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TECHNIQUES IN ASEPTIC RODENT SURGERY

Shelley L. Hoogstraten-Miller¹ and Patricia A. Brown²

¹ *National Human Genome Research Institute, Bethesda, Maryland* ² *Office of Laboratory Animal Welfare, Bethesda, Maryland*

Abstract

Performing aseptic survival surgery in rodents can be challenging. This unit describes some basic principles to assist clinicians, researchers, and technicians in becoming proficient in performing aseptic rodent surgery.

Key Terms

aseptic surgery; technique; rodent; surgical preparation; instrument preparation; suture material; anesthesia; analgesia; surgical gloves

Unit Introduction

Performing aseptic survival surgery in rodents can be challenging. Unlike in larger species where there is a dedicated surgical suite and several personnel to assist the surgeon, rodent surgery is most commonly performed alone. This means the surgeon must induce, maintain, and recover the animal from anesthesia, as well as, surgically prepare the animal, and perform the surgery aseptically.

The following principles described in the Guide (NAS, 1996) apply to rodent surgery:

1. Appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices is required.
2. All survival surgery will be performed by using aseptic procedures, including sterile gloves, masks, sterile instruments, and aseptic techniques.
3. A dedicated surgical facility is not required for rodents but surgery must be performed using aseptic techniques.
4. Research personnel will be appropriately qualified and trained in all procedures to ensure that good surgical technique is practiced.

Shelley Hoogstraten-Miller, DVM, MS, DACLAM, National Human Genome Research Institute/National Institutes of Health, Building 49/3A14 MSC4472, 49 Convent Drive, Bethesda, MD 20892, T: (301) 435-5566, F: (301) 435-8366, E: shoogstr@mail.nih.gov, Patricia Brown, VMD, MS, Office of Laboratory Animal Welfare/National Institutes of Health, RKL1, Suite 360, MSC 7982, 6705 Rockledge Drive, Bethesda, MD 20892-7982, T: 301-451-4209, F: 301-402-2803, E: brownp@mail.nih.gov.

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Good surgical technique includes asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns.

Aseptic surgery is performed using procedures that limit microbial contamination so that significant infection or suppuration does not occur. This includes preparation of the animal, preparation of the surgeon, sterilization of instruments, supplies, and implanted materials, and the use of operative techniques to reduce the likelihood of infection (NIH ARAC, 2005).

Strategic Planning

Aseptic technique is achieved, in part, by the pre-surgical planning that begins during protocol development in consultation with your veterinarian. This includes identification of personnel, their roles and training needs, equipment and supplies required for the procedures planned, the location and nature of the facilities in which the procedures will be conducted, and pre- and post-operative care.

1. Choosing the Surgical Area

Characteristics of a good surgical area include an uncluttered area that is easily organized and disinfected, and free of debris and equipment not related to surgery (figure 1.12.1). The area should be dedicated for the duration of the procedure, but can be used for other purposes when not being used for surgery. Avoid locations that are beneath supply ducts to minimize contamination from dust. Avoid high traffic areas such as those near doorways to prevent unnecessary interruptions and creation of air turbulence and contamination of the surgical field.

2. Considerations for Anesthesia and Analgesia, an Introduction (See Unit 1.4)

Select the anesthetic based on the type of surgical procedure, the length of the surgical procedure, the equipment available and the expertise of those who will be responsible for administering the anesthetic (Flecknell, 1996; Kohn, 1997; Swindle, 2002). Consideration must also be given to the application of pre-, intra-, and post-operative analgesia. Analgesics can be injected, applied topically in a drop-wise fashion to the surgical area and/or supplied in the food or water.

Inhalant gas anesthetics are administered using precision calibrated vaporizers (Figure 1.12.2). When using gas anesthetics you must account for scavenging of waste gases. One acceptable method of scavenging is the use of a downdraft table (Figure 1.12.3). It is important not to completely cover the surface of the downdraft table. This will cause a loss in its ability to effectively scavenge gases. Downdraft tables are usually only effective up to a height of 6–8 inches from the surface. Do not use induction chambers taller than this for induction of anesthesia. Placing the chamber in a chemical fume hood (Figure 1.12.4) or a type IIB biosafety cabinet (Figure 1.12.5) that is vented to the outside are other methods that can be used to scavenge waste anesthetic gases. A charcoal canister (Figure 1.12.6) attached to the part of the breathing circuit for expired gases can also be used for scavenging. Charcoal canisters must be weighed before, and after, each use and must be replaced after an increase in the recommended weight. Depending on the size of the canister and the manufacturer's recommendations, the canister should also be weighed during especially long procedures to assure its continued effectiveness.

Injectable anesthetics are widely used in rodent surgeries (e.g., ketamine/xylazine mixtures, pentobarbital, tribromoethanol). Controlled substances (pentobarbital, ketamine) require additional record keeping. If using injectable anesthetics, it is important to weigh each animal and dose each according to its body weight.

Some anesthetics, such as ketamine, abolish the blink reflex. Anesthetized animals should have their corneas protected with an ophthalmic lubricant. To avoid contamination of the lubricant, do not touch the tip of the tube to the skin or eye surface.

Anesthetized animals must be monitored during the procedure to assure that they stay in the proper anesthetic plane. Do not allow them to get too light or too deep. Once the anesthetic has been given time to take effect, the anesthetic plane can be assessed by pinching the toes, tail or ear of the animal. Any reaction from the animal indicates that the animal is too light and that additional anesthetic should be given.

Monitoring should include inspection of the mucous membranes and exposed tissues. This will give an indication of tissue perfusion and oxygenation. The color should be a bright pink to red and not dusky gray or blue. Respiratory pattern and frequency is also easily monitored and will give an indication of anesthetic depth and other potential complications.

Various types of instrumentation can assist in monitoring the anesthetized patient. Core body temperature can be monitored in rodents, including mice. Pulse oximetry, capnography, and electrocardiograms can be used in larger rodents to monitor pulse, oxygenation, and heart rate. Monitoring instruments must be properly calibrated, as inaccurate information may be misleading and could result in a compromised condition or fatalities.

The most frequent complication of small animal anesthesia is hypothermia resulting in prolonged recovery or death of the animal. Animals should be provided with a heat source during the pre-operative, intra-operative, and postoperative periods. Because of the high airflow, the risk of hypothermia is heightened when using downdraft tables, chemical fume hoods, or biosafety cabinets.

The safest devices for providing heat to anesthetized animals are circulating hot water blankets or instant heat devices. These devices must be covered with a paper towel or other insulation so that the animal does not come in direct contact with the hot surface. Slide warmers can also be used as a heat source during recovery. By placing the recovery cage on the slide warmer it will be pre-warmed and ready to accept the animal once the surgery is complete. Use a thermometer to measure the temperature at the level of the animal. The temperature not exceed 85 to 95°F (29.4 to 32.2°C). Heat lamps and electric heating pads can be very dangerous and should be used with great caution.

3. Instrument Preparation

Planning the surgical procedure requires consideration of the instruments required for the procedure and what method of instrument sterilization will be used. There are three commonly used methods for instrument sterilization:

- a. Steam autoclave or ethylene oxide. When using one of these methods a simple paper peel pack (Figure 1.12.7) or a complex pack (Figure 1.12.8) is used. A simple peel pack contains small numbers of small to medium sized instruments. A complex pack consists of overlapping cloth or paper drapes folded together and sealed with indicator tape. It can contain a large collection of instruments of various sizes (Knecht, 1987). Tip protectors should be added to delicate instruments or those with sharp points. Delicate instruments, materials for implantation such as catheters or items that otherwise may melt or become damaged when heated can be sterilized using ethylene oxide. The packs must be sufficiently aerated to prevent toxic effects from residual gas. This may require 24–72 hours.

- b. Cold sterilization. Glutaraldehyde solutions are effective if instruments are exposed for the proper length of time and expiration dates of prepared solutions are observed (usually 28 to 30 days).
- c. Dry heat sterilization. Hot bead sterilizers (Figure 1.12.9) sterilize only the tips of the instruments. The beads must be pre-heated to the recommended temperature and the instruments exposed for the recommended time. “Flash” dry heat sterilizers (Figure 1.12.10) sterilize the entire instrument and also requires adherence to the recommended temperature and exposure time. For both methods, gross debris must be removed from the instrument before sterilizing and the instruments must be allowed to cool before touching tissues.

Alcohol provides disinfection not sterilization and should not be used to sterilize instruments.

4. Selection of Wound Closure Materials

The selection of the type and size of suture material should be done in advance of the surgical procedure. A 3-0 suture thickness or smaller is best. Cutting and reverse cutting needles have sharp edges and are best used for skin suturing. Non-cutting, taper or round needles are used for suturing easily torn tissues such as peritoneum, muscle or intestine.

If ligation of vessels or suturing of tissues other than skin is necessary during surgery, an absorbable material such as polyglactin 910, polyglycolic acid, polydioxanone, polyglyconate, or chromic gut should be used. For skin closure, non-absorbable suture such as polypropylene, nylon, stainless steel wound clips or staples may be used. Most rodents will gnaw at any externalized sutures, so a buried suture line or wound clips are recommended. Cyanoacrylate surgical adhesives may be used to close incisions or to close the area between sutures. Silk is a non-absorbable suture material that can cause tissue reactions and may wick microorganisms into the wound. It is best used for cardiovascular procedures only and not for closure (Knecht, 1987).

Basic Protocol 1: Surgical Preparation of the Animal

Once everything for the surgery is pre-selected and organized, the animal can be anesthetized and surgically prepped. Surgical preparation includes removal of the hair and disinfection of the surgical site. Surgical preparation of the animal should occur in a location different than that used for performing the surgeries. This will help to prevent hair and dander from getting on the sterile packs. If space constraints or requirements for use of the down draft table, chemical fume hood, or biosafety cabinet necessitates a single location for prepping and surgery, then the bench towel used to prep the animal should be replaced before performing the surgery. The surgical pack, if already open, must be covered with a sterile drape to prevent contamination with hair.

Materials

“Mini clipper” with no. 0000 blade

Gauze pads (2”×2”)

Adhesive tape (1–2” wide)

Cotton-tipped applicators

70% alcohol

Iodophor or chlorhexidine scrub

1. Using the clipper remove the hair from the surgical site.
In mice, an easy alternative to clipping the fur is to remove it by plucking. Hair follicles in mice are usually in telogen or resting phase, and hair can be removed without injury. It is a fast and easy method that does not leave stubble.
2. Dab the clipped or plucked area with a piece of adhesive tape or moistened gauze to pick up loose hair that could otherwise migrate into the incision.
3. Use a gauze pad or cotton-tipped applicator to prep the surgical site with alternating scrubs of an iodophor and 70% alcohol. Use a circular motion beginning at the center of the shaved area and working toward the periphery. . Never go back to the center with the same sponge.

For small incision sites cotton-tipped applicators work best. Alternative scrubs such as chlorhexidine may also be used
4. Repeat the alternating scrubs at least 3 times.

Be careful not to excessively wet the animal as this can exacerbate hypothermia.

Performing Surgery

The pre-surgical preparation should have included consideration of the surgical technique that will be used: sterile surgical gloves (Basic Protocol 2) or clean exam gloves (Alternate Basic Protocol 2) with a “tips-only” technique. Proper surgical attire for both techniques consists of cap, mask, and clean lab coat.

Basic Protocol 2: Sterile Surgical Gloves

Using sterile surgical gloves allows you to touch all areas of the sterile surgical field and surgical instruments with your gloved hand.

Materials

Surgical attire; cap, mask, lab coat

Sterile surgical gloves

Surgical instruments and equipment

Surgical drape

Anesthetized and surgically prepped animal

1. Don cap, mask, and clean lab coat.
2. Ready the sterile surgical instruments on a sterile surgical field.

If using surgical packs, verify that the sterilization indicator has turned the appropriate color before using. Simple-peel packs are opened in a manner that preserves the sterility of the inside surface. Do not touch the inside surface as it can be used as a sterile field on which to keep the instruments. Complex surgical packs are also opened in such a way as to keep the inside surface of the wrapping sterile so that it can be used as a sterile field. Instruments in cold sterilant solutions must be removed from the solution and rinsed with sterile water, saline or alcohol. This is very important, as the sterilization solution is very irritating to tissues. Rinsed instruments must be placed on a sterile field. Dry heat sterilized instrument must also be placed on a sterile surgical field. Remember that bead sterilizers only sterilize the tips of the instruments.

3. Open all other sterile equipment, such as scalpels and suture material. Open these items in such a way as to prevent contamination of the item and the surgical pack.
4. Place the anesthetized and surgically prepped animal on the warming device that has been covered with a clean paper bench towel.
5. Don the surgical gloves as described below (Video 1.12.1) to prevent contamination of the outer surface of the glove (Knecht,1987).
 - a. Open the glove packet in such a way that prevents contamination of the inner surface.
 - b. With one hand, lift a glove from the opened packet by its turned-down cuff.
 - c. Pull the glove onto the opposite hand with a rotating motion. Do not touch the outside surface of the glove.
 - d. Place the gloved fingers beneath the cuff of the other glove.
 - e. With the gloved fingers under the cuff, pull the glove onto the ungloved hand. The folded cuff protects the gloved hand from contamination.
 - f. Pull the cuff of the glove up and over the cuff of the lab coat.
 - g. Slip the fingers under the cuff of the first glove to pull it over the lab coat cuff
6. Organize the instruments. It is helpful to point all the tips in one direction and place them in the order used (Figure 1.12.11). Between surgeries or during breaks in surgeries cover the tips of the instruments with sterile gauze or drapes (Figure 1.12.12).
7. Drape the surgical site.

The most common drape is a paper drape (Figure 1.12.13). It may be precut or one in which you must cut your own hole. The disadvantage of paper drapes is that they usually cover the entire animal, making patient monitoring difficult. Plastic drapes (Figure 1.12.14), usually with an adhesive, offer the advantage of more visibility and better patient monitoring. Sterile gauze sponges (Figure 1.12.15) can also be used for drapes. If using the “tips-only” technique you must handle the drape only by its edges so that it does not become contaminated.
8. Perform the surgical procedure.
9. Close the surgical wound in layers (e.g., body wall, subcuticular space, skin).
10. Move animal to a warm cage for recovery.
11. Sterilize or sanitize instruments between surgeries.

If multiple surgeries are performed each day and multiple surgical packs are not available, the instruments should be rinsed with 70% alcohol or “flash” sterilized between surgeries. Remember, alcohol will disinfect, not sterilize. Alternatively, a glass bead sterilizer can be used to sterilize the tips of the instruments. Remember to allow instruments to cool before touching tissues!
12. Rinse gloves with 70% alcohol between surgeries.

If you have had to handle another animal to anesthetize and prep it, you should change gloves before performing the next surgery.

Alternate Basic Protocol 2: Clean Exam Gloves

Using clean exam gloves and a “tips-only” technique restricts you to using only the sterile working ends of the surgical instruments to manipulate the surgical field. The gloved, but not sterile, hand must never touch the working end of the instruments, the suture, suture needle, or any part of the surgical field. This technique is useful when working alone and manipulation of non-sterile objects (e.g., anesthesia machines, microscopes, lighting) is required.

Additional Materials (also see Basic Protocol 2)

Clean exam gloves (not sterile surgical gloves)

1. Don cap, mask, and clean lab coat.
2. Place the anesthetized and surgically prepped animal on the warming device that has been covered with a clean paper bench towel.
3. Don clean examination gloves.
4. Place the sterile tips of the instruments on a sterile gauze sponge or drape to prevent contamination (Figure 1.12.16).
5. Open all other sterile equipment, such as scalpels and suture material. Open these items in such a way as to prevent contamination of the item and the sterile tips of the instruments.
6. Drape the surgical site. Handle the drape only by its edges so that it does not become contaminated.
7. Continue as described in steps 8 through 12 above

Commentary

When performing multiple rodent surgeries it is a good idea to have staging areas for the different steps of the procedure. Whenever possible, animals waiting for surgery should be kept at a visual and olfactory distance from those animals undergoing surgery.

As you are performing your surgery, you should be aware of the space that is not sterile between your pack and the draped animal (Figure 1.12.17). Do not lay instruments in this space. They will become contaminated.

While performing surgery, be careful not to get paper or cloth instrument drapes wet (Figure 1.12.18). Wet material acts as a wick to pull bacteria through from the non-sterile surface below. When this occurs the instruments should be considered contaminated and re-sterilized before further use.

You must keep the recovering patient warm. Do not lay recovering animals directly on the bedding. They may aspirate and asphyxiate. Recovery from anesthesia can also be aided by the administration of warmed fluids given subcutaneously or intraperitoneally.

In neonates, or animals recovering from prolonged surgical procedures, hypoglycemia can be a problem leading to post-surgical complications. These animals may benefit from the administration of oral glucose. Glucose solutions should never be given subcutaneously (SQ) or intraperitoneally (IP). Animals may be returned to their holding area once they are awake and appear to be making a normal recovery. Be sure to mark the cage card with the surgical procedure performed and the date.

The Guide (NRC, 1996) states that the application of prophylactic antibiotics is not a substitute for the practice of proper aseptic surgery. If prophylactic antibiotics must be used, for example in gastrointestinal surgery or an accidental break in aseptic technique, choose an appropriate antibiotic and give it at the dose and for the length of time recommended by the veterinarian. In guinea pigs and hamsters, the use of inappropriate antibiotics can cause fatalities.

Post-operative care does not end with the return of the animal to its home environment. Animals must be monitored for several days after the surgical procedure for the development of post-surgical complications and for the continued need for analgesics. Food intake may be difficult to monitor in rodents, especially if they are group housed. However, if post-operative animals are singly housed and food rations are supplied in measured amounts this can be a useful monitoring tool. A more practical and sensitive method of monitoring the animal is daily weighing of the animal. While subtle changes in the animal's activity or appetite may not be clinically observed, changes in weight will be quickly detected allowing appropriate clinical intervention to be instituted. It is important to remember that some analgesics may depress the appetite causing secondary weight loss. This weight loss must be differentiated from that which occurs in an animal that is not feeling well.

Supplying a softer, more palatable, easily accessible diet may encourage the animal to eat.

The animal's hydration can be monitored by "tenting" the skin along the back of the animal. In a well-hydrated animal, the skin should quickly fall back into place when released. If an animal is dehydrated, the skin will be slow to return to its original place. When this occurs, your veterinarian should be consulted for the appropriate use of subcutaneous or intraperitoneal fluids.

Wound closures should be removed at 10 to 14 days post-operatively. Suture scissors or staple removers should be used.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.12.1.
Good surgical site (left) and poor surgical site (right).



Figure 1.12.2.
Anesthesia machine with precision calibrated vaporizer.



Figure 1.12.3.
Downdraft table.



Figure 1.12.4.
Chemical fume hood.



Figure 1.12.5.
Type IIB biosafety cabinet.



Figure 1.12.6. Charcoal canister.



Figure 1.12.7.
Simple peel pack.



Figure 1.12.8.
Complex surgical pack.



Figure 1.12.9.
Hot bead sterilizer.



Figure 1.12.10.
Flash dry heat sterilizer.



Figure 1.12.11 .
Organize the surgical instruments.



Figure 1.12.12.
Between surgeries, the tips of the instruments should be covered.

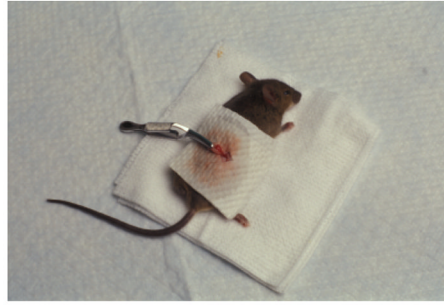
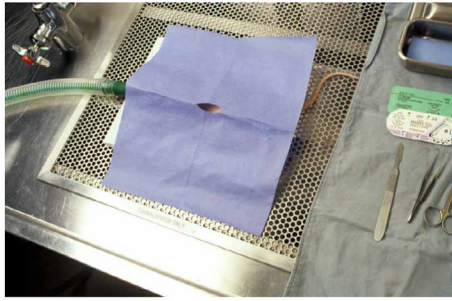


Figure 1.12.13.
Paper surgical drape

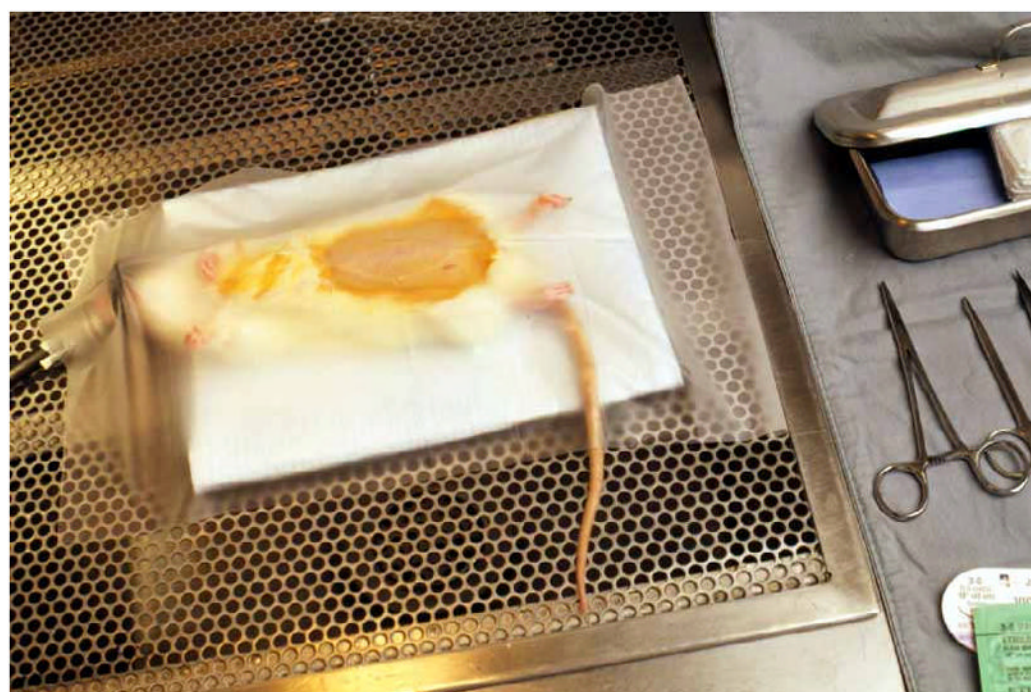


Figure 1.12.14.
Plastic adhesive surgical drape.



Figure 1.12.15.
Sterile gauze pads used for surgical drapes.



Figure 1.12.16.
Sterile tips of the instruments are placed on a sterile field.

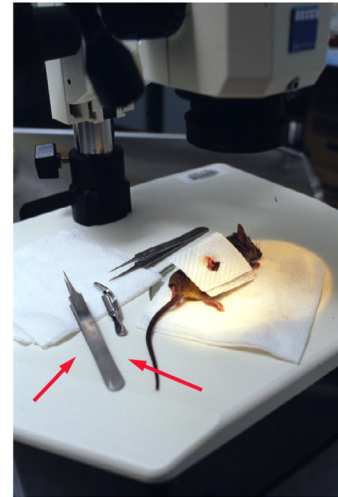
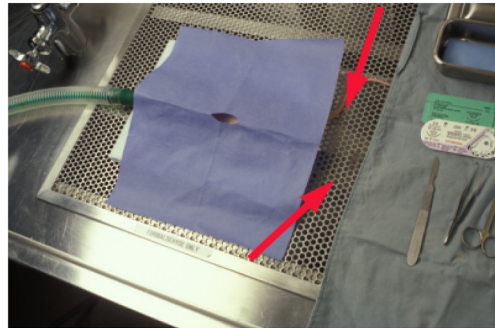


Figure 1.12.17.
Arrows indicate space between drape and instruments that is not sterile.



Figure 1.12.18.
A wet area on a paper or cloth drape acts as a wick to pull bacteria through from the non-sterile surface below.



Video 1.12.1.
Donning sterile surgical gloves.