

Practical guidelines for aseptic surgery in rodents and the management of surgical facilities in a laboratory.

ANZCCART
Mitchell Building
Adelaide University
SA 5005
Telephone 08 8303 7586
Fax 08 8303 7587
E-mail
anzccart@adelaide.edu.au

Gary Martinic

Centre for Transplantation & Renal Research

Westmead Millennium Institute & Research Centres, Westmead NSW 2145, Australia

(Past address: The Heart Research Institute, 145-147 Missenden Road Camperdown NSW 2050, Australia)

Introduction

The daily operation and management of an operating suite in a research laboratory varies considerably with that of a veterinary hospital establishment. Rather than the provision of a series of dedicated rodent operating suites, often such facilities only comprise of a separate room, a portion of a room, or even less, an area of a bench which is thoroughly disinfected and prepared for surgery. This area of research generally requires staff with formal training and experience in laboratory animal science. It also requires competence in the use of microsurgical instruments, often ingenuity in 'manufacturing' novel devices according to special needs, and regimented facility maintenance and management schedules. In scientific organizations, it is commonly the appointed senior animal technologist (AT) or an experienced scientist, rather than veterinary

personnel, who is charged with the responsibility of operating suite management. This article describes the principles used in managing such facilities, how they should be prepared for day-to-day use, how and why they should be routinely maintained and monitored, and reasons why this is necessary. Additionally, it also provides recommendations for the conduct of aseptic surgery on rodents. It focuses on the most commonly used laboratory animals, which include mice, rats, guinea-pigs and rabbits.

The general principles described can also be applied to larger animals. Compliance with these principles pre-, intra and post-operatively should ensure reliable and consistent scientific results.

Discussion

The importance of asepsis

One of the most important fundamental prerequisites for success in any operating theatre environment, is a thorough knowledge of the principles of sterility, and an appreciation that infection of a surgical wound may be the result of microbiological (particularly bacterial) contamination. Essentially, the total absence of any microorganisms (including spore-forming bacteria) is defined by the term 'sterile'. Good theatre technique is therefore dependant on eliminating the introduction of microbiological organisms during the surgical procedure, and in the post-recovery period. Thus, routine application of the principles of 'strict' aseptic technique are not only important in the preparation of an operating room/bench to be used for rodent experimental surgery, but also in obtaining this standard and maintaining it throughout procedures.

Is aseptic technique necessary in rodent surgery?

While it has been past common practice at many laboratory establishments not to follow strict aseptic technique for rodent surgery, as rodents often survive surgical procedures using less than aseptic technique. It is now

generally accepted by most researchers (refer Cunliffe-Beamer 1972-1973 and 1990; Bancroft *et al*, 1989; Abbas *et al*, 1991; Bradfield *et al*, 1992; Committee on Infectious Diseases of

“Good theatre technique is therefore dependant on eliminating the introduction of microbiological organisms during the surgical procedure”.

Laboratory Rats and Mice, 1992) that the use of aseptic surgical technique for rodents is, in fact, recommended. Cunliffe-Beamer (1993) proposed that 'survival alone is not a valid criterion for judgement of acceptability of a rodent surgical technique, but rather, the criterion for acceptability should be the presence of untoward, unplanned alteration of physiological functions or behaviour due to perioperative infection'. Furthermore, ATs and theatre workers should remember that an animal's immune response can be activated due to reaction to bacteria, which may have been inadvertently introduced during surgical procedures, where aseptic surgical technique has not been followed.

Aspects of surgical practice unique to rodents

There are of course aspects which are unique to rodents when used as surgical models. The first of which is the difference in bodyweight and size of these animals as compared to larger animals used in research. It is important to note that there may be significant bodyweight variation depending on the species, age and strain of an animal to be used. For example, a research protocol may require the use of a newborn mouse weighing only 1.0g at birth, or an adult guinea-pig weighing between 600-800g. In percentage bodyweight terms, this disparity can approximately equate to comparing a 1.5 kg Pomeranian dog with a 750-800 kg dairy cow. Because of this body size difference, binocular dissecting microscopes (with zoom focus capability) and fine microsurgical instruments (ie neurosurgery, ophthalmic type) should be used for surgical procedures on rodents.

Furthermore, as microsurgery is, by definition, any surgery involving the use of a microscope, routine surgical procedures performed on rodents could be regarded as 'microsurgery'. Secondly, it is important to remember that the AT or scientist who prepares the animal(s) for surgery, the anaesthetist who induces and maintains anaesthesia, and the surgeon who operates on the animal(s), is often the same person. Thus, it is important to meticulously plan procedures which will be performed and to prepare materials and equipment in advance before the surgery commences, as assistants are often not available. Repetitive surgery is frequently another aspect of surgical procedures conducted on rodents. For example, during a single session, the same surgical procedure may be carried out on individual rodents or on 'batches' of rodents (particularly where relatively simple and acute surgical procedures are used). Also, because of the need to maintain asepsis, it is important to be

able to effectively re-sterilize or disinfect surgical instruments between rodents, as it is uneconomical to open a separate sterilized surgical pack for every individual rodent. Methods which can be used to decontaminate instruments between animals include simply wiping instrument tips with a 70% alcohol-moistened gauze swab between rodents (tissue residue or blood should be removed first), or by using commercially-available glass bead sterilizers (note-these can cause burns to tissue and need to be adequately cooled prior to tissue use). Instruments can be held in a bowl/kidney dish containing 70% ethanol. Sterile forceps can then be used to retrieve particular instruments after rinsing them in a separate bowl of 0.9% physiological saline, to remove the ethanol before re-introduction into animal tissues. Finally, although rodents are omnivorous and have a monogastric stomach, it is important to remember that rodents do not possess a regurgitation reflex, and therefore are incapable of regurgitation. Thus the withholding of both food and water prior to surgery is not generally required. The exception to this rule however, applies where surgical procedures are to be performed on the gastro-intestinal tract, in which case food (but not water) should be withheld, as distended digestive organs increase the risk of potential surgical complications.

What constitutes minor and major surgery?

The main aim of any surgical procedure is that it is, carried out skilfully with the minimum of risk and disturbance to the animals, and secondly, that it is done without the accompaniment of infection (Wayneforth & Flecknell, 1992). The current Australian 'Code of Practice for the Care and Use of Animals for Research Purposes' (6th edition, 1997) does not specifically define what constitutes minor and major surgery, however an earlier document entitled 'A Guide to the Care and Use of Laboratory Animals' (U.S. Public Health Service), as well as part 1.1 of the U.S. Animal Welfare Act (of the same year), do define major survival surgery as 'any surgical intervention that penetrates a body cavity or has the potential for producing a permanent handicap in an animal that is expected to recover', thus it would be reasonable to assume that any procedure that does not penetrate a body cavity or does not produce a permanent impairment of function could be regarded as 'minor surgery'. Although as Cunliffe-Beamer (1993) points out, 'one should remember that even a relatively minor surgical procedure, such as vascular catheterisation, can have life-threatening complications if bacteria are introduced into the blood stream'. Indeed, such

relatively 'minor' procedures may constitute the bulk of the work conducted in many animal research laboratories.

Additional considerations

The success of any experimental surgical procedure on rodents will depend on the competence, skill and experience of the AT or scientist performing them. As the overall welfare of the animal being used is of primary importance, it is imperative that the 'experimental surgeon' has sufficient training in dealing with possible surgical and anaesthetic emergencies (particularly if the surgeon is neither a veterinary or medical physician) and post-surgical complications. This can vary from having a strategy for implementing cardiopulmonary resuscitation, or having the surgical training and/or experience to effectively control internal bleeding, or to prevent potential surgical complications such as wound infection (which can develop through poor aseptic technique). Careful planning is required before any experimental surgery proceeds. It is imperative that experimental surgeon has carefully selected the most appropriate animal model for the research project (which includes having a detailed knowledge of the anatomical features which are characteristic of the species, as well as its metabolic and behavioural requirements). Due consideration should also be given to the surgical intervention to be performed, and if there are any potential health complications likely to occur as a result of it, and how to deal with them. More straightforward issues include deciding on the type of surgical approach and method of anesthesia to be used, as well as the pre-, intra- and post-operative care required for the selected species. Another important issue relates to timing of the surgery. Unless contra-indicated, all surgical procedures should be carried out as early in the day as practically possible, as this will allow that there are sufficient manpower resources to ensure that postoperative care can continue for extended periods of time.

Finally, researchers should consult both the relevant statutory (Animal Research Act, 1995) and national code(s) of practice requirements (Code of Practice for the Care and Use of Animals for Scientific Purposes, 6th edition 1997) when planning any experimental surgical protocols to ensure that they will comply with the accepted, national guidelines.

What is required for aseptic technique?

It is recommended that recovery surgical procedures on rodents be conducted using aseptic principles to minimize microbiological contamination and to prevent transmissible infections between animals. At a minimum, this should include the use of sterilized instruments, gloves and a face mask. However, it also assumes that prior disinfection of equipment such as the dissecting microscope, the bench or table where surgery is to be performed, as well as skin decontamination around the incision site of the rodent, has also been done. Additionally, all surgical instruments should have been sterilized (either by autoclaving or by other means) and that the sterility of instruments/equipment be maintained between animals. It should be remembered that all of these principles are mandatory requirements for recovery surgery on non-rodent and larger species, which additionally require that there are separate areas for surgery, preparation, storage and recovery.

Conditioning, familiarization and health assessment

A baseline of normal animal behaviour and general health should be established prior to any preparation for surgery, particularly where animals have been transported from an external supplier. It is also important to familiarize the animals to frequent handling as often as possible before any laboratory study commences. The ideal situation should be one where the experimental surgeon allows incoming animals to acclimatize for up to one week (minimum of 2-3 days) during which time frequent visits are made to the animal house. Scientists should use this time to observe the animals in their own environment, pet them, practice gentle handling skills on them, weigh them and make a record of any weight gain/loss during the pre-operative period.

The other equally important reason for an acclimatization period is that rodents are given adequate time in which potential subclinical infections can manifest themselves, before affected animals are subject to the stresses of surgery, recovery and post-operative care. The pre-study time should also be spent carrying out a general health assessment. Most reputable suppliers of animals (particularly those sourced from a specific pathogen-free facility) will ensure

that animals obtained from them are in a fit and healthy state for laboratory experimentation or surgery to proceed almost immediately. Rodents are, because of their small size, generally housed in communal group cages, and therefore they are commonly examined as a group rather than as individual animals. Upon opening cage lids housing rodents, researchers should check that the animals appear bright, alert and responsive. Rodents should generally appear very active within their cage (lethargic or immobile animals should be considered suspect) and inquisitive. Many animals will frequently tend to stand up on their hindlegs or look out over the edges of their cage in a rather curious manner. The AT and scientist should carefully listen for the pattern, rate and consistency of breathing, as rodents with chronic respiratory disease will display signs of either sniffing, weezing and/or sneezing (particularly if housed on sawdust or wood shaving bedding material). The quantity of water and food consumed should be checked, recorded and regularly monitored. Low levels of water consumption may be an indicator of dehydration (sometimes seen in transported animals post-arrival). Often the best indicator of this is by observing an animals coat condition and by using the pinch test (ie pinch skin overlying the spine to see if it settles quickly, if it doesn't the animal may well be dehydrated).

A dedicated rodent surgical facility?

Rather than the provision of a dedicated room for rodent surgery (with separate preparation and recovery rooms) in most biomedical research establishments, experimental rodent surgery is generally performed on laboratory benches that are often used for other functions. Due to the small body size of rodents, a separate animal preparation room where animals may be pre-medicated, anaesthetised, clipped and prepared for surgery, is seldom necessary as rodents can generally be prepared for surgery in the same room. However, a physically-separated recovery room or area is required. Some research institutions however, are fortunate to have 'purpose-built' facilities, for example, Gregory (1998) describes in detail specially-designed, multiple rodent operating suites at one biomedical research organization. Rodent surgery is also commonly performed inside class II biological safety cabinets, which have high efficiency particulate absorbency (HEPA) filtration, which has the advantage of protecting both the animals inside the hood and the operator using it, against aerosol hazards. Apart from the many hazards of an infectious nature, such hazards may also include

occupationally-induced asthma (OIA) and laboratory animal allergy (LAA). Generally, most areas of a laboratory (ie room, part of a room, laboratory bench top) that can be easily cleaned and disinfected, with good ventilation and lighting and if possible, scavenging facilities (if gaseous anaesthesia is used), can serve as a useful area for rodent surgery. The important issue is to ensure that areas should be subdivided according to function (ie distinct areas for performing surgery, preparing animals, recovering animals, and for storage of cages and equipment).

Maintaining hygiene of operating rooms

In order to maintain cleanliness and hygiene of the surgery areas, the floors should be damp-mopped using hot water and disinfectant solution and allowed to dry. As in veterinary operating theatres, periodic hygiene 'wash-downs' of the walls, floors and (waterproof) ceilings should be carried out using a disinfectant solution at suitable strength, and allowed to dry. If rooms are not to be used for any period of time, equipment should be covered over with clean drapes. Extraction fans (which should be operating at negative pressure) should be kept running to maintain filtration efficiency, but they should be regularly (ie daily) cleaned of dust particles.

Preparation of the rodent operating area

Bench-tops or other surfaces to be used for recovery rodent surgery should be cleaned of dust by wiping over with a wet cloth that has been soaked with a cleaning agent followed by disinfection with either 70% alcohol or a quaternary ammonium compound. This is best done immediately before surgery, followed by 5-10 minutes drying time. All other equipment to be used in the surgical procedures, such as microscope/s, operating lights, anaesthetic machine (if used), dissection/instrument trays, etc. should also be wiped over with a disinfectant-soaked cloth and allowed to dry. A large sheet of clean 'Bench-roll' (bench covering; Kimberley Clark, NSW) should be laid out to cover the entire bench surface where surgical procedures are performed. Machinery, equipment and theatre furniture should be arranged in a way that is most convenient to the surgeon. For example, placing the sterile instruments on a tray, at the surgeons' side, so he is not required to make repeated un-ergonomic movements in order to reach needed instrumentation. Unlike ceiling-mounted, multiple

head theatre lights as used with larger animals, bifurcated, telescopic light guides (if a fibre-optic light source is used) should be positioned according to the surgeon's preference, particularly if they are to illuminate the subject under a binocular, dissecting microscope. This powerful source of lighting, which is generally used for rodent surgery as it can give precise 'spot' lighting as well as diffuse lighting, without generating any heat. It is also important to ensure that an ample supply of commonly used materials (ie syringes, needles, gauze swabs, cotton-tip applicators) as well as drugs (ie analgesics, antibiotics) and/or emergency medications/equipment (respiratory stimulants) are close to hand. If surgery equipment is not to be used within 10-15 minutes of cleaning, it should be covered over using sterile drapes.

Operating board/table

Recovery surgery performed on rodents is commonly of a repetitive nature and it often involves animals being restrained in dorsal recumbency. Using this postural approach, it is necessary to outstretch (but not overstretch) the limbs and comfortably secure them. Overstretching the limbs can often result in overstraining of the muscles, which can produce post-operative pain. One of the best ways to achieve restraint, is by using some kind of board which has either anchors or ties, to place around an animal's limbs. Adequate padding should be placed between the ties and the limbs for comfort, and so as not to impede the blood circulation. ATs and scientists performing surgery on rodents have the advantage of being able to work in the sitting position, making it much easier to comfortably stabilise their hands and wrists and to reduce the risk of tremor. In most laboratories, an operating board of some kind is used. Simple materials which are commonly used for such boards include cork, rubber, foamy-rubber and polystyrene (lids from cooling boxes), which are all favoured because of the ease with which fixing pins can be used. Such boards measure approximately 415mm x 185mm x 30 mm. They can be thoroughly cleaned of blood and tissue residues using a scrubbing brush, followed by immersion in a suitable disinfectant. But because they can't be sterilised by autoclaving, it is recommended that operating boards only be used for terminal surgical procedures on rodents. The other advantage in using small boards is that they can be placed beneath the viewing objectives of dissecting, binocular microscopes, thus allowing the image of the rodent to be magnified and visualized, thereby enhancing operability.

Commercial animal caging suppliers have recently made available specially-designed rodent operating tables and raised platforms. These products vary in their applicability, design and dimensions, but most are composed of a rigid, moulded, (non-corrosive) polycarbonate plastic material and are fully autoclavable (refer to Index of Equipment). And most come supplied with specially-designed limb holders, anchors or ties.

Care of instruments for microsurgery

The extent of tissue trauma during experimental surgical procedures on animals is directly related to the state and operability of the surgical instruments used (as well as the skill of the surgeon!). Needless to say, the risk of tissue trauma is increased by the use of blunt instruments or instruments which have been inadvertently damaged. Some examples of this include the tips of dissecting forceps which do not meet, blunting of the cutting edge of hinged instruments, and inadvertent curvature of tips, blades and possibly handles. For this reason, it is important to check all instruments regularly, usually during cleaning, before storage, and once again before commencement of procedures. Inspection of instruments is best achieved using either a hand-held magnifying glass or examining the instruments under the low power of a binocular dissecting microscope.

Selection of instruments for rodent surgery

Although it is important to match the size of the surgical instruments to the size of the animal to be operated on, the types of surgical instruments selected for experimental surgical procedures on rodents will vary depending on a number of factors which include, the organ systems and tissues to be manipulated (ie vascular work in rodents requires very fine, delicate microsurgical instruments), the surgeon's preference, and the availability and pricing of particular instruments. There is generally a lack of specially-designed surgical instruments for use in rodent experimental surgery, and for this reason many scientist-surgeons and ATs have often successfully employed the use of specialised microsurgical instruments (ie human neurosurgery, ophthalmics, paediatric medicine). Indeed, many experienced scientist-surgeons and ATs have often enterprisingly devised specifically-designed instruments and other theatre equipment of their own, according to a novel need for them.

An elementary instrument pack for rodent experimental surgery may be assembled for use. This may include microsurgical instruments such as; a size 3 scalpel handle and size 15 blade (not always necessary for murine surgery as small surgical scissors can be used, but recommended for rats and larger species), pointed surgical scissors (straight or curved, for general surgical use and for cutting sutures), long pointed (round-tipped) non-traumatic surgical scissors (for blunt dissection of tissues/muscles), if cannulation work is to be done then fine, pointed or angled microsurgery scissors should be included, as well as very fine, microsurgical dissection forceps (for delicate tissue and vessel dilation work). Also required are straight/curved, toothed, DeBakey dissection forceps (for grasping tissues, but not for delicate tissue handling), non-toothed, DeBakey dissection forceps (tend to slip, but are almost atraumatic and good for tissue handling), 'Mosquito' forceps or haemostats (for clamping vessels before ligation), Microsurgical needleholders (ratchet or non-ratchet type may be used, to aid suturing). Some type of wire speculum or self-retaining retractors (for retracting and making abdominal tissues 'accessible'). If precise murine vascular work is to be performed then it is necessary to also employ the use of small blood vessel clips (for cannulation studies). Work with larger rodents (rats, guinea pigs) should employ the use of 'Bulldog' clamps (which facilitate blood vessel occlusion). Other essential materials include absorbable haemostatic gauze swabs and cotton-tipped applicators. Apart from being a useful general use item, gauze swabs (50 x 50 mm) are indispensable in controlling haemorrhage, and when moistened with physiological saline, can be used to cover tissues or organs when they are required to be exteriorised. They can also be utilised, when rolled-up, to bolster the position of rodents for surgery. Currently, species-customised 'V' shaped thoracic positioners are available (see Index of Equipment). Cotton-tipped applicators are also indispensable in rodent surgery, particularly for the blunt dissection of connective and most delicate tissues, and absorbing minute amounts of blood and other bodily fluids. Alternatively, some experimental surgeons may prefer to use commercially-available skin stapling equipment or the use of skin clip applicator apparatus for the closure of wounds, rather than using 'traditional' suturing methods, which require a moderate degree of skill.

The other item of absolutely essential equipment is lighting. Because of the relatively small surface area which is used in rodent experimental surgery, a strong source of sustainable bright light is imperative. While diffuse lighting is acceptable, preference should be given to precisely positioned 'spot' lighting, particularly for surgical procedures where only one mouse is operated on at a time. Although it is more expensive than other forms of lighting, fibre-optic light sources (see Index of Equipment) should be used wherever possible, as they do not generate excessive heat which risks increased evaporation of body fluids. Recovering animals should be made comfortable on one of a number of commercially-available, purpose-made warming pads (see Equipment Index).

Scrub procedure for the surgeon

In line with formal surgical protocol, and to minimize the risk of external contamination entering the facility, outer 'office' clothing should be removed and replaced with a surgical scrub suit, and a surgical face mask and cap donned. The surgeon is then required to perform a complete scrub procedure prior to donning a sterile surgical gown and gloves. The hands, fingers (including nails) and mid-forearms should be washed thoroughly with an appropriate antiseptic soap and then scrubbed using an iodine surgical scrub solution and a scrubbing brush. Once the surgeon has dried the hands and arms with a sterile handtowel (found inside a sterile instrument pack), he should put on a sterile theatre gown and surgical gloves.

Log/Record of anaesthesia and surgical procedures

For biomedical research purposes, such information should include the type of procedure or operation conducted, the date and time it was conducted, the name of the surgeon, the animal ethics committee approval number and expiry, the name and dosage of anaesthetic and other medications administered, the identification number(s) of the animal(s), the age, sex, strain and weight of the animals, colour of mucous membranes, capillary refill time, the source/supplier of the animals used, etc.

Preparation of rodents for experimental surgery

Apart from acute, non-recovery surgeries in rodents, in any recovery surgical procedure that is performed in a room or part of a room which has been designated for that sole purpose, the surgeon should follow the principles of strict aseptic technique. The rodent should be placed in a position appropriate to the surgery (preferably on a heating pad) and the fur clipped using electric clippers (see Index of Equipment) over the site where the incision is to be made. In mice, approximately 1.0-1.5 cm either side of the incision site should be clipped (if possible, the diameter of clipper blades should be no more than 2.0-2.5 cm). In rats and guinea-pigs, this area should be approximately 4-5 cm, and even larger in rabbits. Fur clippings can be easily removed from the incision site using a small, rechargeable, hand-held vacuum cleaner. All four limbs should be secured to the operating board by one of the methods described earlier. Using either 'Mosquito' haemostats or toothed dissecting forceps, the surgeon can grasp a thick wad of non-sterile gauze swabs with which to disinfect the exposed incision site. Seventy-percent ethanol should be sprayed over the incision site, any excess fluid should then be wiped off with the gauze swabs using the forceps (wiping the site should be done in a circular motion beginning from the centre outwards so that any potential pathogens are transferred to the non-sterile outer region). This step should be repeated using Iodine surgical scrub solution, followed by a spray of surgical Iodine over the site and left for 4-5 minutes to take effect. It is important to remember to use a clean (albeit non-sterile) swab each time. A fenestrated drape (of the appropriate size for the species to be used) should be applied directly over the region where the incision will be made, to maintain a sterile operative field. Fenestrated surgical drapes are commonly used as they avoid surgical instruments or exposed organs from coming into direct contact with parts of the animal which have not been prepared for surgery.

The use of surgical drapes

Drapes for rodent surgery can be fashioned from a variety of different materials including (disposable) paper, cloth or plastic (which can also be transparent and adhesive). One method the author has used has included preparing surgical drapes for mice from the fabric of 'aged' theatre gowns. These were cut to a size

measuring approximately 80mm x 80mm and were easily sterilized (between two handtowels, inside an No.1 double-folded autoclave bag) in a high pressure steam autoclave. Abdominal sponges (as used in human and veterinary surgery) can also be cut to size and used to drape rats or larger species, for aseptic surgery.

Maintaining asepsis of instruments & equipment in between use

In between use, all surgical instruments may be kept in a kidney dish or bowl containing 70% ethanol, and sterile forceps used to retrieve individual instruments.

Post-operative care & analgesia

The provision of long-acting analgesia is of the utmost importance in the management of laboratory animals used in recovery surgical procedures. A long-acting analgesic such as Temgesic (buprenorphine 0.3 mg/kg; Reckitt & Colman West Ryde, NSW Australia) should be routinely administered prophylactically every 8 hrs (up to the first 48 hrs, if required) particularly where very invasive surgical procedures have been performed. These would include any surgical procedures penetrating body cavities, ie the gastrointestinal, respiratory or the urinogenital tract, joint or muscle tissue manipulations, or surgical procedures on or within the skull. Animals should be regularly monitored every 2-4 hours for first 24-36 hours in an effort to relieve any possible post-operative wound pain or discomfort. The temperature of the area or room where the animal will recover should be maintained at a constant rate, for example 21-23°C (depends on the species used). The recovery area or room should be draught-free, dimly-lit and quiet. If possible the animal should be placed on a heating pad, or alternatively, on soft bedding (no sawdust or wood shavings as they may contaminate the wound) to prevent the risk of hypothermia. This should be emphasized, as smaller mammals, because of their faster metabolic rate, are prone to acute falls in body temperature. It is important to check the colour of the mucous membranes of the animal as well as its respiration rate and temperature for 2-3 minutes, and then at regular intervals. The animal should be observed until it has fully regained consciousness and the righting reflex, then returned to its usual pen or cage. Larger animals such as rabbits and guinea pigs, should be monitored every 10-15 min for the first hour, then every 30 minutes for the next

2-3 hours, until full recovery is achieved. On the first day post-operatively (and for a few days thereafter) the wound should be checked for evidence of weeping, bleeding, trauma and/or self-mutilation, and the respiration, pulse and temperature noted. The frequency of urination and defaecation should also be carefully noted, as well as observing any problems with these behaviours. A useful assessment of recovery can generally be made by measuring an animals intake of food and water both pre- and post-operatively. Most research laboratories commonly employ the use of a simple mask circuit, rather than endotracheal intubation (ET) when anaesthetising animals such as rabbits but ET does provide better airway control. If an endotracheal tube has been used, post-operatively, it should be left in place until the animal has regained its swallow/cough reflex, at which point the cuff can be deflated and the tube carefully pulled out. ET is not commonly used with small rodents in research laboratories, although the technique can, with sufficient practice, be performed quite successfully (refer Thet, 1983; Yasaki and Dyck, 1991).

Post-operative care of instruments

All instruments, especially fine microsurgical items should be washed by hand as soon as possible post-operatively, using a fine bristled brush under running warm tap water, followed by ultrasonic cleaning (Lanz & Bennet, 1998; Green & Simpkin, 1987; Serafin & Georgiade, 1986; and Lee, 1987). Soaking instruments in detergent solution is not generally recommended, because instruments frequently tend to develop rust especially those which are hinged. They should be dried using heated forced air or a hand towel (Hoyt, Clevenger and McGee, 2001) and each should be carefully inspected for damage, particularly microsurgical instruments and those with fine tips, before storage. After thorough cleaning and drying, those instruments which are hinged or which have pivot points (scissors, microvascular clips, retractors) should be sprayed with a good quality instrument lubricant. Autoclavable instrument 'tip covers' should be placed on all microsurgical instruments (Hoyt, Clevenger and McGee, 2001) and these instruments should be placed into cushioned (microsurgical) instrument sterilizer trays. They should be placed in between the silicon 'fingers' which will securely hold them in place, and will prevent any contact with other instruments, then the lid should be closed.

Post-operative care of surgical equipment/materials

All drapes and gowns should be collected and washed as soon as possible preferably using an active biological detergent which helps to dislodge and remove blood stains. All workbenches and used equipment should be wiped down with a combined disinfectant solution and the area or room tidied before replacing all equipment to its previous position. Operating lights should be examined to see that they are working properly (ie new bulbs may be needed, if so, a reserve supply should be close to hand) and wiped down. All electrical appliances should be examined to check that they are maintained in good working order, this includes any electrical cables, which should be examined for wear and tear. The AT or scientist should check and make note of any consumables (ie sterile swabs, drapes, syringes, needles, etc) which require re-stocking and re-ordering.

Conclusion

This article has attempted to provide practical recommendations for the maintenance and management of surgical facilities using rodents in medical research laboratories, which are mainly aimed at research scientists and ATs charged with facility management responsibilities. It has outlined the importance of implementing the principles and practices of aseptic surgery for use with small laboratory animals. It also defines the requirements for good surgical technique with particular emphasis being placed on rodents and rabbits which are utilised in the laboratory for medical research purposes. Standard operating procedures (SOPs) should be developed and implemented for small animal surgical facilities, and they should be periodically reviewed by the facility manager, as research programmes can vary from time to time. Appropriate surgical SOPs and the strict application of aseptic technique should ensure that any potential variables in the research data are eliminated.

Acknowledgements

The author gratefully thanks the staff of the Biological Services Unit; K. Berry, M. Khalil and M. Riad for routine animal handling and care. He also thanks Professors Phillip Barter and Mike Davies for their constant encouragement and support.

References

1. Cunliffe-Beamer, T. (1972-73). Pathological changes associated with ovarian transplantation. *44th Annual Report of The Jackson Laboratory*. Bar Harbour, Maine. 104.
2. Bancroft, G., and R. Schreiber, *et al* (1989). T cell independent macrophage activation in SCID mice. *"The Scid Mouse – Characterization and Potential Uses"*. New York, Springer Verlag. 235-242.
3. Abbas, A., Lichtman, A., *et al* (1991). *"Cellular and molecular Immunology"*. Philadelphia, WB Saunders Company. 229, 302-3.
4. Bradfield, J., T. Schactman, *et al* (1992). Behavioural and physiologic effects of wound infections in rats. *Laboratory Animal Science*. 42(6): 572-578.
5. Committee on Infectious Diseases of Laboratory Rats and Mice, I.L.A.R. (1992). *"Infectious Diseases of Laboratory Rats and Mice"*. Washington, D.C., Government Printing Office.
6. Cunliffe-Beamer, T.L. (1993). Applying principles of aseptic surgery to rodents. *AWIC Newsletter*. April-June; Vol. 4, No. 2.
7. Waynforth, H.B., and Flecknell, P.A. (1992). *Experimental and surgical technique in the rat*, 2nd edn. Academic Press, London.
8. NHMRC (1997). *Australian code of practice for the care and use of animals for scientific purposes*, 6th edn. Australian Government Publishing Service, Canberra.
9. Committee on the Care and Use of Laboratory Animals, I.L.A.R. (1985). *Guide for the Care & Use of Laboratory Animals*, N.I.H. Publication No. 85-23. Washington, D.C., Government Printing Office.
10. Gregory, D.J. (1998). Practical aspects of continuous infusion in rats. *Animal Technology*. Vol. 46, No. 2
11. Thet, L.A. (1983). A simple method for intubating rats under direct vision. *Laboratory Animal Science*. Vol. 33, No. 4 August.
12. Yasaki, S., and Dyck, P.J. (1991). A simple method for rat endotracheal intubation. *Laboratory Animal Science*. Vol. 41, No. 6 December.
13. Lanz, O., and Bennett, R.A. (1998). Microvascular instrumentation. In: Bojrab, M.J., Ellison, G.W., and Slocum, B., eds. *Current Techniques in Small Animal Surgery*. Williams and Wilkins, Inc., Baltimore, pp. 713-719.
14. Green, C., and Simpkin, S. (1987). *Basic Microsurgical Techniques*. Surgical Research Group, MRC Clinical Research Centre, Northwich Park Hospital, Harrow, Middlesex, pp. 1-46.
15. Serafin, D., and Georgiade, N.G. (1986). *A Laboratory Manual of Microsurgery*. Division of Plastic, Reconstructive and Maxillofacial Surgery, Duke University Medical Centre, Durham, pp. 1-56.
16. Lee, S. (1987). *Experimental Microsurgery*. Igaku-Shoin Medical Publishers, Inc., New York, pp. 21-26.
17. Hoyt, R.F., Clevenger, R.R., and McGee, J.A. (2001). Microsurgical instrumentation and suture material. *Lab. Animal Europe*, Vol. 1 No.9, pp. 33-34.

Index of Equipment

- Analgesic: Temgesic; (buprenorphine 0.3 mg/ml) Reckitt and Colman, W Ryde, NSW, Australia.
- Antiseptic: Hibiclens; 4% chlorhexidine gluconate, ICI Pharmaceuticals, Villawood NSW 2163
- Bench Covering: Bench-roll; Kimberley Clark Pty. Ltd, 52 Alfred St Milsons Point NSW 2061
- Blood pressure recorder: Harvard Bioscience Equipment Ltd., Australian distributors are Hadland Phototonics, 19A Hampshire Road, Glen Waverley, Victoria 3150
- Chemical sterilising solution: Cidex; 2% glutaraldehyde solution; Johnson and Johnson Ltd, 1-5 Khartoum Road North Ryde, NSW 2113.
- Dissection microscope: Nikon Corporation, Tokyo Japan. Australian distributors are Maxwell Optical Industries Ltd., Unit 4 20-36 Nancarrow Ave Meadowbank NSW 2114
- Fibre-optic illumination: Kyowa Optical Company, Tokyo Japan. Australian distributors are c/o - Coherent Scientific Ltd., 116 Sir Donald Bradman Drive, Hillton S.A. 5033
- Glass bead sterilizer: World Precision Instruments Ltd., Australian distributors are Coherent Scientific Ltd., 116 Sir Donald Bradman Drive, Hillton S.A. 5033
- Heating Pads/Lamps: Austvet Ltd., 5 Trade Place, Vermont Victoria 3133
- Surgical Scrub Solution: VR-Iovone; Coopers Animal Health Aust., 71 Epping Rd N. Ryde NSW
- Operating Boards/Tables: Harvard Bioscience Equipment Ltd., Australian distributors are Hadland Phototonics, 19A Hampshire Road, Glen Waverley, Victoria 3150
- Operating Boards/Tables: Iffa Credo Animal Products Ltd., Australian distributors are Imbros Ltd., 345 Riversdale Rd. Hawthorn, Victoria 3122.
- Povidone-Iodine 10% solution: Poviderm; Martin and Clark Pharmaceuticals, Cnr. Cross & Pittwater Rd, Brookvale NSW Surgical & Microsurgical Instruments: World Precision Instruments Ltd., Australian distributors are Coherent Scientific Ltd., 116 Sir Donald Bradman Drive, Hillton S.A. 5033
- Surgical Scrub Brushes: Becton Dickinson Ltd, 4 Research Park Drive, N. Ryde NSW 2113
- Thoracic positioners: Easyvet Ltd., B1, 26 Powers Road, Seven Hills NSW 2147.