The Optimization of Short-Term Hepatocyte Preservation Before Transplantation

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Background. No optimal methods for short-term hepatocyte preservation have been established. We have recently developed a prominent oxygen-permeable bag (Tohoku Device [TD]) for pancreatic islet culture and transplantation. In this study, we investigated whether TD is also effective for hepatocyte preservation and tried to optimize other conditions. Methods. Hepatocytes were preserved in the following conditions, and their outcomes were observed. First, the effectiveness of TD was investigated. Second, hepatocyte medium (HM) and organ preservation solutions with or without fetal bovine serum (FBS) were compared. Third, as supplementations, FBS and human serum albumin (HSA) were compared. Fourth, low, room and high temperature were compared. And finally, hepatocytes preserved in various conditions were transplanted into the subrenal capsule space of nonabdominal rats and engrafted areas were assessed. Results. The survival rate of hepatocytes preserved in TD tended to be higher and their viability and function were maintained significantly greater than those of non-TD group. Irrespective of FBS supplementation, the survival rate of HM group was significantly higher than those of organ preservation solution group while viabilities and plating efficiency were similar among them. Although survival rates of groups without FBS were extremely low, results of HSA supplemented group were not inferior to FBS supplemented group. Hepatocytes preserved at high temperature had the worst results. The engrafted area of TD group tended to be higher than those of other groups. Conclusions. TD is effective for short-term hepatocyte preservation. HSA is a useful substitute for FBS, and preserving in HM at low temperature is recommended.

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Hepatocyte transplantation (HTx) is expected to be a promising option as a bridging therapy for lethal liver diseases, such as fulminant hepatitis,1-4 or as an alternative therapy for patients suffering from liver-related metabolic disorders.2-4 The introduction of HTx to cure inborn errors of metabolism seems logical since the performance of liver transplantation, which is an established conclusive therapy for liver disease, is technically difficult and too invasive for newborns. In contrast, HTx is a simple and minimally invasive approach. Moreover, HTx has an advantage in that...
livers those are not suitable for liver transplantation due to hepatic steatosis, nonheart beating donors, or other reasons, can be utilized. Considering that the replacement of defective hepatocytes with normal hepatocytes could be sufficient for curing liver-related metabolic disorders, the applications of HTx may extend beyond whole organ liver transplantation soon. However, there are still many issues that remain to be solved before HTx can be used for routine clinical applications.

The poor engraftment of transplanted hepatocytes is undoubtedly one of these problems.2–7 This may be, at least in part, due to the low quality of the transplanted hepatocytes. Isolated hepatocytes are well known to be extremely fragile and it is difficult to maintain their viability.5–11 Indeed, Jorns et al12 showed that the function of hepatocytes that were isolated after the preservation of the liver (as an organ) was clearly higher than the function of hepatocytes preserved in the cell state at the same time after isolation. Furthermore, in the clinical setting, the short-term preservation of hepatocytes before transplantation is inevitable because various preparations must be made, including—but not limited to—conveying isolated hepatocytes, performing quality assessments of the hepatocytes, and the insertion of the intraportal catheter. Thus, establishing a protocol that maintains the viability and function of isolated hepatocytes during the few hours between isolation and transplantation may be of great importance in improving the engraftment of HTx. Several groups have thus far reported on the optimization of short-term hepatocyte preservation12–15 or cryopreservation.16,17 However, the optimal methods remain to be established.

The aim of the present study was to investigate and optimize the effects of several conditions including containers, suspending solutions, supplementation, and the temperature during short-term preservation on the viability and function of isolated hepatocytes. Cell preservation is one of the key issues for the success in the field of pancreatic islet transplantation, which has become an alternative to treatment for diabetic patients.18–20 We previously described the 1st use of a culture bag system for preserving pancreatic islets in the clinical setting.21 We also developed a bag system (the Tohoku device [TD]) by applying a prominent oxygen-permeable and transparent material (made from polyethylene film and its oxygen permeation coefficient is 3000 cm3/m2 atm) to improve and simplify the procedures used in islet culture and transplantation.22 Thus, in the present study, we 1st investigated whether the TD is also useful for short-term hepatocyte preservation, and then attempted to optimize other conditions, including the suspending solutions, supplementation and temperature.

MATERIALS AND METHODS

Animals

Rat livers were obtained from male inbred F344/NSLc rats (age, 10–14 weeks; weight, 280–340 g; Japan SLC Inc., Shizuoka, Japan). The albinomimic rats (age, 6–7 weeks), which were characterized by an extraordinarily low serum albumin level, were kindly provided from Prof Yuji Nishikawa at Asahikawa Medical College and were bred at the animal laboratory in Tohoku University. All of the animals used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health,23 and the guidelines for animal experiments and related activities at Tohoku University (approved protocol ID:2015 NICHe-Animal-001). All surgeries were performed under anesthesia, and maximal efforts were made to minimize suffering.

Hepatocyte Isolation

Rat hepatocytes were isolated by the 2-step collagenase perfusion technique as described previously.24 First, Ca2+-free Hanks’ balanced salt solution (Sigma-Aldrich, St. Louis, MO) containing ethylene glycol tetraacetic acid was perfused through the portal vein at a rate of 14 mL/min for 5 minutes. Second, Ca2+-containing Hanks balanced salt solution with 0.5 mg/mL of collagenase (Sigma type V) (Sigma-Aldrich) was perfused via the same route at the same rate. The isolated cells were suspended in Dulbecco’s modified Eagle’s medium (DMEM) (Sigma-Aldrich) containing 10% fetal bovine serum (FBS) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). The cells were then filtered through a 150 mesh (Ikemoto Scientific Technology, Tokyo, Japan) and purified by gradient centrifugation (50g, 2 minutes). Next, gradient centrifugation (50g, 20 minutes) was performed again using Percoll density gradient centrifugation media (GE Healthcare Biosciences, Pittsburgh, PA) to obtain a highly purified cell population. The yield and viability of the hepatocyte preparations were assessed immediately after isolation using trypan blue exclusion (TBE).

The Investigation of the Effects of the TD and a Commonly Used Plastic Tube on the Efficiency of Short-term Hepatocyte Preservation

Aliquots of 1.3 – 1.5 × 107 hepatocytes were suspended in 200 mL of hepatocyte medium (HM) supplemented with 10% FBS and were preserved in the different containers for 2 hours at room temperature (RT) (approximately 26 °C). HM is based on DMEM supplemented with glutamine, penicillin, streptomycin and HEPES. The following 3 groups (n = 8) were used in the present study: (I) Non-TD group: hepatocytes were quickly preserved in the ordinary tube, (II) TD-shaking group: hepatocytes were preserved in the TD and gently shaken (10/min) on a shaker, (III) TD-quiet group: hepatocytes were quietly preserved in the TD without shaking. The TD was purchased from Nipro Corporation (Osaka, Japan).

The Investigation of the Effects of Solutions With/Without FBS Supplementation on the Efficiency of Short-Term Hepatocyte Preservation

Aliquots of 1.3 to 1.5 × 107 hepatocytes were suspended in 200 mL of each solution (HM, ET-Kyoto solution (ETK), and University of Wisconsin preservation solution (UW) and were quietly preserved in the TD at 4°C for 4 hours. Two experimental groups (with or without FBS supplementation) were constructed for each solution, resulting in a total of 6 groups (n = 8).

The Investigation of the Effects of FBS and Albumin Supplementation on the Efficiency of Short-Term Hepatocyte Preservation

Aliquots of 1.3 to 1.5 × 107 hepatocytes were suspended in 200 mL of HM supplemented with 10% FBS (HM/FBS +) or 1 mL of 25% human serum albumin (HSA) supplementation (HM/Alb+) and were preserved quietly in the TD at 4°C for 4 hours (n = 8).
The Investigation of the Effects of Temperature on the Efficiency of Short-Term Hepatocyte Preservation

Aliquots of 1.3 to $1.5 \times 10^7$ hepatocytes were suspended in 200 mL of HM supplemented with 10% FBS and were preserved quietly in the TD for 4 hours at 3 levels of temperature. The low temperature (LT), RT and high temperature (HT) groups were preserved at 4°C, 26°C, and 37°C, respectively ($n = 8$).

The In Vitro Evaluation of the Viability and Function of the Hepatocytes

After preservation, all the solutions and cells were retrieved and purified by gradient centrifugation (50g, 5 minutes). Subsequently, survival rate of hepatocytes and viability of the hepatocytes assessed. Survival rate was defined as the percentage of viable hepatocytes after the preservation to the viable hepatocytes before the preservation. Viability was defined as the percentage of viable hepatocytes to the whole (viable and dead) hepatocytes. Whether hepatocytes were viable or not was judged by TBE.

Aliquots of $0.5 \times 10^6$ viable cells/mL were seeded on type I collagen-coated 35 mm dishes (AGC Techno Glass, Shizuoka, Japan). The cells were cultured in DMEM supplemented with 10% FBS, glutamine, penicillin, streptomycin, and 1 M HEPES at 37°C in 5% CO₂. The culture medium was changed on days 1 and 3. The plating efficiency (PE) was determined by counting the attached and unattached cells using a light microscope at ×200 magnification at 18 hours after plating.

The PE of hepatocytes was expressed as the percentage of attached cells in comparison to the total number of plated hepatocytes. Six fields were randomly studied for each culture dish. Deoxyribonucleic acid (DNA) quantitation was performed to evaluate the energy status of the cultured hepatocytes at 5 days after plating. The DNA content was measured using a DNA Quantify kit (Primary cell, Ishikari, Japan) twice for each dish, as described previously.²⁰ DNA quantitation was only performed when investigating the use of the TD.

Ammonia-load culture medium was prepared with a known concentration of ammonium chloride solution and Williams Medium E containing 10% FBS, 1 μmol/L insulin, and 1 μmol/L dexamethasone. The concentration of ammonia was adjusted to approximately 2.4 mmol/L. Aliquots of $1 \times 10^6$ hepatocytes in 1 mL of the ammonia-load culture medium were cultured using a 35-mm culture dish for 10 minutes at 37°C in a CO₂ incubator, then the concentration of ammonia at 0, 1, 2, and 3 hours was measured in 20 μL aliquots of the culture medium using an Amicheck Meter (Arkray, Inc., Kyoto, Japan). The ammonia concentration at each point was shown as the percentage in comparison to the concentration at 0 hour.

Immunohistochemical Staining

Hepatocyte pellets (38.4 μL) equivalent to approximately $2.0 \times 10^6$ hepatocytes were collected using a gastight syringe (Hamilton Company, Reno, NV) and were preserved at RT (26°C) for 4 hours under following 4 conditions: (I) HM with no supplementation in an ordinary tube (HM/Tube group), (II) HM with HSA in an ordinary tube (HM + Alb/Tube group), (III) HM with 10% FBS in an ordinary tube (HM + FBS/Tube), (IV) HM with HSA in the TD (HM + Alb/TD), (V) HM with 10% FBS in the TD (HM + FBS/TD group). After preservation for 4 hours, each group of hepatocytes was centrifuged (50g, 2 minutes) and suspended in 1 mL of HM with HSA. The hepatocytes were spun down and transplanted in pellet form into the left renal subcapsular space of albinumemic rats ($n = 8$). Immunosuppressants were not administered because the recipients were congenic with the donors. On day 4, the recipients were sacrificed, and left nephrectomy was performed. The left kidneys were equally divided into 8 slices and stained using hematoxylin and eosin (Muto Pure Chemicals Co., Ltd., Tokyo, Japan) and antialbumin antibodies (MP Biomedicals, Santa Ana, CA) combined with the VECTASTAIN ABC system (Vector Laboratories, Inc., Burlingame, CA) for albumin staining. Then albumin-positive area on each section was measured using the Image J software program (version 1.47) (NIH, Bethesda, MD)²⁵ and the engrafted area was estimated by calculating the area under the curve of the albumin-positive hepatocytes on 8 sections.

Statistical Analysis

All values were expressed as the means and standard deviation. The statistical analyses were performed using the JMP pro 11 software program (SAS Institute Inc., Charlotte, NC). Student t test was used for comparisons between 2 groups. Data from more than 3 groups were compared using an analysis of variance. The Turkey honestly significant difference test was used for multiple post hoc comparisons to determine the significance of differences. P values less than 0.05 were considered to indicate statistical significance.

RESULTS

The Effects of the TD and a Commonly Used Plastic Tube on the Efficiency of Short-Term Hepatocyte Preservation

The survival rate of isolated hepatocytes after 2-hour preservation in the TD group tended to be higher than that in the non-TD group but did not reach significance ($P = 0.149$) (Figure 1A). The viability of the isolated hepatocytes after 2-hour preservation in the TD-shaking and TD-quiet groups was significantly higher than that of the non-TD group ($P = 0.027$ and 0.003, respectively) (Figure 1B). The PE of 18-hour cultured hepatocytes after 2-hour preservation in the TD-quiet group was significantly higher than that in the non-TD group ($P = 0.027$) (Figure 1C). No significant difference in DNA quantitation was observed among any of the groups ($P = 0.312$) (Figure 1D).

The Effects of Solutions With/Without FBS Supplementation on the Efficiency of Short-Term Hepatocyte Preservation

The survival rate and viability of the isolated hepatocytes and the PE of cultured hepatocytes after 4-hour preservation were generally well maintained in the groups that received FBS supplementation in comparison to the groups that did not, irrespective of the solutions that were used in short-term hepatocyte preservation (Figures 2A-C).

The survival rate of isolated hepatocytes after 4-hour preservation in the HM/FBS(+) group was significantly higher than that in the ETK/FBS(+) and UW/FBS(+) groups ($P = 0.0005$ and 0.001, respectively) and that in the ETK/FBS(+) group was significantly higher than that in the UW/FBS(+) group ($P = 0.038$). The survival rate in the HM/FBS(−) group was also significantly higher than that of the ETK/FBS(−) and UW/FBS(−) groups ($P = 0.009$ and 0.016, respectively).
No significant differences were seen in terms of viability (P = 0.681 and 0.584) or PE (P = 0.549 and 0.687) among the groups with or without FBS supplementation (Figures 2B and C).

The Effects of FBS and Albumin Supplementation on the Efficiency of Short-Term Hepatocyte Preservation

No significant differences were observed in the survival rate or PE between the FBS supplementation and albumin supplementation groups (P = 0.881, 0.761) (Figures 3A and C). However, a slight, but significant difference was observed in the viability of isolated hepatocytes in the FBS and albumin groups (89.8 ± 3.2% vs. 86.6 ± 2.7%, respectively; P = 0.046). The 2 groups showed almost the same tendency on the ammonia removal test (1, 2, and 3-hour percentages of remaining ammonia concentration, FBS group: 43.9 ± 7.2%, 25.5 ± 6.8%, and 16.4 ± 6.2%, respectively; albumin group: 46.2 ± 4.3%, 22.6 ± 0.1%, and 16.0 ± 4.6%, respectively). P values of each point were 0.164, 0.160, and 0.869, respectively (Figure 3D).

The Effects of Temperature on the Efficiency of Short-Term Hepatocyte Preservation

The survival rate of isolated hepatocytes after 4-hour preservation in the HT group was significantly lower than that in the LT and RT groups (P = 0.008 and 0.013, respectively) (Figure 4A). Likewise, the viability in the HT group was significantly lower than that in the LT group (P = 0.042) (Figure 4B). In terms of the PE of cultured hepatocytes after 4-hour preservation, exactly same tendency was seen between the HT group versus the LT and RT groups (P < 0.0001, P = 0.0002, respectively) (Figure 4C). In contrast, an ammonia removal test of the isolated hepatocytes revealed a similar pattern among all the groups (1-, 2-, and 3-hour percentages of remaining ammonia concentration, LT group: 31.1 ± 6.4%, 11.2 ± 6.1%, and 8.6 ± 2.2%, respectively; RT group: 25.0 ± 4.0%, 10.9 ± 5.0%, and 8.5 ± 1.6%, respectively, HT group: 23.7 ± 5.3%, 11.3 ± 3.1%, and 8.6 ± 2.2%, respectively). P values of each point were 0.341, 0.986, and 0.994, respectively (Figure 4D).

The In Vivo Evaluation of the Engraftment of Hepatocytes Transplanted Into the Renal Subcapsular Space

The engrafted area was estimated by calculating the area under the curve of albumin-positive hepatocytes on 8 pathologic sections per recipient kidney. Although the difference in the engrafted area did not reach statistical significance (P = 0.101), the use of TD appeared to be beneficial for improving...
hepatocyte engraftment (HM + Alb/TD group: 3.06 ± 0.78 gigapixels vs HM + Alb/Tube group: 2.89 ± 0.94 gigapixels, \( P = 0.743 \) and HM + FBS/TD group: 3.95 ± 1.13 gigapixels vs HM + FBS/Tube group: 3.27 ± 1.32 gigapixels, \( P = 0.200 \)) (Figure 5C).

DISCUSSION

It has been noted that HTx, which is a promising alternative therapeutic option to liver transplantation for patients with liver-based metabolic disorders, shares many aspects with pancreatic islet transplantation. Pancreatic islet transplantation is currently regarded as one of the treatment options for severe diabetes.

Considering that the clinical applications of HTx remain limited, a number of experiences originating from islet transplantation, including cell isolation methods, culture systems, cell preservation, cell graft evaluation, and engraftment could be applicable for improving the outcomes of HTx. Per the above-noted concepts, in the present study, the TD was applied to hepatocytes and was found to be effective for maintaining the viability and function of hepatocytes during a short-term preservation period. The major advantages of the TD include the prominent oxygen permeability and the prevention of cell aggregation due to a surface coating with novel materials. Thus, the use of TD may have contributed to minimizing hypoxic damage to the hepatocytes, resulting in the preferable outcome. Merani et al reported that compaction of cells was detrimental to islet transplantation, thus it is most likely that aggregation is also deleterious to hepatocytes because they are much more fragile than pancreatic islets. Hence, the other advantage of the TD—the prevention of cellular aggregation—could be another possible explanation for the favorable results that were obtained in the case of hepatocytes. In a previous report, Sufiandi et al, found that sheer stress was also detrimental to hepatocytes. In support of this finding, the survival rate, viability, and PE in the TD-quiet group tended to be superior to the TD-shaking group; however, the differences did not reach statistical significance.

FIGURE 2. The effects of solutions with/without FBS supplementation on the efficiency of short-term hepatocyte preservation. A, The survival rate of isolated hepatocytes after 4-hour preservation in the HM/FBS(+) group (black bar: 76.5 ± 15.9%, \( n = 8 \)), the ETK/FBS(+) group (dotted bar: 53.7 ± 18.3%, \( n = 8 \)), the UW/FBS(+) group (gray bar: 33.8 ± 9.8%, \( n = 8 \)), the HM/FBS(−) group (gray bar: 33.3 ± 19.0%, \( n = 8 \)), the ETK/FBS(−) group (horizontal line bar: 7.4 ± 2.9%, \( n = 8 \)) and the UW/FBS(−) group (vertical line bar: 9.4 ± 4.5%, \( n = 8 \)). *\( P < 0.01 \), the HM/FBS(+) group vs. the ETK/FBS(+) and the UW/FBS(+) group; the HM/FBS(−) group vs. the ETK/FBS(−) group. **\( P < 0.05 \), the ETK/FBS(+) group vs. the UW/FBS(+) group; the HM/FBS(−) group vs. the UW/FBS(−) group. B, The viability of the isolated hepatocytes after 4-hour preservation in the HM/FBS(+) group (black bar: 89.5 ± 2.7%, \( n = 8 \)), the ETK/FBS(+) group (dotted bar: 89.1 ± 2.9%, \( n = 8 \)), the UW/FBS(+) group (gray bar: 71.5 ± 9.6%, \( n = 8 \)), the ETK/FBS(−) group (horizontal line bar: 68.0 ± 10.2%, \( n = 8 \)) and the UW/FBS(−) group (vertical line bar: 73.1 ± 10.1%, \( n = 8 \)). C, The PE of 24-hour cultured hepatocytes after 4-hour preservation in the HM/FBS(+) group (black bar: 65.4 ± 10.4%, \( n = 8 \)), the ETK/FBS(+) group (dotted bar: 58.9 ± 11.8%, \( n = 8 \)), UW/FBS(+) group (gray bar: 62.3 ± 12.7%, \( n = 8 \)), the HM/FBS(−) group (gray bar: 52.5 ± 10.2%, \( n = 8 \)), ETK/FBS(−) group (horizontal line bar: 51.0 ± 6.0%, \( n = 8 \)) and the UW/FBS(−) group (vertical line bar: 48.7 ± 8.5%, \( n = 8 \)).
In organ transplantation, preservation solutions are crucial and are intimately associated with the transplant outcome especially in cases with a long cold ischemic time. In liver transplantation, UW solution has been established as the gold standard and is currently used in all over the world. However, in this study, we found that both UW solution and ETK solution, which has been shown to be useful for pancreatic islet preservation, appeared to be undesirable for maintaining the isolated hepatocytes during a short-term preservation period. Although it was previously reported that UW solution seemed to be beneficial to some extent in terms of maintaining the viability of hepatocytes, the effects on the survival rate remain uncertain. In accordance with previous studies, the viability of the hepatocytes that were preserved in UW solution remained at the same level as the other groups in the present study. However, there was a remarkable decrease in the survival rate of the hepatocytes that were preserved in UW solution in comparison to the other groups. The high viscosity and/or high potassium level of the UW solution might have adversely affected the isolated hepatocytes. It was reported by Gómez-Lechón et al. that citrate medium supplemented with glutathione and N-acetyl-cysteine might be useful as a short-term preservation solution for hepatocytes. However, the viability of hepatocytes after 3-hour-preservation in their study (72.0 ± 5.0%) was considerably lower than that of the present our study (86.5 ± 2.7%), suggesting that an additional use of TD may at least in part be beneficial for maintaining the viability of isolated hepatocytes. Poullain et al. also reported that Leivovitz L-15 culture medium supplemented with 4% polyethylene glycol could maintain the viability of isolated hepatocytes during 48 hours (83.0 ± 2.0%). Given that high viability of hepatocytes was kept for rather long period, the use of Leivovitz L-15 culture medium supplemented with 4% polyethylene glycol in combination with TD could be a possible candidate for effective protocol of short-term hepatocyte preservation.

The present examinations also indicated the importance of FBS in maintaining the survival rate and viability of hepatocytes (Figures 2A-C). However, the use of FBS in clinical HTx is not desirable, thus alternative supplements are essential. For this purpose, we investigated the efficacy of albumin. Although only a slight difference was seen in the viability of the isolated hepatocytes and in the engrafted area of the transplanted hepatocytes, the investigation proved that FBS could be replaced by albumin. To the best of our knowledge, this is the first report to show the effectiveness of albumin in
maintaining the quality of isolated hepatocytes during short-term preservation. It is most likely that the beneficial effects of albumin are only limited to its capacity to prevent cellular aggregation. Thus, additional supplements, such as polyethylene glycol or N-acetyl-cysteine, which have been shown to increase the viability of isolated hepatocytes during cold preservation, should be sought to completely compensate for the capacity of FBS.

Regarding the influence of temperature on the isolated hepatocytes, it revealed that a HT (37°C) was less suitable for hepatocyte preservation than LT (4°C) and RT (26°C). Likewise, an LT has also been shown to be beneficial for maintaining the survival rate of pancreatic islets. However, the optimal temperature for islets remains controversial because it was also reported that the islet function is well maintained at 37°C. In the case of hepatocyte preservation, given that both the survival rate and the function of the hepatocytes deteriorated at 37°C, it could be concluded that preservation should not be performed at 37°C. Although the survival rate and viability were equivalent between the LT and RT groups, the PE in the LT group tended to be higher than that in the RT group, suggesting that the LT might be superior to RT for maintaining the function of isolated hepatocytes during short-term preservation. Of note, the viability and function of hepatocytes in the early phase after preservation, which were assessed by TBE and an ammonia removal test, respectively, were similar among the groups, whereas the PE in the HT group was extremely low in comparison to the other 2 groups. This observation suggests that the detrimental effects of HT on hepatocyte function are associated with an onset in a rather late phase.

In the present study, the renal subcapsular space was used as transplant site to accurately assess the whole engrafted area in the in vivo immunohistochemical study. This is one of the most established pancreatic islet models; Ohashi et al. reported that this method was even successful in cases involving HTx. Reflecting the outcomes of the in vitro studies, each group of TD (HM + Alb/TD group and HM + FBS/TD group) tended to have better rate of engraftment than Tube groups which contained same solutions (HM + Alb/Tube group and HM + FBS/Tube group). But differences did not reach statistical significance. This may indicate that several crucial factors other than short-term preservation could be associated with the engraftment of transplanted...
hepatocytes. The possible candidate factors include—but are not limited to—cell density, pressure to the hepatocyte grafts at the transplant area, the transplant site. In addition to short-term preservation, the optimization of these factors is crucial for improving the outcome of hepatocyte transplantation, and will be topics of interest for our next study.

In conclusion, the present data suggest that suspending a TD in common culture media, but not in the organ preservation solutions, under LT conditions is effective for short-term hepatocyte preservation before transplantation. Although FBS is a crucial factor for maintaining the quality of isolated hepatocytes, albumin might be a useful substitute for FBS in the clinical setting.

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