

Surgical Implantation of Mini-Osmotic & Micro-Osmotic Pumps

Equipment For Pump Implantation (Located in B014):

- | | |
|--|---------------------------|
| 1. one pair of large surgical scissors | 6. Skin Staples |
| 2. straight hemostats | 7. Pumps for implantation |
| 3. one pair of forceps | 8. Blue diapers |
| 4. Anesthesia | 9. Shaving implement |
| 5. Betadine | 10. 70% ethanol |

Initial Preparation:

Anesthesia Preparation: 5 ml Ketamine, 0.7 ml Xylazine, and 16.25 ml 0.9% Saline (pre-made anesthesia is located under lock and key in AD lab)

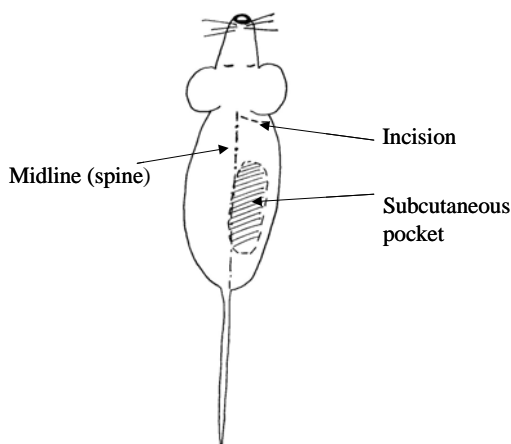
- Using a 3/10 cc insulin syringe, inject mice intraperitoneally. Use caution to avoid injections into the bladder.
- For a 20-25 gram mouse, inject 0.05 cc. For a 25-30 gram mouse, inject 0.06 cc. Mice should become unconscious and non-responsive to pain within 5-10 minutes. Once the mouse is at this level, it would be appropriate to begin pump implantation. Note time that the mouse begins to fall into anesthetized state, and note time when it begins to show signs of waking (i.e., tail twitching, pain response is returning, etc.). This will give you a general idea of the appropriate dose to use on your mice in the future. Finally, record the length of time that it takes the mouse to fully recover, from the initial injection to the point it returns to normal activity. Below is a chart of example times, your results may vary, depending on the anesthetic mixture, injection, and the dynamics of the mouse. Optimal times appear in the chart below.

Mouse Wt	Inject	Time to Unresponsiveness	Time Down	Recovery Time	Total Duration of Anesthesia
20-25	0.05 cc	5-10 min	~ 20 min	~ 1 hour	~ 1.5 - 2 hours
25-30	0.06 cc	7-12 min	~ 20 min	~ 1.5 hours	~ 2 hours

- After you have verified the efficacy and safety of your anesthetic, you may now begin implantation.

Surgical Procedure:

- Instruments should be placed in a bead sterilizer or 70% EtOH prior to surgery.
- Place one blue diaper under hood in a procedure room. This will provide a clean work surface. Roll the second blue diaper, adsorbent side out and place at the side. This will catch the water as you drain it off the pumps prior to implantation.
- Inject one mouse with the appropriate dose of the ketamine/xylazine mixture.
- Once anesthetized, the mouse may be prepped by shaving the area to be implanted.
- Place the mouse on the blue diaper in front of you, with it's head pointing toward your dominant hand. Swab area with betadine.
- Using the surgical scissors, make a 1 cm incision behind the ear, over the shoulder blade of the front leg. This incision should be perpendicular to the tail. Use care to cut only the skin, and not the underlying tissues (SEE DIAGRAM)
- Use the forceps to hold open the incision. Use the straight hemostats to make a subcutaneous tunnel under the skin and enlarge a pocket for the mini-pump.



8. Beginning with the hemostat closed, place the tips into the incision, pointed back toward the opposite leg. Angle the tip of the hemostat up and gently push along the skin as you advance into the incision. Once you have moved them forward, gently rock the tips downward away from the skin. This blunt dissection will separate the skin from the fascia that covers the underlying muscles. If done properly, there will be no bleeding from the subcutaneous pocket, and only a minimum of bleeding will occur at the incision.
9. Continue to dissect the skin away from the muscle layer, advancing the tips of the hemostat back toward the tail. Once it is obvious that you have done this, create a pocket for the pump. This is accomplished by carefully opening the jaws of the hemostat sideways under the skin to open up a pouch. Pull the hemostat back out of the incision just slightly as you close the jaws back together. This will prevent any pinching of tissues inside the mouse. Advance the tip of the hemostat back towards the tail, and repeat this process until you are confident that there is room for pump placement.
10. Remove the hemostat from the incision. Select the appropriate pump for the mouse group. Pumps should be kept in test tubes with saline until ready for use. Pour the water and pump out onto the 2nd blue diaper.
11. With the hemostat, pick the pump up by the end, and insert the pump with the regulator head first into the incision.(Pointed toward the tail end of the mouse).
12. Gently push the pump back towards the pocket that you have made for the pump. The entire pump should fit, with no portion of it remaining visible. There should be enough skin free to close the wound with no tension or stretching of the skin needed. Should the opening be too small for the pump to slide in, very carefully lengthen the incision with the scissors. Take care not to over extend the incision.
13. Once the pump has been inserted, firmly pinch both sides of the incision and staple the incision closed. Carefully inspect the incision site to ensure that there is complete closure of the wound. Apply betadine to the wound site.
14. Return the mouse to its cage, and repeat above steps for the next mouse.
15. All surgical instruments may be dipped or wiped in 70% EtOH between mice. This is only to maintain cleanliness, not for sterility.
16. Be sure to monitor all mice until full recovery is achieved (usually within 1 hour). Twelve to 24 hours post-op, make certain that the skin closures held, and that there is no evidence of the pump pushing out through the incision. Should an incision require additional glue or staples OR a pump appears to be protruding, perform surgical repairs immediately.
17. Continue to monitor recovery and fill in the post-operation form for the allotted 5 days.
18. Remove sutures at Day 14 post-op.

Lab: Alan Daugherty Lab, CVRC
Protocol Developed : Michael W. Manning **Date** : July 10, 2001
Updated : Britteni M. Kane **Date** : March 24, 2008
Updated : Jess Moorleghen **Date** : August 21, 2008

File name: C:\Documents and Settings\earan2\Desktop\Osmotic pump implantation.wpd