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Title: Sharing Experience on Long-Term Survival in a Non-Arterialized Rat Liver Transplantation Model

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 25

Number of Shots: 81

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Zhuoyu Jia**: Our research focuses on the non-arterialized rat liver transplant model to improve survival and enable research on long-term tolerance and chronic rejection.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Xinqiang Li**: Conventional models often result in severe biliary complications that compromise survival; this optimized technique significantly reduces ischemic injuries.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.3. **Kaiyi Yan**: The most critical challenge is preserving the peribiliary tissue and carefully inserting the stent without applying excessive force.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Dongpei Zheng**: After this procedure, researchers can perform long-term immunological monitoring and detailed cytological and histopathological evaluations of the liver graft.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Xueteng Wang:** Future studies can use this robust model to translate experimental immunological discoveries into novel clinical therapeutic strategies.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

~~What problem in your field of research does your JoVE article address?~~

- 1.6. **Jinzheng Cai, Director of the Organ Transplantation Center, Vice President of the Affiliated Hospital of Qingdao University:** (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What is the key takeaway you hope viewers remember after watching your video article?~~

- 1.7. **Jinzheng Cai, Director of the Organ Transplantation Center, Vice President of the Affiliated Hospital of Qingdao University:** (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Experimental Animal Ethics Committee at Qingdao University

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Harvesting the Donor Rat Liver Graft

Demonstrator: Zhuoyu Jia

- 2.1. To begin, place the anesthetized Sprague Dawley rat aged 8 to 10 weeks and weighing 220 to 250 grams in the supine position on a 37 degrees Celsius heating pad to maintain body temperature intraoperatively [1-TXT].
 - 2.1.1. WIDE: Talent positioning the anesthetized rat supine on a heating pad. **TXT: Anesthesia: 5% Isoflurane**
- 2.2. Use surgical scissors to make a cruciate incision extending to the xiphoid process, down to the pubic symphysis, and laterally to the axillary midline to maximize exposure of the surgical field [1]. Wrap the intestines with saline-soaked gauze [2] and place them on the sterile field on the left side of the abdomen to expose the operative area [3].
 - 2.2.1. Talent creating the cruciate abdominal incision.
 - 2.2.2. Talent wrapping the intestines with saline-soaked gauze. **Author's NOTE: 2.2.2 and 2.2.3 were combined in one shot.**
 - 2.2.3. Talent positioning the intestines on the left side of the surgical field.
- 2.3. For rapid hemostasis, use electrocautery to divide the falciform ligament [1]. Clamp and retract the xiphoid process cranially to expose the suprahepatic inferior vena cava, or SHIVC (*S-H-I-V-C*), and the left inferior phrenic vein [2]. Next, divide the left triangular ligament [3]. Use a 7-0 (*seven-oh*) or 8-0 (*eight-oh*) suture to ligate the left phrenic vein close to the SHIVC [4].
 - 2.3.1. Talent dividing the falciform ligament.
 - 2.3.2. Talent retracting the xiphoid process to expose the vessels.
 - 2.3.3. Talent dividing the left triangular ligament.
 - 2.3.4. Talent ligating the left phrenic vein.
- 2.4. Mobilize the caudate and papillary lobes [1]. Use microscopic forceps to remove fat and connective tissue from the surface of the infrahepatic inferior vena cava, or IHIVC (*I-H-I-V-C*) [2].
 - 2.4.1. Talent mobilizing the caudate and papillary lobes.
 - 2.4.2. Talent removing tissue from the infrahepatic inferior vena cava using microscopic forceps.

- 2.5. Ligate the right adrenal vein flush with the IHVC [1]. Isolate the right renal vein [2] and ligate it flush with the IHVC [3]. Continue dissecting the IHVC caudally to just above the left renal vein [4].
 - 2.5.1. Talent ligating the right adrenal vein.
 - 2.5.2. Talent isolating the right renal vein.
 - 2.5.3. Talent ligating the right renal vein.
 - 2.5.4. Talent dissecting the infrahepatic inferior vena cava caudally.
- 2.6. Use a cotton swab to gently elevate the liver [1]. Dissect the ligamentous attachments between the right inferior hepatic lobe and the retroperitoneum [2]. Halt the dissection upon reaching the SHVC [3].
 - 2.6.1. Talent elevating the liver with a cotton swab. Author's NOTE: 2.6.1 and 2.6.2 were combined in one shot.
 - 2.6.2. Talent dissecting ligamentous attachments.
 - 2.6.3. Talent demonstrating the completed dissection at the suprahepatic inferior vena cava.
- 2.7. Dissect the connective tissue between the portal vein, or PV (P-V), and the proper hepatic artery [1]. Isolate the PV [2]. Ligate the right gastric or pyloric vein flush with the PV [3]. Continue the dissection to the root of the splenic vein [4].
 - 2.7.1. Talent dissecting tissue between the portal vein and hepatic artery. Author's NOTE: 2.7.1 and 2.7.2 were combined in one shot.
 - 2.7.2. Talent isolating the portal vein.
 - 2.7.3. Talent ligating the right gastric vein.
 - 2.7.4. Talent extending the dissection toward the splenic vein.
- 2.8. Expose the porta hepatis [1]. Identify the fatty connective tissue containing the common bile duct and hepatic artery [2]. Make a V-shaped incision on the anterior wall of the common bile duct 3 to 5 millimeters distal to the hepatic duct bifurcation [3].
 - 2.8.1. Talent exposing the porta hepatis. Author's NOTE: 2.8.1 and 2.8.2 were combined in one shot.
 - 2.8.2. Close-up of the identified connective tissue.
 - 2.8.3. Talent creating the V-shaped incision in the common bile duct.
- 2.9. Use microforceps to hold the biliary stent [1]. Insert the stent approximately 3 millimeters into the biliary tract while maintaining parallel alignment with the duct [2]. Secure the stent with a circumferential 7-0 silk tie [3]. Ligate the hepatic artery at the incision level and transect it proximally [4].
 - 2.9.1. Talent holding the biliary stent with microforceps.

- 2.9.2. Talent inserting the stent into the bile duct.
- 2.9.3. Talent securing the stent with silk suture.
- 2.9.4. Talent ligating and transecting the hepatic artery.
- 2.10. For liver perfusion, expose the abdominal aorta [1]. Insert a 1-centimeter, 23-gauge scalp vein needle connected to a 20-milliliter syringe containing cold University of Wisconsin, or UW (U-W) solution at 4 degrees Celsius [2]. After removing the air from the syringe, use a vascular clamp to occlude and secure the abdominal aorta and needle [3].
 - 2.10.1. Talent exposing the abdominal aorta.
 - 2.10.2. Talent inserting the needle into the aorta.
 - 2.10.3. Talent securing the setup with a vascular clamp.
- 2.11. Slowly initiate perfusion by depressing the syringe at a steady rate of 3 to 3.5 milliliters per minute [1]. Incise the diaphragm [2]. Fully expose the thoracic IVC and thoracic aorta [3]. Use vascular clamps to occlude both vessels [4]. Transect the thoracic IVC below the clamp to allow efflux [5].
 - 2.11.1. Talent initiating perfusion. Author's NOTE: 2.11.1 and 2.11.2 were combined in one shot.
 - 2.11.2. Talent incising the diaphragm.
 - 2.11.3. Talent exposing the thoracic vessels.
 - 2.11.4. Talent applying vascular clamps.
 - 2.11.5. Talent transecting the thoracic inferior vena cava.
- 2.12. Continue perfusion to a total volume of approximately 40 milliliters until the liver parenchyma becomes completely pale [1]. Transect the IHIVC flush with the left renal vein [2]. Transect the PV at the splenic vein level [3]. Harvest the liver graft with the attached diaphragm [4]. Place the graft in a sterile Petri dish containing cold UW solution at 4 degrees Celsius, ensuring transplantation is completed within 2 hours of procurement [5].
 - 2.12.1. Talent continuing perfusion until liver becomes pale.
 - 2.12.2. Talent transecting the infrahepatic inferior vena cava.
 - 2.12.3. Talent transecting the portal vein.
 - 2.12.4. Talent harvesting the liver graft.
 - 2.12.5. Talent placing the graft into a petri dish with sterile, cold UW solution.

3. Ex Vivo Graft Preparation

- 3.1. Grasp the tail of the PV cuff with mosquito forceps and secure it perpendicular to the petri dish bottom [1]. Pass microforceps through the cuff lumen [2] and pull the PV through the cuff [3]. After adjusting the anterior wall of the PV, evert approximately 3 millimeters of vessel wall over the cuff end [4]. Secure the vessel with a circumferential 7-0 silk tie [5].
 - 3.1.1. Talent securing the portal vein cuff.
 - 3.1.2. Talent passing microforceps through the cuff. Author's NOTE: 3.1.2 and 3.1.3 were combined in one shot.
 - 3.1.3. Talent pulling the portal vein through the cuff.
 - 3.1.4. Talent everting the vessel wall.
 - 3.1.5. Talent securing the vessel with silk suture.
- 3.2. Prepare and cuff the IHVC using the same vessel mobilization, trimming, and cuff fixation procedure demonstrated for the PV [1]. Use a 1-milliliter syringe containing 0.5 milliliters of UW solution to gently flush the bile duct through the biliary stent, ensuring no fluid leakage occurs during flushing [2].
 - 3.2.1. Talent performing cuff preparation of the infrahepatic inferior vena cava.
 - 3.2.2. Talent flushing the bile duct through the stent.
- 3.3. Trim the SHVC by excising the residual diaphragm, ensuring complete vessel skeletonization [1]. Use a 7-0 or 8-0 suture to flatten the vessel stump and anchor the left and right lateral corners [2].
 - 3.3.1. Talent trimming the suprahepatic inferior vena cava.
 - 3.3.2. Talent placing corner sutures.

4. Recipient Rat Surgery for Donor Liver Implantation

- 4.1. Perform a midline laparotomy on the anesthetized recipient rat placed supinely on a 37 degrees Celsius heating pad [1-TXT]. Use four elastic retractors to pull the abdominal wall laterally [2]. Ligate the left inferior phrenic vein [3]. Mobilize the caudate and papillary lobes [4]. Use electrocautery to divide the esophageal and hepatic communicating branches [5].
 - 4.1.1. Talent performing the laparotomy on the anesthetized recipient rat. **TXT: Weight=270-300 g**
 - 4.1.2. Talent positioning elastic retractors.
 - 4.1.3. Talent ligating the left inferior phrenic vein.
 - 4.1.4. Talent mobilizing the liver lobes.
 - 4.1.5. Talent dividing branches with electrocautery.

- 4.2. Bluntly dissect the IHVC to the right renal vein level [1]. Isolate and ligate the left adrenal vein approximately 2 millimeters from IHVC [2]. Pass a rubber band transversely behind the SHVC, leaving it in place for later transection [3].
 - 4.2.1. Talent dissecting the infrahepatic inferior vena cava.
 - 4.2.2. Talent ligating the left adrenal vein.
 - 4.2.3. Talent passing the rubber band behind the vessel.
- 4.3. Isolate the PV from the hepatic hilum to the pyloric vein branch [1]. Isolate the hepatic artery at the hilum, ligate it flush, and transect it proximally [2]. Use a 7-0 Prolene suture to ligate the common bile duct flush with the confluence of the left and right hepatic ducts and transect it [3].
 - 4.3.1. Talent isolating the portal vein.
 - 4.3.2. Talent isolating, ligating, and transecting the hepatic artery.
 - 4.3.3. Talent ligating and transecting the common bile duct.
- 4.4. Apply microvascular clamps to the IHVC and PV, confirming the vascular blockage via liver darkening [1]. Insert a 23-gauge scalp vein needle attached to a 5-milliliter syringe to infuse 2 milliliters of 5 percent glucose into the PV to flush intrahepatic blood [2].
 - 4.4.1. Talent clamping the infrahepatic inferior vena cava and the portal vein. Author's NOTE: 4.4.1 and 4.4.2 were combined in one shot.
 - 4.4.2. Talent inserting the needle into the portal vein.
- 4.5. After exposing the SHVC by pulling the rubber band downward, cross-clamp the vessel [1]. Transect the IHVC, PV, and SHVC, followed by the removal of the native liver completely from the abdominal cavity [2].
 - 4.5.1. Talent cross-clamping the vessel.
 - 4.5.2. Talent transecting the vessels and removing the recipient liver.
- 4.6. After positioning the donor liver graft orthotopically, use the pre-placed 7-0 suture at the left corner to perform continuous suturing of the posterior wall toward the right corner [1], then secure it to the stay suture [2]. Continue the running suture along the anterior wall to complete the anastomosis, tightening each stitch to maintain uniform tension [3].
 - 4.6.1. Talent suturing the posterior wall.
 - 4.6.2. Talent securing the corner suture.
 - 4.6.3. Talent completing the anterior wall.
- 4.7. For PV reconstruction, place 7-0 stay sutures at the left and right corners of the recipient PV stump [1]. Apply three-point traction to create a triangular opening [2]. Insert the cuffed donor PV into the lumen [3]. Secure the anastomosis with a 7-0 ligature tied

around the circumferential groove [4]. Reconstruct the IHVC as demonstrated for PV reconstruction [5].

4.7.1. Talent placing portal vein stay sutures.

4.7.2. Talent creating the triangular opening.

4.7.3. Talent inserting the cuffed donor portal vein.

4.7.4. Talent securing the ligature.

4.7.5. Talent finishing the reconstruction of the infrahepatic inferior vena cava.

4.8. For reperfusion, after sequential release of the vascular clamps on the SHVC, PV, and the IHVC [1], confirm successful reperfusion by observing uniform color restoration of the graft [2].

4.8.1. Talent releasing the vascular clamps. Author's NOTE: 4.8.1 and 4.8.2 were combined in one shot.

4.8.2. Shot of uniform color restoration of the graft.

4.9. For biliary reconstruction, make a small transverse incision on the anterior wall of the recipient common bile duct stump [1]. Insert the donor biliary stent approximately 3 millimeters into the duct lumen [2]. Secure the stent with a circumferential 7-0 suture [3].

4.9.1. Talent making the duct incision.

4.9.2. Talent inserting the biliary stent.

4.9.3. Talent securing the stent with suture.

4.10. Prior to abdominal closure, irrigate the peritoneal cavity with 150 milliliters of normal saline at 37 degrees Celsius [1]. Use a 4-0 absorbable thread to close the musculofascial layer with a continuous running suture [2]. Close the skin with a continuous running suture using 4-0 silk thread, followed by post-operative care [3].

4.10.1. Talent irrigating the peritoneal cavity.

4.10.2. Talent closing the musculofascial layer.

4.10.3. Talent closing the skin.

Results

5. Results

- 5.1. Histological evaluation of liver graft architecture following transplantation at 7 days post-transplantation showed preserved hepatic lobular architecture with intact portal tracts and no evidence of acute rejection or ischemic necrosis [1].
 - 5.1.1. LAB MEDIA: Figure 8A. *Video Editor: Please emphasize the inset image showing the uniform radial distribution of cells surrounding the central area*
- 5.2. At 30 days post-transplantation, sustained structural integrity without chronic inflammation or distortion was observed [1]. At 100 days, the morphology was comparable to that of normal liver tissue, indicating long-term graft stability [2].
 - 5.2.1. LAB MEDIA: Figure 8B. *Video Editor: Please emphasize the inset image showing the uniform distribution of cells in the diagonal patch area.*
 - 5.2.2. LAB MEDIA: Figure 8C. *Video Editor: Please emphasize the inset image showing the uniform and neat distribution of cells.*

Pronunciation guides:

1. Xiphoid
Pronunciation link: <https://www.merriam-webster.com/dictionary/xiphoid>
IPA: /ˈzaiˌfɔɪd/
Phonetic Spelling: zye·foyd
2. Symphysis
Pronunciation link: <https://www.merriam-webster.com/dictionary/symphysis>
IPA: /ˈsɪmfəsəs/
Phonetic Spelling: sim·fuh·suhs
3. Electrocautery
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrocautery>
IPA: /iˌlektrōˈkɒt(ə)rē/
Phonetic Spelling: ee·lek·troh·kaw·tuh·ree
4. Falciform
Pronunciation link: <https://www.merriam-webster.com/dictionary/falciform>
IPA: /ˈfal-səˌfɔrm/
Phonetic Spelling: fal·suh·form
5. Suprahepatic
Pronunciation link: No confirmed link found

IPA: /ˌsuːprəhɪˈpæɪk/

Phonetic Spelling: soo-pruh-hih-pat-ik

6. Phrenic

Pronunciation link: <https://www.merriam-webster.com/dictionary/phrenic>

IPA: /ˈfrenɪk/

Phonetic Spelling: fren-ik

7. Caudate

Pronunciation link: <https://www.merriam-webster.com/dictionary/caudate>

IPA: /ˈkɔːdeɪt/

Phonetic Spelling: kaw-dayt

8. Papillary

Pronunciation link: <https://www.merriam-webster.com/dictionary/papillary>

IPA: /ˈpəpələreɪ/

Phonetic Spelling: pap-uh-lair-ee

9. Infrahepatic

Pronunciation link: No confirmed link found

IPA: /ˌɪnfrəhɪˈpæɪk/

Phonetic Spelling: in-fruh-hih-pat-ik

10. Adrenal

Pronunciation link: <https://www.merriam-webster.com/dictionary/adrenal>

IPA: /əˈdriːnəl/

Phonetic Spelling: uh-dree-nuhl

11. Retroperitoneum

Pronunciation link: <https://www.merriam-webster.com/dictionary/retroperitoneum>

IPA: /ˌreɪtrəˈperətɪˈeəm/

Phonetic Spelling: reh-troh-peh-ruh-tuh-nee-um

12. Portal

Pronunciation link: <https://www.merriam-webster.com/dictionary/portal>

IPA: /ˈpɔːrtəl/

Phonetic Spelling: por-tuhl

13. Hepatic

Pronunciation link: <https://www.merriam-webster.com/dictionary/hepatic>

IPA: /hɪˈpæɪk/

Phonetic Spelling: hih-pat-ik

14. Porta Hepatis

Pronunciation link: <https://www.merriam-webster.com/dictionary/porta%20hepatis>

IPA: /ˌpɔːrtə hɪˈpæɪs/

Phonetic Spelling: por-tuh huh-pat-is

15. Bifurcation

Pronunciation link: <https://www.merriam-webster.com/dictionary/bifurcation>

IPA: /ˌbaɪfərˈkeɪʃən/

Phonetic Spelling: bye-fur-kay-shuhn

16. Biliary
Pronunciation link: <https://www.merriam-webster.com/dictionary/biliary>
IPA: /'bɪli,erē/
Phonetic Spelling: bil·ee·air·ee
17. Perfusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/perfusion>
IPA: /pər'fju:ʒən/
Phonetic Spelling: per·fyoo·zhuhn
18. Parenchyma
Pronunciation link: <https://www.merriam-webster.com/dictionary/parenchyma>
IPA: /pə'reŋkəmə/
Phonetic Spelling: puh·reng·kuh·muh
19. Anastomosis
Pronunciation link: <https://www.merriam-webster.com/dictionary/anastomosis>
IPA: /ə,næstə'moʊsɪs/
Phonetic Spelling: uh·nas·tuh·moh·suhs
20. Ischemic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ischemic>
IPA: /ɪ'ski:mɪk/
Phonetic Spelling: ih·skee·mik