Preparation and Implantation of Alzet Pumps (Osmotic Pumps)

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Abstract

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Protocol

Step 1.

PREPARATION of ALZET Osmotic Pumps for Subcutaneous Implantation

Reagents/Supplies

Step 2.

1. Mini-osmotic pumps (ALZET 2001, 2004, 2006) or Micro-osmotic pumps (ALZET 1004). A filling needle is included in the box with osmotic pumps.
2. Reagents for osmotic pump infusion; as an example: Angiotensin II (AngII) purchased from Bachem, Cat # 1705; store at - 20 EC.
3. Sterile Saline
4. Plastic test tubes for dissolution of the reagent or incubation of osmotic pumps (4 - 15 ml; sterile)
5. Eppendorf tubes to prepare solution for individual mice (0.5 - 1.5 ml, sterile)
6. 1 cc syringe (sterile) to inject solution into osmotic pumps

Pump Information:

Alzet pump model	1004	2001	2004	2006
Duration (days)	28	7	28	42
Pump rate (µl/h) *	0.10	1.06	0.25	0.15
Mean fill volume (µl) *	101	233	246	243
Transient time (h)	48	4 to 6	40	40
Recommended incubation (h)	24	0	24	24

*pump rate and mean fill volume may differ with different lot #.

Calculation of AngII Amount

Step 3.

1. Design the experiment and determine the length of the infusion (e.g. 1, 2, 4, 6 or 12 weeks) and the infusion rate (e.g. AngII 500 ng/kg/min or 1,000 ng/kg/min).

2. Weigh mice before calculating the amount needed for osmotic pump infusion. Weight of a mouse < 20 g is not allowed for osmotic pump implantation (see Daugherty IACUC protocol # 2006-0009)
3. Use Microsoft Excel worksheet as a template to calculate amount of AngII and saline needed.
4. In the template, we assume that mice do not gain weight during infusion of AngII for 4 weeks. For infusion of other reagents, body weight gain should be determined based on the reagent, study duration, mouse model, and diet.

NOTES

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The following steps use AngII as an example, which are also applicable to or can be modified for other reagents.

Dissolution of AngII

Step 4.

1. Equilibrate AngII vial to room temperature before opening.
2. Weigh out the calculated amount of AngII use a sterile plastic tube. **Do not use glass because AngII solution has a high affinity for glass.** Please record the lot number of AngII in your note.
3. Add the calculated volume of sterile saline into the plastic tube with the lyophilized AngII, cap and mix thoroughly until the solution becomes clear.
4. Prepare AngII solution for each mouse based on body weight under a cell culture hood: Label mouse number on Eppendorf tubes (0.5 - 1.5 ml; sterile). Refer to the calculation on the excel worksheet for appropriate volumes. Pipette the calculated amount of sterile saline and amount of AngII solution into each Eppendorf tube.
5. Prepare and label mouse numbers on plastic test tubes (4 ml; sterile). These will be used for incubating pumps.

Osmotic Pump Filling

Step 5.

1. Pumps are supplied in two separate parts: the main body of the pump and the flow regulator. Open only the number of pumps and flow regulators needed for the study, as these cannot be stored once opened. Record lot # in your note. **ALWAYS USE GLOVES! Natural oils from your hands may damage the exterior of the pump casings.**
2. Weigh each pump (main body and flow regulator) individually, and note the weight to 4 decimal places (e.g. 1.1018 grams). This (called as “empty weight”) will be used to calculate the fill volume. Place each weighed pump in weight boat marked with the mouse number.
3. Attach the filling needle to a 1 cc syringe and carefully draw up AngII solution from Eppendorf tube. It is important to minimize the air drawn into the syringe along with the AngII solution.
4. Carefully remove all bubbles from the syringe and invert with the needle aimed at the floor. Keep the needle/syringe in this position to prevent the introduction of bubbles into the pump.
5. Gently insert the filling needle/syringe into the pump body (Figure 1). Advance the tip of the needle into the pump. Ensure the tip of the needle does not rest tightly on the bottom of the pump.
6. Fill the pump slowly. Notice the dark shadow inside the pump indicating the fluid level. Watch

this level rises as you continue to fill the pump. STOP filling the pump as soon as you see a bead of fluid rises out of the pump body. Carefully remove the needle/syringe and draw up the fluid out of the pump body.

7. Insert the flow regulator into the body of the pump. Make sure the regulator is seated tightly against the pump body. As you insert the regulator into the pump body, you may notice some fluid leaking out the opening of the flow regulator. THIS IS NORMAL. Carefully blot up all extra fluid that might have leaked during the regulator placement.
8. Weigh the filled pump. This is now marked as “fill weight”. Filling volume = “fill weight” - “empty weight” (denoted as “ìl”). Assume that 1 ìl of fluid = 1 ìg of weight. Compare this calculated volume to the mean fill volume indicated in the Instruction for the lot #. See the following example for calculation.

Mouse ID	Empty Weight (g)	Fill Weight (g)	Actual Fill volume (µl) = (Fill Weight - Empty Weight) x 1000	% Fill = (Actual Fill Volume/ Mean Fill Volume*) x 100%
1	1.2245	1.4674	= (1.4674 - 1.2245) x 100 = 242.9	= (242.9/243) x 100% = 100%
2	1.2437	1.4934	= (1.4934 - 1.2437) x 100 = 249.7	= (249.7/243) x 100% = 103%

* assume the Mean Fill Volume is 243 µl

9. Place the filled pump into the labeled test tube (see Dissolution of AngII Step 5) with the regulator head facing UPWARDS. Add enough sterile saline to cover the pump. Pumps should be kept in test tubes with saline until ready for use.
10. Place test tubes in a 37 ºC incubator. As a general rule in Daugherty lab, for Alzet 2004 model, incubate pumps overnight (at least 12 hours) to allow partial priming. Pumping of AngII will start 24 hours after the implantation surgery. This allows mice to recover from the surgery prior to the potential stress of AngII infusion. If a second pump is needed to continuously infuse AngII after removal of the first pump, it must ensure that pumping has already started before the surgery. This requires that pumps are incubated in sterile saline at 37 ºC for > 40 hours (for Alzet 2004).

Step 6.

Surgical Implantation of Mini-Osmotic & Micro-Osmotic Pumps

Equipment For Pump Implantation

Step 7.

1. Sterile Pack: one pair of large surgical scissors, straight hemostats, forceps, 9 mm wound clip kit, drapes, cotton swabs, gauze squares
2. Sterile gloves
3. Surgical mask
4. Yellow gown
5. 3 conical tubes: Betadine,70% ethanol, Sterile water.
6. Pumps for implantation
7. Shaving implement
8. Antiseptic handrub
9. Topical 4% Lidocaine
10. Isoflurane vaporizer
11. Induction chamber
12. Nose cone

13. Oxygen
14. 2 charcoal canisters

Preparation of Autoclave Pack

Step 8.

1. Put gauze and cotton swabs on a surgical drape and fold corner to corner and put an autoclave tape on it. Wrap the pack with another drape and tape with autoclave tape.
2. Put all the surgical tools (scissors, hemostat, forceps, staples and stapler) in the sterilization pouches and seal.

Autoclave both the packs in dry cycle.

Preparation of Vaporizer

Step 9.

- Fill Isoflurane and have induction box in the prep area and nose cone in the clean area of laminar flow hood. (See vaporizer details to use the equipment http://www.surgivet.com/catalog/anesthesia-equipment/cds-9000-small-animal.html).

Preparation for Surgery

Step 10.

1. Set up betadine, 70% ethanol, sterile water, bead sterilizer, hand cleaner, swabs, gauze, and the pumps in the laminar hood.
2. Surgeon puts on the mask and gown, and then opens up the outer drape in the laminar hood with clean hands. Put on sterile gloves and open the inside pack.
3. Set up sterile drape with nose cone in the laminar flow hood.

Surgical Procedure

Step 11.

1. Place mouse in induction chamber. Once anesthetized, shave area over the shoulder to be implanted.
2. Place mouse in laminar hood with nosecone. Mouse head points toward your dominant hand.
3. Swab and wipe area with betadine three times, and then 70% ethanol.
4. Use surgical scissors to 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 but not the underlying tissues
5. Use one hand to hold forceps to open the incision, and use another hand to hold a straight hemostat to make a subcutaneous tunnel under the skin.
6. Advance the tip of the hemostat toward the tail. Create a pocket for pump. This is accomplished by carefully opening the jaws of the hemostat under the skin to open up a pouch. Pull the hemostat back out of the incision as you close the jaws back together.
7. Insert pump with the regulator head first into the incision.(Pointed toward the tail end of the mouse).
8. Gently push the pump completely into the pocket. There should be enough skin free to close the wound with no tension or stretching of the skin needed.
9. Once the pump has been inserted, firmly pinch both sides of the incision and staple the incision.

Inspect the incision site to ensure that there is complete closure of the wound.

10. Apply Topical 4% Lidocaine cream with a clean swab.
11. Return the mouse to its cage, and repeat above steps for the next mouse.
12. Place surgical instruments into a bead sterilizer for 10 seconds between mice. Dip in sterile water, be sure to allow instruments to cool before use. Clean hands with Antiseptic handrub between mice.
13. Monitor all mice until full recovery is achieved. Fill the post operation card. Monitor mice closely within 7 days after the surgery.
14. Remove wound clips between 10-14 days