Video Article

Orthotopic Liver Transplantation in Rats

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Abstract

Clinical progress in the field of liver transplantation has been largely supported by animal models[1,2]. Since the publication of the first orthotopic rat liver transplantation in 1979 by Kamada *et al.*[3], this model has remained the gold standard despite various proposed alternative techniques[4]. Nevertheless, its broader use is limited by its steep learning curve[5].

In this video paper, we show a simple and easy-to-establish revision of Kamada's two-cuff technique. The suprahepatic vena cava anastomosis is performed manually with a running suture, and the vena porta and infrahepatic vena cava anastomoses are performed utilizing a quick-linker cuff system[6]. Manufacturing the quick-linker kit is shown in a separate video paper.

Video Link

The video component of this article can be found at https://www.jove.com/video/4143/

Protocol

1. Donor Operation

1. Anesthetize male rats weighing 200±20 grams with isoflurane on cone mask (3% for the induction, 2% during the operation, 1 l/min air flow, FiO2 70%).
2. Perform a large median and transverse laparotomy and cauterize the epigastric vessels on both sides.
3. Cut the falciform ligament, and separate the left diaphragmatic vein from the suprehepatic vena cava. Finally, divide the diaphragmatic vein between two 7-0 silk ligatures.
4. Cut the left triangular and the gastro-hepatic ligaments.
5. Divide the hepato-esophageal ligament and artery between 7-0 ligatures (coagulation is a valid alternative).
6. Isolate the infrahepatic vena cava down to the left renal vein. Separate the right renal vein from the surrounding tissues and divide it between a 10-0 and a 7-0 ligature, on its proximal and distal ends, respectively.
7. Divide the right suprarenal vein between 7-0 ligatures and free the liver from its posterior ligaments by cutting under gentle traction.
8. Isolate the gastrosplenic vein and divide it between a 10-0 and a 7-0 ligature, on its proximal and distal ends, respectively.
9. Divide the proper hepatic artery between 7-0 ties.
10. Isolate the duodeno-pancreatic vein and divided it between a 10-0 and a 7-0 ligature, on its proximal and distal end, respectively.
11. Insert a 22G, 3.5 mm stent into the common bile duct by practicing a small incision at 1 cm distance from the hepatic hilum. Secure the stent in position with a 7-0 ligature.
12. Inject 10 UI of heparin, diluted into 1 ml of normal saline, through the dorsal vein of the penis.
13. Cannulate the vena porta (as far as possible from the hilum) with a 21G needle and gently flush the liver with 20 ml of cold ringer lactate (or other preservation solutions). At the same time, cut the vena cava below the renal veins to allow an adequate outflow. Liver cold perfusion should last between one and two minutes.
14. Cut the vena porta below the duodeno-pancreatic vein.
15. Cut the common bile duct distally to the ligature around the stent.
16. Complete the incision of the infrahepatic vena cava.
17. Cut the suprahepatic vena cava skim to the diaphragm and remove the liver.
18. Place the liver into a basin filled with cold ringer lactate and lay the basin on an ice pad.

2. Graft Preparation

1. Insert the vena porta into its cuff, then evert the vessel around the edge to expose the intima. Secure the everted segment on the cuff with a 7-0 ligature.
2. Repeat the same operation on the infrahepatic vena cava.
3. Position a micro-clamp (4-6 mm length) on the proximal part of the infrahepatic vena cava. This operation avoids blood loss after portal reperfusion.
4. Overturn the liver and insert two prolene 8-0 sutures at the opposite lateral edges of the suprahepatic vena cava, from outside to inside.
5. Store the graft (immersed into ringer lactate solution) in the refrigerator at 0-4 °C.

3. Recipient Operation

1. Anesthetize male rats weighing 200±20 grams with isoflurane on cone mask (3% for the induction, 2% during the operation, 1 l/min air flow, FiO2 70%) and keep on a warm pad. Of note, recipient and donor weights should be matched (±40 grams).
2. Inject 10 ml of normal saline subcutaneously and 0.03 g of piperacillin/tazobactam intramuscular before laparotomy.
3. Perform a midline xipho-pubic laparotomy.
4. Reverse the xiphoid process with an auto-static forceps in order to better expose the liver.
5. Cut the anterior ligaments and divide the left diaphragmatic vein between two 7-0 ties.
6. Divide the hepato-esophageal ligament and artery between 7-0 ligatures (coagulation is a valid alternative). Not shown in the video (identical to donor operation).
7. Isolate the infrahepatic vena cava down to the right renal vein.
8. Divide the right suprarenal vein between 7-0 ligatures and free the liver from its posterior ligaments by cutting under gentle traction (not shown in the video (identical to donor operation)).
9. Place a 7-0 ligature around the common bile duct, just below its division. Put another ligature one millimeter below and cut in between.
10. Isolate the hepatic artery at the hilum and divide it between 7-0 ligatures.
11. Gently separate left and right portal branches. Pay attention not to damage the common hepatic artery or the gastro-splenic vein.
12. Position the caval ring around the vena cava, cranially to the right renal vein. Then secure the vessel wall to the ring by placing four cardinal 9-0 sutures.
13. Repeat the same operation with the portal ring on the vena porta.
14. Inject 10 UI of heparin, diluted into 1 ml of normal saline, through the dorsal vein of the penis.
15. Set the anesthesia on 0.25% and the FiO2 on 100%.
16. Clamp the infrahepatic vena cava just above the right renal vein, then clamp the vena porta just above the gastro-splenic vein. Finally clamp the suprahepatic vena cava being sure to include at least 2 mm of diaphragm.
17. Cut the suprahepatic vena cava close to the parenchyma.
18. Cut the left and the right portal branches and divide the septum in between.
19. Cut the infrahepatic vena cava close to the parenchyma, paying attention not to incise any of the sutures previously positioned. Remove the liver and place the graft into the abdominal cavity.
20. Perform an end-to-end anastomosis between the suprahepatic vena cava of the graft and the recipient. Start with the running suture of the posterior wall from left to right, then proceed on the anterior wall from right to left. Keep the anterior suture loose until the end in order to allow a generous flushing of the interior lumen before tightening the ends.
21. Insert the caudal branch of the approximator into the portal ring handle; then insert the vena porta of the graft into the slit of the cranial branch of the approximator, with the portal cuff finding its position into the slot.
22. Close the approximator while flushing the recipient's vena porta with normal saline and allow the cuff to insert into the vessel. Secure the recipient's vena porta around its cuff with a 7-0 circumferential tie.
23. Remove the approximator. Open the clamp on the suprahepatic vena cava and the vena porta in order: portal flow is now reestablished.
24. Remove the portal ring by cutting the 9-0 ligatures with extra-fine scissors.
25. Insert the caudal branch of the approximator into the caval ring handle; then insert the infrahepatic vena cava of the graft into the slit of the cranial branch of the approximator, with the caval cuff finding its position into the slot.
26. Close the approximator while flushing the recipient's infrahepatic vena cava with normal saline and allow the cuff to insert into the vessel. Secure the recipient's vena cava around its cuff with a 7-0 circumferential tie.
27. Remove the approximator. Open the microclamps on the infrahepatic vena cava of the graft and the recipient in order: venous flow is now reestablished.
28. Perform a partial incision of the recipient's common bile duct, apply a gentle traction towards the head, and insert the free side of the stent previously inserted into the bile duct of the graft. Secure the stent with a circumferential 7-0 tie.
29. Close the animal by layers with 5-0 sutures. Allow free water and food from wakening.
30. Administer effective analgesia according to local institutional protocols. A minimum duration of 24 h is recommended.

Of note: end-to-end hepatic artery anastomosis can be performed, when required by the experimental design (the artery is not required for long-term survival after rat liver transplantation).

4. Representative Results

The described technique could be successfully mastered by our team after approximately 10 training surgeries. It allowed 100% long-term (>21 days) survival on 36 consecutive Lewis-to-Lewis and Dark Agouti-to-Lewis transplantations, after the short training period (a number of syngeneic liver graft recipients have been kept alive for over 12 months now). Post- syngeneic transplant day 1 liver function tests showed median aspartate transaminase (AST): 84 U/l (51.7 - 92.7) and median alanine transaminase (ALT): 172.5 U/l (127.5 - 240.2). The mean

anhepatic phase (from vena porta clamping to graft re-perfusion) was 14 ±2 min, with the longest step being the suprahepatic vena cava anastomosis (running suture) time 9±2 min.

The quick-linker system allowed the positioning of 1.55 mm-bore cuffs on vena porta and 2.40 mm-bore cuffs on vena cava (recipients weighing 200 ±20 g). By contrast, when we tried transplanting using Kamada's technique, they could not exceed 1.40 mm and 2.16 mm, respectively (rats matching for strain and weight). As shown in **Figure 7**, quick-linker rings are designed to keep recipient's vessels on optimal stretching, allowing minimum caliper and length wasting, which results in closer-to-physiological hemodynamic results.

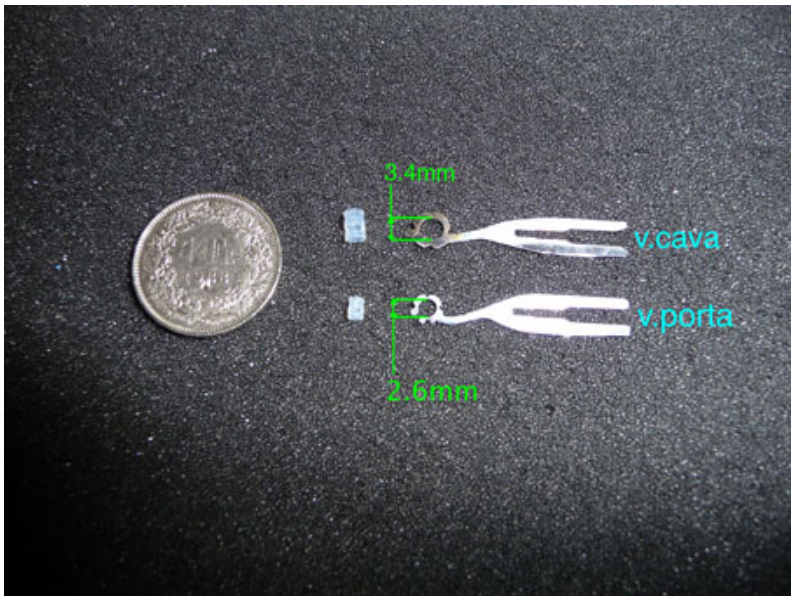

Figure 1. Rings measures.

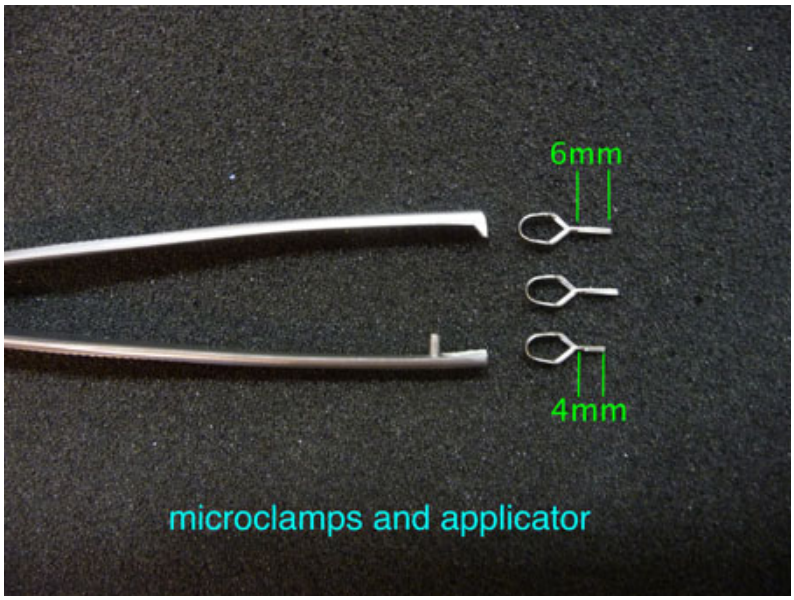

Figure 2. Microclamps measures.

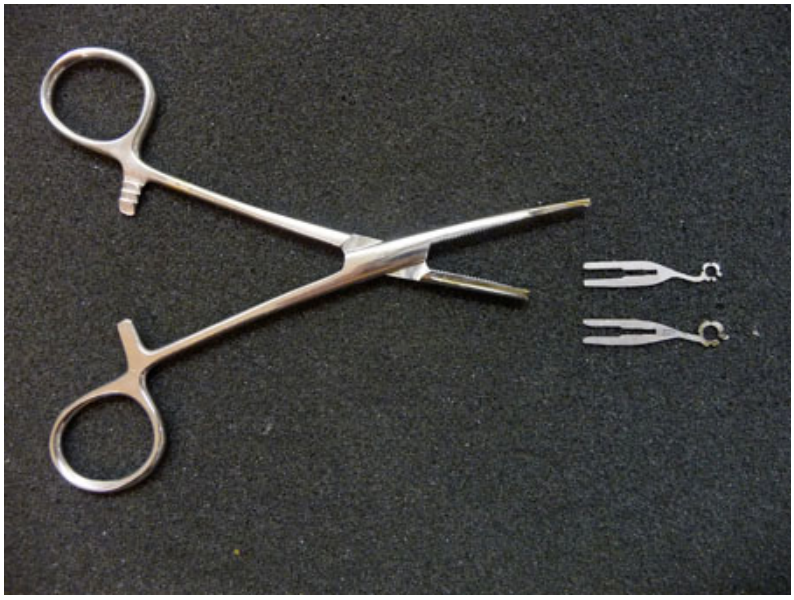

Figure 3. Quick-linker kit.

Figure 4. Quick-linker armed.

Figure 5. Quick-linker closed.

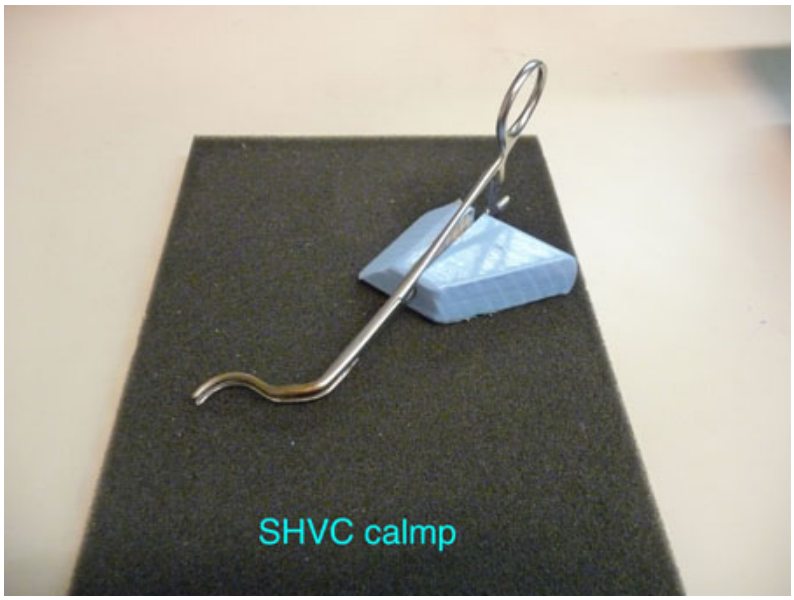

Figure 6. SHVC clamp.

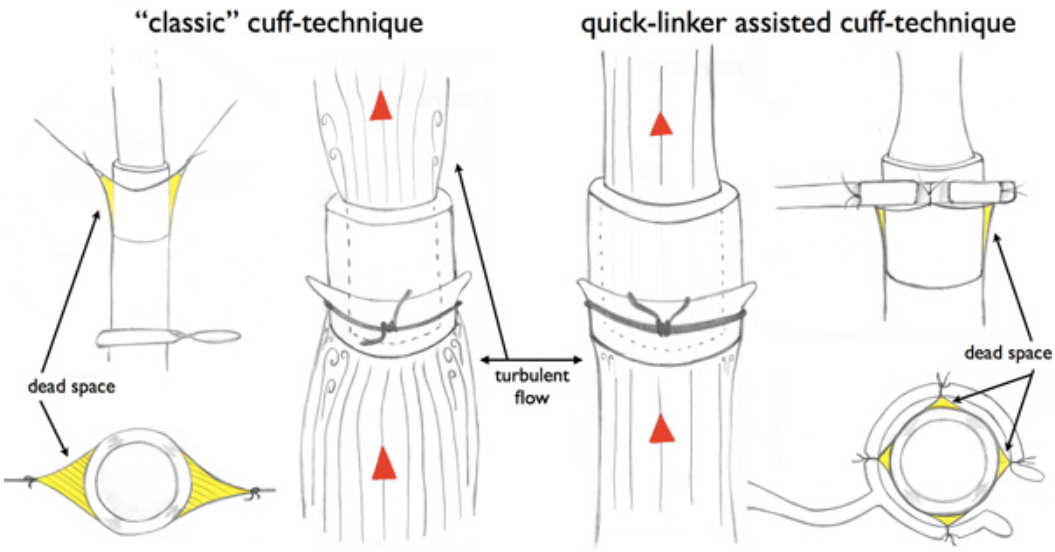

Figure 7. Classic vs. quick-linker assisted cuff-anastomosis hemodynamics.

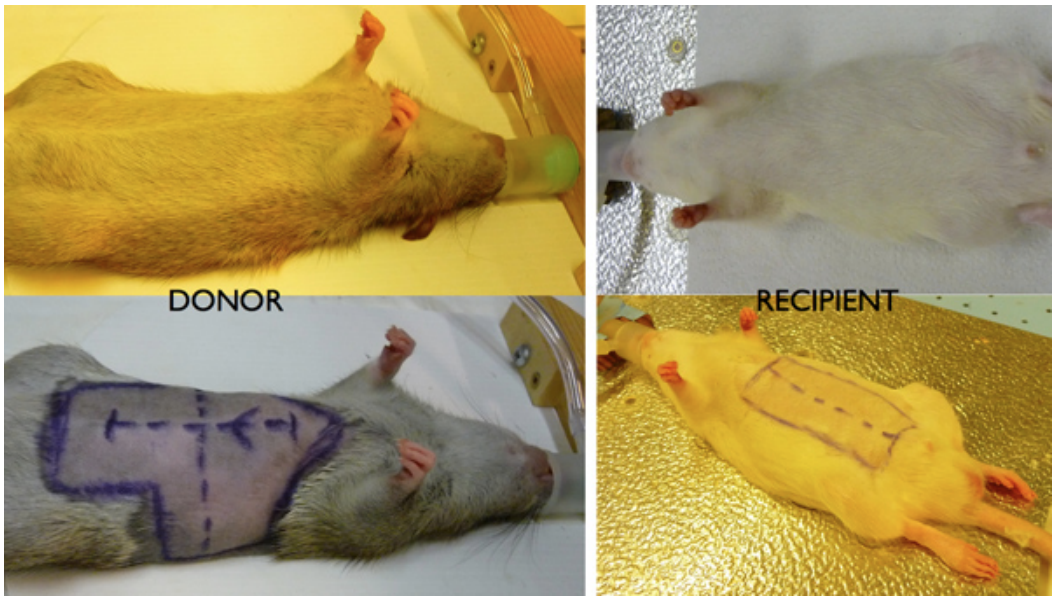

Figure 8. Donor and recipient's skin preparation and incision.

Discussion

The present rat liver transplantation model can be easily established. In our experience, a few key points help minimize the risk of death (≤20%). Early deaths, occurring within the first three hours from reperfusion, are most often due to a bowel infarction, as a consequence of a long portal clamping. It is therefore recommended to keep the anhepatic phase within the ideal limit of 15 minutes (20 minutes still acceptable). Central air embolism can also be a cause of sudden death and it is compulsory to flush all veins prior to anastomosis.

Deaths occurring between postoperative day one and three are often due to liver failure or thrombosis of the vena cava. One should therefore avoid any aggressive graft flushing before the explantation in the donor (we flush with 20 ml over approximately 60 seconds) and ensure an appropriate cold preservation (full immersion into 0-4 °C ringer lactate solution). The risk of thrombosis can be decreased by minimizing the manipulation of the intima and preserving its integrity. We do not recommend the use of anticoagulation after surgery.

Deaths between postoperative day four and nine are often related to bacterial cholangitis[7]. Based on our experience, a single dose of piperacilline/tazobactam (0.03 g) given before laparotomy is enough to minimize the risk of common bile duct infection.

Death is a rare event after day 10. However, due to absence of arterial flow, some bile duct problems can be observed with cholastasis[8]. Depending on the needs of the investigator an arterialization may be considered[9]. Of note, a cold ischemia time up to 24 hours can be considered as acceptable[10].

The quick-linker system was originally designed to perform liver transplantation with three cuff anastomoses (very short anhepatic phase). However, the three-cuff technique requires the use of very small calipers for the suprahepatic vena cava (SHVC) cuff and it has not been widely accepted due to the risk of liver outflow problems[11-14]. We therefore favor a quick-linker assisted three-cuff technique only when warm ischemia times shorter than ten minutes are required. In most studies however, implantation phases up to 15-18 minutes are acceptable, allowing the use of the two-cuff technique, and a more physiological SHVC drainage. As shown in this video paper, we routinely use the quick-linker system in the second part of the implantation only. While some extra surgical time is required to position the quick-linker handles, this part of the procedure is associated with minimal risks and allows for a shortening of the critical anhepatic phase thanks to easier cuffs insertions. Other advantages of the quick-linker system are a better graft-recipient vessel alignment and the use of wider cuffs with better hemodynamic results compared to the previously described techniques (including the one described by Kamada *et al.*)[6](**Fig 7**).

Disclosures

No conflicts of interest declared.

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