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Original article

Surgical guide including innovative techniques for orthotopic liver transplantation in the rat: Key techniques and pitfalls in whole and split liver grafts

T. Hori[1,2], S. Uemoto[2], X. Zhao[3], F. Chen[1], A-M T. Baine[1], L.B.Gardner[1], N. Ohashi[4], F. Conkle[5], M. Castanedes-Casey[1], V.R. Phillips[1], L.G. Rousseau[1], M. Murray[1], N. Kamo[6], J.H. Nguyen[7]

SUMMARY

Background. Although the techniques of orthotopic liver transplantation (OLT) and split OLT (S-OLT) have been well documented in the rat by established microsurgeons, the surgical skills required for this model limits its use by some investigators. Furthermore, alternative artificial OLT/S-OLT models are of limited use in the liver transplantation field. Herein, we describe detailed surgical procedures for our rat OLT/S-OLT model and our surgical learning curves. Methods. We studied the anatomical findings, including inflow and outflow, in 100 rats following OLT/S-OLT, and determined the operator learning curves for this model. We also investigated the discrepancy between survival rates and the rates of reliable samples in OLT and S-OLT, respectively, because surgical issues destroy all experiments. Results. Learning curve data indicated that 50 cases were required for operator training plus sufficient animals to start a study. In making split-liver grafts, thoughtful consideration of inflow and outflow are crucial for successful S-OLT. Our results showed that some complications necessarily disrupt all of the experiments, and we should omit unreliable samples if any complications and/or unreasonable histology are observed, especially at early post-operative period after S-OLT. Conclusion. Although OLT/S-OLT in rats required advanced skills, this is the only liver transplantation model that provides clinically relevant and reliable results.

Keywords: liver transplantation, animal model, microsurgery, ultra-microsurgery, rat, reperfusion injury

[1]Department of Neuroscience, Mayo Clinic Florida, Jacksonville, FL 32224, USA, [2]Department of Transplant Surgery, Kyoto University Hospital, Sakyo-ku, Kyoto, 606-8507, Japan, [3]Innovation Center for Immunoregulation Technologies and Drugs, Transplant Tolerance Unit, Kyoto University Graduate School of Medicine, Sakyo-ku, Kyoto, 606-8507, Japan, [4]Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya, Aichi, 466-8550, Japan, [5]Department of Comparative Medicine, Animal Facility, Mayo Clinic Florida, Jacksonville, FL 32224, USA, [6]Dumont-UCLA Transplantation Research Center, Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA, [7]Department of Transplant Surgery, Mayo Clinic Florida, Jacksonville, FL 32224, USA

Author for correspondence:

Tomohide Hori, Ph.D., M.D., Department of Neuroscience, Birdsall Research Building, 3rd. floor, Rm# 323, Mayo Clinic Florida, Jacksonille, FL 32224, USA, Phone: +1-904-953-2449, Fax: +1-904-953-7117, E-mail: hori.tomohide@mayo.edu & horit@kuhp.kyoto-u.ac.jp

INTRODUCTION

Clinical liver transplantation (LT) is made possible by the phenomena of immunological tolerance even after al-

Abbreviations:

biliary duct, BD; common bile duct, CBD; cold ischemic time, CIT; common hepatic artery, CHA; gastro-duodenal artery, GDA; Graft damage score, GDS; hepatic artery, HA; hepatic inferior vena cava, HIVC; hepatic vein, HV; infra-hepatic inferior vena cava, IHIVC; inferior caudate segment, ICS; left adrenal vein, LAV; left lateral segment, LLS; left median segment, LMS; liver transplantation, LT; left renal vein, LRV; lactate Riner's solution, LRS; lumbar vein, LV; membranate ligament, ML; monofilament nylon suture, MNS; monofilament polypropylene sutures, MPS; normal saline, NS; orthotopic liver transplantation, OLT; proper hepatic artery, PHA; portal reflow, PR; portal vein, PV; preservation solution, PS; right adrenal vein, RAV; right inferior segment, RIS; right median segment, RMS; right renal artery, RRA; right renal vein, RRV; Riner's solution, RS; right superior segment, RSS; superior caudate segment, SCS; supra-hepatic inferior vena cava, SHIVC; split orthotopic liver transplantation, S-OLT; thin silk thread, TST; warm ischemic time, WIT.

lograft transplantation and the ability of the liver to regenerate even after initial insufficient volume. As such, these factors form the prominent focus of studies attempting to further develop the LT field. Murine organ transplantation models, such as cardiac, lung, and kidney grafts, are well established,[1-5] and are commonly used by transplant immunity investigators. New insights into the mechanisms of graft injury after orthotopic liver transplantation (OLT) have also been established from experiments in small animal models. Mice are particularly suitable for laboratory assays due to the growing availability of gene-altered or knock-out animals and the development of specific agents and antibodies. However, murine LT is the most technically difficult animal transplantation model, even when reconstruction of the hepatic artery (HA) is omitted. Furthermore, a validated model of OLT in mice is unavailable, and we consider that the rat OLT model produces more clinically relevant and reliable data. Hence, there is a requirement for a complete rat OLT model, including split OLT (S-OLT), particularly in the field of liver regeneration.

Surgical OLT procedures in the rat were first reported in 1973 using hand-suture techniques,[6] while a modified model without HA reconstruction and temporal shunt of the porto-jugular veno-venous bypass was documented in 1975.[7] However, these models were not widely used as they required prolonged surgeries and advanced techniques. The cuff method was introduced in 1973,[8] and OLT models in rats using this method without HA reconstruction became globally accepted. The pros and cons of each model were recently reported,[9-23] and a combination of hand-suture and cuff methods are considered key for a successful surgery,[24] Innovated and improved techniques of rat OLT/S-OLT model have been used in the previous documents with high citation.[25-34]

Due to the clinical situation of liver donor shortage and donor safety, the main focus of the field of liver regeneration field is on patients with small-for-size liver syndrome and liver reperfusion injury. The development of clinically relevant rat OLT models[9-23] has advanced the clinical liver regeneration field.[35,36] However, pseudo models of fake OLT/S-OLT, such as temporal clamp or simple hepatectomy, are still used experimentally for assessing reperfusion injury and/or small-for-size syndrome after LT due to the technical demands of the LT model.[37-39] The cold ischemic time (CIT) is a critical factor in producing reliable data using the LT model, and also plays an important role in the mechanism of real reperfusion injury and true small-for-size syndrome.[40] Data from pseudo models that omit CIT are clinically irrelevant, and should not be translated into the actual LT field.

Herein, we describe the detailed surgical procedures of our innovative OLT model in the rat based on two decades experience. Furthermore, we present our surgical learning curves for this model, and discuss the key factors for successful OLT/S-OLT in rats. Our OLT model including S-OLT should provide the basis for more reliable and clinically relevant experiments into liver regeneration or transplant immunity.

MATERIALS AND METHODS

Animals

Lewis rats (haplotype in major histocompatibility complex: RT-1^{l} of 8–10 weeks of age were used as recipients. As donors, Lewis rats were used for syngeneic grafts and Wistar rats (RT-1^{u}) was used for allogeneic grafts, at the same age as the recipient. All rats were bred in animal facilities of Mayo Clinic Florida at least one week prior to surgery. The weight is routinely measured before surgery by using electronic scale (LCD Animal Weighing Scale, Kent Scientific Co., Torrington, CT 06790, USA). As a large vessel diameter is necessary for the anastomosis, males rats of 230–250 g body weight were most suitable for surgery; rats with a body weight >300g have a large amount of intra-abdominal fat, making the surgical procedures more difficult, thus outweighing the benefits of the larger vessel diameter.

Animals were deprived of solid food for 12-24 h prior to surgery to obtain enough surgical field without an expanded gastrointestinal tract, and to prevent aspiration under anesthesia. Animals were kept well hydrated prior to surgery as dehydration can easily lead to death during the introduction phase of anesthesia. Adequate injections of infusion solutions are important for a successful surgery, particularly prior to the anhepatic phase and after portal reflow (PR).[39] We used male rats due to the easily accessed penile vein for repeatable intravenous injections whenever required.

All experimental protocols were approved by the ethical committees of Mayo Clinic Florida (Institutional Animal Care and Use Committee, A19609), and animal handling and care met the requirements of the Ethical Guidelines of the Declaration of Helsinki and of our institutional guidelines for animal welfare.

Anesthesia

General anesthesia was performed using a commercial anesthesia system (VetEquip Inc., Pleasanton, CA 94588-0785, USA), including an evacuation canister (f/air, Bickford Inc., New York, NY 14169, USA) and induction chamber (2 Liter Induction Chamber, VetEquip Inc.).

General instruments

A large-sized and round-edged knife (Feather Surgical Blade, stainless steel, No. 21; Feather Safety Razor Co., Osaka, 531-0075, Japan), and an acute-edged knife (Feather Surgical Blade, stainless steel, No. 11; Feather Safety Razor Co.) were used in making the cuffs. Cotton swabs with optimal stiffness (cotton tipped applicators; Hardwood Products Company, Guilford, ME 04443-0149, USA) and soft clay (Color Mounting Clay; Hampton Research, Aliso Viejo, CA 92656-3317, USA) were prepared (**Fig. 1A**). A cylindrical warmer (Mantello, Ambulatory Surgical Warmer, Medium; Kent Scientific Co.) was used immediately after surgery.

Agents and solutions

The chemical agents used were: heparin (Heparin Lithium Salt, 100 unit/mg; MP Biomedicals, Cleveland, OH 44139, USA), antibiotics (Cephalexin Hydrate; MP Biomedicals), analgesic agent (buprenorphine 100 μg/mL; Cerilliant, Round Rock, TX 78655, USA; or buprenorphine 300 μg/mL, Buprenex Injectable; Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA 23235, USA), bicarbonate (8.4% Sodium bicarbonate Injection USP, 1 mEq/ml, 84 mg/ml; Hospira Inc., Lake Forest, IL 60045, USA; or 8.4% Meylon; Otsuka Pharmaceutical Factory, Inc., Tokushima, 772-8601, Japan), and cotton-like regional haemostatic agent (microfibrillar collagen, Avitene; C. R. Bard, Inc., Murray Hill, NJ 07974, USA). The solutions used were: lactate Ringer's solution (LRS; Lactated Ringer's Injection USP; B. Braun Medical Inc., Irvine, CA 92614-5895, USA), Ringer's solution (RS; Ringer's Injection USP, B. Braun Medical Inc.), and isotonic normal saline (NS; 0.9% Sodium Chloride Injection USP; Hospira Inc.).

Preservation solutions for organ storage

The preservation solutions (PS) were used at 4°C. Many storage solutions can be used including Histidine-tryptophan-ketoglutarate solution, sodium-lactobionate-sucrose solution, Euro-Collins Solution, and University of Wisconsin Solution. RS or NS also can be used for more drastic damage during a long CIT. PS should be selected based on the aim of each experiment.

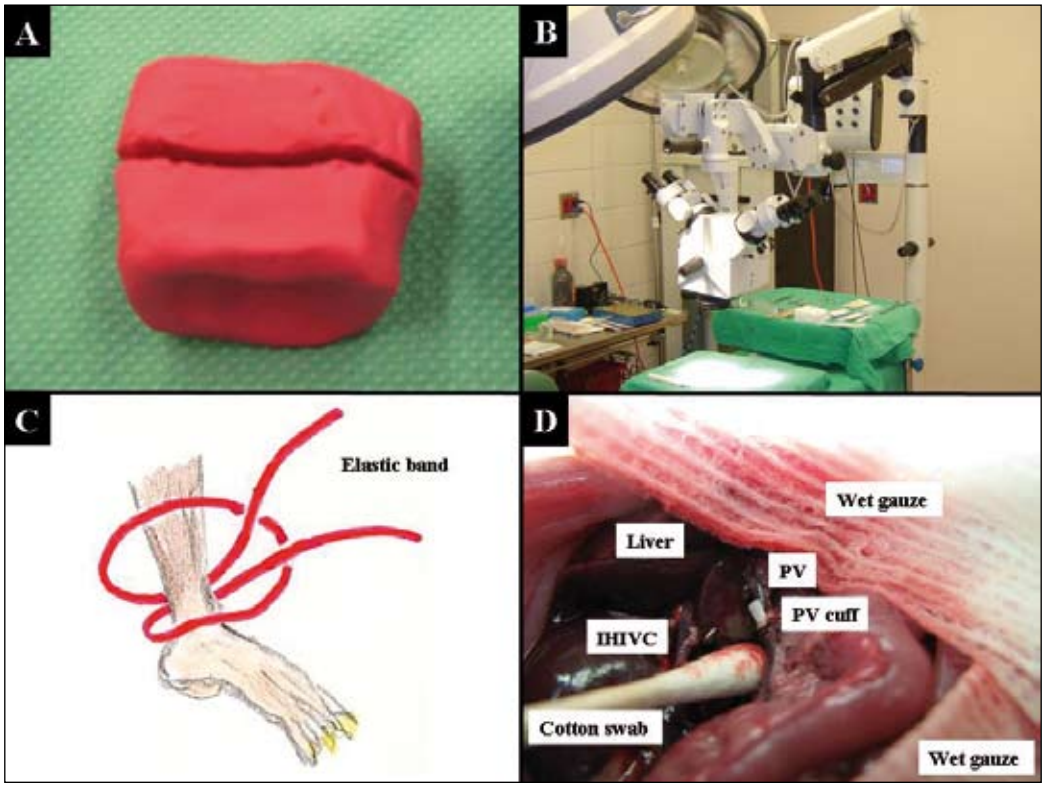


Figure 1. Preparation before surgery and intra-operative procedures. A) A forceps holder is made using soft clay. **B)** A 5–20X magnification surgical microscope is recommended for microsurgery and ultra-microsurgery. **C)** All feet of each rat are fixed onto the operating table using an elastic band and pins. **D)** The liver must be handled delicately by blunt and soft items such as a cotton swab as it is easy to cause damage and bleeding after PR in a recipient operation. To temporarily move tissue to one side of the surgical field, a delicate subtraction using wet gauze works well.

Surgical instruments for microsurgery and ultra-microsurgery

Basic instruments including scissors, forceps (fixation forceps, mosquito forceps, Adson's forceps, Kelly's forceps, and Jeweler's forceps), retractors, and towel clamps were the same as those typically used in small animal surgery, while the microsurgery and ultra-microsurgery instruments were purpose-built. Elementary microsurgery procedures have been well described.[24,39] Ultra-microsurgery refers to microsurgery for the anastomosis of vessels with a diameter under 0.5–1.0 mm.[24,39,41] Surgical instruments should be prepared according to the aim of each experiment.

The manufacturers of the surgical instruments can be chosen according to individual preference. For example, our institutions mainly use instruments made by Takasago Medical Industry Co. (Tokyo, 113-0033, Japan), Kent Scientific Co., Roboz Surgical Instrument Co., Inc. (Gaithersburg, MD 20898-0710, USA), Southpointe Surgical Supply Inc. (Coral Springs, FL 33071, USA), Scanlan International (Saint Paul, MN 55107, USA), Aesculap Inc. (Center Valley, PA 18034, USA), and Fine Science Tools GmbH (Heidelberg, 69121, Germany). For reference, our instruments can be seen in **Table 1**. All instruments were kept on a cork board during surgery to prevent damage to their fine tips.

Table 1. Surgical instruments for microsurgery and ultra-microsurgery

Micro-forceps
- **Straight:** Tweezers, Dumount #5, Dumoxel, INS14098, Kent Scientific
- **Curved:** Micro Dissecting, serrated, slight curve, length 4", RS-5136, Roboz
- **45 degree-angled:** Tweezers, Dumount #5, SS, INS14101, Kent Scientific
- **Right-angled:** Micro Dissecting, full curve, serrated, length 4", RS-5138, Roboz
- **Waved:** Botvin, serrated tips, tip width 0.5mm, length 3", RS-5168, Roboz
- **Comparatively-blunt:** Tweezers & Thumb Forceps, straight, 0.7 mm tips, INS14142, Kent Scientific
- **Tying forceps:** Tying Forceps, forceps with tying platform, straight, INS14356, Kent Scientific
  Colibri forceps, 8.5 cm, INS 14358, Kent Scientific
- **Dilator forceps:** Dilator Forceps, hollow handles, str 0.15mm tips, INS15910, Kent Scientific
- **Tissue forceps:** Singley, heart holding forceps, length 6", RS-5255, Roboz
- **DeBakey's forcepts:** DeBakey, jaw width 1.5mm, length 6", RS-7561, Roboz

Dissecting spring micro-scissors
- **Small-sized**
  - **Straight:** Vannas, straight, 3", RS-5620, Roboz
  - **Curved:** Vannas, curved, 3", RS-5611, Roboz
  - **Reverse:** Catroviejo, angular, sharp, $3^{1/2}$", RS-5658, Roboz
- **Large-sized:** Spring Scissors, straight, 12cm, extra fine straight blades, INS14125, Kent Scientific

Micro-scissors
- **Straight:** Dissecting Scissors, 10cm, straight, INS14393, Kent Scientific
- **Angled:** Micro Dissecting, angled, sharp, length $4^{1/2}$", , RS-5918, Roboz
- **Double curved:** La Grange double curve, sharp serrated, length $4^{1/4}$", RS-5925, Roboz

Micro-needle holders
- **Small-sized:** McPherson, curved, smooth tapered jaws, length 4", RS-6421, Roboz
- **Middle-sized:** Castroviejo, straight without catch, length $5^{3/4}$", RS-6410, Roboz
- **Large-sized:** Needle Holder, straight, round handles, 14cm, INS14080, Kent Scientific
  Needle Holder, curved, round handles, 12.5cm, INS14132, Kent Scientific

Atraumatic vascular micro-clamps
- **Small-sized:** Micro Vascular Clip, diameter 1mm, 10-15gr, length 8mm, RS-6470, Roboz
- **Middle-sized:** Micro Vascular Clip, diameter 1-2mm, 15-20gr, length 11mm, RS-6472, Roboz
- **Large-sized:** S&T Vascular Clamps, straight, diameter 2.15mm, 00325-00, Fine Science Tools
- **For SHIVC clamp:** Baby vascular clamps, Profunda Cooley Jaws, 2002-231, Scanlan International
  Baby-Satinskey, 15cm, TKZ-K1391-15, Takasago Medical Industry

Atraumatic Bulldog clamps
- **Small-sized:** Bull Dog Clamps, micro, serrated jaw, curved, INS14119, Kent Scientific
- **Middle-sized:** Bull Dog Clamps, John Hopkins, serrated jaw, straight, INS14116, Kent Scientific

Bulldog clamp
- **For cuff attachment**
  Glover Bulldog Clamp, straight, 6cm Jaw, length 11.4cm, 645-036, Southpointe Surgical Supply
  DeBakey-Weldon, Micro Vascular Clamp, RU-1278-1, Takasago Medical Industry
- **Small-sized:** Cooley Bulldog clamps, curved, serrated, MB586R, V.Mueller, Aesculap
- **Middle-sized:** Cooley Bulldog clamps, curved, serrated, MB587R, V.Mueller, Aesculap

Dull-tipped injectors
- **Straight:** Feeding Needle, straight needle, 22G, FNS-22-1.5, Kent Scientific
- **Curved or L-shaped :** Feeding Needle, curved needle, 22G, FNC-22-1.5, Kent Scientific

Electrocautery scalpels

Bipolar forceps (Martin ME 102 Electrosurgical unit and Bipolar Accessory Set for ME 102; Harvard Apparatus, Holliston, MA 01746, USA) were used as a conventional scalpel. Fine-tipped and delicate scalpels were prepared separately for microsurgery and ultra-microsurgery. We used monopolar type (Bovie, low temperature cautery kit; Aaron Medical, Clearwater, FL 33710, USA) and bipolar-forceps type (bipolar electro surgery package includes the MV-9 unit and bipolar coagulation forceps, Micro Non-serrated straight tips; Kent Scientific Co.).

Microscopes

A surgical loupe (2.0–3.0X magnification) or a microscope (5–6.25X magnification) is sufficient for microsurgery. We used a surgical microscope at 5–20X magnification (Surgical Scope M680, Type 10445496; Leica Microsystems Inc., Bannockburn, IL 60015, USA) (**Fig. 1B**). Even though a loupe and a microscope with low magnification can be omitted, ultra-microsurgery for HA reconstruction requires high magnification (12.5–20X magnification) and a sufficient light source.

Surgical materials

The sutures used were: thin silk threads (TST) (Silk Suture 7-0; Braintree Scientific Inc., Braintree, MA 02185-0929, USA), monofilament nylon suture (MNS) (10-0 Ethilon, BV130-3, 2820G; Ethicon, Inc., Somerville, NJ 08876-0151, USA), monofilament polypropylene sutures (MPS) (7-0 Prolene, BV-1, 8304H-X, and 8-0 Prolene, BV130-5, 8732H; Ethicon, Inc.), and absorbable thread (5-0 Coated Vicryl Plus; Ethicon, Inc.). Micro-clips were prepared in several sizes (Microclip size: M, ML, and L, Horizon Ligation System; Teleflex Medical, Durham, NC 27709, USA). Several sizes of applying forceps (Microclip applicators; Teleflex Medical) were also prepared.

Micro-tubes

The micro-tubes used were: polyurethane micro-tube (Polyurethane Catheters, Straight Tip, Hydrocoat, 3 French, 20 gauge; Access Technologies, Skoki, IL 60076, USA) and silicone micro-tube (Silicon Catheters, Straight Tip, IntiSil, 2 French, 23 gauge; Access Technologies).

Catheters

The peripheral catheters used were: 14 gauge (14G Cathlon i.v. catheter; Johnson & Johnson Medical, Inc., Arlington, TX 76004-3130, USA), 16 gauge (16G Exel Safelet Cath; Exelint International, Co., Los Angeles, CA 90045, USA), and 24 gauge (24 G Surflo Flush; Terumo Co., Tokyo, 151-0072, Japan).

Hepatic volumes of each segment for split liver grafts

The weight of each segment was measured in a total of 100 normal rats, and segmental percentages were calculated as segmental weight (g)/whole liver weight (g) in each rat.

Learning curves

Until portal reflow (PR) in recipient operation, the procedures of OLT/S-OLT includes critical phases, such as CIT, warm ischemic time (WIT; the time from liver insertion to PR) and anhepatic phase (the time from portal clamp to PR). The surgical timetable for OLT/S-OLT can be seen in **Table 2**.

We examined the learning curves of some surgeons at our institutions. A success rate ≥0.8 in whole liver transplantation was considered enough to learn the basic procedures for this model.

Statistical analysis

Data are presented as mean ± standard deviation. The between-group comparisons was performed by Student's *t* test for unpaired variables. Kaplan-Meier method (the log-rank) was used for analysis of survival rates. Statistical calculations were performed using SPSS Software Version 16.0 (SPSS Inc., Chicago, IL 60606, USA). The p value < 0.05 was considered statistically significant.

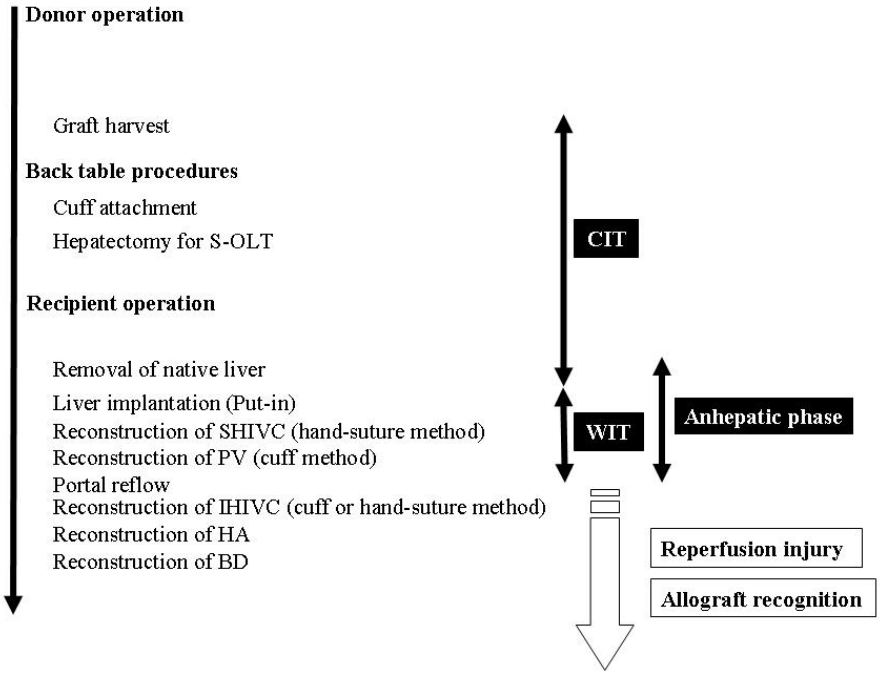
RESULTS

Anatomy

Though mice have the gallbladder, there are no gallbladders in rats. The liver is divided into three lobes (right, left, and caudate lobes) arranged into seven segments (**Fig. 2A**).[42,43] The percentage of total volume of each segment were 26.5±2.6% for the right median segment (RMS), 12.3±1.2% for the left median segment (LMS), 31.4±2.8% for the left lateral segment (LLS), 13.6±1.2% for the right superior segment (RSS), 8.0±1.7% for the right inferior segment (RIS), 4.3±0.9% for the superior caudate segment (SCS), and 3.9±0.8% for the inferior caudate segment (ICS) (**Fig. 3**).

For hepatic vein (HV) reconstruction, anastomosis of the supra-hepatic inferior vena cava (SHIVC) to the SHIVC must be performed, as the HV itself has no extra-hepatic margins for suture. The esophagus is situated near the liver, and the involvement of the esophagus

Table 2. Time table of OLT/S-OLT in rats



at the total clamp of the SHIHC should be avoided. The hepatic inferior vena cava (HIVC) enters into the right lobes of the liver (i.e., RIS, RSS, and RMS). There are no structures behind the portion of the HIVC except for a thin membrane. Segmental drainages of HV can be seen in **Figures 2B and 4A**. Although the LMS is joined with the RMS, the predominant vessels of the LMS make a common channel with those of the LLS. This is a key for making S-OLT grafts.

For portal vein (PV) reconstruction, the large communicating vessels between the PV and the paraesophageal vessels can be detected on the SCS (**Fig. 5**). The segmental PV branches can be seen in **Figure 4B**. The PV flows in the direction from the PV trunk to the segmental PV branches to the LMS and the LLS.

For IHIVC reconstruction, the RIS is fixed to the surface of the IHIVC by the membranate ligament (ML). The right renal artery (RRA) is located behind the IHIVC. The RRA usually branches into dual arteries, and the upper RRA branches into the right adrenal artery. The right adrenal vein (RAV) and the lumbar vein (LV) flow directly into the IHIVC at the point of the lowest edge of the RIS, and these veins occasionally make a common channel before the IHIVC (**Fig. 6**). The LV is derived from the left side of the HIVC, and travels behind the HIVC. The left adrenal vein (LAV) and the lower LV flow into the IHIVC at the junction of the left renal vein (LRV) and the IHIVC (**Fig. 6**).

For HA reconstruction, the common hepatic artery (CHA) is located at the backside of the PV trunk, and the branched proper hepatic artery (PHA) and the gastro-duodenal artery (GDA) are located at the left side of the PV trunk (**Fig. 7**). The PV trunk and these arteries are encased together by a thin sheath.

Stent tube for biliary duct and cuffs for PV and IHIVC

The tube and cuffs are made prior to surgery. Stent tubes for biliary duct (BD) reconstruction were made using 24 gauge peripheral catheters or 2 French silicone/polyurethane micro-tubes. A bevel was cut at each end to allow easy insertion into the BD using a cut-down method (**Fig. 8A**). A total length of 7–8 mm is sufficient; too long a BD stent tube causes biliary obstruction at the points of the first branch from the caudate lobes and the intra-pancreatic BD. We prefer polyurethane tubing as its soft structure is suitable for surgical procedures.

The PV cuff is made using a 14 or 16 gauge peripheral catheter. First, 2–3 mm of the main body and 2 mm of the extension are made using a large-sized and round-edged knife. Next, the detail is adjusted using an acute-edged knife (**Fig. 9A**). The total length of the cuff should be short-

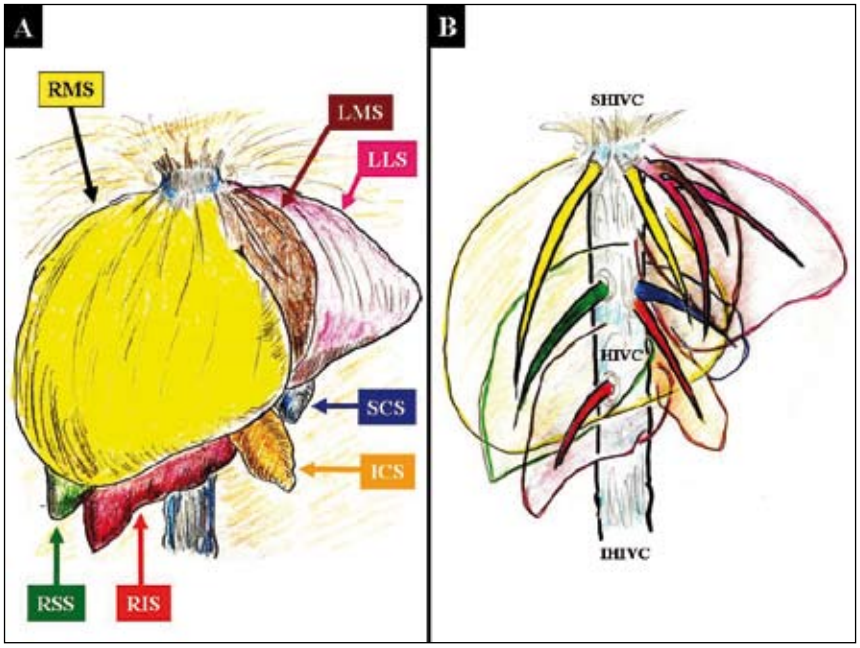


Figure 2. Hepatic segments and HV flows. A) The liver comprises three lobes, which are subdivided into seven segments. **B)** The HV itself has no extra-hepatic margins for suture. The HIVC enters into the RIS, RSS, and RMS. Note that the HVs from four segments (RSS, RIS, SCS, and ICS) flow directly into the HIVC.

ened as much as possible, as a long cuff easily leads to stenosis. Encircled chases are made using these knives.

The IHIVC cuff is made using a conventional sterilized tube with a minimum inner diameter of 2.0-2.5 mm and a thin wall. We use polyurethane tubing rather than polyethylene tubing as it is softer. The basic IHIVC cuff procedure is the same as for the PV cuff. The total cuff length should be 5-6 mm, with a 3-4 ml main body and 2

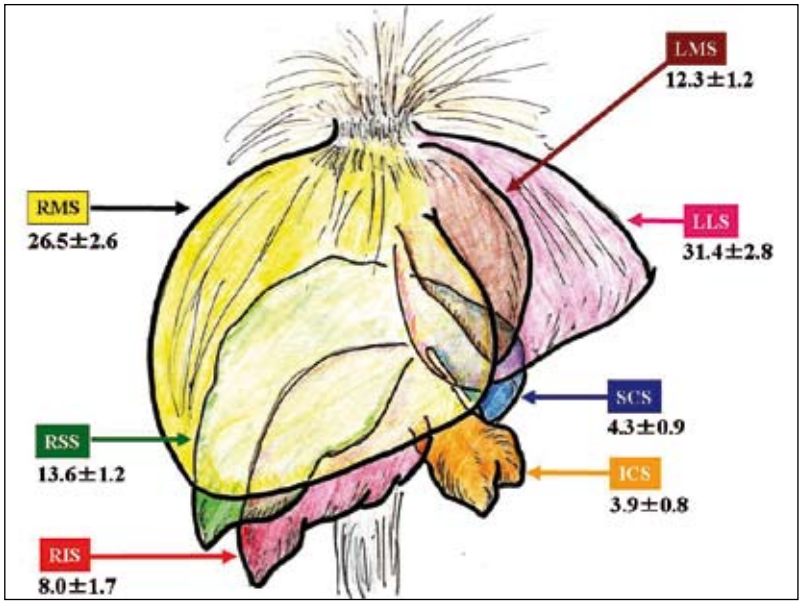


Figure 3. Percentage of each segment in terms of hepatic volume. In basic anatomy, the LMS is joined with the RMS, and an incomplete lobulation is often detected in those segments.

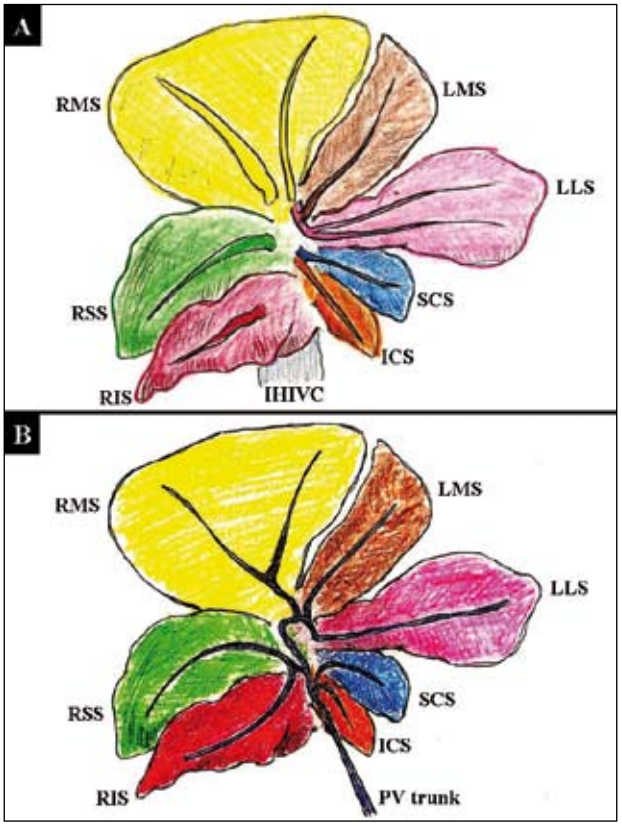


Figure 4. Segmental flows of the HV and PV. A) Segmental drainages of the HV are shown. Although the LMS is anatomically joined with the RMS, the predominant vessels of the LMS make a common channel with those of the LLS. Thus, with respect to out-flow during surgery, the LMS should be handled together with the LLS. **B)** Segmental branches of the PV are shown. The segmental PV to the LMS and the LLS are branched from the same root of the PV.

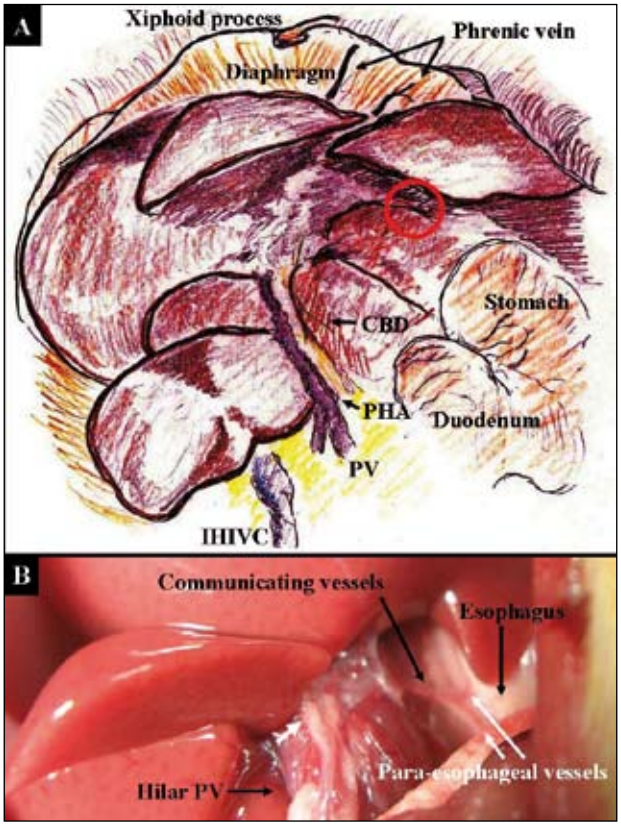


Figure 5. Communicating vessels between the PV and paraesophageal vessels. A) The large communicating vessels between the PV and the paraesophageal vessels are detected on the SCS (circle). **B)** The esophagus is situated near the liver, and the PV and the paraesophageal vessels have large communicating vessels.

mm extension. Encircled chases can be made using fine-tipped mosquito forceps (**Fig. 9A**).

Anesthesia

Barbiturates (pentobarbital sodium injection USP, Nembutal sodium solution; Hospira Inc.) can be used for anesthesia, although we have stopped their use. Though sodium pentobarbital has an advantage of rapid induction and easy procedures, it is difficult to control the depth of anesthesia according to surgical procedures in recipient operation if once introduced. All operative procedures are performed under general anesthesia using isoflurane accompanied with oxygen, and inhalational anesthesia is induced and maintained by isoflurane using an anesthesia system. Body weight is measured after anesthesia. A sufficient depth of anesthesia, but without asystole, asphyxia, or a subsidence of tongue root is required as the operation includes microsurgical procedures. Isoflurane accompanied with oxygen flow at 5 L/min is used in the introduction phase, and is reduced to 0.5-2.0 L/min in the maintenance phase. Diethylether (Ether 99.5%, Extra Dry, over Molecular Sieves, AcroSeal; Acros Organics, Fair Lawn, NJ, USA) with room air can also be used for anesthesia according to institutional equipment. Cotton-absorbing diethylether is set at the bottom of a large-sized tube (50 ml conical tube; BD Falcon, Franklin Lakes, NJ 07950, USA), and anesthetic depth is controlled by the distance from the rats' snout. A large advantage of isoflurane and diethylether is controllable depth of anesthesia according to surgical procedures in recipient operation. Based on our experiences assessed by univariate and multivariate analyses[37], there were no significant differences between isoflurane and diethylether in the influences upon survival rates after OLT. However, we recommended the induction and maintenance of anesthesia by isoflurane accompanied with oxygen, because diethylether is considered by the interna-

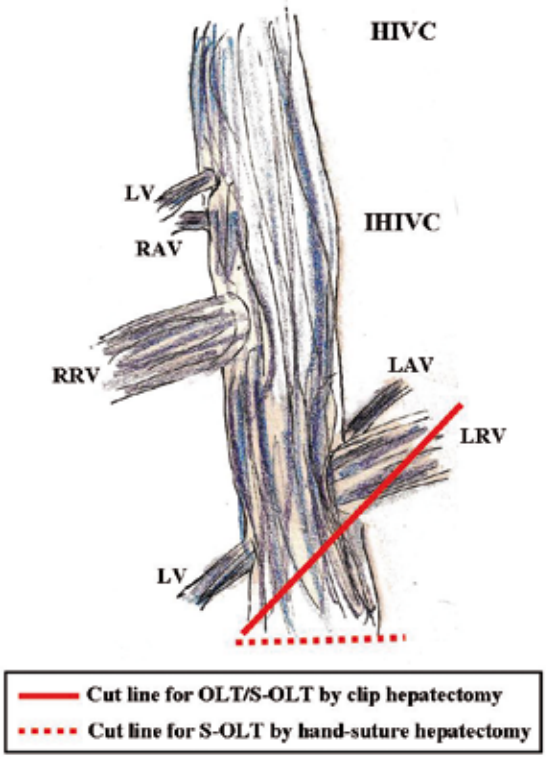


Figure 6. IHIVC branches. The RAV and the LV flow directly into the IHIVC at the point of the lowest edge of the RIS. The LAV and the lower LV flow into the IHIVC at the junction of the LRV and the IHIVC. In the donor operation for IHIVC reconstruction using the cuff method, the IVIHC is cut in a branch patch-fashion in OLT cases and S-OLT cases with hepatectomy by clip method (solid line). By contrast, the IHIVC is cut under the LRV level in S-OLT cases with hepatectomy by hand-suture method (dotted line). Note that hepatectomy made by hand-suture method shortened SHIVC margin for suture. Then, longer IHIVC is required in S-OLT by hand-suture hepatectomy.

tional guidelines of many scientific associations for animal experimentation, as high carcinogenesis.

After the introduction of anesthesia, the abdominal wall is shaven using electric clippers. All feet of each rat are fixed upon the operating table (rubber board) using an elastic band and pins (**Fig. 1C**). Before skin incision, a total of 2.0-2.5 ml/rat of LCR is injected intravenously (penile vein) using a 27 gauge fine needle, and the body position is adjusted again.

Donor operation

Laparotomy

The abdominal wall is sterilized using a surgical scrub including povidone iodine. Any liver damage should be avoided during laparotomy. A long midline skin incision is initially made up to a exteriorization of the xiphoid process

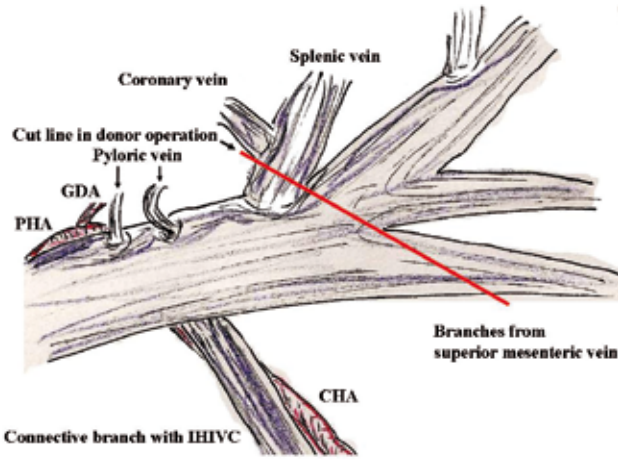


Figure 7. Anatomy of the PV and HA. The splenic vein and the coronary vein flow into the PV trunk at the left side or the posterior side of the PV. The pyloric vein flows into the PV trunk at the left side of the PV. The posterior side of the PV trunk has a branch that connects with the IHIVC. The CHA is located at the back of the PV trunk, and the PHA and the GDA branches are located at the left side of the PV trunk. The PV trunk and these arteries are encased together in a thin sheath. The PV trunk is cut in a branch patch-fashion using the PV trunk and the splenic vein in the donor operation.

of the sternum, and a transverse incision is then made with an anticipated prevention of sarcous bleeding by grasping of the muscle using a Kocher's clamp or a mosquito forceps. Complete homeostasis is made with an electrocautery scalpel, especially around the xiphoid process. This process creates a crosswise incision of sufficient size. An adequate surgical field is achieved using retractors for optimal retention of the bilateral costal bows and the fixation forceps to pull the lower abdominal wall. Warm NS is arbitrarily dripped onto the intraperitoneal organs to prevent drying after laparotomy.

Preparation for graft harvest

A 10 ml syringe is inserted under the back of the rat to allow sufficient view of the SHIVC, if required. The gastrointestinal tract is moistened with warm NS and positioned to the outside of the left abdominal cavity and coated with gauze. The liver must be handled delicately by blunt and soft items such as a cotton swab as it is easy to cause damage and bleeding after PR in a recipient operation (**Fig. 1D**). Note not to touch the liver directly during the donor operation to avoid an unexpected graft injury. To temporarily move tissue to one side of the surgical field, a delicate subtraction using wet gauze works well (**Fig. 1D**).

The falciform ligament is divided into two parts around

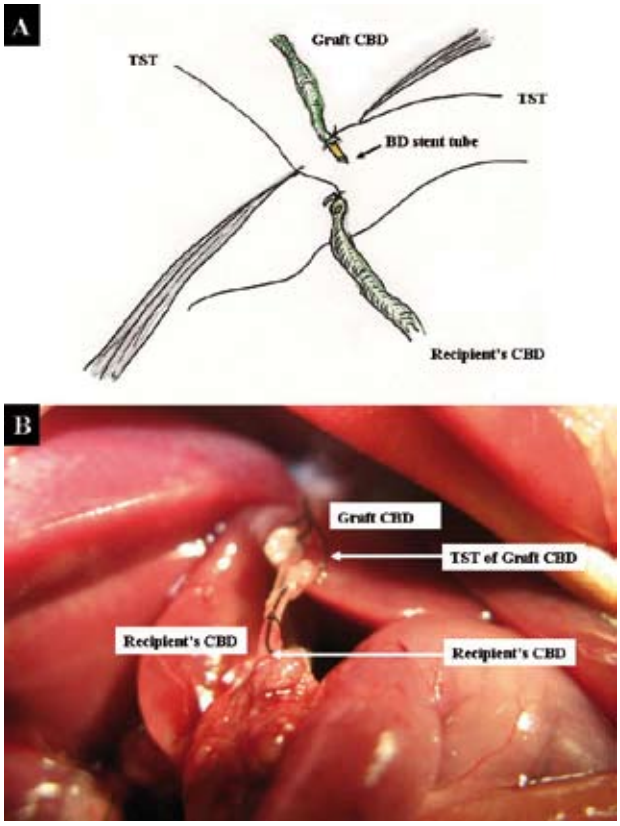


Figure 8. BD reconstruction. A) Both sides of the BD stent tube are beveled beforehand. The previously ligated TST of the recipients' CBD is held by mosquito forceps from the right side. TST is set under the recipients' CBD. The micro-tube is led onto the recipients' CBD. The recipients' CBD is opened using the cut-down method, and the micro-tube is inserted into the recipients' CBD. The micro-tube is then fixed by TST ligation, and one edge of the ligated THT is also reserved for the donor operation. **B)** To prevent removal of the micro-tube, the preserved TSTs in the donor and the recipient are ligated together.

the SHIVC. The falciform and triangular ligaments are sharply cut, and the left inferior phrenic vein can then be found. This vein is skeletonized carefully and is ligated with TST. The complete processing of this vein is important for prevention of massive hemorrhage after PR in the recipient operation and to ensure adaptable out-flow (**Fig. 10A**). The transparent membranes around the liver which fix each lobe to the surrounding organs are cut, and the portal branch communicating to the paraesophageal vessels is cauterized using an electrocautery scalpel or ligated with TST. The ML which fixes the LLS to the retroperitoneum is cut under the careful retention of the LLS.

The retroperitoneum on the IHIVC is dissected, and the IHIVC skeletonization procedures are continued. The right renal vein (RRV) is carefully isolated from the RRA and is ligated with TST, then cut as close to the renal hilus as possible. The fat tissue around the right adrenal gland is dissected. From this side, the back of the IHIVC is skeletonized from the connective tissues and the RRA. The IHIVC is then tunneled using blunt micro-forceps. The root of the LV can be detected at the left side of the HIVC. This root is cauterized beforehand using an electrocautery scalpel to safely cauterize or ligate the LV and the RAV. The right lobes are retracted to the left side using wet gauze, and the ML without vessels can be detected behind the liver. This ML is cut sharply with micro-scissors, allowing easy mobilization of the liver. The micro-forceps are tunneled behind the HIVC from the left side to the right side, and the TST threaded through behind the HIVC. The micro-forceps are then tunneled behind the IHIVC from the right side to the left side, and the edge of the THT returned to the right side (**Fig. 11**). Thus, the vessels and fat tissue under the HIVC and the IHIVC are surrounded by TST. The RAV and the LV are ligated twice by repeating this procedure. The lower LV and LAV are cut after ligation with TST, and the junction of the LRV and the IHIVC is skeletonized. The ligations of the branches described above form good markers for the correct direction of the IHIVC at the cuff attachment.

The hepatoduodenal ligament is sharply cut and the common bile duct (CBD) isolated completely. The CBD is cut at the point on the pancreas, and the stent tube is inserted using the cut-down method. The stent tube is fixed with TST, which are set under the isolated CBD beforehand, and one edge of the TST should remained after this ligation for connection to the recipients' CBD (**Fig. 8**). Bile discharge from the inserted tube will be observed during the donor operation.

Complete isolation of the PV is required for the cuff method as safety fixation of the cuff requires a well-skeletonized PV trunk without any tissue; attached tissues cause stenosis in the cuff. The anatomy around the PV trunk can be seen in **Figure 7**. First, the PHA and GDA are dissected, and the GDA is cut after the ligation with TST. The PHA toward the hepatic hilus is isolated from the PV trunk. The CHA is dissected from surrounding tissues, although the connective tissue around the celiac and superior mesenteric arteries is hard. The PV trunk is constructed from three or four branches of the superior mesenteric vein at the point of the pancreas. The splenic vein and coronary vein flow into the PV trunk at the left side or the posterior side of the PV, and the pyloric vein flows into the PV trunk at the left side of the PV. In the rat, the posterior side of the PV trunk has a branch that connects with the IHIVC (**Fig. 7**). The ligations of these veins form

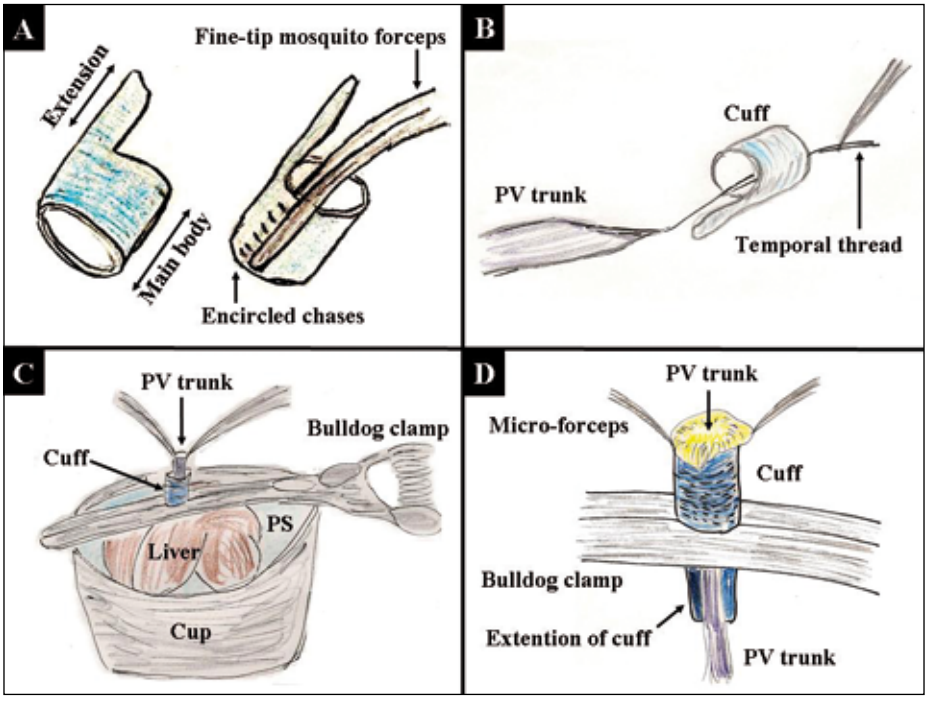


Figure 9. PV cuff attachment. A) The total cuff length should be shortened as much as possible. Encircled chases can be made using fine-tipped mosquito forceps in the IHIVC cuff, although these chases should be made by knifes in the PV cuff. **B)** A temporal suture by MPS is made at the edge of the PV trunk, and the PV trunk is passed through the PV cuff under this guide. **C)** The cuff extension and the PV trunk are grasped using a straight large-sized bulldog clamp. The cuff is set on the cup/glass using the edge of the preservation cup/glass and a long bulldog clamp. **D)** The wall of the PV trunk is completely reversed using micro-forceps.

useful markers for the correct direction of the PV trunk during cuff attachment.

Graft harvest

Heparinization (500 units/rat) is performed via the penile vein or a branch of the superior mesenteric vein. After the restoration from the retention of intraperitoneal organs, we wait one minute after this injection before ligation of the CHA at a point as near to the aorta as possible. Next, the IHIVC is clamped at an upper point of the RRV from the right side using a micro-clamp. The mesenteric PV branches are clamped using micro-clamps, and one of superior mesenteric branches is opened using the cut-down method under RS drips to prevent an air embolism. A 22 or 24 gauge peripheral catheter is subsequently inserted into the V. After confirmation of the tip position in the PV trunk, a 10 ml washout of cold PS (4°C) is started using the left hand. Portal injection should be performed slowly and carefully, without high pressure and any air. Note that any small volume of air will cause air thrombosis at distal branches of PV, and an irregular wash-out will occur. A thoracotomy is performed immediately, and the thoracic SHIVC is cut using the right hand. The IHIVC clamp is maintained during the wash-out procedure and after the cuff attachment. Wash-out is slowly continued without high pressure, and the PV trunk is clamped using a micro-clamp at the hepatic hilus. A change of graft color is verified without any dappled areas (**Fig. 10B**).

After wash-out, a cutting-off process is performed sharply in the order of the PV trunk, diaphragm, remnant ligaments behind the SHIVC, IHIVC, and the RAV/LV. Note that the IVIHC is cut in a branch patch-fashion using the IHIVC and the LRV for an easy insertion of the IHIVC cuff (**Fig. 6**). The PV trunk is also cut in a branch patch-fashion using the PV trunk and the splenic vein (**Fig. 7**). Finally, the whole liver is harvested and immediately placed into cold PS at 4°C. Note that hepatectomy made by hand-suture method shortened SHIVC margin for SHIVC suture. Then, longer IHIVC is required in S-OLT with liver graft made by hand-suture hepatectomy. On the other hand, in IHIVC reconstruction by not cuff method but hand-suture method, the upper level of LRV is enough.

Back table

All procedures should be performed on crushed ice. Any CIT and PS can be used according to the requirements of the liver regeneration experiments. In immunological experiments at our institution, a CIT of 30, 60 or 120 min is generally used.

Plasty of the SHIVC

The white tendon of the dorsal diaphragm is bilaterally grasped using curved bulldog clamps, which are used for retention (**Fig. 10C**). If the retention is insufficient, stay sutures using MPS are added. Trimming of the diaphragm should be performed from the side of the thoracic cavity. The anterior wall of the SHIVC is cut sharply as near to the diaphragm as possible, leaving enough margin for the suture. The anterior diaphragm is completely trimmed. The posterior wall of the SHIVC is carefully detected, and the bilateral edge of the white tendon is removed. Stay sutures using MPS (7-0) are then made on the bilateral edges of the SHIVC to perform the out-in process; a distance double that of the wall thickness is a guide for the size of the bite in the stay suture, while a distance similar to the wall thickness is used for the size of the bite in anastomosis sutures. The bilateral stay sutures are held separately with curved bulldog clamps, and the retention is performed using the stay sutures. The remaining white tendon is then removed with the margin of posterior wall of the SHIVC. A key technique for SHIVC plasty involves leaving enough margin of the wall and retention with the stay sutures (**Fig. 10D**). Under bilateral retention using the stay sutures, the posterior wall straightens, although the anterior wall can be slightly bow-shaped. The points of the stay sutures should be carefully verified again from the viewpoint of the HV and the HIVC flows. If an axis is twisted, the position of the stay sutures must be corrected before liver insertion.

Attachments of cuffs

Any fat tissue on the PV wall is completely removed, particularly in the portion of the cuff, to prevent considerable cuff stenosis. A suture with MPS (8-0) is made at the edge of the PV trunk, and the PV trunk is induced through the PV cuff under the guide of this temporal thread (**Fig. 9B**). The cuff extension and the PV trunk are grasped with a straight large-sized bulldog clamp. The cuff is set on the cup/glass using the verge of the preservation cup/glass and

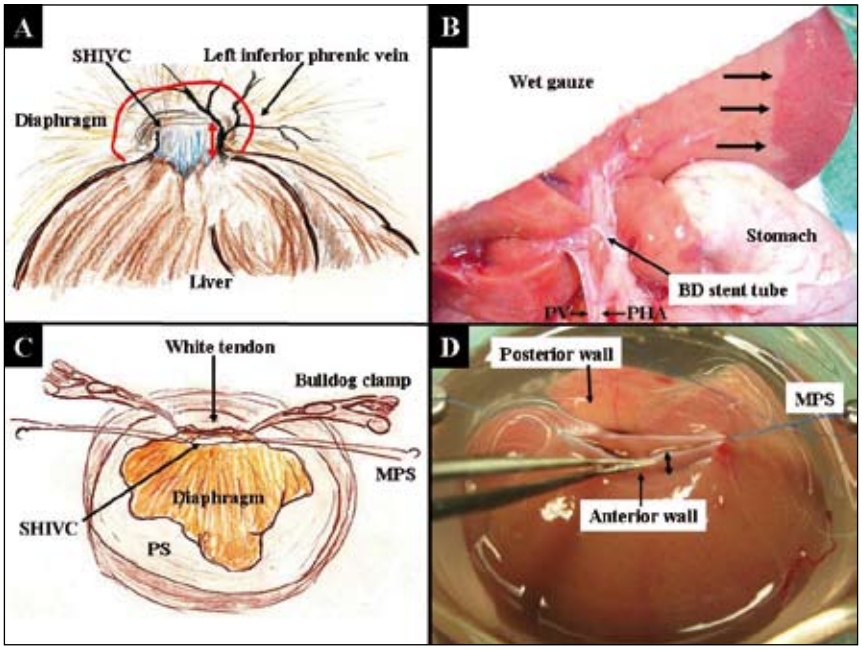


Figure 10. HV preparations in donor operation. A) The left inferior phrenic vein is well developed. This vein is skeletonized carefully and is ligated with TST. The complete dissection of this vein (arrow) is important for preventing massive hemorrhage after PR and to ensure adaptable out-flow. The diaphragm is cut during the wash-out procedure (curved line). **B)** The change in graft color is verified without any dappled areas (arrow), especially after the cutting-off of the thoracic SHIVC. **C)** The white tendon of the dorsal diaphragm is grasped bilaterally using curved bulldog clamps. The trimming of the diaphragm should be performed from the side of the thoracic cavity. **D)** The most important techniques for SHIVC plasty are 1) ensuring enough margin of the wall, and 2) the retention using stay sutures. In particular, sufficient margin of the SHIVC wall (arrow) is indispensable for confirmation of optimal out-flow.

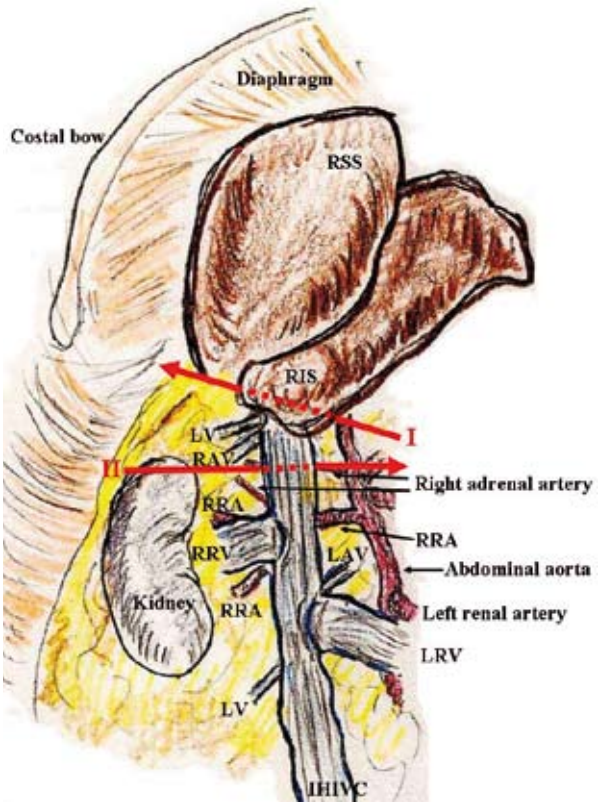


Figure 11. Processing of the RAV and the LV. The micro-forceps are tunneled behind the HIVC from the left side to the right side, and then TST is threaded through behind the HIVC (I). The micro-forceps are tunneled then behind the IHIVC from the right side to the left side, and the edge of the THT then returned to the right side (II). Finally, the RAV and LV are ligated without an engulfment of the HIVC/IHIVC.

a long bulldog clamp (**Fig. 9C**). The bulldog clamp is then fixed by the annular or pinky fingers of both hands during subsequent procedures. The wall of the PV trunk is completely reversed using micro-forceps (**Fig. 9D**). The PV wall is very thin, and the inner side is carefully detected using a PS drip. A circle of TST is made using the tip of a surgical instrument beforehand, and is set at the ligation point on the cuff (**Fig. 12A**). The reversed PV wall is fixed to the chase on the cuff by TST ligation (**Fig. 12B**). We recommend that this fixation is repeated twice at different points. Both fixations are made at the cuff body on the extension side if possible to prevent thrombosis. A clip on the hilar PV is maintained during this procedure.

The IHIVC cuff is also attached using a similar procedure to that for the PV cuff, although during the insertion of the IHIVC into the cuff, fine-tip micro-forceps can be inserted into the cuff to hold the edge of the IHIVC. A clip on distal side is maintained during this procedure. After attachment of the cuffs to the PV and the IHIVC, the patency of the cuffs and the closure of branches up to the clamp points are checked using a flush of PS through a 24 gauge catheter or dull-tip injector.

Graft survey

The rats should be checked for unexpected injury prior to insertion of the liver (**Fig. 13A**). The patency of the PV, IHIVC, and the HIVC are checked again using a cold RS flush of PS into these vessels after removal of the PV and IHIVC clamps. Particular attention should be paid to any thrombosis in the HIVC. The micro-clamps are placed on the hilar PV and the distal IHIVC.

Hepatectomy

The hepatectomy is performed using micro-clips. A key step in this clip method is the margin from the SHIVC (**Fig. 13B**). On the back table, micro-clip application is possible from any direction. The hepatic segment is retracted using tissue forceps when the micro-clip is applied (**Fig. 13C**). Clipping near the SHIVC to produce a strict hepatic volume results in twisting of the SHIVC and an unintended out-flow block, and HIVC occlusion can also occur during hepatectomy of the right lobes. If a strict hepatic volume is required then the hand-suture method is suitable. However, the SHIVC margin becomes shorter when performing a hepatectomy with no margins using hand-suture. As such, an IHIVC cut-off point is set at under the LRV prior to the donor operation (**Fig. 6**). The liver is cut-off sharply, and the cut surface is tightly sutured by continuous suture with MPS (8-0). The cut surface should be carefully checked before liver insertion (**Fig. 13D**).

In our institutions, a 20% graft is made by RSS+RIS, while a 30% graft is made by 'RSS+RIS' + 'SCS+ICS'. As such, an 80% graft and a 70% graft can be made by the removal of these 20% and 30% liver volumes, respectively. For a 40% graft, the liver segments used differ between hepatectomy methods based on the degree of inflow and outflow, previously in our institution. A'RMS+LMS' is used for the clip method, while 'LMS+LLS' is used for the hand-suture method. Currently, we mainly use a 40% graft of 'LMS+LLS' made by clip method, under thoughtful consideration of inflow and outflow. Although the removal of a 40% volume allows a 60% graft volume, this 60% graft should be made using RMS and LLS, and should not include superior and caudate segments.

Recipient operation

Based on our experience,[37] a successful surgery is guaranteed by at least three caveats: (i) anhepatic phase <20

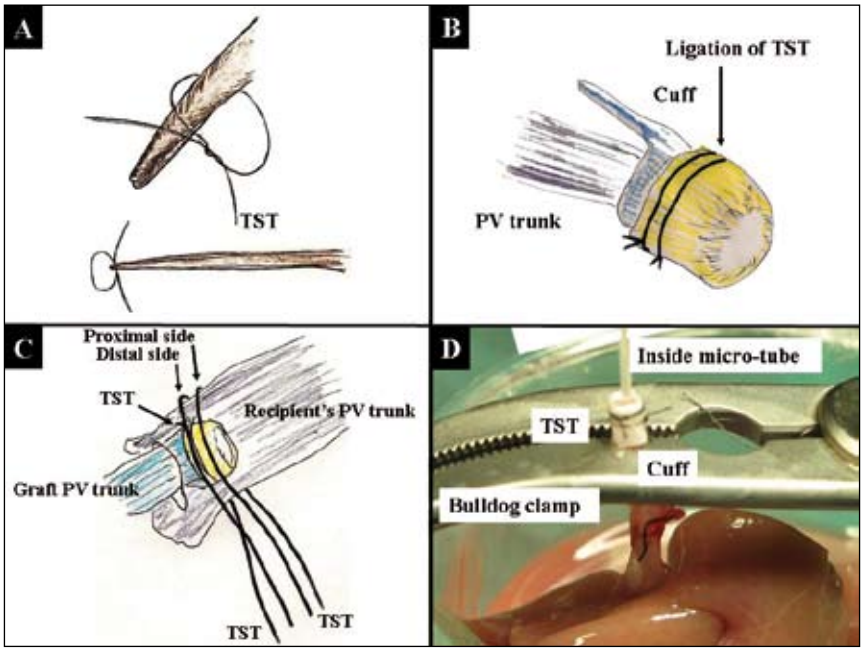


Figure 12. PV cuff attachment and PV reconstruction. A) A circle of TST is made using the tip of the surgical instrument beforehand, and is brought to the ligation point using micro-forceps. B) The reversed PV wall is fixed to the chase on the cuff by TST ligation. C) Direct contact of flowing blood to the TST causes thrombosis. The fixation of the PV cuff to the recipients' PV trunk should be performed as close to the proximal side of the cuff as possible. The fixation of the PV cuff to recipients' PV trunk is first made at the distal side, and then a ligation for the prevention of direct TST contact to blood is made at the proximal side. D) In the back table a bulldog clamp holds the PV trunk, micro-tube, and cuff extension. Because of the micro-tube inside, detection of the inner side is simple. We recommend that double ligation be used to fix the vascular wall to the cuff.

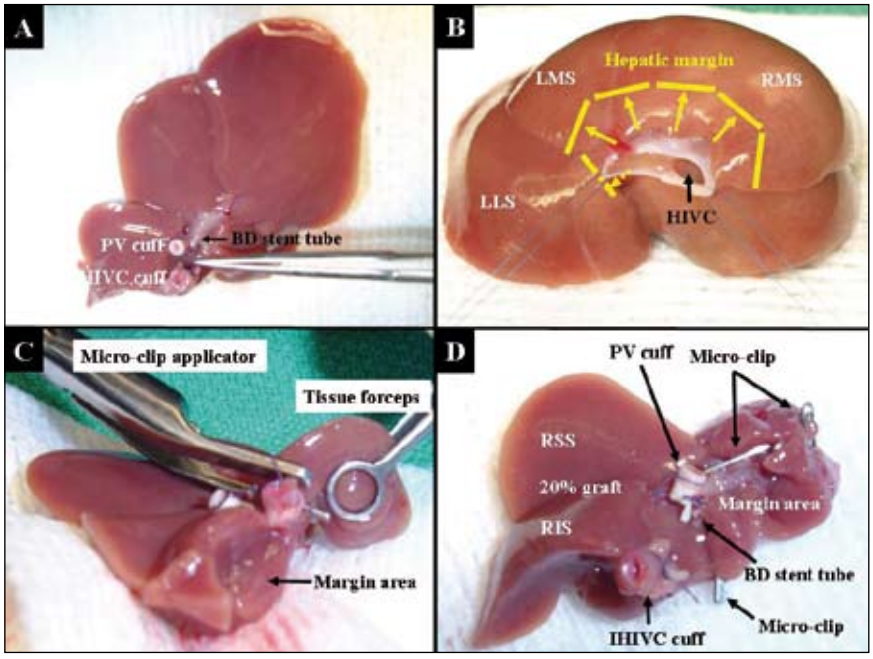


Figure 13. Hepatectomy in back table. A) Unexpected injury and patency of the cuff should be checked before liver insertion. **B)** A key step in the clip method is the hepatic margin from the SHIVC to prevent the occlusion of the SHIVC and HIVC flows. **C)** The hepatic segment is retracted using tissue forceps when the micro-clip is applied. **D)** A split liver graft with a margin area is made using the clip method. Graft survey before put-in procedure is important.

min (anhepatic phase <15 min, if possible), (ii) unstable systemic hemodynamic state <30 min, and (iii) a total of operative time, except for HA reconstruction, within 60 min. Especially, shortened anhepatic phase is a key for success.[37]

Anesthesia

The induction of anesthesia, measurement of body weight, and the injection of LRS are the same as for the donor operation. However, shaving is omitted or performed only over a small as possible area to maintain body temperature after surgery.

Laparotomy

The skin incision is made by either long midline, transverse, or reverse-T-shaped incisions. The surgical field can be obtained in each incision, but we usually chose long-midline skin incision. Based on the observations of post-operative behaviors, we recommend long-midline skin incision. A laparotomy is performed rapidly. Despite NS being indispensable during the donor operation, too great a volume on the intraperitoneal organs causes a low body temperature after recipient surgery. As such, only warm NS is used when necessary. Temporary retention of the abdominal wall by retractors is performed sparingly to prevent limitation of thoracic movements. Direct touch is possible in the recipient operation as the native liver is not use as a graft. The gastrointestinal tract must be moistened with warm NS. Additionally, as for the donor operation, wet gauze is useful when retention of the lobe and intestine are required.

Preparation before anhepatic phase

The procedures used to mobilize the whole liver are basically the same as for the donor operation. The cut-off point of the CBD is the hepatic hilus, and the CBD is isolated to the upper side of the pancreas. The GDA is ligated at the point of the root. The CHA is not dissected up to the root, but the PHA is isolated sufficiently from the PV. The PHA is ligated at the hepatic hilus. The dissection of the hepatic hilus is performed more clearly than in the donor operation to provide enough length and good mobility of the BD, PHA, and PV.

The PV trunk should be isolated to provide enough length for cuff insertion. Skeletonization of the PV trunk is the same as for the donor operation, except for the splenic vein. The PV trunk becomes easily to move after the ligation of the PV branches, as for the donor operation. Liga-

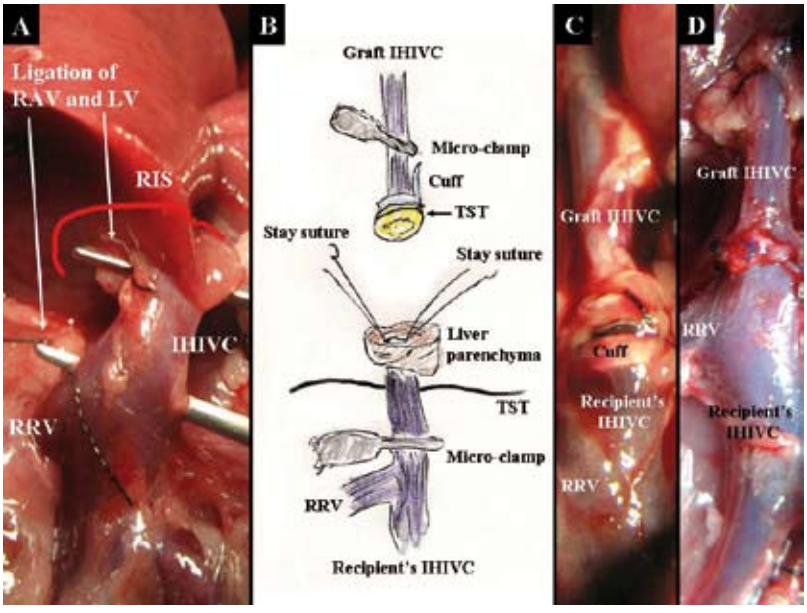


Figure 14. IHIVC reconstruction. A) The portion from the HIVC to the RRV is completely isolated, with preservation of the RRA and the RRV. Bleeding must be avoided around the dissected area. The HIVC in the RIS is cut at the upper point 3–5 mm from the border line of the IHIVC and the HIVC (curved line). **B)** Stay sutures are made bilaterally on the posterior wall, and the anterior wall has some allowance for the cuff insertion. The stay sutures are held by a bulldog clamp and are pulled to the cranial side. TST is set behind the recipients' IHIVC beforehand. Confirmation of the quality of the graft IHIVC is confirmed using an NS flush. The cuff is led towards the recipients' IHIVC, and the cuff is inserted into the IHIVC. **C)** The finding after reconstruction using the cuff method is shown. **D)** The finding after reconstruction using the hand-suture method is shown.

tion of these branches prevents the steal phenomenon in a small-for-size graft, and the expected pattern of damage will be obtained in samples from studies on portal hypertension in a small-for-size graft (**Fig. 7**).

For the IHIVC procedures, the portion from the HIVC to the RRV is completely isolated with preservation of the RRA and the RRV **(Fig. 14A)**. The RAV and LV are removed after specialized ligation, as for the donor operation (**Fig. 11**). After cutting of the dorsal ML of the HIVC, the connective tissues at the boundary line between the SHIVC and the diaphragm are carefully dissected to provide satisfactory extensibility of the SHIVC. The capability of the SHIVC forceps setting is confirmed prior to surgery, and thick threads such as 2-0 threads are temporarily placed behind the SHIVC to act as guides.

Removal of native liver

A total of 2.0–2.5 ml of LRS is injected beforehand. The clamps at the proximal sides are performed in the order of IHIVC from the right side and the PV trunk from the left side. The anhepatic phase then starts, and anesthesia is stopped. The hilar PV is directly grasped using mosquito forceps, or is ligated with TST then holding of the edge of the TST using mosquito forceps. Intra-hepatic blood sometimes disturbs smooth cutting of then SHIVC even after the PV flow is stopped. SHIVC forceps are set-up on the SHIVC including the diaphragm from the right side under the guide of a 2-0 thread. The 2-0 thread guide can be omitted in the approach from the left side as it is easy to avoid the esophagus. Nevertheless, the right-side approach is advantageous due to its HIVC axis orientation.

The SHIVC is cut with the required margin, without the liver parenchyma. Complete draining of retained blood from the liver and cleaning with NS and gauze are required for consistent surgical performance. The SHIVC margin for suture is important, and the SHIVC is carefully removed as close to the liver side as possible. The PV is removed at the most distal side. The HIVC in the RIS is cut at the upper point at 3–5 mm from the border line of the IHIVC and the HIVC (**Fig. 14A**), and removal of the native liver is complete.

Liver implantation (Put-in)

Any coagula are removed by gauze, and the intra-peritoneal cavity is cleaned. The presence of bleeding points should be carefully checked using a cotton swab because secure hemostasis is very difficult after liver insertion. Placement of the graft liver is important, and should be performed based on the anatomical characteristics of the graft liver. Gauze placement can be used for S-OLT cases to prevent a shift of axis and a fall into the subphrenic space. Note that an out-flow block can make the model unusable.

SHIVC reconstruction

At the graft survey, the point of bilateral sutures is so important. A straight posterior wall and an arched anterior wall should be confirmed beforehand **(Fig. 15A)**. A 5 or 10 ml syringe is deployed under the back of recipient at the point of the SHIVC. In some cases, the movement of the thorax stops; however, the heart rate remains stable. A retractor is used for the retention of the costal bows if the respiratory movement is satisfactory. Forceps are used to grasp the diaphragm and expose the ventral surface, then to pull caudally. The forceps are fixed by soft clay at an adequate point for easy and stable sutures, and sutures are placed bilaterally **(Fig. 15B)**. The posterior wall becomes straight and anterior wall is set as an arch. The left side is ligated, and the first thrusting is cleaved from outside of the graft SHIVC to the inside. The posterior wall is sutured from the left side using 5-6 stitches of continuous sutures. The last suture is stubbed to the outside of the recipient SHIVC, and this thread is ligated with a stay suture from the right side avoiding over-tightening **(Fig. 15C)**. The anterior wall is then sutured from the right side using 15–20 stitches of continuous suture. At 2/3's completion of the anterior wall suture, the intra-hepatic air is pushed out by a dull press of the liver around the SHIVC using a cotton swab, and the SHIVC cavity is filled with NS containing heparin using an L-shaped or a curved injector. The anterior suture is then finished, and this thread is ligated not too tightly with the stay suture from the left side (**Fig. 15D**). The back syringe and clay fixation are removed, and the retractors are released.

PV reconstruction

Retention of the recipient PV from the right side is achieved with mosquito forceps, and the forceps are fixed with soft clay. Note that too strong a retention makes it difficult to insert the cuff, despite increasing the PV length. The recipient PV is encircled beforehand with TST, and one knot is made for cuff fixation. The natural form of the PV is confirmed using an NS flush. The cuff is led onto the recipient PV. The PV is opened using the cut-down method at the nearest point of the hepatic hilus, and the inner side of the PV is confirmed with an NS flush (**Fig. 16A**). The cuff is inserted into the recipient PV avoiding any torsion of the PV. Direct contact of flowing blood to the TST causes thrombosis. Fixation of the PV cuff to the PV trunk of the recipient liver should be performed as proximal to the cuff as possible (**Fig. 16B**), while fix-

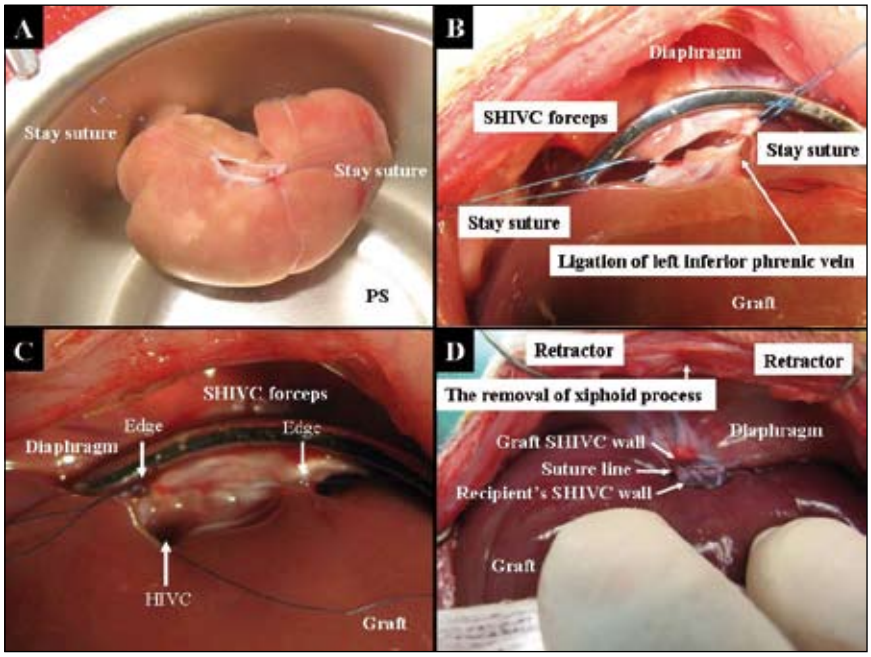


Figure 15. SHIVC reconstruction. A) Before the start of the recipient operation, a stay suture of the graft SHIVC is carefully made. The posterior wall straightens and the anterior wall is set as an arch. **B)** The stay suture is placed bilaterally after careful consideration of the setup. **C)** The finding after suture of the posterior wall is shown. The HIVC is confirmed to enter into the right lobe. **D)** Too tight a ligation causes stenosis of the IHIVC and disturbance of the IHIVC/HIVC flow. Ligations with stay sutures on both sides should be completed, although not too tightly.

ation to the reversed PV trunk is performed at the distal side (**Fig. 12C**).

PR (Graft recirculation)

The IHIVC clamp is checked before PR as massive bleeding occurs if the IHIVC is opened. The clamps are released in the order of the SHIVC then the PV, and the PR then starts. Warm NS is dripped onto graft, and the anhepatic phase ceases. Cardiac and respiratory movements are allowed to recover, particularly in the case of a whole liver graft, and anesthesia is resumed. If bleeding is detected on the cut surface of the liver, additional micro-clips can be used.

IHIVC reconstruction

The inner side of the IHIVC is easily detected by the adherent liver parenchyma (**Fig. 17A**). Stay sutures are made bilaterally on the posterior wall, and the anterior wall has some allowances for the cuff insertion. Stay sutures are held with a bulldog clamp and are pulled to the cranial side. The recipients' IHIVC is encircled beforehand with TST, and one knot is made for cuff fixation. The effectiveness of the graft IHIVC is confirmed using an NS flush, and the inner side is filled with NS. The cuff is led onto the IHIVC of the recipient liver. The cuff is inserted into the IHIVC avoiding any IHIVC torsion (**Fig. 14B**). Fixation of the cuff to the IHIVC should be performed as proximal to the cuff as possible, while fixation to the reversed IHIVC is performed at the distal side. The IHIVC clamps are released in the order of the graft then the recipient (**Fig. 14C**). The congestion of the right kidney and the dilatation of the IHIVC are immediately resolved, and the liver color improves. We can employ hand-suture method in IHIVC reconstruction (**Fig. 14D**).

After the reconstruction, there may be some difficulty in rechecking the point of cuff fixation, the torsion of the donor IHIVC, and the HIVC flow. If the total operative time is less than 60 min, then the hand-suture method is best for IHIVC reconstruction. The cutting-off points of the IHIVC are at the lower point of the RRV in the donor, and at the border line with the liver parenchyma in the recipient. Both IHIVCs are fixed using stay sutures avoiding any torsion, and hand-suture IHIVC reconstruction is performed as a continuous suture using MPS (8-0 or 7-0) as the SHIVC suture.

After IHIVC reflow, 2.0–2.5 ml of LRS is injected via the penile vein to improve blood pressure. If this injection causes the liver to pale, this is evidence of an occlusion in the SHIVC/HIVC. The SHIVC should be rechecked as re-

covery of blood pressure causes additional bleeding if the SHIVC anastomosis is poor (**Fig. 15D**). If required, a total of 0.3–1.0 mEq of bicarbonate can be injected simultaneously for the offset of metabolic acidosis.

HA reconstruction

An atraumatic small-sized clamp is placed on recipients' PHA. A clamp is not needed on the graft CHA as there is usually no back-flow. The connective tissues are completely removed. A sharp cut surface is made at the ends of recipients' PHA and graft CHA. An initial MNS (10-0) is threaded through the whole layer of the CHA from the outside to the inside, and a thrusting is made through the whole layer of the PHA from the inside to the outside. A reverse thrusting from the PHA to the CHA is then made using the same thread. Next, due to the difference in vessel diameter, the recipient PHA is fed into the graft CHA (**Fig. 17B**), and the vessel clamp is released. One or two superficial stitches are added if a bleeding occurs. This completes the 'vest and pant' method. Graft color is slightly improved after HA reconstruction (**Fig. 17C**).

By contrast, complete ultra-microsurgery allows the use of end-to-end anastomosis. First, the sharp surfaces are joined as the vessel diameter in the graft CHA and the recipient CHA are similar. Next, three or four stitches are made through the whole layer using an interrupted suture with MNS (10-0). Although additional superficial sutures are possible if a bleeding occurs, the initial usage of a cotton-like hemostatic agent with subtle compression is better. Intermittent and alternant clamping on the graft CHA and the recipients' CHA also achieves hemostasis. However, we do not recommend this method which depends on thrombosis and spasm. If required, a non-toxic dye can be used for the detection of intima.

BD reconstruction

The previously ligated TST of the recipients' CBD is held using mosquito forceps from the right side, and the mosquito forceps are fixed to a clay holder. The recipients' CBD is encircled beforehand with TST, and one knot is made for micro-tube fixation. The inner side of the recipients' CBD is detected using an NS drip. The micro-tube is led onto the recipients' CBD. The recipients' CBD is open using the cut-down method, and the micro-tube is inserted (**Fig. 8A**). The micro-tube is then fixed with TST ligation, and one edge of the ligated THT is also reserved as for the donor operation. To prevent the removal of the

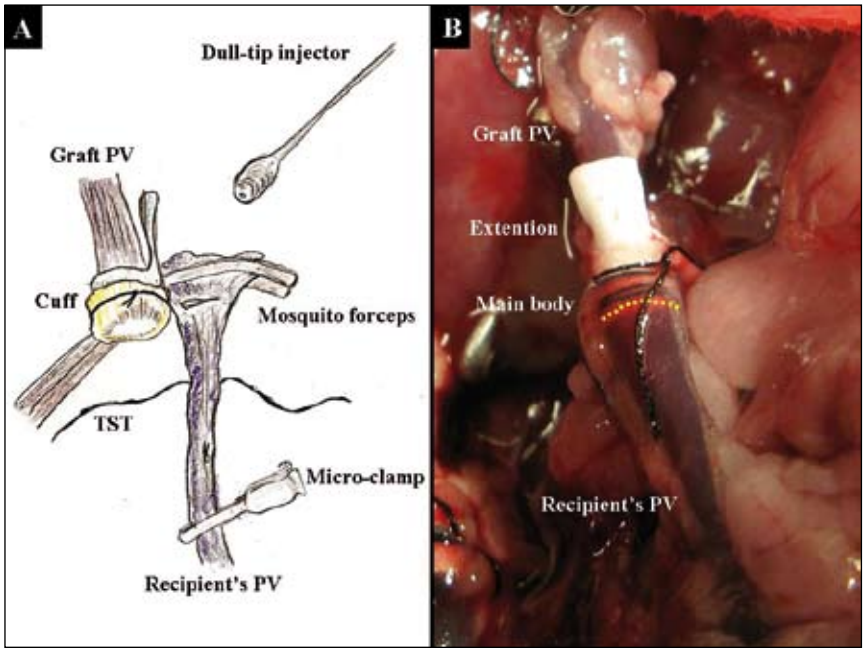


Figure 16. PV reconstruction. A) Retention of the recipient PV is performed using mosquito forceps from the right side. TST is set behind the recipient PV trunk beforehand. The natural form of the PV is confirmed using an NS flush. The cuff is led onto the recipient PV. The PV is opened using the cut-down method at the point nearest the hepatic hilus, and the patency of the inner side is confirmed by NS flush. The cuff is inserted into the recipient PV avoiding any torsion. **B)** The fixation of the PV cuff to recipients' PV trunk is first made at the distal side, and then a ligation for the prevention of direct TST contact to blood should be made at the proximal side (dotted line).

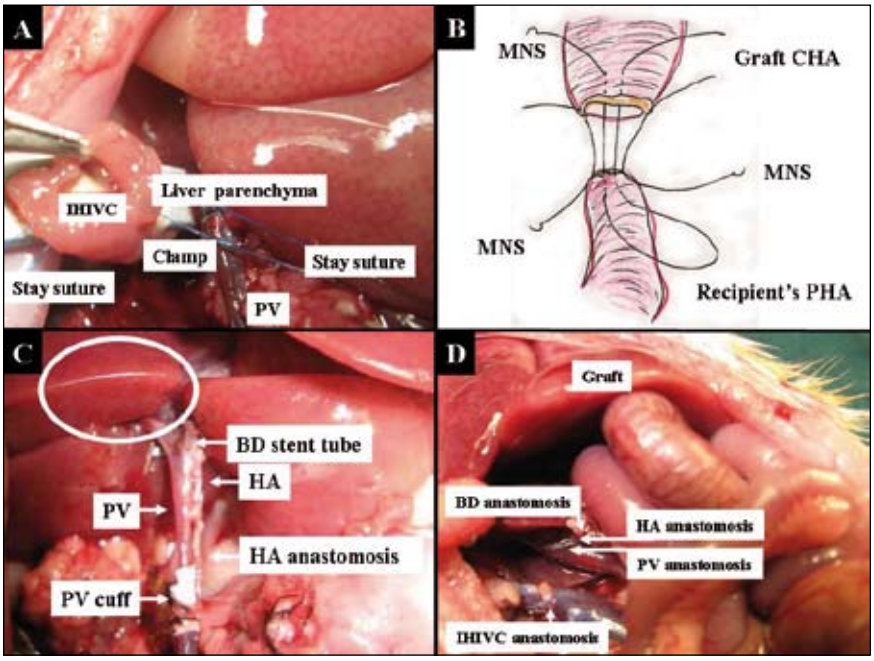


Figure 17. IHIVC and HA reconstructions, and intra-abdominal findings before abdominal closure. A) The inner side of the IHIVC is easily detected by the adherent liver parenchyma. **B)** An initial MNS is made through the whole layer of the CHA from the outside to the inside, and a thrusting is then made through the whole layer of the PHA from the inside to the outside. Subsequently, the reverse thrusting from the PHA to the CHA is made with the same thread. The recipients' PHA is then led into the graft CHA. One or two superficial stitches can be added if bleeding occurs. **C)** Graft color is slightly improved after HA reconstruction (circle). **D)** Anastomoses of PV, IHIVC, HA and BD.

micro-tube, the preserved TSTs in the donor and the recipient are ligated together (**Fig. 8B**).

Abdominal closure

The intraperitoneal cavity and organs are washed with warm NS, and all anastomoses should be carefully checked before abdominal closure (**Fig. 17D**). The point of BD anastomosis is covered with the greater omentum to prevent biliary complications. The peritoneum and fascia are closed with a continuous suture using absorbable thread, and the skin layer is closed separately using the same method to avoid removal of the sutures.

Post-operative care

The recipient is warmed on a hot pad immediately after surgery. A total of 2.0–2.5 ml of LRS or maintenance solution is injected via the penile vein, at least every 2 h after PR until 6 h after surgery. An analgesic agent (0.1 mg/kg) is routinely given intramuscularly every 8 h for 3–5 days after surgery. Use of antibiotics is normally not required, although can be administered intravenously (30 mg/kg) if required. Though post-operative care is tough, thoughtful care and strict observation are so important for successful OLT/S-OLT.

Survival curves and the estimation of graft damage

Each transplanted recipient is allowed to recover in an individual cage to prevent injury by cage mates. Checks for survival check are required every 2 h until 48 h after surgery. Quantitative scores are critical for reliable estimation of graft damage after surgery. Though apoptotic hepatocytes can be estimated based on characteristic features of nuclear and cytoplasmic condensation, the evaluation of apoptosis based on immunohistochemistry, such as terminal-deoxynucleotidyl transferase mediated dUTP nick end labeling (so-called TUNEL) and caspase-3, are usually used in our institution. The graft damage score (GDS) for histopathological analysis in our institution can be seen in **Table 3**.

Learning curves

The importance of learning curves in producing reliable data using the rat OLT/S-OLT model has been previously reported[9]. We have an impression that approximately 30 cases are required for initial achievement of several survivors, and that 50 cases are required to start the study[37]. The survival curves of the first 60 cases from one surgeon can be seen in **Figure 18A**.

Table 1. GDS after OLT/S-OLT

A total of each factor (point)	
1. Neutrophil aggregate	**4. Hepatocyte ballooning**
None = 0	**None = 0**
Minimal = 1	**Mild = 1**
Moderate = 2	**Moderate = 2**
Extensive = 3	**Severe = 3**
2. Monocular cell infiltration	**5. Microvesicular cholestasis**
None = 0	The percentage of involved hepatocytes
Minimal = 1	<5% of hepatocytes = 0
Moderate = 2	5-30% of hepatocytes = 1
Extensive = 3	30-60% of hepatocytes = 2
	>60% of hepatocytes = 3
3. Vacuolization	
None = 0	**6. Hepatocyte necrosis**
Area <10% = 1	None = 0
Area 10-60% = 2	Small foci = 1
Area >60% = 3	Confluent areas = 2
	Bridging necrosis = 3

The survival observation time was at least two days after surgery, and two day survivors were considered confirmation of a successful surgery as surgical problems cause more early deaths in the OLT model[9]. After the first 50 cases of OLT, survival rates > 0.8 were observed.

Critical factors for reliable sampling

As previous researchers described, this model required skillful techniques and surgical issues can not be ignored. Though many complications, such as twisted anastomosis and thrombosis will occur, bleeding is mainly fatal complication. In our experience of 50 cases with surgical issues, the survival time after surgery is 2.8±1.9 h (range: 1-8 h). Note that even SOLT cases may survive for several hours after surgery with complications.

Portal hypertension is one of important factors in the research of small-for-size graft. In S-OLT, the signs of portal hypertension (swelled graft and dilated PV) can be observed immediately after PR (**Fig. 19**).

Immediately after PR, many factors attack allografts in OLT/S-OLT, and we can obtain the liver samples. However, this model is not simple but so complicate. Especially in S-OLT cases accompanied with bleeding, important findings and essential damages were not observed in histopathological analysis (**Fig. 20**).

Some complications necessarily disrupt the data. Currently, autopsy finding and histopathological analysis are routinely performed in all OLT/S-OLT cases. If any complications and/or unreasonable histology are observed, we strictly omit these samples.

As described above, recipients with complication may survive for several hours. Therefore, after enough experiences of over 50 cases, we checked the survival rates and the rates of reliable samples at 6 h and 12 h after surgery. A total of 10 cases were performed for each time point, and each rate was calculated. This examination was repeated three times, and mean and standard deviation were shown in **Fig. 21**. In OLT, survival rates at 6 h and 12 h were 90.0±10.0, though the rates of reliable samples at 6 h and 12 h were 83.3±5.8 and 86.7±5.8. Hence, there were no discrepancy between survival rates and sampling rates in OLT. On the other hand, in S-OLT (40% graft, RS as PS, CIT for 2h), survival rates at 6 h and 12 h were 73.3±11.5 and 46.7±11.5, though the rates of reliable samples at 6 h and 12 h were 43.3±5.8. At 6h after S-OLT, there is significant differences between survival rates and sampling rates (p=0.0158), though no discrepancy are confirmed at 12 h after S-OLT.

DISCUSSION

Although the methods for ligation of the RAV and LV are complicated, we consider this process is important for performing these procedures safely. All procedures for the ligation of the RAV and the LV should not be made by a right side only approach, as this will induce unexpected injury accompanied by massive bleeding around the adrenal gland. Critical bleeding immediately leads to donor death before graft harvest and causes intra-hepatic thrombosis before the wash-out. The best use of the free-space behind the HIVC improves the procedure safety.

The HVs of the four segments flow directly into the HIVC. Note that we never open the IHIVC clamp during

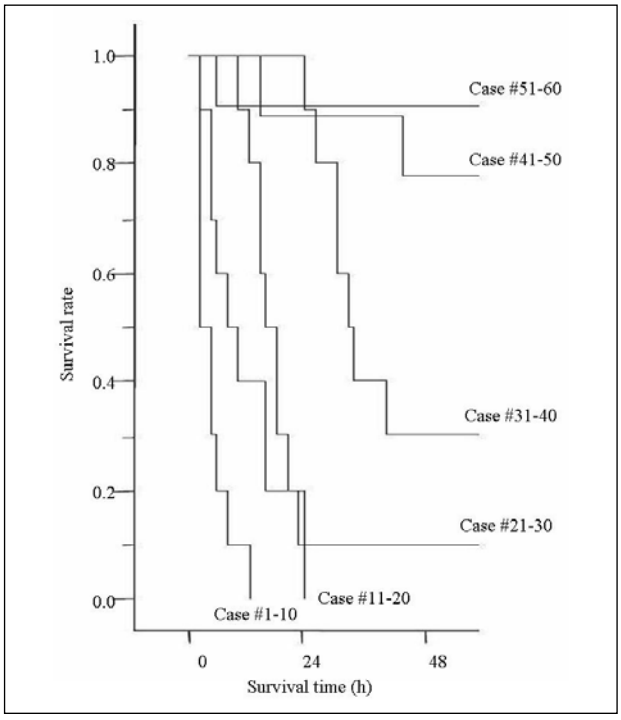


Figure 18. Learning curve in a surgeon. The initial 60 trial surgeries by one surgeon are shown. Whole liver grafts were used in all cases. All cases were accompanied by HA reconstruction. The PS and the CIT are unified as the RS for 2 h. The learning curves of each surgeon showed similar patterns. In order to achieve survival rate >0.8 or to start the sampling, an experiences of 50 OLTs are required at least.

the wash-out procedure as this can cause incomplete wash-out due to drainage from the PV to the HVC via these direct pathways. Hepatectomy for S-OLT can be performed in the donor operation, back table, and the recipient operation. In S-OLT cases, we have experienced unexpected bleeding from the hepatic cut surface and the poor recovery of cardiac and respiratory movements after PR. Thus, hepatectomy in the donor operation is useful for confirmation of hemostasis on the hepatic cut surface during surgery, while hepatectomy in the recipient operation is useful for the immediate recovery of cardiac and respiratory movements after PR. However, the clipping process introduces some problems in donor and recipient operations as the directions of surgical approach for application of the micro-clips are very limited. In the recipient operation in particular, too much retention during creation of the surgical field leads to injury of the anastomoses and the removal of the cuffs and the micro-tube. On the other hand, hepatectomy procedure on back table also has large advantage of an easy application of clip from any directions and no limitations of surgical field. Although continuous suture of the cut surface with an atraumatic needle after sharp cutting is an alternative technique, the most useful point of the micro-clip method is its simplicity. Any strict segmental hepatectomy can be achieved with the tight hand-suture method; however, unexpected massive bleeding often occurs immediately after PR. The areas of the margin for the micro-clip method have no hepatic functions as they do not have sufficient intra-hepatic circulation. These margin areas actually change color in autopsy findings, and always exhibit atrophy in long-term survivors.

A previous study investigating the regenerative capacity of individual lobes after hepatectomy in a murine hepatectomy model demonstrated that the caudate lobes work as well as the remnant liver, especially after a 75% hepatectomy[44]. Furthermore, successful survivals were reported in recipients receiving 20% grafts without HA reconstruction[45]. Thus, some previous researcher suggested that even 20-30 % graft-transplanted recipients can be survive for long term[45], our results in S-OLT with 20% graft (RS as PS and CIT for 2h) will die within 8 h after surgery. One possible explanation is our model makes a complete

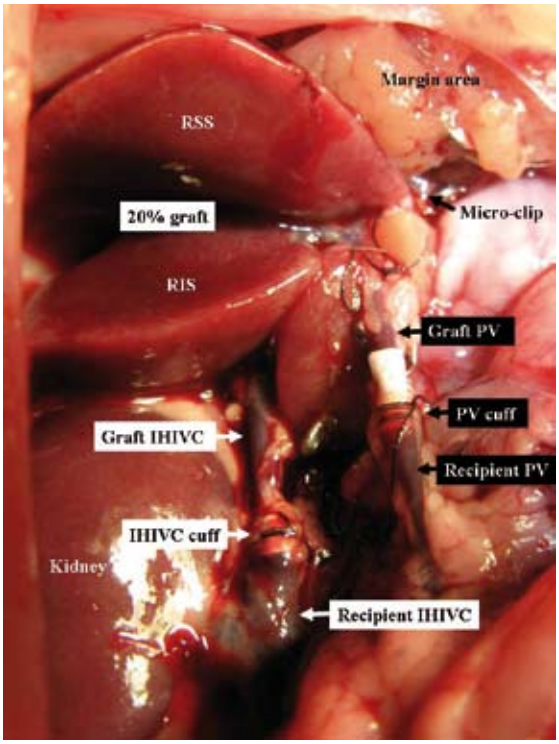


Figure 19. Obvious congestion of a small-for-size graft immediately after PR. Operative findings in SOLT revealed extreme enlargement of a small-for-size graft after PR. Ligation of the PV branches prevents the steal phenomenon in a small-for-size graft, and the expected damage in the rat model occurs in studies focused on portal hypertension due to a small-for-size graft.

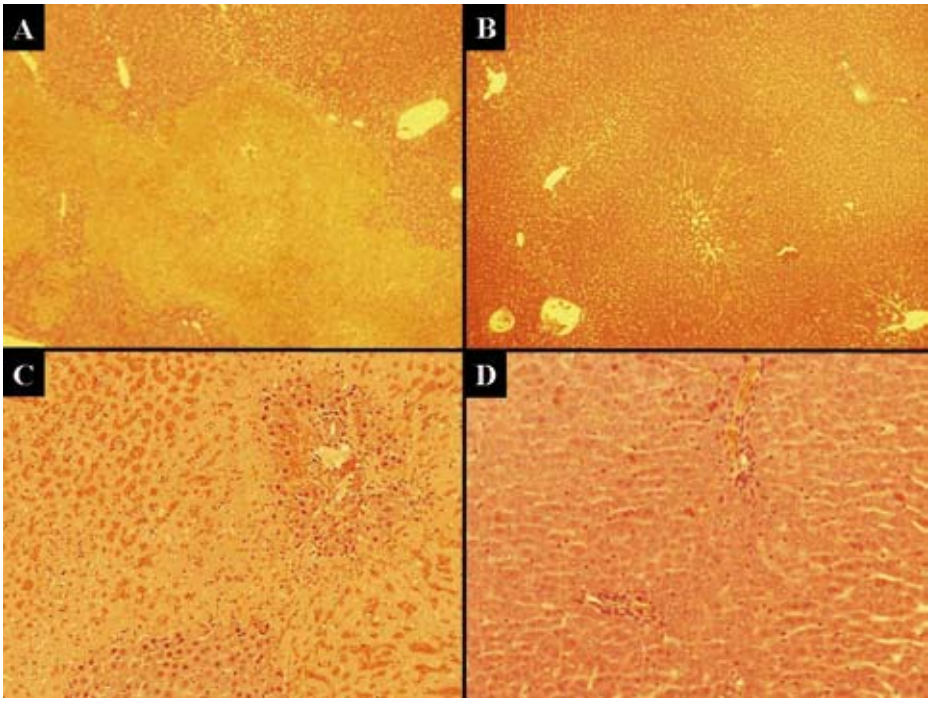


Figure 20. The differences based on histopathological analysis in S-OLT recipients with or without complication. A) Histopathological findings at 6 h after S-OLT (40% graft, RS as PS and CIT for 1 h) without any complications is shown. Massive necrosis was clearly observed. **B)** Histopathological findings at 6 h after S-OLT (40% graft, RS as PS and CIT for 1 h) accompanied with bleeding is shown. This recipient survived at 6 h after S-OLT, even accompanied with bleeding. Although only hepatocyte ballooning was partially observed, this finding lacked an essential graft damage. **C)** Histopathological findings at 6 h after S-OLT (20% graft, RS as PS and CIT for 2 h) without any complications is shown. Allograft was severe damaged. **D)** Histopathological findings at 6 h after S-OLT (20% graft, RS as PS and CIT for 2 h) accompanied with bleeding is shown. This recipient survived at 6 h after S-OLT, even accompanied with bleeding. Although graft damages were partially observed, there was obvious difference in comparison with S-OLT without any complications (**C**).

ligation of porto-caval branch in order to steal phenomenon, and then complete portal hypertension will attack small-sized grafts. Thus, we suggested that our model of S-OLT with 20-30% grafts reflected real portal hypertension without any shunt flow. The 30% graft is made using the caudate segments, while the 10% graft is possible in this model by using the caudate lobe. However, we do not use the caudate lobe for creating 70% or 40% grafts as the caudate lobe showed reduced regenerative capacity in the 30% and 60% hepatectomy model compared with other lobes[44]. Based on the behavior of the caudate lobe in large hepatic remnants, we perform a 60% graft without SCS, ICS, RSS, or RIS. The set-up of 20% and 30% grafts is well established in our model for the studies of small-for-size liver grafts.

The 40% graft is clinically important in liver regeneration due to the split and pediatric grafts in cadaveric donor for donor shortage in the United States, and the shift to left-lobe graft in a living-donor for donor safety in Japan. It should be noted that when using our experimental model, there are distinct differences between basic anatomy and surgical anatomy. In basic anatomy, the LMS is aggregated with the RMS, and an incomplete lobulation is often detected in those segments. However, dominating vessels of the LMS make a common channel with those of the LLS. Thus, the LMS should be handled together with the LLS with respect to in-flow and out-flow in surgery. Actually, we need to distinguish the selection of segments according to each method for the 40% graft. As no-margin hepatectomy by hand-suture did not injure this common channel and did not twist the SHIVC/HIVC, the 'LMS+LLS' is used in this method. Although hepatectomy with parenchymal margin by hand-suture causes a larger cut surface and increases the risk of unintended bleeding, the clip method works well for this method. Paradoxically, no-margin hepatectomy by the clip method causes SHIVC twist, especially during removal of the RMS. The hepatic margin in the clip method prevents the SHIVC twist and common channel injury. The parenchymal margin has no hepatic circulation (these areas are in-

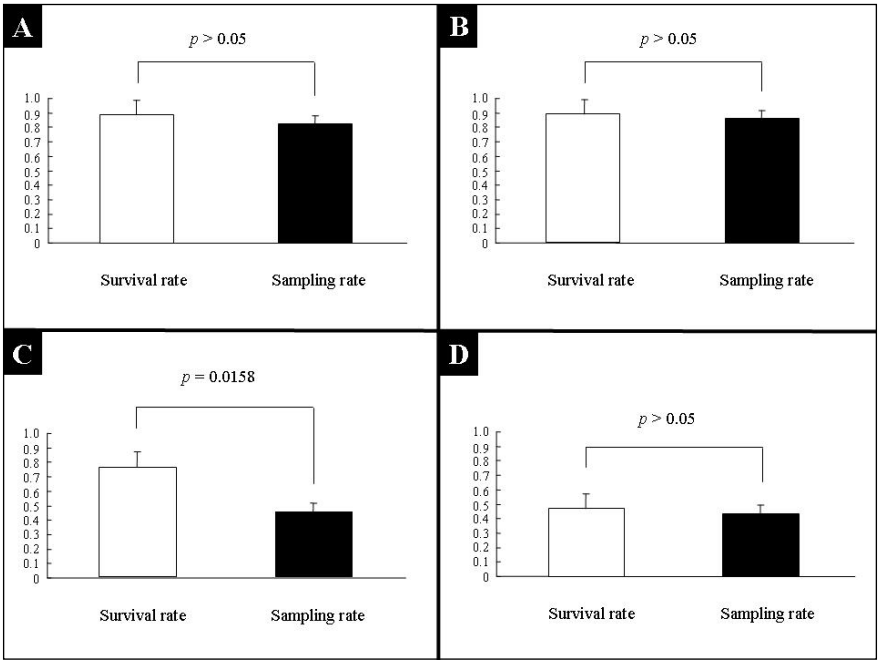


Figure 21. The discrepancy between survival rates and sampling rates, especially at early post-operative period in S-OLT. Currently, autopsy finding and histopathological analysis are routinely performed in all OLT/S-OLT cases. If any complications and/or unreasonable histology are observed, we strictly omit these samples. The OLT/S-OLT recipients with complication may survive for several hours. Therefore, after enough experiences of over 50 cases, we checked the survival rates and the rates of reliable samples at 6 h and 12 h after surgery. A total of 10 cases were performed for each time point, and each rate was calculated. This examination was repeated three times, and mean and standard deviation were shown at each time point and graft type. White bar (□) expresses survival rate, though black bar (■) expresses the rate of reliable samples. (**A**) Each rate at 6 h after OLT (RS as PS and CIT for 1h) is shown. There were no discrepancy between survival rates and sampling rates. (**B**) Each rate at 12 h after OLT (RS as PS and CIT for 1h) is shown. There were no discrepancy between survival rates and sampling rates. (**C**) Each rate at 6h after S-OLT (40% graft, RS as PS and CIT for 1h) is shown. There were significant differences between survival rates and sampling rates ($p<0.05$). (**D**) Each rate at 12 h after S-OLT (40% graft, RS as PS and CIT for 1h) is shown. There were no discrepancy between survival rates and sampling rates. Overall, we have to consider that some complications necessarily disrupt all of the experiments, and we should omit unreliable samples if any complications and/or unreasonable histology are observed.

significant as a functional volume), and we initially use 'LMS+RMS' as a 40% graft accompanied by the margin method. Although removal of the RMS requires several micro-clips, LLS can be usually treated with only one micro-clip. Thus, for procedure simplicity, we recommend a 'RMS+LMS' 40% graft using the clip method of hepatectomy accompanied with a margin, although the 'LMS+LLS' 40% graft without margin is ideal based on surgical anatomy. This simple and useful method with margin areas prevents unintended injury of the common channel, avoids twisting of the SHIVC/HIVC, and allows easy application to incomplete lobulation. Currently, our model shifted to 'LMS+LLS' as a 40% graft accompanied by the margin method. As described above, clip method has a large advantage in S-OLT model. However, the percentage of the actual graft weight to the recipients' native liver cannot be calculated due to margin weight. Therefore, we estimated the percentages of each segment in 100 rats. A previous detailed report in a rat hepatectomy model used a ligation method with a hepatic margin[39], and we strongly recommend the clip method with a hepatic margin in the rat S-OLT model.

The PV cuff procedures are often difficult due to its thin wall and small diameter, while the IHIVC cuff procedures are comparatively easy due to its larger diameter. In small PV cases, we perform the following procedure. The PV trunk is clamped using the micro-clamp near the hepatic hilus to prevent air embolism, and a soft micro-tube is inserted into the PV trunk from the point of the washout injection. Next, another micro-clamp is placed on the PV trunk and the micro-tube. In the back table, a bulldog clamp holds the PV trunk, micro-tube, and cuff extension. Due to the micro-tube inside, detection of the inner side of the vessel is very easy, even in thin and small PV cases (**Fig. 12D**). However, care must be taken to avoid air em-

bolism and injury of the PV trunk during this procedure. The same procedure can be also applied for the attachment of the IHIVC cuff if required.

The cuff method cannot be used in SHIVC reconstruction. The setting for the HV flow after liver insertion should be carefully considered, as the initial setting impacts on all following procedures. An important consideration for the SHIVC setting is that it is different from the human, as the HIVC flows into the right lobes. Bilateral retention using accurate stay sutures at both edges is indispensable from the start of the procedure. With respect to operative time, a running suture of the anterior wall is faster than an interrupted suture. The thread used in the running suture should be ligated at both edges with some allowance to prevent stenosis, although too loose a ligation causes bleeding after PR. The clamp for the SHIVC needs an optimal bite on the thoracic side, as a shallow bite totally disturbs the SHIVC suture and slows down the surgery, although too deep a bite results in cardiac and respiratory arrest. SHIVC reconstruction should be completed using only thin SHIVC walls, and never include the liver or diaphragm in order t out-flow block and thrombosis. As for HV reconstruction, in contrast to humans, rats have no extra-hepatic margins in each HV. Thus, there is no choice except for the anastomosis of the SHIVC to the SHIVC. A twist of the SHIVC causes out-flow block, and out-flow complications destroy the experimental setup. Even under when attempting rapid surgery due to the limitation of the anhepatic phase, all IHIVC reconstruction procedures, including adequate initial setting, smooth hand-suture, and optimal ligation, should be perfectly completed.

We consider that the cuffs may cause the occlusion of blood flow in long-term survivors, although based on our experience, the cuff method produces no survival problems up to 14 days recovery. Since the PV is thin and small, a kink of the trunk, torsion of the axis, and incorrect insertion can easily occur. Shortened anhepatic phase to less than 15 min is critical during both PV reconstruction and SHIVC reconstruction.[37] Although we and others use a hand-suture (MNS 10-0) even for PV reconstruction,[12] we consider the cuff method indispensable for keeping the anhepatic phase <15 min and for obtaining reliable data. Based on the results of the present study, we perform the late phase of the recipient operation on a hot pad to prevent hypothermia. Furthermore, we keep transplanted rats covered with a cylindrical warmer to reduce heat loss immediately after surgery.

The importance of training for liver transplantation was previously reported.[9,16] In the first study, 65 rat OLTs were performed by a single investigator for training. The first 39 OLT were required to master the technique, and included 23 recipients that died in the first 24 hours due technical deficits and 16 OLT to learn the technique. In our experience, 20 OLTs are required to get an overnight survivor, while 40 OLTs are required to get a one week survivor. Thus, we suggest that 40–50 OLTs per surgeon are necessary for complete learning, while more OLTs are required for an amateur microsurgeon or non-surgeon. As previously reported[9,16], surgical issues can occur, even in long term survivors, and we initially estimated a reliable sampling rate for assays of approximately 0.6–0.9, even when experienced microsurgeons perform the surgery. Surprisingly, sampling rate is lower as shown. However, we have to consider that some complications necessarily disrupt all of the experiments. Currently, autopsy finding and histopathological analysis are routinely performed in all OLT/S-OLT cases, and this check system seem to work well. If any complications and/or unreasonable histology are observed, we should omit these samples. Strict elimination of unsuitable rats at the sampling time point is also important for reliable data in this model. Although a large amount of time and labor is required for even a small number of reliable samples, this model will provide drastic data and will produces valuable results, because this model is not fake but real transplantation model including CIT.

Co-instantaneous reconstruction of the HA is ideal for studies focused on liver regeneration, although the omission of HA reconstruction is fine for studies focused on transplant immunity. HA reconstruction requires the most skillful ultra-microsurgery (approximately 0.1–0.3 mm vessel diameter), and extended anesthesia and operative times. Overall, we consider that OLT without HA reconstruction allows more stable sampling, even in studies of liver regeneration in which sampling should be 24 h after PR, due to the following: (i) anesthetic and operative times have a marked impact on survival, and prolongation of these times will cause unexpected problems with the animal preparation; (ii) in our preliminary studies there were no differences in GDS at 24 h after PR between with or without HA reconstruction, although longer term survivors do exhibit GDS differences; and (iii) the frequency of anesthetic complications is increased. For the surgeon, both a finely-honed concentration and a non-nervous distraction are required in microsurgery and ultra-microsurgery. If a microscope is employed, we recommend limiting its use to for reconstructive procedures only to avoid fatigue. In particular, ultra-microsurgery is introduced at only limited times, while continuous microsurgery during all steps is unadvisable due to the likely loss of mental focus. If possible, the preparation of the surgical equipment

is better than repeating a surgery in the same day to obtain enough samples.[39]

In summary, although the rat OLT/S-OLT with HA reconstruction model is difficult and complicated, this model is well established[9-23] and provides clinically relevant data. We hope that our surgical guide will also help many researchers with an interest in the LT field.

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