While performing the procedures in an approved animal protocol, everyone strives to stay compliant. There are several effective techniques that can be used during the process of creating and implementing an animal protocol which can help prevent non-compliances.

Composing a protocol that allows for flexibility is one way to prevent non-compliances. Using words such as: approximately, about, no more than, up to, and other key phrases to describe procedures can create natural flexibility in the protocol. If an exact time is given, a non-compliance issue will arise if the time point is missed. Providing ranges for certain values can also add to the flexibility in the protocol. For example, an investigator details a procedure to be performed on an animal at a certain age. If this investigator states that the procedure will be done between a range of ages this will give the investigator a little breathing room that would not have been there if he would have chosen an exact age. Personnel issues are also very common compliance issue identified. Ensuring that all personnel listed on the protocol are trained for the task they will be performing as well as having all the necessary IACUC training is also important for maintaining compliance. Assistance with writing the protocol is always available from either the IACUC or OLAC offices and may be helpful identifying issues that can create non-compliant issues later.

There are several basic steps that can be taken during implementation to ensure compliance. First, all personnel involved should read the protocol before implementation. Reviewing the protocol during a lab meeting can help answer questions early and ensure that all personnel are familiar with their roles. This is especially helpful if some time has passed between procedures. Periodically reviewing the protocol and procedures throughout the three year approval period can be done to prevent drift away from the original protocol’s intention. Secondly, having a copy of the protocol close at hand during the project can be helpful if any questions arise and can often help in preventing mistakes. If a change needs to occur in the protocol, being proactive about submitting amendments can prevent a non-compliance from occurring. Amendments should be submitted when there is any change to procedures or personnel involved on the protocol.

Finally, if one realizes that they are doing something that does not match the original protocol, self-reporting can be done through either the IACUC or OLAC office. Both offices are available to help discuss the issue with the PI to try and make the right adjustment, which may include writing and submitting an amendment. Remember that if we all work together, compliance can be easily achieved.
The Principles of Suture Selection

Joleen Adams, DVM

Absorbable multifilament sutures are catgut, Vicryl®, and Polysorb®. Monofilament sutures are smoother and tend to cause less tissue drag. Consequently, they are associated with less tissue damage and irritation than braided sutures. Also, monofilament sutures have historically been believed to be more appropriate to use in contaminated wounds. Studies have shown that closure of contaminated wounds with monofilament suture had less bacterial colonization of the suture.\(^1,2\)

The suture selected for a surgical procedure can play an important role in the outcome. In general, tissue closure can be obtained by staples, wound clips, or suture. Wound clips and staples are primarily used for closure of skin incisions or incisions with low tension. Tissue glue can be used for minor surgical procedures or as an adjunct to another method of skin closure. Sutures are broadly grouped into those that are absorbed by the body and those that are not readily broken down. Absorbable sutures are broken down after a period of time so when used, tissue healing needs to occur prior to suture degradation. Some examples of absorbable sutures are catgut, Vicryl®, Monocryl®, and PDS®.

Non-absorbable sutures are utilized for skin closure, tissues that heal slowly, or tissues in which more permanent sutures are desired. When used for skin closure, the sutures are usually taken out 10-14 days following the surgical procedure or when sufficient skin healing has occurred. Suture removal requires restraint but typically does not require the animal to be under anesthesia. Examples of non-absorbable suture include Prolene®, Ethibond®, Ethilon®, and stainless steel.

Sutures can also be braided or monofilament. Examples of monofilament sutures are Monocryl®, PDS®, Ethilon®, and Prolene®. Examples of multifilament sutures that are non-absorbable are Ethibond® and silk. Monofilament sutures are not necessarily the best choice over multifilament for every surgical procedure. Suture choice is typically based largely upon preference of the surgeon or experience. Some prefer multifilament as this suture can be easier to handle, and knots are less likely to slip off. Multifilament sutures are also now coated to reduce tissue drag and consequently cause less irritation to the tissue. Incisions or wounds caused in a sterile surgical setting are not going to be contaminated unless a break in surgical technique has occurred. Consequently use of multifilament in such a situation may not be contraindicated. A recent contaminated wound study performed on rats showed that use of a multifilament suture (Vicryl®) in the peritoneal cavity resulted in significantly fewer adhesions than other suture material tested which included monofilament sutures.\(^3\) Some also prefer use of coated multifilament not only in intestinal surgeries but cardiovascular procedures as well.\(^5,6\)

Size of suture is another important criterion to consider. The smallest suture size appropriate for the procedure is usually the one to use. In regards to size, suture is labeled with a number, with the smaller number being equivalent to the smaller size. In other words, PDS® 7 would be larger than PDS® 3. When the number is followed by “-0”, the larger the number, the smaller the suture size. Prolene® 2-0 would be larger in size than Prolene® 8-0. Typically for delicate tissue, a smaller size suture is used. The smaller the suture size, though, the more prone it is to being broken if too much tension is applied. Careful handling of finer gauge sutures is recommended.

The type of material that the suture is comprised of is another important factor that may need to be considered. Depending on the type, some suture materials can be associated with a more pronounced tissue inflammatory response than others. Stainless steel, a relatively inert material, has very low tissue reaction. However, it can be very difficult to work with, so it is not ideal to use in every situation. Silk is relatively easy to work with, however, tissue reaction can be significant.
Every suture has both advantages and disadvantages. Maintaining current on recent trends and best practices in surgery can assist tremendously in making informed choices. Ultimately, the choice of material to use will be based upon the type of surgery, personal experience, and preference of the surgeon.

*All brand names mentioned are strictly for example purposes only and not an endorsement of any product.

References:

The Animal Care Advisory Council (ACAC) was formed by the Institutional Official (IO) in 2006. The ACAC is chaired by the Associate Vice Chancellor for Research and composed of key Program personnel including deans and department heads of faculty utilizing dedicated animal facilities. The ACAC provides advice and perspective to the IO regarding the UTK Area animal care and use program. A primary goal of the ACAC is to enhance communication and ensure an efficient mechanism to administer all aspects of the decentralized Program. Additionally, Council members are able to identify resources which the IO is then able to allocate to ensure the Program’s overall effectiveness. All required regulatory functions are maintained by the IO, IACUC, and Attending Veterinarian; the ACAC is only advisory to the IO.

Due to the varied nature of teaching and research activities conducted and the size and dispersion of the UTK Area Animal Care and Use Program across the campus and state, management of the UTK Area animal facilities is decentralized and several satellite facilities are maintained. Animal facility managers are directly supervised by the facility’s responsible dean, department head, or REC director. The ACAC serves as a resource to assist the IO and keep the various facilities informed about the Animal Care and Use Program of the entire institution.

Items that are discussed during the monthly meetings include updates from the IACUC and OLAC. Post-approval monitoring reports are provided. Budgetary items from per diems to technical service rates have been presented. Facility needs, renovations, new plans and grant proposals for dedicated animal facilities are also on the agenda. The new Tennessee Electronic Research Administration (TERA) Animal Care Protocol Application (ACAP) plan has been a large topic of discussion for the past several months. The ACAC serves an important role for the IO as well as keeping all of the different animal user groups updated on the status of the UTK area animal care and use program.
Environmental monitoring is an important part of the overall health surveillance program in the laboratory animal facility. Monitoring key areas in the laboratory animal environment ensures that the current measures in place for reducing or eliminating microbial load on surfaces are sufficient. To effectively achieve sanitation, laboratory animals rely on mechanical and manual methods which usually incorporate the use of hot water, chemicals, or a combination of both to achieve sanitation. Environmental monitoring assesses the effectiveness of this process.

The Guide for Care and Use of Laboratory Animals, devotes a small section to environmental monitoring and gives several ways to achieve this objective. One method is through the use of organic detection systems such as ATP bioluminescence. This method uses a chemical reaction to generate light from ATP, which is present in all living cells. The quantity of light generated is directly proportional to the amount of ATP present. As a result, the light units can be used to estimate the biomass of the cells in a sample. Another method and the one that is used in the UTK environmental monitoring program is microbiological culture. The Office of Laboratory Animal Care conducts the environmental “audit” and the swabs of environmental surfaces are plated and cultured in the bacteriology lab at the Veterinary Medical Center. Special attention is given to surfaces which come in direct contact with the animal such as primary enclosures, watering devices, and enrichment. A limit of 50 colonies is set as the maximum number of bacterial colonies that is acceptable. Swabs that exceed this value are interpreted as areas with significant bacterial growth. Depending on the item and sanitation process, a significant result could lead to corrective actions put in place to ensure that the material is successfully sanitized in the future. Corrective actions take the place in the form of re-cleaning and re-testing, changing sanitizing chemicals, or disposing of the item altogether.
Located at the University of Tennessee Medical Center, the Vascular Research Laboratory is a basic and translational research laboratory dedicated to the study of peripheral vascular disease and the development of therapeutic interventions to prevent development of vascular pathologies. Currently, one in every 20 Americans over the age of 50 has peripheral arterial disease, making vascular procedures some of the most common surgical interventions nationwide. Common vascular interventions result in mechanical damage to the diseased vessel, initiating the process of vascular remodeling and oftentimes contributing to development of secondary vascular pathologies. The most widely used intervention is balloon angioplasty. While the procedure is temporarily beneficial for revascularization, complications of intimal hyperplasia (IH) restenosis occur in 30-60% of all cases. Stenting the vessel at the time of angioplasty to maintain a luminal gain and reduce vessel recoil has become common practice, but helps reduce the occurrence of restenosis in only about 20-30% of all patients. This leaves IH a very serious problem where the recognition of risk factors and/or co-morbidities to accurately predict the incidence of restenosis in patients prior to intervention would be extremely beneficial. Furthermore, the delineation of the contributing mechanisms could lead to new therapeutic targets for the prevention of post-intervention complications. Therefore, the Vascular Research Laboratory is involved in projects focused on 1) delineating the cellular and molecular mechanisms of vascular restenosis, intimal hyperplasia, and graft failure following vascular intervention and 2) the development of therapeutic methods of intervention targeting these mechanisms.

Instrumental in our laboratory’s ability to study vascular pathology in a clinically relevant light is the access to animal models of vascular disease. We use two different rodent models of vascular disease. Our primary model is a microsurgical technique of balloon-induced injury of the rodent carotid artery. In this technique the endothelial cell layer on the luminal side of the vessel is denuded using a neonatal balloon catheter, and the underlying elastic lamina and smooth muscle cell layers are exposed to the shear stress of blood flow through the lumen. This is a widely accepted experimental model for examining intimal hyperplasia development because it closely mimics the most common clinical vascular intervention, balloon angioplasty. Additionally, hyperplasia development in vascular grafting is in response to a much different vascular injury caused by stitching the vessel and graft ends together, a process called anastomosis, resulting in hemodynamic changes and turbulent flow. Therefore, we have worked to create a microsurgical anastomotic model of intimal hyperplasia of the rodent carotid artery as our secondary model of vascular pathology and to establish its equivalence to the...
balloon-injury model as an accepted experimental design.

Our laboratory is currently focused on several objectives involving the use of these animal models. Our primary focus is investigating the role of sex hormones in vascular wall remodeling post-injury. Postmenopausal women receiving hormone replacement therapy (HRT) have more adverse outcomes after vascular reconstruction, including IH and decreased graft patency, which can ultimately lead to multiple surgical interventions and limb loss. In contrast, men with low serum testosterone levels appear to be at increased risk for the development of vascular disease, and though the role of androgen deficiency is unclear, animal studies have shown a significant reduction in the development of IH following vascular injury when testosterone therapy is initiated. A key group of enzymes involved in vascular remodeling is matrix metalloproteinases (MMPs), and our group has previously shown a correlation between sex hormone levels and differentially-regulated MMP activity in cultured vascular cell types. Therefore, we are currently using ovariectomized female and orchiectomized male rodent models of balloon-induced carotid artery injury to examine the seemingly opposite roles of estrogen and androgen deficiency and to investigate the effect of HRT and testosterone supplementation in MMP-modulated IH development. Our objective is to identify critical rate-limiting steps where pharmacological interventions could prevent or hinder dysfunctional vascular remodeling.

In a separate but complementary study we are using animal models for the development of a non-toxic, non-viral method of gene therapy in the prevention of vascular disease. Targeted-inhibition of genes or mechanisms that could prevent or hinder development of vascular pathologies would allow physicians to employ ameliorative strategies pre- or intra-operatively to reduce the risk of post-intervention complications. Gene therapy shows promise in the treatment of a multitude of clinical entities, including vascular disease, but a formidable challenge has been delivery of genetic material in a safe and non-toxic way. Traditional transfection methods commonly used in the laboratory are poorly translatable to the in vivo environment, and to date there are still no FDA-approved gene therapy products. Biodegradable polymers have shown promise as a safe, predictable, and non-toxic alternative to viral gene therapy, relying on endocytosis of synthetic polymeric carriers bioconjugated to targeted genetic/protein material of choice. In this study we are aiming to establish polymeric transfection as a feasible non-viral, non-toxic method for gene therapy in vascular tissue and in animal models of vascular disease. If successful, this study may provide a rationale for therapeutic regimens in molecular genetic therapy in the prevention of vascular pathologies and complications.

In short, the University’s commitment to research, the collaborative efforts of OLAC, and all those behind the scenes ensuring the accreditation and success for our animal care and use program are invaluable to the advancement of biomedical research through access to animal models of disease. As for our laboratory, these models are helping us answer some very important questions in terms of risk factors of vascular pathology, mechanisms of development, and viable methods of targeted intervention. The long-term impact of this work could be therapeutic regimens of molecular genetic therapy in the prevention and treatment of vascular pathologies. In terms of risk factors for vascular disease, such as smoking, obesity, diabetes, hypertension, and physical inactivity, Tennessee ranks within the bottom 5 states on all measures. This makes the clinical, social, and economic burden of vascular disease and surgical vascular intervention a very serious problem for the people of this region and our University’s commitment to helping us find solutions for prevention and advanced treatment all the more invaluable.
Tracking Animal Usage
Jane Czarra, BS, LATg

When writing an IACUC protocol, one of the necessary steps is to justify the species and number of animals necessary for the scope of the project. Tracking animal use should always begin at the onset of the project.

The tracking of animal usage for non-agricultural research animals is commonly tracked by the Principle Investigators (PIs) and facility managers. To acquire non-agriculture research animals and begin the tracking process, PIs will need to fill out the “Non-Agricultural Research Animal Requisition Form” found at http://www.vet.utk.edu/olac/forms.php, and submit the form to the facility manager. The manager will determine if these animals meet the criteria of your protocol, assess the number of animals needed, evaluate the potential health status of the animals, and determine if housing is available in the “preferred facility”.

It is essential that PIs track usage in terms of animal movement from one protocol to another. Examples of this would be movement between a breeding protocol and a research protocol. PIs will need complete the “Animal Transfer Request Form”, found at http://www.vet.utk.edu/olac/forms.php, and submit the form to the facility manager. Animals moving from one protocol to another are only counted once on an annual basis to USDA and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). They are, however, counted against the approved animal numbers for each protocol.

A note to field studies and satellite facilities: these PIs are responsible for tracking all animal usage. The PI must set up a system for tracking research animals both to the IACUC for annual renewal of your protocol and also to OLAC for annual reports to the USDA and AAALAC.

Welcome a New Member to the Biosafety Office

Jonathan Phipps, PhD, joined the office of Biosafety in November 2012 as Biological Safety Specialist. Prior to this, he received his undergraduate degree in Biology from Lincoln Memorial University in 2001 before moving to UT as a research assistant in the laboratory of Dr. Michael Karlstad. That fall he began work on his Master’s degree in the Comparative and Experimental Medicine Program with Dr. Karlstad. Upon completion of the degree requirements, Dr. Phipps joined the Human Immunology and Cancer Program at UT Medical Center as a PhD student in 2005. This degree was awarded in 2011, under the guidance of Dr. Jonathan S. Wall for work using small interfering RNAs along with lentiviral delivery vectors to silence the production of human light chain genes in myeloma cells, using both in vitro and in vivo model systems, demonstrating the feasibility of this approach as a therapeutic intervention in Primary Amyloidosis and other plasma cell dyscrasias. From September 2011 until October 2012 Dr. Phipps worked as a Post-Doctoral research associate with Dr. Daniel Kestler, before transitioning to the UT Office of Biosafety.