Video Article

# Cardiopulmonary Bypass in a Mouse Model: A Novel Approach

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## Abstract

As prolonged cardiopulmonary bypass becomes more essential during cardiac interventions, an increasing clinical demand arises for procedure optimization and for minimizing organ damage resulting from prolonged extracorporal circulation. The goal of this paper was to demonstrate a fully functional and clinically relevant model of cardiopulmonary bypass in a mouse. We report on the device design, perfusion circuit optimization, and microsurgical techniques. This model is an acute model, which is not compatible with survival due to the need for multiple blood drawings. Because of the range of tools available for mice (*e.g.*, markers, knockouts, *etc.*), this model will facilitate investigation into the molecular mechanisms of organ damage and the effect of cardiopulmonary bypass in relation to other comorbidities.

## Video Link

The video component of this article can be found at https://www.jove.com/video/56017/

## Introduction

Since the introduction of cardiopulmonary bypass (CPB) into the clinic, it has played an essential role in cardiac surgery[1]. In modern cardiac surgery, prolonged CPB time is essential to perform extensive aortic reconstructions and combined procedures. Although technological advances have been tremendous, the use of extracorporal circulation is associated with intra- and postoperative systemic and local organ damage[2,3].

Large animal models have been developed to investigate the role of CPB on physiological processes[4,5]. Although these models have provided insight into some of the CPB associated complications, they are extremely costly and molecular tools (e.g., antibodies) are very limited. A more cost-efficient alternative has been developed in small animals. Since their development, multiple studies have been conducted to optimize a CPB model in rats and rabbits[5,6,7,8,9]. These models provide a good basis for measurements of pathophysiological disease processes; however, they are still insufficient to investigate cellular and humoral immunology due to the lack of relevant antibodies and reagents. This impairs their role in this field of research.

We have recently developed a mouse model of CPB. Due to a wide variety of mouse-specific reagents and genetically-modified mice, mouse models are in general the model of choice for physiological, molecular, and immunological research[10,11]. Therefore, our model will facilitate the study of CPB in relation to various comorbidities as there are many mice strains available with clinically-relevant diseases[12,13]. Accordingly, this paper describes, in detail, how to perform CPB in mice. Oxygen and hemodynamic parameters are closely monitored after deep respiratory and circulatory arrest.

## Protocol

All animal experiments were performed in compliance with the German Animal Protection Law (TierSchG) and were approved by the local animal welfare committee (Lower Saxony State Office for Consumer Protection and Food Safety, Protocol TSA 14/1556). The minimal weight of mouse suitable for this model is 25 g.

## 1. Preoperative Preparations

NOTE: All procedures are carried out under clean, non-sterile conditions, with autoclaved instruments.

 

1. **Place 50-60 8-cm long propylene hollow fibers in parallel in a tube and connect with a T-adapter on both sides. Fill the space between the hollow fibers and the external branch of the T-adapter with glue.**
    1. Allow at least 24 h for the glue to harden. Cut the hollow fibers extending out from the T-adapter using a standard microtome and pull the silicone tube over to the connection sites.

2. **Insert a 27 G metal needle (from a 26G branule) into a 2 Fr polyurethane cannula. Use a surgical blade and micro-scissors under a microscope (8-12X magnification) to make three to four fenestrations of about 0.15 cm each within the distal third of the cannula to assure optimal venous return flow.**
    1. Remove the wire when completed. Use cotton swabs for blunt dissection and retraction of structures. Use gauze swabs (5 x 5 $cm^2$) to soak up excess fluid to prevent tissue dehydration.

3. Prepare the priming volume. For completion, add 30 IU of heparin per mL priming solution and 2.5% v/v of an 8.4% solution of $NaHCO_3$ for buffering. Store this solution at 4 °C until used.
4. Fill the CPB circuit *(i.e.*, pump, air trapper, tubing and both cannulas) with 850 µL priming solution via the venous cannula. Once filled, keep the CPB circulating until the animal is ready for cannulation.

## 2. Animal Anesthesia

1. To administer anesthesia, place the animal in an induction chamber under 2.5% v/v isoflurane/oxygen mixture. Confirm proper anesthesia by assessing pedal withdrawal reflex, tail pinch reflex, and eye blink reflex. Apply veterinary eye ointment to avoid eye dryness.
2. Place the animal on a warming pad with temperature-regulating function. Measure body temperature with a probe inserted rectally and connected to the data acquisition system.
3. After full anesthesia is achieved, intubate the animal orotracheally using a 20G plastic braunule, inserting it orally and pushing it into the trachea under visual control. Start mechanical ventilation using an isoflurane vaporizer connected to a mouse ventilator. Depending on the animal weight, adjust the ventilation so that a tidal volume of 250 - 350 µL is achieved.
4. To assure complete analgesia, inject 5 mg/kg animal bodyweight carprofen subcutaneously. It is recommended that isoflurane concentration be kept between 1.3 - 2.5% in oxygen. Isoflurane concentration may be manually adjusted depending on the stage of the procedure.

## 3. Surgical Procedures

1. After full anesthesia and intubation is achieved, perform a midline skin incision to the neck with sharp fine scissors, separate muscles in a blunt fashion, and expose the right jugular vein and left carotid artery. During preparation, coagulate small vessels using a veterinary coagulator connected to bipolar forceps to ensure minimal blood loss.
2. After preparation of the jugular vessels, cranially ligate the distal segment of the left common carotid artery to its bifurcation using 8-0 silk sutures.
3. Connect the distal end of a 27 G cannula to the arterial inflow tubing using a silicone connector (0.5 mm ID, 1 mm OD), place 8-0 silk suture slip knots at the proximal segment of the artery, and insert the tip of the cannula into the carotid artery.
4. After correct placement of the cannula tip, advance the cannula tip so that it lays 3 - 4 mm proximally to the aortic arch. Fix the cannula tip by securing with 8-0 silk knots.
5. Using microsurgical forceps and scissors, perform blunt and sharp dissection, expose the right jugular vein by blunt preparation of tissue laterally to the sternocleidomastoid muscle, close to clavicle, and place an 8-0 silk knot at the distal end and an 8-0 loop at the proximal end.
6. After ligation of the distal end of the jugular vein, place the tip of the venous cannula in the right jugular vein and progress towards the right atrium and secure using the silk knot. For optimal venous return, it may be necessary to advance the venous tip into the right ventricle.
7. Once the correct cannula position is achieved, carry out systemic heparinization by adding 2.5 IU of heparin per gram of the animal bodyweight via direct intravenous injection into the jugular vein.
8. **For real-time invasive pressure monitoring, cannulate the left femoral artery. Expose the groin area and gently separate the common femoral artery from the femoral vein and femoral nerve under 25X magnification.**
    1. Ligate the distal part of the femoral artery. Temporarily occlude the proximal part with a slip-knot and make a small incision on the anterior wall using micro-forceps.
    2. Afterwards, insert a 1 Fr polyurethane cannula into the femoral artery and secure it with a silk 10-0 suture. This cannula is used to extract blood samples for blood gas analysis.

9. After successful placement of the arterial and venous cannulas, initiate cardiopulmonary bypass by turning on the pump. The time from intubation to starting of CPB is approximately 45 min. Start using a flow rate of 0.5 mL/min, depending on systemic pressure measurements, and increase blood flow within 2 min to full flow (4 - 6 mL/min) by increasing the pump speed.
10. Under full monitoring, perform an upper sternotomy using sharp scissors starting from the manubrium and going 5 mm in the caudal direction. Coagulate the sternal edges immediately to avoid bleeding. Expose the aortic arch by pulling the right carotid artery in the cranial direction.
11. For optimal control, circumferentially free the aortic arch from the thymus and surrounding tissue to facilitate clamping. Place a 8-0 silk loop around the ascending aorta. To place the cross clamp for cardioplegia, pull the silk loop in the cranial direction to better expose the ascending aorta.

## 4. Cardiopulmonary Bypass and Blood Gas Analysis

1. **For blood gas analysis (BGA), use glass capillary tubes to collect 60 - 90 µL arterial blood via the femoral artery catheter.**
    1. Use a small clamp on the silicone tubing and detach the tubing from the catheter. Release pressure slowly on the clamp to allow controlled filling of the capillary tube.
    2. Reattach the silicone tube to the catheter. Insert capillary tubes into the blood gas analyzer for measurements at the following time points:

10 min after initiation of CPB with ventilation (BGA1)
after 25 min of CPB and 15 min of respiratory arrest (BGA2)
after 40 min of CPB and 30 min of respiratory arrest (BGA3)
after 55 min of CPB, 45 min of respiratory, and 20 min of cardiac arrest (BGA4)

2. Under stable CPB flow, initiate respiratory arrest by stopping ventilation.
3. After termination of ventilation, set the warming pad to 28 ºC and start topically cooling the animal to 28 ºC body temperature within the first 20 min of respiratory arrest using gauze soaked in cold saline.
4. Once a body temperature of 28 ºC is achieved, and after a total of 30 min of respiratory arrest, administer 0.3 mL of 7.45% KCl solution into the CPB circuit to initiate cardioplegia.
5. For cross clamping of the aorta, pull the previously placed silk loop (step 3.10) in the cranial direction for better exposure and place a micro-serrefine clamp on the ascending part of the aorta.
6. Perform a total of 60 min respiratory arrest and 30 min of cardiac arrest. Remove the micro-serrefine clamp from the ascending aorta to initiate reperfusion of the heart. Simultaneously, re-ventilate and warm the animal to 37 °C.
7. After reperfusion is completed and the animal has reached normothermia, terminate the CPB by turning off the pump and continue to monitor physiological measurements (*e.g.* body temperature, ECG, and invasive blood pressure) for 20 min.
8. At the end of the experiment, exsanguinate the animal under full anesthesia (5% isoflurane) and collect blood and organs for further analysis[14].

## Representative Results

This protocol describes the perfusion circuit, surgical procedures, and monitoring of physiological parameters during CPB of a mouse. When performed by an adequately skilled microsurgeon, the results are consistently and reproducibly obtained.

To maintain adequate tissue perfusion, the mean arterial pressure is always kept between 40 and 60 mmHg by adjusting the CPB blood flow and adding of extra volume. Depending on the weight of the animal, its volume status, and body temperature, the extracorporal blood flow is maintained between 2.3 - 6.5 mL/min. Correction of volume loss during the experiment is achieved by adding of 0.1 mL of priming solution to the circuit during CPB. Systemic pH stabilization is achieved by adding 8.4 mmol/L of $NaHCO_2$. All fluids are administered via the air-trapping reservoir to reduce risk of air embolization.

Physiological parameters were assessed using BGA at four different time points (**Figure 1**) and the measurements from a representative successful CPB procedure are presented in **Table 1**.

Hematocrit measurements show hemodilution due to the addition of priming volume to the circuit (**Table 1**). There was, however, no need for blood transfusion as hemoglobin levels were maintained at sufficient levels during the course of the experiment (**Table 1**). Systemic arterial $pO_2$, oxygen saturation, $pCO_2$ expiration values validated excellent functioning of the micro-oxygenator (**Table 1**). $pCO_2$ expiration was optimal using an oxygen/air mixture at $FiO_2$ 0.8.

BGA also provided insight into the metabolic status of the animal during CPB. After initiation of CPB with ventilation, arterial pH was elevated (**Table 1**). This effect is often lessened once respiratory arrest is initiated. A gradual reduction in pH during the course of the experiment was seen (**Table 1**). Continuous buffering of arterial pH and lactate was necessary to compensate for acidosis.

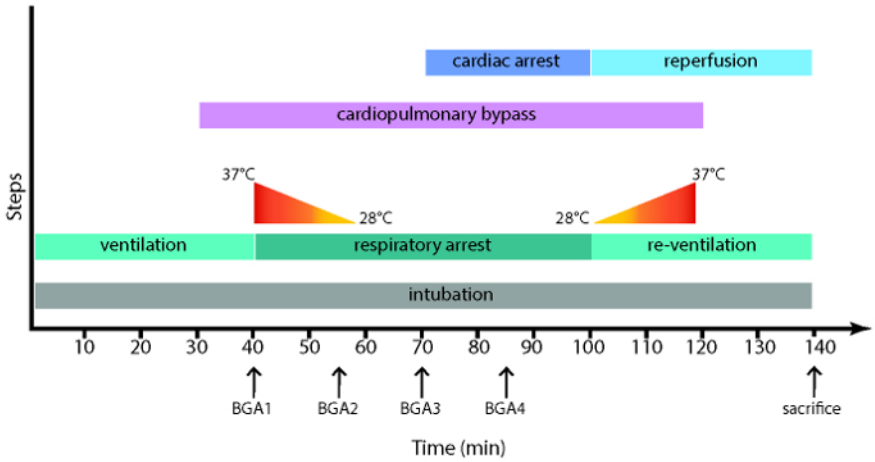


**Figure 1: Experimental Timeline.** Timing of intubation, ventilation, respiratory arrest, CPB, cardiac arrest, reperfusion, cooling/re-warming, and BGA sampling points. Please click here to view a larger version of this figure.

| | BGA1 | BGA2 | BGA3 | BGA4 |
|---|---|---|---|---|
| **Hemoglobin (g/dl)** | 8.9 | 6.8 | 6.8 | 5.6 |
| **Hematocrit** | 27.5 | 21.2 | 21.3 | 17.7 |
| **pO2 (mmHg)** | 508 | 506 | 504 | 271 |

| | | | | |
|---|---|---|---|---|
| **pCO2 (mmHg)** | 24.5 | 20.3 | 20 | 36.4 |
| **sO2 (%)** | 100 | 100 | 100 | 100 |
| **pH** | 7.56 | 7.65 | 7.36 | 7.32 |
| **Lactate (mmol/L)** | 2.6 | 3.1 | 3 | 6.9 |

**Table 1: Representative BGA Results from a Successful CPB in a Mouse.** BGA taken at four different time points over the course of an experiment.

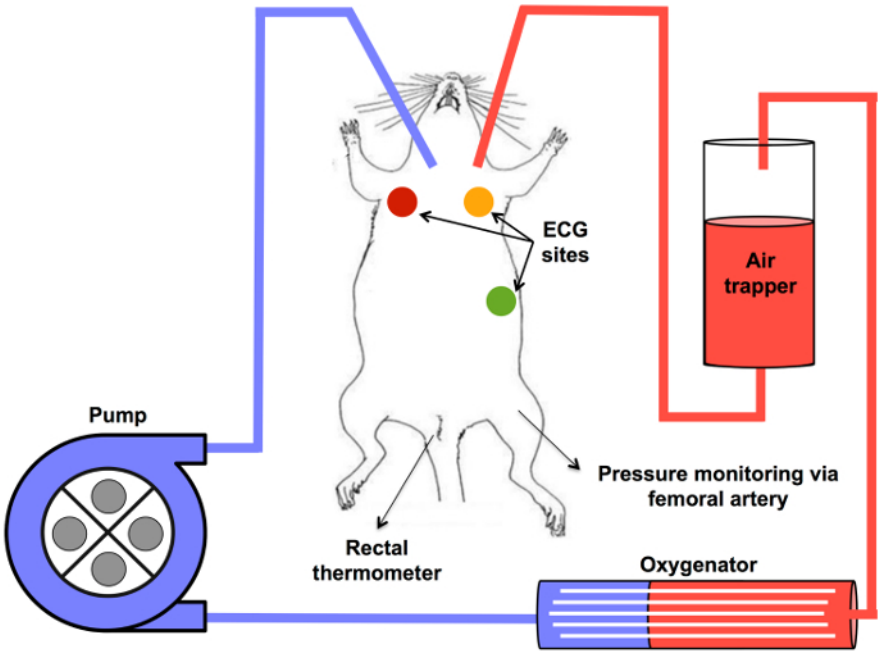


**Figure 2: Schematic Diagram of the CPB Circuit in the Mouse.** A venous cannula (blue) is placed in the inferior vena cava *via* the right jugular vein and an arterial cannula (red) in the aorta *via* the left carotid artery. Oxygenated blood is pumped through the air-trapper reservoir into the left carotid artery. ECG electrodes are placed subcutaneously, body temperature is measured rectally, and pressure is monitored via the femoral artery. Please click here to view a larger version of this figure.

## Discussion

We have developed a fully-functioning clinically-relevant model of CPB in a mouse. With more than thirty strains of mice having cardiovascular diseases, our model could be a starting point for development of new prospective protocols related to CPB. Moreover, due to the plethora of mouse-specific reagents and knockout-out mice, this model can not only replace the current rat model of CPB but will facilitate dissection of the molecular mechanisms involved in CPB-related organ damage. To date, CPB has not been applied in mice due to microsurgical challenges in cannulation technique, as well as technical challenges including the development of a micro-oxygenator having a sufficiently small priming volume. Approximately 90 trails and the diligent work of an experienced microsurgeon were needed to achieve a stable model. Over 15 prototypes of the CPB machine having different roller pumps, tubing, and diverse reservoirs were tested and constantly improved. More than 10 versions of different oxygenators were built and tested to achieve the current results. Our novel model required a complete redesign of the existing rat model setup of extracorporal circulation. Firstly, we built the smallest possible micro-oxygenator allowing priming volumes <0.3 mL. The oxygenator was redesigned using an inverted system where blood flows through the hollow fibers and not between them, allowing for a significant reduction in priming volume.

During the development of our model we encountered several technical difficulties. Our first prototype circuits required large priming volumes of up to 6 mL. This resulted in extreme hemodilution with hemoglobin values below 4 g/dL and hematocrit values of approximately 15. Despite BGA showing good oxygenation we observed signs of hypoxemia leading to rapid cardiac arrest during the procedure. In order to achieve proper tissue oxygenation, hematocrit values should be above 25. By adjusting the tubing size, altering the design of the roller-pump, producing a miniaturized air trapper, and optimizing venous and arterial cannulas, the priming volume was significantly reduced to <0.9 mL.

Despite adequate perfusion flow of 4 - 6 mL/min, providing sufficient venous back flow is essential. Placement of the venous cannula in the right atrium or, even better, in the right ventricle, alleviates this problem. Increasing the perfusion flow leads to either sucking of the venous cannula or overperfusion-related loss of fluid and tissue edema. To avoid $CO_2$ retention in the mouse, which has a fast metabolism, we found that keeping the oxygen supply through the oxygenator at $FiO_2$ 80% with a flow of 600 mL/min is optimal for tissue oxygenation.

Another issue one can encounter is gradual loss of intravascular volume to the interstitium, necessitating repeat volume substitution every 30 - 40 min. The hyperosmolarity of electrolyte solutions containing hydroxyethyl starch (HES) prevents intravascular volume loss, but when used exclusively, its high viscosity causes an enormous increase in systemic pressure during CPB. This leads to leakage in the oxygenator and the

tubing proximal to the arterial cannula. Therefore, to achieve a balance between hyperosmolarity and moderate viscosity, a 1:1 mixture of HES-containing solutions and another isotonic balanced fluid was found to be optimal.

The driving electronics of the roller pump were modified to increase the rotation speed thus allowing sufficient flow within the small diameter tubes.Under the strong suction produced by the roller-pump, it is typical to have microscopic air-bubbles in the venous system. Construction of a miniaturized air trapper with priming volume below 0.15 mL resolved this problem. Adding 0.1 mL of extra volume and reducing CPB flow in addition to checking the correct placement of the venous cannula eliminated air embolism in the circuit.

To test the technical feasibility of a novel cardiopulmonary bypass model, multiple blood samplings are required. Removing more than 0.2 mL of blood is usually lethal for a healthy mouse. To assure oxygen and hemodynamic stability, the amount of blood samplings in our experiment was far beyond this value and almost reached 0.9 mL at the end of the experiment. Nevertheless, stable oxygenation and hemodynamic parameters were observed despite the constant decrease in hematological values. Therefore, our initial feasibility model was primarily designed as an acute, non-survival protocol. We are now developing a minimally invasive survival model that, by necessity, will have less blood sampling.

## Disclosures

The authors have nothing to disclose.

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