GUIDELINE ON RODENT SURVIVAL SURGERY

General Guidelines

Surgery on rodents must be performed using aseptic techniques.

Surgical Facilities

For most rodent surgery, a facility may be small and simple, such as a dedicated space in a laboratory appropriately managed to minimize contamination from other activities in the room during surgery.

In general a rodent surgery facility should have the following components:

- A place for cages of rodents awaiting surgery.
- An animal preparation area, away from the surgery area, for hair removal and initial skin preparation.
- A surgery area.
- A holding and recovery area should be a quiet location where the animals can be observed.
- A rodent surgical area can be any room or portion of a room that is uncluttered and easily sanitized.
- Limit access to people involved in performing the procedure.

Aseptic Procedures

The basic concept of aseptic surgery is to insure that anything that comes in contact with the surgical site is sterile. The procedures outlined here are to insure that sterility is achieved when beginning the procedure and maintained throughout the procedure.

Instruments

- Instruments, supplies and devices must be sterilized (includes: catheters, osmotic pumps, telemetry transmitters, trocars, etc). See Table 1.
- Often rodent surgeries are done on multiple animals in a single session; instruments must be sterilized between animals. Two sets of sterile instruments facilitate re-sterilizing instruments between animals.
- Segregation of instruments according to function helps maintain aseptic technique (i.e., instruments used on skin should not be used within the abdominal cavity).
- Instruments should be wiped clean of blood and tissues with sterile gauze and then sterilized either chemically or mechanically. See Table 1.
- Do not use toothed or crushing instruments if it is not necessary.
- Close surgical wounds using appropriate techniques and materials (see Table 2).
### Table 1

<table>
<thead>
<tr>
<th>Agents</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical: Steam Sterilization</td>
<td>Autoclave</td>
<td>Effectiveness dependent upon temperature, steam pressure, and time. Always use temperature tape to ensure sterilization.</td>
</tr>
<tr>
<td>(moist heat)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry Heat</td>
<td>Hot Bead sterilizer</td>
<td>Rapid sterilization. Instruments must be cooled before contacting tissue. Only the parts of the instrument that comes in contact with the beads are sterile.</td>
</tr>
<tr>
<td>Dry Chamber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionizing Radiation</td>
<td>Gamma radiation</td>
<td>Requires special equipment.</td>
</tr>
<tr>
<td>Chemical (Gas sterilization)</td>
<td>Ethylene Oxide</td>
<td>Requires 30% or greater relative humidity for effectiveness against spores. Requires safe airing time.</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Chlorine Dioxide</td>
<td>Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.</td>
</tr>
<tr>
<td>Glutaraldehydes</td>
<td>Cidex</td>
<td>Requires many hours of contact time for sterilization. Corrosive and irritating. Must rinse equipment with sterile water before use.</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Suture</th>
<th>Properties</th>
</tr>
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<tbody>
<tr>
<td>Vicryl ®(polyglactin 910)</td>
<td>Absorbable; 60-90 days.</td>
</tr>
<tr>
<td>Dexon® (polyglycolic acid)</td>
<td></td>
</tr>
<tr>
<td>PDS® (polydiaxanone), Maxon® (polyglyconate)</td>
<td>Absorbable; 6 months.</td>
</tr>
<tr>
<td>Prolene® (polypropylene)</td>
<td>Nonabsorbable, Inert</td>
</tr>
<tr>
<td>Ethilon® (nylon)</td>
<td>Nonabsorbable, Inert</td>
</tr>
<tr>
<td>Silk</td>
<td>Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound).</td>
</tr>
<tr>
<td>Chromic Gut</td>
<td>Absorbable. Versatile material.</td>
</tr>
<tr>
<td>Stainless Steel Wound Clips, Staples</td>
<td>Nonabsorbable. Requires special instrument to remove.</td>
</tr>
<tr>
<td>Surgical Adhesive, Vet Bond</td>
<td>Area needs to be dry and free of blood.</td>
</tr>
</tbody>
</table>

Suture material used for skin closure must be removed in 10-14 days post surgery.
Preparation of Surgical Site

- Prepare animal in a location separate (bench or room) from the surgical area.
- Remove hair using electric clippers with a #40 blade. Avoid skin abrasions and thermal injuries. (Brand names of electric clippers are Oster, Andis, etc.) Dispose of hair (e.g., vacuum) to avoid hair from becoming airborne.
- Sterile gauze / Q-tips soaked with skin disinfectant can be used for scrubbing. See Table 3.
- Do not wet large areas of animal; it will exacerbate hypothermia.
- Begin along the incision line with disinfectant soaked Q-tip or gauze and extend outward in a circular pattern. Never from outward (dirty) towards the center (clean). Do not go over the incision site twice with the same gauze/Q-tip.

Table 3

<table>
<thead>
<tr>
<th>NAME</th>
<th>EXAMPLES</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodophors</td>
<td>Betadine®, Prepodyne®,</td>
<td>Inactivates a wide range of microbes but their activity is reduced in the presence of organic matter.</td>
</tr>
<tr>
<td></td>
<td>Wescodyne®</td>
<td></td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Hibiclens®, Nolvasan®</td>
<td>Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.</td>
</tr>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol</td>
<td>Not adequate for skin disinfection. Not a disinfectant. Not a sterilant.</td>
</tr>
<tr>
<td></td>
<td>70-99% isopropyl alcohol</td>
<td></td>
</tr>
</tbody>
</table>

Surgeon Preparation

The purpose of the surgeon's scrub is to remove dirt, and as much as is possible, decrease the bacterial flora on the surgeon's hands and arms. Some of the agents used during a surgeon's scrub have residual activity which helps decrease bacterial levels under surgical gloves.

Depending on the availability of assistants, the surgeon often opens a sterile towel pack, gown and gloves before scrubbing.

- Put on surgical mask, clean lab coat or scrubs. Surgical cap is recommended.
- Scrub hands with disinfectant scrub
- Put on sterile gloves (not exam gloves out of the box)

Gloving Procedures

- Remove outer paper package from gloves,
- Unfold inner paper covering on the gloves; careful not to touch gloves with non-sterile objects (e.g. your fingers)
- Fold the bottom fold (near the cuffs) – under, to prevent it from refolding
- Touch only the folded cuff while putting gloves on
- Do not touch any non-sterile surfaces. If the gloves touch non-sterile surfaces - re-glove.
Draping

- Drape the surgical site using sterile material such as towels, stockinet, drapes, gauze, or plastic drape.

Maintaining Sterility

- The surgeon should restrict his/her contact with the surgical site, using only sterilized equipment until the incision is closed.
- The surgeon should not touch anything that is not sterile once gloves are put on.
- ALWAYS put sterile equipment on sterile surface (sterile surgical tray, sterile towel or drape, or sterile gauze) when not in use. Do not let equipment (instruments, catheters, implants, etc.) become contaminated.
- The tips of sterilized instruments should be used to manipulate and handle tissues. Minimize exteriorizing organs, but if necessary, place on sterile gauze and keep moist with sterile saline.
- Sterile gloves must be changed if they come in contact with a non-sterile surface.
- If instruments are being used on multiple animals a Glass bead sterilizer may be considered for use to sterilize instruments between animals.

Anesthesia

- Do not fast rodents unless specifically mandated by the protocol. (The high metabolic rate depletes reserves quickly.)
- Analgesia should be given pre-operatively; see Analgesia section below.
- Following induction of anesthesia, apply ophthalmic ointment to the eyes to prevent corneal drying.
- The animal must be maintained in a surgical plane of anesthesia throughout the procedure. Vital signs of the animal must be monitored throughout the procedure (such as: breathing, skin color, not responsive to toe pinch).
- Anesthetic technique must be on approved Animal Use Form (AUF)
- Gas anesthetics: appropriate scavenging must be used to avoid personnel exposure (Not all hoods provide scavenging; therefore, contact EHS for assistance evaluating your scavenging system)

Antibiotics

Prophylactic antibiotics (given immediately prior to surgery in order to maintain a therapeutic blood level during surgery and continued for up to 12 hours post op) decrease wound infection and may be considered for use.

Analgesia

Adherence to federal policies and regulations as well as Burch and Russell’s 3Rs necessitate the use of drugs to alleviate or ameliorate pain and distress associated with research procedures. The basic assumption is that if a procedure would be likely to cause pain or distress in a human, then it is likely to also do so in animals. The MSU IACUC has determined that analgesia must be provided in all such cases unless non-administration has been scientifically justified in the animal use protocol and approved by the MSU IACUC. See MSU IACUC guideline IG003: http://www.iacuc.msu.edu/iacuc/iacuc_index.htm

Analgesia is most effective if it is administered prior to commencement of the surgery, followed by additional analgesics given post-operatively. Multi-modal therapy (e.g., use of a local skin anesthetic and a NSAID or opioid) provides broader coverage of pain receptors and is recommended. The level, type and duration of analgesia will depend upon the procedure performed. For post-operative analgesia, minimally invasive procedures need less potent or shorter-acting analgesics and may respond to a single dose of analgesic. More invasive procedures (e.g. laparotomy, orthopedic surgery) may require multiple doses of analgesic for up to 72 hours post-operatively. Specific criteria for evaluating pain and provision of analgesia must be developed through veterinary consult and described in the IACUC Animal Use Form (AUF). It is the responsibility of the investigator and staff to evaluate the animal on a routine basis and determine whether additional treatment is needed based upon the criteria described in the IACUC AUF.
Local Anesthetics:

These agents (e.g. lidocaine, bupivacaine) have been shown to provide effective analgesia by blocking nerve impulse conduction. They can be used topically (e.g. bathe exposed nerves or cut tissues) or injected locally using a fine needle. For thoracic and abdominal surgery they can be instilled post-operatively. Local anesthetics have not been shown to significantly delay tissue healing. These agents can be cardiotoxic, so it is important that a maximum safe dose should not be exceeded (4 mg/kg lidocaine; 1-2 mg/kg bupivacaine). Bupivacaine has a longer duration of action (4-12 hours) than lidocaine (1-2 hours) but takes slightly longer to take effect (5-10 minutes vs. 1-2 minutes). These agents can be diluted in a balanced electrolyte solution to provide more volume for injection or instillation. ULAR recommends the use of bupivacaine (0.25-0.5%) over lidocaine due to its longer duration of action. Local anesthetics should not be used as sole analgesic agents except in minor procedures.

Non-steroidal Anti-inflammatory Drugs (NSAIDs):

NSAIDs are an alternative class of compounds for pain management. These drugs act by reducing prostaglandin synthesis by inhibiting one or both of the COX isoenzymes. These compounds have analgesic activity both locally and centrally and are synergistic with opioids. In addition to aspirin and acetaminophen, newer compounds used extensively for managing pain include carprofen, ketoprofen, ketorolac and meloxicam. The advantage of the newer compounds is that they are not controlled substances and can be dosed once daily. These drugs can be given post-operatively, pre-operatively or intra-operatively. To avoid renal problems be sure the animal remains hydrated (warmed fluids injected subcutaneously during anesthesia is effective: 5-10ml for rats and 0.5-1.0 ml for mice). Combinations of NSAIDs should not be used because adverse side effects of GI ulceration or renal failure can occur. These compounds may be used as sole analgesic agents in both minor and some major procedures. For rats, ULAR is currently recommending the use ketoprofen at a dose of 5 mg/kg subcutaneously once daily (for no longer than 5 days) or meloxicam once daily (0.2 mg/kg IM, IP, SQ). If repeated injections are required, the oral preparation of meloxicam can be provided in drinking water (Mice: 1.2 ug/ml [0.2 mg/kg/day] and rats: 2.0 ug/ml (0.2mg/kg)).

For significantly invasive procedures or procedures which are expected to have severe pain, these agents should be combined with an opioid for maximum effectiveness. For these types of procedures, ULAR recommends at least one dose of opioid (either butorphanol or buprenorphine, see below) and 1-5 days of ketoprofen.

Opioids:

Long known for their potent analgesic activity, opioids remain the gold standard for analgesic therapy. Opioids are the drug of choice for treatment of moderate to severe pain. Side effects such as respiratory depression and bradycardia are dose-dependent and are reduced with the new mixed agonists and agonist-antagonist compounds (buprenorphine, butorphanol). Pica may be seen with buprenorphine, but again is dose dependent and transient. Metabolic rates for opioids are high for rodents, making several of the opioids (morphine, meperidine and pentazocine) impractical for use, however, buprenorphine is effective for 8-12 hours after administration.

Opioids are ideal for using pre-operatively, which reduces the overall amount of anesthetic needed and allows analgesia to be available when the animal wakes up. While analgesia is excellent with these compounds, there are disadvantages. The primary disadvantage is that all of these drugs are controlled substances and subject to federal regulations for acquisition, record keeping and disposal. Of the opioids, ULAR recommends buprenorphine due to its long duration of effectiveness and few side effects. Buprenorphine, however, comes in 1 ml glass ampules and there are problems with proper storage of the drug after opening an ampule. On average, one - 1ml ampule will dose 6 - 250 gram rats once each. The dose for buprenorphine in rats is 0.01-0.05 mg/kg subcutaneously every 8-12 hours (mice - 0.05 – 0.1 mg/kg every 6-12 hours). If combining an opioid with an NSAID, a good combination for short duration opioid is butorphanol (1-4 mg/kg subcutaneously every 4- hours for mice and 2mg/kg every 4 hours for rats) or buprenorphine for longer duration + either meloxicam, ketoprofen, carprofen or flunixin (2.5 mg/kg subcutaneous for both rats and mice). For a multiple dosing alternative, the oral preparation of meloxicam (dose is 0.2mg/kg orally) can be mixed with Jello (strawberry, grape or cherry appear to be preferred flavors) and the dose provided daily in a very small cube per rat or in a very small amount of peanut butter (use the smallest amount possible so that the animal will eat the entire amount at one time). To provide analgesia in food the animal must be singly housed, at least while consuming the food with the medication. Contact a ULAR veterinarian for specifics on this mixture.
Monitoring of the animal

Heat Loss

- Hot water blankets, warmed (body temperature) fluid bags, warming blankets, and warming discs must be used or the animal will become hypothermic. When using warmed fluid bags, monitor the fluid temperature frequently and remove when it falls below body temperature. Hypothermia kills. Heat lamps are not recommended because of the difficulty of regulating the amount of heat applied.

Fluid Loss

- Fluid loss occurs as a result of evaporation from body cavities and blood loss.
- All animals (including humans) lose fluid during anesthesia.
- Rodents are vulnerable to the effects of fluid loss because of their small size.
- Reduce fluid loss by:
  - Irrigating the operative field with warm sterile saline (be careful not to wet drapes).
  - Control blood loss.
  - Administer warm sterile fluids post procedurally.

Postoperative Care

- Monitor the animal continuously during the procedure and until the animal is conscious and then every 10-15 minutes until fully ambulatory. Body temperature must be maintained. Place animal in a clean, dry, warm area on a padded surface (not animal bedding) and provide warmth with a circulating water blanket, warm water bottle, heat discs, or other method.
- House rodents individually until they are ambulatory to prevent cannibalism or suffocation. Do not return them to the animal holding room until they are stable and awake.
- Post-surgical animal should be seen at least daily by a member of the investigator's staff or other identified trained individual to ensure that there are no complications.
- If principle investigator or staff finds that an animal appears ill, or the surgical wound appears abnormal, contact the veterinarian immediately.
- Animals should be monitored for signs of pain and distress (see below). Administration of analgesics must be consistent with the description provided in the IACUC approved AUF.

Potential signs associated with pain or distress in rodents:

- Decreased food and water consumption, weight loss
- Self-imposed isolation/hiding
- Rapid, open mouth breathing
- Biting, aggression
- Increased/decreased movement
- Unkempt appearance (rough, dull hair coat)
- Abnormal posture/positioning (hunched back, head-pressing)
- Dehydration, skin tenting, sunken eyes
- Twitching, trembling

Medical Records

The IACUC requires that peri-operative records be maintained on all vertebrate animals undergoing survival surgery.

Surgical Records will include the following information:

- Animal identification
- Surgical Procedure and date procedure was performed.
Postoperative observations and treatments must be recorded and initialed on each date performed. At a minimum, there must be daily entries as to the animal’s condition for 3 days following the procedure and must also include information describing analgesic drugs as they are administered. The weight of the animal must also be recorded in the daily record if so indicated in the IACUC approved protocol. If the MSU IACUC approved protocol states that food and water consumption will be monitored post-operatively, these observations will be recorded in the daily log. All daily entries must be legible. Sutures or staples must be removed (unless exempted in the IACUC approved protocol), and the date of removal recorded in the record. The surgical records will be maintained as long as the animal is housed in MSU facilities. After the death of the animal the surgical records for non-USDA covered species will be maintained as the PI deems appropriate. The PI will maintain records for USDA covered species for 3 years after the animals’ termination.

There are two (2) options for maintaining perioperative records. 
1. The records may be kept on 3” x 5” cards maintained behind the cage card in the cage cardholder. 
2. Records may be maintained in a notebook which is kept in the animal room.

If for some reason option 1 or 2 is not possible, other arrangements must be described in the protocol and approved by the IACUC

3” x 5” record or notebook format. Cards may be obtained from CAR/ULAR.:

<table>
<thead>
<tr>
<th>ULAR Rodent Sx Record</th>
<th>Post Operative Notes and Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal ID:</td>
<td>Date</td>
</tr>
<tr>
<td>Date of Surgery:</td>
<td></td>
</tr>
<tr>
<td>Name:</td>
<td></td>
</tr>
<tr>
<td>Procedure:</td>
<td></td>
</tr>
<tr>
<td>Anesthetic(s):</td>
<td></td>
</tr>
<tr>
<td>Analgesic: include drug/ duration/ dose/ route</td>
<td></td>
</tr>
<tr>
<td>Sutures / Staples: yes no</td>
<td>Date Removed:</td>
</tr>
<tr>
<td>Include postoperative notes on back</td>
<td>ULAR09102013</td>
</tr>
</tbody>
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REFERENCES

1. AALAS Training Manual, Technician Level, 121-138