1.0 PURPOSE:

Perfusion of Microfil® into the blood vessels of a specimen will increase the contrast for imaging modalities, especially micro-computed tomography (MicroCT).

2.0 SCOPE:

2.1 Proceeding methods concern mice, but can be applied to rats with variations to doses and instruments.

2.2 Intermediary procedures unrelated to the perfusion process can be conducted with reference to their respective SOPs.

2.3 Procedure is to be conducted in an animal preparation area in a containment level 2 (CL2) laboratory.

3.0 RESPONSIBILITIES:

3.1 A licensed veterinary technician performs all handling of the animal. The veterinary technician is responsible for supporting safe handling and laboratory practices in compliance with ACVS and UCAC.

3.2 Any deviations from the general procedure or additional intermediary procedures can be made with discretion from the veterinary technician along with joint approval from ACVS.

4.0 DEFINITIONS:

ACVS – Animal Care and Veterinary Services

Microfil – silicone injection compound that becomes solid when mixed with MV-Curing Agent.

Perfusion – pumping a liquid into an organ or tissue (especially by way of blood vessels)

Heparin – a drug given directly into a vein which thins the blood.

IP – Intraperitoneal.

UCAC – The University Council on Animal Care

5.0 REFERENCES:

None.

6.0 MATERIALS AND EQUIPMENT:

6.1 MATERIALS

- Crazy-glue (Cyanoacrylate)

- Heparin
- Isotonic saline (0.9%)

6.2 EQUIPMENT

- 30 gauge needle
- 23 gauge butterfly catheter
- IV bags
- IV stand
- Styrofoam bed (*with* tray, pins).
- Surgical tools (small dissecting scissors, non-tooth forceps)

7.0 PROCEDURES:

Animals are to be handled and perfused one at a time.

7.0.1 PRELIMINARY SETUP

1. Keep mice on counter inside screened cages. A maximum of 4 mice can be kept in each cage.
2. Position an IV stand next to the area where perfusion will occur.
3. Use an empty screened container to hold a mouse during an anaesthetizing process.
4. Lay absorbent cloths on the countertop of the working area.

7.0.2 ANAESTHETIZE THE MOUSE

1. Anaesthetize (overdose) a mouse from a screened cage with an IP injection of Ketamine/xylazine (0.03mL/10g x 2 of body weight).
2. Place mouse in an empty screened container and wait 5-10 minutes for anaesthetic to take effect.
3. Using a 30 gauge needle, inject a 0.075mL bolus of heparin into the tail vein of the anaesthetized mouse.
4. Confirm that the mouse is completely anaesthetized by determining the quality and rate of respiration and heartbeat, colour and condition of mucous membranes, and reaction to reflex stimulation as in the toe pinch test. The mouse respirations will begin to get slower. Once the animal is taking its last breaths you may proceed to perfuse.

7.0.3 PERFUSION

1. Move the overdosed mouse from the screened container onto the countertop.
2. Using a 30 gauge needle, inject a 0.075mL bolus of heparin into the tail vein of the anaesthetized mouse.
3. Lay the mouse on it's back on a Styrofoam bed.
4. Pin the mouse to the Styrofoam bed through the webbings of all four paws.
5. Place the Styrofoam bed inside a tray at a slight tilt to allow excess fluids to run down.
6. Make wide incisions to open the rib cage.
7. Pin back the rib cage and apply crazy-glue wherever a rib cage incision was made to prevent bleeding.

A. SALINE FLUSH

1. Place an IV bag of 0.9% isotonic saline mixed with 10% heparin on the IV stand 160cm above the surface of the animal, this will set the pressure to 120mmHg.
2. Flush the IV line with the butterfly catheter already attached with the saline.
3. Insert a 23 gauge catheter into the left ventricle of the heart.
4. Make an incision on the right atria of the heart to allow blood to drain.
5. Open the regulating clamp on the IV line slowly to about ¾ of the way.
6. Allow the saline to perfuse until it begins to drain from the right atria as a clear substance (approx. 10min).
   NOTE: The liver should change from red to brown if the catheter is in the right position. Otherwise, the lungs will inflate with saline indicating that you have crossed the left side of the heart into the right side. At this point the experiment has failed and cannot continue.

B. MICROFIL PERFUSION

1. Mix Microfil® into an IV bag (SOP: *"Preparing Microfil for Perfusion"*.)
2. Shut off the flow from the saline.
3. Remove the butterfly catheter from the IV saline line and connect it to the new Microfil IV line.
4. Allow the Microfil to perfuse until it begins to drain from the right atria. This may take a variable amount of time, but should not exceed 30min.
5. Wait 1 hour for Microfil to set. The Microfil in the IV bag will feel like a hard gel.

7.0.4 PRESERVATION

Preservation should only be done on mice that have had a successful Microfil perfusion.

1. Using proper surgical tools and methods, remove any organs or tissues that require preservation.
2. Place organs in 10% formalin inside a 50mL conical centrifuge tube.
3. Place mouse in 10% formalin inside a 50mL conical centrifuge tube.
4. Samples are then stored for further analysis and scanning.

7.0.5 CLEANUP

1. Dispose all fluids into the biohazard container.
2. Dispose all used needles and pins into the sharps container.
3. Wash and clean all surgical tools for reuse (even when exposed to Microfil). IV lines and bags exposed to Microfil must be placed in a box housed in the flammables cabinet until Chemical Waste disposal personnel picks up and disposes of the contents.
4. Waste carcasses not being used may be incinerated, but **no more than 3 per day**. Waste Saline bags containing set Microfil, gloves or towels exposed to Microfil must be put aside in a container to be disposed of by Chemical Waste disposal personnel.

7.0.6 DECONTAMINATION

1. Anything exposed by the mouse, including the countertop, is to be sprayed and cleaned with a disinfectant (eg. Clidox). Allow for disinfectant to be in contact with surface for 10min. Then proceed to wipe surfaces with a clean cloth or towel.

7.2 SAMPLE STORAGE AND HOLDING TIMES:

The organs can be stored indefinitely at room temperature in a fixative solution (i.e. 10% buffered formalin).

7.3 RISKS TO PERSONNEL AND PRECAUTIONS FOR RISK REDUCTION:

7.3.1 Impervious examination gloves must be worn at all times.

7.7 CONTINGENCIES:

Accident Reporting is done by completing an accident report form which should be filled out within 24h of an incident and given to the supervisor within 3 days.

7.7.1 CATHETER OVERSHOOT

During the perfusion process, if the 23 gauge butterfly catheter is overshot into the left ventricle of the heart, the lungs of the mouse will inflate and consequently results in a failed experiment.

1. Perform the cleanup procedure (7.0.5 CLEANUP)
2. Dispose of the mouse carcass into the biohazard container.

7.7.2 SPILL OR LEAK

The hazardous material user is responsible for cleaning up a spill. If the spill is beyond the resources or abilities of the users to cleanup, contact security at x34041 who will then notify the Robarts Emergency Response Team as well as the supervisor. During off-hours and weekends call the Emergency Pager at 14111 or 9-911 if the situation warrants immediate attention. This duty must not be delegated to other staff such as caretakers.

Follow the general spill procedure outlined in "section 10.6: Chemical Spills" of the Robarts Research Health and Safety Manual.

A. MICROFIL

Wipe, scrape or soak up in an inert material (eg. paper towel) and place in a box housed in the flammables cabinet until Chemical Waste disposal personnel picks up and disposes of the contents.

B. ISOTONIC SALINE

Wipe or soak up in an inert material (eg. paper towel). This is non-hazardous and may be disposed as normal waste.

7.7.3 INCOMPLETE MICROFIL PERFUSION

If surface vessels in the feet, nose, and tail are not perfused, then you probably do not have a complete fill. Allow more time to perfuse. However, if the time has exceeded 30min, the experiment has failed because the Microfil will have set.

7.7.4 ANIMAL BITES

1. The bite wound should be washed immediately in warm soapy water.
2. If the wound is bleeding, attempt to encourage bleeding by expressing the wounded area.
3. After washing, cover the wound with a dry sterile dressing.
4. Go to “ROOM #’ for further assessment and treatment. After hours go to University Hosptial Emergency Department.

   [Room number must be specified. This exact procedure is specified by ACVS.]

8.0 REPORTING AND DOCUMENTATION:

N/A

9.0 REVIEWS AND REVISIONS:

This procedure shall be reviewed for compliance and effectiveness and revised as necessary.

10.0 ATTACHMENTS and REFERENCE FORMS:

None.