

PURPOSE: To passage or create orthotopic patient derived xenografts

SURGICAL SITE PREP

1. Clean the surface of the surgery area and isoflurane chamber with Vimoba including the nose cone, turn on the bead sterilizer and heat pad.
2. Cover the surgical surface with an absorbent pad. Do not lay an animal directly on a stainless steel surface. *The cold temperature of the metal surface may exacerbate the hypothermia associated with anesthesia.*
3. All instruments, and supplies that come into contact with the surgical site must be sterile and maintained in sterile packaging prior to surgery. *Surgifoam will be alloquated into autoclaved bottles upon first opening.*
4. If a ready surgical pack is not available, instrument tips may be sterilized prior to surgeries using a glass bead sterilizer. The bead sterilizer should be turned on 20 minutes prior to use. The surgical instrument should first be rinsed clean of organic material using sterile water and carefully dried with sterile gauze. The instruments are placed in the glass bead sterilizer for 10 seconds. The instruments are then removed and allowed to cool on a sterile surface or alternatively cool by dipping in sterile water.
5. When operating outside of a hood/downdraft table the surgeon should be wearing sterile gloves, masks, cap.

ANIMAL PREP

Surgical preparation (i.e. hair removal and initial skin cleansing) will be performed on a separate surface and the animal will then be moved into the surgical field.

1. Animal recipients (typically 5 Nu/Nu or NSG immune compromised mice) for these xenografts are anesthetized with inhaled Isoflurane or injected Ketamine-Xylazine. *The following instructions are for Isoflurane.*
2. Put food down in their regular housing cage.
3. If using furred mice shave the fur in a large area between the ears and partially down the neck using clippers. *Alternatively a depilatory or plucking can be used.*
4. Protect the eyes with a petrolatum ophthalmic ointment to prevent corneal drying. *To avoid contamination, do not touch the tip of the tube to the skin or eye surface, use q tip.*
5. Tail mark for surgical notes/chipping.

TRANSPLANTATION

1. Wash gloves with Viomba or 70% alcohol between mice. *Sterile surgical gloves do not have to be worn, if the following criteria are followed: The gloved hand does not touch the prepped field, and only the tips (of the tools) handle the animal tissues.*
2. Betadine and alcohol will be used for skin preparation prior to surgery. Scrub the skin with a single pass of a q tip impregnated with betadine in one direction and repeat with an impregnated 70% alcohol wipe. The skin should be scrubbed at least three times using this method. *Be careful not to excessively wet the animal, as it can exacerbate hypothermia. Alternative antiseptics to the iodophor may be considered after consultation with a Center veterinarian. Clorhexidine, Nolvasan, Hibiclens.*
3. Depth of anesthesia will be monitored by foot pinch. The procedure will not begin until there is no foot pinch response. Respiration and heart rate will be monitored as they are good measures of depth.
4. Using a scalpel an incision is made starting near the eyes, between the ears and ending near the back of the skull. *Keeping the incision as small as possible will expedite healing.*
5. Using a cotton tip applicator push the muscle and lining on top of the skull aside until the intended drilling region is dry and bare.
6. A 0.9 mm diameter burr hole is placed in the calvarium with a hand held surgical drill.



X Right cerebellum- 2 mm lateral (right) to the sagittal suture, 2 mm posterior of the lambdoid suture
O Right cerebral hemisphere- 1 mm lateral (right) of the sagittal suture, 1.5 mm anterior to the lambdoid suture

7. 2-3 uL of human brain tumor cells (1×10^5 cells in single cell suspension) will be intracranially injected into the brain parenchyma 2mm under the dura using a p20 pipet and a 0.2-10ul tip. The tip will be inserted gently until it can go no further. When the tip stops it will be 2 mm deep. Cells suspension will be slowly injected, leaving the plunger depressed with the tip in place for approximately 5-10 seconds. Pipette tip will be slowly removed from the burr hole while keeping the plunger depressed. *(2ul normal, 3ul for low cell count/frozen).*
8. Using forceps, a small piece of SurgiFoam should be added to the burr hole site as soon as the pipette tip is removed to minimize spillage and perform proper hemostasis.
9. Incision will be gently closed using forceps.
10. Tissue-mend/vetbond will be administered to close the incision.
11. Gloves should be washed with Vimoba or 70% alcohol between surgeries, and tools that cannot be placed in the bead sterilizer should be rinsed between surgeries with 70% alcohol.

SURGICAL ASSISTANT

Take record of any surgical notes on the back of the cage card

1. Inject 80-100ul of the Bupivacaine-Lidocaine subcutaneously around the incision line (40ul on each side) to provide a final total dose of 8mg/kg. (see Bupivacaine-Lidocaine SOP for preparation)
2. Inject up to 500ul of sterile 1X PBS: sub-cutaneous injection between the shoulder blades.
3. Ear punch if required by the study. (#1 is no punch, #2 is left, #3 is right, #4 is left right, and #5 is left left). Otherwise chip for PDX upon recovery (see Chip SOP for directions).
4. Place mouse in clean cage, with half of the cage on a heated mat and the bedding removed. *Do not lay recovering animals directly on any bedding they may asphyxiate or aspirate.*
5. Allow the animal to recover, while maintaining observation. The animal must be conscious and able to right itself and move about normally before being returned to its regular housing cage, and must not recover in a cage containing conscious animals. *When using isoflurane anesthesia, mice will most often be awake and ready to return to their home cage by the time the following surgery has been completed. When using injectable anesthetics (Avertin, Ketamine-Xylazine) the mice will be observed once per hour during their recovery period.*
6. After the all mice recover, return the cages to the racks.

SOURCES

- 1457 IACUC Protocol, Olson Lab 7/16/2012.
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- Guide for the Care and Use of Laboratory Animals Ch. 3, Veterinary Medical Care pp 56-70, Institute of Laboratory Animal Resources, National Research Council, National Academy Press, Washington, DC, 1996. (<http://www.nap.edu/readingroom/books/labrats/>)
- Guidelines for Survival Rodent Surgery, NIH Animal Research Advisory Committee, Revised 9/12/12. (http://oacu.od.nih.gov/ARAC/documents/Rodent_Surgery.pdf)
- SOP2: Making PDK mice, Karina Bloom, Sally Ditzler, Kyle Pedro, 12/12/12.
- http://www.mbl.org/anatomy_images/fresh/bigfresh/Step2.jpg

Authored by: Madison Wise

Previous editions approved/authored by: Emily Girard, Michelle Cook Sangar VMD, PhD, Kyle Pedro, Andrew Richards