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Sterile Tissue Harvest

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Overview

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In 1959 The 3 R's were introduced by W.M.S. Russell and R.L. Burch in their book *The Principles of Humane Experimental Technique*. The 3 R's are replacement, reduction, and refinement of the use of animals in research.¹ The use of cell lines and tissue cultures that originated from research animals is a replacement technique, as it allows for many experiments to be conducted *in vitro*. Harvesting tissues and organs for use in cell and tissue cultures requires aseptic technique to avoid contamination of the tissues. Sterile harvest is also necessary for protein and RNA analysis and metabolic profiling of tissues. This manuscript will discuss the process of sterile organ harvest in rats and mice.

Principles

Aseptic technique must be followed to ensure the sterility of the tissues. The harvest is performed in a laminar flow hood that has been disinfected with alcohol. All materials used must be sterilized. The technician performing the harvest must don personal protective equipment, including a mask, bouffant, sterile surgical gown, and sterile surgical gloves. The gown must cover the wrists to prevent the shedding of skin cells into the opened body cavity, petri dishes, or sterile medium. To maintain sterility of the technician, this procedure requires that a second, nonsterile technician assist the sterile technician with the euthanasia of the animals.

In experiments that require the metabolic profiles of tissues, the method of tissue harvest has a great impact on the quality of the data collected.² Both the euthanasia procedure and the length of time the harvest takes can affect the integrity of tissues. Hypoxia, a consequence of euthanasia with carbon dioxide, has been shown to have a profound effect on the metabolites in tissues. Hemorrhaging of the vessels in lung tissue is also caused by the use of carbon dioxide for euthanasia. The order of tissue removal when sequential dissection of targeted tissues is performed can cause metabolic changes, as the tissues begin to age or decay during the harvest.

While most sterile harvests target a single organ, there may be times that more than one organ is needed. During sequential dissection, the tissues required will dictate the order in which they should be removed.² Tissues that rapidly degrade must be harvested as quickly as possible following euthanasia. To maintain sterility of the instruments, organs harvested from the abdominal cavity are removed in the following order: spleen, liver, kidney, adrenal glands, ovary or testes, accessory sex organs (prostate, seminal vesicles, uterus, fallopian tubes), pancreatic tissue, lymph nodes, urinary bladder, and intestinal tract (stomach, small and large intestine). The order of the organ harvest from the thoracic cavity is to first remove the heart and lungs followed by the collection of thymus, lymph nodes, and esophagus.³ New gloves are required for each mouse.

Some of the sterile harvested organs may need to be preserved. Preservation of the tissues is accomplished by flash freezing with liquid nitrogen (LN₂) or with a combination of LN₂ and 2-methylbutane. For LN₂ alone, the tissues are rinsed in sterile saline, blotted on a sterile absorbent cloth to deplete excess moisture, placed in sterile aluminum foil squares, and immediately submerged into the LN₂.² For immunological specimens, 2-methylbutane is added. The tissue is actually submerged into the 2-methylbutane that has been cooled down by the LN₂. When the tissues have turned white, they are removed from the 2-methylbutane, placed in sterile packs, and then placed directly into the LN₂.

Procedure

1. Preparation

1. Materials commonly used include 70% alcohol in a squirt bottle, sterile 20-25 gauge needles, two packs of sterile instruments of iris scissors and thumb forceps (one for the skin and one for the internal tissues), paper towels, sterile surgeon gloves, a sterile petri dish, sterile medium, and sterile saline.
2. The hood fan should be running for at least 15 minutes prior to the procedure, and it should be disinfected with 70% alcohol.
3. If the hood is equipped with a UV sterilization lamp, it can be used to further disinfect the work surface.
4. Clean gloves are worn for the setup of the hood. The equipment is loaded into the hood in the order in which they will be used, usually from left to right. This allows for the work to be conducted in a unidirectional workflow, preventing dirty items from contaminating clean items. This will protect the harvested tissues or organs from accidental contamination from the animal carcasses.
5. The sterile instrument packs are opened, respecting the sterile interior of the packages. To maintain sterility, the pack is placed in the center of the table, and each flap is opened without crossing over the sterile field or the inside of the pack. The nonsterile person opens the first top flap by reflecting it away for him/her. The side flaps are then opened using both hands-one on each flap-and pulling them away from the pack. The final flap is then pulled toward the person opening the pack, taking care to avoid touching any of the inner surfaces of the pack.
6. Place the paper towels into the hood, and wet them with alcohol.
7. The nonsterile person will euthanize the animals.
8. The sterile person wears two sets of sterile surgical gloves.
9. The nonsterile person places the carcass on the paper towels and completely soaks the carcass with 70% alcohol.
10. Using one set of sterile scissors and forceps, make a horizontal cut in the skin just anterior to the prepuce (in males) or the pelvic bone (in females). Avoid cutting through the abdominal musculature.

11. Set the instruments aside.

2. Harvesting tissues

1. Make a small cut just anterior to the pelvis in females and above the prepuce in males.
2. Place the thumb under the cut and grasp the cranial edge of the cut. Using the other hand, securely hold the hind limbs and tail while pulling the skin toward the head.
3. The skin will tear away from the body just above the hind limbs and can be turned inside out over the head and forelegs. This effectively removes hair contaminants during organ harvest.
4. Soak the body again with 70% alcohol, and remove the superficial pair of gloves that are no longer sterile.
5. Open the abdominal cavity using sterile instruments.
 1. Spleen
 1. To harvest the spleen, the body is positioned in right lateral recumbency.
 2. The spleen can be visualized through the abdominal muscle.
 3. A scissor cut is made directly over the spleen.
 4. The spleen is gently retracted from the body cavity.
 5. When cutting all attachments between the spleen, stomach, and pancreatic tissue, avoid puncturing or tearing the organ.
 2. Liver
 1. To harvest any other abdominal organs, the body is placed in dorsal recumbency.
 2. The abdominal muscle is lifted and cut away, leaving an oval opening directly over the abdominal cavity.
 3. After exposing the abdominal cavity, reflect the liver caudally away from the diaphragm.
 4. Carefully sever any membranes attaching the liver to the diaphragm.
 5. Make a cut perpendicular to the spine through the blood vessels visible between the liver and the diaphragm.
 6. Lift the liver and reflect it toward the diaphragm to visualize the attachments beneath it.
 7. There will be thin membranes attaching the smaller liver lobes to the stomach and small intestine, which will need to be disrupted.
 8. Grasp the fibrous node in the center of the liver on its underside, and lift the organ to find all the attachments. Separate the liver from the underlying organs and tissues.
 9. Note that the liver is fragile and can be easily punctured, resulting in the release of blood; therefore, it must be handled carefully.
 3. Kidneys
 1. After exposing the abdominal cavity, push the intestines to the left to visualize the right kidney, or to the right to visualize the left kidney.
 2. To remove the kidney, use forceps blades to grasp beneath the kidney to isolate the organ from the body. Using scissors or a scalpel blade, cut the kidney away from the body. Make the cut under the forceps to avoid damaging the kidney.
 3. Once the kidney is removed, any fat can be trimmed from the organ.
 4. Ovary
 1. After exposing the abdominal cavity, move the intestines cranially to visualize the uterus.
 2. Follow the left, and then the right, horns of the uterus to the ovaries.
 3. The ovaries are small. In older animals, they may be imbedded in fat.
 4. Slide the forceps under the ovary to isolate it from the body, and cut it free from the fat and the fallopian tube.
 5. Testes
 1. After opening the abdominal cavity, gently grasp the inguinal fat pad and pull it anteriorly.
 2. This will pull the testis from the scrotum.
 3. Once the testis is exposed, cut the attachments to the scrotum and the vas deferens to free it from the body.
 4. This is done for each testis.
6. The thoracic cavity is opened to remove the thoracic organs
 1. The body is placed in dorsal recumbency. It is imperative that the skin be reflected well above the head if thoracic cavity organs are to be harvested.
 2. Grasp the xiphoid process and lift to allow a cut through the abdominal muscle just caudal to the ribs. Extend the cut along the lower curve of the rib cage on both sides. This will allow visualization of the diaphragm.
 3. Puncture the diaphragm by making a small cut in the rib cage at the most lateral point possible on each side. Cut the diaphragm away from the ribs.
 4. Lift the ribs at the xiphoid to visualize the thoracic organs.
 5. Extend the lateral cut to the top of the sternum on both sides, and open the chest.
 6. The heart and lungs are most easily removed together.
 1. Place the forceps perpendicular to the trachea, and grasp the trachea firmly.
 2. Using the scissors, make a cut perpendicular to the trachea just anterior to the forceps. This cut should sever the trachea and esophagus.
 3. Without loosening the grip on the trachea, lift the trachea up and caudally. Snip any attachments of the lungs to the spinal surface in the rib cage.
 4. The esophagus may need to be cut to be able to lift the heart and lungs free of the chest cavity.
7. Heart only
 1. Lift the heart, and carefully cut through the pericardial sac.

2. The aorta is at the cranial aspect of the heart on the animal's left.
 3. Holding the aorta with the forceps, make a cut distal to the forceps, retaining a hold on the aorta and heart.
 4. Grasp the aorta as you sever the anterior vena cava and pulmonary arteries and veins.
 5. Lift the heart free of the thoracic cavity by the aortic artery.
8. Other thoracic organs
1. Organs are harvested in the following order to maintain sterility of the instruments: thymus, lymph nodes, and esophagus.
7. Brain
1. In special circumstances, the brain will need to be harvested for sterile culture.
 2. The brain is harvested last after any other organ harvests.
 3. In instances where the brain is the only organ to be collected, the skin is not stripped from the body, but instead a cut is made at the nape of the neck and extended along the midline from the dorsal cervical area to the tip of the nose.
 4. After pulling the skin away from the skull laterally, the exposed bone is soaked with 70% alcohol and the soiled top gloves are removed.
 5. Using a dedicated pair of sterile brain harvest scissors, make a cut through the spine at the base of the skull.
 6. Open the skull by placing the point of the scissors in the foramen magnum and cutting along the midline.
 7. Use the flat end of the scissor blade to lever the parietal bones away from the brain.
 8. Carefully disrupt the nerve attachments at the brain stem, and the optic chiasm beneath the brain, using the closed point of thumb forceps.
 9. In a similar manner, slide the closed forceps anteriorly beneath the brain and break the attachments at the olfactory bulbs.
 10. The brain can then be dropped from the skull into sterile medium.
 11. When harvesting the brain, one hand will be considered nonsterile as it grasps the skin from the skull reflected beneath the chin to stabilize the head, while the skull is cut and the brain removed.
8. Skin
1. Mammary tissue
 1. Mammary tissue is found from the top of the sternum at the manubrium to the genital opening on the ventral surface, and extends laterally up both sides, nearly touching on the dorsal surface at the hips and shoulders.
 2. Examine the mammary tissue to look for masses, discoloration, or abnormalities. Lactating or pregnant females will have increased mammary tissue volume, and milk may be present.
 3. Excision of the mammary glands requires grasping the edge of the gland with thumb forceps and using blunt dissection to loosen attachments to the skin. Once the gland has been separated from the skin, iris scissors can be used to cut any remaining attachments.
 4. Soak the exposed surfaces with 70% alcohol and begin the harvest of tissues using a new set of sterile instruments.
 5. The harvested tissue is rinsed with sterile saline prior to placing it in culture medium.
 2. Subcutaneous tumors
 1. Oftentimes, tumor cells are repeatedly implanted into animals, and then the resulting tumor is harvested to maintain the tumor cell line.
 2. It is imperative that sterile tissue harvest of subcutaneous tumors be performed prior to the breach of the skin's integrity.
 3. If the mass has abraded or is necrotic, the tissue collected will not be of good quality and may not be sterile.
 4. The body is positioned to expose the area of interest. The skin is cut along one side of the tumor.
 5. Carefully reflect the skin to expose the tumor. Place the skin instruments to the side and soak the exposed tissues with 70% alcohol.
 6. With new sterile instruments, dissect the mass from the underlying attachments and sever any blood vessels feeding the mass.
 7. The tumor is then placed in culture medium.

Summary

Although many of the procedures for sterile organ harvest are similar to a standard necropsy, the use of sterile technique is imperative for tissues to be of value for experimental procedures, which include harvesting cells from the excised tissues. Profiling or collecting RNA from tissues can be compromised if sterility is not maintained.

References

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