UNIVERSITY OF SASKATCHEWAN

**University Animal Care Committee (UACC)**
**Standard Operating Procedure (SOP)**

| SOP # | Title |
|---|---|
| 20220094 | *In vivo* evaluation of rat nerve repair tissue scaffolds |

## 1. Purpose

To induce injuries in sciatic nerve in rat and then implant hydrogel scaffold for evaluating its performance for helping regrowth of injured sciatic nerves.

## 2. General Information

In many mammalian species, axon regeneration readily occurs, and sensory and motor function is restored. In human clinical cases, however, peripheral nerve regeneration can be less successful. This is due in part to the greater distances which must be traveled. A more immediate problem, however, is that human nerve injury often results in the creation of a gap that separates the surviving proximal and distal nerve stumps. If this gap is substantial, axons that are poised to regenerate will fail to find a supportive peripheral pathway, and regenerative growth is blocked. Implantable tissue scaffold is known in the literature to aid in the regeneration and repair of injured nerves. Scaffolds fabricated with different biocompatible biomaterials introduce different recovery performances. Therefore, tissue scaffolds implantation surgery is necessary to evaluate this effect. The nervous system biology of rats is similar to that of humans and rats are large enough to easily carry out sciatic nerve surgery and tissue scaffold placement.

## 3. Equipment/Materials

| **Consumables** | # Per rat | Supplier | Catalogue # |
|---|---|---|---|
| Tissue scaffolds | 1 | - | - |
| Sutures for nerve reconnection | 1 | | 8-0 Ethilon |
| Sutures for muscle | 1 | | 6-0 Vicryl |
| Sutures for skin | 1 | | 4-0 Perma-hand Silk |
| Scalpel blades | 1 | | |
| Cotton Applicator | 1 | | |
| Gauze | 2 | | |
| Cotton cloths | 6 | | |
| Oxygen for machine | N/A, from wall | LASU | |
| Isoflurane for machine | N/A, from wall | LASU | |
| Eye lube | 1 bottle | LASU | |

| | | | |
|---|---|---|---|
| Lubricating gel | 1 bottle | LASU | |
| Buprenorphine Sustained-Release (Bup SR) 1 mg/kg | 0.3 ml | | |
| Syringes, 1 mL | 1 | | |
| Syringes, 3 mL | 1 | | |
| 23-gauge needle | 1 | | |
| Gel food | 1 for each day | LASU | |
| 70% ethanol | 5 ml | | |
| Bitter apple spray | 1 | | |

| **Equipment** | Quantity | Manufacturer |
|---|---|---|
| Scalpel handle, size 3 | 1 | |
| Forceps, straight, grey handle, 3 inches | 2 | |
| Forceps, curved, grey handle, 3 inches | 2 | |
| Forceps, curved, grey handle, 4 inches | 1 | |
| Forceps, curved, grey handle, 5 inches | 1 | |
| Forceps, 1x2 teeth, straight | 1 | |
| Forceps, broad shanks, standard tips, straight | 1 | |
| Needle Holder, grey handle | 2 | |
| Needle Holder, gold handle | 1 | |
| Haemostat, straight, serrated | 1 | |
| Scissors, straight, sharp, black handle | 1 | |
| Scissors, straight, fine, gold handle | 1 | |
| Scissors, straight, blunt | 1 | |
| Bone scissors, curved with gold handle | 1 | |
| Nerve scissors, gold handle | 1 | |
| Retractor | 1 | |
| Wound clip applicator | 1 | |
| Wound clip remover | 1 | |
| Wound clips | 3 | |
| Shaver (from LASU in induction area) | 1 | |
| Anesthetic machine with induction box (from LASU in surgical suite) | 1 | |
| Physiosuite monitoring system with heat pad and thermometer probe (from LASU in surgical suite) | 1 | |
| Glass bead sterilization (from LASU in surgical suite) | 1 | |
| Overhead surgical lights (from LASU in surgical suite) | 1 | |
| Empty cage with paper towel (from LASU in recovery area) | 1 | |
| Electric heat pad (from LASU in recovery area) | 1 | |
| Elizabethan cone | 5 | |

## 4. Procedure

### 4.1. Planning for the surgeries:

Surgeries should be planned for mornings so that animals can be monitored after surgery for several hours in the afternoon. Surgeries cannot be undertaken on Friday, Saturday, or Sunday without both: 1) specific permission granted by LASU director (Michele Moroz), and 2) adequate postsurgical monitoring as described below. The Lab Animal Services Unit (LASU) - Health Sci Bldg, surgical suite, performs this surgery on 8-week-old rat, therefore 8-week-old rat must be ordered (to allow for more than 1 week of acclimatization in LASU).

### 4.2. Setting up

Set up all equipment needed for the surgery onto the surgical tables. Scrub the surgical tools, dry them well to avoid water spots, and autoclave them (takes about 1.5h). This can even be done the day prior. On the morning of the surgery, plug in and turn on all equipment. Allow 30 min to heat up the homoeothermic blanket system and the recovery hot water blanket. Weigh the animals and aseptically draw up all sterile solutions and medications prior to the start of anesthesia.

### 4.3. Preparing solutions

#### 4.3.1. Buprenorphine

Sustained-Release (Bup SR): Bup SR is a controlled substance so after purchasing, it is kept directly at LASU and will be provided by the technician. Remove from fridge on morning of surgeries and let warm up at room temperature. Calculate dose using formula below and draw into syringe immediately before administering (during shaving) – no need to dilute. Place back in the fridge after last dose of the day.

$$\frac{\text{Body Weight [kg]} \times \text{Dose } \left[\frac{\text{mg}}{\text{kg}}\right]}{\text{Concentration } \left[\frac{\text{mg}}{\text{mL}}\right]} = \frac{\_\_\_\_\_\ \text{kg} \times 1.2\ \frac{\text{mg}}{\text{kg}}}{1\ \frac{\text{mg}}{\text{mL}}} = \_\_\_\_\_\ \text{mL}$$

#### 4.3.2. Saline

The saline we purchased doesn't need to be diluted. For each animal undergoing surgery that day, calculate the amount of saline you will inject at the end of each surgery (assuming 1h per surgery). Write the amount on the surgical monitoring sheet and draw up the saline amount in a syringe.

$$10\frac{\text{mL}}{\text{kg}\cdot\text{hr}} \times \text{Body Weight [kg]} \times \text{Time[hr]} = 10\frac{\text{mL}}{\text{kg}\cdot\text{hr}} \times \_\_\_\_\_\ \text{kg} \times 1\text{hr} = \_\_\_\_\_\ \text{mL}$$

### 4.4. Before the surgery

1) Acclimation (~7 days before surgery) for getting familiar to procedures and equipment.
2) 2-3 days before surgery, tape the dish for the gel food to the bottom of the group cage (we usually tape one facing down and then mount the second dish to the first one – this allows the dish to be at the same level as the bedding). Give gel food every day until the day of surgery.
3) Autoclave surgical tools not in sterilized packaging.

### 4.5. On the day of the surgery:

1) Check the rat conditions to identify whether it's appropriate for anesthesia and surgery.
2) Enter the induction area.
   a) Turn on water-heated surface.
   b) Ensure the LASU anesthetic machine has sufficient isoflurane and is properly set up. Isoflurane is refilled regularly by LASU.
   c) Ensure induction box is present.
   d) Ensure the nose cone fits our animal.
   e) Ensure shaver is present.
3) Enter the recovery room.
   a) Turn on the heat pad in the recovery room.
   b) Place half of the recovery cage on top with paper towel inside.
4) Enter the surgical suite.
   a) Ensure the LASU anesthetic machine has sufficient isoflurane and is properly set up.
   b) Turn on glass bead sterilizer 20-30 minutes before surgery.
   c) Ensure heat pads in are turned on 10-15 mins before starting and tape down to table. Keep at 37 Celcius.
   d) Place surgical pad on top of heat pad and tape down.
   e) Ensure temperature probe is functional and position probes.
   f) Position surgical microscope (if used)
   g) Position surgical lights and surgical gooseneck lights (if used)
   h) Place all surgical tools neatly on the table.
5) Weigh all the rats that are to undergo surgeries that day and immediately place them alone in new cages. Ensure they have water bottles, dishes for gel food, a fresh serving of gel food, and plenty of normal dry food
6) Fill syringes with the correct amount of saline and label each syringe with the rat number for each animal. Place the syringe for the first animal in close proximity to the heat pad on the surgical table.
7) Ensure each team member is wearing a green LASU jacket once in the surgical suite with gloves, a face mask, and a head covering (bouffant).
8) Keep a record of all the LASU personal protective equipment used i.e., masks, gloves, surgical masks, and bouffant caps.

### 4.6. Preparing the rat in the induction area:

1) Put the rat in the induction box. Induce rats with 5% isoflurane and oxygen level 1 until the righting reflex is lost.
2) Reduce isoflurane to 2% or 3% while keeping oxygen level at 1 and move animal to the nose cone. Make sure the valves along the tubing are correct.
3) Place temperature assurance material on top of isoflurane tube to keep it in place.
4) Apply eye lube to the rat.
5) Place rat on its abdomen to shave the lateral surface of left hindlimb and clip fur around the surgical site.

6) Prepare incision site by doing 3 surgical scrubs with gauze and soap and scrubbing in circles. Follow up with 70% ethanol with gauze also by scrubbing in circles. If the alcohol wipe is showing pink soap residue, repeat the alcohol wipe until no pink is seen on the wipe.
7) Ensure anesthesia depth with toe and tail pinches before proceeding.
8) Move the rat back into the induction box and take to the surgical suite.
9) Record anaesthetic machine usage in induction area.

### 4.7. Prepare for rat in the surgical suite:

1) If the animal has woken up, repeat 4.6 steps 1 through 4.
2) Use lubricating jelly and insert rectal temperature probe to measure temperature. Flip rat onto its back while keeping the nose in the nose cone and the rectal probe in place.
3) Tape down the animal's forelimbs
4) Tape down the nose cone to keep it in place. Make sure it is loose enough so that the rat can breathe freely, but that it still holds a little bit of tension on the skin in the neck area.
5) Take another piece of tape and place under the chin of the rat and secure the tape to the nose cone – this keeps the chin of the rat in the nose cone, which helps keep the neck area stretched.

### 4.8. Sciatic nerve lesion induction and tissue scaffolds implantation surgery:

1) Inject Bup SR subcutaneously and make note on anaesthesia record the time of injection. Bup SR is thick so draw with a large gauge needle (18-gauge) and then inject with a smaller gauge needle (23-gauge).
2) Continue to fill out the anesthesia and surgery record every 15 min.
3) A skin incision (around 3 cm long) will be made just behind the femur of left hindlimb using a scalpel and forceps, extend incision with scissors if necessary.
4) Using the straight blunt forceps to hold the edge of the skin at the incision, the sciatic nerve will be exposed by gently separating muscle groups along the fascial plane with haemostat.
5) Use retractor to keep the surgical site open.
6) While keeping track of the anaesthesia record, humidify the skin and gland every 20-30 mins with saline and a cotton applicator. Also make sure the rat doesn't get too warm from surgical lights. If temperature goes too high (above 38°C), rub 50% ethanol on the legs and tail of the rat with a cotton applicator. If surgery is running long, douse the surgical site with saline to keep area moist.
7) Swing the surgical microscope (if used) into place.
8) Sciatic nerve lesion induction: sciatic nerve will be resected using scissors around 10 mm above the sciatic nerve trifurcation.
9) Tissue scaffold implantation: the tissue scaffold, which has already been placed in a 3D printed stent, will be implanted, and connected to the two nerve stumps to mediate reconnection of the cut nerve ends.
10) The repaired section of nerve will be enclosed in a 3D printed stent.
11) The nerve-stent will be closed with resorbable 8-0 sutures.
12) The muscles will be sutured closed using 6-0 sutures.
13) The skin incision will be closed with wound clips as preferred. Use 4-0 sutures as backup.

14) Spray the closed skin incision with bitter apple to prevent the animal from biting at the wound clips or the suture. Consider using gauze dabbing iodine at the closed incision because iodine is an antiseptic agent and bitter tasting.
15) Inject saline subcutaneously
16) Expected time under anesthesia is 1 hr.
17) For sham-operated control rats, the sciatic nerve will be left intact after step 3).
18) For transection control rats, the sciatic nerve will be transected using scissors, then the cut ends of the nerve will be sutured back together using resorbable 8-0 Ethilon.
19) In between surgeries, sterilize surgical instrument tips in the glass bead sterilizer for 20 seconds.
20) Clean all instruments. First in ultrasonic cleaner for 5 min and then with soap and water to remove blood, tissue, and debris.
21) Record how long anaesthesia machine was used in the surgical suite.

### 4.9. Recovery and post-op monitoring:

1) Turn off anesthesia so that only oxygen is flowing to the nose cone.
2) Remove the rectal probe, remove the tape keeping the animal down, move animal to the recovery area and it in the pre-warmed recovery cage with paper towel. The recovery cage is partially on a heat pad set to low.
3) Elizabethan cone is an option if animals are shown to biting at the closed wound. If used, it will restrict the animal's movement in cage.
4) Once the animal is waking, move it to its home cage. Place cage partially on a heat pad for the next 4 hours.
5) Fill out the procedure card and contact info card (yellow and green cards) and tuck behind white cage card for each animal post-op.
6) Monitor the animal every hour for a minimum of 4 hours following the procedure.
7) Monitor the animal every 8 hours for the first 2-3 post-operative days and twice (12 hours) each day during the remainder of post-operative week 1, once each day during post-operative week 2-6.
8) Refer to Humane Intervention Point (Endpoint) Checklist for Rat, for Grading Scheme during monitoring.
9) Insert scores in the Post-Surgical Monitoring Checklist Document. One checklist should be printed off for every animal who underwent surgery (even shams).

## 5. Safety

- Please read MSDS and ERP regarding proper use of Isoflurane and Bup SR (TOXIC).
- Be familiar with proper handling of medical oxygen tanks.
- Be familiar with proper rat handling techniques to minimize the amount of stress placed on the animal and the chance of being bitten or having an animal escape from its cage.
- Be aware that the scalpel blades, fine forceps, scissors, and needles are very sharp.

## 6. Potential Complications and Troubleshooting

- If animal is showing signs of pain or distress (defined by HIP scoring sheet) or infection (swelling, pus or discharge, red, inflamed tissue, self-trauma), consult the emergency vet.

- In the event of cases of morbidity or mortality during or after surgery write notes on the surgical sheets, and fill out the required form, submit to the LASU director (Michele Moroz), and file another copy in our lab records.

## 7. Revision History

List the changes made during the most recent revision, as well as the reasons for the changes.

| **Date Created: April 16th, 2022** | | **Written by: Xiaoman Duan, Xiao Fan Ding** |
|---|---|---|
| **SOP Review and Revision History** | | |
| **Revision Number** | **Revision Date** | **Reviewer** |
| **1** | **2022-11-18** | **Xiaoman Duan** |
| **2** | **2022-11-28** | **Xiaoman Duan** |
| **3** | **2023-10-02** | **Xiaoman Duan** |
| **4** | **2023-12-01** | **Xiao Fan Ding** |