TITLE: Rodent Surgery

SCOPE: All Animal Program Personnel

RESPONSIBILITY: Veterinary Staff, Facility Manager, All Animal Personnel and Research Staff

PURPOSE: To Outline the Proper Procedures for the Performance of Rodent Surgery

I. PURPOSE

1. In accordance with the Guide for the Care and Use of Laboratory Animals and 9 CFR, the Animal Welfare Act (AWA), all survival surgical procedures on rodents must be performed using aseptic procedures. This includes the use of sterile instruments, and the aseptic preparation of the surgical site in order to prevent postoperative infections. A rodent surgical area can be a room or portion of a room that is easily sanitized, but must not be used for any other purpose during surgery.

2. Surgery can be classified as minor or major in nature.

II. RESPONSIBILITIES

1. The Veterinarians oversee all aspects of animal health, and are assisted by all program staff. Facility Managers ensure implementation of all procedures. Research staff is required to follow these guidelines.

III. PROCEDURES

1. OVERVIEW
   a. Preoperative preparation includes assessment of the animal’s health, induction of anesthesia, preparation of the surgical site for aseptic surgery, the use of sterilized instruments, and adherence to aseptic technique.
   b. Survival surgical procedures require aseptic technique which is used to reduce microbial contamination to the lowest possible practical level. Survival procedures are those from which an animal recovers from anesthesia.
   c. Non survival surgical procedures do not require aseptic technique. For these procedures, at a minimum the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean.
   d. Minor surgery does not expose a body cavity and causes little or no physical impairment (e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy). These procedures are routinely done on an “outpatient” basis in veterinary clinical practice.
   e. Major surgery (e.g. laparotomy, thoracotomy) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection.
2. SURGERY AREA PREPARATION
   a. Rodent surgery is conducted in a dedicated area free of clutter, large enough to accommodate all supplies and equipment, easily sanitized, used only for operative purposes during surgery, and separated into the four functions of:
      1. Pre-operative rodent preparation
      2. Surgeon preparation
      3. Area for rodent surgery
      4. Post-operative rodent recovery
   b. Exposed cork or Styrofoam boards must not be used during survival procedures.
   c. The area immediately surrounding the area for surgery should be disinfected prior to surgery to decrease dust borne contamination.
   d. The equipment used for rodent surgery (e.g. anesthesia equipment, lights, surgical microscopes, stereotaxic equipment), which cannot be sterilized by other means, should be thoroughly wiped clean with Oxivir Tb, Clidox or Sporicidin in accordance with SOP #1015, “Decontamination of Common Procedural Areas.”
   e. Surgical instruments must be sterile or subject to high level disinfection when appropriate in accordance with SOP #1017, “Surgical Pack Preparation” and SOP #011, “Reagents, Solutions and Decontaminants – Labelling and Use.” Heat sterilization using an autoclave is recommended. Non autoclavable instruments and supplies may undergo high level disinfection with chemical agents (i.e., Metricide OPA 0.6% ortho-Phthaldehyde). Chemical disinfectants must be thoroughly rinsed off of instruments with sterile saline or sterile water before use.

3. ANIMAL AND SURGEON PREPARATION
   a. A pre-surgical weight should be recorded and an evaluation should be performed (within 24-48 hours prior to surgery) to ensure the animal is not exhibiting unexpected signs of illness. The animal should be alert, and have a smooth hair coat and clear eyes. The withholding of food is not necessary in rodents unless specifically mandated by the protocol. Water should NOT be withheld unless required by the protocol. Withholding of food for more than six hours should be described in the animal use protocol.
   b. Preparation of the animal should include application of a bland ophthalmic ointment to protect the corneas from drying and removal of hair by clipping or shaving the operative site with enough border area to keep hair from contaminating the incision site. Hair removal should be performed in a location remote from the surgical area. The surgical site will be painted with an alcohol based skin sanitizer containing 2% Chlorhexidine, being careful to begin application in the center and move out towards the periphery. The site can then be dried using sterile gauze. (Note that excessive saturation with liquid will contribute to hypothermia if used too liberally.) Finally, the area should be draped. This not only helps prevent stray hair from entering the surgical field, but also provides an area on which to lay sterile instruments during surgery. (Note: Sterile, adhesive plastic drape material (Bioclusive®), may be useful as the primary/initial drape of the surgical site.)
   c. Preparation of the surgeon must include a thorough scrub his or her hands with a bactericidal scrub. The use of sterile, surgical gloves is required. A surgical mask and clean lab coat are required for use during survival surgeries. The wearing of a hair net or surgeon’s cap is encouraged.
d. Surgical instruments, gloves* and other paraphernalia may be used on more than one animal. Items used on multiple animals must be carefully cleaned and disinfected between animals. One method commonly employed is the hot-bead or glass-bead sterilizer (“Germinator”). Prior to use in the next/subsequent surgery, surgical items are cleaned with sterile water and the tips re-sterilized using a hot-bead sterilizer. Instruments should then be cooled prior to handling animal tissue. **Alternating sets of instruments** is another way in which to allow adequate time for instruments to be exposed to a disinfectant or sterilant solution.

*Sterile surgical gloves may be worn in multiple surgeries except when sterile technique has been compromised.

4. **INTRAOPERATIVE AND POST OPERATIVE MONITORING AND CARE.**

   a. **Monitoring of the animal** during surgery is critical. Evaluation of anesthetic depth is usually of first importance. Techniques for monitoring anesthetic depth vary somewhat with the agent used. A quiet animal that does not move when a painful stimulus is applied is the most certain indicator of adequate anesthesia. However, the zone between quiet and too quiet is very narrow in rodents. Depth of anesthesia will be monitored via inter-digital pinch, palpebral/eye blink reflex, and depth and rate of respiration.

   b. Maintaining body temperature is next in importance. A warm water blanket or hot water bottles provides supplementary warmth without being too hot. Bubble wrap may help a small rodent maintain body temperature. During long surgeries, warmed sterile fluids (saline or lactated Ringer’s solution) should be provided. These can be administered subcutaneously or intravenously. Any tissues exposed for extended periods during surgery should be kept moist with these same warmed solutions.

   c. **Closure of body cavities** is typically accomplished in at least two layers, with an absorbable inner layer(s), and a nonabsorbable skin layer, or absorbable subcuticular layer. A continuous pattern is used to close the inner layer, while the outer skin/layer is closed with a simple interrupted pattern. For external closure, skin wound edges should first be approximated with nonabsorbable suture material, to eliminate skin edge tension, before tissue adhesive is applied. Note - If clinical and/or research experience indicates that external wound edge approximation is not warranted in a wound of this size, at this anatomical location, and that the use of tissue adhesive only for surgical skin closure has been satisfactory, this would be indicated in the animal use protocol. If present, nonabsorbable skin sutures or staples should be removed 10-14 days post-operatively.

   d. Observation and **attendance during postoperative recovery** is important. A recovering animal should be watched closely until securely in sternal recumbency, and capable of purposeful movement. The animal, in or out of its cage, must be kept warm. Ancillary heat can be provided by using warm-water circulation heating pads, heated saline bottles/bags, heated (static) isothermal warming pads or blue “underpads.” The use of electric heating lamps is limited to post-ultrasound imaging and post-behavioral water maze tests. Protocols with this exception should clearly state that monitoring of the heat lamp’s use is through a thermometer at the cage level. If electric heating pads are used as an indirect source of heat, approximately ½ of the cage should be in contact with the heating pad. During the continuous observation until capable of purposeful movement, the animal should be turned on its opposite side at least every 20 minutes so that it will not overheat in
accordance with SOP #028 entitled Thermal Regulation of the Anesthetized Patient. This provision ensures that when an animal has recovered from anesthesia, it can move away from the heat source. Warmed fluids can be administered subcutaneously or intravenously to ensure that the animal is adequately hydrated. Over hydration is not generally a problem in animals with normal kidney function.

e. Signs indicative of postoperative complications that require resolution include the following: lack of activity/inquisitiveness, labored or abnormal breathing, unkempt appearance, increased or decreased movement, self-mutilation, abnormal posturing, dehiscence of surgical site, redness or swelling around the surgical site.

f. Postoperative observations must include a minimum of a daily observation of the general condition of the animal and of the surgical site until sutures or staples are removed at 10-14 days. For procedures involving the use of surgical glue and/or subcuticular sutures only, the animals should be observed for a minimum of 10 days. Any foreign substance left in the incision for a long period of time serves as a nidus of irritation and infection. A veterinarian should examine incisions that do not appear to be healing.

g. Recordkeeping is required for rodent surgery. For survival surgical procedures, the Rodent Surgery/Procedural Record (CMDC #139) must detail preemptive analgesic, anesthesia, recovery, post-operative analgesic, observations, complications, treatments, and suture/staple removal, if necessary. Monitoring should be documented every fifteen minutes for all surgical procedures including terminal procedures lasting longer than fifteen minutes using the Rodent Record of General Anesthesia (CMDC #206).

IV. SUTURE SELECTION

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<tr>
<th>SUTURE</th>
<th>CHARACTERISTICS AND FREQUENT USES</th>
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<tbody>
<tr>
<td>Polygalactin (Vicryl®, Dexon®)</td>
<td>Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.</td>
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<tr>
<td>Polydioxanone (PDS® or Maxon®)</td>
<td>Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable</td>
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<tr>
<td>Chromic Gut</td>
<td>Absorbable. Inflammatory.</td>
</tr>
<tr>
<td>Silk</td>
<td>Nonabsorbable. Caution: Tissue reactive and may wick organisms into the wound. Useful for securing catheters/devices.</td>
</tr>
<tr>
<td>Polyester (Ethibond ®)</td>
<td>Multifilament. Nonabsorbable. Useful for ligation or suturing where nonabsorbable material is desirable.</td>
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<tr>
<td>Stainless Steel Wound Clips, Staples</td>
<td>Nonabsorbable. Requires instrument for removal from skin.</td>
</tr>
<tr>
<td>Polypropylene (Prolene®)</td>
<td>Nonabsorbable. Inert.</td>
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* The use of common brand names as examples does not indicate a product endorsement.
NOTE: Suture gauge selection: Use the smallest gauge suture material that will perform adequately. Cutting and reverse cutting needles: Provides edges that will cut through dense, difficult to penetrate tissue, such as skin. Non-cutting, taper point or round needles: Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as the peritoneum or intestine.

IV. HELPFUL CONSIDERATIONS

1. Be aware that much rodent research is performed within human medical centers and that implants or instruments can contaminate rodents with human pathogens if improper technique is used.
2. Techniques that are important and often difficult to perfect:
   a. Touch only "prepped" areas with instruments and hands.
   b. Keep operating fields draped.
   c. Do not let catheters or implants become contaminated.
   d. Use sterile solutions.
   e. Disinfect the tops of containers of solutions.
   f. Use sterile technique to access implanted catheters.
3. Aseptic surgical procedures in rodents are ethically and scientifically justified to ensure the uneventful post-operative recovery of the patient animal, and the highest level of data integrity, uncomplicated by intercurrent pathologies.