



Organ Structure and Vascularization

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What You will Learn in This Chapter and Associated Exercises

Students will be briefed about different organ systems, organ ultrastructure, and problems associated with organ transplantation. Students will be required to explore online resources for more details about specific organ of their choice. They will learn differences in organ's vascularization routes and practice cannulating an organ of their choice.

2.1 Main Organs and Their Function

The human body consists of ten organ systems: nervous, cardiovascular, respiratory, digestive, musculoskeletal, endocrine, immune/lymphatic, urinary, reproductive, and integumentary. A failure of any of these systems disturbs the homeostasis and can lead to death. Each organ system usually has one central or key organ. *Heart*, for example, is the central organ of the cardiovascular system, while the *lungs* are the main organs of the respiratory system. *Kidneys* are part of the urinary system and serve to excrete waste from our body with urine. Esophagus, stomach, intestines, liver, and pancreas work with each other as parts of the *digestive system* that takes in food, digests it, absorbs nutrients, and excretes waste with feces. Each organ system is affected by the functions of all other organ systems in the body and is a very complex hierarchical structure, governed by its own homeostatic principles. Organs are made of several types of tissue. Four main tissue types include: *epithelial, connective, muscle, and nervous*. Students who need a refresher or don't have a previous biology background are strongly encouraged to read about the general structure and basic functions of main mammalian organs using available online resources.

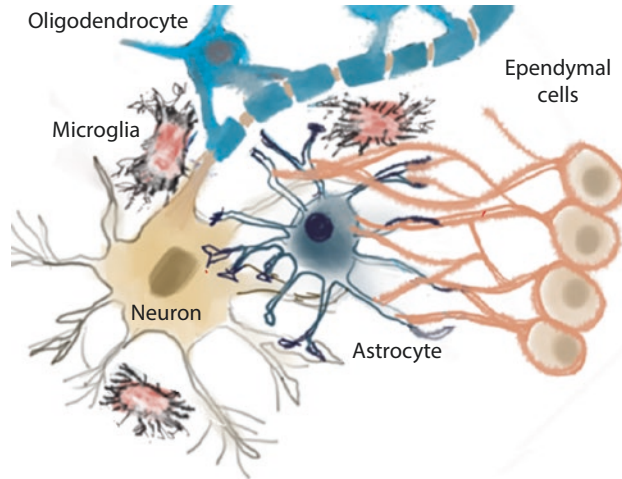
2.2 Organ Transplantation

Acute injury or chronic disease can lead to irreversible organ damage. Until recently, the only option for patients with end-stage failing organs was their replacement with healthy organs from another individual. To do so, a healthy organ has to be extracted from a donor and transplanted into a patient, who is then called an organ recipient. Today, the transplantation of most vital organs is possible, including the heart, kidneys, liver, and even lungs [1]. There are three main shortcomings to transplantation. The first is a severe shortage of organs that can be transplanted, so more often than not patients die from organ failure before being matched to a suitable organ donor. Secondly, despite being a life-saving surgery, organ transplantation is not without intraoperative or postoperative complications. Lastly, organ recipients must take immunosuppressive drugs for the rest of their lives, and even that is not guaranteed to prevent the recipient's immune system to reject the implanted organ. Tissue engineering offers a hope to create new healthy organs using a patient's own cells, thus resolving most of the above-mentioned shortcomings [2].

2.3 Tissue Ultrastructure

Each organ contains main cells specific to its function. For example, fundamental functional blocks of the liver are hepatocytes, for the heart – cardiac myocytes, for skin – keratinocytes and fibroblasts, for cartilage – chondrocytes, and so on. Yet, the

■ **Fig.2.1.** A cartoon of brain tissue with multiple cell types



same way a skyscraper cannot be built solely from one type of bricks, complex organs cannot be made by simply putting together a large number of cells specific to that particular organ function. In fact, each organ has its own very complex 3D architecture, composed of smaller, yet very complex, functional units, being it nephrons, alveoli, hepatic lobules, etc. ■ Figure 2.1, for example, shows a cartoon of brain tissue in which one can see at least five types of different cells organized in a very specific manner. To build such functional macroscopic structures is one of the major challenges of the tissue engineering and regenerative medicine (TERM) field.

An analogy one can use to characterize the current state of the TERM field is to compare it to architecture. Today we know how to build a simple cabin from one or two types of bricks. We also know that it should be possible to build a skyscraper since we have seen one (skyscraper being our own body). The challenge is to learn how to use multiple materials to create a much more complicated inner and outer structure of the building with all its parts: plumbing, electrical wiring, disposal routes, heating, etc. working perfectly together. Moreover, such a structure must be adaptable to the environment and be able to self-heal—as our body does. In their efforts to create complex tissues, multiple labs are currently experimenting with layering or printing structures composed of two or three different cell types. Yet, the TERM field is still very far from reaching its ultimate goal of creating functional macroscopic organs made from multiple cell types.

2.4 Vasculature and Its Role

The second major challenge of the TERM field is to provide cells within dense 3D tissue with nutrients while removing cell waste. Evolution was able to solve this task by creating a vast network of tiny blood vessels around almost every cell of the body (■ Fig. 2.2). The size of the smallest capillaries is so small that ~200 of them can fit into one human hair. Considering this, it is extremely challenging to create a similar network of such fine vessels *in vitro*. Today a number of promising vascularization strategies is being explored, including seeding scaffolds with endothelial cells

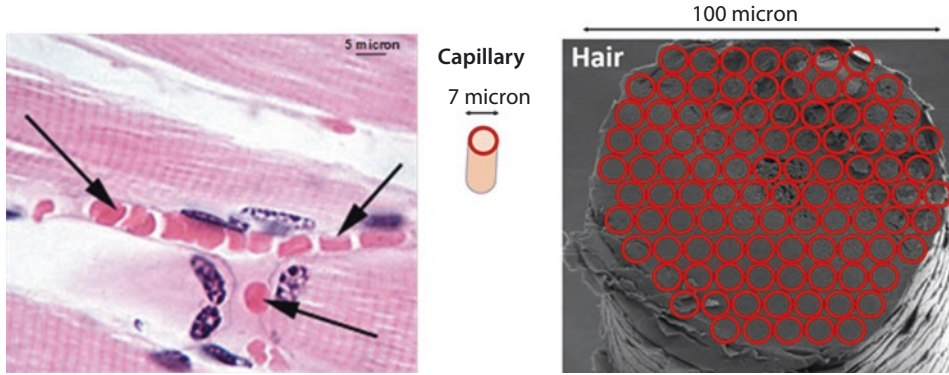


Fig. 2.2 Left: A histology of a muscle showing red blood cells squeezing through thin capillaries between muscle cells. Right: a relative diameter of a capillary versus human hair. Red circles show how many individual capillaries can “fit” inside a single hair

progenitors, 3D printing of small vessels, use of microfluidic chambers, in vivo implantation of multiple thin layers, and many other emerging techniques (more in ► Chap. 11). In addition, each of the red blood cells that squeeze through these capillaries is filled with a highly concentrated solution of hemoglobin that serves as an oxygen and carbon dioxide carrier. The presence of hemoglobin increases the amount of oxygen available to surrounding cells nearly 50 times when compared to a plasma-based solution. So even if vasculature routes are created, continuous perfusion with fluids carrying a sufficient amount of oxygen will be needed to sustain the metabolic needs of engineered tissue that is close to the composition of the native tissue.

2.5 Organ Cannulation

For the purpose of this course, it is important for students to understand the basic anatomy of an animal to be dissected, how to cannulate selected organs to gain access to their major vascular beds, and how to properly use dissection tools. These procedures will become useful for both cell isolation and organ decellularization protocols described in more detail in the following chapters. To isolate live cells, a cannulated organ first undergoes blood removal, followed by collagen digestion using collagenase, trypsin, or other proteolytic enzymes. For organ decellularization, a sequence of detergents in perfusate removes cells, leaving behind only scaffold material. Not every organ can be cannulated, but for organs in which vascular access can be gained, much more efficient cell isolation or organ decellularization can be achieved by proper perfusion.

In general, blood flows from major arteries to smaller arteries, then to arterioles, and finally into what is called a capillary bed. Blood then returns to the heart via a venous system. However, the best way to cannulate a particular organ for perfusion is not necessarily via its main artery. Let’s consider three specific cases that illustrate the importance of understanding the vascular anatomy of each organ for its proper cannulation. Specifically, we will discuss cannulation of three different organs of an adult rat, the most commonly available laboratory animal.

Fig. 2.3 A simplified cartoon of gastrointestinal tract circulation showing portal vein being the main vessel to carry blood to the liver

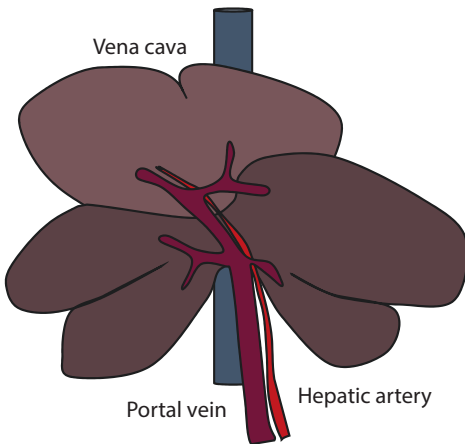
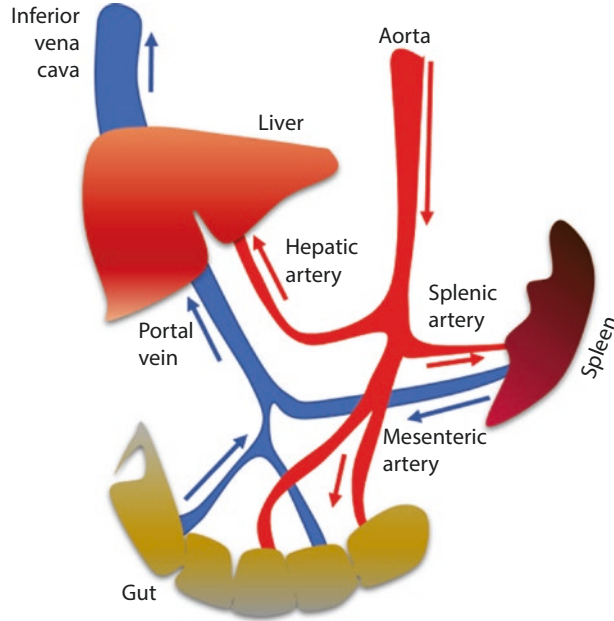


Fig. 2.4 Rat liver cannulation. Left: a cartoon showing the appearance of the portal vein into which cannula has to be inserted. Right: if cannulated properly, liver lobes immediately begin blanching upon blood removal

LIVER cannulation via the portal vein Most organs can be cannulated through their artery. However, in the case of the liver, cannulation is commonly done via the portal vein. This is because of the peculiarity of hepatic circulation shown in **Fig. 2.3**. The portal vein is the biggest visible vessel that enters the liver. After opening the animal's abdomen, liver lobes can be flipped upward toward the heart exposing a dark colored portal vein (**Fig. 2.4**). The cannula is then inserted into a small incision within the vein and tied 1–2 mm above the edge of its tip. If cannulated properly, immediately after saline solution starts to flow through the cannula, liver lobes begin to blanch (i.e., lose their dark color).

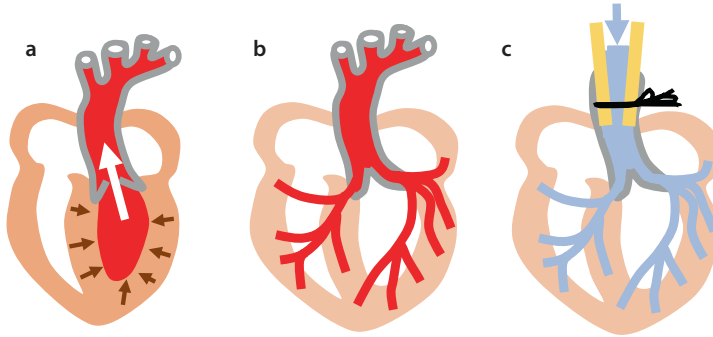


Fig. 2.5 Basics of Langendorff technique to perfuse heart muscle. **a** A cartoon showing how blood is ejected from the left ventricle into the aorta during the systole. **b** During diastole aortic valve is closed, while the muscle is relaxed. This makes aortic blood flow into coronary vessels. **c** When the aorta is cannulated above the aortic valve, perfusate flows into coronary arteries enabling perfusion of the heart muscle

HEART cannulation via the aorta Coronary arteries supply the heart muscle with blood. The openings of coronary arteries are located right behind leaflets of the one-way aortic valve. Therefore, when a cannula is placed slightly above the aortic valve, fluid pressure keeps the aortic valve closed while pushing flow into coronary arteries. This is called Langendorff preparation [3] (■ Fig. 2.5). In the case of the rat surgery, the animal is first anesthetized, its chest is open, and the heart is removed (■ Fig. 2.6). This is best done by positioning scissors as close as possible behind the heart and cutting it out together with lungs and large vessels. These extra tissues can be then trimmed outside the animal in a Petri dish filled with cold saline. Upon removing these extra tissues, the white arch of the aorta becomes visible due to its size and color. The aorta is then cut down to about 3–4 mm. A cannula is inserted and tied twice using surgical thread. If cannulated properly, immediately after saline solution starts to flow through the cannula, the heart muscle begins to blanch (i.e., lose its pink color).

LUNG cannulation Lung consists of two systems comprising vascular tissue and airway or respiratory tissue. Therefore, for most efficient access to all lung compartments, both pulmonary artery and trachea are commonly cannulated [4]. This enables inflating the lungs that increase the accessibility of vascular beds to digestive enzymes. For protocols in which cellular content of lungs needs to be simply removed (a process called decellularization to be covered in ► Chap. 9), the detergents can be introduced directly through the tracheal route without the need for vascular access. Therefore, for ventilation-based perfusion, the trachea is commonly used and not pulmonary blood vessels.

2.6 Sources of Animal Organs

Different protocols are involved in obtaining freshly excised organs that can serve as sources of live cells. In the case of large agricultural animals, such as cows, sheep, chicken, or goat, a local abattoir is the most common starting place. The excised organ needs to be immediately washed from blood and placed in ice-cold phosphate-

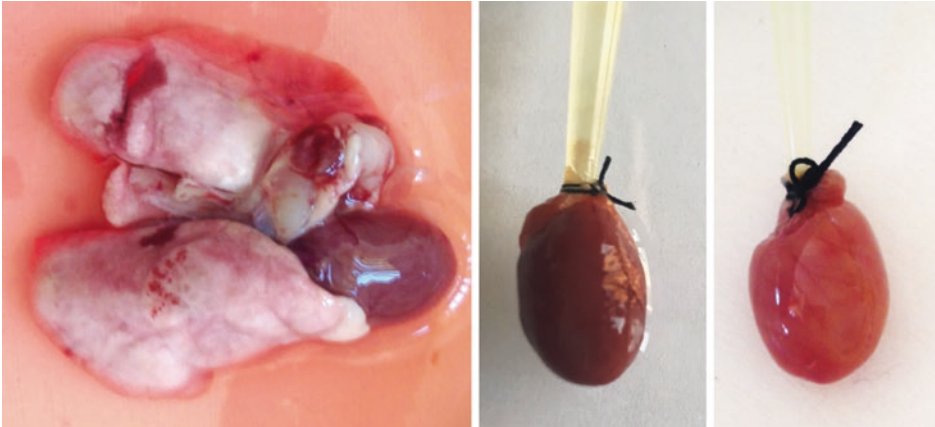


Fig. 2.6 The heart is removed by cutting it out together with lung tissue. After aortic cannulation, lungs and other tissues nearby are trimmed. The heart muscle becomes more pink upon blood washout and continues to contract

buffered saline (PBS) or any other kind of organ-preservation solution. Time to deliver animal tissue to the lab for further steps is crucial for successful cell isolation—the quicker the better. Age, sex, and weight of the animal, as well as tissue collection date and the location of the abattoir, all have to be properly recorded.

In the case of most common small laboratory animals, such as mice, rats, or guinea pigs, tissue or organs are taken from fully anesthetized animals. Necessary steps to minimize stress and pain to the animal must be taken with all the key steps approved by the Institutional Animal Care and Use Committee or its equivalent. Before an organ is extracted, the animal needs to be heparinized (to prevent blood clotting) and anesthetized (to minimize pain and distress). Heparin disrupts blood coagulation cascade preventing the formation of small blood clots that can block sections of vascular beds. Heparin is injected either intravenously or intramuscularly with exact amounts depending on the weight and age of the animal. After heparin injection, the animal is left in an animal cage/container for an additional 10–20 min for heparin to be evenly distributed within the circulation. Then a specified amount of anesthetic agents such as ketamine or pentobarbital is injected. Alternatively, in case of vapor anesthetics, a cotton ball saturated with isoflurane or chloroform can be placed next to the animal while making sure that the container is hermetically closed. In the case of live fish, several drops of clove oil into a water container containing the fish can be used for anesthesia or a standard fish anesthetic such as MS222 can be used. For more detailed information on animal anesthesia, students are referred to open sources such as ► <https://animal.research.uiowa.edu/iacuc-guidelines-anesthesia> or online reviews [5].

For the purposes of this introductory course, it is expected that a trained instructor or teaching assistant will be conducting any of the above-mentioned procedures involving live animals. Students can proceed with dissection and organ removal only after the animal is fully anesthetized and unresponsive to tail pinch or similar sensitivity tests. Clean and sharp dissecting instruments such as ones shown in ► Fig. 2.7 are prepared for dissection including scalpels, scissors, and

■ **Fig. 2.7** Commonly used dissection instruments. From left to right: rat teeth forceps, small straight tweezers, small surgical scissors, curved and straight self-locking forceps, curved tweezers. A scalpel is on the top. Teams should keep their color labeled instruments sharp and clean for each new dissection



forceps. Surgical scissors are used for general cutting of skin or fascia. They are also useful for spreading tissue layers. Tissue forceps, called “rat teeth,” are used to hold thick skin. Instruments must be sterile and so are the gloves of the person who performs the surgery. The surface area can be disinfected using 70% ethanol spray. For dissection, one must know ahead of time the external and internal anatomy of an animal to be dissected. A convenient way to make a custom size cannula for organ perfusion is to cut both sides of the yellow tip at denoted places (■ Fig. 2.8). A narrow side of the cannula is then briefly placed near a candle flame to create a lip. Such a lip enables tying a thread around it preventing its slippage. Having a small custom-fitted wooden or plastic block or platform that holds the syringe with cannula and thread around it can greatly help to perform speedy organ cannulation.

For fish dissection, a line from anus to all the way up to the gills is cut, enabling to expose the internal organs of fish. Note: Due to differences between the anatomy of fish and mammalian species, students should not assume that methods of cannulation described above will work for the fish heart and need to do their homework to understand how to gain access to its vasculature and/or major organ cavities. For rat or mice dissection, pins are used to spatially fix the extremities of the fully anesthetized animal on dissecting tray, after which the abdominal skin area is sterilized using 70% ethanol. Students need to study the anatomy of the selected animal to be dissected ahead of time using available online resources. This will enable them to better understand the relative positions of the organs and blood vessels that lead to them. This is important since the color, the appearance, and the positions of actual organs in a dissected animal can be quite different from what students see in textbook cartoons (■ Figs. 2.9 and 2.10).

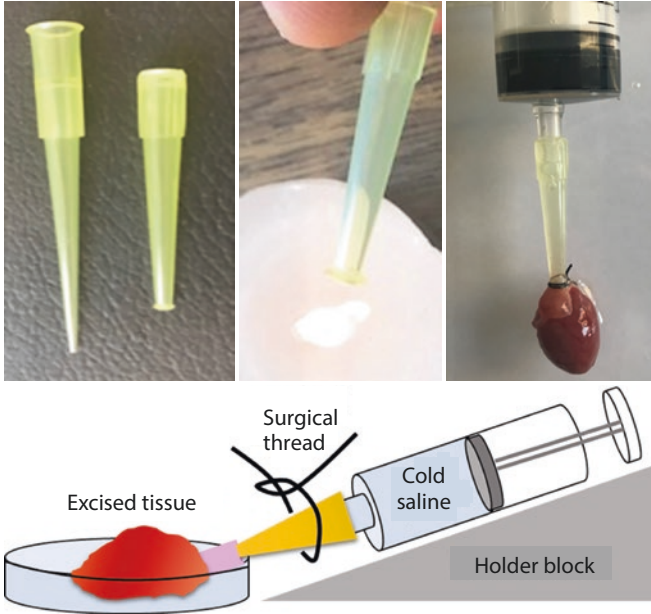


Fig. 2.8 Cannulating organs using cannulas made from yellow pipette tips. The narrow end of the cannula is cut and flamed to create a small lip. The wide end of the cannula is also cut to enable attachment to a standard-sized syringe. The bottom cartoon shows the positioning of the cannula and thread relative to the vessel to be cannulated. A prism-shaped holder is used to support the syringe in a slightly tilted position

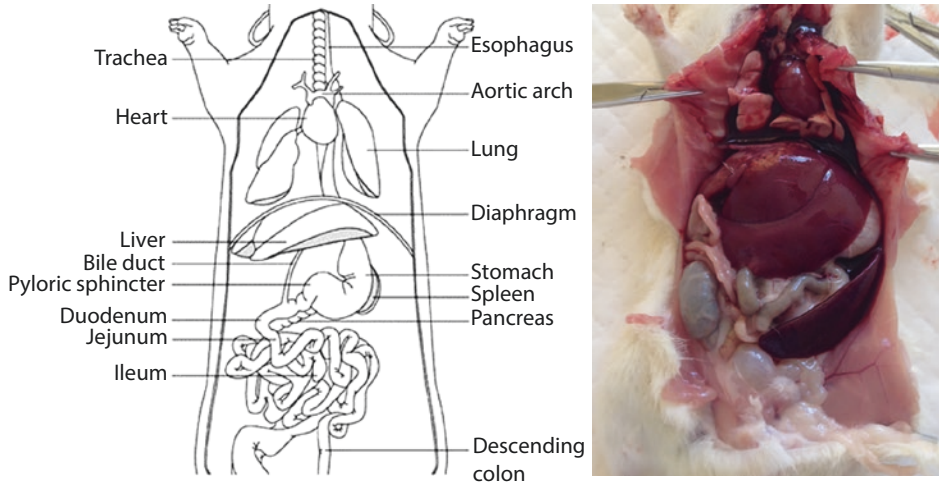
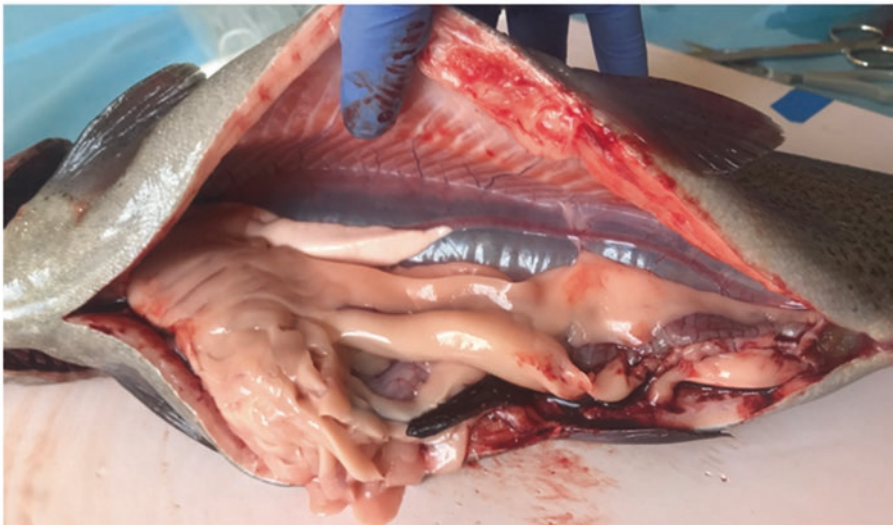
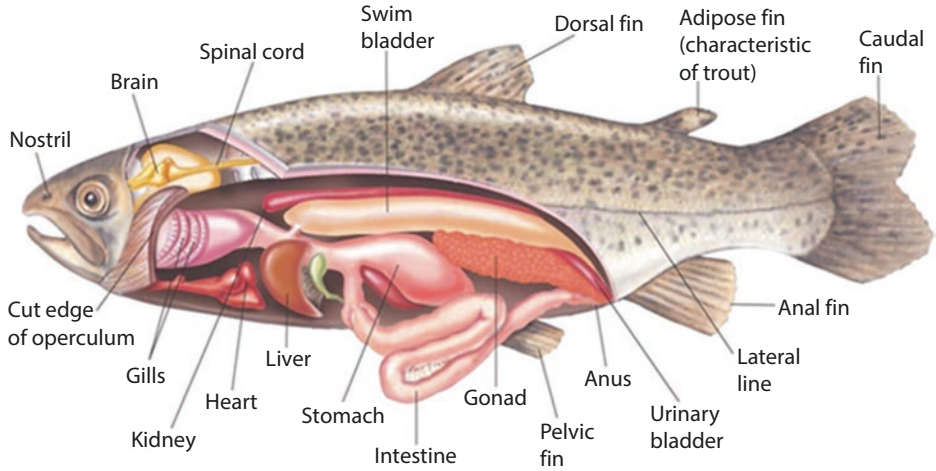


Fig.2.9 A cartoon versus real image of a dissected rat



■ Fig.2.10 A cartoon versus real image of a dissected fish

Session I

Demonstration

The instructor shows how to make the cannula, heparinize and anesthetize the animal, followed by cannulation of one of the internal organs. The selection of an animal is based on local availability. For rat anesthesia instructor should follow animal protocol guidelines established by their institution, being it an intraperitoneal injection of ketamine/xylazine, barbiturates, or isoflurane inhalation. To immobilize a live fish, one to two drops of clove oil per liter of water can act as an effective and natural anesthetic. The clove oil should be mixed with a small amount of warm water before adding it to the bucket with cold water containing the fish. Alternatively, fish

anesthetic such as MS222 can be used as per the product manual. After a few minutes, the fish becomes motionless and can be dissected.

Homework

Teams are asked to decide on the organ they want to cannulate and read about its function, anatomy, and vascular supply.

Session II**Team Exercises**

Surgical area, anesthetics, all required instruments, custom-made cannula, and perfusion solutions have to be prepared by students. After tissue becomes blanched upon blood removal, cannulated organs are placed in the freezer (with cannula attached) to be used later in the course. Alternatively, dissection and cannulation can be done using internal organs of the whole chicken purchased from farmer market or freshly sacrificed large fish. Students are asked to take images throughout the process to document organ appearance before and after cannulation, and after perfusion with saline.

Note - in case of live rat, the animal should be heparinized and anesthetized by the instructor. The following steps can be performed by the students. After the rat is anesthetized, it is important to perform all the surgical procedures rapidly.

Homework

Teams are tasked with the creation of a detailed protocol to describe performed procedures that include images taken at every step of their cannulation experiments.

i Sample Protocols**Rat liver cannulation**

- Check animal responsiveness using tail pinch.
- Fix animal extremities to dissection board using pins or strong tape.
- Sterilize the abdomen with 70% ethanol spray.
- Cut the skin and fascia with surgical scissors.
- Remove any visible hairs not to appear in the working area.
- Stretch and fix the skin and fascia with forceps.
- Open the abdomen to expose the brown-colored liver.
- Gently cut all the ligaments that fasten the organ to other tissues.
- Lift liver lobes up to reveal portal vein, a big bluish-red vessel that goes to the liver's central area.
- Depending on animal weight, the portal vein can vary in size. Several cannulas have to be prepared ahead of time so students can choose the one that fits the best.
- Insert the cannula in vein. Successful insertion is confirmed by seeing blood flowing into the cannula.
- Tie the cannula with surgical thread.
- Carefully lift the liver and cannula out and put them in Petri dish with ice-cold saline for further experimentation.

Ventilation-based rat lung cannulation

- Check animal responsiveness using tail pinch.
- Fix animal extremities to dissection board using pins or strong tape.
- Remove the hair on the chest and abdominal area of the animal.
- Make an incision from the epigastric area through the sternum extending to jugular fossa.
- Open the chest of the animal and locate the lungs, the main bronchi, and the trachea.
- Dissect and remove the lungs together with the main bronchi and the trachea. Be very careful in order to avoid damage and keep these organs intact.
- Identify the trachea. It appears as a small tube with a narrow lumen and cartilaginous consistency. Use a custom-made cannula to attach it to the trachea using a double thread.
- Using a syringe, test if the placement of the cannula is correct and organ is intact. If so, lungs should inflate upon pushing syringe in, and they should shrink back when creating a vacuum by the syringe. Be careful not to damage the lungs with high positive pressure.

Take-Home Message/Lessons Learned

After reading this chapter and performing the requested assignments and exercises, students should:

- Understand the complexity of each organ and main differences between organ systems
- Be familiar with the ways different organs can be cannulated for perfusion
- Be able to make their own cannula and solutions to perfuse organ of their choice
- Know the importance of following approved protocols to minimize stress and pain to the animal used in any experimental procedure

Self-Check Questions

- ❓ Q.2.1. To effectively cannulate and perfuse an organ, one needs to use major _____ leading to that organ
- A. Artery
 - B. Vein
 - C. Both at the same time
 - D. Depends on the organ
- ❓ Q.2.2. Average capillary has a diameter of about _____
- A. 1 mm
 - B. 100 micron
 - C. 10 micron
 - D. 1 micron

- Q.2.3. To dissociate live cells from the extracellular matrix for future culturing, organs are commonly perfused with
- A. Collagenase
 - B. Heparin
 - C. Detergents
 - D. High calcium concentration solution
 - E. Ketamine/xylazine
- Q.2.4. One can anesthetize a rat or mice before dissection using
- A. Collagenase
 - B. Heparin
 - C. Detergents
 - D. High calcium solution
 - E. Ketamine/xylazine
- Q.2.5. Blood coagulation can be slowed down by injection of
- A. Collagenase
 - B. Heparin
 - C. Detergents
 - D. High calcium solution
 - E. Ketamine/xylazine

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