Video Article

Re-Arterialized Rat Partial Liver Transplantation with an *in vivo* Vessel-Oriented 70% Hepatectomy

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Abstract

Split liver transplantation and living liver donor liver transplantation were developed in the clinic to utilize liver organs in a more efficient manner. To better understand the mechanism behind these surgical procedures, a rat partial liver transplantation (PLTx) model was established for relevant surgical studies. Because of the complexity of the rat PLTx model, a protocol with detailed descriptions is required. An article published previously reported a protocol in which *ex vivo* hepatectomy was used to achieve 50% rat PLTx. In contrast to this protocol, we introduced a re-arterialized PLTx with an *in vivo* 70% hepatectomy. An updated vessel-oriented hepatectomy was incorporated into the rat PLTx to refine the microsurgical procedure. The portal veins and hepatic arteries of the left lateral lobe and the median lobe were individually dissected and ligated before removal of the liver parenchyma, thereby decreasing the probability of bleeding in the remnant liver stump. Furthermore, an end-to-side vessel anastomosis between the common hepatic artery and the enlarged proper hepatic artery was introduced to re-arterialize the hepatic artery. By using this end-to-side vessel anastomosis technique, the diameter of the anastomosis was enlarged, thereby decreasing the difficulty of hand suture and maintaining a high rate of anastomotic patency. Moreover, the cuff anastomosis of the infrahepatic vena cava was slightly modified. A section of circumferential liver parenchyma around the vena cava of a recipient was preserved during cuff anastomosis to maintain the three-dimensional shape of the vascular lumen. This section of liver parenchyma was removed after completing the anastomosis. With this modification, the step involving placement of stay sutures was omitted, thereby further shortening the cuff anastomosis time. By using this protocol of rat PLTx, a low liver enzyme level, an intact liver lobular architecture and a high survival rate were achieved after microsurgery.

Video Link

The video component of this article can be found at https://www.jove.com/video/56392/

Introduction

Currently, there is a large discrepancy between the number of donated liver organs and the number of patients waiting for a donated liver. The shortage of liver organs is a global problem. To expand the donor pool, split liver transplantation (LTx) and living donor LTx were developed to use a partial liver as a graft[1].

To further investigate the mechanism behind partial liver transplantation (PLTx), relevant animal models have been established[2,3,4,5]. In rat PLTx, the liver lobes are resected *in vivo* and *ex vivo* to mimic the conditions of the living donor LTx and split LTx, respectively, in human. A paper published in the *Journal of Visualized Experiments* presented a detailed protocol involving a 50% rat PLTx using an *ex vivo* hepatectomy[5]. However, a rat PLTx with an *in vivo* hepatectomy has not yet been reported in the visualized literature.

In addition to the difference between *in vivo* and *ex vivo* hepatectomies, the technique of performing a hepatectomy itself also plays an important role in determining the outcome of PLTx. Currently, in many surgical studies using the rat PTLx model, the liver lobes were resected after placing a simple ligation at the pedicle of the liver lobe[2,3,6,7,8,9]. However, placing a simple ligation before resection is not suitable for all liver lobes, as different liver lobes have different shapes and sizes. A simple ligation at the base of the median lobe carries a high risk of causing a constriction of the vena cava, which might eventually affect the outflow of the partial liver graft[10,11]. Therefore, an update hepatectomy technique based on knowledge of the rat hepatic anatomy is required in the field of rat PTLx.

In the protocol described in this study, an updated vessel-oriented 70% hepatectomy was incorporated into the procedure of the rat PLTx. The portal veins and hepatic arteries of the left lateral lobe (LLL) and median lobe (ML) were dissected and divided individually before removal of the liver parenchyma. Then, the hepatic veins of the LLL and ML were ligated by piercing sutures. By using individual ligations and multiple piercing

sutures rather than placing a simple ligation, the remnant stump of the ML was able to spread over the vena cava. Hence, the constriction of the infrahepatic vena cava caused by a surgical ligation was avoided. Additionally, occlusion of the blood supply of the LLL and ML by individual ligations before removal of the liver parenchyma decreased the rate of bleeding in the remnant liver stump, thereby minimizing the influence of blood loss on the experiments[11].

For microsurgeons, it is a significant challenge to reconstruct the proper hepatic artery (PHA) of a liver graft because of the extremely small diameter of this vessel. Although the question of whether re-arterialization in LTx is truly necessary is still under debate[12,13,14], numerous microsurgical techniques for reconstructing the hepatic artery have been proposed[14]. Here, we introduce a novel technique for re-arterialization of the liver graft, which anastomoses the common hepatic artery (CHA) to the enlarged PHA in an end-to-side manner. By using this end-to-side technique, two hepatic arteries are connected with an anastomosis of a larger diameter. The larger the diameter of the anastomosis is, the easier hand suturing is to perform and the greater the improvement in the patency of the anastomosis becomes.

Protocol

All of the procedures followed the guidelines of rodent surgery approved by the Wenzhou Medical University Animal Policy and Welfare Committee [15].

1. Animals

1. Use male Lewis rats weighing 250-350 g as donors and recipients.

2. Operative Environment

1. Perform all microsurgical procedures under a surgical microscope with a magnification ranging from 7X to 20X. Sterilize all surgical instruments before use in operation.
   NOTE: The operating room and operating table are clean but not sterile.

3. Basic Microsurgical Maneuvers

1. Free, dissect, and mobilize the vessel.
   1. First, use straight micro-forceps to lift the surrounding tissue on the vessel.
   2. Second, repeatedly and gently open and close the tips of the curved micro-forceps such that the fine tips can cut open and peel the surrounding tissue on the anterior wall of the vessel.
   3. Third, use straight micro-forceps to clamp and lift the vessel to expose the posterior space. Then, use the tips of the curved micro-forceps to peel the surrounding tissue on the posterior wall of the vessel. Finally, a bald vessel without any surrounding tissue connected should be presented.

2. Divide or double ligate and divide a vessel or tissue.
   1. First, tie two square knots on the vessel or tissue using two micro-forceps[16]. Second, cut off the vessel or tissue between the two square knots using micro-scissors.

4. Preparation of the Cuff and Biliary Stent

1. Make the cuffs of the portal vein and infrahepatic vena cava using 12-gauge and 14-gauge intravenous catheters, respectively, while the biliary stent used is a 22-gauge intravenous catheter. The cuffs consist of a body part and handle part. Use a fillister on the body part to secure the vessel wall onto the cuff (**Figure 1**).

5. Anesthesia and Fixation of the Rats

1. Gently place the Lewis rat in an induction box with 5% isoflurane mixed with 0.5 L/min oxygen to induce inhalation anesthesia. Monitor the respiratory frequency of the rat to prevent unexpected death caused by an overdose of isoflurane. Reduce the concentration of isoflurane to 2% to maintain anesthesia when the rat does not respond to pain stimulation.
2. Move the rat to a 30 cm x 30 cm oak plank covered by a sterilized surgical mat towel, and place the rat in a supine position. Tie the four limbs of the rat using medial tape, and subsequently fix the tape with four thumbtacks. Disinfect the skin using 70% alcohol one time and iodophors three times.

6. Donor Operation

1. Exposing the operative field
   1. Cut a small transverse incision on the upper abdomen to transect the bilateral inferior epigastric vessels using an electric coagulator.
   2. Extend the transverse incision close to the costal margin on the upper abdomen using surgical scissors.
   3. Reverse and fix the upper abdomen wall and xiphoid process, respectively, to the cranial side.
   4. Move the intestine out of the abdominal cavity with two wet cotton sticks. Cover the intestine using a wet piece of gauze.

2. Cutting off the ligaments and trimming the suprahepatic vena cava (SHVC)

1. Cut off the falciformal ligament, coronary ligament and triangular ligament in sequence.
2. Free the left diaphragmatic vein from the diaphragm using micro-forceps. Then, double ligate it using 6-0 silk sutures.
   NOTE: Do not divide this vein in this step.
3. Cut off the gastrohepatic ligament using micro-scissors to free the inferior caudate lobe and the hepatoesophageal ligament and artery.

3. Occluding the blood supply of the LLL and ML before resection (**Figure 2**)
   1. First, use curved forceps to carefully dissect the posterior wall of the common trunk of the PV that supplies the LLL and left median lobe (LML) from the parenchyma. Second, double ligate and divide it with the corresponding bile duct and hepatic artery together.
   2. Dissect and divide the PV of the RML gently because this vein adheres closely to the liver parenchyma.
      NOTE: 14X magnification is recommended. Do not injure the PV of the right superior lobe, which is close to the PV of the RML.
   3. Identify the hepatic artery and bile duct of the RML located between the PVs of the RML and LML. Double ligate and divide the hepatic artery and bile duct of the RML using 6-0 silk sutures.
      NOTE: A change in the color of the LLL and ML from red to dark red is an indicator of successful occlusion of the blood supply. Thorough knowledge of the vascular anatomy is essential to identifying and dissecting the corresponding vessels. A previous study in rat clearly illustrated the hepatic anatomy by corrosion casts[10].

4. Resecting the LLL
   1. Ligate the pedicle of the LLL using a circumferential 3-0 silk suture to occlude the blood flow of the left lateral hepatic vein. Using micro-scissors, cut the liver immediately above the ligation to remove the liver mass.

5. Resecting the LML
   1. Clamp the left median hepatic vein by mosquito hemostatic forceps placed along the median fissure (**Figure 3A**). Remove all of the liver parenchyma of the LML immediately above the forceps using micro-scissors.
   2. Place a piercing suture that penetrates the liver parenchyma immediately under the forceps surrounding the left median hepatic vein. Then, tie a square knot to ligate it before releasing the forceps.
   3. Place another two piercing sutures under the forceps to surround the rest of the open incision of the LML. Then, tie two square knots to close the rest of the incision. Release the mosquito hemostatic forceps to check for bleeding.

6. Resecting RML (**Figure 4**)
   1. Place mosquito hemostatic forceps to surround the base of the RML at a distance of 0.5 cm to the SHVC (**Figure 4B**). Then, cross-clamp the base using the mosquito hemostatic forceps to occlude the right median hepatic vein.
   2. Using micro-scissors, remove all of the liver parenchyma of the RML along the upper surface of the forceps. Be sure to work very close to the surface of the forceps, leaving a plain and thin stump of the ML.
   3. Place the first piercing suture penetrating the liver parenchyma under the forceps to surround the middle median hepatic vein. Then, tie a square knot to ligate the middle median hepatic vein. Place the second piercing suture to ligate the right median hepatic vein using the same method. Place another two piercing sutures close to the rest of the incision following the description in 6.5.2.

7. Trimming the infrahepatic vena cava (IHVC)
   1. Cut open the retroperitoneum to expose the IHVC. Use curved micro-forceps to free the IHVC and right renal vein from the surrounding tissue. Dissect and ligate the right adrenal vein, which drains into the IHVC from the rear.
   2. Perform a right nephrectomy after ligating the right renal vein and right renal artery to save enough vascular length to install the cuff onto the IHVC in a later step.

8. Installing the biliary stent
   1. Mobilize the common bile duct (CBD) from the first hepatic hilum using micro-forceps. Ligate the CBD at the cross-point of the pyloric vein and CBD using a 6-0 silk suture.
   2. Cut a transverse incision on the anterior wall of the CBD on the proximal side of the ligation. Insert a biliary stent, made of a 22-gauge intravenous catheter, into the lumen of the CBD via the incision. Secure the biliary stent with a circumferential 6-0 silk suture. Transect the CBD between the two 6-0 silk sutures.

9. Trimming the PV
   1. Ligate the pyloric vein using a 6-0 silk suture distal to the PV. Next, ligate the pyloric vein using a 7-0 polypropylene suture close to the PV. Transect the pyloric vein between these two ligations. Double ligate and divide the splenic vein in the same way.
      NOTE: Use a 7-0 polypropylene suture to ligate the vessel to make a smaller ligation knot compared with that of the 6-0 silk suture. A large knot may interfere with the cuff installation in the last step. The vascular wall of the pyloric vein and splenic vein is thin, so gentle manipulations are essential during the dissection. A previously published article with more technical descriptions and illustrations about dissecting and dividing the pyloric vein and splenic vein should be read before operation[17].

10. Trimming the common hepatic artery (CHA)
    1. Carefully free the CHA and its two bifurcations, the PHA and the gastroduodenal artery (GDA), from the surrounding tissue using two micro-forceps. Double ligate the GDA using 6-0 silk sutures at the bifurcation of the CHA to GDA.

11. Heparinizing and perfusing the liver graft
    1. Inject 50 IU of heparin in 1 mL of saline via the penile vein. Wait for 5 min to achieve systemic heparinization.
    2. Cross-clamp the PV, IHVC and CHA using three curved micro-serrefines to occlude hepatic blood flow. Perform a small incision on the anterior wall of the PV to insert a 22-gauge catheter. Perfuse the partial liver graft with saline at 4 °C via the catheter under a pressure of 20 cmH_2O.
    3. Rapidly cut open the thoracic cavity and intrathoracic vena cava using surgical scissors to drain out the blood and perfusate. Do not stop the perfusion until the color of the entire partial liver graft becomes uniformly yellow.

12. Explanting the partial liver graft

1. Transect the left diaphragmatic vein between the two ligations (described in 6.2.2) using micro-scissors.
2. Next, transect the SHVC close to the diaphragm to keep the vascular length as long as possible. Transect the PV at the level of the splenic vein using micro-scissors. Divide the GDA between the double ligations, and transect the CHA close to the celiac trunk.
3. Transect the adrenal vessels on the distal side of the 6-0 silk ligation placed in 6.7.2. Transect the IHVC at the level of the left renal vein using surgical scissors.
4. Excise and transfer the partial liver to a back-table dish (30 mm culture dish) filled with saline at 4 °C.

7. Back-Table Operation (Graft Preparation)

1. Installing the vascular cuffs (**Figure 5**)
    1. Pull the PV through the lumen of a cuff made with a 12-gauge intravenous catheter using micro-forceps. Temporarily fix the cuff handle and PV together using a vessel clamp. Evert the PV vessel wall to cover the outside surface of the cuff. Secure the vessel wall and cuff in position with a circumferential 6-0 silk suture.
    NOTE: Do not twist the vessel. The cuff handle should be kept in the 12 o'clock direction.
    2. Install the IHVC cuff following the descriptions in 7.1.1.

2. Presetting the two stay sutures onto the SHVC
    1. Penetrate the vessel wall of the SHVC from outside to inside using two 7-0 polypropylene sutures along the 3 o'clock and 9 o'clock directions, respectively.
    NOTE: Do not tie a knot.

3. Preserving the graft
    1. Immerse the partial liver graft in saline at 4 °C for preservation after the back-table operation.

8. Recipient Operation

1. Repeat the procedures in 6.1-6.2 and 6.7, except for the right nephrectomy, in the recipient to expose the operative field, transect the ligaments and trim the vessels.
2. Preparing the IHVC, bile duct, hepatic artery and portal vein in the recipient
    1. First, mobilize the section of the IHVC down to the right renal vein. Second, ligate the right adrenal vessels using a 6-0 silk suture. Third, mobilize the CBD from the first hepatic hilum using micro-forceps.
    NOTE: Do not trim off the fatty tissue around the CBD.
    2. Transect the CBD at the first bifurcation in the hepatic hilum after double ligation using a 6-0 silk suture. Then, transect the PHA using micro-scissors subsequent to the double ligation using a 6-0 silk suture. Mobilize the section of the PV from the hepatic hilum to the pyloric vein.

3. Mobilizing the rear of the liver
    1. Retract the recipient liver to the left side using two cotton sticks. Then, cut off the ligament in the rear of the liver from the SHVC to the right adrenal vein as described in a previous visualized article[18]. After this, introduce a rubber band through the back space of the SHVC.

4. Preparing the instruments and materials during the anhepatic phase
    1. Recheck the availability of all of the instruments and materials required in the anhepatic phase, such as a 5 mL syringe with a curved needle, micro-scissors, micro-needle holder, micro-forceps, two micro-hemostatic forceps, two 7-0 polypropylene sutures and 6-0 silk suture material.
    NOTE: Searching for materials or instruments may prolong the anhepatic phase.

5. Excising the liver in the recipient
    1. Cross-clamp the IHVC immediately above the renal vein using a vessel clamp and the PV immediately above the pyloric vein. Record the duration of the anhepatic phase from this moment until the clamp on the PV is released.
    2. Reduce the concentration of isoflurane to 0.5% immediately.
    3. Pull the SHVC and diaphragm down by the preset rubber band. Clamp the parts of the diaphragm together with the intrathoracic vena cava using a Bulldog clamp, maintaining a distance of 0.5 cm to the SHVC.
    NOTE: Do not clamp the lung of the rat.
    4. Using micro-scissors, cut the vessel wall of the SHVC carefully along the upper edge of the liver parenchyma to keep the vascular wall as long as possible.
    5. Transect the PV at the first bifurcation in the hepatic hilum using micro-scissors. Then, transect the inferior vena cava in the parenchyma of the right inferior lobe, rather than in the vessel wall, leaving the circumferential liver parenchyma around the vena cava. Keep the cutting edge in the right inferior lobe at a distance of 4 mm to the IHVC. After transecting the vessels, move the whole liver out of the abdominal cavity.

6. Reconstructing the SHVC
    1. Place the partial liver graft in the abdominal cavity orthotopically. Pierce the vessel wall of the SHVC from inside to outside along the 3 o'clock and 9 o'clock directions using two preset stay sutures. Pull the two stay sutures to the right and left after they are tied into a knot with their own ends.
    2. Pierce the SHVC vessel wall from outside to inside close to the knot in the 3 o'clock direction to introduce the needle of the right stay suture into the vascular lumen.

3. Anastomose the posterior wall of the SHVC by a running suture inside the vascular lumen from the 3 o'clock direction to the 9 o'clock direction.
4. At the corner of the 9 o'clock direction, pierce the vessel wall from inside to outside close to the knot to introduce the needle out of the vascular lumen.
5. Use this suture to anastomose the anterior wall using a running suture outside of the vascular lumen from the 9 o'clock direction to 3 the o'clock direction. Finally, tie the suture with its own end 3 times (**Figure 6**).
   NOTE: Inject 3-5 mL of saline into the lumen of the reconstructed SHVC to force out air bubbles before the last 2-3 stitches. A more detailed description of SHVC anastomosis can be found in the article by Delriviere *et al.*[19].

7. Reconstructing the PV
   1. Place two 7-0 polypropylene stay sutures at the end of the PV of the recipient following the description in 7.2. Pull two stay sutures in the opposite direction to extend the vessel wall. Fix two stay sutures using two micro-mosquito hemostatic forceps.
   2. Inject approximately 1 mL of saline into the vascular lumen of the PV in the recipient to force out air bubbles. Quickly insert the portal vein cuff into the vascular lumen of the PV in the recipient to force when it is opened using micro-forceps. Secure the cuff anastomosis using a circumferential 6-0 silk suture. Cross-clamp the CHA.

8. Reperfusion to liver graft
   1. Release the clamps on the SHVC and PV in sequence to reperfuse the liver graft. Record the time of the anhepatic phase. Check for bleeding of the anastomosis. Increase the concentration of isoflurane to 1-2% according to the reaction of the rat to the pain stimulation.

9. Reconstructing the IHVC
   1. Inject 1 mL of saline into the vascular lumen of the IHVC in the recipient to force out the air bubbles. Insert the cuff into the cylindrical vascular lumen of the IHVC, which is supported by the circumferential liver parenchyma (**Figure 7**). Introduce a 6-0 silk suture through the back space of the IHVC. Quickly secure the cuff anastomosis using a 6-0 silk suture.
   2. Release the two vessel clamps in the IHVC to restart the perfusion. Trim off the surrounding liver parenchyma carefully above the circumferential silk suture using micro-scissors.

10. Reconstructing the hepatic artery
    1. Penetrate the vessel wall of the CHA in the liver graft from outside to inside using two 11-0 polypropylene sutures in the 3 o'clock and 9 o'clock directions, respectively. Occlude the blood flow of the CHA and GDA in the recipient using two curved micro-serrefines.
    2. Transect the PHA of the recipient at its root to expose the vascular lumen using micro-scissors. Enlarge the vascular lumen of the PHA in the recipient by cutting part of the vessel wall longitudinally to accommodate the diameter of the CHA in the liver graft.
    3. Anastomose the CHA of the liver graft to the enlarged PHA in an end-to-side manner using the running suture technique described for the SHVC anastomosis (8.6.2-8.6.7).
       Note: Detailed illustrations can be found in the article by Huang et al[14].

11. Reconstructing the CBD
    1. Move the intestine back into the abdominal cavity to reduce the distance between the two ends of the bile duct. Perform a transverse incision on the anterior wall of the bile duct in the recipient.
    2. Pull the biliary stent in the partial liver graft down to insert it into the lumen of the bile duct in the recipient via the transverse incision. Secure the stent using a 6-0 circumferential silk suture.
    3. Tie the two 6-0 circumferential silk sutures in the bile duct to each other to reduce the tension of the anastomosis.

12. Closing the abdomen
    1. Close the abdomen in two layers using 3-0 silk suture in the running suture pattern.

9. Postoperative Treatments (Analgesia and Antibiotics)

1. Treat all recipients with 0.1 mg/kg buprenorphine subcutaneously. Administer cefuroxime (16 mg/kg) subcutaneously in addition to the analgesic drug. Offer tap water and standard laboratory animal chow to the rats *ad libitum*.

Representative Results

In total, 31 cases of syngeneic arterialized rat PLTx were completed using this protocol. All of the recipients survived until the end of the observation time. The body weight of the recipients began to recover after postoperative day (POD) 4. The slope of the body weight was close to that of a normal Lewis rat after POD 6 (**Figure 8**), which indirectly implied the recovery of the recipient. Histologically, a slight proliferation of the bile duct was observed in the recipients, and the liver lobular architecture of the recipients was intact. No necrosis or obvious sinusoidal dilatation was observed (**Figure 9**). The serum level of alanine aminotransferase (ALT) was within the normal range at POD 30 (**Figure 10**). No cases of yellow urine caused by jaundice were observed in these recipients, which was indicated by checking the cage bedding daily, indicating the patency of the biliary stent. The normal serum level of bilirubin on POD 30 further confirmed the successful reconstruction of the bile duct (**Figure 11**). The patency of the hepatic artery was checked by transecting the PHA on the proximal side of the anastomosis during sacrifice. Bleeding from the PHA was observed in all of the recipients, implying the patency of the anastomosis. All of these results suggested that the liver grafts functioned well.

The average duration of the anhepatic phase was 23.03 ± 2.3 min. The cuff anastomosis of the PV took 4.68 ± 0.77 min, while the cuff anastomosis of the IHVC only took 2.05 ± 0.71 min. Compared with the cuff anastomosis of the PV, the cuff anastomosis of the IHVC saved the step of placing stay sutures and thereby further shortened the anastomosis time (**Table 1**).

Two cases of bleeding in the remnant stump of the ML during the 70% hepatectomy were observed among the 31 cases of vessel-oriented hepatectomy. In a previous experiment, bleeding in the remnant stump of the ML was observed in 8 out of 18 cases of parenchyma-preserving vessel-oriented hepatectomy (**Figure 12**). This result indicated a lower rate of bleeding in the remnant liver stump of the ML after using vessel-oriented 70% hepatectomy.

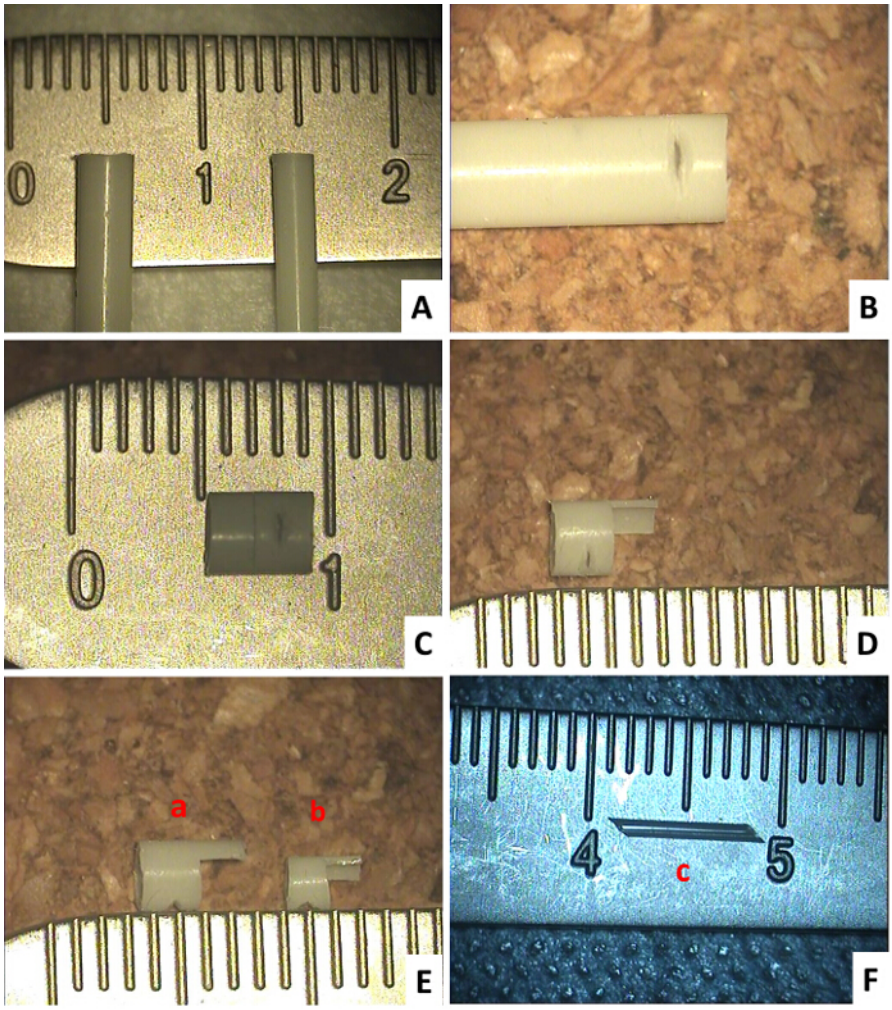


Figure 1. The cuffs and biliary stent.
(A,C) The size of cuffs. (B) The fillister on the cuff. (D) The body part and handle part of the cuff. (E) (a) IHVC cuff; (b) PV cuff. (F) Biliary stent. Scale bar = 5 mm. Please click here to view a larger version of this figure.

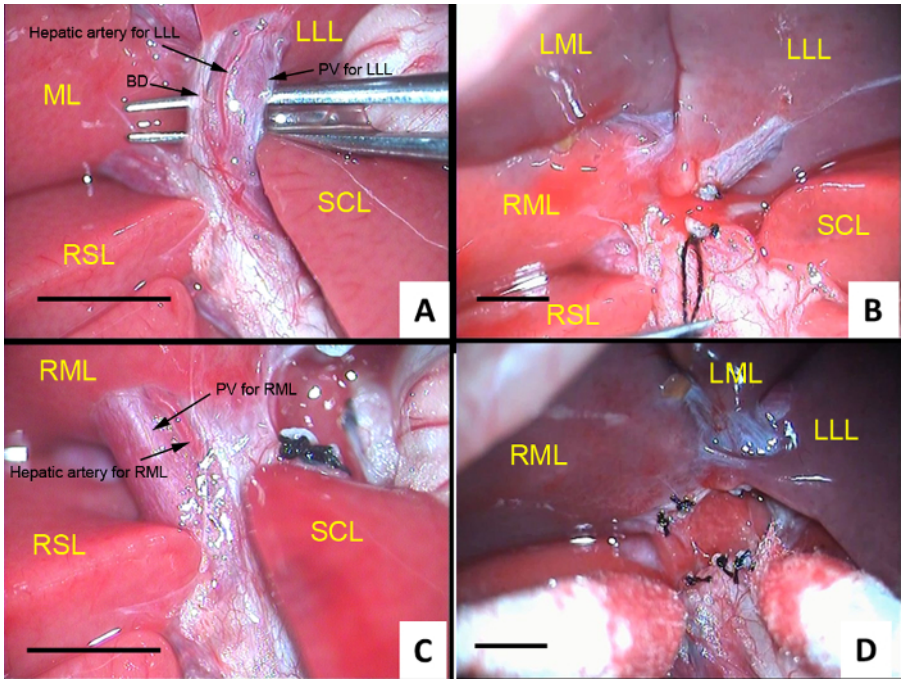


Figure 2. To occlude the blood supply of LLL and ML.
(A)The common trunk of the PV, hepatic artery and bile duct of the LLL and LML.
(B)The discoloration of the LLL and LML after transecting the common trunk.
(C)The portal vein of the RML.
(D)All PVs, hepatic arteries and bile ducts of the LLL and the ML were transected.
Scale bar = 5 mm. Abbreviations: LLL left lateral lobe, ML median lobe, LML left median lobe, RML right median lobe, RSL right superior lobe, RIL right inferior lobe, SCL superior caudate lobe. Please click here to view a larger version of this figure.

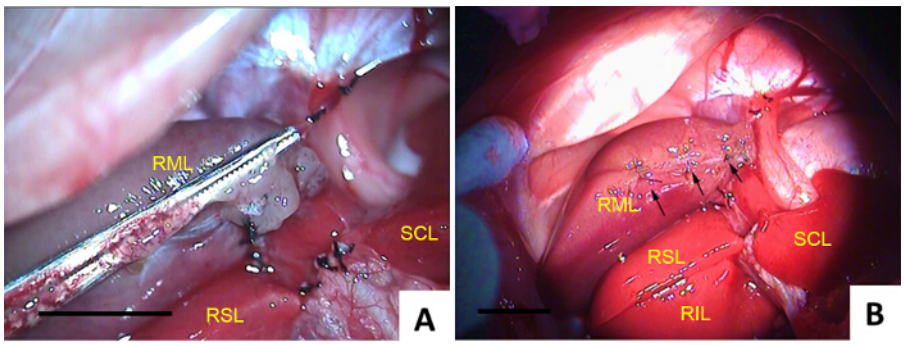


Figure 3. To resect the left median lobe.
(A) Resect the liver mass of the LML immediately above the forceps.
(B) Place three piercing sutures on the incision. (↖)
Scale bar = 5 mm. Abbreviations: RML right median lobe, RSL right superior lobe, RIL right inferior lobe, SCL superior caudate lobe. Please click here to view a larger version of this figure.

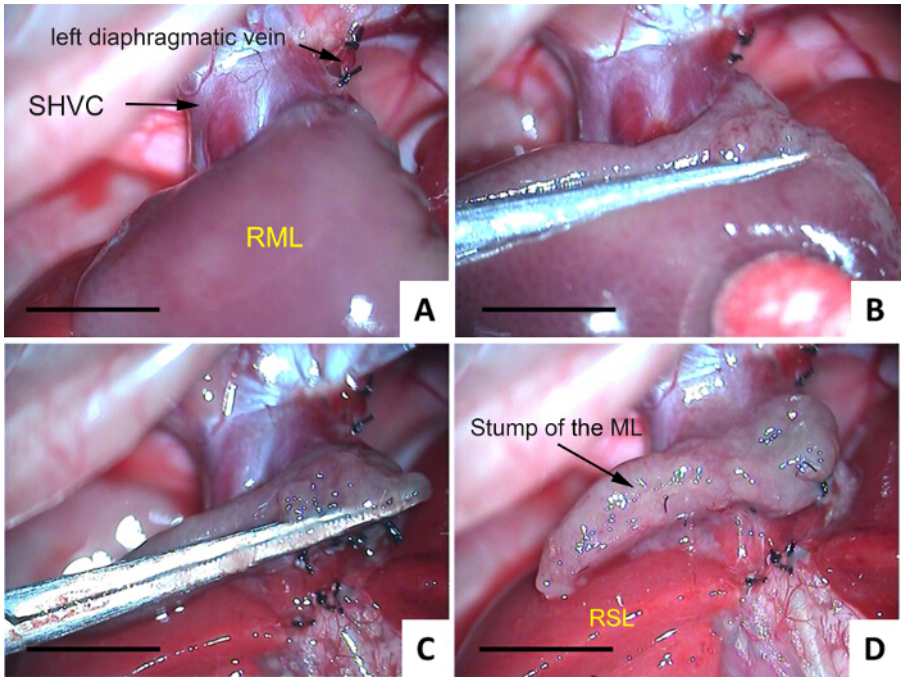


Figure 4. To resect the right median lobe (RML).
(A)The base of the RML.
(B)Place mosquito hemostasis forceps around the base of RML.
(C)Resect the liver parenchyma of RML immediately above the forceps.
(D)Leave a plain and thin layer of the remnant liver tissue on the SHVC.
Scale bar = 5 mm. Abbreviations: ML median lobe, RSL right superior lobe. Please click here to view a larger version of this figure.

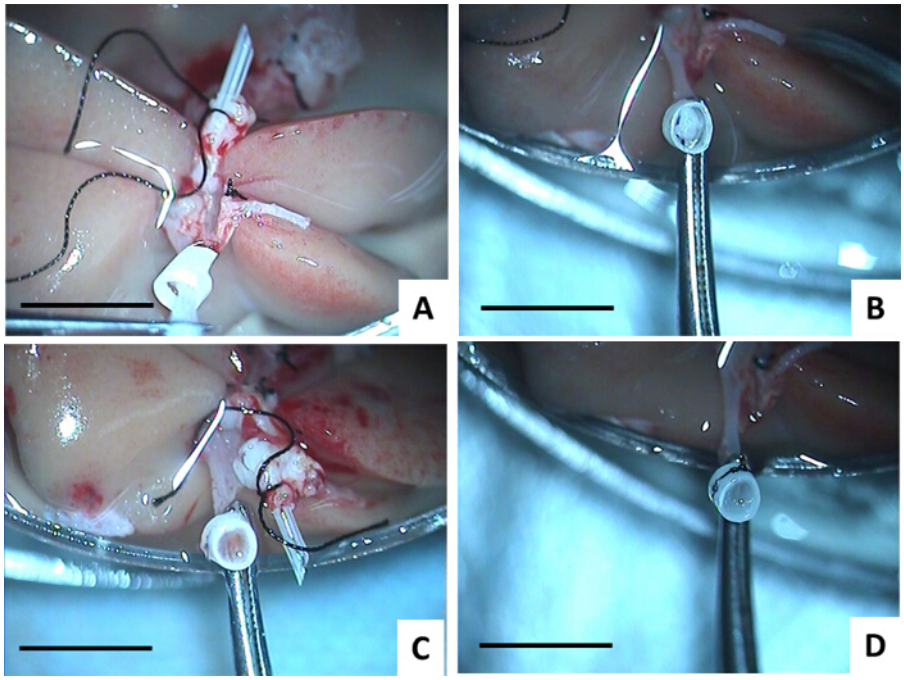


Figure 5. To install the cuff.
(A) Pull the PV through the lumen of a cuff.
(B) Fix the cuff handle and PV together by a vessel clamp.
(C) Evert the PV vascular wall to cover the outside surface of the cuff.
(D) Secure the vascular wall and the cuff in position.
Scale bar = 5 mm. Please click here to view a larger version of this figure.

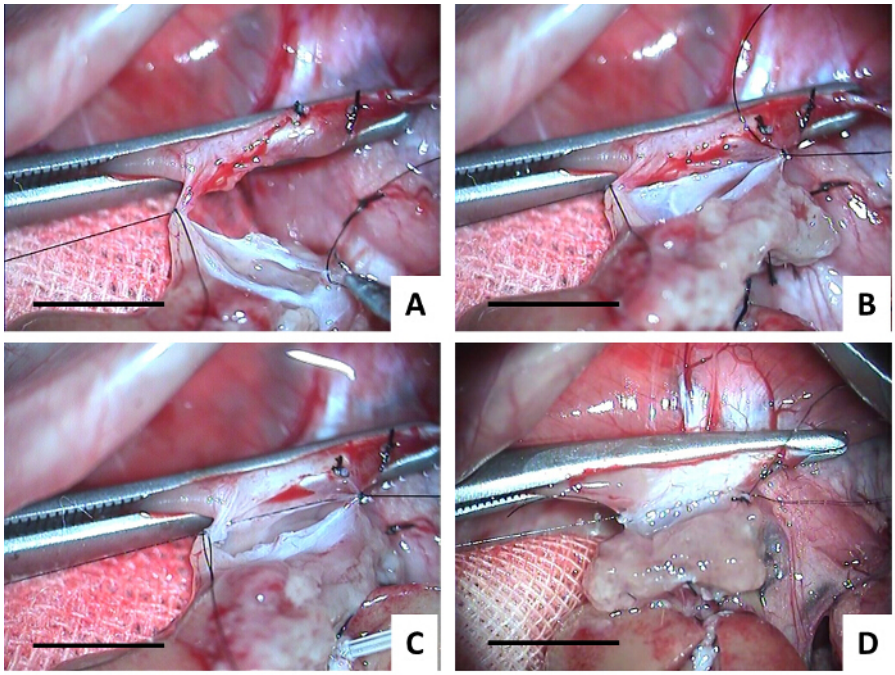


Figure 6. To reconstruct the SHVC.
(A&B) Pull two stay sutures to the right and left, respectively.
(C) Anastomose the posterior wall of the SHVC by a running suture in the vascular lumen.
(D) Anastomose the anterior wall of the SHVC out of the vascular lumen.
Scale bar = 5 mm. Please click here to view a larger version of this figure.

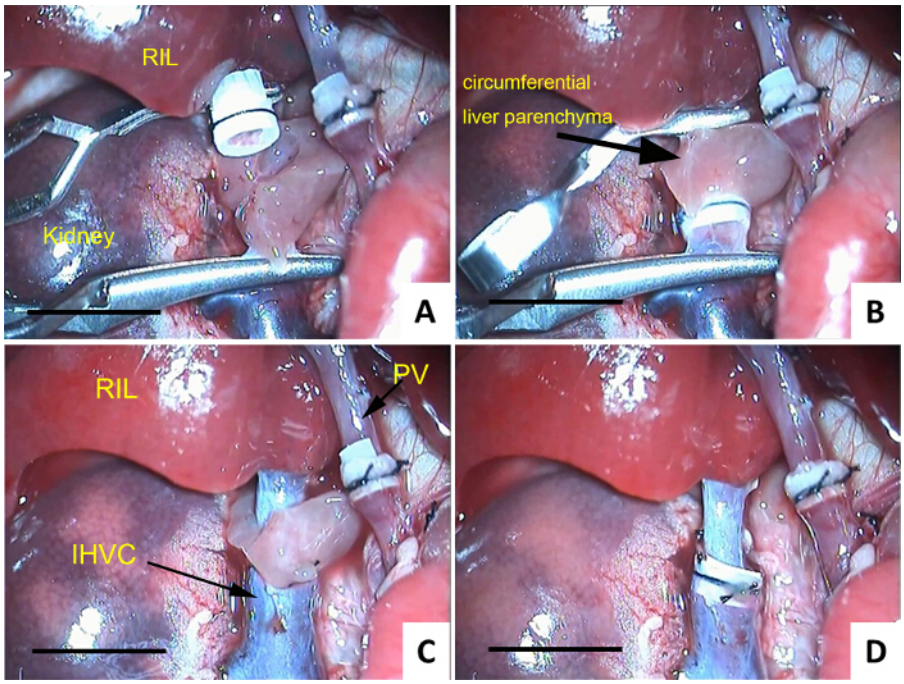


Figure 7. To reconstruct the IHVC.
(A) A cylindrical vascular lumen of the IHVC in the recipient.
(B) Secure the cuff anastomosis of the IHVC.
(C) Start the reperfusion.
(D) Trim off the surrounding liver parenchyma above the circumferential silk suture.
Scale bar = 5 mm. Abbreviations: PV portal vein, IHVC inferior infrahepatic vena cava, RIL right inferior lobe. Please click here to view a larger version of this figure.

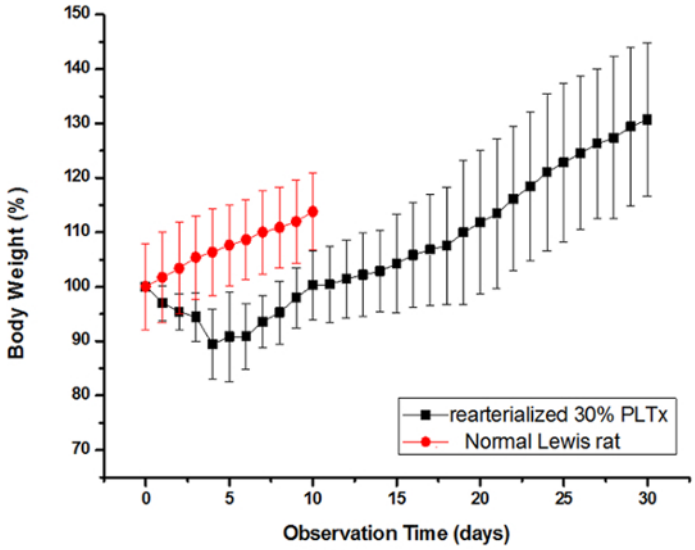


Figure 8. Postoperative body weight recovery.
The body weight of recipients began to recover after postoperative day 4. The slope of the body weight was close to that of a normal Lewis rat after postoperative day 6, which indirectly implied the recovery of the recipient.

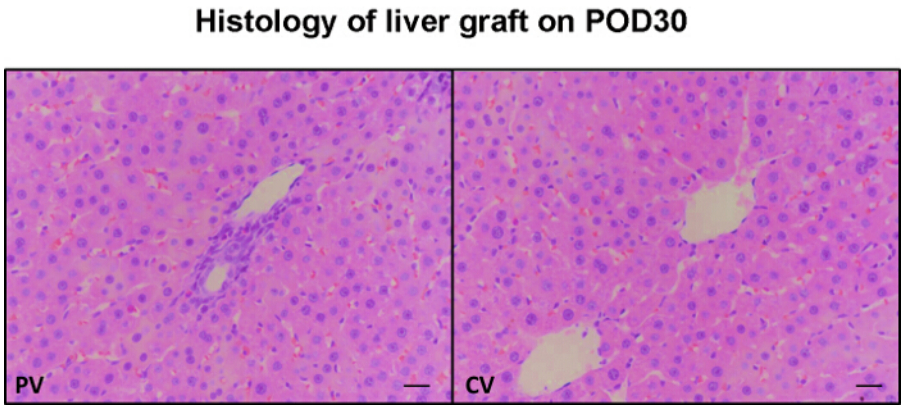


Figure 9. Histology of liver graft after 30 days.
In histology, a slight proliferation of the bile duct was observed in recipients, and the liver lobular architecture of recipients was intact. No necrosis or obvious sinusoidal dilatation was observed (400X). Scale bar = 20 um Please click here to view a larger version of this figure.

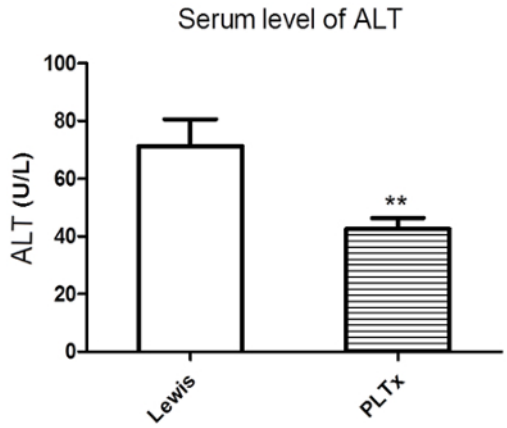


Figure 10. Serum level of ALT.
The serum level of ALT was within the normal range on POD 30. (**, P<0.05).

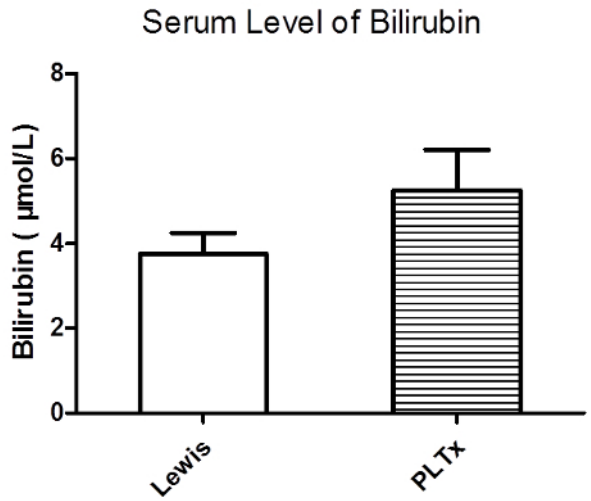


Figure 11. Serum Level of bilirubin.
The serum level of bilirubin on POD 30 was comparable to that of a normal Lewis rat (P>0.05).

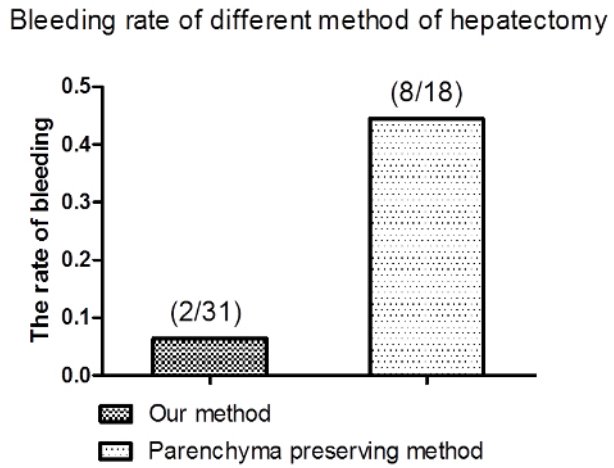


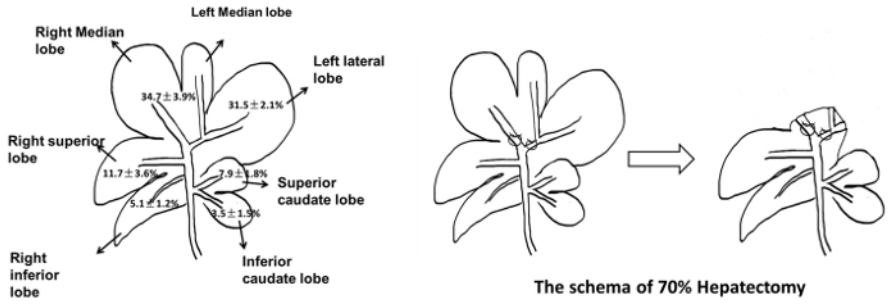
Figure 12. Bleeding rates of different methods of hepatectomy.
A lower rate of bleeding in the remnant liver stump of the ML was observed in the method using the vessel-oriented 70% hepatectomy.

Procedures	Removal of liver	SHVC anastomosis	PV anastomosis	IHVC anastomosis	Anhepatic time
Time (minutes)	2.62±0.46	15.73±2.05	4.68±0.77	2.05±0.71	23.03±2.30
Data were expressed in Mean±SD					

Table 1. Time of individual surgical procedures during anhepatic phase

Authors	Year	Laparotomy incision	Heparin used in donor	Perfusate	Hepatectomy	Graft Weight to recipient liver weight	IHVC anastomosis	Hepatic artery anastomosis	Sequence of vascular anastomosis	Relevant clinical operation
Nagai et al.	2013	Midline incision	500IU/2mL saline	HTK solution	Hepatecomy ex vivo using a simple ligation	About 50%	End-to-end anastomosis by Hand suture	Stent technique	PV→Hepatic artery→IHVC	Split liver transplantation
Chen et al. our protocol	2017	Transverse incision	50IU/1mL saline	Normal saline	Hepatectomy in vivo using vessel-oriented technique	About 30%	Modified cuff anastomosis	End-to-side anastomosis by hand suture	PV→IHVC→Hepatic artery	Living donor liver transplantation

Table 2. Difference between two visualized protocols of the rat PLTx. Please click here to view a larger version of this table.



Supplementary Materials

Discussion

Rat PLTx is a sophisticated microsurgical procedure with a training program that is high in cost and long in duration[20]. The complexity of the rat PLTx protocol has prevented researchers from using this animal model. Compared with full-size rat LTx, rat PLTx presents the microsurgeon not only with the challenge of a transplantation procedure but also with the challenge of liver resection in a small animal. Therefore, a visualized microsurgical protocol describing the whole procedure in detail is essential for establishing this complex model. A previously visualized article reported by Nagai *et al.* presented a protocol of 50% partial orthotopic LTx with hepatic arterial reconstruction in rats[5] (**Table 2**). This protocol used an *ex vivo* hepatectomy, which is clinically similar to the procedure of split LTx.

Compared with split LTx, living donor LTx resects the partial liver graft from the living donor rather than splitting the liver in the back-table operation. The *in vivo* liver resection may lead to more surgical manipulations, longer portal hypertension, and even more warm ischemia injuries to the partial liver graft. Hence, several rat PLTx models utilizing *in vivo* hepatectomy have been established in relevant studies[6,8,21,22]. Many protocols involve resection of the liver lobes after simple ligation at the pedicle or base[2,3,6,8,21,23]. However, different liver lobes are of different shapes. The ML of the rat has a wide base that semi-surrounds the vena cava[10,24]. Placing a simple ligation at the base of the ML easily constricts the inferior vena. To avoid the constriction of the vena cava, Madrahimov *et al.* developed a parenchyma-preserving vessel-oriented hepatectomy to remove the ML in 2 steps after four piercing sutures in the parenchyma were made, leaving the remnant stump of the ML flat[10]. However, the PVs of the liver lobes ligated by the piercing sutures were hidden in the parenchyma. Bleeding in the remnant stump caused by missing or injuring the small branches of the PV could not be completely avoided [11]. In our protocol, the corresponding PVs and hepatic arteries were further dissected first. Then, the blood supply of the resected liver lobes was individually occluded by ligations before the resection. Therefore, the rate of bleeding in the remnant stump was lowered using our protocol (**Figure 10**). The technique of placing four piercing sutures in the stump of the ML was also employed in our protocol to ligate the hepatic veins but not the PV and hepatic arteries. With these ligations using multiple piercing sutures and a step-wise resection, the flat shape of the remnant liver stump was maintained, which avoided the constriction of the vena cava.

For the IHVC reconstruction, a slight modification was made to the cuff anastomosis described by Kamada *et al.*[25]. The inferior vena cava of the recipient was transected in the parenchyma of the right inferior lobe, rather than on the IHVC vessel, which left a circumferential liver parenchyma around the vena cava. The liver parenchyma around the vena cava helped maintain a cylindrical shape of the vascular end of the IHVC of the recipient rather than a flat shape. Hence, the lumen of the IHVC was naturally opened without the help of stay sutures and forceps. This modification helped save the step of placing stay sutures and facilitated the insertion of the IHVC cuff into the vascular lumen of the recipient. Moreover, this modification prolonged the vascular length of the IHVC, thereby reducing the tension between the two ends during the cuff anastomosis.

With the advantage of fast vascular anastomosis, the two-cuff technique is frequently used in the rat orthotopic LTx for anastomosing the PV and IHVC. Miyata *et al.* even proposed a protocol involving a three-cuff technique to anastomose the PV, IHVC and SHVC. However, the cuff technique also has its disadvantages. First, a cuff with a fixed size cannot perfectly fit the size of the corresponding vessel. In addition, the cuff technique disturbs blood flow, resulting in a higher incidence of thrombosis and foreign body reaction to the cuff[26]. The SHVC, a main vessel in the rat, shows high blood flow. Any constriction or thrombosis in this vessel may cause severe postoperative complications. Therefore, in our PLTx protocol, we choose to use the hand suture technique to reconstruct the SHVC, which allows for the adjustment of the anastomosis as close as possible to the physical setting. However, high-quality SHVC anastomosis completed by the hand suture technique requires high microsurgical skill. For beginners, Carrel's triangulation technique is recommended for anastomosing vessels [27]. By using this technique, three stay sutures are placed at regular intervals around the circumference of the SHVC. The 3D shape of the SHVC is maintained by retracting these three stay sutures along three directions, which can prevent the SHVC from stenosis during anastomosis.

Re-arterialization of the liver graft is a controversial topic. In our opinion, re-arterialization is not the key step for ensuring a high survival rate of the recipient, but it might be the key step for ensuring a high-quality liver graft. In the traditional protocol of the rat LTx involving the two-cuff technique, the hepatic artery was not reconstructed, as the lack of perfusion of the hepatic artery did not affect the outcome of the rat LTx[12]. Moreover, a previous study on mouse LTx was conducted to investigate the effect of re-arterialization on long-term graft survival, histological alterations, ischemic liver damage and early immunologic activation pathways[13]. The researchers concluded that the re-arterialization of liver grafts did not have a major effect on the survival rate or the degree of immunologic activation. However, a recent study reported by Huang *et al.* suggests that re-arterialization is important for the recovery of liver parenchyma subjected to hepatectomy, especially with the obstruction of outflow[28]. Non-arterialization of the partial liver graft aggravated liver damage and delayed the recovery from focal necrosis. Therefore, re-arterialization of the partial liver graft is recommended. As illustrated here, an end-to-side anastomosis technique was used to reconstruct the hepatic artery, which was described previously by one of our authors [14]. The advantage of this technique is the choice to anastomose two thick

arteries, the CHA and GDA, rather than the thin PHA, which reduces the difficulty of reconstructing the hepatic artery and maintains a high rate of patency of anastomosis.

The classical rat LTx protocol proposed by Kamada *et al.* suggests controlling the anhepatic phase such that it is less than 26 min, as no animal survived when the anhepatic phase lasted longer than 26 min[25]. Our experience is consistent with this suggestion, although we previously observed several survival cases with an anhepatic phase time longer than 26 min. In our opinion, a stable anhepatic phase takes priority over a shorter anhepatic phase in surgical research. In our group, the anhepatic phase is precisely controlled to within a specific time. The anhepatic phase is not stopped until the preset anhepatic time is reached, although the anastomosis of the SHVC and PV could be completed much earlier. However, in a training project, a shorter anhepatic phase is encouraged.

Although many improvements have been made to shorten the training phase and eliminate unnecessary operations, rat LTx and PLTx are still complex microsurgical operations[20]. Therefore, in addition to a visualized protocol, a step-wise training protocol based on the Plan-Do-Check-Action cycle is important for achieving a high survival rate and high operation quality. Our preliminary experience in using this training protocol showed that 40-73 operations were required to master the rat orthotopic LTx. An additional 9-13 operations were required to master the rat re-arterialized PLTx. The training protocol proposed by Czigány *et al.* claims that 50-60 LTxs are sufficient to master the orthotopic LTx and the PLTx[29].

In our Plan-Do-Check-Action training protocol, quality control at each training step and in each individual manipulation must be emphasized, especially in such a complex model. A high survival rate is not tantamount to a high-quality procedure. In the representative results, several criteria for evaluating the quality of the microsurgery were listed, including histology, liver enzyme, body weight recovery, jaundice (serum bilirubin and yellow urine), duration of the anhepatic phase, and patency of the hepatic artery. High quality is achieved only when all of the criteria are met.

Disclosures

All authors have no conflicting interests to disclose.

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