at 25 mL/min).

16 When perfusion is complete, remove all needles, tubes, clamps attached to rat and dissect out stomach, leaving generous lengths of both esophagus and duodenum.

17 Place the stomach in a glass jar with ~100 mL of 4% paraformaldehyde and store at 4 °C for about 24 hours.

Staining

18 Prepare a stock solution of I_2 / KI by adding 10 g of I_2 and 20 g of KI to 1 L of ultrapure water and stir for at least an hour until both fully dissolved.

19 Prepare 0.1% I_2 + 0.2% KI solution by mixing 100 mL of stock solution and 900 mL of ultrapure water.

20 Once the stomach has been fixed for about 24 hours it can be flushed and stained. Flushing of stomach contents is

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not required but does give better results at micro-CT. 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 tube during perfusion). If 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.

21 Put each stomach into a glass jar containing the prepared 0.1% I_2 / 0.2% KI solution, close the lid, and place onto a shaker table. To ensure that staining is as even as possible, gently squeeze out any air if the stomach is floating, flip stomachs every couple of hours or so, and replace solution as it becomes less tinted. Leave the stomach to absorb iodine for 24 hours.

Scanning Preparation

22 In order to hold the stomach in a stable yet floating orientation during scanning prepare ahead of time a thick solution of gelatin. 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 exertion of excess force on the tissue and with enough flow so that there will be no air pockets around the stomach.

23 An hour or so before scanning, remove the stomach from the iodine solution and rinse in PBS. In the following steps keep the stomach submerged in PBS as far as possible.

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

23.2 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.

23.3 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.

23.4 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.

23.5 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.

23.6 Return the stomach to iodine solution.

Scanning

24 The instrument used for microCT scanning is

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Quantum GX2
MicroCT imaging system
Perkin Elmer CLS149276

25 Warm up the scanner. If possible, set the warmup voltage to 90 kV and current to 88 µA.

26 Under a ventilated hood, remove the stomach from iodine 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.

27 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.

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

29 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.

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

31 Once back in the lab, remove tie-off threads and return all stomachs to 4% PF and restore at 4 °C (samples can always be rescanned though they may require restaining with iodine starting at step 18).

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