Preconditions for warm organ preservation
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A novel oxygenated machine perfusion system for preservation of the liver

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Abstract

Machine perfusion (MP) is a potential method to increase the donor pool for organ transplantation. However, MP systems for liver grafts remain difficult to use because of organ-specific demands. Our aim was to test a novel, portable MP system for hypothermic preservation of the liver.

A portable, pressure-regulated, oxygenated MP system designed for kidney preservation was adapted to perfuse liver grafts via the portal vein. Three porcine livers underwent 20 hour of hypothermic perfusion using Belzer MP solution. The MP system was assessed for perfusate flow, temperature, venous pressure, and \( pO_2 / pCO_2 \) during the preservation period. Biochemical and histological parameters were analyzed to determine post-preservation organ damage.

Perfusate flow through the portal vein increased over time from \( 157 \pm 25 \text{ mL/min} \) at start to \( 177 \pm 25 \text{ mL/min} \) after 20 hours. Portal vein pressure remained stable at \( 13 \pm 1 \text{ mmHg} \). Perfusate temperature increased from \( 9.7 \pm 0.6 \text{ °C} \) at the start to \( 11.0 \pm 0.0 \text{ °C} \) after 20 hours. AST and LDH increased from \( 281 \pm 158 \text{ and } 308 \pm 171 \text{ U/L} \) after 1 hour to \( 524 \pm 163 \text{ and } 537 \pm 168 \text{ U/L} \) after 20 hours, respectively. Blood gas analysis showed a stable \( pO_2 \) of \( 338 \pm 20 \text{ mmHg} \) before perfusion of the liver and \( 125 \pm 14 \text{ mmHg} \) after 1 hour perfusion. The \( pCO_2 \) increased from \( 15 \pm 5 \text{ mmHg} \) after 1 hour to \( 53 \pm 4 \text{ mmHg} \) after 20 hours. No histological changes were found after 20 hours of MP.

This study demonstrated the feasibility of a portable MP system for preservation of the liver and showed that continuous perfusion via the portal vein can be maintained with an oxygen-driven pump system without notable preservation damage of the organ.
Introduction

Static cold storage (SCS, 4 °C) is currently the gold standard for preservation of abdominal organs\(^1,2\), allowing preservation times of more than 12 hours for healthy liver grafts.\(^3,4\) However, expanded criteria donor (ECD) organs, such as those from the elderly and non-heart-beating donors as well as steatotic livers, are known to be more prone to preservation-induced damage than healthy donor organs.\(^5\) Continuous artificial circulation through an organ during machine perfusion (MP) has the potential to keep organs from ECD in a better functional condition. This preservation technique, however, often requires complex pump systems and trained personnel and is subject to logistical constraints.

For decades, MP of the liver was primarily confined to the experimental setting and reports of clinical application were scarce.\(^6\) However, MP has evolved into an accepted clinical preservation method for kidneys, especially from ECD kidneys. A large multi-center clinical trial has confirmed the benefits of MP over CS, showing enhanced functional and survival outcomes.\(^7\) With respect to livers, however, only one clinical (phase 1) series has been reported thus far.\(^8\) Although this trial has presented encouraging results for MP of liver grafts, widespread implementation tends to be constrained by the difficulty to practically implement the technology necessary for the preservation of livers. Currently, the major obstacle preventing large scale clinical application of MP for liver grafts is the lack of portable, oxygenated hypothermic (4 °C) MP systems that are compatible with the size of liver grafts and the associated perfusion demands.\(^9\)

We have developed a disposable oxygenated MP system (Airdrive\(^\text{®}\)) that has demonstrated its value in experimental hypothermic kidney preservation.\(^10-12\) The system is based on an oxygen-driven, positive displacement pump that allows pressure-controlled pulsatile perfusion, continuous heat convection, and oxygenation of the perfusion medium. With the weight of <15 Kg and all components fully integrated into a single module, this system is also portable. In this study, the settings, perfusion dynamics, and organ quality were assessed in the Airdrive MP system during 20-h hypothermic preservation of porcine liver grafts.
Methods

Airdrive system

The Airdrive system (Figure 1) is based on pressurized oxygen as the driving force, which is used for three purposes: 1) to drive a positive displacement pump, 2) to supply the oxygenator, and 3) to induce overpressure in the organ chamber to support sterility. The oxygen is provided by a standard onboard 2-L pressurized cylinder containing medical grade oxygen (Medidis, Lelystad, The Netherlands). A 12-volt, non-rechargeable battery powers the gas valves and pressure feedback systems via a proportional-integral-derivative (PID) controller.

The pump design is based on a low energy, reciprocating positive displacement (membrane-)pump that separates the pump chamber in a lower half containing pressurized oxygen and an upper half containing perfusate (Figure 2A and 2B-11). The pump has a maximum output of 13.9 mL perfusate per cycle.

The pump cycle (Figure 2C, phase A) starts with the opening of gas valve $V_2$ (Figure 2A), driving the $O_2$ pressure to a maximum in the oxygen buffer vessel $B_1$ (Figure 2A) and the sub-membrane space. During phase B (Figure 2C), $V_2$ closes and the stored $O_2$ pressure in $B_1$ continues to elevate the membrane by further expansion of the sub-membrane space. During this phase, the produced perfusate flow is primarily restricted by the resistance of the tubing and tube connectors. As $B_1$ is no longer continuously refilled, the $O_2$ pressure gradually decreases, represented by the declining trend observed in phase B. At the start of phase C (Figure 2C), the membrane reaches maximum deflection,
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Figure 2.

A. Schematic view of oxygen and perfusate flow in the Airdrive system. Oxygen pressure is reduced to 12.5 (± 2.5) kPa from the oxygen cylinder (160 kPa). Gas valves V₁ and V₂ are controlled by a PID controller. Expansion chambers are denoted as B₁ (10-15 kPa) and B₂ (1.6 kPa). Transducers P₂ and T are monitored and their data stored during the preservation period, whereas P₃ and P₄ are used for feedback control.

B. Anatomy of the Airdrive system. 1, porex gas outlet; 2, transparent lid; 3, perfusion outlet to which the afferent vasculature is connected to; 4, organ suspension hammock; 5, sample port; 6, display and control panel; 7, bubbletrap; 8, expansion chamber; 9, oxygenator; 10, polystyrene housing module; 11, pump system.

C. Pressure profile of a pump cycle. The cycle is comprised of 3 phases indicated by A, B, and C, where the white plane demarcates on cycle. The arrow designates closure of V₂ coinciding with maximum O₂ pressure build-up of B₂.
at which no more perfusate is infused into the system. During this phase, the organ is continued to be supplied by the stored pressurized perfusate in the buffer vessel $B_2$ (Figure 2A). Due to the relatively small volume capacity of $B_1$, the $O_2$ pressure drops rapidly during this phase. The perfusate flow is now primarily restricted by the vascular resistance of the organ. When a low-pressure threshold is passed at the end of phase C, $B_1$ is refilled by re-opening of $V_2$, initiating a new cycle.

During the pump cycle, a software-embedded maximum pressure limit prevents overshoot of pressure and flow to the organ. Transducer $P_2$ (Figure 2A) can limit the pressure to 6.0 kPa (45 mmHg). When $P_2$ exceeds the maximum pressure, the system switches to a burst of short pump actions to relieve the oxygen in the system, lowering $P_2$ to within the designated limits. Once baseline levels are reached, normal operation is resumed. Excess usage of the oxygen supply and batteries are prevented by flow limitation. The flow rate is limited to 200 mL/min for renal artery perfusion and to 250 mL/min for portal vein perfusion.

The oxygenator is comprised of a coil of porous silicone tubing that is placed inside a pressurized oxygen container (Figure 2B-09). To maintain pressure in the afferent tubing and remove air bubbles (which could lead to intra-organ emboli), the oxygenator is placed before an expansion chamber and a bubble trap (Figure 2B-11 and 10, respectively). The oxygen used for the pump and oxygenator eventually dissipates into the chamber to induce overpressure. This pressure is limited by two outlets in the lid covered with breathable fabric (Porex), maintaining an overpressure in the organ chamber during perfusion.

During preservation, the flow, pressure, intra-organ vascular resistance (VR), temperature, and preservation time are continuously displayed and stored. All components are embedded in polystyrene (Neopor) to provide optimal isolation. Four pre-cooled packs (stored at -20 °C) are placed underneath the organ chamber inside the transporter box for temperature control.

**Experimental procedure**

All animal experiments were approved by the institute's animal ethics committee and animals were treated in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. Three female landrace pigs weighing $50.3 \pm 3.1$ kg underwent a liver procurement procedure under general anesthesia. After induction anesthesia by intramuscular administration of ketamine (10-15 mg/ kg, Eurovet Animal Health, Bladel, The Netherlands), midazolam (1-1.5 mg/ kg,
Actavis Group, Baarn, The Netherlands), and atropine (1.5 mg/ kg, Centrafarm, Etten-Leur, The Netherlands), the animal was intubated and ventilated. Maintenance anesthesia was provided by intravenous infusion of sufentanil (5-10 μg/ kg/ h, Hameln Pharmaceuticals, Hameln, Germany), ketamine (8-15 mg/ kg/ h, Eurovet, Bladel, The Netherlands), midazolam (1-2 mg/ kg/ h, Actavis, Hafnarfjordur, Iceland), and rocuronium bromide (2-2.5 mg/ kg/ h, Sandoz, Princeton, NJ).

The liver was approached via a midline laparotomy. The celiac trunk was dissected and prepared for cannulation. All splenic and gastric branches of the celiac trunk were dissected and ligated. The common bile duct was ligated and transected. The portal vein was mobilized and prepared for cannulation. The infrahepatic caval vein was prepared for ligation. Before cannulation, 25,000 IU of heparin (Leo Pharma BV, Breda, The Netherlands) was given intravenously and allowed to circulate for at least five minutes. A washout system with 4 L cold (4 °C) histidine-tryptophan-ketoglutarate solution (Custodiol, Dr. Franz Köhler Chemie, Germany) was used at 100-cm hydrostatic pressure. The celiac trunk and portal vein were cannulated using a 9-French (3-mm) polyamide luer lock cannula (VBM Medizintechnik, Sulz am Neckar, Germany). Upon simultaneous start of the flush, the infrahepatic caval vein was ligated and the suprahepatic caval vein was dissected. Ice slush was placed in the abdomen to facilitate cooling of the organ. After 2 L of washout solution had passed through the liver, the organ was removed and the washout continued on the back-table with an additional 2 L, during which a peripheral biopsy was obtained and the gallbladder was flushed with saline solution via a puncture. The gallbladder was canulated and left in place in this experimental setup to serve as a bile collection reservoir.

The Airdrive system was primed with 1 L of Belzer MPS (Bridge to Life, Colombia, SC) according to the manufacturer’s instructions. One gram of ceftriaxone (Fresenius Kabi, Bad Homburg, Germany) was added to the perfusion medium. The liver was placed in a suspended polyurethane hammock (Inoac USA, Bardstown, KY) inside of the organ chamber of the Airdrive and subsequently, the portal vein was connected to the pump tubing. After connecting the organ, the liver software program was initiated and perfusion of the organ was commenced.

Data management and sample collection

Blood gas analysis (pO₂ and pCO₂) was performed on the circulating perfusate using an ABL80 Flex gas analyzer (Radiometer, Brønshøj, Denmark) before placement of the liver in the Airdrive (t =0 h) and at 1, 3, 5, 10, and 20 h of perfusion. Perfusate samples for
biochemical assessment (lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and total bilirubin) were obtained at similar time intervals as for the blood gas analysis. The flow was calculated by the Airdrive system and recorded from the display. Pump output was calculated by multiplication of the number of pump strokes and stroke volume (13.9 mL of perfusate). Pressure was measured at \( P_2 \) (Figure 2A). Vascular resistance was calculated by dividing the pressure \( P_2 \) (mmHg) by the calculated flow (mL/ min). Temperature was measured by an imbedded temperature sensor inside the pump.

**Histological analysis**

Biopsies for histological examination were obtained at two time points: a peripheral biopsy after the flush but before placement in the Airdrive (control sample) and a peripheral and core biopsy after 20 h of perfusion in the Airdrive. Biopsies were fixed in ethanol, formaldehyde, acetic acid, and water in a 10: 2: 1: 7 ratio and dehydrated in graded steps of ethanol (70-80-90-100%) and xylene over 5 days prior to paraffin embedding. Histological slides were stained with hematoxylin and eosin and examined under a Leica DMLB microscope (40x objective, Leica Microsystems, Wetzlar, Germany) equipped with a Leica DC200 CCD camera that was controlled with QWin software (Leica Microsystems). The sections were scored in a randomized blinded fashion by a liver pathologist, according to a modified liver scoring system (Figure 4). The data was analyzed using Matlab (Mathworks, Natick, MA) and presented as mean ± standard deviation (SD).

**Results**

**Perfusion dynamics**

Portal vein flow exhibited a gradual increase over time from 157 ± 25 mL/ min at start to 177 ± 25 mL/ min after 20 h of perfusion (Figure 3A). The mean portal vein pressure remained constant throughout the entire preservation period (13 ± 1 mmHg, Figure 3B). Vascular resistance remained constant with 0.08 ± 0.02 at start of perfusion and 0.07 ± 0.02 mmHg/ min/ L after 20 h (Figure 3C). Perfusate temperature showed an increase from 9.7 ± 0.6 °C at start to 11.0 ± 0.0 °C after 20 h of perfusion (Figure 3D). Bile, probably residual, had accumulated in the gall bladder after 20 h of perfusion, totaling 7 ± 2 mL.

**Biochemical assessment**

Biochemical parameters showed an increase in AST and LDH from respectively 281 ± 158 U/ L and 308 ± 171 U/ L after the first hour to respectively 524 ± 163 U/ L and
Figure 3.  
A, perfusion flow (mL/ min); B, pressure (mmHg); C, vascular resistance (mmHg/ min/ mL); D, temperature (°C); E, perfusate pO₂ and pCO₂ (mmHg); F, perfusate AST and LDH concentrations (U/ L). All values are depicted as mean±SD.
Histology scores and representative H&E-stained liver sections. Sinusoidal dilatation: 0, absent; 1, less than 1/3 of parenchyma; 2, less than 2/3; 3, more than 2/3. Portal edema: 0, absent; 1, less than 25%; 2, between 25-50%; 3, between 50-75%; 4, more than 75%. Areas of confluent parenchymal necrosis: 0, absent; 1, affecting less than 25% of parenchyma; 2, affecting 25-50%; 3, affecting 50-75%; 4, affecting >75%. Hepatocellular mitosis: 0, absent; 1, in case of <2 foci per 8 fields (40x objective); 2, in case of 2-4 foci; 3, in case of 5-10 foci; 4, in case of >10 foci. Councilman bodies (cytosegresome formation): 0, absent; 1, in case of <2 foci (10x objective); 2, in case of 2-4 foci; 3, in case of 5-10 foci; 4, in case of >10 foci. See Appendix 1, page 203 for the color image.

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537 ± 168 U/ L after 20 h (Figure 3F). The bilirubin concentration remained below detection levels at all times (<1 μmol/ L), indicating no bile leakage.

Blood gas analysis before placement of the liver yielded a pO₂ of 338 ± 20 mmHg and a pCO₂ of <1 mmHg (Figure 3E). After organ placement in the Airdrive, the pO₂ dropped to 125 ± 14 mmHg and the pCO₂ increased to 15 ± 5 mmHg. After 20 h of perfusion, the pO₂ remained stable at 124 ± 14 mmHg and the pCO₂ increased further to 53 ± 4 mmHg.
Histology

None of the biopsies showed evidence of portal edema, parenchymal necrosis, or mitosis (score = 0, Figure 4). All preserved livers showed signs of sinusoidal dilation (2 ± 0.7 out of 3), with no apparent differences after MP (pre-MP of 2 ± 1 versus post-MP of 2 ± 1). Three post-MP biopsies (2 peripheral, 1 core) showed signs of apoptosis, as evidenced by the presence of Councilman bodies (all scored 1 out of 4).

Discussion

Matching organ supply with demand has been a major focus in the transplantation field in an effort to reduce the organ demand-supply gap. To this end, MP is increasingly employed to utilize more ECD organs. However, MP technology for liver grafts falls behind when compared to kidney grafts. A particular challenge for liver MP is the portability and dependability of the flow and pressure systems. Here we demonstrated the feasibility of an MP system for liver preservation that meets these challenges, and showed that liver perfusion via the portal vein can be achieved by an oxygen-driven pump system while remaining portable. Moreover, no notable damage to the organs was observed after a 20-h preservation period.

Graft assessment

This study sought to demonstrate the feasibility of a portable MP device that meets the flow and pressure demands considered suitable for preservation of a liver graft. We examined both biochemical (AST, LDH) and histological parameters to assess the damage profile after MP. During perfusion, the biochemical parameters showed a gradual increase, as usually found in a recirculating perfusion device. These values are in agreement with earlier reports on porcine and clinical liver MP when corrected for circulating volume, indicating minimal hepatocellular injury.8,13

Histologically, we have shown preservation of architectural integrity of the parenchyma and absence of edema after 20 h MP. The ultimate test is graft outcome in a large-animal liver transplantation model. In large animal kidney transplantation, MP using the Airdrive has proven to be beneficial over conventional cold storage in pre-damaged kidney grafts.12 However, large animal liver transplantation encompasses surgical restrictions (e.g. absence of collateral hepatic circulation) that make the model less comparative.
Pump system

One of the main concerns in the use of an MP system, as reflected by the technical diversity of the experimental and commercial systems produced to date, is implementation of the pump. Currently, most available MP systems are based on a roller pump mechanism to recirculate the perfusate. However, a significant technical obstacle associated with roller-type pumps is their extensive power consumption. As demonstrated by the commonly used liver MP systems, it is evident that these pump systems cannot support larger organs while maintaining portability. To circumvent this major hurdle, the Airdrive makes use of a 2-L oxygen cylinder to drive the pump while sustaining an average portal vein flow of >150 mL/min for up to 20 h.

The current configuration of perfusion dynamics is aimed at perfusion via the portal vein only. It is arguable that a dual pump system could provide flow for both hepatic vascular systems, i.e., the portal venous system and the hepatic artery system. It can been conceived that dual MP can be beneficial due to oxygenation of the biliary system. However, studies assessing this effect in a clinically representative model remain to be conducted.

Flow

The required flow through a liver graft during MP remains a subject of debate and can be of critical importance for optimal preservation of the graft. Reports suggest that 25% of physiological flow is safe for hypothermic MP, based on the resulting shear stress. Given the fact that physiological portal flows at normothermic temperatures are typically around 1000 mL/min, the recorded perfusate flow of 177 ± 25 mL/min in our study is consistent with a safe range.

The second element governing intragraft flow is graft temperature and its corollary effects on cumulative diameter of the microvasculature. Hypothermia causes an initial afferent vasoconstriction (i.e., reduction of total vascular diameter) resulting from surgical handling and a reduced Ca²⁺-ATPase activity in vascular smooth muscle cells. In a pressure-controlled MP setting such as the Airdrive, a reduction in diameter causes a proportional decrease in flow at constant pressure, providing an additional rationale for using subphysiological flow rates.

Temperature

Preservation of organs at 4 °C constitutes the gold standard in organ preservation. In this study, the MP system showed moderately higher temperatures during
perfusion, reaching an average of 10 ± 0.5 °C. Earlier experiments with kidney grafts using MP in the Airdrive report temperatures of 5.8 ± 0.8 °C. This difference may arise from the difference in organ mass needed to cool, and the residual metabolic activity.

The main premise for hypothermic preservation temperatures is based on van ‘t Hoff’s principle, which states that metabolism is reduced by 50 % for every 10 °C decline in temperature. Theoretically, this would amount to less than 10 % residual metabolism at 4 °C, enabling the organ to withstand the low levels of oxygen and nutrients during cold storage. In our experiments, metabolic activity was evident from the increase in perfusate pCO$_2$ and decrease in pO$_2$, collectively typifying a metabolically active organ. Taking this into consideration, it is arguable whether extreme lowering of metabolism is required during MP as nutrients and oxygen are continuously available. And, in line with this view, there is growing consensus on the use of (sub)n normothermic preservation temperatures during MP. The preservation of sub-optimal organs, such as those from elderly or non-heart-beating donors as well as steatotic livers, may benefit from MP and from preservation at higher temperatures. In that respect, the Airdrive MP system is capable of operating at any temperature within the spectrum of hypothermic and (sub)n normothermic perfusion.

**Portability**

Allocation of organs is rarely restricted to a single center, making portability of an MP system essential to its implementation. By using disposable lightweight materials, the Airdrive weight remains below 15 kg when operating, which is below the maximum one-person manual handling weight enforced by occupational health codes in many countries.

**Conclusion**

This study has demonstrated the feasibility of a portable MP system for preservation of the liver. Using an oxygen-driven pump system, continuous perfusion via the portal vein can be maintained for a clinically relevant preservation time without notable preservation damage of the organ.
References