

RESEARCH ARTICLE

The ultrastructural characteristics of bile canaliculus in porcine liver donated after cardiac death and machine perfusion preservation

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Abstract

The effects of each type of machine perfusion preservation (MP) of liver grafts donated after cardiac death on the bile canaliculi of hepatocytes remain unclear. We analyzed the intracellular three-dimensional ultrastructure of the bile canaliculi and hepatocyte endomembrane systems in porcine liver grafts after warm ischemia followed by successive MP with modified University of Wisconsin gluconate solution. Transmission and osmium-maceration scanning electron microscopy revealed that lumen volume of the bile canaliculi decreased after warm ischemia. In liver grafts preserved by hypothermic MP condition, bile canaliculi tended to recover in terms of lumen volume, while their microvilli regressed. In contrast, midthermic MP condition preserved the functional form of the microvilli of the bile canaliculi. Machine perfusion preservation potentially restored the bile canaliculus lumen and alleviated the cessation of cellular endocrine processes due to warm ischemia. In addition, midthermic MP condition prevented the retraction of the microvilli of bile canaliculi, suggesting further mitigation of the damage of the bile canaliculi.

Introduction

The shortage of brain dead donors for liver transplantation is a serious problem worldwide [1]. Although donors with circulatory arrest have the potential to expand the transplanted liver pool [2,3], post-circulatory arrest liver grafts induce high rates of primary nonfunction and ischemia-reperfusion injury after transplantation [4]. In particular, a high risk of acute and chronic rejection, including ischemic bile duct damage, and biliary complications has been reported [5]; thus, the development of liver graft preservation methods after circulatory arrest is required to overcome these problems [1].

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Existing cold storage organ preservation techniques fail to preserve marginal donor grafts [6]. On the other hand, machine perfusion (MP) of post-circulatory arrest donor liver grafts has been reported to have numerous advantages [6–25], and the optimal conditions of MP, including perfusion temperature, oxygenation status, flow rate, steady flow and pulsatile flow, have been discussed [26–33]. Recently, hypothermic MP (HMP) has been established to maintain the functions of liver grafts, and its application in clinical practice has begun [34–46]. On the other hand, warm perfusion has also been reported as an advanced MP method that maintains the liver graft functions [2,38,47–56]. Warm perfusion had introduced to maintain liver grafts at a more physiologic temperature compared with HMP to offers the opportunity to assess and possibly repair a metabolically active liver graft. Our previous reports indicated that the midthermic MP (MMP), one type of warm perfusion [57], reduces the hepatocellular enzyme release [58,59]. In addition, we confirmed that hepatocytes of DCD liver grafts after MMP retain a functional ultrastructure compared to HMP, by using the observation method of scanning electron microscopy after osmium-maceration (OM-SEM) [60,61]; ultrastructural characteristics of hepatocytes are reported to reflect the function of the transplanted liver [60].

One of the important physiological functions of hepatocytes is the production and secretion of bile [62]. For liver grafts, MP has the potential to not only inhibit the development of post-transplant biliary complications, including ischemic cholangiopathy [17,63–67], but also to protect the bile canaliculus [68]. We evaluated the ultrastructural changes in the bile canaliculi and hepatocytes around them at four hours after HMP or MMP using OM-SEM and transmission electron microscopy (TEM). As a result, the bile canaliculi that regressed one hour after warm ischemia showed a strong tendency to recover after MP, especially in MMP, suggesting the preventative effects of HMP and MMP on bile canaliculi-related functions in liver grafts.

Materials and methods

Animals

We purchased domestic female pigs (cross-bred Large White, Landrace, and Duroc pigs; age, 2–3 months; body weight, approximately 25 kg) from Taisetsusanroku-sya Co., Ltd. (Asahikawa, Japan). The pigs were kept in a well-ventilated room with a 12-h light: dark cycle, controlled temperature and humidity, and *ad libitum* access to food and water. All experiments were performed according to the Guide for the Care and Use of Laboratory Animals at Asahikawa Medical University, and the procedures were approved by the Institutional Animal Ethics Committee of the Clinical Research Center, Asahikawa Medical University (permit no. 14172).

Machine perfusion preservation

Livers harvested from pigs were connected and perfused with a MP system (Fig 1), as described previously [61]. The system was composed of two separate circulating perfusion circuits, which had a roller pump, for the hepatic artery (HA) and portal vein (PV). Each circuit had a flow meter and a pressure sensor, allowing pulsatile and non-pulsatile flow, respectively. Additionally, an oxygenator was installed in the the upstream of the circuits for the PV and HA were connected via plastic connectors to each of the hepatic vessels. The MP systems had waterproof thermocouples that measured the solution and organ temperatures, and a dissolved oxygen meter. The flow conditions and temperatures of the preservation solution were recorded by a system-installed computer. In the systems, the organ chamber temperature was controlled by ice-cold water and a heat exchanger. The flow rate was mainly set to 0.22 mL/min/g for the PV and 0.06 mL/min/g for the HA, as described previously [61].

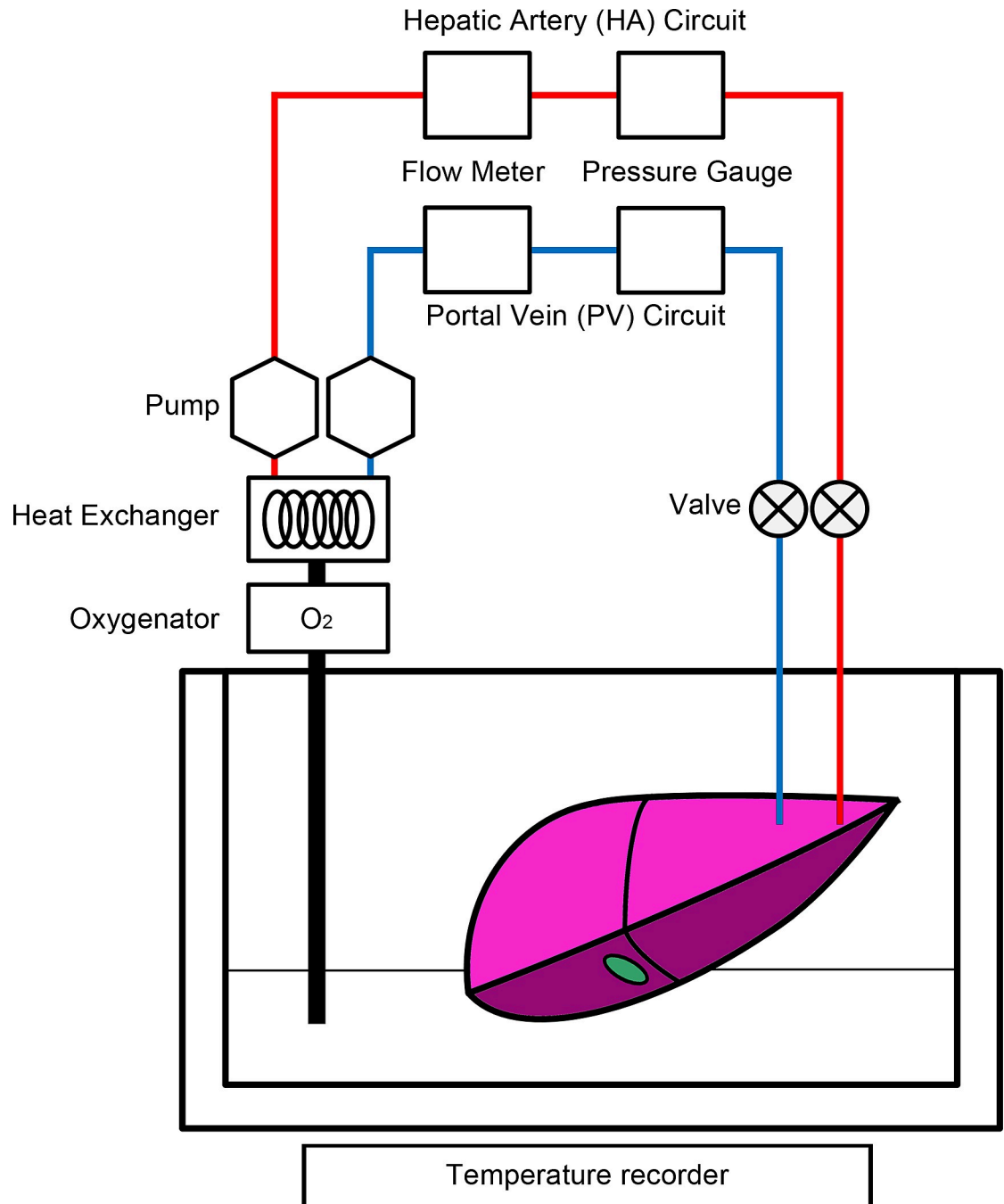


Fig 1. Schematic representation of the continuous machine perfusion system.

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Preparation and preservation of the liver donated after cardiac death

Pigs were used as liver graft donors. These pigs were intubated and ventilated with inhalation anesthesia by isoflurane (Forane; Abbott, Japan), and laparotomized. Immediately after laparotomy, liver tissue samples from the liver surface were obtained by biopsy as a control. Then the pigs were intravenously injected with potassium chloride to induce circulatory arrest followed by the withdrawal of ventilation, as described previously [61]. The time point of the

induction of circulatory arrest was defined as 0 minutes of warm ischemia. During warm ischemia, hepatic artery and portal vein were isolated to connect with organ flush lines, and at 60 minutes of warm ischemia, the liver tissue samples were obtained from distinct regions of the liver surface. Immediately after tissue sampling, the liver grafts were harvested and subsequently flushed with Euro-Collins solution via the HA and PV routes at 8°C on the back table. After the initial flushing, the flush routes were connected to the perfusion preservation machine and the liver grafts were continuously perfused for four hours with modified University of Wisconsin gluconate solution (sodium gluconate 17.5 g, KH₂PO₄ 3.4 g, trehalose 10 g, glutathione 0.9 g, adenosine 1.3 g, HEPES 4.7 g, penicilline 200,000 U, dexamethasone 16 mg, MgSO₄ 1 g, caffeine 4 g, polyethylene glycol 10 g, and glycine 1 g per 1 L). The liver grafts were conserved at a constant temperature of 8°C as HMP (n = 4) or gradually warmed from 8°C to 22°C during perfusion as MMP (n = 5). After MP, the liver tissue samples were biopsied from the well-perfused region of graft surface in each group. Liver sample blocks were immediately fixed with an appropriate fixative for the analysis, as described below. The degree of biliary injury at four hours after MP were evaluated based on the alkaline phosphatase level in perfusate collected from the suprahepatic vena cava of liver grafts in each MP group, as described previously [69]. These alkaline phosphatase data were presented as the mean ± SEM, and unpaired two-tailed *t*-tests were used to compare the significance of differences between groups A and B.

Transmission electron microscopy

The liver tissue samples were trimmed into small blocks and fixed with 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (PB) for two hours at 4°C. After fixation, the blocks were washed 3 times with PB containing 7.5% sucrose and post-fixed with 1% osmium tetroxide (OsO₄) in PB for two hours at 4°C. After washing thoroughly with PB containing 7.5% sucrose, the blocks were dehydrated with a graded series of ethanol. After dehydration, the samples were transferred in propylene oxide, infiltrated and then embedded in epoxy resin (Epon 812). Ultrathin section (80 nm thick) were cut, stained with uranyl acetate and lead citrate, and observed using an HT7700 transmission electron microscope (Hitachi High Technologies, Tokyo, Japan).

Osmium-maceration for SEM

For SEM observation, the osmium maceration method was applied to the liver tissue samples, as described previously [61]. In brief, liver samples cut into small pieces were fixed with 0.5% glutaraldehyde and 0.5% paraformaldehyde in PB for 30 min at 4°C. After fixation, the liver blocks were directly immersed in 1% OsO₄ in PB for four hours at 4°C. The samples were then washed thoroughly with PB and transferred into dimethyl sulfoxide solution in order of 25 to 50% each for 30 min for cryoprotection. The samples were then frozen on a deeply chilled aluminum metal plate with liquid nitrogen, and cracked into two particles with a screwdriver and a hammer. After freeze cracking, the samples were thawed in 50% dimethyl sulfoxide solution, washed thoroughly in PB and transferred into 0.1% diluted OsO₄ in PB for 96 hours at around 20°C under light for maceration. After the maceration period, the samples were immersed into 1% OsO₄ in PB for one hour for post-fixation and then thoroughly washed with PB. The samples were transferred into 1% tannic acid in PB for two hours, subsequently washed with PB, and then immersed in 1% OsO₄ in PB for one hour for conductive staining. The liver samples were dehydrated in graded series of ethanol and immersed in tert-butyl alcohol. After freezing, the samples were lyophilized in an ES2030 freeze-dryer (Hitachi Koki Co., Ltd., Tokyo, Japan). The dried specimens were then mounted onto a metal plate and lightly coated

with platinum-palladium in an E1010 ion sputtering device (Hitachi Koki). These finally processed specimens were observed in secondary electron-mode by a field emission S4100 scanning electron microscope (Hitachi High Technologies).

Results

Ultrastructure of the normal bile canaliculi observed by OM-SEM and TEM

First, we established the overall shape of bile canaliculus by OM-SEM and TEM. In control livers, OM-SEM revealed that hepatocytes mutually formed the bile canaliculi with microvilli between the plasma membranes of contiguous hepatocytes (Fig 2A and 2B). The bile canaliculi were often accompanied by several small stacks of Golgi apparatus around the cytoplasm of the constituent hepatocytes (Fig 2B). The small vacant spaces without any other endomembrane organelles around the bile canaliculi were often found (Fig 2B). The corresponding findings were also obtained by TEM observation (Fig 2C). The vacant space around the bile canaliculi observed by OM-SEM corresponded to the cytoplasmic region without endomembrane organelles (Fig 2C). These findings showed that the details and three-dimensional conformation of the bile canaliculi and the related intracellular components could be visualized by OM-SEM with complementary TEM observation.

Changes in the ultrastructure of the bile canaliculi after warm ischemia

The continuous hypoxic exposure of liver grafts induced by one hour of warm ischemia caused the cessation of bile production and morphological abnormalities of the bile canaliculi. After warm ischemia, OM-SEM revealed the large vacuoles in hepatocytes (Fig 3A, colored red), as described previously. Although the bile canaliculi seemed normal at low magnification (Fig 3A), the cross-sectional area of the lumen of the bile canaliculi after warm ischemia tended to become smaller in comparison to controls (Figs 2B and 3B). In contrast with the controls, small stacks of Golgi apparatus were rarely detected around these bile canaliculi (Figs 2B and 3B). The small vacant spaces around the bile canaliculi were observed similarly to controls, and these space corresponded to the cytoplasmic area without any endomembrane organelles observed by TEM (Fig 3B and 3C). These findings showed that warm ischemia causes ultrastructural destruction of bile canaliculi and the related intracellular subsets, reflecting the decreased bile production induced by hypoxic exposure.

Recovery of the ultrastructure of bile canaliculi by HMP and MMP

Even after liver graft preservation with four hours of HMP or MMP, almost no bile was collected from the bile ducts of the liver grafts. However, ultrastructural restoration of the bile canaliculi was found to have occurred, particularly after MMP.

After four hours of HMP, OM-SEM revealed swollen mitochondria in many hepatocytes (Fig 4A). The cross-sectional area of the lumen of the bile canaliculi after HMP was restored from the changes that were observed in warm ischemia (Figs 3B and 4B); however, the restoration was decreased in comparison to that in the controls (Figs 2B and 4B). This restoration of the bile canaliculi was more clearly indicated by TEM (Fig 4C), whereas the microvilli in the bile canaliculi tended to regress after HMP (Fig 4C).

OM-SEM revealed that the hepatocytes after MMP included macro-autophagosomes and functional forms of mitochondria (Fig 5A), indicating that MMP was more protective for hepatocytes than HMP, as described previously. Additionally, after MMP, the cross-sectional area of the lumen of the bile canaliculi almost recovered to the control level (Fig 5B and 5C), and the microvilli in the bile canaliculi were also maintained (Fig 5B and 5C).

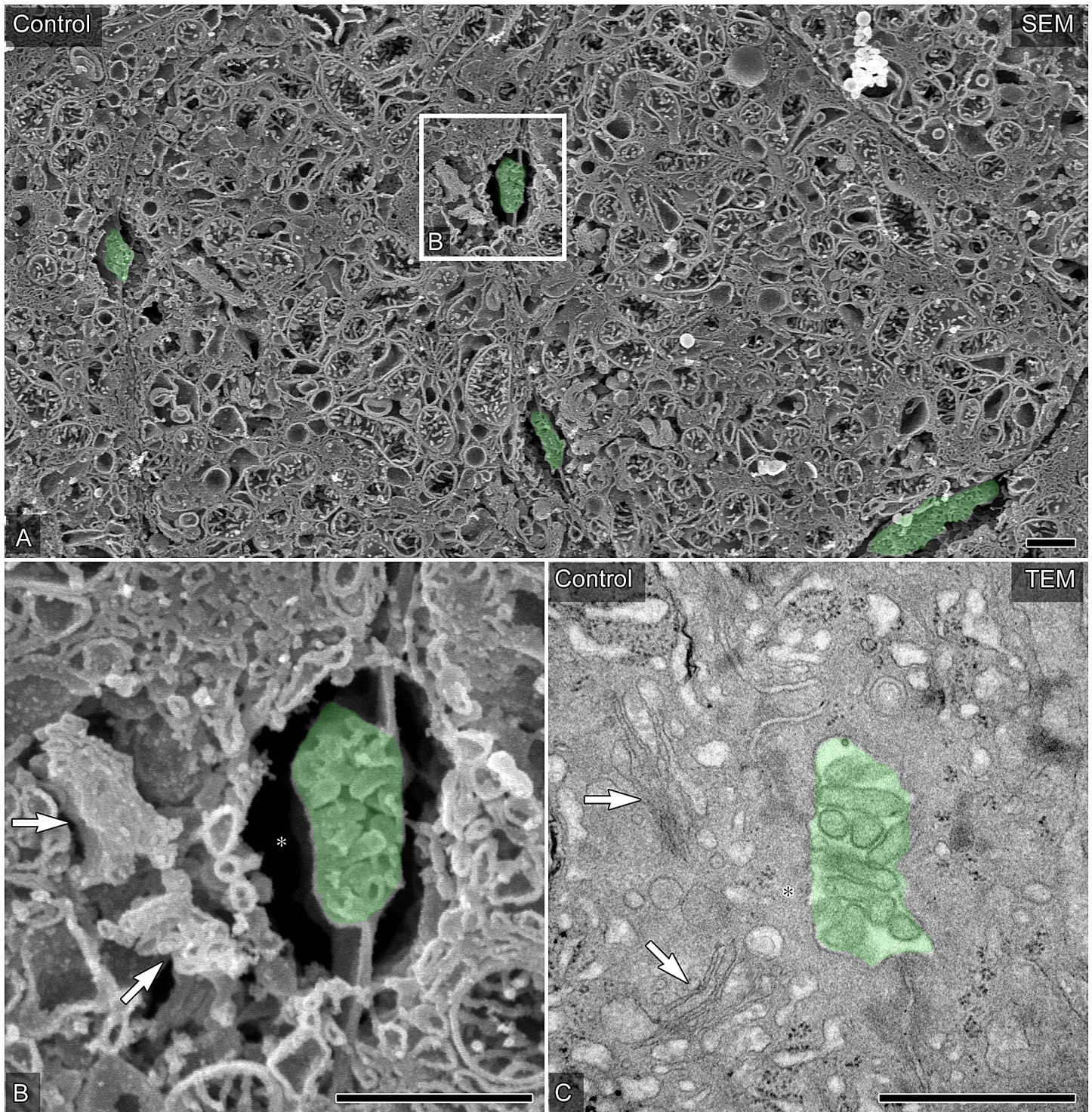


Fig 2. The ultrastructure of the bile canaliculi in porcine hepatocytes of the control liver. (A and B) Representative hepatocytes and bile canaliculi were observed by SEM in osmium-macerated control porcine liver graft samples. The partial area indicated in A was further photographed at a higher magnification (B). (C) Typical bile canaliculi were identified in the ultrathin sections of the Epon 812-embedded control liver tissue. Bile canaliculi are colored green. Arrows indicate the Golgi apparatus, and asterisks indicate vacant spaces without any other endomembrane organelles around the bile canaliculi. Bars = 1 μ m.

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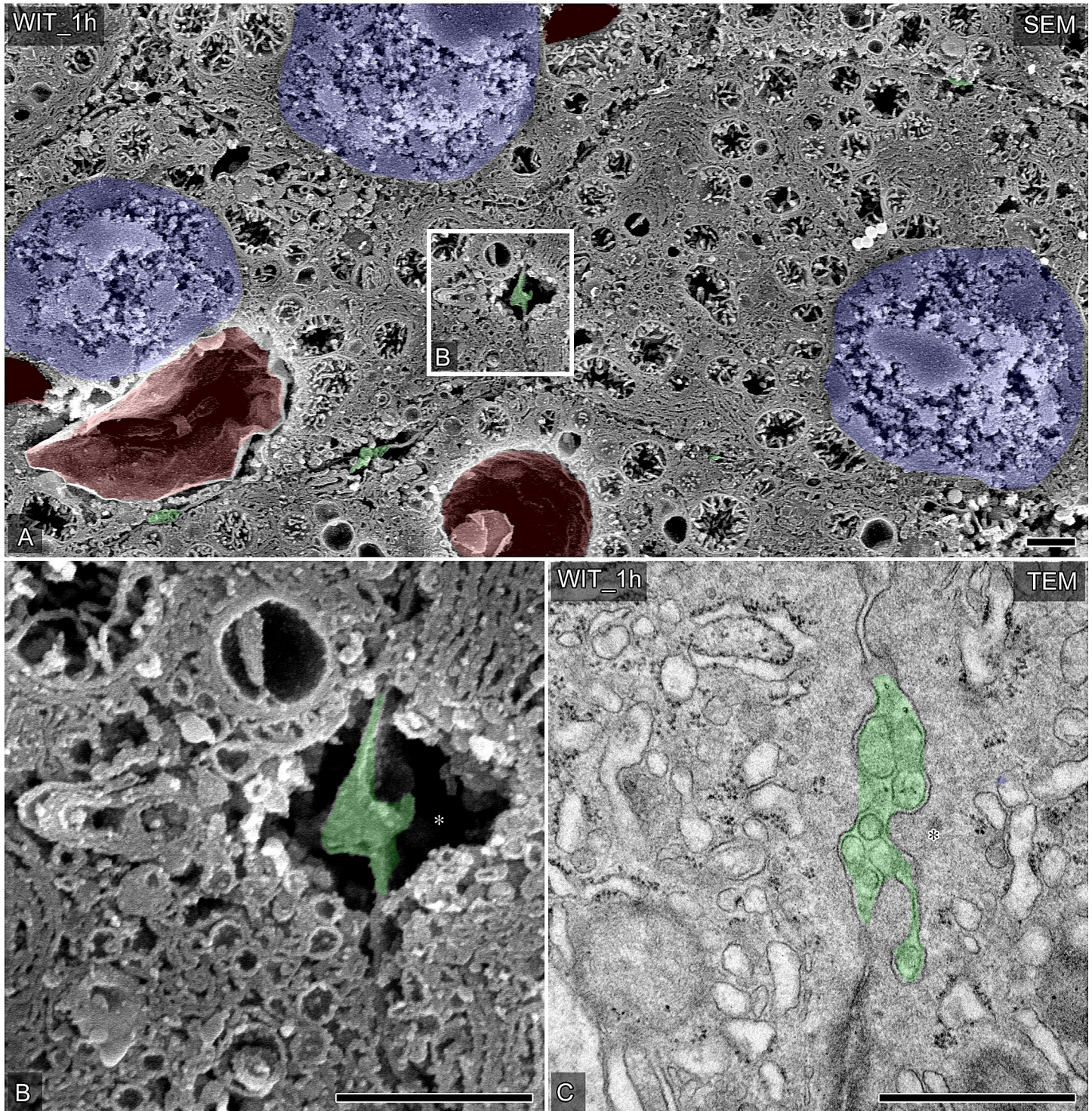


Fig 3. Changes in the ultrastructure of the bile canaliculi in porcine hepatocytes after warm ischemia. (A and B) Representative hepatocytes and bile canaliculi were observed by SEM in osmium-macerated porcine liver graft samples after warm ischemia for 60 minutes. The partial area indicated in A was further photographed at a higher magnification (B). Bile canaliculi are colored green, nuclei are colored blue, huge vacuoles are colored red. Asterisks indicate vacant space without any other endomembrane organelles around the bile canaliculi. (C) Typical bile canaliculi were identified in the ultrathin sections of Epon 812-embedded tissues from liver graft samples after warm ischemia for 60 minutes. Bile canaliculi are colored green and asterisks indicate the vacant space without any other endomembrane organelles around the bile canaliculi. Bars = 1 μ m.

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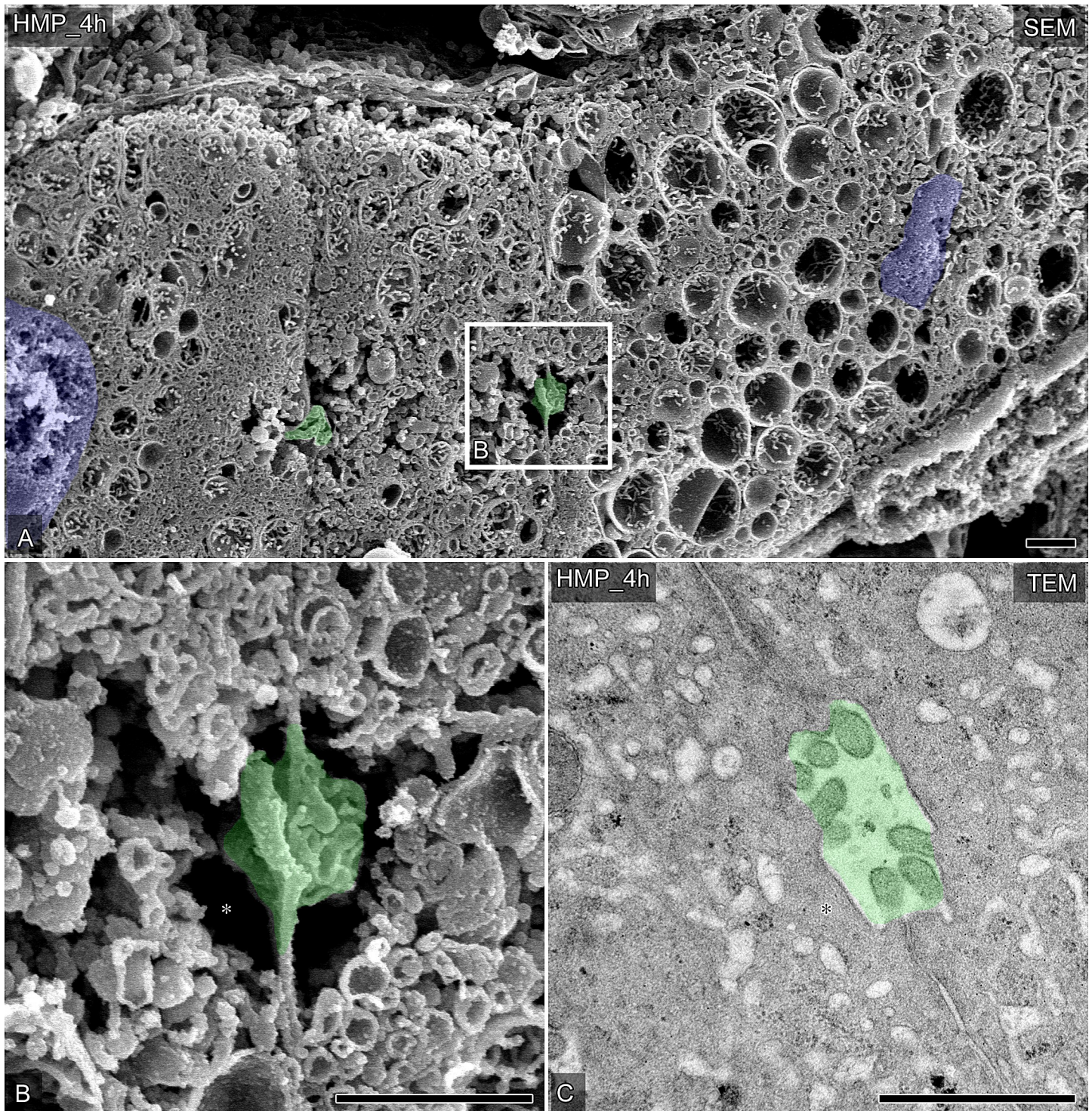


Fig 4. The ultrastructural changes of the bile canaliculi in porcine liver grafts preserved by HMP. (A and B) Representative hepatocytes and bile canaliculi were observed by SEM in osmium-macerated porcine liver graft samples preserved by HMP for 4 h after 60 minutes of warm ischemia. The partial area indicated in A was further photographed under higher magnification (B). Bile canaliculi are colored green, nuclei are colored blue. Asterisks indicate vacant space without any other endomembrane organelles around the bile canaliculi. (C) Typical bile canaliculi were identified in the ultrathin sections of Epon 812-embedded tissues from liver graft samples preserved by HMP for 4 h after 60 minutes of warm ischemia. Bile canaliculi are colored green. Asterisks indicate the vacant space without any other endomembrane organelles around the bile canaliculi. Bars = 1 μ m.

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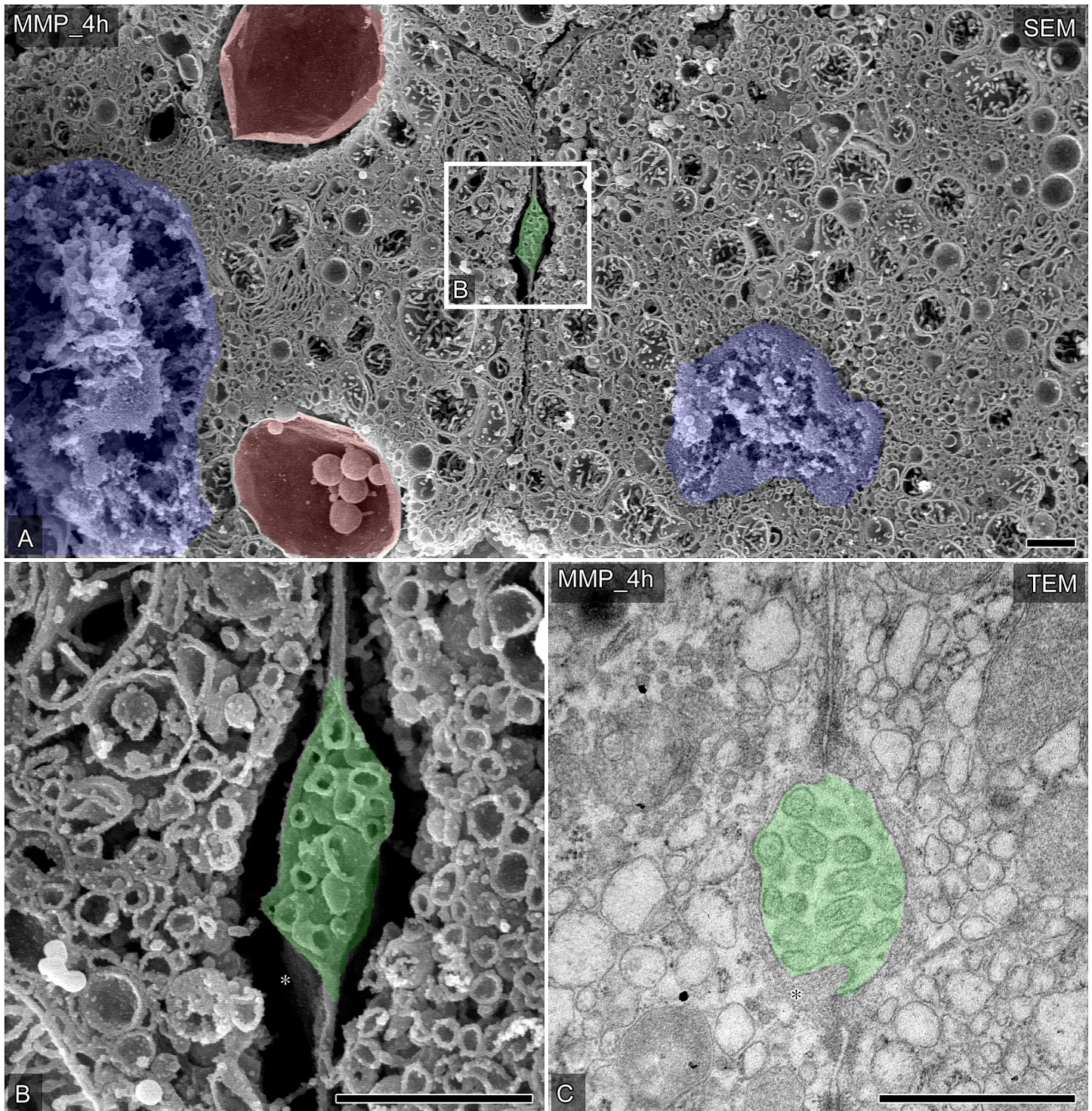


Fig 5. The ultrastructural changes of the bile canaliculi in porcine liver grafts preserved by MMP. (A and B) Representative hepatocytes and bile canaliculi were observed by SEM in osmium-macerated porcine liver graft samples preserved by MMP for 4 h after 60 minutes of warm ischemia. The partial area indicated in A was further photographed under higher magnification (B). Bile canaliculi are colored green, nuclei are colored blue, huge vacuoles are colored red. Asterisks indicate vacant space without any other endomembrane organelles around the bile canaliculi. (C) Typical bile canaliculi were identified in the ultrathin sections of the Epon 812-embedded tissues from liver graft samples preserved by MMP for 4 h after 60 minutes of warm ischemia. Bile canaliculi are colored green. Asterisks indicate vacant space without any other endomembrane organelles around the bile canaliculi. Bars = 1 μm.

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Correspondingly, the value of alkaline phosphatase in perfusate after four hours of MMP (18.0 ± 3.1 IU/L) was significantly lower in comparison to HMP (26.3 ± 1.0 IU/L) (S1 Fig), indicating that MMP suppressed the increased biliary enzyme release in comparison to the HMP. Nevertheless, after preservation with both HMP and MMP, small stacks of Golgi apparatus were rarely detected around the bile canaliculi (Figs 4B and 5B). These findings indicated that, MP preservation, especially MMP, of liver grafts after warm ischemia enabled the ultrastructure of the bile canaliculi to be maintained and restored.

Discussion

In the present study, the porcine bile canaliculi after warm ischemia and after HMP and MMP preservation were analyzed by OM-SEM and complementary TEM methods. Okouhchi et al. [70] analyzed the ultrastructure of the rat liver after HMP preservation by OM-SEM, but did not describe the bile canaliculi. Our previous study using OM-SEM [61] described the bile canaliculi after MMP preservation under low magnification, but the detailed ultrastructure of the bile canaliculi was not established at that stage. The present study revealed the detailed ultrastructural changes of the bile canaliculi after both HMP and MMP preservation.

Morphological abnormalities of the bile canaliculi after warm ischemia were alleviated by subsequent MP, and this preventative effect was greater in MMP than in HMP. These findings were consistent with previous physiological reports [2,38,47–56], which suggested that the ultrastructure of the bile canaliculi is important from both morphological and functional perspectives.

One hour of warm ischemia was associated with regression of the bile canaliculus lumen; this change is consistent with our previous findings [61]. Moussa et al. [71] also reported the regression and disappearance of the bile canaliculi during warm ischemia based on observation by TEM. These findings may reflect autophagic changes in hepatocytes after warm ischemia suppressed the intracellular trafficking pathway [72]. In addition, small stacks of Golgi apparatus around the bile canaliculi were not found after warm ischemia. This distorted arrangement of Golgi apparatus was probably caused by hypoxia because the hepatocellular polarity is maintained by mitochondrial energy [73,74]. On the other hand, the organelle-poor cytoplasmic region of hepatocytes around the bile canaliculi and functional microvilli in the bile canaliculi was retained, even after warm ischemia. The accumulation of actin, a cytoplasmic component concentrated in the peri-biliary region and microvilli, occurs in hepatocyte cytoplasm around the bile canaliculi [75], and the actin in this region is disrupted under reperfusion rather than during warm ischemia [76], although the microtubules in hepatocytes are distorted after warm ischemia [77]. The present study supported the opinion that warm ischemia does not largely affect the cytoplasmic components around the bile canaliculi.

The cross-sectional area of the bile canaliculus lumen in liver grafts that regressed from warm ischemia tended to recover after both HMP and MMP preservation, suggesting the resumption of the secretion of bile canaliculi contents, namely bile salts. The secretion of bile salts from hepatocytes into the bile canaliculi is an important factor for activating bile production [78]. HMP and MMP did not seem to irregularly increase the bile duct pressure, although irregular peri-biliary actin accumulation and biliary dilatation caused by the ischemia-reperfusion treatment—reflecting increased bile duct pressure—has been reported [79]. These findings suggested that the initial cold perfusion phase present in both MMP and HMP may prevent mitochondrial damage in hepatocytes and lead to the resumption of bile salt secretion [65].

The bile canaliculus microvilli after HMP preservation tended to regress more in comparison to after MMP. The ultrastructure of the microvilli in the bile canaliculi is associated with

the bile secretion function [73,80,81], and ultrastructural changes of bile canaliculus microvilli during ischemia-reperfusion injury, drug injury, and cholestasis are characterized by microvilli withdrawal [81–87]. Retraction of the microvilli occurs due to damage of the cell membrane forming the bile canaliculi by protonated hydrophobic bile salts [65,88]. Since HMP consumes less oxygen than warm perfusion [89], hepatocytes may reduce ATP production due to decreased metabolism during HMP preservation. ATP depletion in hepatocytes alters transporter functions, such as the bile salt export pump and disrupts the balance between bile salts and phospholipids [65]. It is therefore considered that the neutralization of salts by phospholipids is weakened and that the cell membrane of the bile canaliculi may be more damaged during HMP than during MMP. Actually, it was also confirmed that the level of ALP, a marker of capillary bile duct damage, was lower in MMP than in HMP [90].

The present study was associated with several limitations. First, this study did not confirm the ultrastructural changes in the bile canaliculi in the liver transplanted or monitored after perfusion storage. Like a previous study of allogeneic liver transplantation of porcine liver grafts under conditions that were similar to the conditions in this study [91], the present MMP results are still in the preclinical stage. In addition, this study did not evaluate the effect in reperfusion at normothermia. Thus, the present results should be investigated by further studies using liver transplant models that are clinically suitable for transplantation and reperfusion at normothermia *ex situ*.

Second, among the components of the biliary system, bile duct cells are very sensitive to ischemia [4]; however, this study did not examine the bile ducts. Future studies are needed to examine the ultrastructure of the bile duct in order to investigate the optimal conditions for clinical transplantation [65].

In conclusion, MP preservation alleviated the cessation of intracellular trafficking processes of hepatocytes caused by warm ischemia and restored the retracted bile canaliculus lumen. In addition, MMP temperature conditions prevented the retraction of the microvilli in the bile canaliculi by mitigating the damage to the cell membrane forming the bile canaliculi. In future study, more clinically appropriate MP conditions to preserve the functions of the liver grafts should be established by using normothermically reperfusion systems. To achieve the above objectives, further physiological studies are required to reveal the ultrastructural changes in liver constituent cells under various conditions, including different temperatures and different levels of oxygenation, during perfusion storage.

Supporting information

S1 Fig. Changes in the perfusate enzymes after warm ischemia and subsequent preservation by HMP or MMP. The levels of alkaline phosphatase (ALP) in the perfusate at 4 hours after hypothermic and midthermic machine perfusion preservation. Data are shown as the mean \pm SEM. Unpaired two-tailed *t*-tests were used ($p < 0.05$). (TIF)

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Supervision: Naoto Matsuno.

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Writing – review & editing: Yo Ishihara, Hiroki Bochimoto, Daisuke Kondoh, Hiromichi Obara, Naoto Matsuno.

References

1. Dutkowski P, Linecker M, DeOliveira ML, Müllhaupt B, Clavien P-A. Challenges to liver transplantation and strategies to improve outcomes. *Gastroenterology*. 2015; 148: 307–23. <https://doi.org/10.1053/j.gastro.2014.08.045> PMID: 25224524
2. Goldaracena N, Barbas AS, Selzner M. Normothermic and subnormothermic ex-vivo liver perfusion in liver transplantation. *Curr Opin Organ Transplant*. 2016; 21: 315–21. <https://doi.org/10.1097/MOT.000000000000305> PMID: 27093224
3. Okamura Y, Hata K, Tanaka H, Hirao H, Kubota T, Inamoto O, et al. Impact of Subnormothermic Machine Perfusion Preservation in Severely Steatotic Rat Livers: A Detailed Assessment in an Isolated Setting. *Am J Transplant*. 2017; 17: 1204–1215. <https://doi.org/10.1111/ajt.14110> PMID: 27860296
4. Mourad MM, Algarni A, Lioussis C, Bramhall SR. Aetiology and risk factors of ischaemic cholangiopathy after liver transplantation. *World J Gastroenterol*. 2014; 20: 6159–69. <https://doi.org/10.3748/wjg.v20.i20.6159> PMID: 24876737
5. Chan EY, Olson LC, Kisthard JA, Perkins JD, Bakthavatsalam R, Halldorson JB, et al. Ischemic cholangiopathy following liver transplantation from donation after cardiac death donors. *Liver Transpl*. 2008; 14: 604–10. <https://doi.org/10.1002/lt.21361> PMID: 18433032
6. Vogel T, Brockmann JG, Pigott D, Neil DAH, Muthusamy ASR, Coussios CC, et al. Successful transplantation of porcine liver grafts following 48-hour normothermic preservation. *PLoS One*. 2017; 12: e0188494. <https://doi.org/10.1371/journal.pone.0188494> PMID: 29176869
7. Tchilikidi KY. Liver graft preservation methods during cold ischemia phase and normothermic machine perfusion. *World J Gastrointest Surg*. 2019; 11: 126–142. <https://doi.org/10.4240/wjgs.v11.i3.126> PMID: 31057698
8. Banan B, Chung H, Xiao Z, Tarabishy Y, Jia J, Manning P, et al. Normothermic extracorporeal liver perfusion for donation after cardiac death (DCD) livers. *Surgery*. 2015; 158: 1642–50. <https://doi.org/10.1016/j.surg.2015.07.016> PMID: 26294088
9. Op den Dries S, Karimian N, Westerkamp AC, Sutton ME, Kuipers M, Wiersema-Buist J, et al. Normothermic machine perfusion reduces bile duct injury and improves biliary epithelial function in rat donor livers. *Liver Transpl*. 2016; 22: 994–1005. <https://doi.org/10.1002/lt.24436> PMID: 26946466
10. Bral M, Gala-Lopez B, Bigam D, Kneteman N, Malcolm A, Livingstone S, et al. Preliminary Single-Center Canadian Experience of Human Normothermic Ex Vivo Liver Perfusion: Results of a Clinical Trial. *Am J Transplant*. 2017; 17: 1071–1080. <https://doi.org/10.1111/ajt.14049> PMID: 27639262
11. Monbaliu DR, Debbaut C, Hillewaert WJ, Laleman WJ, Sainz-Barriga M, Pirenne J, et al. Flow competition between hepatic arterial and portal venous flow during hypothermic machine perfusion preservation of porcine livers. *Int J Artif Organs*. 2012; 35: 119–31. <https://doi.org/10.5301/ijao.5000038> PMID: 22388941
12. Kim JS, Boudjema K, D'Alessandro A, Southard JH. Machine perfusion of the liver: maintenance of mitochondrial function after 48-hour preservation. *Transplant Proc*. 1997; 29: 3452–4. [https://doi.org/10.1016/s0041-1345\(97\)00975-5](https://doi.org/10.1016/s0041-1345(97)00975-5)
13. Nasralla D, Coussios CC, Mergental H, Akhtar MZ, Butler AJ, Ceresa CDL, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature*. 2018; 557: 50–56. <https://doi.org/10.1038/s41586-018-0047-9> PMID: 29670285
14. Bessems M, Doorschodt BM, van Marle J, Vreeling H, Meijer AJ, van Gulik TM. Improved machine perfusion preservation of the non-heart-beating donor rat liver using Polysol: a new machine perfusion

- preservation solution. *Liver Transpl.* 2005; 11: 1379–88. <https://doi.org/10.1002/lt.20502> PMID: 16237689
15. Fondevila C, Hessheimer AJ, Maathuis M-HJ, Muñoz J, Taurá P, Calatayud D, et al. Superior preservation of DCD livers with continuous normothermic perfusion. *Ann Surg.* 2011; 254: 1000–7. <https://doi.org/10.1097/SLA.0b013e31822b8b2f> PMID: 21862925
 16. Jia J, Li J, Zhang S, Xie H, Zhou L, Zheng S. A promising ex vivo liver protection strategy: machine perfusion and repair. *Hepatobiliary Surg Nutr.* 2019; 8: 142–143. <https://doi.org/10.21037/hbsn.2019.03.07> PMID: 31098362
 17. Bellini MI, Nozdrin M, Yiu J, Papalois V. Machine Perfusion for Abdominal Organ Preservation: A Systematic Review of Kidney and Liver Human Grafts. *J Clin Med.* 2019; 8. <https://doi.org/10.3390/jcm8081221> PMID: 31443179
 18. van Rijn R, van den Berg AP, Erdmann JI, Heaton N, van Hoek B, de Jonge J, et al. Study protocol for a multicenter randomized controlled trial to compare the efficacy of end-ischemic dual hypothermic oxygenated machine perfusion with static cold storage in preventing non-anastomotic biliary strictures after transplantation of liver gra. *BMC Gastroenterol.* 2019; 19: 40. <https://doi.org/10.1186/s12876-019-0956-6> PMID: 30866837
 19. Burlage LC, Karimian N, Westerkamp AC, Visser N, Matton APM, van Rijn R, et al. Oxygenated hypothermic machine perfusion after static cold storage improves endothelial function of extended criteria donor livers. *HPB (Oxford).* 2017; 19: 538–546. <https://doi.org/10.1016/j.hpb.2017.02.439> PMID: 28351756
 20. Schlegel A, Rougemont O De, Graf R, Clavien PA, Dutkowsky P. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. *J Hepatol.* 2013; 58: 278–286. <https://doi.org/10.1016/j.jhep.2012.10.004> PMID: 23063573
 21. Jain S, Lee CY, Baicu S, Duncan H, Xu H, Jones JW, et al. Hepatic function in hypothermically stored porcine livers: comparison of hypothermic machine perfusion vs cold storage. *Transplant Proc.* 2005; 37: 340–1. <https://doi.org/10.1016/j.transproceed.2004.12.069> PMID: 15808637
 22. Zhang Y, Zhang Y, Zhang M, Ma Z, Wu S. Hypothermic machine perfusion reduces the incidences of early allograft dysfunction and biliary complications and improves 1-year graft survival after human liver transplantation: A meta-analysis. *Medicine (Baltimore).* 2019; 98: e16033. <https://doi.org/10.1097/MD.000000000016033> PMID: 31169745
 23. Guarrera J V., Estevez J, Boykin J, Boyce R, Rashid J, Sun S, et al. Hypothermic machine perfusion of liver grafts for transplantation: technical development in human discard and miniature swine models. *Transplant Proc.* 2005; 37: 323–5. <https://doi.org/10.1016/j.transproceed.2004.12.094> PMID: 15808631
 24. Nassar A, Liu Q, Farias K, D'Amico G, Tom C, Grady P, et al. Ex Vivo Normothermic Machine Perfusion Is Safe, Simple, and Reliable: Results From a Large Animal Model. *Surg Innov.* 2014; 1553350614528383-. <https://doi.org/10.1177/1553350614528383> PMID: 24694840
 25. Nickkholgh A, Nikdad M, Shafie S, Dezfouli SA, Mehrabi A, Eason JD, et al. Ex Situ Liver Machine Perfusion As An Emerging Graft Protective Strategy In Clinical Liver Transplantation: The Dawn of A New Era. *Transplantation.* 2019; 1. <https://doi.org/10.1097/TP.0000000000002772> PMID: 31022148
 26. Barbas AS, Goldaracena N, Dib MJ, Selzner M. Ex-vivo liver perfusion for organ preservation: Recent advances in the field. *Transplant Rev (Orlando).* 2016; 30: 154–60. <https://doi.org/10.1016/j.tre.2016.03.002> PMID: 27158081
 27. Yuan X, Theruvath AJ, Ge X, Floerchinger B, Jurisch A, García-Cardeña G, et al. Machine perfusion or cold storage in organ transplantation: indication, mechanisms, and future perspectives. *Transpl Int.* 2010; 23: 561–70. <https://doi.org/10.1111/j.1432-2277.2009.01047.x> PMID: 20074082
 28. Marecki H, Bozorgzadeh A, Porte RJ, Leuvenink HG, Uygun K, Martins PN. Liver ex situ machine perfusion preservation: A review of the methodology and results of large animal studies and clinical trials. *Liver Transpl.* 2017; 23: 679–695. <https://doi.org/10.1002/lt.24751> PMID: 28240817
 29. Dutkowsky P, de Rougemont O, Clavien P-A. Machine perfusion for “marginal” liver grafts. *Am J Transplant.* 2008; 8: 917–24. <https://doi.org/10.1111/j.1600-6143.2008.02165.x> PMID: 18416733
 30. Verhoeven CJ, Farid WRR, de Jonge J, Metselaar HJ, Kazemier G, van der Laan LJW. Biomarkers to assess graft quality during conventional and machine preservation in liver transplantation. *J Hepatol.* 2014; 61: 672–84. <https://doi.org/10.1016/j.jhep.2014.04.031> PMID: 24798616
 31. Guarrera J V. Are We Emerging From the Ice Age of Liver Preservation? *Am J Transplant.* 2016; 16: 1647–8. <https://doi.org/10.1111/ajt.13756> PMID: 26880347
 32. Hessheimer AJ, Billault C, Barrou B, Fondevila C. Hypothermic or normothermic abdominal regional perfusion in high-risk donors with extended warm ischemia times: impact on outcomes? *Transpl Int.* 2015; 28: 700–7. <https://doi.org/10.1111/tri.12344> PMID: 24797796

33. Boteon YL, Afford SC. Machine perfusion of the liver: Which is the best technique to mitigate ischaemia-reperfusion injury? *World J Transplant.* 2019; 9: 14–20. <https://doi.org/10.5500/wjt.v9.i1.14> PMID: 30697517
34. Li P, Liu YF, Yang L. Advantages of dual hypothermic oxygenated machine perfusion over simple cold storage in the preservation of liver from porcine donors after cardiac death. *Clin Transplant.* 2015; 29: 820–828. <https://doi.org/10.1111/ctr.12586> PMID: 26147375
35. Dutkowski P, Polak WG, Muiesan P, Schlegel A, Verhoeven CJ, Scalera I, et al. First Comparison of Hypothermic Oxygenated PERfusion Versus Static Cold Storage of Human Donation After Cardiac Death Liver Transplants: An International-matched Case Analysis. *Ann Surg.* 2015; 262: 764–70; discussion 770–1. <https://doi.org/10.1097/SLA.0000000000001473> PMID: 26583664
36. van Rijn R, van Leeuwen OB, Matton APM, Burlage LC, Wiersema-Buist J, van den Heuvel MC, et al. Hypothermic oxygenated machine perfusion reduces bile duct reperfusion injury after transplantation of donation after circulatory death livers. *Liver Transpl.* 2018; 24: 655–664. <https://doi.org/10.1002/lt.25023> PMID: 29369470
37. Dutkowski P, Furrer K, Tian Y, Graf R, Clavien P-A. Novel short-term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. *Ann Surg.* 2006; 244: 968–76; discussion 976–7. <https://doi.org/10.1097/01.sla.0000247056.85590.6b> PMID: 17122622
38. van Leeuwen OB, de Vries Y, Fujiyoshi M, Nijsten MW, Ubbink R, Pelgrim GJ, et al. Transplantation of High-risk Donor Livers After Ex Situ Resuscitation and Assessment Using Combined Hypo- and Normothermic Machine Perfusion: A Prospective Clinical Trial. *Ann Surg.* 2019; XX: 1. <https://doi.org/10.1097/SLA.0000000000003540> PMID: 31633615
39. Schlegel A, Kron P, Graf R, Dutkowski P, Clavien P-A. Warm vs. cold perfusion techniques to rescue rodent liver grafts. *J Hepatol.* 2014; 61: 1267–75. <https://doi.org/10.1016/j.jhep.2014.07.023> PMID: 25086285
40. Zeng X, Wang S, Li S, Yang Y, Fang Z, Huang H, et al. Hypothermic oxygenated machine perfusion alleviates liver injury in donation after circulatory death through activating autophagy in mice. *Artif Organs.* 2019; 1–13. <https://doi.org/10.1111/aor.13525> PMID: 31237688
41. Zeng X, Li M, Fan X, Xue S, Liang W, Fang Z, et al. Hypothermic Oxygenated Machine Perfusion Alleviates Donation After Circulatory Death Liver Injury Through Regulating P-selectin-dependent and -independent Pathways in Mice. *Transplantation.* 2019; 103: 918–928. <https://doi.org/10.1097/TP.0000000000002621> PMID: 31033856
42. Schlegel A, Graf R, Clavien P-A, Dutkowski P. Hypothermic oxygenated perfusion (HOPE) protects from biliary injury in a rodent model of DCD liver transplantation. *J Hepatol.* 2013; 59: 984–91. <https://doi.org/10.1016/j.jhep.2013.06.022> PMID: 23820408
43. Schlegel A, Muller X, Kalisvaart M, Muellhaupt B, Perera MTPR, Isaac JR, et al. Outcomes of DCD liver transplantation using organs treated by hypothermic oxygenated perfusion before implantation. *J Hepatol.* 2019; 70: 50–57. <https://doi.org/10.1016/j.jhep.2018.10.005> PMID: 30342115
44. Dutkowski P, Schlegel A, de Oliveira M, Müllhaupt B, Neff F, Clavien P-A. HOPE for human liver grafts obtained from donors after cardiac death. *J Hepatol.* 2014; 60: 765–72. <https://doi.org/10.1016/j.jhep.2013.11.023> PMID: 24295869
45. Dutkowski P, Krug A, Krysiak M, Dünschede F, Seifert JK, Junginger T. Detection of mitochondrial electron chain carrier redox status by transhepatic light intensity during rat liver reperfusion. *Cryobiology.* 2003; 47: 125–42. <https://doi.org/10.1016/j.cryobiol.2003.08.004> PMID: 14580847
46. Dondossola D, Lonati C, Zanella A, Maggioni M, Antonelli B, Reggiani P, et al. Preliminary Experience With Hypothermic Oxygenated Machine Perfusion in an Italian Liver Transplant Center. *Transplant Proc.* 2019; 51: 111–116. <https://doi.org/10.1016/j.transproceed.2018.04.070> PMID: 30736971
47. Ciria R, Ayllon-Teran MD, González-Rubio S, Gómez-Luque I, Ferrín G, Moreno A, et al. Rescue of Discarded Grafts for Liver Transplantation by Ex Vivo Subnormothermic and Normothermic Oxygenated Machine Perfusion: First Experience in Spain. *Transplant Proc.* 2019; 51: 20–24. <https://doi.org/10.1016/j.transproceed.2018.04.092> PMID: 30655130
48. de Vries Y, Berendsen TA, Fujiyoshi M, van den Berg AP, Blokzijl H, de Boer MT, et al. Transplantation of high-risk donor livers after resuscitation and viability assessment using a combined protocol of oxygenated hypothermic, rewarming and normothermic machine perfusion: study protocol for a prospective, single-arm study (DHOPE-COR-NMP tri. *BMJ Open.* 2019; 9: e028596. <https://doi.org/10.1136/bmjopen-2018-028596> PMID: 31420387
49. Hoyer DP, Mathé Z, Gallinat A, Canbay AC, Treckmann JW, Rauhen U, et al. Controlled Oxygenated Rewarming of Cold Stored Livers Prior to Transplantation: First Clinical Application of a New Concept. *Transplantation.* 2016; 100: 147–52. <https://doi.org/10.1097/TP.0000000000000915> PMID: 26479280

50. Minor T, Efferz P, Fox M, Wohlschlaeger J, Luer B. Controlled oxygenated rewarming of cold stored liver grafts by thermally graduated machine perfusion prior to reperfusion. *Am J Transplant*. 2013; 13: 1450–60. <https://doi.org/10.1111/ajt.12235> PMID: 23617781
51. Olschewski P, Gass P, Ariyakagorn V, Jasse K, Hunold G, Menzel M, et al. The influence of storage temperature during machine perfusion on preservation quality of marginal donor livers. *Cryobiology*. 2010; 60: 337–43. <https://doi.org/10.1016/j.cryobiol.2010.03.005> PMID: 20233587
52. Vairetti M, Ferrigno A, Carlucci F, Tabucchi A, Rizzo V, Boncompagni E, et al. Subnormothermic machine perfusion protects steatotic livers against preservation injury: a potential for donor pool increase? *Liver Transpl*. 2009; 15: 20–9. <https://doi.org/10.1002/lt.21581> PMID: 19109848
53. Horn C Von, Baba HA, Hannaert P, Hauet T, Leuvenink H, Paul A, et al. Controlled oxygenated rewarming up to normothermia for pretransplant reconditioning of liver grafts. *Clin Transplant*. 2017; 31: 1–7. <https://doi.org/10.1111/ctr.13101> PMID: 28871615
54. Hoyer DP, Paul A, Luer S, Reis H, Efferz P, Minor T. End-ischemic reconditioning of liver allografts: Controlling the rewarming. *Liver Transpl*. 2016; 22: 1223–30. <https://doi.org/10.1002/lt.24515> PMID: 27398813
55. Goldaracena N, Echeverri J, Spetzler VN, Kathis JM, Barbas AS, Louis KS, et al. Anti-inflammatory signaling during ex vivo liver perfusion improves the preservation of pig liver grafts before transplantation. *Liver Transpl*. 2016; 22: 1573–1583. <https://doi.org/10.1002/lt.24603> PMID: 27556578
56. de Vries Y, Matton APM, Nijsten MWN, Werner MJM, van den Berg AP, de Boer MT, et al. Pretransplant sequential hypo- and normothermic machine perfusion of suboptimal livers donated after circulatory death using a hemoglobin-based oxygen carrier perfusion solution. *Am J Transplant*. 2019; 19: 1202–1211. <https://doi.org/10.1111/ajt.15228> PMID: 30588774
57. Karangwa SA, Dutkowski P, Fontes P, Friend PJ, Guarrera J V., Markmann JF, et al. Machine Perfusion of Donor Livers for Transplantation: A Proposal for Standardized Nomenclature and Reporting Guidelines. *Am J Transplant*. 2016; 1967: 2932–2942. <https://doi.org/10.1111/ajt.13843> PMID: 27129409
58. Obara H, Matsuno N, Shigeta T, Hirano T, Enosawa S, Mizunuma H. Temperature controlled machine perfusion system for liver. *Transplant Proc*. 2013; 45: 1690–2. <https://doi.org/10.1016/j.transproceed.2013.01.087> PMID: 23769025
59. Matsuno N, Obara H, Watanabe R, Iwata S, Kono S, Fujiyama M, et al. Rewarming preservation by organ perfusion system for donation after cardiac death liver grafts in pigs. *Transplant Proc*. 2014; 46: 1095–8. <https://doi.org/10.1016/j.transproceed.2013.12.035> PMID: 24815137
60. Meng L, Matsuno N, Watanabe K, Furukori M, Obara H, Bochimoto H, et al. Scanning Electron Microscopy Findings of Machine Perfused Liver Graft After Warm Ischemia Between Hypothermic and Rewarming Machine Perfusion in Pigs. *Transplant Proc*. 2016; 48: 2467–2470. <https://doi.org/10.1016/j.transproceed.2016.03.059> PMID: 27742324
61. Bochimoto H, Matsuno N, Ishihara Y, Shonaka T, Koga D, Hira Y, et al. The ultrastructural characteristics of porcine hepatocytes donated after cardiac death and preserved with warm machine perfusion preservation. *PLoS One*. 2017; 12: e0186352. <https://doi.org/10.1371/journal.pone.0186352> PMID: 29023512
62. Imber CJ, St Peter SD, de Cenarruzabeitia IL, Lemonde H, Rees M, Butler A, et al. Optimisation of bile production during normothermic preservation of porcine livers. *Am J Transplant*. 2002; 2: 593–9. <https://doi.org/10.1034/j.1600-6143.2002.20703.x> PMID: 12201359
63. Boteon YL, Boteon AP, Attard J, Wallace L, Bhogal RH, Afford SC. Impact of machine perfusion of the liver on post-transplant biliary complications: A systematic review. *World J Transplant*. 2018; 8: 220–231. <https://doi.org/10.5500/wjt.v8.i6.220> PMID: 30370232
64. Schlegel A, Kron P, Dutkowski P. Hypothermic machine perfusion in liver transplantation. *Curr Opin Organ Transplant*. 2016; 21: 308–14. <https://doi.org/10.1097/MOT.0000000000000303> PMID: 26918882
65. Schlegel A, Dutkowski P. Impact of Machine Perfusion on Biliary Complications after Liver Transplantation. *Int J Mol Sci*. 2018; 19. <https://doi.org/10.3390/ijms19113567> PMID: 30424553
66. Op den Dries S, Sutton ME, Karimian N, de Boer MT, Wiersema-Buist J, Gouw ASH, et al. Hypothermic oxygenated machine perfusion prevents arteriolonecrosis of the peribiliary plexus in pig livers donated after circulatory death. *PLoS One*. 2014; 9: e88521. <https://doi.org/10.1371/journal.pone.0088521> PMID: 24551114
67. Westerkamp AC, Mahboub P, Meyer SL, Hottenrott M, Ottens PJ, Wiersema-Buist J, et al. End-ischemic machine perfusion reduces bile duct injury in donation after circulatory death rat donor livers independent of the machine perfusion temperature. *Liver Transpl*. 2015; 21: 1300–11. <https://doi.org/10.1002/lt.24200> PMID: 26097213

68. Bellomo R, Marino B, Starkey G, Fink M, Wang BZ, Eastwood GM, et al. Extended normothermic extracorporeal perfusion of isolated human liver after warm ischaemia: a preliminary report. *Crit Care Resusc.* 2014; 16: 197–201. Available: <http://www.ncbi.nlm.nih.gov/pubmed/25161022>
69. Furukori M, Matsuno N, Meng LT, Shonaka T, Nishikawa Y, Imai K, et al. Subnormothermic Machine Perfusion Preservation With Rewarming for Donation After Cardiac Death Liver Grafts in Pigs. *Transplant Proc.* 2016; 48: 1239–43. <https://doi.org/10.1016/j.transproceed.2015.12.076> PMID: 27320595
70. Okouchi Y, Sasaki K, Tamaki T. Ultrastructural changes in hepatocytes, sinusoidal endothelial cells and macrophages in hypothermic preservation of the rat liver with University of Wisconsin solution. *Virchows Arch.* 1994; 424: 477–84. <https://doi.org/10.1007/BF00191432> PMID: 8032528
71. Moussa ME, Sarraf CE, Uemoto S, Sawada H, Habib NA. Effect of total hepatic vascular exclusion during liver resection on hepatic ultrastructure. *Liver Transpl Surg.* 1996; 2: 461–467. S1527646596000512 [pii]
72. Khambu B, Li T, Yan S, Yu C, Chen X, Goheen M, et al. Hepatic Autophagy Deficiency Compromises Farnesoid X Receptor Functionality and Causes Cholestatic Injury. *Hepatology.* 2019; 69: 2196–2213. <https://doi.org/10.1002/hep.30407> PMID: 30520052
73. Gissen P, Arias IM. Structural and functional hepatocyte polarity and liver disease. *J Hepatol.* 2015; 63: 1023–37. <https://doi.org/10.1016/j.jhep.2015.06.015> PMID: 26116792
74. Treyer A, Musch A. Hepatocyte polarity. *Compr Physiol.* 2013; 3: 243–87. <https://doi.org/10.1002/cphy.c120009> PMID: 23720287
75. Kachi K, Okanoue T, Morioka H, Ohta M, Ohta Y, Kanaoka H, et al. Immunoelectron microscopic localization of actin in normal and cholestatic rat hepatocytes. *Gastroenterol Jpn.* 1989; 24: 523–7. <https://doi.org/10.1007/bf02773879> PMID: 2680743
76. Benkoel L, Doderio F, Hardwigsen J, Campan P, Botta-Fridlund D, Lombardo D, et al. Effect of ischemia-reperfusion on bile canalicular F-actin microfilaments in hepatocytes of human liver allograft: image analysis by confocal laser scanning microscopy. *Dig Dis Sci.* 2001; 46: 1663–7. <https://doi.org/10.1023/a:1010693218680>
77. Shinohara H, Tanaka A, Fujimoto T, Hatano E, Satoh S, Fujimoto K, et al. Disorganization of microtubular network in posts ischemic liver dysfunction: its functional and morphological changes. *Biochim Biophys Acta.* 1996; 1317: 27–35. [https://doi.org/10.1016/0925-4439\(96\)00031-2](https://doi.org/10.1016/0925-4439(96)00031-2)
78. Monbaliu D, Libbrecht L, De Vos R, Vekemans K, Walter H, Liu Q, et al. The extent of vacuolation in non-heart-beating porcine donor liver grafts prior to transplantation predicts their viability. *Liver Transpl.* 2008; 14: 1256–65. <https://doi.org/10.1002/lt.21513> PMID: 18756467
79. Yasui H, Yoshimura N, Kobayashi Y, Ochiai S, Matsuda T, Takamatsu T, et al. Microstructural changes of bile canaliculi in canine liver: the effect of cold ischemia-reperfusion in orthotopic liver transplantation. *Transplant Proc.* 1998; 30: 3754–7. [https://doi.org/10.1016/s0041-1345\(98\)01222-6](https://doi.org/10.1016/s0041-1345(98)01222-6)
80. Tamaki I, Hata K, Okamura Y, Nigmat Y, Hirao H, Kubota T, et al. Hydrogen Flush After Cold Storage as a New End-Ischemic Ex Vivo Treatment for Liver Grafts Against Ischemia/Reperfusion Injury. *Liver Transpl.* 2018; 24: 1589–1602. <https://doi.org/10.1002/lt.25326> PMID: 30120877
81. Cutrin JC, Cantino D, Biasi F, Chiarpotto E, Salizzoni M, Andorno E, et al. Reperfusion damage to the bile canaliculi in transplanted human liver. *Hepatology.* 1996; 24: 1053–7. <https://doi.org/10.1002/hep.510240512> PMID: 8903374
82. Falasca L, Tisone G, Palmieri G, Anselmo A, Di Paolo D, Baiocchi L, et al. Protective role of tauroursodeoxycholate during harvesting and cold storage of human liver: a pilot study in transplant recipients. *Transplantation.* 2001; 71: 1268–76. <https://doi.org/10.1097/00007890-200105150-00015> PMID: 11397961
83. Nigmat Y, Hata K, Tamaki I, Okamura Y, Tsuruyama T, Miyauchi H, et al. Human Atrial Natriuretic Peptide in Cold Storage of Donation After Circulatory Death Rat Livers: An Old but New Agent for Protecting Vascular Endothelia? *Transplantation.* 2019; 103: 512–521. <https://doi.org/10.1097/TP.0000000000002552> PMID: 30461725
84. Okumura S, Uemura T, Zhao X, Masano Y, Tsuruyama T, Fujimoto Y, et al. Liver graft preservation using perfluorocarbon improves the outcomes of simulated donation after cardiac death liver transplantation in rats. *Liver Transpl.* 2017; 23: 1171–1185. <https://doi.org/10.1002/lt.24806> PMID: 28650112
85. Walker RM, Racz WJ, McElligott TF. Scanning electron microscopic examination of acetaminophen-induced hepatotoxicity and congestion in mice. *Am J Pathol.* 1983; 113: 321–30. Available: <http://www.ncbi.nlm.nih.gov/pubmed/6650662>
86. Baiocchi L, Tisone G, Russo MA, Longhi C, Palmieri G, Volpe A, et al. TUDCA prevents cholestasis and canalicular damage induced by ischemia-reperfusion injury in the rat, modulating PKCalpha-ezrin pathway. *Transpl Int.* 2008; 21: 792–800. <https://doi.org/10.1111/j.1432-2277.2008.00682.x> PMID: 18435680

87. Phillips MJ, Azuma T, Meredith S-LM, Squire JA, Ackerley CA, Pluthero FG, et al. Abnormalities in villin gene expression and canalicular microvillus structure in progressive cholestatic liver disease of childhood. *Lancet (London, England)*. 2003; 362: 1112–9. [https://doi.org/10.1016/S0140-6736\(03\)14467-4](https://doi.org/10.1016/S0140-6736(03)14467-4)
88. Hessheimer AJ, Cárdenas A, García-Valdecasas JC, Fondevila C. Can we prevent ischemic-type biliary lesions in donation after circulatory determination of death liver transplantation? *Liver Transpl*. 2016; 22: 1025–33. <https://doi.org/10.1002/lt.24460> PMID: 27082839
89. Morito N, Obara H, Matsuno N, Enosawa S, Furukawa H. Oxygen consumption during hypothermic and subnormothermic machine perfusions of porcine liver grafts after cardiac death. *J Artif Organs*. 2018; 21: 450–457. <https://doi.org/10.1007/s10047-018-1063-0> PMID: 30046934
90. Bertone V, Tarantola E, Ferrigno A, Gringeri E, Barni S, Vairetti M, et al. Altered alkaline phosphatase activity in obese Zucker rats liver respect to lean Zucker and Wistar rats discussed in terms of all putative roles ascribed to the enzyme. *Eur J Histochem*. 2011; 55: e5. <https://doi.org/10.4081/ejh.2011.e5> PMID: 21556120
91. Shigeta T, Matsuno N, Obara H, Kanazawa H, Tanaka H, Fukuda A, et al. Impact of rewarming preservation by continuous machine perfusion: improved post-transplant recovery in pigs. *Transplant Proc*. 2013; 45: 1684–9. <https://doi.org/10.1016/j.transproceed.2013.01.098> PMID: 23769024