

Innovative Magnetic Anastomosis Enables Immediate Liver Transplantation Without Cold Preservation

Hang Liu

First Affiliated Hospital of Xi'an Jiaotong University

Peng Liu

First Affiliated Hospital of Xi'an Jiaotong University

Jiawei Yu

First Affiliated Hospital of Xi'an Jiaotong University

Qiang Lu

First Affiliated Hospital of Xi'an Jiaotong University

Yerong Qian

First Affiliated Hospital of Xi'an Jiaotong University

Junxi Xiang

First Affiliated Hospital of Xi'an Jiaotong University

Aihua Shi

First Affiliated Hospital of Xi'an Jiaotong University

Shasha Wei

First Affiliated Hospital of Xi'an Jiaotong University

Yi Lyu

First Affiliated Hospital of Xi'an Jiaotong University

Lifei Yang

yanglifei826@126.com

First Affiliated Hospital of Xi'an Jiaotong University

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Abstract

Background

Living donor liver transplantation (LDLT) traditionally relies on static cold preservation, which can trigger significant ischemia-reperfusion injury due to abrupt temperature shifts. This study investigated the feasibility of immediate liver transplantation without cold storage using rapid magnetic anastomosis techniques.

Methods

Eighty-eight adult male Sprague-Dawley rats were randomly divided into two groups: Cold Storage Transplantation Group (CSTG) and Immediate Transplantation Group (ITG). Partial liver transplantation was performed using magnetic anastomosis, with the ITG group undergoing direct transplantation without cold preservation. Thermal imaging, histological analysis, oxidative stress markers, cellular proliferation, apoptosis, liver function tests, and inflammatory responses were evaluated at 1- and 5-days post-transplantation.

Results

The ITG demonstrated several key advantages: more stable temperature profiles during transplantation, significantly reduced oxidative stress during the ex vivo phase, enhanced hepatocyte proliferation and decreased cellular apoptosis ($P < 0.05$ at day 5). Liver function markers showed improved recovery in the ITG, with significantly lower alanine aminotransferase (ALT) and higher albumin (ALB) levels on day 5 ($P < 0.05$ at day 5). The 14-day survival rates were comparable between groups (80% ITG vs. 70% CSTG, $P = 0.629$).

Conclusions

Immediate liver transplantation using magnetic anastomosis appears feasible and potentially beneficial, offering a novel approach that mitigates ischemia-reperfusion injury by avoiding cold storage. This method demonstrates promising outcomes in terms of graft viability, cellular regeneration, and functional recovery. Further research in large animal models is warranted to validate these findings for potential clinical application.

1. Introduction

Living donor liver transplantation (LDLT) has emerged as a vital solution to alleviate the shortage of donor livers^{1,2}. Similar to traditional liver transplantation, current LDLT practices involve the static cold

preservation of liver grafts prior to transplantation³. However, the abrupt shift in temperature upon warm reperfusion of the cold organ can in itself constitute a genuine trigger for significant impairments in the respiratory control ratio and subsequent graft dysfunction after transplantation (temperature paradox)^{4,5}. Unlike traditional liver transplantation, LDLT is typically performed within the same medical center, allowing for controlled surgical timelines and eliminating the need for logistics and transportation. This raises a crucial question: Is cold preservation truly necessary? Given the inherent vulnerability and insufficient size of the liver, is it essential for the organ to undergo cold preservation and subsequent rewarming? Could the liver avoid the detrimental ischemia-reperfusion injury caused by the temperature shift?

In traditional liver transplantation, one of the primary reasons for the necessity of cold preservation is the prolonged duration vascular anastomosis time. During vascular anastomosis, following cold preservation, the liver is placed into the recipient's abdominal cavity. This process typically lasts approximately 40 to 80 minutes⁶, during which the liver lacks blood supply. Despite being wrapped in ice gauze, the liver's temperature tends to rise⁷, resulting in ischemic and hypoxic conditions that can lead to hepatic injury⁸. Therefore, effective cold preservation is essential to mitigate these risks. However, our team's novel magnetic anastomosis technology significantly alters this process. Magnetic anastomosis is a swift vascular reconstruction technique that has been applied in rapid liver transplantation^{9,10} and laparoscopic liver transplantation^{11,12}. Compared to traditional manual suturing, it facilitates faster organ transplantation^{9,10}. By utilizing magnetic anastomosis, the entire procedure can be reduced to approximately 20% of the traditional duration, with our team achieving an anhepatic phase lasting only 9 minutes¹¹. This brief period without blood supply is entirely tolerable, as the hepatic portal blocking time during hepatectomy significantly exceeds this duration¹³. Thus, the entire living donor liver transplantation process can be simplified to resemble the replacement of a Lego part: removing the donor liver and immediately placing it into the recipient's abdominal cavity for rapid implantation.

Based on the aforementioned considerations, we hypothesize that utilizing the rapid magnetic anastomosis technique, it is feasible to directly anastomose the liver graft with the recipient's vasculature without prior flushing and cold preservation, thereby avoiding the ischemia-reperfusion injury process. Following hepatectomy, the liver graft can be promptly placed into the recipient's abdominal cavity and quickly revascularized using magnetic anastomosis.

In this study, we employed a rat model of partial liver transplantation to simulate LDLT. Our primary objective was to test our hypothesis by comparing the outcomes of the cold storage transplantation group (CSTG) with the immediate transplantation group (ITG). Specifically, we evaluated oxidative stress, cellular proliferation and apoptosis, immune rejection, liver function, inflammatory response, survival rate, and other critical parameters. Our findings demonstrate that the ITG effectively reduces oxidative stress, enhances hepatocyte proliferation, decreases cellular apoptosis, and facilitates a more rapid recovery of liver function compared to traditional cold storage methods.

2. Methods

2.1. Animal grouping

A total of 88 male Sprague-Dawley rats (44 donors and 44 recipients), aged 8 weeks and weighing 250–300 g, were obtained from the Experimental Animal Center of Xi'an Jiaotong University. The rats were randomly allocated into two groups. The CSTG (n = 22) underwent standard procedures where donor livers were flushed with cold (4°C) Histidine-Tryptophan-Ketoglutarate (HTK) solution (Custodiol HTK; Dr. Franz Köhler Chemie GmbH, Germany) and preserved before transplantation. In contrast, the ITG (n = 22) had donor livers transplanted directly without flushing or cold preservation. Recipient rats were euthanized at 1- and 5-days post-transplantation, with 6 animals per group at each time point, to obtain graft livers and blood samples for further analysis. The rats were anesthetized and euthanized by exposure to 5% isoflurane in oxygen in accordance with institutional guidelines. All rats were maintained under standard laboratory conditions throughout the study. Any animals that experienced unsuccessful procedures at any stage were excluded from the study. The animal experiments were conducted in accordance with regulations and guidelines. The study protocols were approved by the Committee on the Ethics of Animal Experimentation at Xi'an Jiaotong University.

2.2. Liver transplantation

2.2.1. Donor liver harvest

The procedure was conducted following a previously established protocol¹⁰, with slight modifications. All rats underwent a 12-hour fast with access to water before surgery. Anesthesia was induced and maintained using isoflurane, with meticulous attention given to minimizing animal distress. To optimize graft viability and reduce ischemia time, donor and recipient surgeries were conducted concurrently by two separate surgeons.

A cruciform incision was used to access the abdominal cavity. The liver was mobilized by dissecting surrounding connective tissues and falciform ligaments. The common bile duct (BD) was transected and cannulated with a modified 24-gauge intravenous cannula. The infrahepatic inferior vena cava (IHVC) was isolated to the left renal vein level, with the right renal and suprarenal veins ligated and divided. A 50% hepatectomy (Fig. 1) was performed on the donor rat using Omura et al.'s method¹⁴, removing the left lateral (LL), caudate lateral (CL), and left median (LM) lobes (Fig. 1). The donor was then heparinized via the dorsal penile vein with 2 mL of heparinized saline (50 U/mL).

In the CSTG group, the remnant donor liver was perfused through the abdominal aorta with 10 mL of HTK solution containing 25 U/mL heparin (4°C) at a rate of 3 mL/min using an infusion pump. The graft was then excised and placed in a 4°C saline bath for graft preparation.

In the ITG group, the suprahepatic vena cava (SHVC), portal vein (PV), and IHVC were immediately divided without initial flushing. The livers were placed in room temperature (20–25°C) saline solution and

directly transplanted into the recipient's abdominal cavity following graft preparation.

2.2.2. Graft preparation

The graft preparation involved two critical steps: cuff fixation and magnetic ring installation for the SHVC. The PV and IHVC cuff fixation was performed following the method established by Kamada and Calne¹⁵. Polyethylene cuff tubes were applied to the PV (outer diameter: 2.1 mm, inner diameter: 1.8 mm) and IHVC (outer diameter: 2.8 mm, inner diameter: 2.6 mm). For the SHVC, a titanium oxide-neodymium-iron-boron (Ti-NdFeB) magnetic ring, with a major axis diameter of 8 mm and a minor axis diameter of 5.5 mm, was secured using a 6 – 0 silk suture through a diaphragm loop.

2.2.3. Recipient operation

With the collaboration of two operators, the preparation of the graft liver and the removal of the recipient liver were nearly completed simultaneously. A magnetic ring was applied to the recipient's SHVC until the vessel adequately covered its edge. SHVC reconstruction was accomplished by coupling the magnetic rings embedded in both donor and recipient SHVCs through magnetic force. The PV and IHVC were reconstructed using the cuff technique. Following the release of clamps on the PV and SHVC, hepatic blood flow reperfusion was initiated. BD anastomosis was performed using a stent technique. Finally, the abdominal incision was closed.

2.3. Thermal monitoring of liver grafts

During donor liver procurement and transplantation, a thermal imager (T630SC, Teledyne FLIR LLC, USA) was employed to record temperature changes at several key time points: *in vivo*, *ex vivo*, pre-reperfusion, and at 1- and 5-minutes post-reperfusion. The average temperature of the entire liver area was calculated, along with the rate of temperature change per unit time.

2.4. Vascular patency assessment post-transplantation

Inferior vena cavography (PLX7000B High Frequency Mobile C-arm System, Nanjing Perlove Medical Equipment LLC, China) and duplex ultrasonography (ACUSON X 150 Diagnostic Ultrasound System, Siemens Medical Solutions Inc., USA) were performed 5 days post-transplantation to evaluate the patency of the inferior vena anastomosis.

2.5. Histological and molecular analysis

Rats were euthanized on days 1 and 5 post- transplantation for histological analysis. Hematoxylin and eosin (H&E) staining was performed to assess pathological changes. The rejection activity index (RAI) was calculated based on the Banff scoring system¹⁶, with blinded assessments of the animal tissue samples conducted by expert pathologists.

Immunofluorescence techniques were employed to analyze reactive oxygen species (ROS), cell proliferation, and apoptosis. Specifically, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) staining

served as a ROS marker, Ki67 indicated cellular proliferation, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was utilized to detect apoptotic cells.

2.6. Serum analysis

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), and total bilirubin (TBIL) were measured using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. To evaluate the immune response following transplantation, serum levels of interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) were quantified using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's protocols.

2.7. Statistical analysis

All analyses were conducted in a blinded manner. Data are presented as means $\pm$ standard error. Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Comparisons between two groups were conducted using two-tailed Student's *t*-tests, while multi-group comparisons employed one-way analysis of variance (ANOVA). Semi-quantitative analysis of fluorescence images was carried out using ImageJ software (version 1.51f, National Institutes of Health, USA)¹⁷ via an automated script. Statistical significance was set at $P < 0.05$. In all figures, asterisks denote levels of significance as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

3. Results

3.1. Surgical outcomes of liver transplantation

This study utilized a specialized surgical approach with custom components (SHVC magnetic ring, IHVC cuff, PV cuff, and BD stent) for vascular and biliary anastomoses, as shown in Fig. 2A. Liver implants in the ITG appeared bright red in vitro due to the absence of flushing, while those in the CSTG exhibited a khaki color resulting from flushing (Fig. 2B).

Figure 2C demonstrates the typical surgical procedure for magnetic anastomosis liver transplantation. The SHVC magnetic anastomosis technology enabled suture-free, instantaneous anastomosis (Fig. 2B-ii). Following the reconstruction of the PV (Fig. 2B-iii), liver blood flow rapidly recovered, and the organ color transitioned to dark red (Fig. 2B-iv). The mean total operation time was 29.97 ± 2 minutes for the ITG and 28.8 ± 2 minutes for the CSTG, with anhepatic periods of 9.97 ± 2 minutes and 9.8 ± 2 minutes, respectively. No statistically significant differences were observed in total operation time or anhepatic period between groups (Fig. 2D).

Vascular patency and blood flow were evaluated 5 days after transplantation using angiography and Doppler ultrasonography. Angiography confirmed patency of the PV and IVC and verified the proper positioning of the paired elliptical magnetic rings (Fig. 2E). Doppler ultrasonography revealed robust blood flow signals in the IHVC, with a measured velocity of 3 cm/s (Fig. 2F). These findings confirmed

successful liver transplantation procedures across all experimental groups, with no significant surgical variations, thereby establishing a comparable baseline for subsequent analyses.

3.2. Thermal profiles of liver grafts during transplantation

Rapid temperature fluctuations significantly contribute to oxidative stress. Temperature changes during the surgical procedure were monitored using thermal imaging, with results detailed in Fig. 3. In the ITG, liver graft temperatures initially remained stable, gradually decreasing from $35.4 \pm 1^\circ\text{C}$ to room temperature ($21.4 \pm 0.7^\circ\text{C}$) *ex vivo*, at a cooling rate of $1.4^\circ\text{C}/\text{min}$ (Fig. 3B-C). After placement in the recipient's abdominal cavity, the graft temperature increased slowly, reaching $29.0 \pm 0.5^\circ\text{C}$ just prior to reperfusion, with a warming rate of $0.76^\circ\text{C}/\text{min}$. Post-reperfusion, the temperature rose more rapidly, returning to $34.3 \pm 0.3^\circ\text{C}$ at a rate of $0.58^\circ\text{C}/\text{min}$. In the CSTG, liver grafts were maintained at $7.8 \pm 0.9^\circ\text{C}$ during cold preservation, with an initial cooling rate of $2.7^\circ\text{C}/\text{min}$. After transfer to the recipient's abdominal cavity, graft temperature gradually increased to $25.4 \pm 0.3^\circ\text{C}$ before reperfusion, at a rate of $1.76^\circ\text{C}/\text{min}$. Following reperfusion, temperature steadily rose to $34.0 \pm 0.3^\circ\text{C}$ at $1.36^\circ\text{C}/\text{min}$.

Comparative analysis of thermal imaging demonstrated that liver grafts in the ITG group maintained a more stable temperature profile, characterized by a gradual decline and subsequent increase, thereby avoiding the abrupt thermal shock observed in CSTG grafts. In contrast, CSTG liver grafts experienced a pronounced initial temperature differential and exhibited a slower rate of temperature normalization following reperfusion compared to ITG grafts.

3.3. Histopathological Evaluation

H&E staining (Fig. 4) revealed no significant differences in liver morphology between the ITG and CSTG groups. Both groups exhibited well-preserved cellular structures and lobular architecture with minimal inflammation or necrosis. The RAI scores, evaluated using the Buff system, showed a trend toward being lower in the ITG group on days 1 and 5 post-transplantation, although the difference was not statistically significant (Fig. 4B). These results suggest that direct transplantation does not elevate the risk of liver rejection, indicating its potential safety and feasibility in this context.

Additionally, the liver weight increase (Fig. 4C) showed no significant difference between ITG and CSTG on days 1 and 5, suggesting normal hepatic regeneration unaffected by direct transplantation.

3.4. Oxidative stress dynamics

Using DCFH-DA as an H_2O_2 probe, we found that ROS production in the ITG group was significantly lower during the *ex vivo* stage, suggesting reduced oxidative stress due to gradual temperature changes (Fig. 5A, C). Both groups showed a sharp ROS increase upon reperfusion, peaking at 1-hour post-reperfusion, reflecting acute oxidative stress. By days 1 and 5 post-transplantation, ROS levels were comparable between the groups.

3.4.2. Cellular proliferation analysis

After partial liver transplantation, graft regeneration restores liver mass and function. Ki67 immunofluorescence staining, a marker of proliferation, revealed distinct patterns of Ki67-positive nuclei in ITG and CSTG (Fig. 5B). Quantitative analysis showed a significantly higher proliferation index in ITG on day 5 post-transplantation ($P < 0.05$, Fig. 5D), consistent with the trend in liver weight gain (Fig. 4C), indicating enhanced regeneration in ITG.

3.4.3. TUNEL staining

TUNEL assays were used to assess apoptosis in the transplanted liver grafts. As shown in Fig. 5B and quantified in Fig. 5E, significantly fewer TUNEL-positive cells were observed in the ITG compared to the CSTG on days 1 and 5 post-transplantation ($P < 0.05$), indicating reduced apoptosis and cellular damage. Combined with enhanced proliferation (Ki67 staining), these findings demonstrate the superiority of immediate transplantation in preserving graft viability and promoting regeneration.

3.4.4. Liver function

Liver function was evaluated using serum AST, ALT, ALB, and TBIL levels at 1- and 5-days post-transplantation. AST and ALT levels decreased over time, indicating reduced liver injury (Fig. 6A). ALT levels were significantly lower, and ALB levels higher, in the ITG compared to the CSTG on day 5 ($P < 0.05$), suggesting improved recovery and protein synthesis in the ITG. TBIL levels showed no significant difference, though slightly elevated in the CSTG on day 5. These findings highlight that immediate transplantation promotes faster and more stable liver function recovery.

3.5. Inflammatory response

As shown in Fig. 6B, inflammatory cytokine levels (IL-1 β , IL-6, and TNF- α) varied between CSTG and ITG over time. At 1-day post-transplant, IL-1 β levels were elevated in CSTG but not significantly different from ITG. By day 5, IL-1 β levels significantly increased in ITG ($P < 0.05$), suggesting a delayed inflammatory response. IL-6 levels were similar between groups at 1 day but rose significantly in ITG on day 5 ($P < 0.05$). TNF- α levels showed no significant differences between groups at either time point. The delayed increase in inflammation in ITG by day 5 may be due to the lack of flushing, leaving residual donor blood in the liver, which could contribute to elevated inflammatory markers.

3.6. Survival Rate

Figure 6C shows the Kaplan-Meier survival curve for ITG and CSTG over 14 days. The 14-day survival rate was 80% for ITG and 70% for CSTG, with no significant difference between the groups ($P = 0.629$). This suggests that immediate transplantation without cold storage does not compromise survival.

4. Discussion

This study aimed to investigate the feasibility and potential benefits of immediate liver transplantation without cold preservation in a rat model of partial liver transplantation. Our findings support our initial hypothesis that utilizing rapid magnetic anastomosis technique, it is possible to directly anastomose the

liver graft with the recipient's vasculature without prior flushing and cold preservation, thereby mitigating the ischemia-reperfusion injury process. The ITG demonstrated several advantages over the CSTG, including reduced oxidative stress during the *ex vivo* phase, enhanced hepatocyte proliferation, decreased cellular apoptosis, and more rapid recovery of liver function. These results suggest that immediate transplantation without cold storage may be a viable alternative to traditional methods in living donor liver transplantation, potentially mitigating ischemia-reperfusion injury and improving graft function.

Our findings reveal a crucial link between temperature management and liver graft viability. The thermal imaging data (Fig. 3) demonstrated that ITG liver grafts experienced a more gradual temperature change compared to the CSTG. This temperature stability appears to be the key factor in reducing oxidative stress during the *ex vivo* phase, as evidenced by lower levels of ROS production in the ITG (Fig. 5). This reduction in initial oxidative stress likely set the stage for the cascade of positive outcomes observed in the ITG^{7,18}. The lower oxidative burden may have preserved cellular integrity and mitochondrial function, creating a more favorable environment for hepatocyte survival and function. As a consequence of this reduced initial damage, we observed enhanced hepatocyte proliferation and decreased apoptosis in the ITG at 5 days post-transplantation (Fig. 5). This improved cellular health and regenerative capacity ultimately contributed to the faster recovery of liver function in the ITG, as indicated by the lower ALT and higher ALB levels on day 5 (Fig. 6). These findings are consistent with previous research highlighting the detrimental effects of temperature management on liver regeneration and function^{19,20}. Our study extends this understanding by demonstrating that avoiding cold storage and maintaining a more stable temperature profile can mitigate the initial oxidative insult^{21–26}, thereby promoting a chain of events leading to improved graft viability and function in the context of living donor liver transplantation²⁷.

The feasibility of immediate liver transplantation without cold storage is predicated on the ability to perform the procedure rapidly, minimizing the time the liver spends outside the body. This approach draws inspiration from the Pringle maneuver^{13,28} in clinical practice, where intermittent clamping of hepatic blood flow (currently considered safe for 15 to 20 minutes) is used to reduce bleeding during liver resection without causing serious side effects. The rationale is that if liver transplantation can be completed quickly, similar to temporarily blocking hepatic blood flow, it theoretically would not cause severe damage to the transplant. This concept is supported by previous studies using magnetic anastomosis technology, which have achieved a record 9.5-minute anhepatic phase^{6,29}, suggesting that cold preservation may be unnecessary within such a short duration. In our current study, we performed magnetic anastomosis reconstruction of the SHVC only, which significantly shortened the anastomosis time^{30,31}. This rapid anastomosis technique is crucial for the success of the no cold storage method, as it directly addresses the primary concern of prolonged ischemia time. If the liver remains anoxic for too long, the resulting damage could outweigh the benefits of avoiding hypothermia. Additionally, prolonged exposure to higher temperatures requires adequate nutrients and oxygen to maintain normal metabolism.

In this study, we used partial liver transplantation to simulate LDLT, where only a portion of the donor's liver is transplanted. LDLT offers advantages such as better graft quality control, surgical timing, and location. Additionally, being typically performed between close relatives, it involves milder immune rejection, making it more suitable for immediate transplantation. Our findings of reduced oxidative stress and improved cellular proliferation in ITG support its potential in LDLT. In contrast, DCD liver transplantation may be less suitable due to poorer graft quality and ischemia-reperfusion injury. However, controlled donor liver procurement may allow immediate transplantation in such cases. The magnetic anastomosis technique used in our study also reduces LDLT complexity, lowering the steep learning curve, and could facilitate broader adoption, especially in regions with lower surgical volumes^{32–34}.

Another important issue is the elevated inflammatory markers (IL-1 β and IL-6) on day 5 post-transplantation, despite no pathological signs of rejection in H&E staining or RAI (Fig. 4). This is likely due to the lack of liver flushing during graft procurement. To save surgery time and reduce ischemia, we omitted flushing, leaving donor blood, including immune cells, in the graft. This residual blood likely contributed to the inflammation. Notably, no anti-rejection treatments were used in this study. We believe immunosuppressive therapy or initial flushing with room-temperature solutions (e.g., HTK or Ringer's) could improve liver function recovery and proliferation. Future studies should prioritize room-temperature flushing to mitigate immune responses caused by donor blood in the recipient.

Rapid magnetic anastomosis liver transplantation with immediate transplantation shows promise for clinical application, especially in LDLT. This approach simplifies the surgical procedure, potentially making LDLT more accessible while reducing oxidative stress, promoting hepatocyte proliferation, and facilitating faster liver function recovery. To optimize this method for clinical use, future research should explore room-temperature flushing, oxygenated perfusion, and ATP-rich preservation solutions³⁵. While particularly suited for LDLT, this technique could also benefit traditional liver transplantation when donor procurement is controllable. Further studies are needed to refine the protocol and validate its efficacy and safety in clinical settings. By streamlining the procedure and improving graft viability, this approach could significantly advance liver transplantation, ultimately benefiting patients awaiting this life-saving procedure.

This study has several limitations that should be addressed in future research. Primarily, we used a small animal model, which presents some inherent differences from human liver transplantation. The smaller liver volume in rat results in more rapid temperature changes compared to human livers, where larger volume leads to slower temperature fluctuations, especially in the core³⁵. Additionally, the liver transplantation procedure in small animals differs significantly from clinical LDLT. These factors may limit the direct translation of our findings to human applications. Therefore, further validation of this immediate transplantation approach in large animal models of living donor liver transplantation is necessary before considering clinical trials.

Conclusion

In conclusion, this study demonstrates the feasibility and potential advantages of immediate liver transplantation without cold preservation using magnetic anastomosis techniques. Our findings reveal that the immediate transplantation exhibited several significant benefits compared to traditional cold storage methods, including more stable temperature profiles, reduced oxidative stress during the ex vivo phase, enhanced hepatocyte proliferation, and decreased cellular apoptosis. The improved outcomes were reflected in better liver function recovery, particularly evident in lower ALT and higher ALB levels by day 5 post-transplantation. These results suggest that immediate transplantation could be a viable alternative to traditional cold storage methods, particularly in living donor liver transplantation settings where surgical timing can be precisely coordinated. The comparable survival rates and absence of significant rejection further support the potential of this innovative approach. Future research should focus on validating these findings in large animal models and exploring the clinical applicability of this novel transplantation technique.

Abbreviations

ALT, alanine aminotransferase

ALB, albumin

ANOVA, analysis of variance

AST, aspartate aminotransferase

BD, bile duct

CL, caudate lateral (lobe)

CSTG, Cold Storage Transplantation Group

DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate staining

ELISA, enzyme-linked immunosorbent assay

HA, hepatic artery

H&E, Hematoxylin and eosin staining

HTK, Histidine-Tryptophan-Ketoglutarate solution

IHVC, infrahepatic inferior vena cava

IL-1 β , interleukin-1 β

IL-6, interleukin-6

ITG, Immediate Transplantation Group

LDLT, Living donor liver transplantation

LL, left lateral (lobe)

LM, left median (lobe)

PV, portal vein

RAI, rejection activity index

ROS, reactive oxygen species

SHVC, suprahepatic vena cava

TBIL, total bilirubin

Ti-NdFeB, titanium oxide-neodymium-iron-boron

TNF- α , tumor necrosis factor- α

TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling assay

Declarations

Authorship

Hang Liu: Contributed to research design, participated in data analysis, and was involved in the writing of the paper.

Peng Liu: Participated in data analysis, and was involved in the writing of the paper.

Jiawei Yu: Participated in the performance of the research, contributed to data analysis.

Qiang Lu: Participated in data analysis.

Yerong Qian: Participated in the writing of the paper.

Junxi Xiang: Contributed to the research design, assisted in data collection, and participated in the analysis of findings.

Aihua Shi: Participated in the analysis of findings.

Shasha Wei: Participated in the analysis of findings.

Yi Lv: Contributed to the research design and participated in the analysis of findings.

Lifei Yang: Contributed to the research design, participated in the performance of the research and helped in reviewing and revising the manuscript.

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest.

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References

1. Raia, S. & Neto, J. O. S. Living donor liver transplantation: 35 years of saving lives. *Lancet* **403** (10427), 614–615 (2024).
2. Vasavada, B. Living-donor liver transplant for patients with end-stage liver disease. *JAMA Surg.* **158** (4), 427 (2023).
3. Takizawa, D. et al. Changes in apparent systemic clearance of propofol during transplantation of living related donor liver. *Br. J. Anaesth.* **95** (5), 643–647 (2005).
4. Minor, T., Efferz, P., Fox, M., Wohlschlaeger, J. & Lüer, B. Controlled oxygenated rewarming of cold stored liver grafts by thermally graduated machine perfusion prior to reperfusion. *Am. J. Transpl.* **13** (6), 1450–1460 (2013).
5. von Horn, C., Zlatev, H., Kathis, M., Paul, A. & Minor, T. Controlled oxygenated rewarming compensates for cold storage–induced dysfunction in kidney grafts. *Transplantation* **106** (5), 973–978 (2022).
6. Zhang, X. G. et al. Fast vascular reconstruction with magnetic devices in liver transplant: a novel surgical technique. *Liver Transpl.* **27** (2), 286–290 (2021).
7. Olthof, P. B. et al. Protective mechanisms of hypothermia in liver surgery and transplantation. *Mol. Med.* **21** (1), 833–846 (2015).
8. Nasralla, D. et al. A randomized trial of normothermic preservation in liver transplantation. *Nature* **557** (7703), 50–56 (2018).
9. Lu, Q. et al. Liver transplantation using magnetic anastomosis in pigs. *Sci. Rep.* **13** (1), 20143 (2023).
10. Yang, L. et al. A rat model of orthotopic liver transplantation using a novel magnetic anastomosis technique for suprahepatic vena cava reconstruction. *J. Vis. Exp.* 2018;(133).

11. Liu, X. M. et al. Laparoscopic-assisted full-sized liver transplantation with magnetically fast portal vein anastomosis: an initial cohort study. *Int. J. Surg.* **110** (9), 5483–5488 (2024).
12. Feng, Z. et al. Magnetic-assisted laparoscopic liver transplantation in swine. *Hepatobiliary Pancreat. Dis. Int.* **21** (4), 340–346 (2022).
13. Imamura, H. et al. Pringle's manoeuvre in living donors. *Lancet* **360** (9350), 2049–2050 (2002).
14. Omura, T., Ascher, N. L. & Emond, J. C. Fifty-percent partial liver transplantation in the rat. *Transplantation* **62** (2), 292293 (1996).
15. Kamada, N. & Calne, R. Y. Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. *Transplantation* **28** (1), 47–50 (1979).
16. Demetris, A. J. et al. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* **25** (3), 658–663 (1997).
17. Jensen, E. C. Quantitative analysis of histological staining and fluorescence using imagej. *Anat. Rec (Hoboken)*. **296** (3), 378–381 (2013).
18. Zabala, V. et al. Transcriptional changes during hepatic ischemia-reperfusion in the rat. *PLoS One*. **14** (12), e0227038 (2019).
19. Vajdová, K. N., Smreková, R. T., Mišlanová, C., Kukan, M. R. N. & Lutterová, M. Cold-preservation-induced sensitivity of rat hepatocyte function to rewarming injury and its prevention by short-term reperfusion. *Hepatology* **32** (2), 289–296 (2000).
20. Schlegel, A., Kron, P., Graf, R., Dutkowski, P. & Clavien, P. E. R. A. Warm vs. Cold perfusion techniques to rescue rodent liver grafts. *J. Hepatol.* **61** (6), 1267–1275 (2014).
21. Wang, S. et al. Ischemia-free liver transplantation improves the prognosis of recipients using functionally marginal liver grafts. *Clin. Mol. Hepatol.* **30** (3), 421–435 (2024).
22. Guo, Z. et al. A randomized-controlled trial of ischemia-free liver transplantation for end-stage liver disease. *J. Hepatol.* **79** (2), 394–402 (2023).
23. Choi, H. J. The impact of ischemia-free liver transplantation on improving the prognosis of recipients using functionally marginal liver grafts: editorial on ischemia-free liver transplantation improves the prognosis of recipients using functionally marginal liver grafts. *Clin. Mol. Hepatol.* **30** (4), 702–704 (2024).
24. Lin, J. et al. Substantial decline of organ preservation fluid contamination following adoption of ischemia-free liver transplantation: a post-hoc analysis. *Int. J. Surg.* **110** (5), 2855–2864 (2024).
25. He, X. et al. The first case of ischemia-free organ transplantation in humans: a proof of concept. *Am. J. Transpl.* **18** (3), 737–744 (2018).
26. Guo, Z. et al. Abrogation of graft ischemia-reperfusion injury in ischemia-free liver transplantation. *Clin. Transl Med.* **12** (4), e546 (2022).
27. Liu, J. & Man, K. Mechanistic insight and clinical implications of ischemia/reperfusion injury post liver transplantation. *Cell. Mol. Gastroenterol. Hepatol.* **15** (6), 1463–1474 (2023).

28. Sugawara, Y. et al. Increased nitric oxide production in the liver in the perioperative period of partial hepatectomy with pringle's maneuver. *J. Hepatol.* **28** (2), 212–220 (1998).
29. Song, J. S., Wang, F. M., Zhang, J. W. & Zhang, X. F. Magnetic compression anastomosis in vascular surgery: a comprehensive review of techniques and clinical applications. *Magn. Med.* **1** (2), 100023 (2025).
30. Jamshidi, R., Stephenson, J. T., Clay, J. G., Pichakron, K. O. & Harrison, M. R. Magnamosis: magnetic compression anastomosis with comparison to suture and staple techniques. *J. Pediatr. Surg.* **44** (1), 222–228 (2009).
31. Shi, Y. et al. Magnetic ring anastomosis of suprahepatic vena cava: novel technique for liver transplantation in rat. *Transpl. Int.* **28** (1), 89–94 (2015).
32. Rela, M. & Rammohan, A. Why are there so many liver transplants from living donors in asia and so few in europe and the us? *J. Hepatol.* **75** (4), 975–980 (2021).
33. Hibi, T., Wei Chieh, A. K., Chi-Yan Chan, A. & Bhangui, P. Current status of liver transplantation in asia. *Int. J. Surg.* **82**, 4–8 (2020).
34. Ikegami, T., Balci, D., Jung, D. H., Kim, J. M. & Quintini, C. Living donor liver transplantation in small-for-size setting. *Int. J. Surg.* **82**, 134–137 (2020).
35. Chullo, G. et al. Focusing on ischemic reperfusion injury in the new era of dynamic machine perfusion in liver transplantation. *Int. J. Mol. Sci.* **25** (2), 1117 (2024).

Figures

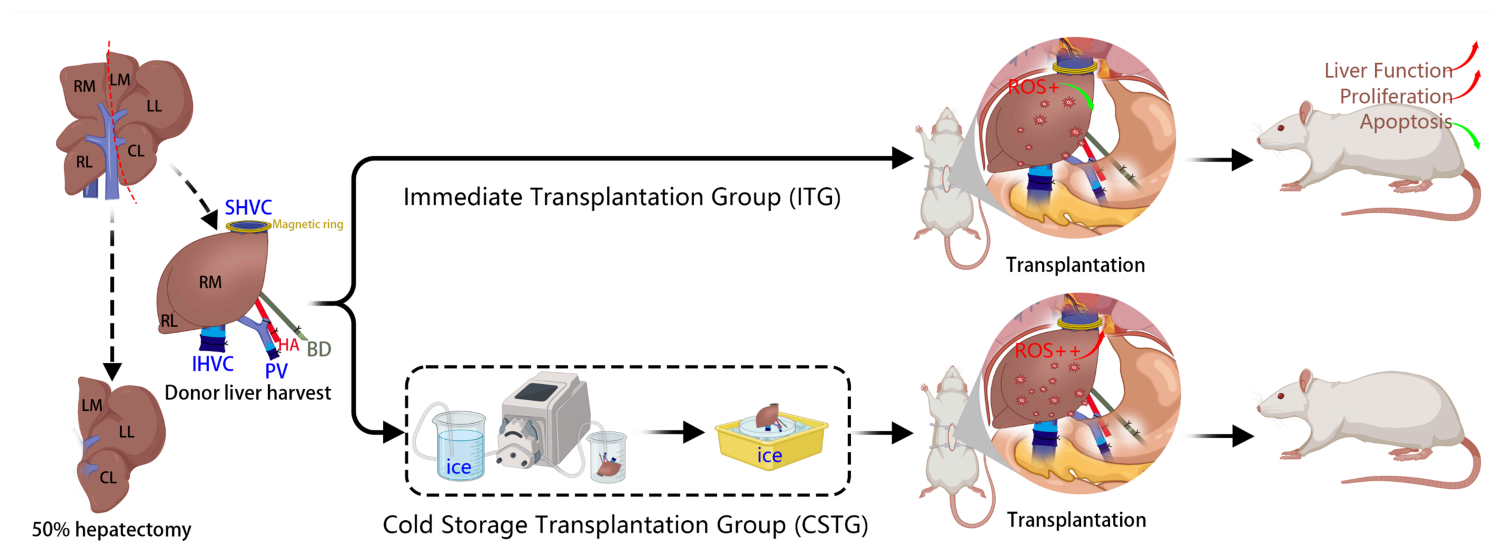


Figure 1

Schematic illustration of experiment design

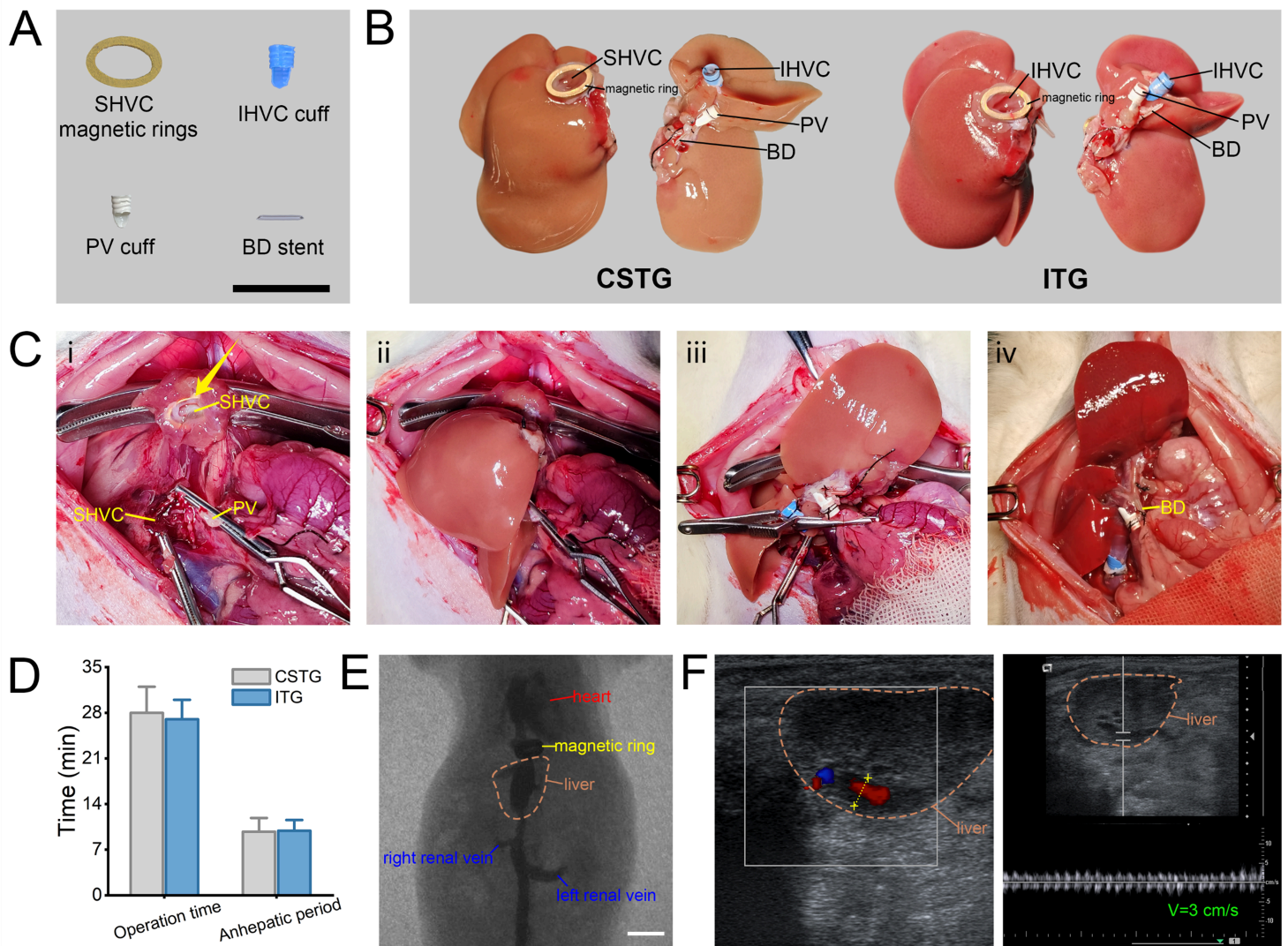


Figure 2

Surgical components, procedures, and outcomes of liver transplantation

(A) Custom-designed components for vascular and biliary anastomoses. Scale bar = 10 mm. (B) Ex vivo liver implants: Immediate Transplantation Group (ITG, bright red) and Cold Storage Transplantation Group (CSTG, khaki). (C) Typical surgical procedure for magnetic anastomosis liver transplantation: (i) Recipient liver removal, exposing SHVC, IHVC, and PV ends; magnetic ring on SHVC stump (yellow arrow). (ii) Completed SHVC reconstruction. (iii) Completed IHVC and PV reconstruction. (iv) Post-reperfusion liver appearance. (D) Comparison of surgical time and anhepatic period between ITG and CSTG (not significant). (E) Angiography 5 days post-surgery showing renal vein, liver, magnetic anastomosis rings, and heart. (F) Doppler ultrasound 5 days post-surgery demonstrating inferior vena cava blood flow (velocity: 3 cm/s).

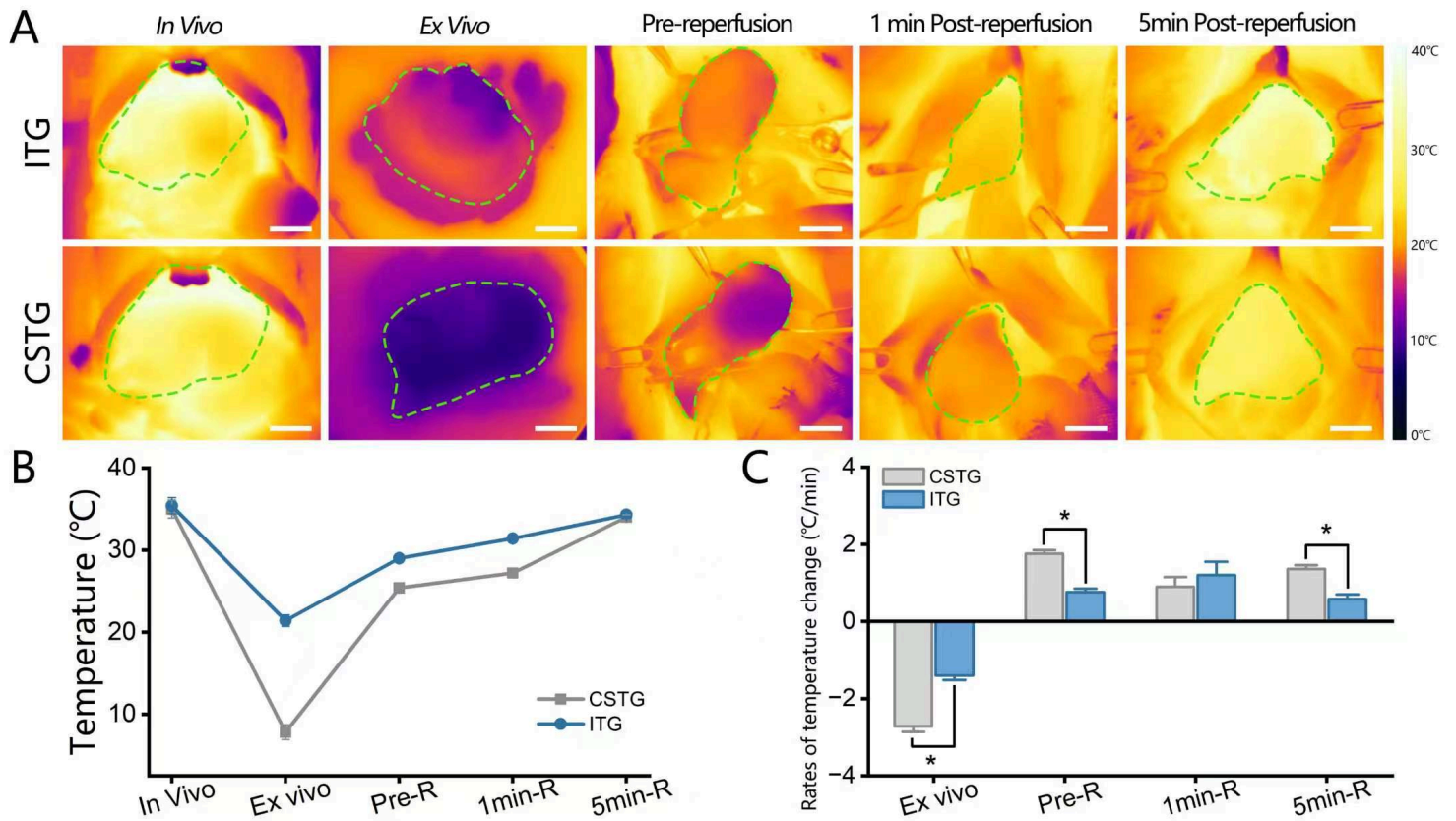


Figure 3

Thermal imaging and analysis of temperature changes

(A) Representative thermal images of ITG and CSTG at different time points: *In Vivo*, *Ex Vivo*, Pre-reperfusion, 1 min Post-reperfusion, and 5 min Post-reperfusion. The dotted green outlines highlight the graft regions. The color scale represents temperature (°C), with warmer colors indicating higher temperatures. Scale bar = 10 mm. (B) Temperature profiles of ITG and CSTG during each stage, showing a significant drop in the *Ex Vivo* phase, followed by gradual temperature recovery during reperfusion. (C) Rates of temperature change (°C/min) in ITG and CSTG at each time point. * $P < 0.05$ and ** $P < 0.01$.

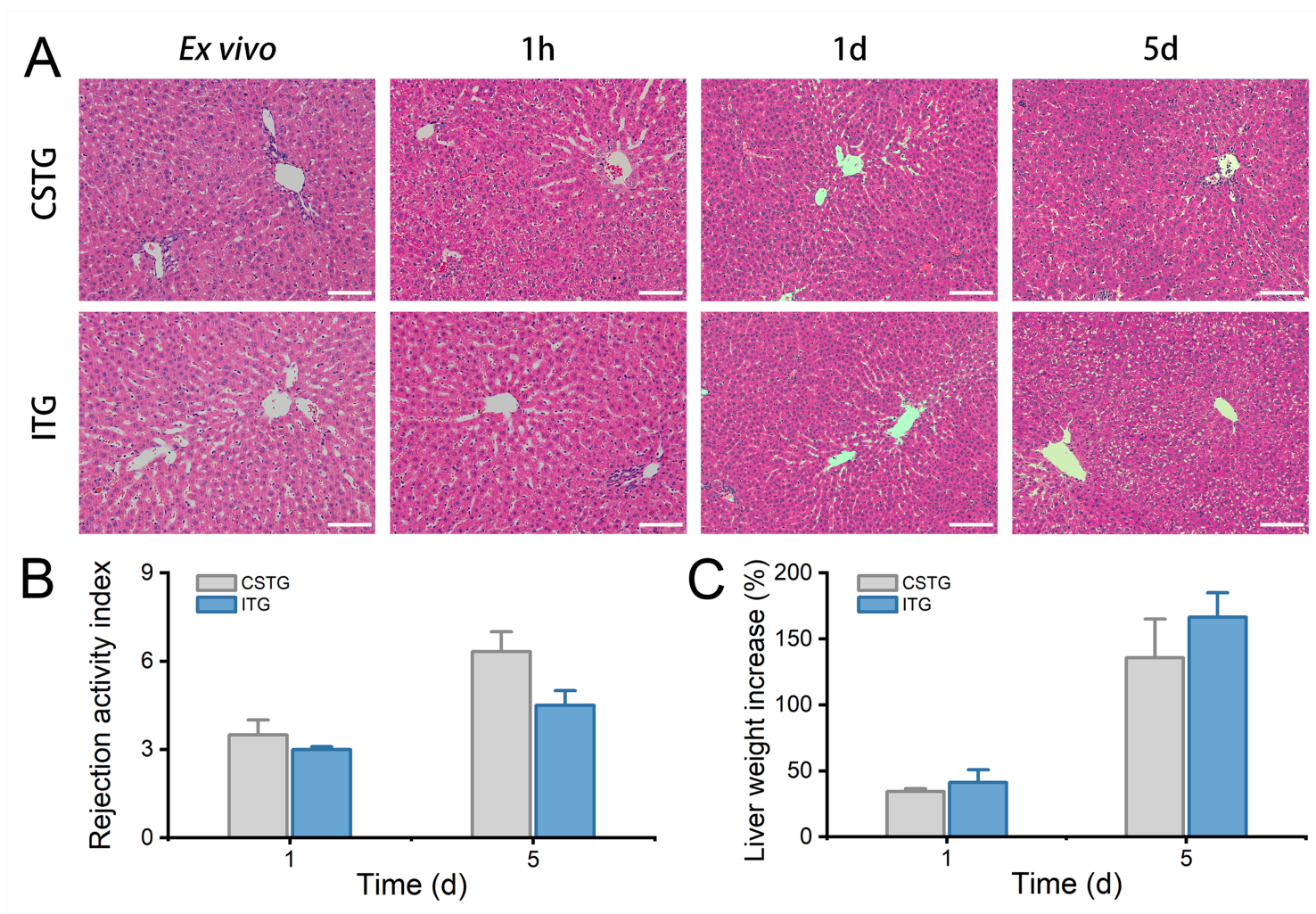


Figure 4

Histopathological and quantitative analysis of liver grafts post-transplantation.

(A) Representative H&E staining images of liver grafts from CSTG and ITG groups, showing well-preserved cellular structures and minimal inflammation across all time points. Scale bar = 50 μ m. (B) Quantitative comparison of rejection activity index (RAI) scores on days 1 and 5 post-transplantation, indicating no significant differences between groups, with ITG displaying a trend toward lower scores. (C) Liver weight gain assessed on days 1 and 5, demonstrating no significant differences between CSTG and ITG, suggesting that direct transplantation preserves hepatic regenerative capacity and does not increase rejection risk.

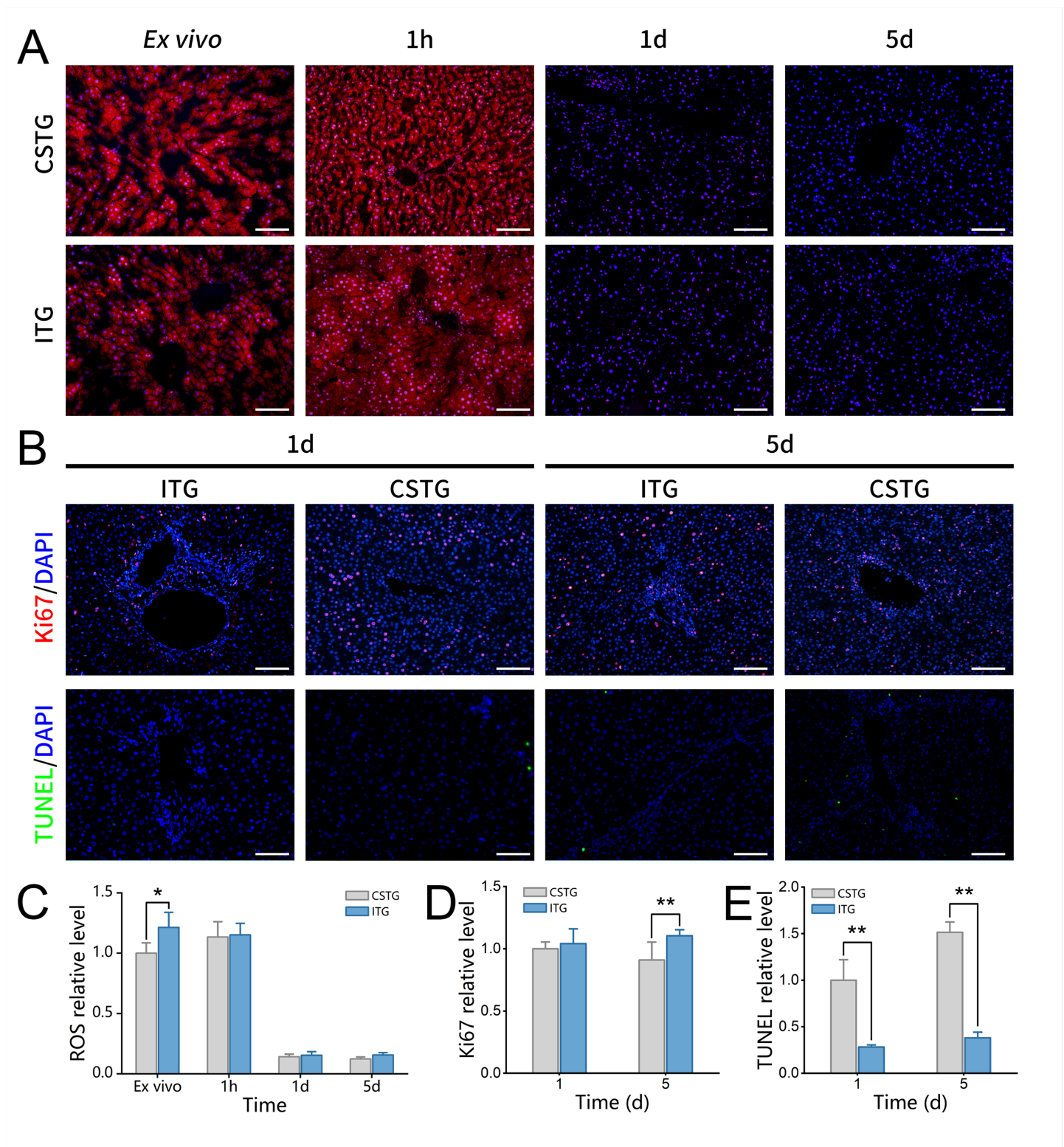


Figure 5

Comparative analysis of oxidative stress, cellular proliferation, and apoptosis in liver grafts

(A) Representative images of DCFH-DA staining in liver grafts from the CSTG and ITG at various time points. Scale bar = 50 μ m. (B) Immunohistochemical staining for Ki67 and TUNEL in liver grafts from ITG and CSTG at 1- and 5-days post-transplantation. Scale bar = 50 μ m. (C) Quantification of relative ROS

levels across time points, showing significantly higher ROS levels in the ITG compared to the CSTG at the *ex vivo* stage. (D) Ki67 relative levels at 1 and 5 days, with significantly higher levels in ITG compared to CSTG at day 5. (E) TUNEL relative levels at 1 and 5 days, with CSTG showing significantly more apoptotic cells than ITG. * $P < 0.05$ and ** $P < 0.01$.

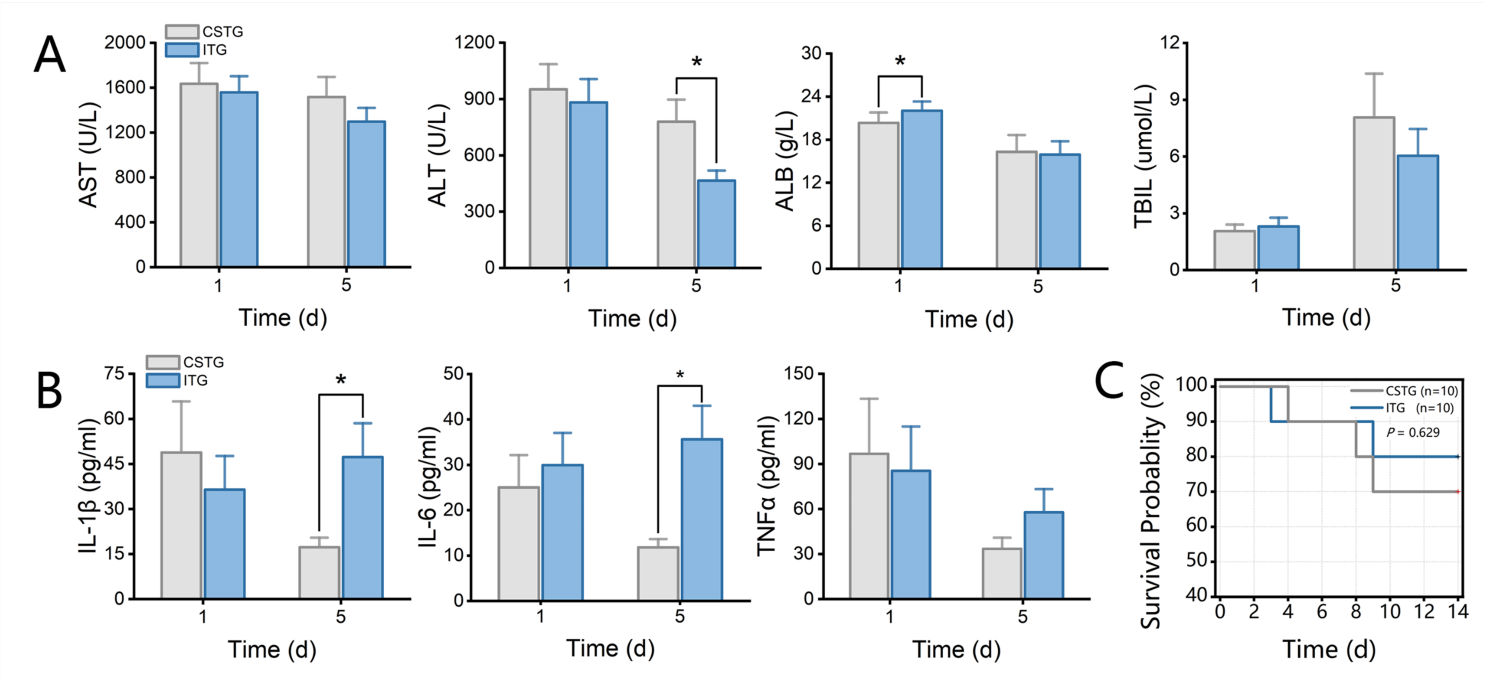


Figure 6

Comparison of liver function, inflammatory response, and survival

(A) Serum markers of liver injury (AST, ALT) and function (ALB, TBIL) at 1- and 5-days post-transplant. ALT and ALB levels were significantly improved in ITG on day 5 ($P < 0.05$). (B) Inflammatory cytokines (IL-1 β , IL-6, TNF- α) at 1- and 5-days post-transplant. IL-6 and IL-1 β levels were higher in ITG on day 5 (* $P < 0.05$), indicating a delayed inflammatory response. (C) Kaplan-Meier survival curve over 14 days, showing no significant difference in survival between groups ($P = 0.629$).