

Technical Note

Micro SMP-200



Recommendations for Intrathecal Administration

INTRODUCTION

iPRECIO® is a completely implantable, programmable micro-infusion pump system for experimentation in small laboratory animals. The pump has a built-in microprocessor which can be programmed to administer small volumes, in vivo, for extended durations. Additionally, the pump houses a septum designed for percutaneous access, through which filling and exchange of solutions is made possible. The iPRECIO® system's highly precise, in vivo-, capabilities uses a patented, high accuracy, mechanical pump technology, the "Rotary Finger Method", which was developed by Primetech.

Intrathecal administration is a useful in-vivo method which allows a quick drug efficacy test with a low-dose agent to the spinal cord or brain for central nervous system studies. It is expected that chronic intrathecal administration using iPRECIO® in a free-moving animal model contributes to anticancer drug development, pain research, and the study of the central nervous system including stroke research, spinal cord injuries (SCI) and regeneration.

The total volume of cerebrospinal fluid in rat is around 150-300µl and it is replaced at least 6 times per day. For chronic administration, it is recommended that the infusion flow rate does exceed 3.0µl/h. Also, the body weight of the animal should be at least 250g. Successful surgery for intrathecal catheterization requires specialist surgery techniques and instruments such as stereotaxic apparatus. It is recommended that the adequacy and location of the intrathecal catheter is confirmed by lidocaine tests or another appropriate method prior to implanting and using the iPRECIO® pump with the catheter.

WARNING : iPRECIO® Micro – Infusion Pump is not intended for human use.

REQUIREMENTS

Perioperative Care (Recommended products are shown in parentheses)

- Antibiotics and the treatments
- Anesthetic agents and the techniques
- Heating Pad
- Surgical glove, mask, cap and gown
- Sterilized gauze
- 70% Isopropyl alcohol
- Disinfectant soap
- Sterile PBS solution or Saline
- Electric shaver or Hair remover
- Stereotaxic apparatus
- Surgical retractor
- Intrathecal catheter (Supplied by ReCathCo, LLC.)
- 3-0 to 5-0 non-absorbable suture with curved needle
- Surgical scissors, tweezers, forceps, and sterile scalpel blade with handle
- Scalprum
- 20-gauge needle

- Needle introducer (for more detail, see below)
- Sealing putty
- Wound clips and wound clips applier
- Tissue adhesive (3M™ Vetbond™ Tissue Adhesive supplied by 3M)

METHODS

1. Perioperative Care

Careful attention to sterile techniques and the use of sterile equipment are crucial to successful surgery. Additionally, antibiotics are most effective to administer pre- and post-surgery in order to maximize blood levels during surgery and recovery (e.g. 0.5mg/ml gentamicin 1ml i.p.). Primetech recommends the use of a heating pad to prevent decreased body temperature in the peri- and post-operative animal.

2. Anesthetize the Animal

Anesthesia must be used to ensure a reliable experimental result. General anesthesia should be maintained for around 60 minutes. Primetech recommends using an anesthetic method that supports prompt postoperative recovery.

3. Animal Placement ⋯See Fig.1

1. Remove hair on the occipital area.
2. Scrub with disinfectant soap and isopropyl alcohol. A series of three scrubs with both the disinfectant soap and alcohol is recommended.
3. Place the animal in the stereotaxic apparatus.
4. Put the animal head in the stereotaxic apparatus above the heart and ensure that respiration is not restricted in anyway. Adjust the animal position on to the mid-spinal line.

4. Intrathecal Catheter Preparation ⋯See Fig.2

1. Adjust the length* of the intrathecal catheter according to the animal size and administration site. For guidance, refer details below;
 - 7.5cm from first to fifth cervical vertebra in 250-300g B.W.
 - 8.0cm from first to fifth cervical vertebra in 300-350g B.W.
 - 8.5cm from first to fifth cervical vertebra in above 350g B.W.
 * Adjust for animal strain, body weight, and infusion site.
2. Make a knot with a suture on the beads of the catheter. Keep 5 cm of suture on both sides of the knot.
3. Fill sterile PBS or saline solution into the catheter with the guide-wire.
4. Maintain clean and sterile conditions at all times and take special care before insertion.

5. Access to First Cervical Vertebra ⋯See Fig.3

1. Palpate the occipital area to find the boundary line between the occipital bone and muscle.
2. Put the edge of the scalpel blade at 2-3mm cranially from the boundary line.
3. Pull the skin up to the top of the head to make a 3cm incision on the dorsal midline of the occipital area.
4. Make an incision on the muscle layers to expose the posterior atlanto-occipital membrane. Separate the dissected muscle and tissue with a retractor.
5. Chip off remaining tissues with scalprum to expose a cisternal membrane.

6. Intrathecal Catheter Insertion ⋯See Fig.4

1. Make a needle introducer by bending the tip of a new 20 gauge needle to 90 degree angle using forceps. The needle introducer should be smoothed with a grinder before use.
2. Scrub the membrane delicately with the tip of needle introducer. Cerebral spinal fluid (CSF) will appear when the membrane has been incised. Take special care to minimize CSF loss.
3. Place the catheter on the mid-spinal line, then guide the catheter along the incision on the head. Make sure the tip of guide-wire is covered by the catheter to prevent damaging the spinal cord.
4. Guide the tip of the catheter with the needle introducer into the incision site of membrane.
5. **GENTLY** insert the catheter into the spinal space until the beads of the catheter.
6. Make sure the incision site is free of bleeding/blood. **IF** blood/bleeding is seen, it is recommended that the animal is sacrificed. Re-start from the beginning with a new animal and be more careful when inserting the catheter. Also, ensure that the guide wire is always covered by the catheter during the insertion process.

7. Intrathecal Catheter Fixation ⋯See Fig.5

1. Suture the catheter on to the inner muscle layer with a suture between the beads.
2. Remove the retractor, suture an inner muscle layer, an outer muscle layer and a skin across the catheter.
3. Remove the guide wire.
4. Flush the catheter with 10µl PBS or Saline.
 - If the catheter is placed properly, the animal shows no-abnormal behavior.
 - If the catheter is **NOT** placed appropriately, it cannot be flushed correctly, and/or the animal behaves abnormally with twisting of the lower body, seizure response, and/or behavior which suggests pain. If this is found, the animal should be sacrificed.
5. Putty the catheter, and remove the animal from the stereotaxic apparatus.
6. Allow 3-7 days for the animal to recover after surgery and then conduct a recovery test to confirm location of catheter tip.

8. Recovery Test with Intrathecal Catheter ⋯See Fig.6

Lidocaine test

This lidocaine test assumes that the catheter tip is located in the lumbar vertebrae domain. For example, in case the catheter is located in the thoracic vertebra domain that is near to the apneustic center, breathing of the animal may be inhibited by the test. It is recommended that the appropriate test is performed to confirm the region where the catheter tip is located.

1. Fill 10µl PBS solution and 7-10µl 2% lidocaine across a small air gap in 50µl Hamilton syringe.
2. Lightly-anesthetize the animal.
3. Remove the putty protecting the catheter, and gently infuse the filled solution.
 - If the animal recovered properly and the catheter is correctly located, paralysis of the lower part of the body is observed. The paralyzed animal will recover within around 5 minutes.
 - If the catheter is **NOT** placed appropriately, the catheter cannot be flushed correctly, and/or the animal behaves abnormally with twisting of the lower body, seizure response, and/or behavior which suggests pain. If this is found, the animal should be sacrificed.
4. After confirming that the catheter tip location and the animal condition are fine, the iPRECIO® pump may be implanted and connected to the catheter.

9. Connecting Intrathecal Catheter with iPRECIO, and Pocket Making ⋯See Fig.7

1. Position the animal in sternal recumbency on the heating pad.
2. Remove the hair from incision site and scrub with disinfectant soap and isopropyl alcohol. A series of three scrubs with both the disinfectant soap and alcohol is recommended.
3. Make an incision on the skin sutured previously.
4. Using blunt dissection, create a pouch under the skin from the point of the incision to the

- caudal area by separating the skin from the underlying tissue with scissors.
5. Cut the outlet tube to around 10cm under sterile conditions.
 6. Fill the solution to the tip of the outlet tube.
 7. Connect the catheter with the outlet tube of the pump. Any part of the catheter outside of the animal's body should be sterilized as a precaution by wiping the surface with disinfectant soap and isopropyl alcohol.
 8. Activate the pump.

The pocket should be the appropriate size (not too large and not too small) for pump fixation, low-stress implantation for a successful long-term infusion.

10. Pump Fixation ⋯See Fig.8

1. Place the pump into the pocket with a stress loop.
2. Suture the pump through the muscle layer.

Over tightening of the sutures may induce a tissue necrosis and laceration due to compression.

11. Wound Closing

1. Close the skin incision with non-absorbable suture or wound clips. Once closed, seal the incision with tissue adhesive.

Proper pocket closing will help in wound healing and help to prevent infection. Apply iodine on sutured skin to reduce risk of infection.

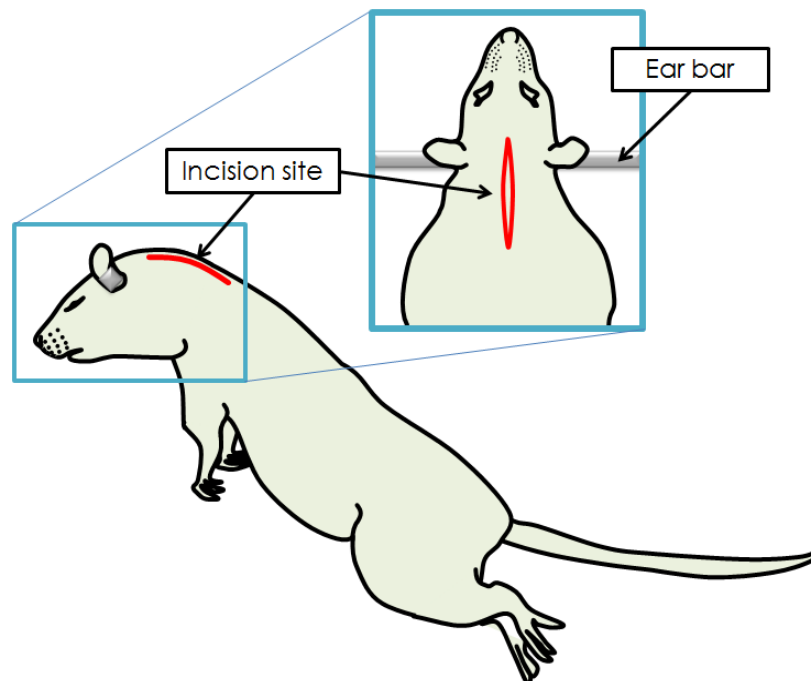


Fig. 1 Animal Placement

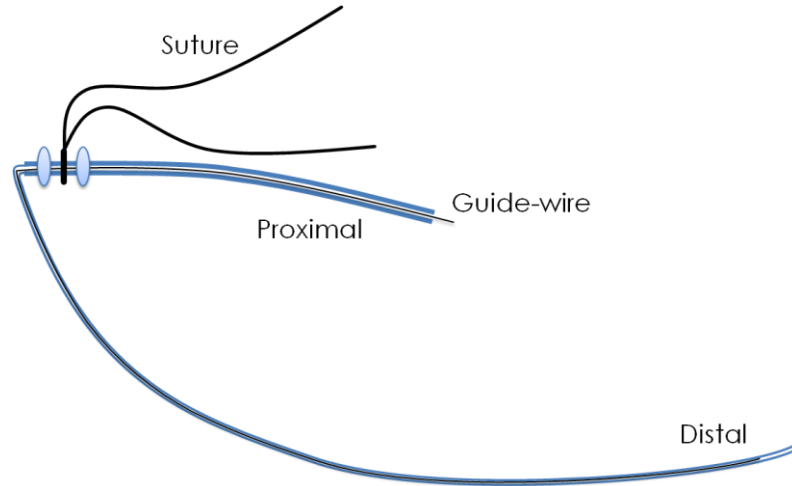


Fig. 2 Intrathecal Catheter Preparation

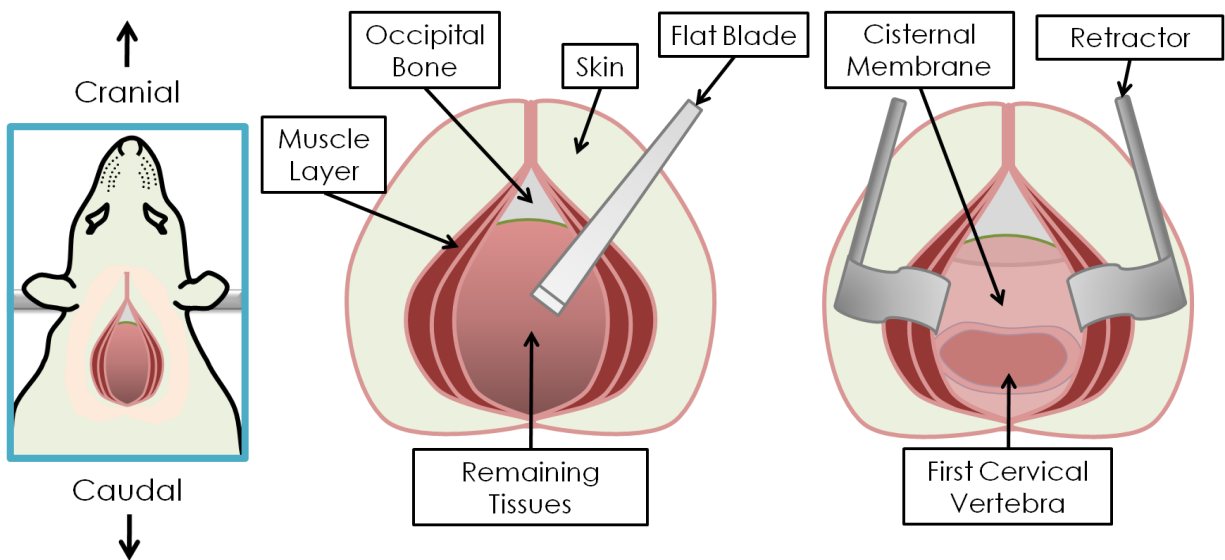


Fig. 3 Access to First Cervical Vertebra

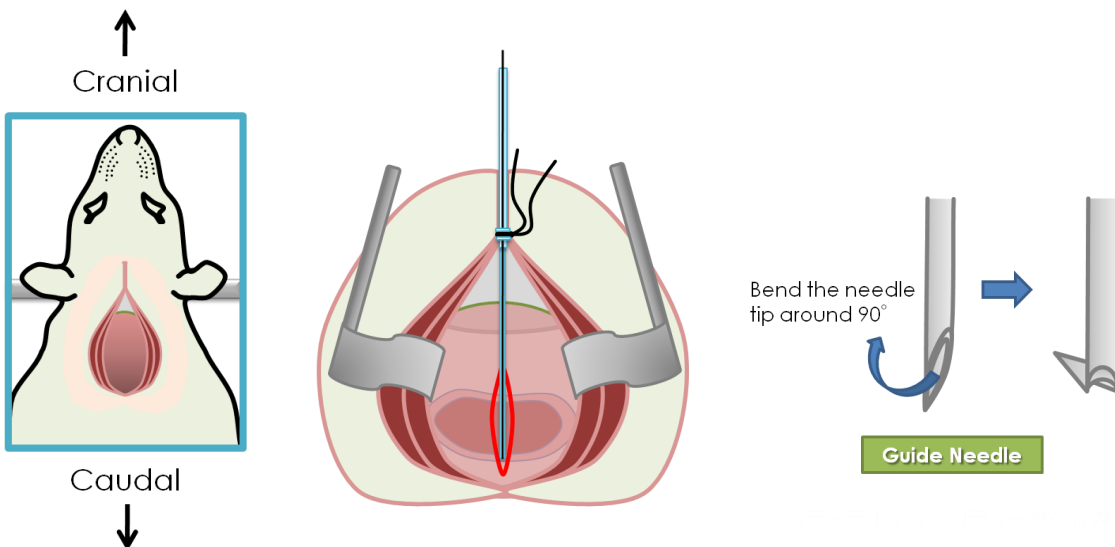


Fig. 4 Intrathecal Catheter Insertion

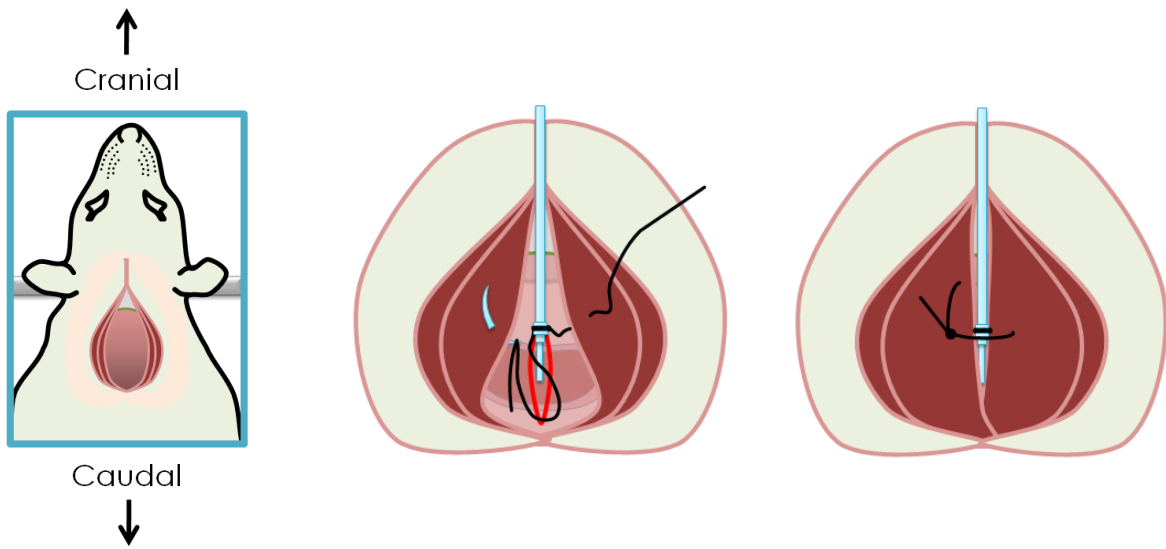


Fig. 5 Intrathecal Catheter Fixation

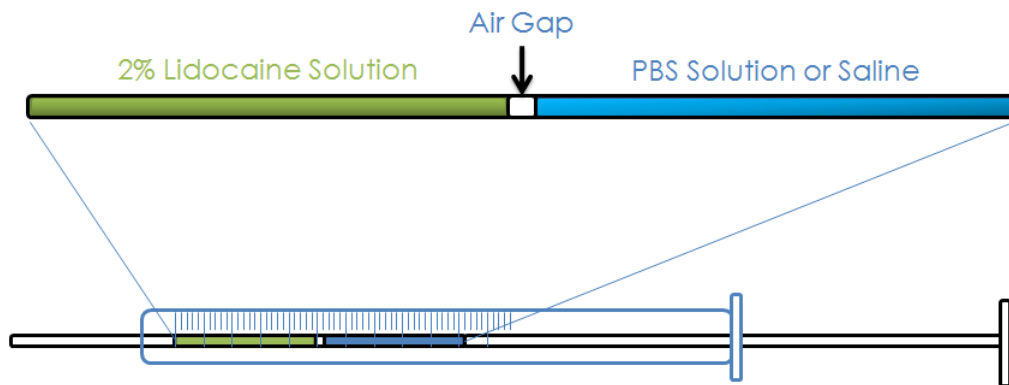


Fig. 6 Recovery Test of Intrathecal Catheter

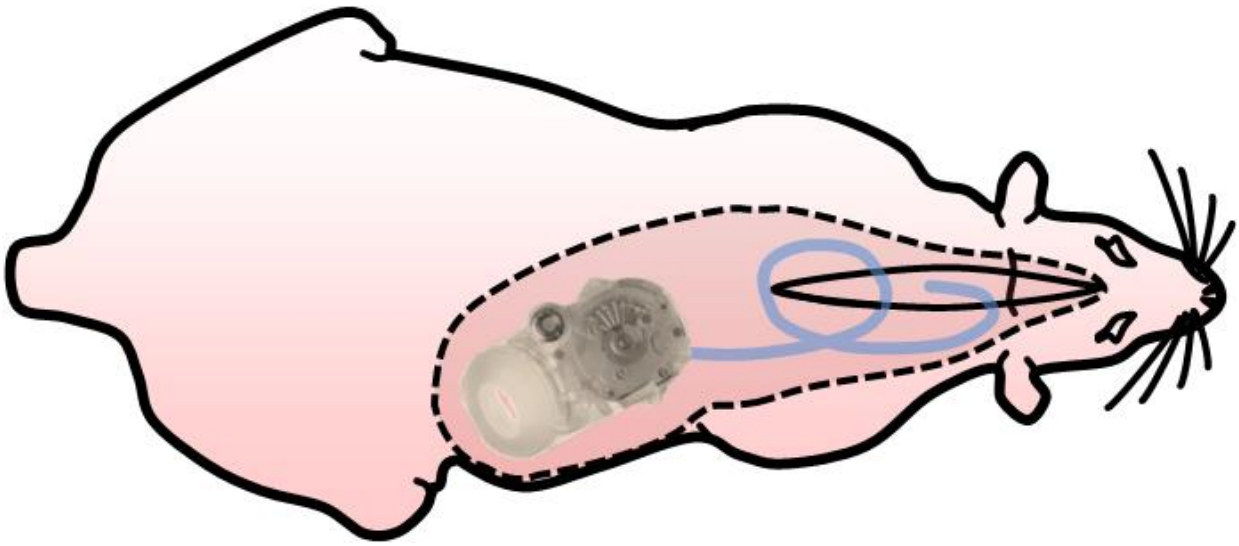


Fig. 7 Connecting Intrathecal Catheter with iPRECIO, and Pocket Making

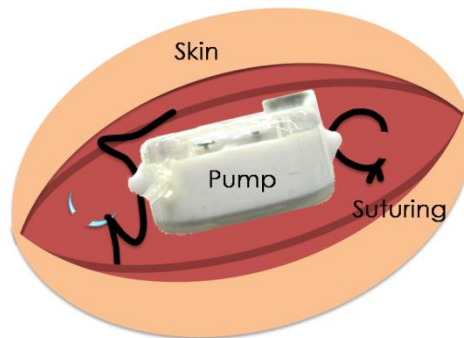


Fig. 8 Pump Fixation: View from the back of the pump with septum port on the right hand side

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