Procedure Type: Custom - Other

Procedure Title: Performing Skin Grafts in Mice

Species: Mouse

Pain/Distress Category: D

Procedure Description Tab:

Procedure Description:

Obtaining Skin from Donor Mouse:

Note: Since this is a terminal procedure used to collect tissue, ensure that the method of euthanasia specified here is also described in the Animal Disposition section of this protocol.

- 1. Euthanize mouse outlined in the Animal Disposition section and confirm death.
- 2. Remove hair from tissue harvest site with electric clippers.
- 3. Aseptically prepare the skin according to the ACUC Guidelines for Surgical Procedures (http://www.acuc.berkeley.edu/guidelines/surgical_procedures.pdf).
- 4. Using sterile technique, remove skin with a sterile scalpel blade and sterile forceps; prepare small skin grafts by placing them dermis side down in a petri dish on top of a sterile filter paper disc saturated with sterile PBS.

Grafting of Recipient Mouse:

- 1. Anesthetize mouse per regimen outlined in the Anesthetic Regimen tab. Record weight on blue postsurgical cage card. Administer analgesia per Peri procedure Care/Analgesics tab.
- 2. Place ophthalmic ointment in both eyes.
- 3. Remove hair from the tissue grafting site(s), with electric clippers or approved depilatory cream. Remove at least a 2 mm border of hair from planned surgical margin.
- 4. Place mouse on pre-warmed circulating water heating pad, covered with clean absorbent material to reduce hypothermia.
- 5. Aseptically prepare the shaved skin according to the ACUC Guidelines for Surgical Procedures (http://www.acuc.berkeley.edu/guidelines/surgical_procedures.pdf).

- 6. Before making an incision, check anesthetic depth (i.e., toe-pinch to verify absence of a withdrawal reflex).
- 7. Using sterile technique, prepare the graft bed by cutting an area of skin slightly larger than the skin graft, not to exceed 5mm2, as superficially as possible, using care not to cut into the panniculus. Gently pull it off the graft bed.
- 8. Apply a local anesthetic (e.g., Lidocaine, Bupivacaine) topically to the grafting site.
- 9. Transfer the graft from the donor mouse onto the prepared graft bed, fitting it well within the prepared area. Use a sterile Q-tip to remove excess PBS and smooth the graft to ensure the edges are not curled. If necessary, trim excess donor graft using sterile curved scissors to remove overhanging skin.
- 10. Cover the entire graft bed and small margin of surrounding skin with sterile non- adherent gauze (e.g., Telfa™ or Adaptec® pad).
- 11. Firmly bandage the grafted mouse using a sheer adhesive strip (e.g., Band-Aid®) or self-adhering bandage (e.g., Vetwrap®/Coban®), being careful not to wrap the animal too tightly. Consult the OLAC veterinary staff on the best bandaging technique for the type of graft being performed.
- 12. Return mouse to clean cage once fully recovered from anesthesia.

How does this procedure fit into or address your overall research goals?

[Insert protocol-specific rationale here]

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

While negative clinical effects from this procedure are not expected, inappropriate bandage placement (e.g., too tight or too loose), infection, dehiscence at the surgical site, or skin graft rejection may occur.

Describe post procedure monitoring that will be performed.

The grafted mouse will be monitored daily (e.g., Body Condition scoring index, body weight, mobility, etc.) for the first 5-8 days, then every 2 days for 2 weeks, then weekly thereafter. The bandage will be removed and replaced on day 10, and then every 2 days when scoring the graft for rejection or acceptance; the bandage will be permanently removed by day 21, at which point, the graft should be fully developed.

Mice will be monitored following transplantation for general appearance, activity level, mobility, weight loss, signs of infection, and rejection of skin graft.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

If the mouse appears moribund/lethargic, or if any other abnormal signs are noted (i.e., tissue rejection), an OLAC veterinarian will be contacted or the mouse will be euthanized immediately.

Anesthetic Regimen Tab:

Parameters that will be monitored during anesthesia (check all that apply):

- ✓ Respiratory Rate
- ✓ Pedal Reflex
- ✓ Other (Describe): Skin color or mucous membrane color.

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

Stable respiratory rate, lack of pedal reflex, and skin color or mucous membrane color will be monitored during anesthesia and recorded initially, as well as every fifteen minutes if necessary, until the animal has fully recovered. Anesthetic records will be kept in the lab notebook.

Anesthetic Agents:

Agent Name: Isoflurane

Dosage (in mg/kg if possible) and volume: Induce 3-4%; Maintain 1-2%

Route: Inhalation (IN)

Describe timing, frequency and duration of administration: Mice are initially anesthetized with 3-4% isoflurane by placing them in an induction chamber. Once anesthetized, the mouse is then transferred to a nose cone respirator connected to a precision vaporizer that delivers 1-2% isoflurane for maintenance.

Agent Name: Ketamine hydrochloride

Dosage (in mg/kg if possible) and volume: 80-100 mg/kg

Route: Intraperitoneal (IP)

Describe timing, frequency and duration of administration: Administered once in conjunction with

Xylazine for induction of anesthesia prior to procedure.

Agent Name: Xylazine

Dosage (in mg/kg if possible) and volume: 5-10 mg/kg

Route: Intraperitoneal (IP)

Describe timing, frequency and duration of administration: Administered once in conjunction with

Ketamine for induction of anesthesia prior to procedure.

Other premedications not already listed above:

Agent Name: Ocular Lubricant

Dosage (in mg/kg if possible) and volume: N/A

Route: Topical (Topical)

Describe timing, frequency and duration of administration: A thin strip of ointment is applied to each

eye upon induction of anesthesia.

Peri Procedure Care/Analgesics Tab:

Pre-emptive Agents (analgesics given prior to/during procedure) – Select those that apply:

Agent Name: Bupivicaine (local block)

Dosage (in mg/kg if possible) and volume: 1-2 mg/kg local infiltration

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: Lidocaine or bupivacaine is applied once

during the procedure at the site of the graft placement.

Agent Name: Lidocaine (local block)

Dosage (in mg/kg if possible) and volume: 2-4 mg/kg local infiltration

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: Lidocaine or bupivacaine is applied once

during the procedure at the site of the graft placement.

Agent Name: Carprofen

Dosage (in mg/kg if possible) and volume: 5 mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: NSAID analgesia (e.g., carprofen or

meloxicam) is administered once prior to recovery from anesthesia.

Agent Name: Meloxicam

Dosage (in mg/kg if possible) and volume: 5 mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: NSAID analgesia (e.g., carprofen or

meloxicam) is administered once prior to recovery from anesthesia.

Agent Name: Buprenorphine

Dosage (in mg/kg if possible) and volume: 0.1 mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: Buprenorphine is administered once upon

induction of anesthesia prior to initial skin incision.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Stable respiratory rate, lack of pedal reflex, and skin color or mucous membrane color will be monitored.

Post-procedure Analgesics:

Agent Name: Carprofen

Dosage (in mg/kg if possible) and volume: 5 mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: NSAID analgesia (e.g., carprofen or meloxicam) is administered 24 hours post-operatively. Animals will be checked 48 hours post-operatively for signs of pain or distress as outlined in post procedure monitoring. If animals exhibit any signs of pain or distress, another dose will be administered and the OLAC veterinary staff consulted.

Agent Name: Meloxicam

Dosage (in mg/kg if possible) and volume: 5 mg/kg

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: NSAID analgesia (e.g., carprofen or meloxicam) is administered 24 hours post-operatively. Animals will be checked 48 hours post-operatively for signs of pain or distress as outlined in post procedure monitoring. If animals exhibit any signs of pain or distress, another dose will be administered and the OLAC veterinary staff consulted.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.):

Mice will be monitored following surgery for general appearance, activity level, weight loss, signs of infection, or dehiscence at the surgical site. Monitor bandage application closely to ensure that the bandage was not applied too tightly and the mouse can resume its normal activity.

Monitoring Duration:

Mice will be monitored every 15 minutes until upright and ambulatory. Once mice are fully recovered from anesthesia, they are placed back into a clean cage and returned to the housing room.

Monitoring Frequency:

Mice will be monitored every 15 minutes until fully awake. Animals will be monitored daily for the first week after surgery or more frequently if indicated (e.g., Body Condition Scoring index, body weight) while bandaged, and sutures removed in 7-14 days.

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be consulted or the mouse will be euthanized.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

1.0 ml of prewarmed fluids (pharmaceutical grade 0.9% NaCl or LRS) will be administered subcutaneously. Mice will be allowed to recover in a paper-lined cage on top of a heating pad.

Mice that have difficulty accessing food and water, appear dehydrated, and/or are anorexic, will be provided with moistened food on the cage floor. Nutritional supplements (e.g., NutriCal) may be provided to help stimulate appetite.

Describe record keeping/documentation methods for post-procedure monitoring:

A surgical record must be kept for each surgical patient (e.g., blue post-surgical cage card, anesthesic record, surgical notes in the lab notebook) per ACUC Guidelines on Recordkeeping for Surgical Procedures on Laboratory Animals. Mice will be weighed prior to surgery, as well as daily for 7 days post-operatively. Weights and observations will be recorded on the blue post-surgical cage card during the immediate post-op period, and the lab notebook thereafter.

Other Agents Utilized Tab:

Agent Name: Sodium chloride (fluid therapy)

Dosage (in mg/kg if possible) and volume: 1.0 ml

Route: Subcutaneous (SC)

Describe timing, frequency and duration of administration: Administer 1.0 ml of prewarmed fluids

(0.9% NaCl or equivalent) SC to mouse after procedure.