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Nerve Sample Preparation for MicroCT Scanning

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This protocol describes the preparation and staining process of pig vagus nerves for microCT scanning.

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- Sutures or cotton thread
- 10% neutral buffered formalin
- 50ml tubes
- Lugol's iodine solution (total iodine 1%; 0.74% KI, 0.37% I) (Sigma Aldrich L6141)
- Superglue
- Cling film
- Sponge
- Cylinder or 3D-printed mount
- Forceps
- Scalpel and blade

Vagus nerve dissection and fixation

1 Following euthanasia, dissect the left vagi of the pigs carefully from cervical region (with both the stimulating and EIT electrodes attached) down to the branching regions of cardiac, recurrent laryngeal and pulmonary branches, with all the branches left attached to the main trunk of the vagus nerve.

2 Each sample will be approximately 28 cm in length from the upper cervical level (above electrode cuff placement) to beyond the pulmonary branches at the lower thoracic level.

3 Place sutures around the vagus nerve prior to any branching region (i.e., the region where a branch leaves the main vagal trunk) as well as to demarcate the positioning of the VNS cuffs.

4 Place nerves in neutral buffered formalin (10%) to allow for fixation for a minimum of 24 hours.

Nerve preparation

5 After fixation, measure the nerve samples and superglue sutures of 1 cm length to the vagal trunk in 4 cm intervals to allow for co-registration in the tomography images - use a variation in number of suture pieces to easily identify matching ends.

6 Cut into 4 cm lengths at the level of suture placement leaving half of the sutures on the end of each section as a marker for subsequent co-registration.

7 Keep an order of the nerve sections from cranial to caudal - labeling them from first to last, respectively.

8 Note which branches are coming off specific pieces of the nerve to assist with locating them in

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the scans.

Contrast staining

9 Place two to three 4cm sections into a tube of 50 ml Lugol's solution (total iodine 1%; 0.74% KI, 0.37% I) (Sigma Aldrich L6141) for five days (120 hours) prior to scanning to achieve maximum contrast between fascicles and the rest of the nerve tissue.

10 Label each tube with which nerve sections it contains.

11 On day 3 of staining, empty the tube of Lugol's solution and replace with fresh solution.

MicroCT sample preparation

12 On the day of the microCT scan, remove the nerve samples from the tubes and blot dry on paper towel to remove any excess Lugol's solution.

13 Maintain the order of the nerves and decipher the direction of each piece (cranial to caudal) using the variation in number of superglued sutures and branching regions as markers.

14 Place the nerve sections next to each other onto a piece of cling film (10 cm x 5 cm) (Tesco, United Kingdom) in order from cranial to caudal along the length of the nerve, with cranial ends at the top, and sealed with another piece of cling film to retain moisture during the scan as to avoid shrinkage of the nerve tissue.

15 Roll the sealed nerve samples around a cylinder of sponge (0.5 cm D x 4.5 cm) and wrapped in another two layers of cling film to form a tightly wound cylinder with a diameter of ~1.5 cm in order to fit within the field of view at the required resolution - take note of which end contains the cranial ends of each nerve section.

16 Place the wrapped cylinder inside a 3D-printed mount, or cylinder (~2 cm diameter) that fits into the microCT sample holder, filled with sponge around the edges, ensuring a tight fit and seal the ends with tape (Transpore™, 3M, United Kingdom).

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