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Avoiding Ischemia Reperfusion Injury in Liver Transplantation

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Corresponding Author:	Yixi Zhang Organ transplant center, the first affiliated hospital of SYSU. guangzhou, Guangdong CHINA
Corresponding Author's Institution:	Organ transplant center, the first affiliated hospital of SYSU.
Corresponding Author E-Mail:	yixi0816@163.com
Order of Authors:	Yixi Zhang
	Changjun Huang
	Weiqiang Ju
	Qiang Zhao
	Maogen Chen
	Linhe Wang
	Dongping Wang
	Linwei Wu
	Zhiheng Zhang
	Caihui Zhu
	Zebin Zhu
	Shanzhou Huang
	Rongxing Xie
	Yinghua Chen
	Anbin Hu
	Xiaofeng Zhu
	Zhiyong Guo
	Xiaoshun He
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TITLE:

Avoiding Ischemia Reperfusion Injury in Liver Transplantation

AUTHORS AND AFFILIATIONS:

Yixi Zhang[1,2,3*], Changjun Huang[1,2,3*], Weiqiang Ju[1,2,3*], Qiang Zhao[1,2,3], Maogen Chen[1,2,3], Linhe Wang[1,2,3], Dongping Wang[1,2,3], Linwei Wu[1,2,3], Zebin Zhu[4], Shanzhou Huang[5], Zhiheng Zhang[1,2,3], Caihui Zhu[1,2,3], Rongxing Xie[1,2,3], Yinghua Chen[1,2,3], Anbin Hu[1,2,3], Xiaofeng Zhu[1,2,3], Zhiyong Guo[1,2,3], Xiaoshun He[1,2,3]

[1]Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

[2]Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology, Guangzhou, China

[3]Guangdong Provincial International Cooperation Base of Science and Technology, Guangzhou, China

[4]Organ Transplant Center, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, China

[5]Department of General Surgery, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China

[*]These authors contributed equally to this article and are co-first authors.

Corresponding author:

Xiaoshun He (gdtrc@163.com)

Zhiyong Guo (rockyucsf1981@126.com)

E-mail address for each author:

Yixi Zhang: (yixi0816@163.com)

Changjun Huang: (hchjun@126.com)

Weiqiang Ju: (weiqiangju@163.com)

Qiang Zhao: (522511042@qq.com)

Maogen Chen: (maogen2000@163.com)

Linhe Wang: (646390804@qq.com)

Dongping Wang: (dpwangcn@163.com)

Linwei Wu: (lw97002@163.com)

Zhiheng Zhang: (luckzzh993104@126.com)

Caihui Zhu: (1030985212@qq.com)

Zebin Zhu: (867141061@qq.com)

Shanzhou Huang: (575932993@qq.com)

Rongxing Xie: (1374417146@qq.com)

Yinghua Chen: (417255760@qq.com)

Anbin Hu: (huab@mail.sysu.edu.cn)

Xiaofeng Zhu: (740803277@qq.com)

Zhiyong Guo: (rockyucsf1981@126.com)

Xiaoshun He: (gdtrc@163.com)

KEYWORDS:
liver transplantation, ischemia reperfusion injury, ischemia-free, normothermic machine perfusion, organ preservation, static cold storage

SUMMARY:
Presented here is a protocol to provide a step-by-step ischemia-free liver transplantation protocol under ex situ normothermic machine perfusion (37 °C) of human livers from donors to recipients.

ABSTRACT:
Currently, ex situ machine perfusion is a burgeoning technique that provides a better preservation method for donor organs than conventional static cold preservation (0–4 °C). A continuous blood supply to organs using machine perfusion from procurement and preservation to implantation facilitates complete prevention of ischemia reperfusion injury and permits ex situ functional assessment of donor livers before transplantation. In this manuscript, we provide a step-by-step ischemia-free liver transplantation protocol in which an ex situ normothermic machine perfusion apparatus is used for pulsatile perfusion through the hepatic artery and continuous perfusion of the portal vein is provided from human donor livers to recipients. In the perfusion period, biochemical analysis of perfusates is conducted to assess the metabolic activity of the liver, and a liver biopsy is also performed to evaluate the degree of injury. Ischemia-free liver transplantation is a promising method to avoid ischemia-reperfusion injury and may potentially increase the donor pool for transplantation.

INTRODUCTION:
Ischemia reperfusion injury (IRI) is a well-known and widespread complication in organ transplantation. Obvious nonimmunological events lead to poor graft outcomes and delayed graft function, which are related to the high proportions of organ failure, re-transplantation, and recipient death[1]. Conventional cold storage (CCS) of organs was previously identified as a classic method to slow down metabolism but it does not have an influence on preventing progressive dysfunction and damage to cellular integrity. Furthermore, leukocyte accumulation is induced by reactive oxygen metabolites in the reperfusion phase. All of these biological processes become even more relevant when we use extended criteria donor (ECD) grafts such as fatty livers and those from donors older than 65 years. These ECD grafts are more vulnerable to damage and some other detrimental impacts, especially those from CCS[2]. The technology of normothermic ex vivo liver machine perfusion to preserve donor organs has achieved great progress over the past few decades and is entirely feasible in clinical practice[3]. The safety and viability of warm perfusion techniques in donor organs have been evaluated in preclinical studies, and some study groups have designed new type of perfusates and rewarming tactics in animal models. Some clinical trials of warm perfusion to preserve donor livers have been launched in East Asia, Europe and North America[4,5].

Normothermic machine perfusion (NMP) facilitates a metabolically active scenario in which

organs can achieve homeostasis with continuously provisioned oxygen and nutrients. The metabolism of grafts is activated, and we can judge during perfusion whether the donor organs are suitable for transplantation to recipients according to the biochemical index of the perfusate or biopsy of the perfused organs. Available parameters during the preservation period also offer a means for surgeons to treat grafts or restore ECD grafts[6,7].

Whole blood is the most important component due to its feature of carrying oxygen as well as some other essential ingredients, including antibiotics, antithrombotic agents, and nutrients[8]. Blood is transported and prepared in a cold environment surrounded by ice after a liver has been retrieved. Then, the cold liver is perfused in the already prepared NMP apparatus for several hours for assessment and restoration. However, the liver sustains double vital attacks of IRI prior to NMP and before implantation, although the liver is protected and repaired to some extent during the NMP process[9,10]. Therefore, we attempted to reevaluate the process and reflect on avoidance of the two IRI attacks. We carefully reviewed the process and developed an approach to avoid reverting the liver from a cryopreservation state to NMP, and then after several hours, the warm liver was cooled down by a cold preservation solution prior to transplantation into the human abdominal cavity at normal temperature. Therefore, we hypothesized that IRI was avoidable if a continuous blood supply was provided to the liver. To verify this hypothesis, we changed the conventional double conversion source to an uninterrupted hepatic artery (HA) and portal vein (PV) supply using a Liver-Assist device. This novel transplant procedure was named ischemia-free liver transplantation (IFLT). The first case of IFLT has previously been published and has attracted considerable attention from organ transplantation experts[11].

Two rotary pumps providing pulsatile hepatic arterial flow and a continuous portal vein supply were used in the perfusion device in which the flow was controlled by relevant pressure. The system is controlled by pressure and allows the flow through the liver to be automatically adjusted according to the resistance in the liver. Oxygenation and CO_2 elimination of the perfusate are regulated by two hollow fiber membrane oxygenators. We can set different temperatures according to different types of machine perfusion (ranging from 10 °C to 37 °C). We can monitor and record the real-time pressure, temperature, flow and resistance index in the instrument panel during the perfusion process. Liver assist is not a transportable device. Therefore, the donors used for IFLT should be transferred to the transplant center.

This article aimed to offer a step-by-step IFLT protocol in which an ex situ NMP apparatus is used to provide pulsatile perfusion to the HA and maintain continuous perfusion of the portal vein from human donor liver procurement to implantation.

PROTOCOL:

This protocol was reviewed and approved by the ethics committee of The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. Informed consent was obtained from all the participants. All the procedures in studies involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments or

revisions.

1. Preparation of the perfusion solution and device

NOTE: The total volume of the perfusion solution prepared for normothermic machine perfusion (NMP) according to this protocol is approximately 3,000 mL, as reported previously[1], and the final hemoglobin concentration is 6–10 g/L. The components of the perfusion solution are listed in **Table 1**.

1.1. Add the components of the perfusate to the organ reservoir of artificial hepatic assist device (**Table of Materials**) through the connector at the top of the oxygenator and remove all bubbles from the pipeline.

1.2. Turn on the venous pump on according to the manufacturer's instructions, which is displayed on the screen. Turn on the arterial pump in a similar manner. Null the pressure according to the instructions on the device screen. Click on the **Pressure** button to set the HA pressure within the range of 50–65 mmHg and PV pressure within the range of 4–11 mmHg during the whole process of normal temperature mechanical perfusion.

1.3. Start oxygenation using a mixture of O_2 and air (30% O_2) at a combined flow rate of 400 mL/min. Warm the perfusion solution to 37 °C.

1.4. Obtain a sample of perfusate solution from the T-branch pipe of arterial perfusion line for microbial culture (8 mL), blood gas analysis (0.5 mL) and liver function test (3 mL) after the device has been primed (pO_2, pCO_2, pH and electrolyte within normal range, and temperature near 37 °C), and monitor the biochemical parameters accordingly.

NOTE: The perfusate should be prepared fresh before use in a laminar flow operating room. Bicarbonate or insulin is added, if necessary.

2. Ischemia-free procurement of donor liver

2.1. Conduct the abdominal cruciate incision as follows: vertical, from the sternal notch to the symphysis pubis, and transverse, laterally to both flanks at the level of umbilicus. When procurement of the lung or heart is needed, a sternotomy can be utilized. Use a large C-shaped retractor to provide exposure.

2.2. Perform a detailed inspection of the abdominal viscera. Take a liver biopsy specimen for histological observation and clinical research. Mobilize the liver with a precision technique.

2.3. Place a cannula in the common bile duct for bile drainage and ligate the cystic duct. Cut a full-circumference tissue sample (width: 3–5 mm) from the end of the common bile duct for histological observation and clinical research.

2.4. Dissect the celiac artery (CA), gastroduodenal artery (GDA), splenic artery (SA), inferior vena cava (IVC), and PV. Insert an 8 Fr/12 Fr arterial cannula into the GDA or SA. Ensure that there is no interruption of the arterial supply for the liver from the CA. Connect the arterial cannula to the HA perfusion line of the Liver Assist device.

2.5. Harvest a 3 cm-long right external iliac vein and anastomose the vessel to the PV in end-to-side fashion with partial blockage of the PV for making an interposition vein. Connect a straight 24 Fr cannula to the PV perfusion line of the device and then, via the interposition vein, completely insert into the PV.

2.6. Place a 32–34 Fr caval cannula in the infrahepatic inferior vena cava (IHIVC) for outflow to the organ reservoir of the device. Block the suprahepatic inferior vena cava (SHIVC) thereby blocking the venous drainage to the right atrium. Then, start NMP, and establish the circuit in situ.

2.7. Harvest the liver and transfer to the organ reservoir under continuous NMP. Immediately after the liver is removed from the abdominal cavity, cold-flush the kidneys via the preplaced cannula within the abdominal aorta and procure the kidneys in the conventional manner.

NOTE: In the process of procurement, fully isolate the common hepatic artery (CHA), ligate the left gastric artery (LGA), and isolate the CA to the abdominal aorta. In the case of the accessory HA, bypass the artery in situ before NMP starts.

3. Ischemia-free preservation of the donor liver

3.1. Transfer the liver to the perfusion device. Remove the caval cannula immediately when the liver is moved to the organ reservoir. Continuous ex situ NMP the liver graft until allograft revascularization. Nearly submerge the liver by perfusate. Cover any dry surfaces with wet sterile gauze to prevent dehydration.

3.2. Set the PV perfusion pressure at 6–10 mmHg with a targeted flow rate higher than 500 mL/min. Set the HA pressure at 50–60 mmHg with a targeted flow rate higher than 150 mL/min. During the NMP, ensure that the perfusion parameters are stable, and monitor the pressure and flow rate within an appropriate range.

3.3. Remove redundant tissues from the liver and blood vessels. Transiently block the overall IVC to examine SHIVC and IHIVC for leaks.

3.4. Collect the bile tubing into a 15 mL collection container. Place the opening of the bile drain below the liver to allow bile to run out freely. Record the amount of bile production, and monitor the biochemical parameters every 30 min.

3.5. Obtain a perfusion sample (1 mL) for blood gas analysis every 15–20 min, liver function tests (3 mL) every 30 min and monitor the biochemical parameters accordingly.

3.6. Assess the viability of the liver by blood gas analysis and liver function tests of the perfusate, as well as bile biochemical parameters as previously reported[2].

NOTE: For patient safety, confirm the graft viability during NMP before initiation of the recipient surgical procedures. Add 1 mL of papaverine to reduce vascular resistance, if necessary.

4. Ischemia-free implantation of the donor liver

4.1. Resect the recipient's diseased liver using a conventional technique. Recannulate the donor IHIVC a 32–34 Fr caval cannula and block the SHIVC with a clamp. Then, move the donor liver from the reservoir to the recipient's abdominal cavity so that an NMP circuit in situ can be re-established.

4.2. Suture the donor SHIVC to the recipient counterparts using 3–0 non absorbable polypropylene sutures with a bi-caval or piggy-back technique.

4.3. Suture the donor PV and HA to the recipient's counterparts in an end-to-end fashion using 5–0 and 7–0 non absorbable polypropylene sutures, respectively. Perform these anastomoses under continuous NMP of the allograft as both HA and PV contains branches both native and artificial in nature.

4.4. Collect the liver and common bile duct biopsy specimens before reperfusion. Afterward, release the clamps on the PV and HA in order to re-establish the native dual blood supply for the liver. At the same time, cease the NMP after removal of the HA and PV cannula. Then, remove the cannula within the IHIVC, and release of the clamp on the SHIVC to flush out approximately 200 mL perfusate within the liver. The anhepatic phase is over. Obtain a perfusate sample (8 mL) for microbial culture again.

4.5. Ligate the donor SA or GDA and close the interposition vein with suture. Anastomose the donor's IHIVC to the recipient IHIVC or ligate it according to the surgical procedure. Anastomose the donor's common bile duct to the recipient's common bile duct with end-to-end fashion after withdrawal of the draining tube.

4.6. Collect liver and common bile duct biopsy specimens again after meticulous hemostasis. Close the abdominal wall in the routine procedure.

NOTE: During the implantation process, monitor the portal and arterial cannula closely to avoid twisting or bending, and scrutinize the flow rate parameters in real time to ensure the blood supply of the HA and PV. Increase the perfusion pressure slightly when necessary to ensure that the flow rate is sufficient to the liver. During the anastomosis of the SHIVC, PV, or IHIVC, shorten the venous stump as much as possible to avoid postsurgical obstruction of the venous flow.

REPRESENTATIVE RESULTS:

In April 2018, a 66-year-old male donor with brain death was not considered by local transplant centers because of the high risk of graft loss in such donors. The reasons for discarding the liver, at the time of procurement were older age and macroscopic appearance of moderate firmness, round liver edges and suboptimal liver graft perfusion along with major donor comorbidities, which included hypertension, hypertensive heart disease, and the following associated factors: hypernatremia (sodium, 156 mmol/L) and hemodynamic instability with the need for amine administration (dopamine, 1.5 µg/kg/min, noradrenaline, 0.12 µg/kg/min). Normothermic perfusion of the human donor liver grafts was performed for 5 h as described in the presented protocol. Macroscopic homogeneity of liver perfusion was evaluated to assess the quality of the liver graft. (**Figure 1A–D**). The hemodynamics of the liver was also studied by monitoring the changes in the arterial and portal flows. Stable hemodynamics of the liver grafts was observed during perfusion (**Figure 2A**). Blood gas analysis of the perfusate samples collected from arterial perfusion fluid was used to monitor the oxygenation status in the perfusion fluid. Oxygenation with a mixture of O_2 and air (30% O_2) at a flow rate of 400 mL/min resulted in a continuous O_2 saturation of 100%. **Figure 2B** displays the oxygenation of the perfusion fluid and subsequent extraction of carbon dioxide in our experience. Notably, the perfusate maintained a physiological pH during the whole perfusion process. Lactate levels subsequently decreased rapidly and were normal at 2.5 h of NMP (**Figure 2C**). An increase in the quantities of total bilirubin represented an improvement in the quality of the bile produced during NMP (**Figure 2D**).

FIGURE LEGENDS:

Figure 1: Representative procedures of ischemia-free liver transplantation. (**A**) The arterial cannula is inserted into the spleen artery, and the venous cannula is inserted into the portal vein patch. The bile duct is cannulated with a silicon biliary catheter. (**B**) Sixty minutes after the start of normothermic machine perfusion. (**C**) Four hours after the start of normothermic machine perfusion. (**D**) The donor liver is implanted into the recipient (the suprahepatic vena cava anastomosis is completed). During the operation, the organ chamber is covered by a nontransparent cover to maintain a sterile moist environment for the liver (not shown in these images).

Figure 2: Graphical presentation of perfusion parameters of both the perfusion fluid and bile during 5 h of normothermic machine perfusion. (**A**) Changes in arterial and portal flow. (**B**) Evolution of oxygenation characteristics and pCO_2 during 5 h of normothermic perfusion. (**C**) pH and lactate levels during 5 h of normothermic perfusion. (**D**) Increasing quantities of bilirubin in bile samples taken during machine perfusion.

DISCUSSION:

The article provides a step-by-step IFLT protocol for ex situ normothermic machine perfusion (37 °C) from donor to recipient. The equipment provides pulsatile perfusion of the HA and continuous perfusion of the PV. This technique was established to completely avoid ischemia

reperfusion injury.

Based on NMP, IFLT provides an uninterrupted supply of blood and oxygen to grafts from procurement and perseveration to implantation. Numerous studies have shown that NMP has significant advantages in reducing IRI, improving organ viability, and repairing graft damage compared to static cold preservation[12]. Through the innovation of surgical techniques and the advancement of NMP technology in various organs, the concept of ischemia-free organ transplantation (IFOT) is expected to extend to all solid organ transplants, significantly improving the early and long-term prognosis of organ transplantation and maximizing the use of marginal organs. IFOT technology is currently only used in organ transplantation derived from donation after brain death (DBD), but it is also applicable to transplantation of relative living organ donation (LDOD) by selecting reasonable vessel intubation and perfusion parameters. Donation after cardiac death (DCD) can be divided into two categories: manipulation of DCD (stopped after intentional recall of life support in patients with mechanical ventilation who do not meet brain death criteria, cDCD) and to a lesser extent uncontrolled DCD (unsuccessful resuscitation after cardiac arrest, uDCD)[13]. In uDCD-derived grafts in which organ warm ischemia injury has occurred, regional NMP should be established rapidly prior to organ harvesting. In this case, although the technique cannot completely avoid IRI, the damage to the organ can be maximally repaired. Notably, cDCD-derived grafts are widely used in most countries. With the support of regional NMP technology, IFOT can also be applied to organ transplants derived from such donations to avoid the subsequent occurrence of IRI. Since the IRI of a DCD organ is more severe than that of DBD and LDOD organs, this type of organ will likely benefit the most from IFOT. Therefore, IFOT is a promising method for organ transplants from almost all sources of donation, and its great application prospects warrant exploration.

There are several aspects to be aware of during this procedure. During the procurement process, the CHA is fully dissociated, the LGA is ligated, the celiac trunk is freed to the abdominal aorta, and the variant accessory HA needs to be reconstructed in the body.

During the preservation process using machine perfusion, the perfusion parameters are ensured to be stable, and the pressure and flow rate of the HA and PV are controlled in the physiological state range. The perfusion pressure can be slightly increased to ensure that the flow is sufficient to supply the liver during implantation.

For the process of donor liver implantation, attention should be paid to intubation of the PV and HA. The flow parameters should be monitored in real time to ensure the supply of arterial and portal blood flow. When the hepatic superior vena cava anastomosis is performed, the anastomotic venous fistula should be shortened as much as possible to prevent excessive intraoperative blood loss.

A continuous blood supply throughout the transplant process and the opportunity to add additional agents to the perfusion fluid during organ perfusion offer the potential to assess and improve organ quality prior to transplantation. Therefore, this method can considerably

increase the number of available organs for transplantation.

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DISCLOSURES:

The authors have no competing interests to declare.

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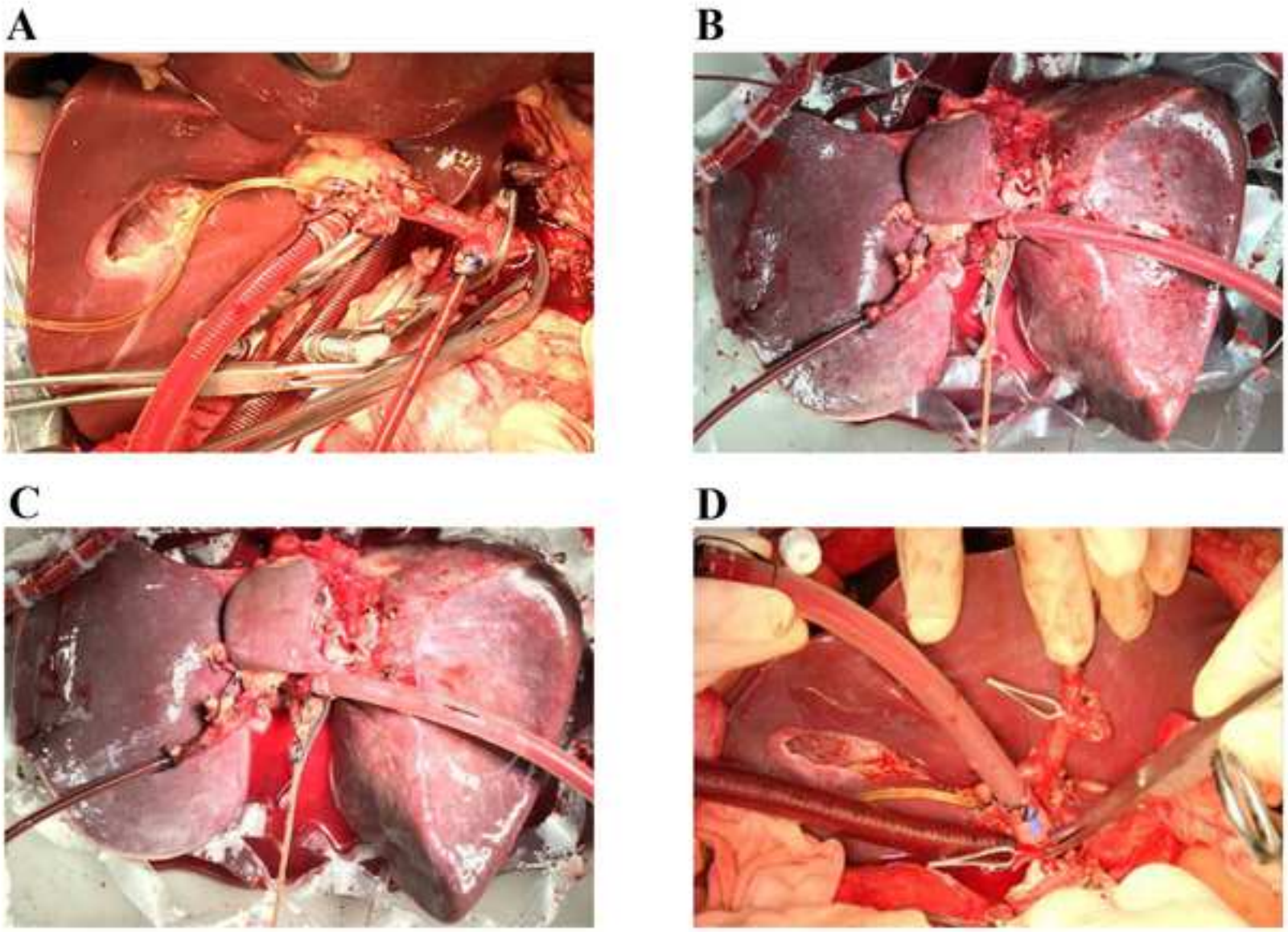
Figure



Figure

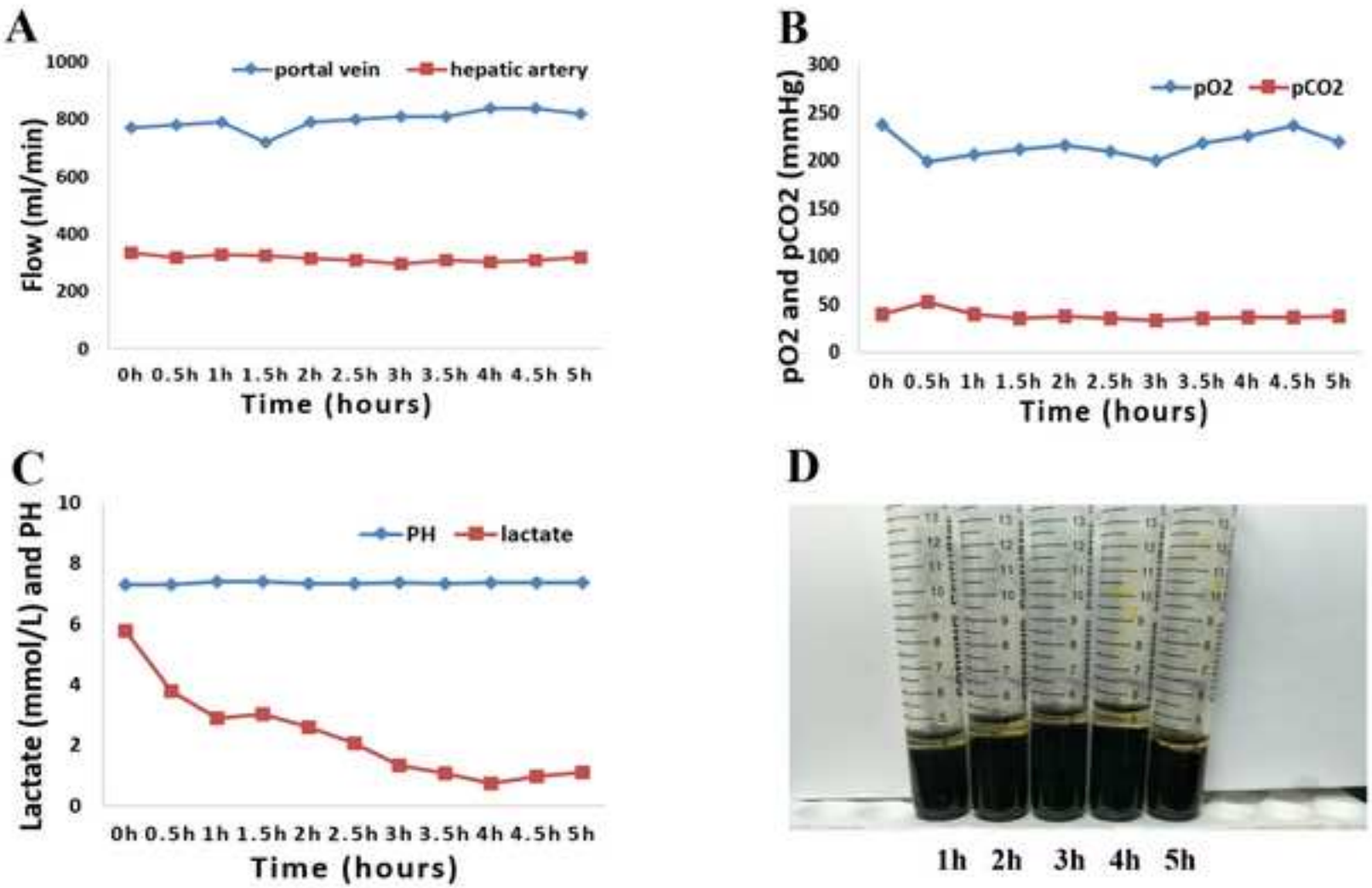


Table 1

Components	Amount
Crossed-matched leucocyte-depleted washed red cells	1.3 L
4% succinylated gelatine	1.4 L
5% sodium bicarbonate	200 mL
Heparin	37,500 U
Metronidazole	0.5 g
Cefoperazone sodium and sulbactam sodium	1.5 g
10% calcium gluconate	30 mL
25% magnesium sulphate	3 mL
Compound amino acid injection	250 mL

Name of Material/Equipment	Company
10% calcium gluconate	Hebei Tiancheng Pharmaceutical Co, Ltd
25% magnesium sulphate	Hebei Tiancheng Pharmaceutical Co, Ltd
5% sodium bicarbonate	Huiyinbi Group Jiangxi Dongya Pharmaceutical Co, Ltd
Cefoperazone sodium and sulbactam sodium	Pfizer
Compound Amino Acid Injection	Guangdong Litai Pharmaceutical Co., Ltd
Crossed-matched leucocyte-depleted washed red cells	Guangzhou Blood Center
Heparin	Chengdu Hepatunn Pharmaceutical Co., Ltd
Liver Assist	Organ Assist
Liver Assist disposable package	Organ Assist
Metronidazole	Shanghai Baxter Healthcare Co., Ltd.
scalp acupuncture	Wuhan W.E.O.Science & Technology Development Co., Ltd
Succinylated gelatinor	B. Braun Medical Suzhou Co., Ltd

Catalog Number	Comments/Description
1S181124101	30 mL
H20033861	3 mL
H36020283	The amount depends on the pH
H20020597	1.5 g
H20063797	250 mL
H20033739	1300 mL
H51021209	37500 U
OA.Li.Li.140	Perfusion device
OA.Li.DP.540	Disposable set and cannulas
H20003301	0.5 g
WEO-JX-32B-5.0 0.7*25mm	Bile duct cannula
H20113119	1400 mL

Dear editor:

On behalf of my co-authors, we thank you so much for giving us an opportunity to revise our manuscript again, " A method to avoid ischemia reperfusion injury in liver transplantation", and we appreciate the editors' and reviewers' positive and constructive comments. We have highlighted our revisions and responded point by point to the comments and suggestions from the reviewers and editors, as listed below. Attached please find the revised version, which we would like to submit for your kind consideration.

Therefore, we are submitting this revised manuscript to *Journal of Visualized Experiments* and hope that it is acceptable for publication in the journal. We look forward to hearing from you soon.

With kindest regards,

Xiaoshun He

Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University; Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology; Guangdong Provincial International Cooperation Base of Science and Technology, Guangzhou 510080

Editorial comments:

1. The editor has formatted the manuscript to match the journal style. Please retain and use the attached version for revision.
2. Please address all the specific comments marked in the mansucript.
3. In the protocol section, please ensure all actions are detailed. Please include all specifics associated with the step and include how each step is performed. Presently it is too general.
4. In this revision the entire introduction, lines 179-200, 208-219, 237-260, entire result section show overlap with previously published literature. Please see the attached ithenticate report and reword.
5. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."
6. Please proofread the manuscript well.
7. Please highlight 3 pages of the protocol text including headings and spacings to be used for filming purpose.

Response to editor: Thank you for your exceptionally useful suggestion. We have made corresponding modifications in the original text according to your advise. If you have any questions about this manuscript, please do not hesitate to tell me. Thank you.

Reviewers' comments:

Reviewer #2:

Thank you for submitting a revised version of this manuscript. I do not see that any information regarding recovery of other organs has been added to the text. I think this information is relevant. Also, only information regarding kidney utilization and results was provided. Given the age of the donors under consideration, they should have also been largely heart and lung donors, as well. It is still unclear to me how IFLT is coordinated with thoracic organ extraction (the explanation provided is nebulous), and no thoracic organ results are presented (number of hearts and lungs evaluated, number ultimately transplanted, graft loss due to ischemia associated with IFLT, post-transplant outcomes, etc.). I would appreciate that this information be provided and mentioned in the text of the manuscript, as well.

Response to Reviewer 1: Thank you for your thoughtful suggestion. Currently, we are also making statistics on the utilization of thoracic organ results, including number of hearts and lungs evaluated, number ultimately transplanted, graft loss and post-transplant outcomes. When our data are released, we will show it in the video. Before the IVC was blocked, the aorta

abdominalis was separated and the ligation line was left. After the IVC was blocked, the abdominal aorta was intubated rapidly perfused with UW solution. Surgeons who were ready to procure thoracic organ completed the preliminary steps before the IVC was blocked. Then the key steps were accomplished once the IVC was blocked and heart and lungs could be procured continuously. So far, we have completed 62 cases of IFLT and welcome you to visit our center to observe the IFLT on site. Beautiful Guangzhou is looking forward to your arrival. Thank you.