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End-stage liver diseases are nowadays effectively treated by transplantation of the affected liver. The transplantation procedure includes procurement of the liver from the donor and subsequently transport of the liver from donor to receiving patient (Chapter 1). To bridge the timespan of transport between donor operation and actual implantation of the organ in the receiving patient, the liver has to be optimally stored and preserved in order to maintain viability of the organ.

To date, the conventional method of preservation is the Cold Storage (CS) preservation technique. The CS method implies a single flush of the liver in situ with an ice-cold preservation solution to wash-out remaining blood and immediately cool the organ. Subsequently, the liver is stored in a plastic bag containing cold preservation solution and transported in a cooling box filled with melting ice to maintain a lowered metabolism during hypothermia (0-4°C). The University of Wisconsin cold storage (UW-CS) solution is nowadays the golden standard in preservation solutions. Although CS preservation shows good results in preserving livers from brain-dead donors, who have an intact circulation, expansion of the donor pool with an important potential group of non-heart-beating donors (NHBDs), after cardiac arrest, requires improved preservation techniques.

Hypothermic machine perfusion (HMP) is a dynamic preservation method that actively perfuses the liver. With HMP a continuous supply of oxygen and removal of waste products is obtained which improves preservation outcome. Especially marginal, older and NHB donor livers will benefit from this improved quality.

The aim of this thesis was to develop a hypothermic machine perfusion system which is able to optimally preserve donor livers.

In Chapter 2 the liver anatomy and physiology are described also concentrating on the efferent vessels of the liver: the portal vein and hepatic artery. The portal vein supplies the liver with blood from the intestine. This nutrients-enriched blood
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enters the liver with a low pressure (12 mmHg) and high flow (± 1000 ml/min). The hepatic artery, on the other hand, supplies oxygenated blood originating from the aorta under a high pulsatile pressure (120/80 mmHg) at a lower flow rate (± 500 ml/min). Both blood streams mix in the sinusoids where physiological interactions with the hepatocytes take place to enable the liver to perform its numerous functions. For an optimal preservation, it is of major importance to keep ischemic periods of interrupted blood flow as short as possible.

In Chapter 3, flow and pressure patterns in the liver are analyzed more in-depth using a numerical simulation model of the hepatic circulation. Such a model could also be helpful in better defining optimal settings of the HMP system. Hemodynamics in the hepatic arterial, portal venous and hepatic venous compartments of the hepatic vascular tree was modelled using an electrical analogue. Calculated pressure and flow profiles throughout the liver were in accordance with physiologic profiles in the total circulatory system. Comparison of calculated flow values with normal control values showed a discrepancy that was explained by inaccurate diameter input data. Until more precise methods for determining vascular dimensions become available, redefining vessel diameter makes the simulation model perfectly suitable for predicting influences of temperature and/or viscosity on hepatic hemodynamics and is thereby an excellent tool in defining optimal settings for our hypothermic liver perfusion system.

In conventional cold storage organ preservation the donor organ is flushed with 0-4°C UW-solution. The initial flush is used to wash-out blood components from the microcirculation to allow an optimal preservation with the UW-solution. The component Hydroxy Ethyl Starch (HES) of UW, which is necessary to maintain osmolarity, is known to cause an increase in viscosity and a possible interaction with blood: i.e. increased RBC aggregation. The aim of Chapter 4 was to investigate the influence of the HES-component on the viscosity of UW and the aggregation behaviour of blood during wash-out. The experiments were performed with whole rat blood and mixtures of whole rat blood with UW-solution or UW without HES (UWmod) at 4°C. The viscosity of blood at 4°C was found to be 2 times higher than at 37°C, the UW/blood mixture at 4°C was 1.3 times higher more viscous than blood at 37°C; the 4°C UWmod/blood mixture equaled the viscosity of 37°C blood. The UW/blood mixture showed a 9 fold increased aggregation compared to whole blood. A mixture of whole blood and UWmod showed a decreased aggregation compared to blood. Apart from an increased viscosity, HES in UW caused increased RBC aggregation. In addition it was found that the size of the aggregates is larger than the diameter of the sinusoids.
In Chapter 5 human RBC aggregability and deformability were investigated in vitro at 4°C. The study of red blood cell aggregation in a binary HES-HES system gave an indication about the nature of HES-RBCs interactions. Bright field microscopy and atomic force microscopy were used to morphologically characterize the aggregates size and form. High molecular weight HES and UW solution had a potent hyperaggregating effect; low molecular weight HES had a hypoaggregating effect on RBC. RBC aggregates were of large size and their resistance to dissociation by flow induced shear stress was high. Our in vitro experiments conclusively showed that the physiological function of red blood cells to form aggregates was significantly affected in the presence of hydroxyethyl starch. It was concluded that the use of high molecular weight HES in UW solution accounts for extended and accelerated aggregation of erythrocytes that may result in stasis of blood and incomplete wash-out of donor organs prior to transplantation.

After studying the initial wash-out procedure, focus was now directed towards the actual preservation phase. In hypothermic machine perfusion of the liver as a dynamic preservation method, the three most important aspects were the type of preservation solution, the characteristics of perfusion dynamics and the oxygen supply. Reviewing hypothermic liver machine perfusion experiments in Chapter 6, the University of Wisconsin machine preservation (UW-MP) solution is the solution most used. It was also found that nothing conclusive can be said about the optimal perfusion characteristics, since either perfusion pressure or perfusion flow has been reported. The best educational guess is perfusion of the liver in a physiological manner, i.e. pulsatile arterial perfusion and continuous portal venous perfusion. The applied pressures were chosen to be lower than physiological pressures to prevent possible endothelial cell damage. Oxygen supply was necessary to achieve optimal preservation of the liver. Incorporating these features in a system based on existing standard surgical and organ sharing procedures and to ability to for 24 hours and weighing less than 23 kg, could successfully implement this technique into routine clinical practise.

Because of the necessity of additional oxygen during preservation, in Chapter 7 four miniature hollow fibre membrane oxygenators were tested for their ability to sufficiently oxygenate the UW-MP solution under hypothermic conditions (0°C). The HILITE and Baby-RX oxygenator showed comparable oxygenation capacity, both higher than the FiberFlo and MiniModule. At the estimated working flow range, oxygenation capacity of the FiberFlo and MiniModule was critical, the capacity of the HILITE and Baby-RX was sufficient. Pressure drop was lowest for
the HILITE and Baby-RX, for the FiberFlo and MiniModule pressure drop was a factor 7 or more higher. Based on the low pressure drop, the HILITE oxygenator was selected for application in the HMP system.

Remaining components for the HMP system were selected in Chapter 8. Two rotary pumps, which are commercially available, were selected based on their ability to run both in a continuous or a pulsatile manner. Tests with ice-cold UW-MP solution showed the pumps to be capable of perfusing the liver through portal vein and hepatic artery with a continuous pressure of 4 mmHg and a pulsatile pressure of 30/20 mmHg, respectively. Pressure and temperature sensors are included, flow is derived from the rotational speed of the pump motors. Perfusion pressure is kept constant by a proportional integral (PI) controller, with a reaction time on pressure changes of less than one minute. These components were incorporated in a design that encompasses a disposable unit and an electro-mechanical unit. The disposable unit includes organ chamber, oxygenator, two pump heads, pressure and temperature sensors, tubing and canulas and cooling box. The electro-mechanical unit is composed of two electro-motors, oxygen cylinder, electronics unit, handheld computer and battery pack. Briefly, the preservation solution is pumped in pulsatile manner from the reservoir and through the oxygenator to the hepatic artery of the liver submerged in preservation solution in the organ chamber. The other pump perfuses the portal vein in a continuous manner, without additional oxygenation. Special features of this design (patent pending) include back-up of cold storage in case of total power failure, an option of normothermic perfusion by using a heat exchanger and blood compatible disposable components, an option of a kidney perfusion system by using only the pulsatile pump, and an additional weight compared to cold storage of 5.3 kg.

A prototype of this design was build and evaluated on functionality in Chapter 9. Evaluation criteria included 24 hours of adequate pressure controlled perfusion, sufficient oxygenation, a maintained 0-4°C temperature and sterile conditions. Porcine livers were perfused with pump pressures that were set at 4 mmHg (continuous, portal vein) and 30/20 mmHg, 60 BMP (pulsatile, hepatic artery). During the preservation time of 24 hours, pressure, flow and temperature were continuously monitored. At time-points t=0, 2, 4, 8, 12 and 24 hr samples of UW-MP solution were taken for measurement of partial oxygen pressure (pO₂) and lactate dehydrogenase (LDH). Biopsies in every lobe were taken for histology and electron microscopy; samples of ice, preservation solution, liver surface and bile were taken and cultured to determine sterility. Results showed that temperature was maintained at 0-4°C; perfusion pressure was maintained at 4 mmHg and 30/20 mmHg.
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mmHg for portal vein and hepatic artery, respectively. Flow was approximately 350 ml/min and 80 ml/min, respectively, but decreased in mainly the portal vein, probably due to edema formation. Arterial pO\textsubscript{2} was kept 100 kPa. Histology showed complete perfusion of the liver with no major damage to hepatocytes, bile ducts and non-parenchymal cells.

In conclusion, the developed prototype proved to comply with the list of required specifications previously defined. The Groningen hypothermic liver perfusion pump prototype was able to dually perfuse the liver for 24 hours in both a pulsatile (hepatic artery) and continuous (portal vein) manner. The system maintained flow at a controlled pressure. Although an increasing resistance of the liver was observed, no major endothelial cell or hepatocyte damage was induced. The hollow fibre oxygenator oxygenated the arterial line satisfactorily, and even supplied the portal venous line with a sufficient amount of oxygen. Melting non-sterile ice kept the temperature of the disposable section, including reservoir, oxygenator, pump heads and liver, below 4°C during 24 hours, and did not affect the sterility inside the disposable section. The complete portable system now weighs 17.3 kg, 5.3 kg more than when the CS method is applied using the same cooling box. User-friendlyness still has to be improved before transplantation experiments are performed to prove the superiority of dual HMP over CS. During the evaluation of the Groningen HMP system a sterile person had to assemble the sterile disposable module from scratch, including connection of tubes and sensors, securement with tie-wraps, filling of the reservoir and air bubble-free priming. In the prototype this procedure was cumbersome and has to be simplified. First, to make the Groningen HMP system an easy-to-handle preservation system, it is of importance to introduce as little (extra) actions as possible compared to the situation when static cold storage is used. A large improvement would be a ready-to-use sterilized disposable module, which can be filled with UW-MP solution without opening the reservoir. In this way, the inside of the disposable module remains sterile and the entire configuration can be primed using the rotary pumps. In the existing situation, the module has to be primed using gravity, which is time-consuming and cumbersome. A consequence of active priming using the pumps is that the pumps have to be placed below the UW-MP fluid level instead of the current placement on top of the lid. A second improvement lies in the cannulation of both hepatic artery and portal vein. Both cannulas were now inserted into the blood vessels, but it is of importance for transplantation not to damage the vessels. For that purpose, a
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special cannulation technique has to be developed that induces minimal damage to the blood vessels. Finally, attention has to be paid to the user-convenience when the liver is taken out of the reservoir after preservation. In the prototype the cover of the reservoir formed the only barrier between non-sterile ice and the sterile inside of the disposable module. After preservation, the outside of the cover was not sterile any more and it was difficult to maintain sterility while removing the liver. This problem can be solved by adding one or more plastic bags/sheets to the module that allow putting the liver into the reservoir, closing the cover and tying a plastic bag around it. For removal of the liver after preservation, the bag can be opened as in the CS-procedure while the reservoir cover is still sterile.

Figure 9.9: Concept of a next generation Groningen HMP system, with the electromechanical module and the disposable module in separate polystyrene cooling box compartments (isometric and top view).

These measures implemented in the next generation Groningen HMP system (Figure 10.1) will result in an improved design, that complies to all functionality requirements and is userfriendly. The Groningen HMP system is based on the cold storage technique and procedures, with in addition a dual pumping system that is capable of perfusing the liver with a constant 4 mmHg and 30/20 mmHg through portal vein and hepatic artery, respectively. Temperature of the liver and oxygenated UW-MP solution is maintained at 0-4°C by crushed ice in a disposable cooling box. A prototype has been tested positive as regards functionality and the proof of concept will be evaluated in a large animal liver transplant model.
Certification and introduction into the clinic will make the Groningen HMP system available for human transplantation. Improved preservation with the HMP system will result in better organ viability and outcome after transplantation despite longer preservation times. This will make transplantation a semi-emergency procedure which will better fit into the day-to-day planning of operation room and clinical logistics. Also, when the incidence of delayed graft function and primary non-function are lower, it will facilitate the use of marginal, older and non-heart-beating donors on a more regular basis and thus contribute to an increase of the donor pool.