Video Article

A Small Animal Model of *Ex Vivo* Normothermic Liver Perfusion

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Abstract

There is a significant shortage of liver allografts available for transplantation, and in response the donor criteria have been expanded. As a result, normothermic *ex vivo* liver perfusion (NEVLP) has been introduced as a method to evaluate and modify organ function. NEVLP has many advantages in comparison to hypothermic and subnormothermic perfusion including reduced preservation injury, restoration of normal organ function under physiologic conditions, assessment of organ performance, and as a platform for organ repair, remodeling, and modification. Both murine and porcine NEVLP models have been described. We demonstrate a rat model of NEVLP and use this model to show one of its important applications — the use of a therapeutic molecule added to liver perfusate. Catalase is an endogenous reactive oxygen species (ROS) scavenger and has been demonstrated to decrease ischemia-reperfusion in the eye, brain, and lung. Pegylation has been shown to target catalase to the endothelium. Here, we added pegylated-catalase (PEG-CAT) to the base perfusate and demonstrated its ability to mitigate liver preservation injury. An advantage of our rodent NEVLP model is that it is inexpensive in comparison to larger animal models. A limitation of this study is that it does not currently include post-perfusion liver transplantation. Therefore, prediction of the function of the organ post-transplantation cannot be made with certainty. However, the rat liver transplant model is well established and certainly could be used in conjunction with this model. In conclusion, we have demonstrated an inexpensive, simple, easily replicable NEVLP model using rats. Applications of this model can include testing novel perfusates and perfusate additives, testing software designed for organ evaluation, and experiments designed to repair organs.

Video Link

The video component of this article can be found at https://www.jove.com/video/57541/

Introduction

There are 14,578 patients on the waiting list for liver transplantation and approximately 7,000 transplants are performed per year[1,2]. In response to this significant donor shortage, the criteria for liver donors have expanded; these are often referred to as marginal organs or extended criteria donors and are expected to perform less well after transplantation than standard criteria allografts, with higher rates of primary graft dysfunction and delayed graft function[3,4,5,6]. As a result, NEVLP has been introduced as a method to evaluate and modify organ function[6,7]. We have designed a rat model of NEVLP and used this model to demonstrate one of its important potential applications – the testing of novel molecule additives to liver perfusate.

NEVLP has been evaluated in both murine (rat) and porcine models, as well as in discarded human organs[6,8,9]. The results of the first human trials of NEVLP have also recently been published[10]. Although hypothermic machine perfusion has clearly become the standard for kidney preservation, the temperature at which liver machine perfusion should occur is still controversial. NEVLP has many proposed advantages in comparison to hypothermic and subnormothermic perfusion. These include reduced preservation injury, restoration of normal organ function under physiologic conditions, the ability to assess organ performance, and as a platform for organ repair, remodeling, and modification[7,11,12,13,14,15,16,17].

A significant number of studies have been completed using porcine NEVLP models. Although these models are comparatively inexpensive when considering models using discarded human organs or human clinical trials, they are very expensive when compared to our small animal NEVLP model. A significant component of the per experiment cost is the perfusate. We are able to complete a 4 h perfusion with 300 mL of perfusate at a relatively low cost. Additionally, the cost of small animals including rats is very low in comparison to the cost of pigs.

In comparison to other models of NEVLP in the rat, the model presented here is relatively simple to implement and has a broad range of applications. The perfusion circuit can be seen in **Figure 1**. The perfusate starts in the perfusate reservoir (1), which is a water jacketed container. Perfusate is pulled from the reservoir by a roller pump (2) and pushed into a windkessel (3) and then the oxygenator (4). The oxygenator is set for countercurrent gas and perfusate flow to provide maximum gas exchange. The perfusate then proceeds to a heating coil (5) inside the perfusion chamber to ensure it is at physiologic temperature, and a bubble trap (6) to prevent perfusion of air bubbles There are pre-organ (7) and post-organ (8) sample ports, which allow the perfusate to be sampled. The perfusate then enters the liver through the portal vein cannula. The portal vein cannula is attached to a pressure monitor that charts the values on the data collection software. The perfusate then exits the liver through the IVC cannula and flows into the pressure equalizer block (9). Finally, the perfusate is pulled from the pressure block back through the roller pump and emptied into the reservoir. This model includes continuous perfusion to the portal vein and leaves out the pulsatile flow to the hepatic artery and dialysis used in some other models, each of which requires a separate and additional circuit, but have previously been demonstrated to not be required[9,13].

To explore the addition of a novel therapeutic molecule to the perfusate, we chose the enzyme catalase. Catalase is an endogenous ROS scavenger that is part of the cells internal defense mechanism to mitigate the effects of ROS[18]. Catalase expression is increased in hepatic ischemia reperfusion injury[19]. Experimental addition of catalase has been demonstrated to decrease ischemia-reperfusion in the eye, brain, and lung[20,21,22,23,24]. Pegylation has been shown to target catalase to the endothelium and aid in catalase uptake into endothelial cells[25]. PEG-CAT has been administered systemically with limited efficacy in reducing hepatic ischemia-reperfusion injury; however, we hypothesized that adding PEG-CAT to an isolated organ perfusion circuit would lead to improved results[26,27,28]. Here, we add PEG-CAT to our base perfusate and demonstrate its ability mitigate liver preservation injury.

Protocol

All procedures were performed according to the guidelines of the Institutional Animal Care and National Research Council's Guide for the Humane Care and Use of Laboratory Animals (IACUC) and has undergone approval by the Ohio State University IACUC committee.

1. Initial Set-up

1. **Prepare the perfusion solution by combining the following: 86 mL of 25% albumin, 184 mL of Williams' media, 30 mL of penicillin/streptomycin (10 U/mL penicillin and 0.01 mg/mL streptomycin), insulin (50 U/L), heparin (0.01 U/mL), L-glutamine (0.292 g/L), and hydrocortisone (0.010 g/L) to a total volume of 300 mL. For the base perfusate and PEG-CAT group, add 625 U/mL of PEG-CAT.**
    1. Buffer the perfusate solution using tris(hydroxymethyl)aminomethane (THAM) to pH 7.4. Use an arterial blood gas machine to confirm the perfusate pH.

2. **Set up the circuit (Figure 1).**
    1. Turn on the water bath warmer and set it to 37 °C. Allow the organ chamber to warm up.
    2. Pour the mixed and buffered perfusate into the reservoir and start circulation.
    NOTE: The perfusate mentioned in this step was prepared in step 1.1.1.
    3. Turn on the oxygenating gas (95% oxygen and 5% carbon dioxide) to the flow counter through the in-line oxygenator.
    4. Turn on the data collection software and click "start" to record for the duration of the experiment.

3. **Set up the surgical microscope and the operating room (Figure 2).**
    1. Turn on all equipment including the warming board, electrocautery, and the anesthesia and vital signs (heart rate and oxygen saturation) monitoring equipment.
    NOTE: The microscope settings will vary based on the microscope used and can be adjusted to the user's comfort.
    2. Fill the 10 mL anesthesia syringe with 10 mL of liquid isoflurane for inhalation (molecular weight 184.5 g/mol) and place in the anesthesia unit.
    3. Position a 0.5 mL syringe of heparin (50 U), the surgical instruments, 4-0 and 7-0 silk suture, sterile cotton swabs, and 4 cm x 4 cm non-woven gauze sponges appropriately (**Figure 2**).

4. Prepare the isoflurane chamber.

2. Induction of Anesthesia

1. Wear the following personal protective equipment (PPE): surgical mask, surgical gloves, disposable gown.
2. Weigh the rat.
NOTE: We use Sprague-Dawley rats between 250–350 g.
3. Turn on the oxygen compressor and isoflurane. Place the rat, after being weighed, into the isoflurane chamber and secure the lid. Induce anesthesia using 6% isoflurane delivered with 2 L/min of oxygen.
NOTE: The exact isoflurane dose used will depend on the specific anesthesia system being used.
4. Use electronic clippers to clip the animal's abdominal hair.
5. Replace the animal in the isoflurane chamber.
6. Turn on the anesthesia unit located in the operating room.
7. Remove the rat from the isoflurane chamber when anesthesia is fully induced. Confirm the depth of anesthesia using a toe pinch.

3. Procurement Procedure

1. **Prepare the 16 G portal cuff (Figure 3).**

1. Begin with a 16 G angiocatheter. Cut a 7 mm section of tubing. Determine the midpoint of the 7 mm section by measuring 3.5 mm. Incise at the midpoint and remove the anterior half of the tubing.
2. Use a hemostat to crush this now flat portion. Use a lighter to melt the other end of the angiocatheter to create a lip. Do not place the tip directly into the flame or it will ignite.

2. **Prepare the bile duct cannula.**
   1. Take a 27 G angiocath and cut off the injection port leaving only the catheter. Connect this to a 10 cm section of the 27 G cannular tubing.

3. Position the rat with its nose in the anesthesia nose cone, and its four extremities immobilized. Monitor the vital signs by attaching the monitor to the left hind extremity. Perform a toe pinch to confirm appropriate depth of anesthesia. Continue anesthesia at 4% isoflurane (for animals that weigh >250 g).
4. Spray the animal's abdomen with 70% isopropyl alcohol. Allow to dry. Place a sterile drape over the animal.
5. Make a midline incision from the xiphoid to the pubis using sharp scissors and extending through the skin (**Figure 4**). Gently enter the peritoneum and incise the muscle. Take care to avoid damaging the bladder in the inferior aspect of this incision and the liver in the superior aspect of this incision.
6. Extend the incision laterally to the left and right to form a cross at the level of the inferior border of the liver.
7. Turn the anesthesia down to 2% (for animals weighing >250 g).
8. Retract the xiphoid process using a curved mosquito clamp and the ribs using rib retractors (**Figure 5**).
9. Cut the falciform, phrenic, and gastrohepatic ligaments with sharp scissors.
10. Locate and tie-off the phrenic vein with a 7-0 suture as close to its origin as possible to prevent leaking.
11. Eviscerate the rat using a sterile moistened cotton tip applicator and wrap the bowel in gauze moistened with 0.9% normal saline. Take care not to stretch the vasculature of the small bowel.
12. Dissect over the inferior vena cava (IVC) to remove excess tissue. Dissect behind the IVC just superior to the bifurcation and pass a loop of a 4-0 silk suture for later use (**Figure 6**).
13. Retract the right kidney to provide exposure to the right adrenal vein. Retract the right lobe of the liver superiorly with gauze. Tie off the right adrenal vein with a 7-0 silk suture as close to the IVC as possible and cauterize across it distal to the tie (**Figure 7**).
14. Carefully dissect out the splenic vein, tie it off using two 7-0 silk sutures and cut across it between the two sutures.
15. Tie off and ligate additional veins using 7-0 silk suture for additional length on the portal vein, if needed.
    NOTE: There are sometimes small branches of the infrahepatic IVC between the right adrenal vein and the inferior liver.
16. Dissect around the gastroduodenal artery, tie off the gastroduodenal artery with a 7-0 silk suture, and ligate the gastroduodenal artery.
17. Dissect around the hepatic artery and then place a 7-0 silk suture tie around it (**Figure 8**).
18. **Dissect out the bile duct.**
    1. Check the length of the bile duct. Tie off the bile duct at the distal end using a 7-0 silk suture. Place a loop of a 7-0 silk suture around the bile duct as proximally as possible.
    2. Cut a hole that is half the diameter of the duct with small scissors and place a 27 G catheter into the bile duct proximally. Tie the catheter in place using a roman sandal tie suture (**Figure 9**).

19. Inject 0.5 mL of heparin (50 U) into the penile vein or IVC of the animal using a 27 G needle.
    NOTE: A 27 G insulin syringe can also be used instead.
20. Clamp and tie off the IVC using the previously placed 4-0 silk suture.
21. Tie off the hepatic artery using the previously placed 7-0 silk suture.
22. Clamp off the portal vein using a microsurgical clip. Cannulate the portal vein using a 22 G angiocatheter. Flush the portal vein with 60 mL of cold 0.9% normal saline with 1 mL heparin (100 U) until the liver blanches (**Figure 10**).
    NOTE: If the liver does not blanch immediately it can be massaged with sterile cotton tip applicators.
23. Expose the suprahepatic IVC and cut across it as high in the chest as possible.
24. Perform a hepatectomy as follows. Cut around the diaphragm, cut the hepatic artery, cut the IVC, cut the portal vein, cut any additional ligaments, and take the liver out. Place the liver in ice cold 0.9% normal saline (**Figure 11**).
25. Place a 16 G vascular cuff in the portal vein (**Figure 12**). Place the liver on the *ex vivo* normothermic liver perfusion circuit.

4. *Ex Vivo* Normothermic Liver Perfusion

NOTE: The perfusate used here was prepared in protocol step 1.1.1.

1. Place the portal vein cannula into the cuffed portal vein (**Figure 13**).
2. During the portal vein cannula placement, maintain flow of the perfusate through the circuit at 2 mL/min to start. Watch the monitor for any spikes in portal vein pressure; this may indicate the vessel has become occluded and repositioning of the cannula is needed.
3. Suture in the IVC cannula for the return flow of the perfusate using a 7-0 silk suture.
4. Once both cannulas are in place, begin turning up the flow at 1 mL/min until a physiological pressure in the range of 10–16 cmH_2O is reached.
5. **Take a 1 mL sample from pre- and post-ports at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min of perfusion. Divide the 1 mL sample into two 0.5 mL samples.**
   NOTE: 0.5 mL of this will be used in protocol step 4.5.1, and 0.5 mL will be used in protocol step 4.5.2.
   1. Snap freeze 0.5 mL of this sample in cryogenic tubes in liquid nitrogen.
   2. Run an arterial blood gas analysis using the remaining 0.5 mL of perfusate.
   3. After running the blood gas analysis at each time point (0, 30, 60, 90, 120, 150, 180, 210, and 240 min) examine the pH levels and buffer the perfusate as needed to return to pH 7.4.

6. At the conclusion of 4 h of perfusion, disconnect the liver from the perfusion circuit. Divide the liver into 0.5 g segments. Snap freeze the liver tissue in cryogenic tubes in liquid nitrogen.

5. Post-experiment Analysis

1. **Determine alanine aminotransferase (ALT) level in the perfusate at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min using a commercial colorimetric assay kit.**
    1. In brief, incubate the perfusate with the reaction mix reagents at 37 °C for 60 min. Measure the optical density values at 570 nm using a microplate reader.

2. **Homogenize 0.5 g of liver tissue with 100 µL of lysis buffer and analyze the tissue lysate for adenosine triphosphate (ATP), glutathione (GSH), and malondialdehyde (MDA).**
    1. In brief, measure the ATP levels of the liver tissue samples using a commercial assay kit. Mix the sample with the reaction buffer and incubate at room temperature for 30 min. Measure the optical density at 570 nm using a microplate reader.
    2. Measure the GSH levels of the liver tissue samples using a commercial assay kit. Mix the tissue samples with the assay cocktail. Measure the optical density values at 405–414 nm.
    3. Measure the MDA levels of the liver tissue samples using a commercial assay kit. Mix the samples with TBA and heat to 95 °C for 60 min. Centrifuge the reactant and transfer the supernatant to a 96-well plate. Measure the optical density at 532 nm.

3. **Homogenize 0.5 g of liver tissue with 100 µL of lysis buffer and analyze the tissue lysate for relative caspase-3/7 activity using a commercial assay kit.**
    1. Mix the tissue lysate with the caspase-3-7 reagent assay buffer and incubate at room temperature for 30 min.
    2. Measure the fluorescence level in each well using a microplate reader.

4. **Determine the level of apoptotic cells in the liver tissue samples using a commercial *in situ* death detection kit.**
    1. Pre-treat the 0.5 g tissue sections with 10 U/mL Proteinase K for 10 min and then incubate with the reaction mixture at 37 °C for 60 min. Perform the analysis using a fluorescent microscope.

Representative Results

A sample size of three rats per group was used. ALT was measured at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min of perfusion. We used Student's *t*-tests to compare results between the base perfusate and base perfusate plus PEG-CAT groups at each time point. In comparing the base perfusate and base perfusate plus PEG-CAT groups, there is significantly less (p <0.05) ALT in the base perfusate plus PEG-CAT group at 150, 180, 210, and 240 min (**Figure 14A**).

Liver tissue was procured in order to analyze tissue damage from both the base perfusate and base perfusate plus PEG-CAT groups. We used Student's *t*-tests to compare results between the base perfusate and base perfusate plus PEG-CAT groups. Tissue ATP was maintained in the base perfusate plus PEG-CAT group in comparison to the base perfusate alone group (**Figure 14B**, p <0.05). Tissue MDA production was significantly higher in the base perfusate group than in the base perfusate plus PEG-CAT group (**Figure 14C**, p <0.05). Total GSH was maintained in the base perfusate plus PEG-CAT group in comparison to the base perfusate alone group (**Figure 14D**, p <0.05).

To analyze apoptosis, liver tissue caspase 3/7 activity was compared between the groups. Fluorescence was measured in each well. We used Student's *t*-tests to compare results between the base perfusate and base perfusate plus PEG-CAT groups. Caspase 3/7 activity was significantly decreased in the base perfusate plus PEG-CAT group in comparison to the base perfusate alone group (**Figure 15A**, p <0.05). Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) staining was used to compare apoptosis between the groups. The percentage of apoptotic cells was significantly less in the base perfusate plus PEG-CAT group in comparison to base perfusate alone group (**Figure 15B**, p <0.05).

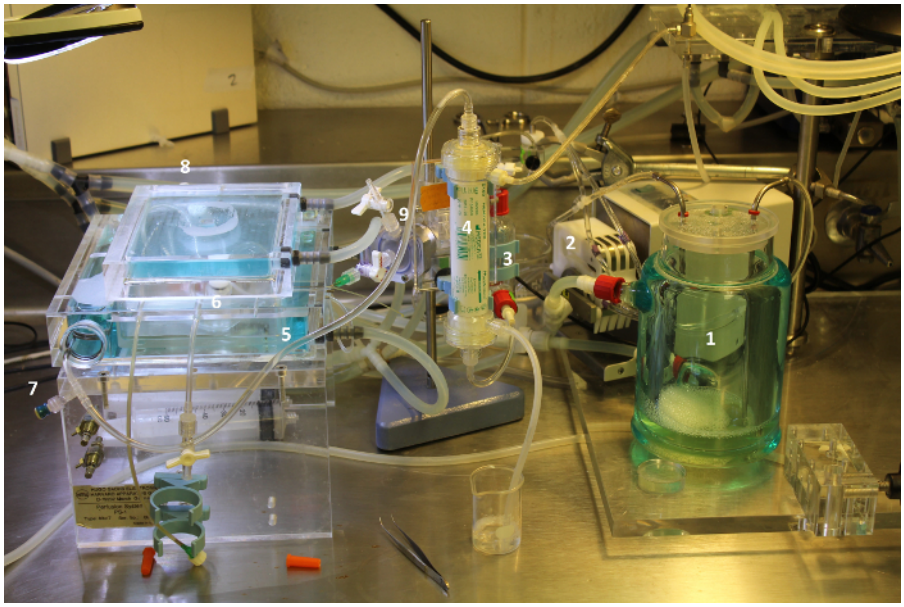

Figure 1: Perfusion Circuit. Components of the circuit are labeled. The perfusate starts in the perfusate reservoir (1), which is a water jacketed container. Perfusate is pulled from the reservoir by a roller pump (2) and pushed into a windkessel (3) and then the oxygenator (4). The oxygenator is set for countercurrent gas and perfusate flow to provide maximum gas exchange. The perfusate then proceeds to a heating coil (5) inside the perfusion chamber to ensure it is at physiologic temperature, and a bubble trap (6) to prevent perfusion of air bubbles. There are pre-organ (7) and post-organ (8) sample ports, which allow the perfusate to be sampled. The perfusate then enters the liver through the portal vein cannula. The portal vein cannula is attached to a pressure monitor, which regulates the pressure equalizer (9). Finally, the perfusate is pulled from the pressure block back through the roller pump and emptied into the reservoir. Please click here to view a larger version of this figure.

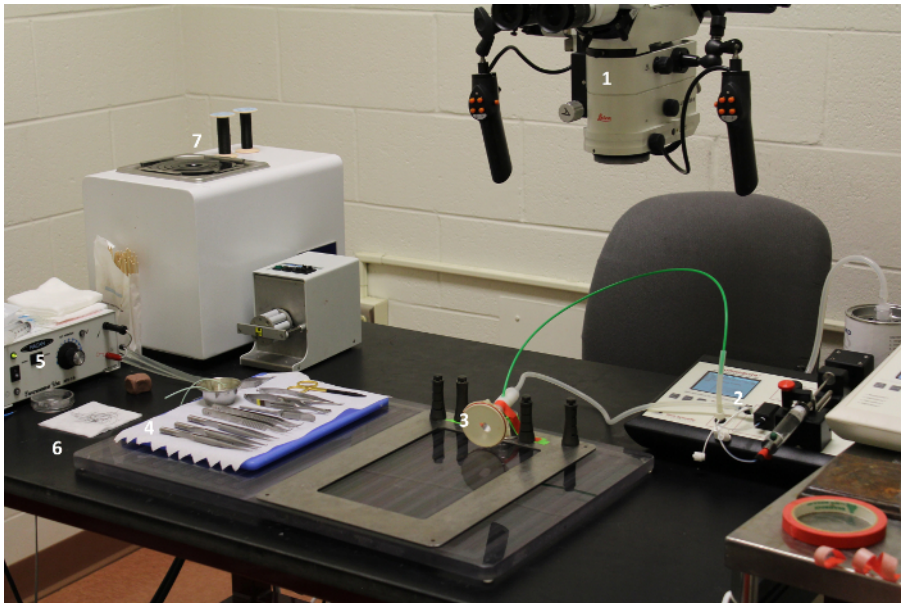

Figure 2: Operating room and surgical instrument set-up. The surgical microscope (1) should be adjusted to the appropriate height and magnification for the user. Isoflurane can be pre-loaded into the anesthesia machine (2). The animal's nose is placed in the nose cone (3). Surgical instruments should be laid out where they can be easily accessed (4). Having electrocautery (5) nearby is helpful. Sutures (6) should be pre-cut so pieces can be obtained quickly when needed, and extra should be available (7). Please click here to view a larger version of this figure.

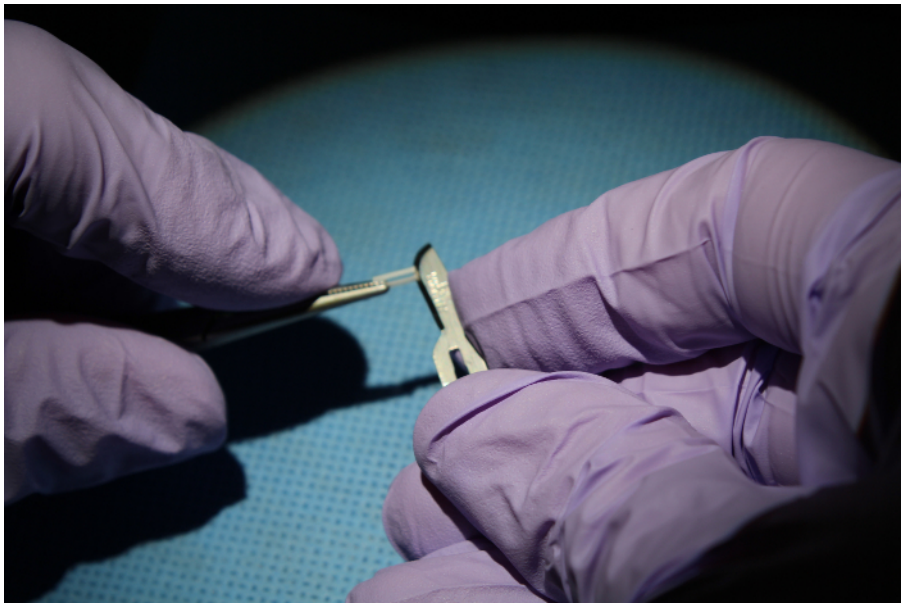

Figure 3: Prepare the 16 G portal vein cuff. Begin with a 16 G angiocatheter. Cut a 7 mm section of tubing. Determine the midpoint of the 7 mm section by measuring 3.5 mm. Incise here and remove the anterior half of the tubing. Use a hemostat to crush this now flat portion. Use a lighter to burn the other end of the angiocatheter to create a lip. Do not place the tip directly into the flame or it will ignite. Please click here to view a larger version of this figure.

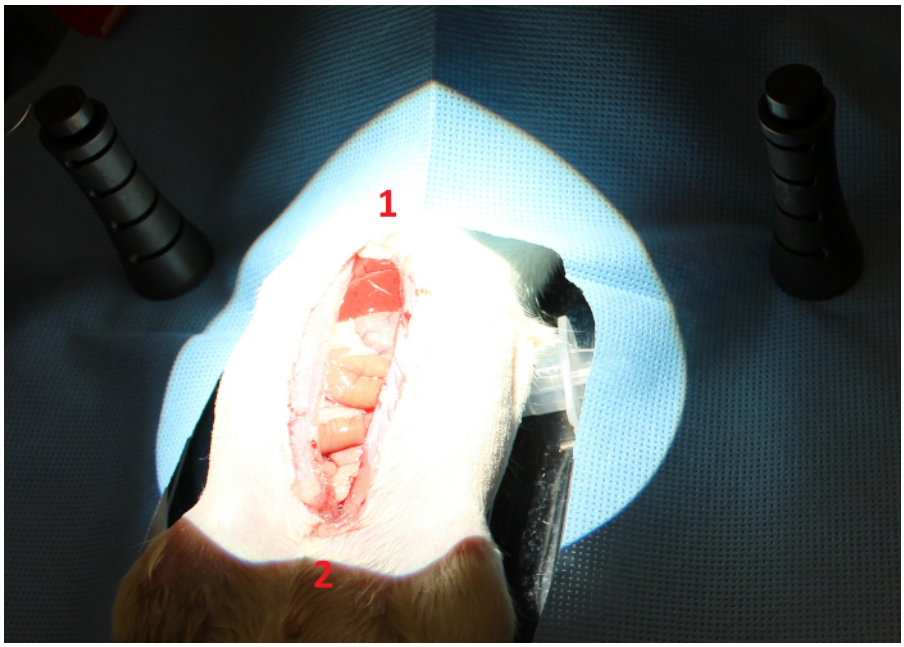

Figure 4: Midline incision. Make a midline incision from the xiphoid (1) to the pubis (2) using sharp scissors and extending through the skin and muscle. Please click here to view a larger version of this figure.

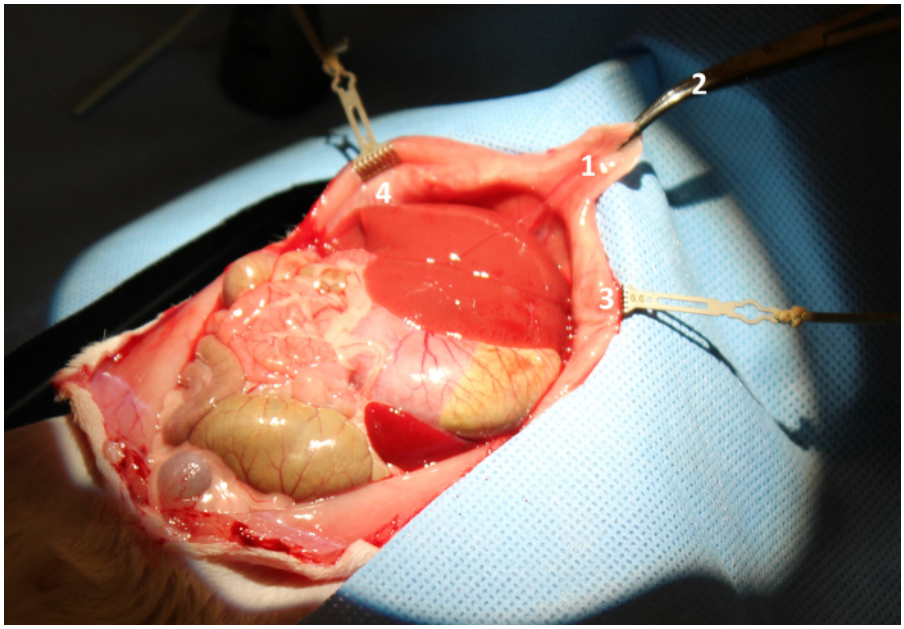

Figure 5: Obtain adequate retraction. Retract the xiphoid process (1) using a curved mosquito clamp (2) and the rib by placing rib retractors (3, 4). Please click here to view a larger version of this figure.

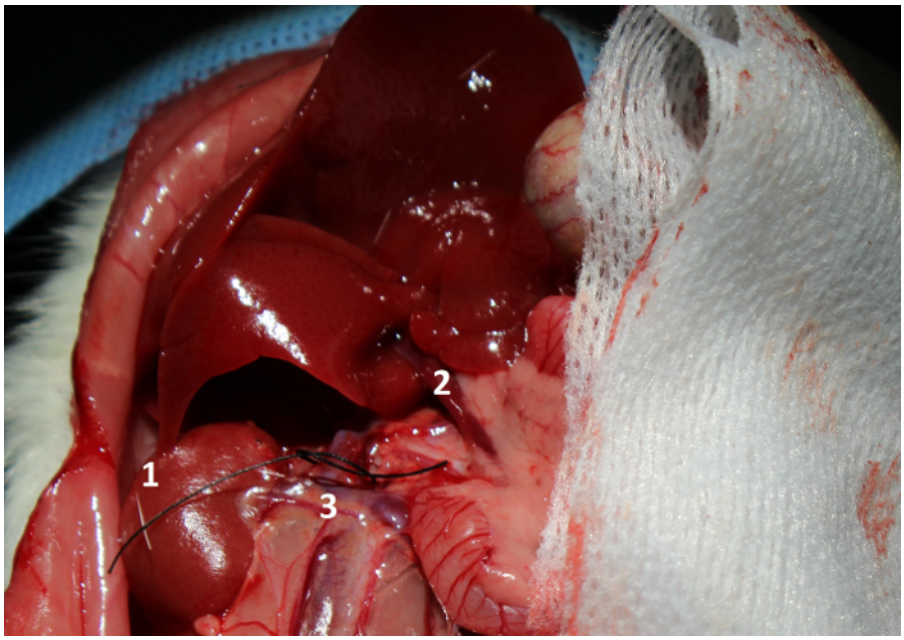

Figure 6: Inferior vena cava (IVC) dissection. Flip the liver up to expose the right kidney (1) and portal vein (2). Dissect around the IVC (3) and place a loop of 7-0 suture for future use. Please click here to view a larger version of this figure.

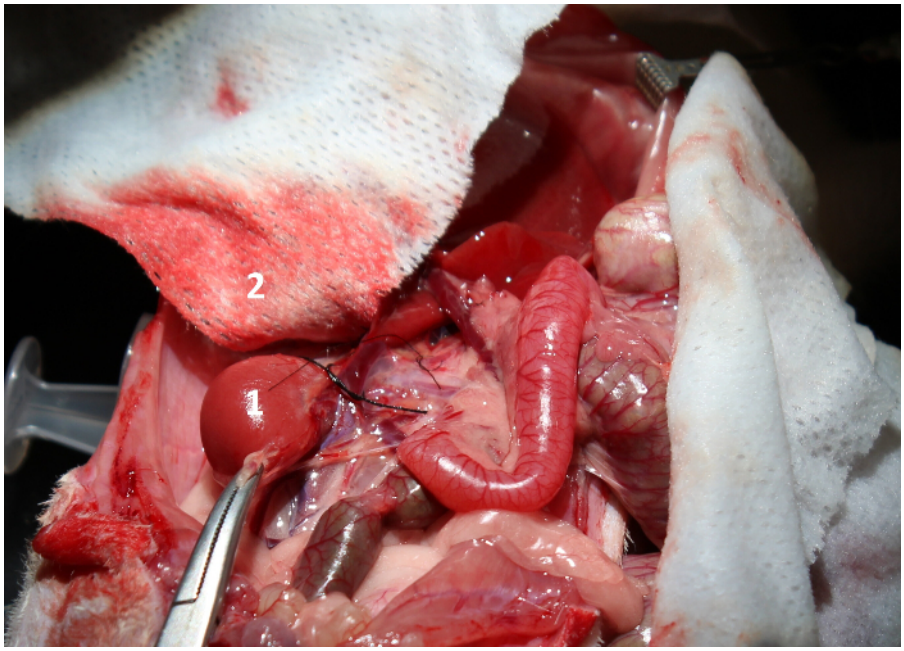

Figure 7: Ligation of the right adrenal vein. Retract the right kidney (1) to provide exposure to the right adrenal vein. Tie off the right adrenal vein and cut across it. A moistened gauze (2) can be used to protect the liver during this maneuver. Please click here to view a larger version of this figure.

Figure 8: Hepatic artery dissection. Dissect around and place a tie around the hepatic artery (1) near where it passes under the portal vein (2). Please click here to view a larger version of this figure.

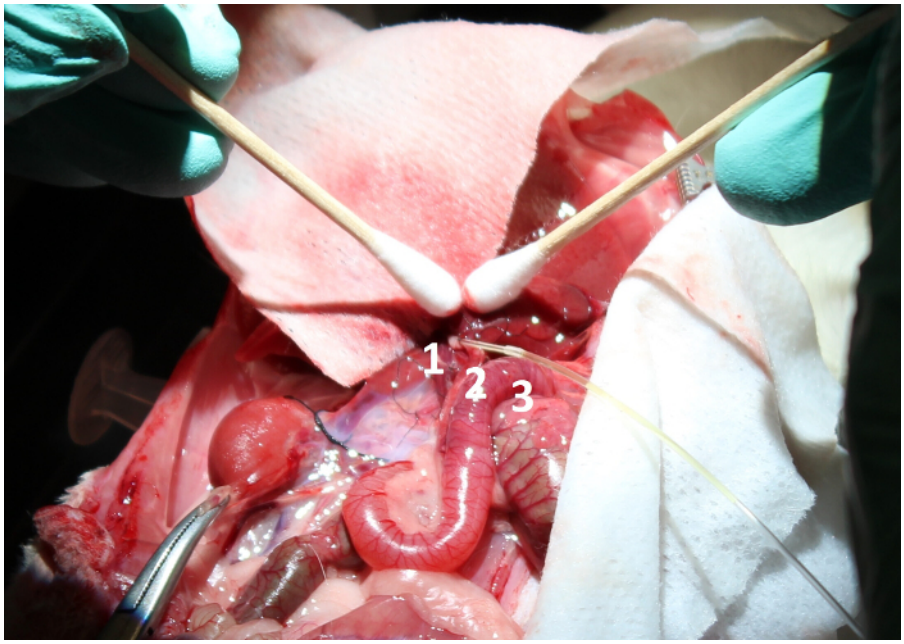

Figure 9: Bile duct cannulation. Cannulate the bile duct (1) using the 27 G angiocatheter (2) connected to the 27 G tubing (3). This will help to collect the bile during perfusion. Please click here to view a larger version of this figure.

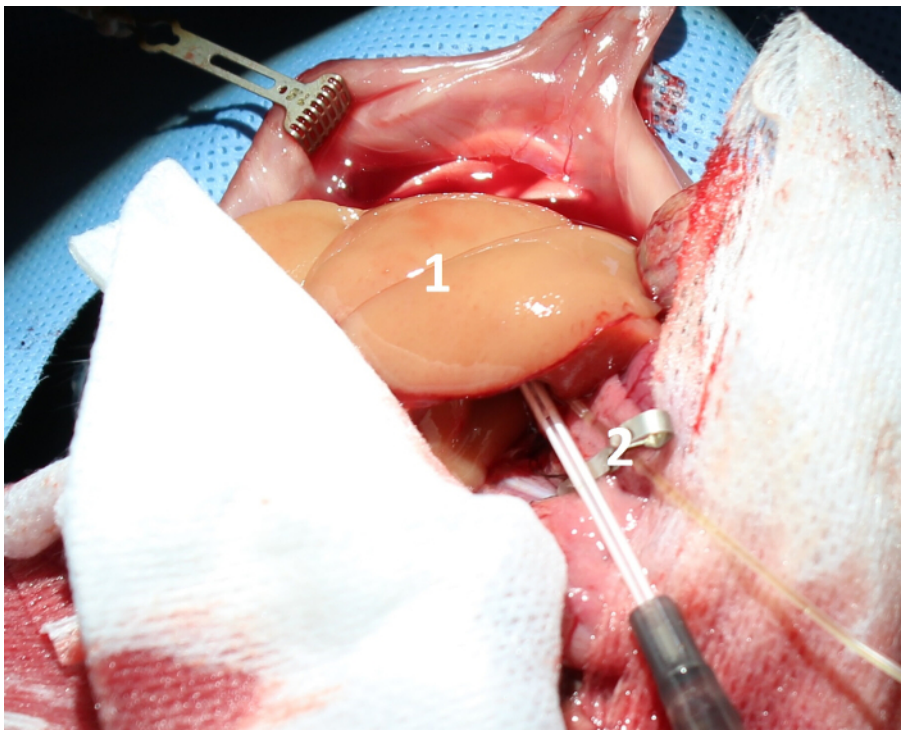

Figure 10: Liver flush. Flush the liver (1) with 60 cc of cold 0.9% normal saline with 100 U (1 mL) of heparin using a 16 G angiocatheter (2). Please click here to view a larger version of this figure.

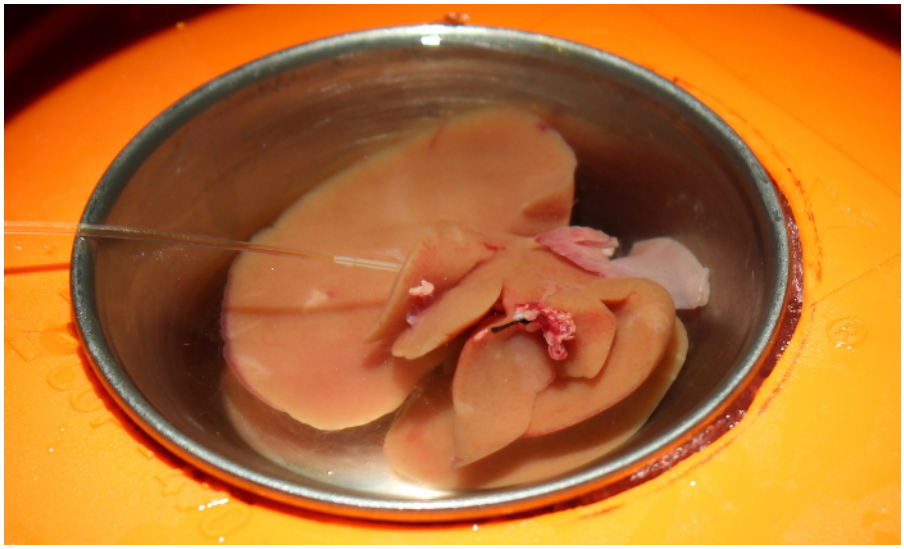

Figure 11: After the hepatectomy. Perform a hepatectomy and place the liver in cold saline. Take care not to dislodge the bile duct cannula. Please click here to view a larger version of this figure.

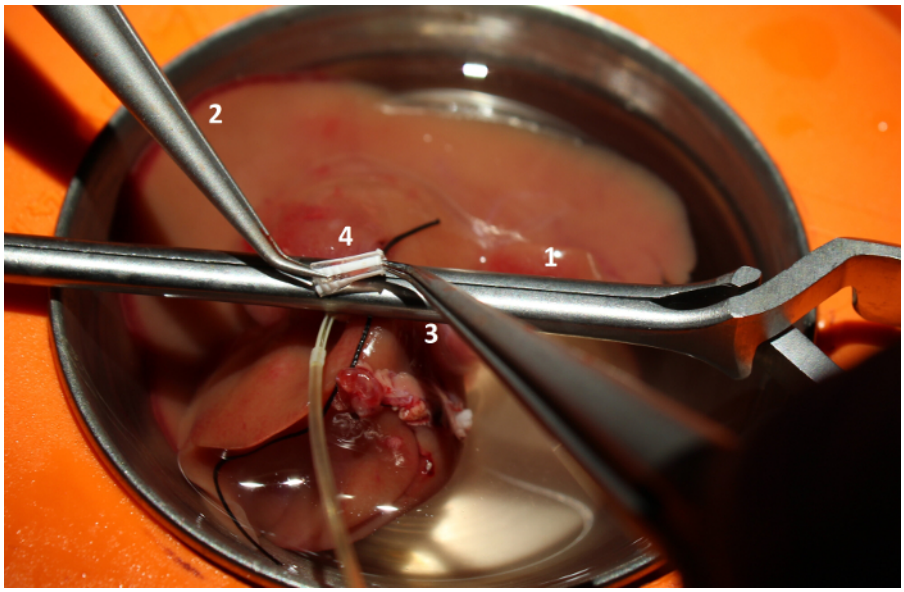

Figure 12: Portal vein cuffing. Locate the portal vein. Use a large clamp (1) to hold up the vein leaving a several millimeter-lip of vein above the clamp. Use microsurgical forceps (2, 3) to place a 16 G vascular cuff (4) in the portal vein. Please click here to view a larger version of this figure.

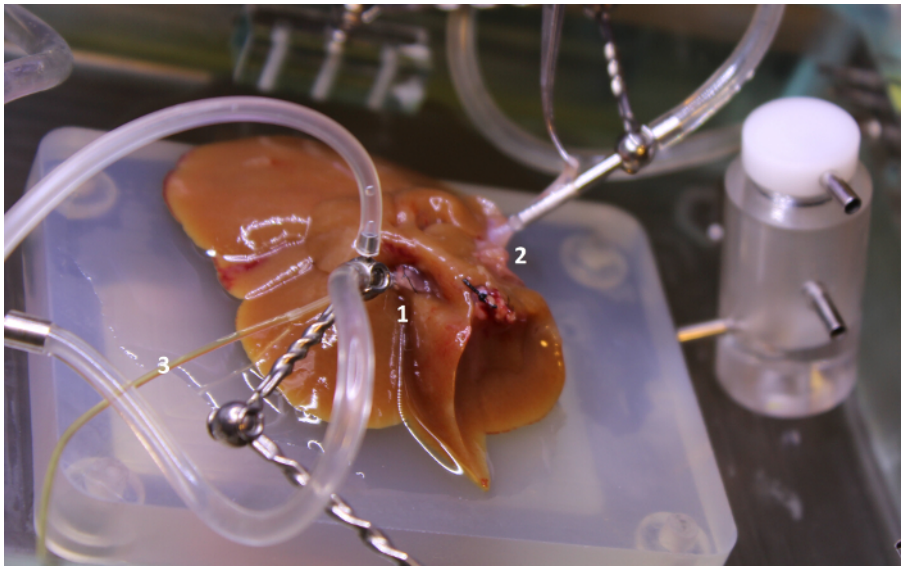

Figure 13: Portal vein cuff and superior IVC cannulation. Cannulate the portal vein cuff (1) and superior IVC (2). Great care must be taken not to dislodge the bile duct cannula (3). Additionally, take care not to twist the superior IVC. Please click here to view a larger version of this figure.

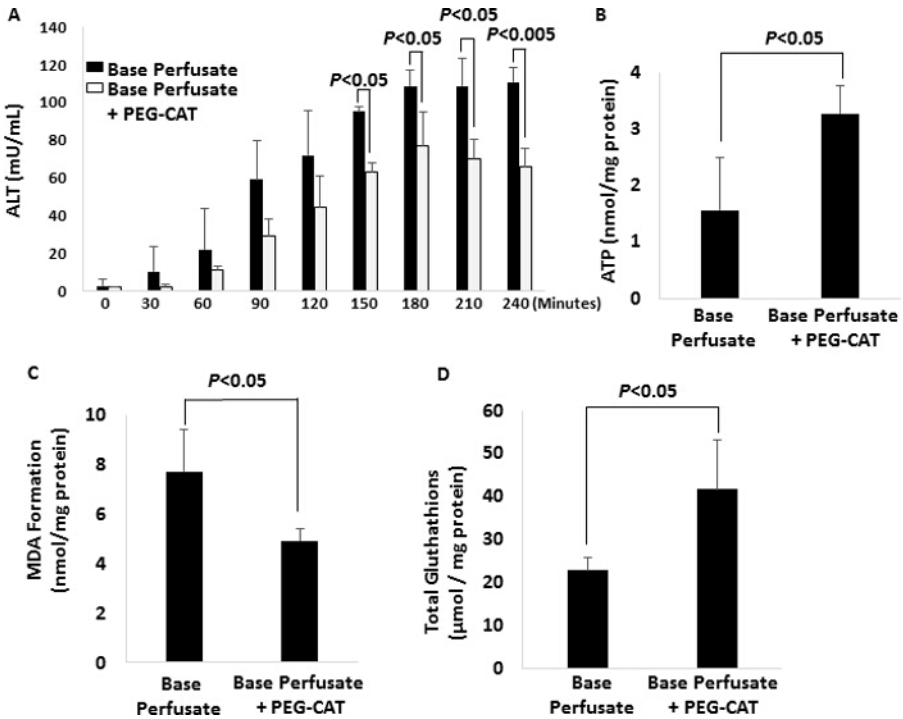

Figure 14: Analysis of tissue damage in base perfusate-only and base perfusate and plus PEG-CAT groups (N = 3/group). Error bars represent standard deviation. (**A**) Alanine aminotransferase (ALT) Levels. In comparing ALT levels between the base perfusate and base perfusate plus pegylated-catalase (PEG-CAT) group, there is significantly less ALT in the base perfusate plus PEG-CAT group at 150, 180, 210, and 240 min (p <0.05). (**B**) Adenosine Triphosphate Levels. Tissue adenosine triphosphate (ATP) was maintained in the base perfusate plus PEG-CAT group in comparison to the base perfusate alone group (p <0.05). (**C**) Malondialdehyde Levels. Tissue malondialdehyde (MDA) production was significantly higher in the base perfusate group than in the base perfusate plus PEG-CAT group (p <0.05). (**D**) Glutathione Levels. Total glutathione (GSH) was maintained in the base perfusate plus PEG-CAT group in comparison to the base perfusate alone group (p <0.05). Please click here to view a larger version of this figure.

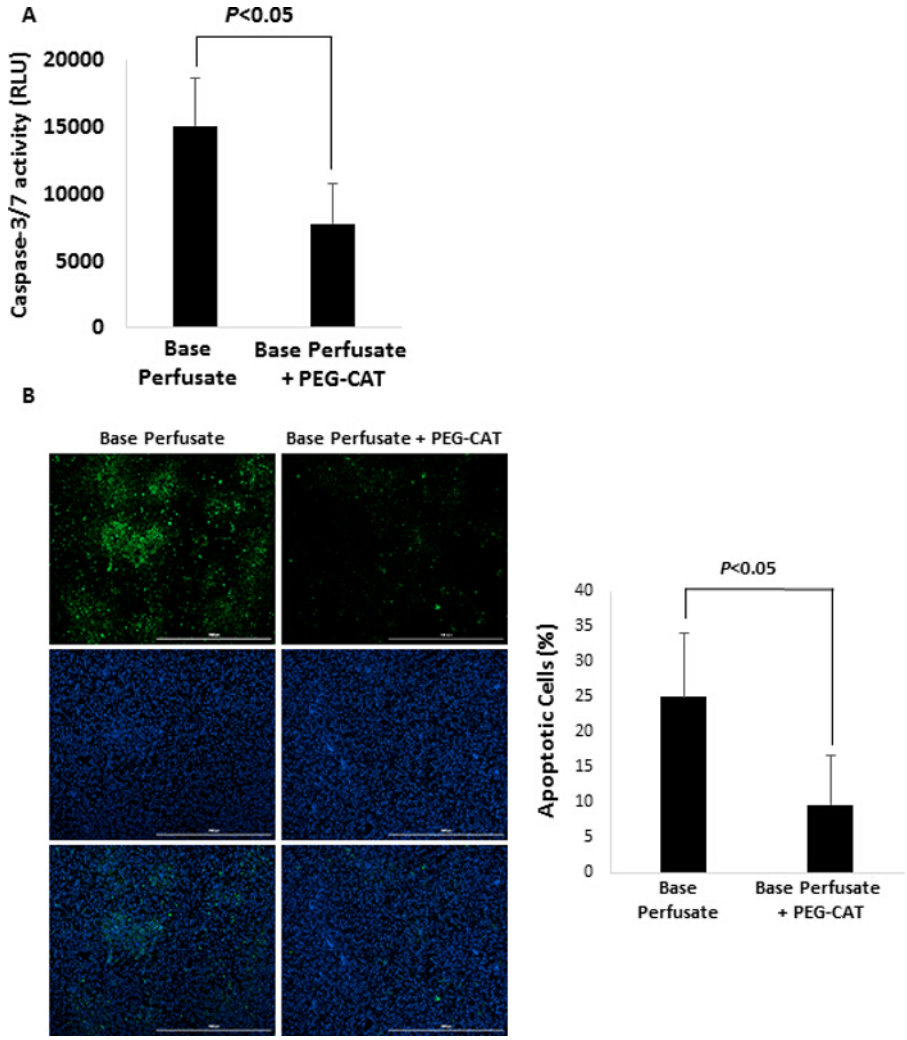

Figure 15: Analysis of apoptosis in base perfusate-only and base perfusate and plus PEG-CAT groups (N = 3/group). Error bars represent standard deviation. (**A**) Caspase-3/7 Activity.Caspase 3/7 activity was significantly decreased in the base perfusate plus PEG-CAT group in comparison to the base perfusate alone group (p <0.05). (**B**) Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) staining. Images were taken using a 4X fluorescent microscope. The percentage of apoptotic cells was significantly less in the base perfusate plus PEG-CAT group in comparison to base perfusate (p <0.05). Green: apoptotic cells. Blue: nuclear. Scale bars = 1,000 µm. TUNEL positive cells were quantified by counting cells from 4 random microscopic fields. Please click here to view a larger version of this figure.

Discussion

There is a significant shortage of liver allografts available for transplantation and in response donor criteria have been expanded[1,2,3,4,5]. As a result of the donor shortage, NEVLP has been introduced as a method to evaluate and modify organ function[6,7]. We have designed a rat model of NEVLP. Furthermore, we have used this model to demonstrate one of its important potential applications — the testing of novel molecule additives to liver perfusate. Here, we added PEG-CAT to the base perfusate and demonstrated its ability to mitigate liver preservation injury.

Critical Steps

The liver perfusion circuit was purchased and used without modification. The circuit can be visualized in **Figure 1**. The perfusate reservoir used is a water-jacketed container that is used to keep the perfusate at physiological temperature. From the reservoir perfusate is pulled out by a roller pumped and pushed into a windkessel chamber. This chamber helps to dampen the pulsatile flow of the perfusate and make it more laminar for entry into the organ. After the windkessel chamber the perfusate flows to the oxygenator. The oxygenator is set for countercurrent gas and perfusate flow providing maximum gas exchange to the perfusate with 95% oxygen. The perfusate then proceeds to a heating coil to ensure that it is still at physiological temperature. A bubble trap right before the organ prevents perfusion of air bubbles. The perfusate is then pumped out of the bubble trap and through the portal vein cannula into the liver. The portal vein cannula has a small branch off of it for the pressure monitor. The tubing to the sensor should be primed with fluid not air so there is no loss of pressure to the sensor. After oxygenating the organ, the perfusate flows out of the liver through the inferior vein cannula to a pressure equalizer. The pressure equalizer block helps prevent over pressurization of the circuit or organ. Finally, the perfusate is pulled from the pressure block back through the roller pump and emptied into the reservoir.

Before beginning each perfusion visual inspection of the circuit should be performed to identify any damage or buildup on circuit components or tubing. If there is a buildup of bacteria or other substances on the circuit, parts should be replaced or cleaned, if possible. Next, the detergent solution maintaining the internal components should be rinsed out. As the components are being rinsed out the pressure sensor and pressure line should be purged of any air bubbles with deionized water. Also, flow should be adjusted at regular intervals to make sure the pressure reading is responding appropriately to changes. If the pressure sensor is not responding appropriately, all items in the line to the sensor should be checked and recalibrated if necessary. At the start of a perfusion it is crucial to make sure the vessels of the liver do not become kinked or twisted when connecting the liver to the circuit. If this has occurred there will be an immediate pressure spike seen on the monitor in a logarithmic trend. The most common error is a kink in the portal vein with a poorly positioned cannula. This issue can be resolved by moving the vessel into a more natural position by pulling it slightly out and straightening the portal vein. The pressure monitor indicates resolution of this problem with a drop in pressure and improved consistency. Next, when connecting the portal vein cuff to the portal vein cannula the vessel can become twisted impeding perfusion of the organ. Adjusting the cuff and correcting this error will result in a sudden spike in portal vein pressure that should then immediately return to a lower pressure and level off at a consistent flow. A kinked or twisted IVC can quickly be identified by no flow from the cannula and a bulging of the vessel. Both of these errors in the vena cava will also result in an increased pressure, however unlike portal vein troubles this pressure is exerted on the organ and should be resolved quickly. This issue should be resolved within 10 min or the experiment should be canceled. An immediate indication for canceling the experiment is seeing clear edema in the organ within the first 20 min.

If there is a leak from the liver or from one of the cannula connections it will be important to monitor perfusate reservoir level. Running out of perfusate and pumping air can be catastrophic to the experiment. Once air is pumped into the lines it is not possible to pause the experiment and re-prime the tubing lines. The only possible correction is for the bubble trap to capture the injected air.

Modifications and Troubleshooting

Once the circuit is flushed the oxygenator can be put in line and then the circuit can be primed with perfusate. Properly purging air from the oxygenator can take a few min but is a crucial step in making sure an air embolism is not generated in the middle of a perfusion. After the oxygenator is fully primed with perfusate the bubble trap should be filled next to capture any air bubbles that do form. At this point the circuit flow should be set to a flow of 1 or 2 mL/min to keep the perfusate moving until the liver is ready for cannulation.

After cannulating the portal vein and the IVC of the liver, the pressure should increase and then level off. As flow is increased to a normal physiological pressure the recorded pressure should start to increase in a similar stepwise manner. Once the desired flow (8–16 mmHg) has been achieved the pressure should remain fairly constant. We aim for a pressure of 10 mmHg, and adjust the flow accordingly. The required flow to reach a pressure of 10 mmHg may vary by organ. There may be a slight leak of perfusate from the organ but this perfusate can be collect and returned to the reservoir.

The circuit should be cleaned after every perfusion to maintain the chamber and reservoir and to preserve the disposable tubing and ports. All perfusate should be removed from the circuit. The circuit should be immediately flushed with a minimum of 300 mL of deionized water. While the deionized water flushes the circuit tubing the external parts should be cleaned appropriately. External components should be rinsed off or gently wiped down and allowed to air dry. Circuit components are fragile and can be damaged easily. It is therefore of utmost importance to clean it gently. The internal circuit should be preserved in a 5% solution of alkaline detergent in deionized water when not in use. The detergent in the circuit helps to extend the life of the tubing and prevent buildup on other components, such as the bubble trap and pressure equalizer.

Most difficulties with the circuit can be prevented with thorough cleaning and maintenance of the circuit after each use. This helps to ensure there is no buildup of residual perfusate that can lead to clogged tubing or cannulas. Circuit components and tubing should be inspected regularly and replaced as needed before each use to make sure there is no contamination or restriction in flow.

Limitations

A limitation of this small animal NEVLP model is that it does not currently include post-perfusion transplantation. It is therefore impossible to evaluate liver graft function after transplantation. This is an important area for future research. Additionally, utilizing the small animal circuit requires both knowledge and skill.

Significance with Respect to Existing Models

Porcine and murine (rat) models of hypothermic, subnormothermic, and normothermic *ex vivo* liver perfusion have been described in the literature. Although controversy still exists regarding perfusion temperature, it has been shown that that machine perfusion can improve function of liver grafts regardless of temperature[9]. The NEVLP model presented here is simple, easily replicable, low cost, and has a broad range of applications. This model does not include dialysis or pulsatile flow to the hepatic artery, which are included in some other models, as they have been shown to be unnecessary[9,13]. Furthermore, the results of the first human trials using NEVLP have shown this to be an effective method of liver preservation — therefore, this model is ideal for testing future applications of *ex vivo* liver perfusion[10].

Future Applications

A variety of future applications for NEVLP have been proposed in the literature. Each of these will need to be methodically tested in animal models prior to testing in discarded human organs and then in human livers. The model presented here is ideal for testing these novel future applications as it is easily replicable, eliminates extraneous steps, and is low cost. One of the most important potential applications of this model is the one demonstrated here - the testing of novel pharmacologic perfusate additives. Other proposed applications include repair of damaged organs, defatting of livers to allow transplantation of steatotic organs, introduction of hepatitis C viral resistance, mesenchymal stem cell therapy, gene modification, and perfusion with immunosuppressant agents[11,31,32,33,34,35,36,37,38].

Conclusions

In conclusion, we have demonstrated an inexpensive, easily replicable NEVLP model using rats. Use of this model requires careful preparation, practice, and knowledge, but can be implemented at low cost. Applications of this model can include testing novel perfusate additives, as was demonstrated in the representative results. Additional applications of this model may include testing software designed for organ evaluation, different perfusates, and artificial or hemoglobin-based oxygen carriers and agents designed to repair organs.

Disclosures

All authors report that they have no relevant disclosures.

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