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RESEARCH

Protection of liver sinusoidal endothelial cells using different preservation solutions

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Abstract

Donor liver preservation methods and solutions have evolved over the last years. Liver sinusoidal endothelial cell (LSEC) barrier function and integrity during preservation are crucial for outcomes of liver transplantation. Therefore, the present study aimed to determine optimal preservation of LSEC barrier function and integrity using different preservation solutions. Human umbilical vein endothelial cells (HUVECs) and LSECs were incubated in either University of Wisconsin machine perfusion solution (UW-MPS), histidine-tryptophan-ketoglutarate, or endothelial cell growth medium 2 (EGM2) (as a gold standard for cell culturing). Endothelial integrity was assessed by measurement of cellular morphology and expression of membrane proteins: PECAM-1, ICAM-1 and Fc-gamma receptor 2b (FCGR2B). Endothelial barrier function was measured by electric cell-substrate impedance sensing. Cellular response to inflammatory stimuli with tumor necrosis factor-alpha (TNF-α) was tested by studying trans-endothelial migration (TEM) under flow conditions. Differences in these parameters were analyzed between the different preservation solutions. PECAM-1 expression was high for all preservation solutions in HUVECs and LSECs. ICAM-1 expression was increased in both LSECs and HUVECs in all preservation solutions plus TNF-α. UW reduced PECAM-1 expression, whereas EGM2 medium promoted barrier function in LSECs and HUVECs, and monolayer recovery after wounding was best achieved in cells incubated in EGM2. LSECs and HUVECs incubated with EGM2 plus TNF-α both supported neutrophil adhesion and TEM, but much less to none when incubated in UW plus TNF-α. Overall, EGM2 showed the best results in preserving endothelial barrier function for both HUVECs and LSECs.

Keywords: preservation; organ transplant; endothelium; sinusoids; liver

Introduction

Liver transplantation is a life-saving treatment for patients suffering from end-stage liver disease. However, due to donor shortage, the waiting list continues to increase. Therefore, prevention of donor liver discard is needed to achieve more donor livers suitable for transplantation. In recent years, donor organ preservation methods have improved substantially. Machine perfusion (MP) preservation has taken place for cold static storage (SCS). Both methods use a preservation solution to maintain cellular integrity under hypothermic or (sub)-normothermic conditions, and consequently, each preservation method may need

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a different type of preservation solution. To date, the University of Wisconsin (UW) solution is used for SCS and in a modified form, also for MP either with or without the addition of oxygen (1).

Hypothermic MP (HMP) preservation is now the standard for donor kidney preservation (2, 3). Current HMP systems are portable, enabling the donor kidney to be transported and mounted into the HMP apparatus. For donor liver preservation, MP systems clinically used today are either sub-normothermic (SNMP) or normothermic MP (NMP) systems and need a perfusate that is rich in additives with a high oxygen-binding capacity, such as erythrocytes, making it possible to carry oxygen as a supply when dissolved oxygen is consumed (4). (S)NMP can help to flush out metabolites and cytokines and enables monitoring of cellular function before implantation into the recipient patient (5, 6). The critical injury still occurs; however, at the time the donor liver is reperfused with circulating, warm and oxygenated blood of the recipient at reperfusion, a process characterized as ischemia-reperfusion injury (IRI) (7).

IRI particularly affects the liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) of the liver graft (7). LSECs are important endothelial cells that line the sinusoids in the liver (8). LSECs regulate vascular tone and blood flow in the sinusoids, maintaining a low pressure in the portal venous liver circulation and function as immunomodulating and nutrient doorkeepers for hepatocytes (8). Cold ischemia time causes adenosine triphosphate (ATP) depletion in LSECs, leading to mitochondrial dysfunction and cellular swelling (9, 10). Upon reperfusion in the recipient, reactive oxygen species (ROS) are formed, which damage LSECs even more. Eventually, a lack of nitric oxide occurs, which increases vascular tone and restricts blood flow to the hepatocytes (11, 12, 13). The damaged LSECs express vascular adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) (7, 14). ICAM-1 is a membrane molecule that, once activated on the LSEC membrane, can bind and allow trans-endothelial migration (TEM) of leukocytes through the endothelium. ICAM-1 induces the production of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) from KCs, which by itself also enhances the activation of ICAM-1 and other inflammatory pathways. Previous research found that donor livers produce TNF-α during preservation and after reperfusion (15). Furthermore, the recipient's own TNF-α serum levels before and after reperfusion of the donor liver have also been shown to predict post-liver transplantation complications (15). Previous research by Post *et al.* showed that incubation at 20 °C with UW solution resulted in optimal preservation of human umbilical vein endothelial cells (HUVECs) (16). However, as LSECs morphologically, functionally and phenotypically differ from HUVECs, it is important to study optimal preservation conditions for LSECs.

The present study aimed to investigate which preservation solution most successfully maintains LSEC barrier function and LSEC monolayer integrity under normothermic conditions. Hereby, we analyzed the response of the LSECs to inflammation induced by TNF-α by measuring the expression levels of intercellular adhesion molecules and changes in physiological identity markers before and after applying vascular endothelial (VE) monolayer damage by wounding experiments. In addition, we analyzed and quantified neutrophil adhesion and TEM across LSECs that were incubated in the different preservation solutions.

Methods

HUVECs isolated from umbilical cord

Human umbilical cords were obtained after written informed consent of the mothers at the obstetrics and gynecology department of the Amsterdam UMC and transported in a flask of phosphate-buffered saline (PBS) to the molecular cell biology research laboratory of Sanquin. For isolation of endothelial cells from the cords, the following protocol was followed: the outer ends of the cords were cut off and a cannula was inserted into the umbilical vein that was tied up with a tie-rip. To remove old blood from the cord, the vein was gently flushed with warm PBS. HUVECs were removed by short incubation with 0.05% trypsin–EDTA solution (Sigma–Aldrich, USA) after closing the outer end of the vein. Then, the flush-out was collected into 50 mL tubes and rinsed three times with PBS. The effluent was centrifuged at 1500 rpm, 9↑ 9↓ for 5 min. The first supernatant was removed, and the pellet was resuspended, and this suspension was plated out on fibronectin-coated petri dishes.

Cell culture of HUVECs and LSECs

HUVECs were isolated from umbilical cords (see above), and LSECs (Zenbio, USA; LSEC) were obtained from donor livers not suitable for organ transplantation, delivered in passage 3. Each donor had signed documentation confirming approval for research of any non-transplantable organ or tissue, and all donors were screened for important viral diseases. LSECs were also obtained from human livers provided by ScienCell, delivered in passage 1. LSECs were thawed from cryopreservation and incubated in EGM2 medium (Promocell, Germany) supplemented with 1% PenStrep (Invitrogen, Thermo Fischer Scientific, USA) under culture conditions (95% air, 5% CO_2, 37 °C). Ninety-five percent of air contains a partial oxygen pressure (pO2) of 150 mmHg, which is higher when compared to the pO2 in human blood, ranging between 75 and 100 mmHg. LSECs were seeded on fibronectin-coated T25 flasks following manufacturing advice. Cells were cultured onto passages

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3 to 5 of a confluent monolayer and then were incubated on fibronectin-coated wells and coverslips for different experiments.

Experimental design

Both HUVECs and LSECs were cultured in endothelial growth medium 2 (EGM2) medium (Promocell) in fibronectin-coated flasks or petri dishes, until passage 5. After reaching a confluent cell layer in EGM2 medium, LSECs and HUVECs were incubated in different preservation media for up to 18 h and for some experiments, measurements were taken after 1, 6 and 10 h of incubation. HUVEC incubated in EGM2 medium, which is the optimal standard for culture of HUVEC, was used as a control. IR injury was imitated by causing cellular damage using cell electroporation in the electric cell-substrate impedance sensing (ECIS) experiment and by adding 10 ng/mL TNF-α to the cells. Since IR injury is associated with upregulation of ICAM-1 while TNF-α also induces upregulation of ICAM-1, we tried to mimic the same circumstances for cells after IR injury. In the liver, KCs produce TNF-α after IR injury, infection or impaired microcirculation. However, in the current experiment, HUVECs and LSECs were cultured singularly without KC. Therefore, HUVECs and LSECs were stimulated with added TNF-α, except for controls, and every experiment was performed in duplicate and repeated at least three times. Three clinically used organ preservation solutions were compared with each other and with the standard medium for cell culture, EGM2. For the experiments, fetal calf serum and glucose were added to the clinical preservation solutions and EGM2. Experiments were designed to test the capability of the different preservation solutions in preserving the cellular barrier function and integrity of LSECs with HUVECs as a control. The four solutions used for preservation were EGM2, UW MP solution (UW-MPS) (Belzers Bridge to Life, USA), RL and histidine-tryptophan-ketoglutarate (HTK) (Custodiol, USA) (Table 1). To all solutions, antibiotics were added to prevent bacterial overgrowth during cell culture.

Immunofluorescence (IF) staining

Cellular integrity and morphology were analyzed by visualizing intercellular adhesion molecules using IF with the following markers: Phalloidin conjugated with Texas Red 568 (Invitrogen) for F-actin filaments, VE-cadherin conjugated with AF488 (Invitrogen) for cell–cell junctions, ICAM-1 conjugated with AF405 (Santa Cruz Biotechnologies, USA) for testing the appearance of pro-inflammatory adhesion receptors, platelet endothelial cell adhesion molecule-1 (PECAM-1) conjugated with AF647 (Becton Dickinson, USA) for endothelial identity and specificity and Hoechst staining for cell nuclei. HUVECs and LSECs were plated on fibronectin-coated coverslips and incubated with the four different preservation solutions for at least 18 h, respectively. Thereafter, coverslips were fixed and incubated with primary antibodies (indicated above) for 1 hour, then washed with PBS and mounted using Mowiol (10% Mowiol 4-88, 2.5% Dabco, 25% glycerol and pH 8.5). Images were acquired using a confocal laser scanning microscope (Leica SP8) with a 20×, 40× or 60× NA 1.4 oil immersion objective and HyD detectors.

Expression of specific endothelial cell molecules

With fluorescence-activated cell sorting (FACS), LSECs and HUVECs were analyzed for the expression of ICAM1, PECAM and FCGR2B. ICAM1 was analyzed for

Table 1 Components of preservation media.

	Components	Additives*
EGM2	Fetal calf serum 0.05 mL/mL, epidermal growth factor (recombinant human) 5 ng/mL, basis fibroblast growth factor (recombinant human) 10 ng/mL, insulin-like growth factor (long R3 IGF-1) 20 ng/mL, VE growth factor 165 (recombinant human) 0.5 ng/mL, ascorbic acid 1 μg/mL, hydrocortisone 0.2 μg/mL, Promocell®	1% PenStrep (Invitrogen) and glucose
UW-MPS	Adenine 5 mmol/L, CaCl 0.5 mmol/L, dextrose 10 mmol/L, reduced glutathione 3 mmol/L, HEPES buffer 10 mmol/L, hydroxyethyl starch N/A, Mg gluconate 5 mmol/L, mannitol 30 mmol/L, KH2PO4 25 mmol/L. Ribose 5 mmol/L, sodium gluconate 80 mmol/L, NaOH N/A, sterile water N/N, Belzers Bridge to Life®	1% PenStrep (Invitrogen) and glucose
RL	NaCl 6.0 g/L, sodium lactate 3.1 g/L, KCL 0.3 g/L, calcium chloride 0.2 g/L, 6.5 pH, Na^+ 130 mEq/L, K^+ 4 mEq/L, Ca^{2+} 2.7 mEq/L, Cl^- 190 mEq/L, lactate 28 mEq/L, caloric content 9 kcal/L, osmolarity 273 mOsmol/L, Baxter® (USA)	1% PenStrep (Invitrogen) and glucose
HTK	NaCl 15 mmol/L, KCl 9.0 mmol/L, C5H5KO5 1.0 mmol/L, MgCl . $6H_2O$ 4.0 mmol/L, Histidine. HCL·H_2O 18.0 mmol/L, histidine 180.0 mmol/L, tryptophan 2.0 mmol/L, mannitol 30.0 mmol/L, CaCl $2H_2O$ 0.015 mmol/L, in sterile water, CL-50 mval, Custodiol®	1% PenStrep (Invitrogen) and glucose

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the ability of inflammatory activation, and PECAM and FCGR2B were analyzed for changes in cellular identity. After incubation of at least 8 h in preservation media, cells were harvested for FACS analysis. To investigate differences in maintaining cellular integrity and expression of these markers under a pro-inflammatory state, TNF-α was added to half of the cells and the other half was not treated with TNF-α as a control.

Endothelial cell monolayer barrier function

ECIS was used to analyze the cellular barrier by measuring the electrical resistance of the cellular layer. In addition, the function and ability of cells to recover from a wound or pro-inflammatory trigger were analyzed, while cells were preserved in different preservation media. LSECs and HUVECs were cultured on 8-well-fibronectin-coated ECIS slides. All cells were first incubated in EGM2 for 1 h and then were replaced with the other preservation media.

ECIS induced a wound in the cellular monolayer by applying high voltage through the EC monolayer, resulting in damage of the monolayer. Recovery was monitored in real time for an additional 8 h in the same preservation media to investigate any differences in the wound-healing capacity of the LSECs, with HUVECs as a control.

Neutrophil adhesion and TEM across endothelium under physiological flow

Ibidi flow chambers were used to study the role of LSECs and HUVECs on the efficiency of neutrophil TEM (flow: 0.5 mL/ min). TNF-α treatment was used to activate the endothelial cells. Differences in the amount of adhering and transmigrating neutrophils were analyzed for all different preservation media.

Neutrophil isolation

Neutrophils were obtained from healthy volunteers. Whole blood with heparin was diluted (1:1) with 5% trisodium citrate in PBS; thereafter, the diluted blood was pipetted on 12.5 mL Percoll (1.076 g/mL) at room temperature. The blood was centrifuged at 800 *g* at room temperature for 20 min. The peripheral blood molecular cell ring fraction and blood plasma were discarded, and erythrocytes were lysed using ice-cold lysis buffer (155 nM NH_4CL, 10 mM KHCO and 0.1 mM EDTA). Thereafter, the neutrophils were centrifuged for 5 min at 450 *g* at 4 °C. The neutrophils were suspended in HEPES buffer (20 mM HEPES, 132 mM NaCl, 6 mM KCL, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 1.2 mM K_2HPO_4, 5 mM D-glucose (Sigma-Aldrich) and 0.4% human serum albumin (Sanquin reagents, the Netherlands). Neutrophils were labeled with calcein red-orange (Invitrogen) according to the manufacturer's protocol. Neutrophils were kept at room temperature for a maximum of 4 h prior to the experiment.

Statistical analysis

Data from IF staining and Ibidi flow experiments were analyzed using Fiji/ImageJ software (https://imagej.net/software/fiji/downloads). Images with labeled neutrophils were thresholded and analyzed using the 'Analyze particles'. To quantify neutrophils rolling and translocating, the manual 'Tracking ImageJ plugin' was used. A turn was defined as a change in the direction of more than 90 degrees in the track of a specific neutrophil. Adhered and transmigrated neutrophils were quantified manually by counting the number of neutrophils and labeling them in the ImageJ software. ECIS data were analyzed using Microsoft Excel. The mean of the resistance in Ohm was calculated for each preservation fluid and for each different measurement time point (after 1, 6 and 18 h of incubation before and after wounding or TNF-α treatment). These means were plotted in a bar chart. For flow cytometry, cells were identified and counted using a BD FACS Aria^{TM} cell sorter. Student's *t*-test is used as the statistical test to test whether the difference between the responses of the groups is significant or not.

Results

Identifying endothelial cells

To identify the morphological response of HUVECs and LSECs to preservation solutions with and without TNF-α, we performed IF staining on the endothelial monolayer for F-actin, VE-cadherin and PECAM-1 (Fig. 1). In LSECs and HUVECs incubated in EGM2 or HTK showed normal distribution of F-actin. Upon TNF-α, the actin filaments showed a more elongated phenotype, in line with the literature (Fig. 1A) with VE-cadherin and PECAM-1 present at cell–cell junctions (Fig. 1B and C).

Protein surface expression

After incubation with the preservation media for at least 8 h, ECs were harvested and analyzed for the expression of surface membrane proteins ICAM-1, PECAM-1 and FCGR2B. We measured the expression of the surface molecules using flow cytometry. This is an accurate measurement of the expression levels of the proteins that are actually on the surface of the endothelium and excludes the presence of proteins expressed intracellularly. This latter issue may interfere with the analysis. For both cell types, we detected

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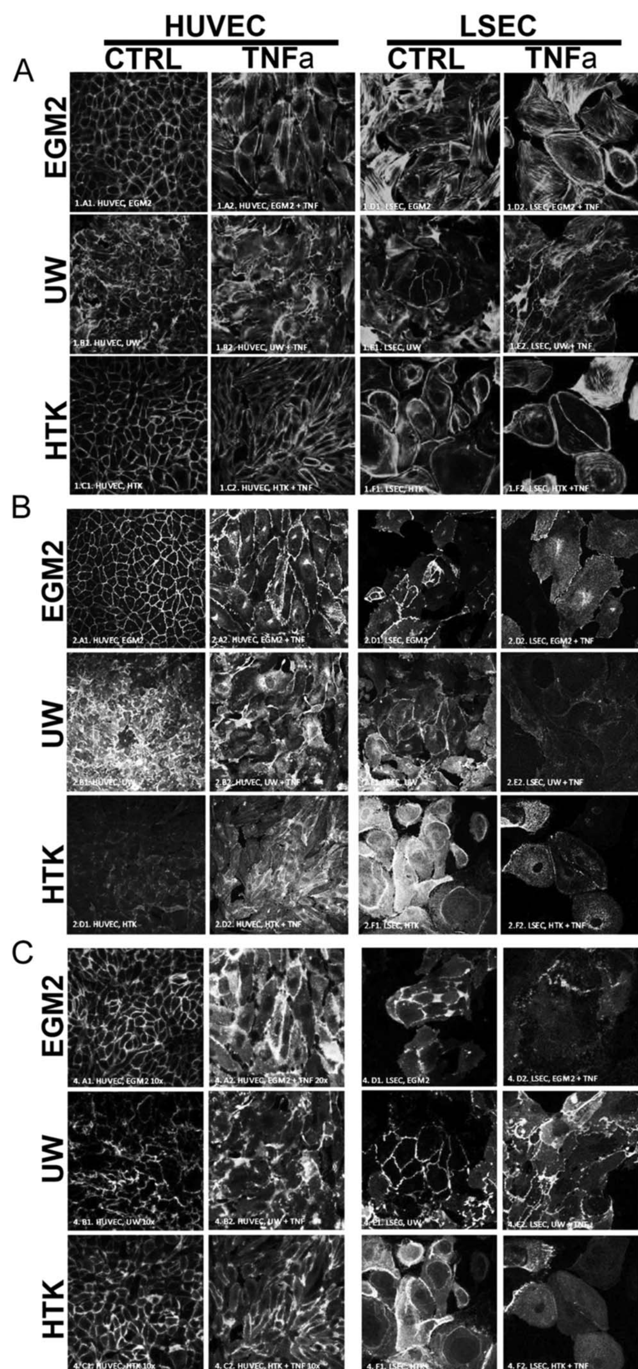

Figure 1

Immunofluorescent staining of endothelial monolayers. Endothelial monolayers from umbilical cord origin (HUVECs) or sinusoids from liver origin (LSECs) were cultured in solutes and left untreated (CTRL) or treated overnight (18 h) with TNF-α as indicated. (A) Endothelial cells are stained for F-actin, (B) VE-cadherin and (C) PECAM-1.

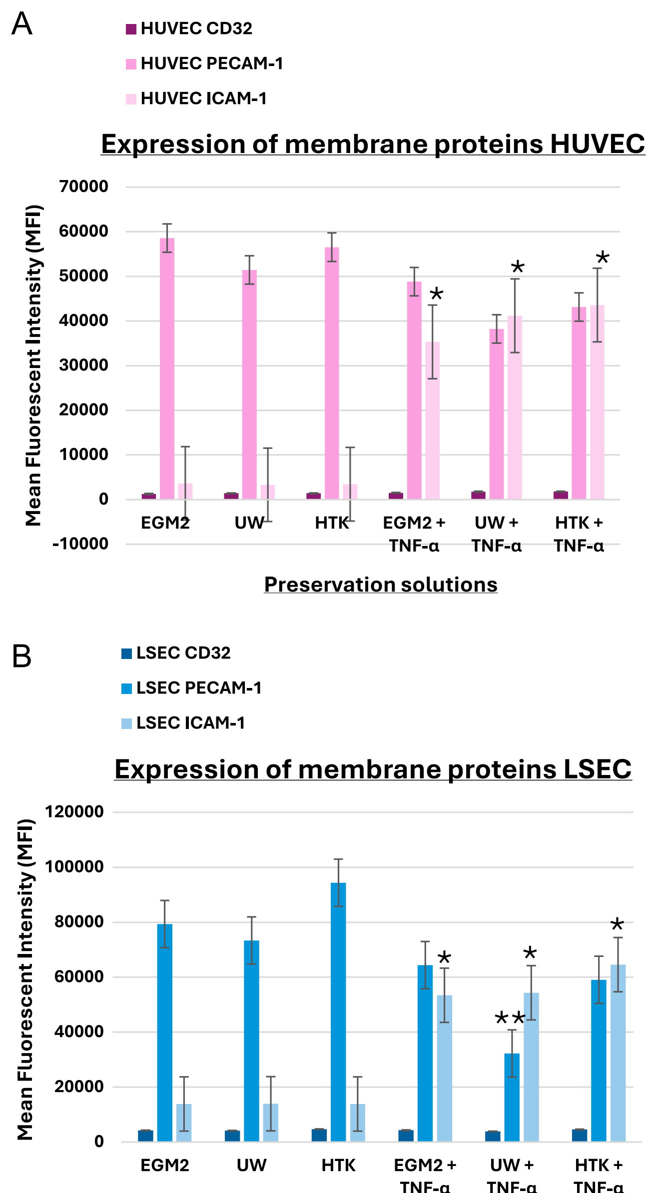

Figure 2

(A) FACS protein expression results of CD23, PECAM-1 and ICAM-1 in HUVECs preserved in EGM2, UW or HTK and treated with or without TNF-α. ICAM-1 expression was significantly increased upon TNF-α for all conditions measured. Experiments were done three times independently from each other. *P < 0.01. (B) FACS protein expression results of CD23, PECAM-1 and ICAM-1 in LSECs preserved in EGM2, UW or HTK and treated with or without TNF-α. ICAM-1 shows a significant increase in expression after TNF-α treatment. Experiments were done three times independently from each other. *P < 0.01; **P < 0.05.

increased ICAM-1 levels upon TNF-α (Fig. 2A and B), whereas PECAM-1 expression levels did not change much between cell types and treatments. For FCGR2B, expression was low in both HUVECs and LSECs.

Endothelial monolayer functionality

To monitor the functionality of the monolayers of HUVECs and LSECs, we used ECIS. In particular, EGM2 medium showed stable barrier values over time before wounding (Fig. 3A).

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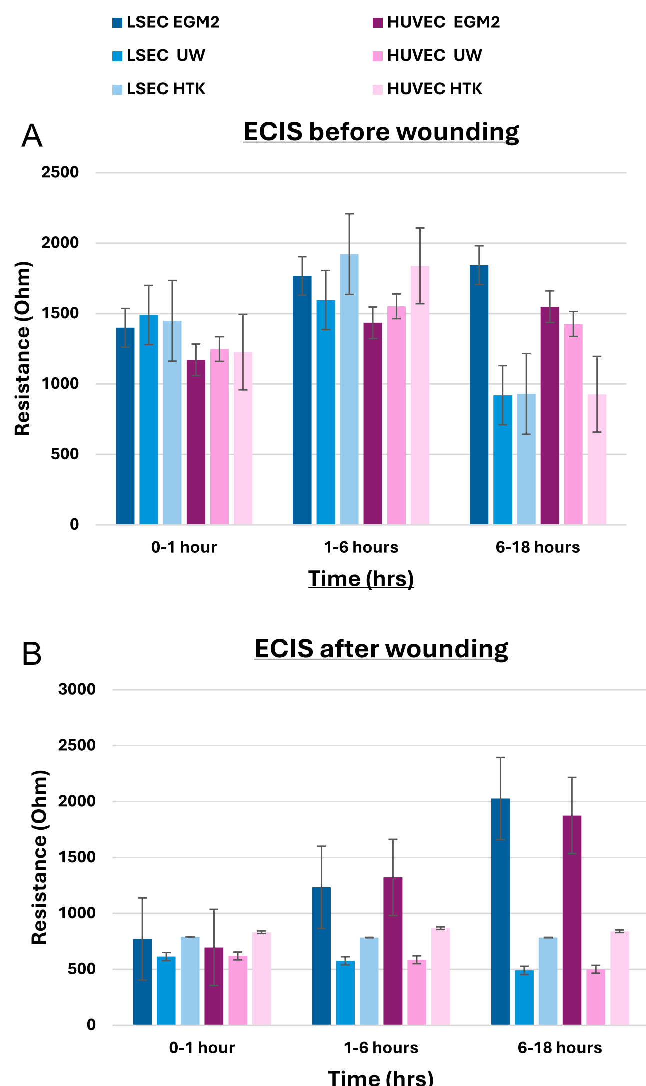

Figure 3

(A) Electric cell impedance sensing results of both LSECs and HUVECs during 18 h of incubation in either EGM2, UW or HTK. (B) Electric cell impedance sensing results after wounding by electroporation of both LSECs and HUVECs during 18 h of incubation in either EGM2, UW or HTK. Both LSECs and HUVECs showed the highest resistance after wounding, especially after 18 h of incubation.

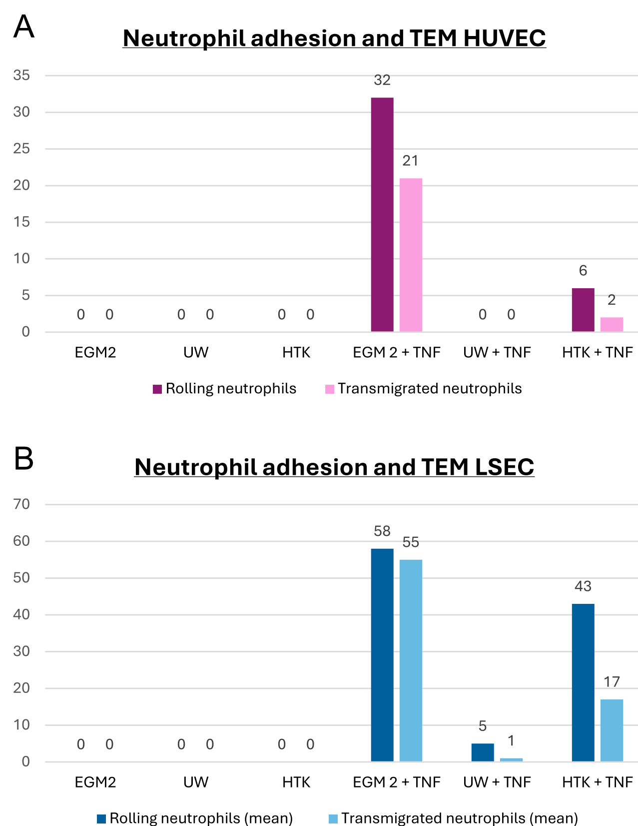

Figure 4

(A) Results of amounts of adhering and transmigrating neutrophils in HUVECs. The number of adhering and rolling neutrophils is depicted in dark purple, and transmigrated neutrophils are depicted in light purple. For all preservation solutions without TNF-α, no rolling nor transmigration was found. After TNF-α treatment, rolling and transmigrating neutrophils were found mostly in HUVECs incubated in EGM2 or HTK. (B) Results of amounts of adhering, rolling and transmigrating neutrophils in LSECs. The number of adhering and rolling neutrophils is depicted in dark purple, and transmigrated neutrophils are depicted in light purple. For all preservation solutions without TNF-α, no rolling nor transmigration was found. After TNF-α treatment, rolling and transmigrating neutrophils were found mostly in HUVECs incubated in EGM2 and HTK. Less transmigration of neutrophils was found in LSECs incubated in UW.

Next, we assessed the capacity of the cells to recover from an artificial wound induced by the ECIS technique (see Methods section). For both cell types, treatment with EGM2 medium quickly promoted recovery of the induced wound compared to the other fluids (Fig. 3B).

Inflammation

Neutrophil adhesion and TEM

Upon inflammation, neutrophils bind to ECs and extravasate from circulation. To study this in cultured HUVECs and LSECs, ECs were treated with TNF-α. In our system, we found that neutrophils efficiently crossed inflamed HUVECs and LSECs incubated with EGM2 or HTK, but not in HUVECs and a lot less in LSECs incubated in UW. However, non-significantly, UW medium prevented efficient TEM, indicating that UW can dampen leukocyte extravasation and prevent proper inflammation responses (Fig. 4A and B).

Discussion

This study aimed to investigate the efficacy of several preservation solutions on endothelial integrity,

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inflammation and leukocyte TEM in HUVECs and LSECs. Our data show that HUVECs and LSECs respond well to EGM2 and show the expected response to classic inflammatory mediators such as TNF-α. For the recovery, LSECs showed a more sensitive behavior compared to HUVECs. This might be due to the nature of the cells, i.e. a different epigenetic imprint. However, for the other parameters, both cell types responded equally well.

This paper can also be helpful to successfully culture LSECs, endothelial cell types that are notoriously known to be difficult to culture. Here, we describe a culture protocol giving detailed information on how to culture endothelial cells derived from the liver. It is important to note that these culture conditions can most likely also be used for other endothelial cell types. However, it is important to note that cultured LSECs rarely fully replicate the fenestration seen *in vivo*, and prolonged culture of such cells, and for that matter any cell type cultured *in vitro*, can lead to dedifferentiation. Here, we used culture methods to study the effects of different preservation solutions on LSECs. Differences between these preservation solutions are mainly the presence of growth factors or antioxidants. EGM2 medium contains an abundance of growth factors (17) while UW solution contains antioxidants, such as glutathione and allopurinol (18). Growth factors can be useful for culturing a monolayer and passing cells into different culture dishes. In addition, we found that cells incubated in EGM2 medium are most capable of repopulating the cellular monolayer after wounding. However, it can be debated whether growth factors in culture media best mimic a physiological environment for LSECs considering liver transplantation purposes. HUVECs are, in contrast to LSECs, physiologically exposed to placental growth factor (PlGF) in a constant manner (19). LSECs are exposed to epidermal and hepatic growth factors, as well as VE growth factors mostly in response to infection, surgery, or functional and oxidative stress (20, 21).

Antioxidants can be helpful in the prevention of oxidative stress and IRI. We did not use an ischemic model. However, cellular stress can also occur when oxygen levels are above physiological levels. The temperature in the cell incubator is 37 °C, which is similar to human blood, but the oxygen pressure in the cell incubator is much higher when compared to human blood (22). We expected that cells incubated with UW were better protected against oxidative stress.

Other additives in UW that are important for the preservation of donor organs and cells are adenine and ribose (18). Adenine and ribose can help to prevent ATP depletion and the formation of ROS in the cells of organs preserved under hypoxic conditions (23). However, we did not find specific differences in cellular damage between the preservation solutions. Upon inflammation, pro-inflammatory cytokines such as TNF-α activate neutrophils, causing them to adhere and transmigrate through the endothelial membrane via protrusions, which are formed in the presence of ICAM-1 (24, 25). We found that ICAM-1 expression was upregulated after TNF-α treatment in all cells. However, not significantly, we found different amounts of adhered and transmigrated neutrophils in between the different preservation media that were used. Neutrophil adherence and TEM were highest in cells incubated in EGM2 and not or much less in cells incubated in UW, which was seen in both HUVECs and LSECs, respectively. Interestingly, it was previously reported that glutathione can impair the TEM of neutrophils after IRI in the brain (26). Another study found that glutathione peroxidase inhibits the effects of TNF-α on the cytoskeleton structure and on the expression of ICAM-1, which are two separate processes (27). We did not find a lower or altered expression of ICAM-1 after TNF-α treatment, but we did find lower TEM of neutrophils in TNF-α-treated cells that were incubated in glutathione-rich UW solution. This implies that glutathione may impair neutrophil TEM by altering the cytoskeleton structure of the endothelium. We recently reported the importance of actin-driven endothelial membrane structures in supporting leukocyte extravasation (24). We did find that LSECs incubated in UW with added TNF-α showed activated and stressed actin filament arrangement with holes between cell membranes. Furthermore, TNF-α induces intracellular production of H_2O_2, which activates NF-kB-dependent gene transcription, upregulating different adhesion molecules. Glutathione peroxidase was recently shown to downregulate this process (28). Therefore, it might be possible that other, not measured, membrane adhesion proteins were downregulated by glutathione in the present study.

We additionally tested Ringer's lactate solution (RL), also known as sodium lactate solution. However, although HUVECs tolerated RL just fine, culturing LSECs in this medium was unsuccessful. Therefore, we have decided not to continue using RL for this study.

This study has its limitations. We commercially obtained LSECs from donor livers that were not suitable for transplantation, which means LSECs may already have been subjected to damage before isolation. Culturing LSECs is challenging as these cells tend not to replicate easily and thus are not suitable for multiple passages in culture, and typical identity and function may change over time (29, 30). Nevertheless, PECAM-1 and VE-cadherin expression were also found to be reliable in identifying LSECs in culture (31). It is worth mentioning that our previous research confirmed that VE-cadherin expression on the surface of endothelial cells, as well as PECAM-1, does not alter upon different inflammatory treatments (17, 18). In addition, this also holds true for endothelial cells from different origins, including lung, pancreas, bone marrow and arterial (19). In conclusion, our study demonstrates that cellular integrity, endothelial barrier function and response to

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pro-inflammatory triggers in HUVECs and LSECs were best provided in EGM2 medium but UW may prevent leukocyte infiltration in LSECs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study. J D van Buul served as an Editor of Vascular Biology. He was not involved in the review or editorial process for this paper, on which he is listed as an author.

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