Company of Primate Veterinarians  
Cranial Implant Care Guidelines for Nonhuman Primates in Biomedical Research

PURPOSE

Use of nonhuman primates (NHPs) in biomedical research may include performing invasive cranial surgeries with chronic implantation of research devices. The Association of Primate Veterinarians (APV) supports the responsible use of NHPs in neurobiological research. Such research must meet specific criteria, such as the institutional animal care and use committee (IACUC) review and approval, verification of the investigator’s skill and experience, and establishment of a close working relationship with institutional veterinary staff (Guide 2011). The following text aims to provide nonhuman primate researchers, IACUCs, and veterinary staff with guidelines for conducting research involving chronic cranial implants and for assessing their routine and non-routine care.

BACKGROUND

Success in maintaining a chronic cranial implant in operational condition is a function of how the implant is placed and the types of materials used, coupled with the animal’s physiology and healing responses. The laboratory animal veterinarian should interface closely with the research group to ensure the adequacy of training and use of optimal surgical technique. Cranial implantation surgery must be conducted with consideration of normal host anatomy and physiology, as well as the maintenance of aseptic technique. With some surgical implant procedures, it is common to stage placement of cranial implants. Waiting to place each attachment (e.g. recording chamber) until the data recording is required helps to preserve the integrity of the chamber and safeguard the health of the animal. The total number of allowable cranial surgeries should be reviewed and approved by the IACUC.

GUIDELINES

Pre-surgical procedures

Clipping the hair liberally around the surgical site while avoiding clipper burns and cuts will help minimize unwanted irritation and infections. Small scissors or commercial depilatory products can be used in areas inaccessible for clipping. The skin must be surgically prepared, disinfected, and draped in a sterile fashion.

Surgical procedures

1. Cranial implantation surgeries must employ techniques that minimize trauma and preserve tissue architecture. A combination of aseptic technique, appropriate instrument and suture use, isotonic fluid lavage, and skillful and gentle tissue handling is highly recommended.

2. A neat and sterile cranial surgical site provides the best bonding surface, promotes bone remodelling, and facilitates anchoring of the cranial implant to the skull.
3. Use of a high-powered drill by an inexperienced surgeon may lead to thermal cranial damage and secondary local bone necrosis with loosening of the screws and eventual implant detachment. Hand drills do not cause thermal damage, but their use can lead to larger than necessary holes due to their poorer stability. Continuous lavage with cold isotonic fluids during drilling or application of thin layers of exothermic compounds (e.g., methacylate) may help prevent or minimize thermal damage to the bone and periosteum. This kind of damage may be particularly important in younger or smaller NHPs with a thinner cranium.

4. Titanium or high quality stainless steel orthopedic screws are often used to anchor cranial implants. Drilling pilot holes combined with the use of bone taps and blunt tipped screws will minimize or even eliminate bone damage while contributing to implant longevity (Abee 2012).

5. Hemostatic materials such as Gelfoam®, are effective in stopping acute bleeding but they must not be left inside the cylinder indefinitely. To remove Gelfoam® the cylinder should be filled with sterile saline for approximately 10 min to soften residual foam pieces for removal and the process repeated, if needed. Forceful removal of Gelfoam® residue may produce additional hemorrhage and should be avoided. Cranial bone edges are the most common source of bleeding within the cylinder and this can be controlled by sealing the edges with bone wax. The implanted cylinder may be opened after the surgery for visual examination and carefully cleaned 1-2 days after surgery. After assuring adequate hemostasis, 2-3 ml of sterile saline should be placed in the cylinder followed by aseptic replacement of the cap for another 2 to 3 days. Routine cleaning and maintenance of a non-infected cylinder should be initiated in 1 week post-op.

Post-surgical procedures

While tending to the newly placed or chronic cranial implants one should be vigilant about potential pain. If there is any evidence of pain or distress associated with routine cleaning the underlying cause should be investigated, addressed, and appropriate analgesia given. One or a combination of the following agents is recommended: EMLA cream, lidocaine jelly, lidocaine or bupivacaine local block, or systemic NSAIDs or opioids.

1. Wound Margin Care

   a. An uninfected surgical wound that is healing well is best left alone for a period of 7-14 days post-operatively. Sterile saline rinses can be used if needed to clean the wound. Use of H₂O₂ is not recommended for 2-3 weeks post-op as it can interfere with normal healing process. Dry, non-infected, hard and crusty scabs formed during normal healing may cause local irritation or pruritus, inviting self-trauma. Petroleum jelly or wet dressings applied every 2-3 days will keep the scabs soft and facilitate healing. There is no universally recommended frequency of cleaning. Rigorous, over exuberant, unwarranted cleaning can result in inflammation and infection. Wound margins should be closely inspected a minimum of once each week and cleaned as often as needed.
b. Re-growing hair should be carefully removed on an as-needed basis.

c. The wound margin adjacent to an implant requires regular observation and attention as it may become infected leading to suture dehiscence or necrosis, resulting in areas of skin devitalization or retraction away from the implant. Daily cleaning may be necessary as the serous, serosanguinous or purulent secretions will dry up at the wound margin producing a protein-rich crust that may serve as a nidus of infection. Cleaning of the skin/implant interface involves gentle removal of loose crusts and of unwanted hair with a scissors and rinsing wound margins. The following solutions or their combinations should be considered for cleansing: sterile saline, chlorhexidine diacetate 0.05% solution (1:40 dilution of stock chlorhexidine with water) (Slatter 2003), povidone-iodine 1-2% solution (1:10 – 1:5 dilution of stock povidone solution to saline), Dakin’s solution (0.5% sodium hypochlorite in water) can be used particularly in the presence of necrotic tissue or 1.5 - 3% hydrogen peroxide (to remove dried up blood and other secretions followed by copious saline irrigation). None of the above compounds is effective indefinitely or against all pathogens and a 7 to 10 day rotation of different disinfectants should be employed.

d. Enzymatic debriding compounds facilitate the process by which devitalized tissue is softened or liquefied and removed (e.g. Trypzyme®, an enzymatic soaking solution).

e. Infected sites should be cleaned frequently (e.g. daily). Where mild but chronic skin/implant problems are evident, a minimum twice a week inspection and cleaning 3-4 days apart, are recommended. Culture and sensitivity should be done to ascertain the nature of the infectious agent. The indiscriminate use of systemic or local antibiotics may contribute to the development of bacterial resistance and is strongly discouraged.

2. Cranial Head-post Care

The skin may retract away from the head-post over a period of weeks to months post-operatively and this is usually a gradual process. In the absence of local infection, skin repair surgery may be attempted. If the skin retraction is significant, an addition of bone cement may be considered.

3. Routine Recording Cylinder Care

Most recording cylinders are anchored with screws and methacrylate products and have a tight fitting cap secured with 1-3 small screws. The inside of a chronic recording cylinder is not sterile but it must be maintained aseptically. Recording cylinders are routinely opened in the non-sterile environment of the research laboratory or procedure room. Careful cleaning of the recording cylinder as described below has been demonstrated to minimize or prevent active cylinder infections. Ideally, no smell should be detectable in the recording cylinder and the underlying dura should appear creamy white, smooth, and shiny.

a. The outside of the cylinder is typically contaminated and must be cleaned before the cylinder is opened for cleaning and/or recording. Povidone-iodine scrub (soap) should be
used for the initial scrub and washed off with saline or 70% alcohol. Residual blood may be removed with 0.75% to 3% hydrogen peroxide (H₂O₂). Care must be taken to avoid contact between alcohol or H₂O₂ and viable soft tissues that are in the process of re-epithelialization.

b. Sterile draping and aseptic techniques while opening a recording cylinder are recommended.

c. Uninfected cylinders should be cleaned as often as possible, but no less than twice a week 3-4 days apart. Sterile instruments (e.g., aspirator/suction tips, forceps) and supplies (e.g., gauze, drapes, gloves) should be used while working inside the recording cylinder. After cleaning, it is recommended that the old cap be replaced with a new sterilized (i.e. autoclave, Cidex®) cap each time. Although it may be less effective, but cleaning of the used cap with povidone-iodine scrub, alcohol, and rinsing or soaking it in a 1:10 sodium hypochlorite solution is used by some programs.

d. Known or suspect infected cylinders should be cleaned 5 to 7 days a week (Gografe & Niekrasz 2009), regardless of whether animals are treated with antimicrobial agents. If there are multiple cylinders, they should each be thoroughly cleaned sequentially rather than simultaneously. No materials (e.g. forceps, suction tips, etc.) should be shared between cylinders during multiple cylinder care. Uninfected cylinders should always be cleaned before suspect or known infected cylinders. Cleaning should always begin with a sterile saline lavage followed by suction. The dura must be carefully examined for the presence of focal infection, necrosis, cuts or tears before any cleaning agents are applied. Disinfectants and antibiotics (e.g. cephalosporins) may contribute to unwanted toxic events that manifest clinically as neurological deficits. The following compounds have proven useful:

i. Use of a 3% H₂O₂ solution or a 1:1 mixture of H₂O₂ and povidone-iodine facilitates removal of biofilm and proteinaceous material from the interior surface of the cylinder wall.

ii. Rinsing several times with a dilute povidone-iodine solution at 1-2 % (dilution is necessary for ionization of bound iodine). After cleaning, a few drops of 2% povidone-iodine solution may be left inside the cylinder.

iii. Although some programs have reported the use of chlorhexidine inside the recording cylinder for routine maintenance without problems, its use is controversial, as the compound has been demonstrated to have neurotoxic properties (Henschen & Olson 1984, Perez, et. al. 2000, Lai, et. al. 2011). The US Physician Desk Reference (PDR), as of 1984, warns that "chlorhexidine gluconate is for external use only. Keep out of eyes and ears and avoid contact with meninges". Since other disinfectants (i.e. chlorine, iodine, etc.) have been demonstrated to be efficacious for cylinder maintenance, the use of chlorhexidine should be carefully evaluated. At a minimum, care should be taken to evaluate the
dural integrity prior to using chlorhexidine and to thoroughly rinse the cylinder free of the compound after each use. Leaving residual chlorhexidine in the cylinder for extended periods of time is also not recommended.

iv. Dakin’s solution may be used cautiously when addressing infections refractory to other treatments and when the integrity of the dura has not been compromised.

v. Chlorine dioxide is typically not used in routine cleaning but it may be effective in short-term treatment of mycotic infections (Lee 1998).

vi. In the majority of cases involving a durotomy or durectomy, the underlying cortex is covered with artificial dura combined with the use of silicone membranes, collagen matrix, or aliphatic polyether polyurethane sheets. Where the dura has been cut, it should be sutured to protect the cortex. The cylinder cleaning process is the same as with intact dura. It is critical to rinse with copious volumes of sterile water or saline if any disinfectant was used.

4. Granulation tissue.

Granulation tissue (GT) formation is part of the normal healing process, but it is not always desired when maintaining chronic cranial implants. Budding GT on the wound margin and the dura is typically highly vascular and bleeds easily, oozes serum, and may interfere with healing if it becomes infected. Dural GT that is not removed on a regular basis may bleed and eventually result in dural fibrosis. Thick granulation tissue pads can harbor bacteria and become a source of chronic chamber infections.

a. 5-Fluorouracil (5-FU) may be helpful in reducing or delaying the GT growth (Spinks 2003). 5-FU is an antimetabolite, antimitotic agent that reduces tissue re-growth, vascularization, and bacterial overgrowth by interfering with nucleic acid synthesis, thus preventing mitosis. 0.5-1.0 ml of 25 mg/ml aqueous 5-FU should be instilled into the cylinder three times weekly to bathe the dura for 5 minutes. At the end of 5 minutes the cylinder should be rinsed with copious volumes of sterile saline. 5-FU must never be used on compromised dura as subdural leaks may contribute to complications. 5-FU decreases fibrinolytic activity and enhances the risk of thromboembolic events (Kessler 1994). Care must be used when handling 5-FU because it is a known carcinogen.

b. Early GT deposits may be removed using suction. Local anesthesia can be provided via instillation of 0.25-0.5 ml of 1-2% lidocaine or 0.25% bupivacaine or a 50:50 mixture for a few minutes before removal. Post-procedural systemic analgesics should be considered.

c. Chronic growth of GT typically leads to the formation of a firm fibrous layer requiring “dural scraping”, which must be conducted under general anesthesia with the post-operative use of analgesics. GT deposits on the wound margin may be addressed by surgical debridement followed by a V-plasty, regular cleaning, treatment of local infections, and chemical or electrical cauterization under systemic or local anesthesia.
5. Treatment of Implant Margin and Cylinder Infections

While assessing the skin/implant interface, care must be taken to determine if the interface infection is topical or originating from under the cranial implant. In addition, the inability to retain fluid within the recording cylinder is often the result of open tracts between the cylinder and wound margin. The ideal interface should be smooth and free of “pockets” and abrupt changes in the contour of the implant. Reshaping of the interface and performing a “V-plasty” should be considered. Culture of purulent exudate, cleaning/debridement of the area, and administration of topical or systemic antibiotics have also been used.

Infections inside the recording cylinders are common and can be prevented and treated with careful cleaning and maintenance as outlined. Systemic antibiotics should be reserved for treating cylinder infections in which the dura or bone are severely compromised or where the infection has been unsuccessfully treated with the frequent cleanings and use of halogen solutions. Indiscriminate use of antibiotics can result in bacterial resistance and additional problems. The use of halogen solutions within the cylinder (e.g. Povidone-iodine, Dakin's solution, etc.) has been demonstrated to clear infections in many cases. These solutions have been used as part of the cleaning regimen and have been left in the chamber after cleaning for extended periods to treat chronic infections. Chronic infections should always be treated in consultation with a veterinarian.

REFERENCES:


