Q&A Report:

The Trainer's Toolkit for Osmotic Pumps: Supporting Researchers in Infusion Studies

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Training

- **When training how many individuals would you train at a given time?**
  - Typically, 3-4 individuals per trainer.

- **How much time would you allocate for training for subcutaneous implantation and then for intraperitoneal implantation?**
  - It typically depends on the skill sets and experience of the individuals being trained.
  - The first step is learning the component parts of what the procedure requires.
    - Skills in aseptic surgical techniques, anesthesia, gentle tissue handling, and wound closure are pertinent. It is appropriate to learn the basic skill sets first prior to learning to perform the actual surgery. University of Kentucky offers different workshops in these basic skill sets to prepare individuals to conduct surgical procedures.
  - Subcutaneous implantation is considered a minor surgery (does not involve a major body part) and is less invasive. Intraperitoneal implantation, is considered a major surgery procedure, involves opening a body cavity and the outcome could be more critical if closure is not done well. We have individuals practice all initial components and develop competencies first on them, then let them pursue the actual surgical training. Practice is done on inanimate objects, then carcasses.

- **Is there anything that you especially try to make sure you do when supporting people as they prepare for a study with the pumps?**
  - Verify individuals possess all skill sets prior to embarking on surgery. Ensure plenty of practice opportunities are available to use inanimate objects and carcasses initially. Assure individuals understand critical follow-up and post operative care. Make certain folks understand challenges that can occur, prepare to intervene early and know how to obtain assistance if needed.

Preparing the Pumps

- **I would like to know a little bit about priming the pump, if you could share:**
  - All ALZET pumps have a start-up gradient during which the pumps absorb fluid to reach the constant osmotic pressure required to pump at their predetermined rate. To avoid this start-up period happening *in vivo* the pumps can be "primed" *in vitro*. Priming is required when immediate pumping is required, a catheter is used, a viscous solution is delivered, or if the drug solution may have acute toxic effects. Priming times vary based on the model.
    - Recommended priming times and a full description of how to prime can be found on the ALZET website: https://www.alzet.com/guide-to-use/filling-priming-alzet-pumps/
    - Information about the start-up gradient and how the pumps work can also be found on the ALZET website: https://www.alzet.com/products/alzet_pumps/how-does-it-work/

- **Are there any activation procedures required before loading the drug into ALZET pumps, are they the same for aqueous and lipid soluble?**
  - There is no activation procedure required before loading the pumps with any solution. Just make sure the solution is compatible with the pumps and remains homogenous at 37°C for the entire duration it is in the pumps.

- **Are there any specific types of drug/compounds not suitable for delivery through the pump?**
  - ALZET pumps can deliver most compounds provided they are stable and homogenous in solution for the entire time they would be in the pump. To check if a specific compound has been delivered before, please email ALZET Technical Support: techsupport@alzet.com

- **How should you be formulating a solution for a Brain Infusion Kit delivery? Do you formulate solutions the same way as an osmotic pump that's implanted subcutaneously or intraperitoneally?**
  - Formulating the solution for use with a Brain Infusion Kit Delivery is the same as without one. The compound must be stable and homogenous in solution for the entire time it would be in the pump and Brain Infusion Kit. You should also consider solution compatibility with the vinyl tubing of the Brain Infusion Kit.

Surgical Technique and Procedure

- **Does the direction of the pump matter for subcutaneous implantation?**
  - Port away from incision is preferable so that wound healing is not affected, especially if the compound being delivered is an irritant.

- **Do you have any advice on the position of the pump subcutaneously if the animal needs to be handled during the experimental period, for performing intraperitoneal injections for example?**
  - It might be helpful to consult a staff surgeon/veterinarian, but the pump can be implanted further down the back and on the opposite side of where the intraperitoneal injection would be in the abdomen.

- **Do you have any suggestions for how to prevent the wound clips from slipping?**
  - Use of good technique and proper application. This requires practice. Also, ensure proper size of wound clips for the animal. For instance, rats many need a different size (9 mm). Mice and young rats may need 7 mm size clips. Space wound clips appropriately and make sure the clips align. Practice on inanimate objects, such as making a slit in a drape, etc. and practice use of wound clips. Additionally, practice use of wound clips on carcasses, prior to use on valuable study animals.

- **How tight should the pocket be for a subcutaneous pump connected to a Brain Infusion Kit?**
  - The pocket should be large enough to accommodate the pump and permit some pump movement, but not so large as to allow the pump to slip down into the flank of the animal.

- **Can you expand on removing the osmotic pumps after the drugs/agents are delivered?**
  - To totally remove an osmotic pump, use the following procedure:
    - Anesthetize the animal and prep the surgical site, according to your institution's guidelines on conducting survival surgery procedures.
    - Make an incision in the skin over the surgical site.
    - Use a sterile hemostat or similar tool to grasp and gently pull the pump out of the subcutaneous tissue.
    - If pump is tightly adhered to connective tissue, it may need to carefully be separated from the surrounding connective tissue to remove it.
    - Close the incision with wound clips, suture, or surgical glue (if very small).
    - Recover the animal following your institution's anesthesia recovery guidelines.
    - Provide analgesia as listed in your approved IACUC protocol.
- The ALZET website offers detailed information regarding explanting in this Technical Tip: https://www.alzet.com/resources/alzet-technical-tips/#1560370752388-6cf26883-eff2

- **What type of analgesic would you use for a subcutaneous ALZET pump surgery?**
  - Analgesic treatment should be provided under the direction of the staff Veterinarian.

- **Are there any major differences between subcutaneous implantation and intracerebroventricular implantation that we should be aware of?**
  - These are 2 distinct methods of drug delivery to animals. Subcutaneous implantation involves delivery into the subcutaneous tissue, and absorption of an agent to provide systemic administration. Intracerebroventricular implantation is a procedure where a minipump is implanted subcutaneously and is connected to a cannula that delivers a substance to the cerebral ventricles or brain tissue. Drugs are delivered directly to the brain for targeted effects.
    - See this webpage for written surgical procedures: https://www.alzet.com/resources/downloads/#surgical-sheets
    - Also see the video of all surgical procedures: https://vimeo.com/channels/alzet/139614384

ALZET Pump Product Information

- **How delicate is the outer membrane of the pump while handling with e.g. forceps?**
  - Handling with forceps should be fine. The pumps can withstand quite a bit of force (when pinching with fingers they are quite hard).

- **How many refills of the subcutaneous implanted pumps do you consider to be safe?**
  - ALZET pumps are **one-time use only** and cannot be refilled. They can be serially replaced. Please refer to the Tech Tip on the ALZET website: https://www.alzet.com/resources/alzet-technical-tips/#1560370752388-6cf26883-eff2

- **Most of the videos showed the osmotic pumps in rats. Are they smaller for mice?**
  - The 100 µl and 200 µl models can be used in mice. We always recommend using the smallest pump possible for your study. Information about animal size requirements for each pump size can be found on the ALZET website: https://www.alzet.com/pump-selection/#animalsize

Specific Use Cases

- **We insert a telemetric probe in the abdominal area; can we make a subcutaneous pocket around that surgical site for inserting pump or it needs to be a separate surgical site on the back?**
  - Usually, telemetry involves surgically placing the pump and associated telemetry devices into the animal's body for continuous drug delivery and data collection. We recommend consulting with the vendor of the telemetry system / apparatus being used. Use a separate surgical site on the dorsum, or pump can be inserted near telemetry device.

- **Can we do echocardiography before the end of study or before the removal of pump?**
  - Echocardiography can be done before the end of study/removal of pump. There are over 1000 studies in the ALZET database of publications that mention echocardiography. If you are interested in a list of these studies, please contact ALZET Technical Support techsupport@alzet.com

- **Is intermittent release possible with any of the pumps?**
  - Intermittent release can be done by connecting catheter tubing to the pump. The pump itself is filled with saline, and the catheter tubing would have alternating drops of drug solution and non-miscible compound. Please refer to the Catheter Use page on the ALZET website for more details.

- **Do you have any specific examples of adhesives to keep the pumps attached to the mouse skull?**
  - Dental cement and cyanoacrylate adhesive are the most common. ALZET sells Loctite, a cyanoacrylate adhesive, which can be used for this purpose.

Troubleshooting

- **When using the Brain Infusion Kit in mice, the skin around the cannula may gradually harden and become exposed. Is there any way to prevent this?**
  - Adhesion of cannula to skull can be improved if care is taken to clean tissue from the skull surface around the cannula site and if the site is dry. Try to ensure that the cyanoacrylate adhesive or dental cement is fully dry before closing the skin over the cannula.

- **How do you make sure the pump doesn't get clogged?**
  - The drug solution should be stable and homogenous for the entire time it would be in the pump to prevent precipitation and clogging.

- **Have you seen any explanation or migration issues with the pumps in specific strains?**
  - We have not.

Housing/Animal Care

- **Do you social house animals after the wound heals/clips removed or just after they recover from anesthesia?**
  - Once the animals have fully recovered from anesthesia and can move freely about the cage environment on their own, the animals can/should be re-joined with familiar cage mates. Monitoring must still occur as described in the approved animal protocol to ensure the incision sites are healing well and animal welfare is appropriate (e.g. pain relief; return to normal behavior).

- **How do you manage pressure sores that form around the pump?**
  - Most individuals using minipumps have no skin lesions. Skin lesions are not expected. If this does occur, likely the pump is too large for the animal (e.g. outside manufacturer weight guidelines). Additionally, problems may occur if the subcutaneous pocket is not made large enough for the pump when the solution being delivered is very irritating or a possible post-surgical infection.
  - When implanted correctly (subcutaneously on back over shoulder blades): sterile technique; appropriate sized subcutaneous pocket, right size animal for pump model, a pump should not cause any skin irritation. If the solution in the pump may be irritating, it may be better to either put the pump in the peritoneal cavity (if the animal is large enough) or attach a catheter such that a pump placed subcutaneously delivers the solution intraperitoneally through the catheter.
  - Below are also some tips to help prevent skin irritation and pressure necrosis:
    - Ensure use of aseptic surgery principles (sterile technique; sterile instruments; appropriate surgical site preparation).
    - Ensure the subcutaneous tunnel is at least 1 cm longer than the pump and insert the pump with the **delivery portal facing away from the incision**.
    - Use analgesia pre-op, prior to procedure, per your IACUC protocol.
    - Avoid direct pressure and ensure pump does not rest directly beneath the incision.
    - Regularly monitor animals post-procedure to ensure the incision site is healing well and take appropriate action as directed by your veterinary staff / follow IACUC protocol for managing complications.

- **If animals are subject to disease induction that leads to weight loss, what are the considerations if their weight falls below the minimum recommended for the pump after treatment begins?**
  - It may be dependent on how significant the weight loss is (5% vs. 15-20%). Perhaps pre-planning for the estimated/typical weight loss observed in the study and pair with a pump that will accommodate that situation may help. It is pertinent to follow your IACUC protocol regarding complications and endpoints and this would be a good area to consult with your institution's veterinary staff on.