
1. PURPOSE

The intent of this Standard Operating Procedure (SOP) is to describe procedures for mammary fat pad injection surgery in rodents.

2. RESPONSIBILITY

Principal investigators (PI) and their staff, veterinary care staff or any individual performing surgery on rodents, or assisting in those procedures.

3. MATERIALS

- 3.1. Analgesics
- 3.2. Anesthetic
- 3.3. Sterile ophthalmic ointment
- 3.4. Electric clipper or depilatory cream
- 3.5. Gauze (sterile and non-sterile)
- 3.6. Antiseptic solution for skin (e.g., chlorhexidine 2% solution or povidone-iodine solution used alternatively with 70% alcohol, or 2% chlorhexidine in 70% alcohol solution)
- 3.7. Heating disc, warming pad or warm-water circulating pad. Do not use electric heating pads unless specifically designed for use with laboratory rodents.
- 3.8. Sterile isotonic saline solution
- 3.9. Sterile cotton-tipped swabs
- 3.10. Sterile surgical instruments
- 3.11. Dry bead sterilizer
- 3.12. Surgical tissue glue (Vetbond®), absorbable suture or wound clips

4. PROCEDURES

- 4.1. Refer to Rodent Surgery SOP.
- 4.2. Document the details of the surgical procedure in the Rodent Procedure Log.
- 4.3. Administer analgesia to recipient animals as per Rodent Analgesia SOP.
- 4.4. Anesthetize donor (if applicable) and recipient animal as per Anesthesia SOP.
- 4.5. Apply ophthalmic ointment in both eyes of recipient animal to prevent corneal desiccation. Reapply as needed.
- 4.6. If applicable, obtain donor animal tissues (small piece of tumour) for implantation. Place sample in sterile PBS until implantation. Euthanize donor by cervical dislocation.

Note: the donor animal is anesthetized as opposed to euthanized prior to tumor collection to maintain the sterility of the samples and minimize time to transplant.
- 4.7. Remove hair over the area surrounding the abdominal (#4) mammary gland of the animal using a clipper or depilatory cream. Remove loose hair with gauze.
- 4.8. Wipe the skin surface with 70% alcohol followed by 2% chlorhexidine solution or povidone-iodine solution.
- 4.9. Mammary Fat Pad Injection:
 - 4.9.1. Place recipient animal in dorsal recumbency.

- 4.9.2. Make a 0.5cm incision between the abdominal (#4) mammary gland/nipple and the midline. Alternatively, the incision can be made in the flank, cranial to the abdominal (#4) mammary gland/nipple.
- 4.9.3. Gently separate the skin from the underlying fascia using a sterile cotton swab or by blunt dissection. Care should be taken not to damage the peritoneal cavity.
- 4.9.4. Injection of cell suspension:
 - 4.9.4.1. Locate the mammary fat pad, grasp it at its base and stabilize it using jewelers or micro-dissecting forceps.
 - 4.9.4.2. Inject cells directly into the fat pad in a maximum volume of 100µL.
- 4.9.5. Implantation of tissue fragments:
 - 4.9.5.3. Locate the mammary fat pad, grasp it at its base and stabilize it using jewelers or micro-dissecting forceps.
 - 4.9.5.4. Prepare a pocket in the fat pad by carefully inserting the closed points of a jeweler's forceps into the fat pad.
 - 4.9.5.5. Remove forceps points from fat pad, and insert donor tissue to be transplanted into the prepared pocket. Tissue fragments should exceed 3x3x3mm.
- 4.9.6. Apply topical local analgesic to the incision.
- 4.9.7. Hold the edges of the incision together with forceps and use one drop of tissue glue to close the skin. Alternatively, suture the incision or use wound clips.
- 4.9.8. Repeat on contralateral side.
- 4.10. Disinfect the instruments between each animal by dipping them in a hot glass bead sterilizer for approximately 30 seconds after removing any blood and debris (let cool completely).
- 4.11. Allow animals to recover in a clean cage. Provide supplemental heat (use a heating disc or pad, heating lamp or incubator) for approximately 30 minutes and monitor the animals until they have fully recovered prior to returning them to their housing room.
- 4.12. Administer analgesics post-operatively as per Rodent Analgesia SOP.

5. REFERENCES

- 5.1. Tavera-Mendoza, L. E., & Brown, M. (2017). A less invasive method for orthotopic injection of breast cancer cells into the mouse mammary gland. *Laboratory Animals*, 51(1), 85–88. <https://doi.org/10.1177/0023677216640706>
- 5.2. Kocatürk B, Versteeg HH. Orthotopic injection of breast cancer cells into the mammary fat pad of mice to study tumor growth. *J Vis Exp*. 2015;(96):51967. Published 2015 Feb 8. doi:10.3791/51967
- 5.3. Zhang, G. L., Zhang, Y., Cao, K. X., Wang, X. M. Orthotopic Injection of Breast Cancer Cells into the Mice Mammary Fat Pad. *J. Vis. Exp.* (143), e58604, doi:10.3791/58604 (2019).

SOP REVISION HISTORY

DATE	REVISION
2019.02.21	4.6. Remove hair over the area surrounding the inguinal abdominal (#4) mammary gland of the animal using a clipper or depilatory cream. Remove loose hair with gauze.
2019.02.21	4.8.2. Make a 1cm 0.5cm incision through the skin longitudinally over between the inguinal abdominal (#4) mammary gland/ nipple and the midline .
2019.03.29	4.8.1. Place recipient animal in lateral dorsal recumbency.
2019.03.29	4.8.3. Gently separate the skin from the underlying fascia using a sterile cotton swab or by blunt dissection. Care should be taken not to damage the peritoneal cavity.
2019.03.29	4.8.4. Injection of cell suspension: 4.8.4.1. Locate the mammary fat pad, grasp it at its base and stabilize it using jewelers or micro-dissecting forceps. 4.8.4.2. Inject cells directly into the fat pad in a maximum volume of 100µL.
2019.03.29	4.8.5. Implantation of tissue fragments: 4.8.5.3. Locate inguinal lymph node in fat pad and prepare a pocket about 0.5 cm from the lymph node for tissue implantation by holding the points of a jeweler's forceps together and carefully insert them into the fat pad the mammary fat pad, grasp it at its base and stabilize it using jewelers or micro-dissecting forceps. Tension on the fat pad can be produced by holding fat pad near lymph node with another forceps, making it easier to insert jeweler's forceps. 4.8.5.4. Prepare a pocket in the fat pad by carefully inserting the closed points of a jeweler's forceps into the fat pad. 4.8.5.5. Remove forceps points from fat pad, and insert donor tissue to be transplanted into the prepared pocket. Tissue fragments should exceed 3x3x3mm.
2019.11.22	4.8.2. Make a 0.5cm incision between the abdominal (#4) mammary gland/nipple and the midline. Alternatively, the incision can be made in the flank, cranial to the abdominal (#4) mammary gland/nipple.
2020.11.17	3.6. Antiseptic solution for skin (e.g., chlorhexidine 2% solution or povidone-iodine solution used alternatively with 70% alcohol, or 2% chlorhexidine in 70% alcohol solution) 3.7. 70% Alcohol 3.8. Chlorhexidine 2% solution or povidone-iodine solution
2020.11.17	3.7. Heating disc, warming pad or warm-water circulating pad. Do not use electric heating pads unless specifically designed for use with laboratory rodents. 3.13 Heating disc/pad, red heat lamp or incubator.
2020.11.17	4.7. Wipe the skin surface with 70% alcohol followed by 2% chlorhexidine solution or povidone-iodine antiseptic solution.
2020.12.07	4.2. Document the details of the surgical procedure in the Rodent Procedure Log.

Investigator:	Protocol:
Procedure: Mammary Fat Pad Injection	Performed by:

Instructions: complete this log for rodent procedures requiring anesthesia, analgesia or post-procedure care (ex. surgeries, experimental infection). Keep the log in the housing room while active and in your files for 3 years for future review by the Quality Assistant and/or the FACC.

ANALGESIA

- carprofen: mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- buprenorphine: mouse: 0.1mg/kg SC or IP every 4-8 hrs;
rat: 0.05mg/kg, SC or IP, every 8-12 hrs
- lidocaine/bupivacaine (local analgesic)
- other: _____

ANESTHESIA

- isoflurane 2-2.5%
- ketamine/xylazine/acepromazine*:
mouse: 100 mg/kg (K)- 10 mg/kg (X)- 3 mg/kg (A) IP
rat: 50 mg/kg (K)- 5 mg/kg (X)- 1 mg/kg (A); IP or IM
- other: _____

OTHER AGENTS ADMINISTERED

- _____
- _____
- _____

Animal ID	Date	Anesthesia		Analgesia		Other		Heat Source Provided		Recovery time	Comments/observations	Initials
		dose	time	dose	time	dose	time	procedure	recovery			
1								<input type="checkbox"/>	<input type="checkbox"/>			
2								<input type="checkbox"/>	<input type="checkbox"/>			
3								<input type="checkbox"/>	<input type="checkbox"/>			
4								<input type="checkbox"/>	<input type="checkbox"/>			
5								<input type="checkbox"/>	<input type="checkbox"/>			
6								<input type="checkbox"/>	<input type="checkbox"/>			
7								<input type="checkbox"/>	<input type="checkbox"/>			
8								<input type="checkbox"/>	<input type="checkbox"/>			
9								<input type="checkbox"/>	<input type="checkbox"/>			
10								<input type="checkbox"/>	<input type="checkbox"/>			
11								<input type="checkbox"/>	<input type="checkbox"/>			
12								<input type="checkbox"/>	<input type="checkbox"/>			
13								<input type="checkbox"/>	<input type="checkbox"/>			
14								<input type="checkbox"/>	<input type="checkbox"/>			

Comments/footnotes:

*Dose can vary with the sex, the age, the strain, and the body condition of the animal.

ANALGESIA

- carprofen: mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- buprenorphine: mouse: 0.1mg/kg SC or IP every 4-8 hrs; rat: 0.05mg/kg, SC or IP, every 8-12 hrs
- OTHER _____

Initial the appropriate boxes when completed

Animal ID	Date	Analgesia			SC fluids			Wet food			Time			Remove Sutures (Day 7-10)
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														

Comments/footnotes: