Chapter 1

Introduction

Organ transplantation is nowadays an effective therapy for patients with end-stage organ diseases. However, organ transplantation had to come a long way before it was successfully introduced in the clinical setting worldwide. Transplantation-related research finds its origin in the development of the first successful vascular anastomosis technique by Carrel in the beginning of the 20th century. His new technique was a major step forward for vascular surgery but it was not until 1954 that the first human kidney transplant was finally successfully performed. Murray transplanted a kidney into the donor’s identical twin brother, with the advantage of having no immunoreaction, although at that time the system of immunoresponse was not yet recognized. In the 1960’s the basis was laid for modern transplantation with the successful introduction of cadaveric donation. Harvesting organs from diseased patients was first performed with kidneys in 1962, but lung (Hardy 1963), liver (Starzl 1963) and heart (Barnard 1967) would soon follow. Graft and patient survival rates were initially not very high, but drastically increased with the discovery in the 1980’s of cyclosporin as an immunosuppressive agent that inhibits graft rejection. Since then, survival rates almost doubled, resulting in one-year graft survival of 80 to 90% in general and 81% for liver, which is the organ we have studied in this thesis.
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1.1 Donor Pool

Despite the major achievements in organ transplantation we are nowadays still facing the persistent problem of shortage of donor organs. Eurotransplant, the organization responsible for the network of organ donation and sharing in Austria, Belgium, Germany, Luxembourg, the Netherlands and Slovenia, reported that the number of patients waiting for a liver transplantation in 2003 was 1714; in the Netherlands alone, the waiting list included 123 patients (Table 1.1). UNOS (United Network for Organ Sharing), which organizes donor procedures in the United States, reported a waiting list of 17,319 candidates for a liver transplantation.

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<th>The Netherlands</th>
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Table 1.1: The amount of procured livers, transplanted livers and patients on the waiting list for 2002 and 2003 in The Netherlands, the Eurotransplant countries and the USA.

To date, the majority of donor livers used for transplantation originates from brain dead cadaveric donors. In these donors circulation is still intact, but brain death has been already diagnosed. Since blood (and oxygen) flow to the liver is still functioning, these livers show a good quality with high success rates after preservation and transplantation. Other new techniques including domino transplantation, split liver and living-(un)related transplantation nowadays are used and have a positive effect on expanding the number of transplantations. Domino transplantation is used for certain liver diseases with incubation times longer than the graft survival time, i.e. patients receive a ‘sick’ liver, but need a retransplantation before the liver disease endangers their health. Splitting the donor liver into two liver segments allows both parts to be
transplanted in two receiving patients. Because of the liver’s regenerative properties, both liver segments will grow into a full functioning liver again. Using the same principle, also living-(un)related transplantation has become possible, when for example a child receives part of the liver of one of its parents.

However, even with these new techniques the waiting list is still increasing, which expresses the need for additional sources of organ donors. Improvements in donor preparation and organ preservation could allow sub-optimal donor groups, e.g. marginal donors and non-heart-beating donors, into the donor pool. Livers from so-called marginal donors are supposed to have a lesser quality due to various causes including age, hypotension or long warm ischemic time due to for example procurement complications. Marginal livers are rarely used for transplantation due to an expected decreased organ viability resulting in an increased initial non and never function and increased delayed graft function with associated technical complications.

Another large group of possible organ donors is formed by the non-heart-beating donors (NHBD). These are cadaveric donors of which the heart has already stopped at the time of procurement. Kootstra\textsuperscript{18} classified the NHBDs into 4 categories:

- **Maastricht category 1:** Dead on arrival at the hospital. These patients suffered cardiac arrest before they arrived at the hospital. The major problem in these patients is that usually the time of cardiac arrest, and thus the exact extent of warm ischemia, is unknown.

- **Maastricht category 2:** Unsuccessful reanimation. These are mostly patients at the emergency department, brought in after myocardial infarction or severe trauma and have to be resuscitated. In contrast to category 1 NHBDs, the exact time of cardiac arrest is know.

- **Maastricht category 3:** Awaiting cardiac arrest. In these patients brain dead is not diagnosed, but their condition is so weak that any further
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treatment is stopped, with a cardiac arrest as consequence. From these patients, the time of cardiac arrest is exactly known and any arrangements for donation procedures and procurement can already be taken.

- Maastricht category 4: Cardiac arrest while brain dead. These NHBDs are already diagnosed brain dead and family approval for donation is already obtained. However, these patients are often very unstable and cardiac arrest can occur. Since all donation procedures and preparations are already arranged these NHBDs have the shortest warm ischemic times.

A consequence of using organs from NHBDs is the time of warm ischemia after cardiac arrest. During this period of stagnating blood flow all kinds of processes, including blood coagulation, will damage the organ and successful transplantation after preservation is no longer guaranteed. The aim is therefore to keep the warm ischemic time as short as possible. Initially, in dedicated centers a small amount of category 4 and in some cases category 3 NHBDs was included to expand the liver donor pool, since warm ischemic times were relatively short. Results were acceptable as far as primary non-function, short-term graft survival and patient survival were concerned, although still inferior to brain dead donors.

To include a large group of donors, i.e. category 2 non-heart-beating donors, and successfully use category 3 on a more regular basis, preservation, as the bridge from donor to recipient, has to be optimized to improve the quality of these donor livers. Adding these donor organs to the transplantation pool would significantly decrease the number of patients on the waiting list.

1.2 Organ Preservation

The removal of an organ from the donor’s circulation and subsequent transport to the recipient initiates a cascade of events that leads to the death of the organ. To bridge this ischemic period, the organ has to be optimally
preserved to minimize any resulting ischemic injury. Methods of clinically preserving organs, and kidneys in particular, were developed in the late 1960’s by Belzer\textsuperscript{3}, who used continuous hypothermic machine perfusion and by Collins\textsuperscript{9}, who used a cold solution for flushing and cold storage of the kidney. These preservation methods became applicable for liver preservation as well.

1.2.1 Cold storage preservation

Only shortly after Belzer started machine perfusion preservation Collins\textsuperscript{9} developed a new acellular preservation solution, mimicking intracellular electrolyte concentration. With this solution it became feasible to hypothermically preserve organs in a static manner, without continuous perfusion. In organ preservation, three phases can be defined: the procurement phase, the preservation phase and the reperfusion phase. In the procurement phase, the donor liver is freed from its surrounding ligaments and the aorta is canulated. An initial flush is performed through a cannula with an ice cold preservation solution to wash-out all blood components from the donor liver and rapidly cool the organ. After the initial wash-out, the liver is dissected from the donor and packed in an organ storage bag filled with the same preservation solution. At this stage, the preservation phase is already started, and is continued by storing the organ inside a cooling box filled with melting ice. To prevent the organ from direct contact with ice and subsequent freezing damage a second bag filled with saline is used around the first bag. Finally, a third bag covering the former two secures sterile conditions. In this configuration, the liver is transported to the receiving patient by car or airplane. During the transport period, the receiving patient is already being called to the hospital and prepared for the actual transplantation phase. Throughout the years, 0-4°C has been shown to be an adequate temperature for static CS. At this lowered temperature, cellular metabolism is reduced to ± 10% of its normal activity at 37°C\textsuperscript{6}. As a consequence, the need of nutrients and oxygen decreases and the production of waste products diminishes. This temperature is easily achieved by cooling with melting ice.
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The CS preservation method directed attention away from organ perfusion preservation and towards identifying the optimal CS preservation solution. In the late 1960’s and early 1970’s, CS solutions like Ringer’s lactate solution, Marshall’s hypertonic citrate solution, Collins solution and later Euro-Collins solution were beneficial in improving organ viability outcome after preservation. But not until the 1980’s, when Belzer and his co-workers developed the University of Wisconsin preservation solution (UW), the CS preservation technique was optimized. In contrast to the previous preservation solutions, the UW solution included so-called impermeants to prevent cell swelling. The success of this UW-solution is illustrated by preservation of donor livers beyond 48 hours in laboratory experiments and for 12 to 18 hours in the clinical setting compared to approximately 6 hours in EuroCollins. Together with the introduction of improved and more specific immunosupression, a better understanding and treatment of complications were factors that improved results in liver transplantation and made CS of the liver using UW solution the golden standard.

1.2.2 Hypothermic machine preservation

A method that could allow better and longer preservation of a donor organ is the so-called hypothermic machine perfusion (HMP). In HMP the donor liver is continuously perfused with a 0-4°C preservation solution, in contrast to CS, which is a static preservation method. The advantage of an active perfusion preservation method is a continuous supply of nutrients to the liver cells and a continuously removal of waste products, resulting in a more optimal preservation method. HMP thus combines the advantages of hypothermic preservation, resulting in a decreased metabolism and thus a decreased demand for nutrients and production of waste products with the above described advantage of continuous perfusion, resulting in an optimal balance of demand and supply. In addition, HMP allows monitoring of liver quality by e.g. intravascular organ resistance measurements, bio-impedance measurements, and perfusate analysis, and incorporates the possibility of adding medication to the preservation solution. On the other
hand, in comparison to the simple CS method, disadvantages including high costs and the need for extra and skilled personal have to be overcome as well.

After the introduction of his successful anastomosis technique in the beginning of 1900, Carrel developed a method to preserve organs outside the body in order to make organ transplantation feasible. In the 1930’s, cooperation between Carrel and Lindbergh resulted in the first organ perfusion apparatus, with which whole organs could be perfused (Figure 1.1). It was not until the 1960’s when Lindbergh resumed working with this technique, and also Belzer set foot into organ preservation research. Belzer built an organ perfusion machine, that perfused organs at hypothermic temperatures (Figure 1.2). In contrast to Lindbergh’s perfusion apparatus with a gas-driven pump in combination with a gravity-based perfusion, Belzer’s perfusion apparatus incorporated two mechanical pumps. This made the apparatus much larger than Lindbergh’s pump, but still remained transportable, all be it by means of an somewhat adjusted truck (Figure 1.2). At first, preservation experiments with kidneys were performed, but later also livers could be preserved with his machine. As
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perfusion solution, Belzer initially used whole blood, but then switched to plasma since that allowed longer preservation times. Due to a coincidence, he discovered that cryoprecipitated plasma was even more successful and allowed preservation of canine kidneys for 72 hours and 100% survival after transplantation. In the same period, the groups of Starzl\textsuperscript{7} and Slapak\textsuperscript{30–32} experimented successfully with liver perfusion preservation as well. Already in the 1960’s, Belzer\textsuperscript{3} started experiments with hypothermic machine perfusion by studying continuous perfusion of kidneys. His efforts, along with others, resulted in improved clinical results using continuous perfused kidney preservation in comparison to CS. With HMP, the clinical use of non-heart-beating and marginal kidney donors is now feasible\textsuperscript{2,11,26}. In the laboratory even 5-7 days successful preservation of canine kidneys can be achieved\textsuperscript{24,25}. Based on these successes of HMP of the kidney, research efforts are now focused on continuous machine perfusion of the liver as well.

In the late 1960’s Belzer\textsuperscript{4}, Slapak\textsuperscript{30} and Brettschneider\textsuperscript{7} experimented with continuous hypothermic machine perfusion of the liver in the experimental setting with results comparable or even better than livers preserved with static CS. In 1986 D’Alessandro and later Pienaar, both from Belzer’s group, managed to preserve\textsuperscript{10,27} and transplant\textsuperscript{27} good quality canine livers after 72 hours preservation in a HMP dog model. HMP is also shown to be beneficial in the preservation of NHBD livers\textsuperscript{19,20,23,33}. Lee et al\textsuperscript{20} obtained 5 out of 6 rats surviving 5 days after...

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\includegraphics[width=\linewidth]{belzer.png}
\caption{Prof. Belzer with his kidney perfusion machine.}
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transplantation of a liver that experienced 30 minutes of warm ischemia (Maastricht category 2) and that was preserved for 5 hours using HMP. Animals receiving a liver that was preserved with CS did not survive. HMP therefore seems to be the method to enable the use of these NHBDs into the liver donor pool. The combination of hypothermia and continuous perfusion appears to be superior to static CS, in prolonging preservation time and improving graft viability. Especially the NHBD livers of Maastricht category 2, which forms the largest group NHBDs, could benefit from the improvement in preservation quality, since these donors are not used at this moment. Brain-dead donors and category 3 and 4 NHBDs are successfully included today in the transplantation process using CS, but results regarding delayed graft function of these livers can be improved by using HMP.

However, despite successful experience with continuous perfusion of kidneys and the results obtained by Pienaar\textsuperscript{27} with healthy donor livers and Lee\textsuperscript{20} with NHBD livers, HMP has not become a standard procedure in every day practice. Moreover, until today, there is no commercially available liver perfusion machine to improve organ viability and seriously challenge the limits of liver preservation by optimizing perfusion and transportation during cold storage as is done with machine preservation of kidneys in dedicated centers.

Therefore, it is our aim to develop a portable hypothermic liver perfusion system, with which donor livers can be continuously cooled, perfused and transported. This thesis describes the design process of the Groningen Portable Hypothermic Liver Perfusion System.

1.3 Rationale

The development process, as described in this thesis, is divided in three sections concerning the liver, the requirements for optimal HMP settings including initial wash-out, the perfusion characteristics and oxygenation, and the prototype design that incorporates these optimal settings.
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The first section concentrates on the liver itself. To fully understand structure and functioning of the liver, under normothermic and hypothermic conditions, chapter 2 deals with the anatomy and physiology of the normal liver and addresses shortly the physiological principles of hypothermic preservation. In addition, chapter 3 describes our efforts to develop a numerical simulation model to allow a more in-depth analysis of the internal blood flow and pressure distribution in the liver. This could be a helpful tool to define strategy and settings of the pump system to be developed.

The second section of this thesis focuses on the procurement phase of the transplantation procedure. In the procurement phase, the initial cooling and wash-out of blood takes place. In order to optimally preserve the liver using HMP, we reasoned that the procurement phase should be as optimal as possible. In preliminary initial wash-out experiments with pig livers using the clinical protocol of organ procurement (i.e. wash-out solution at 100 cmH$_2$O above patient level) we observed a substantial amount of blood still present in the liver after the wash-out. This observation was confirmed in rat experiments performed in our laboratory showing decreased viability after this low-pressure wash-out and subsequent CS compared to high-pressure wash-out. From the literature, it is known that the hydroxyethyl starch component of the preservation solution has some drawbacks, especially in combination with blood cells. Chapter 4 describes an analysis of these possible drawbacks, including viscosity and aggregation. In addition, in chapter 5 a more in-depth analysis of the influence of starch on red blood cell aggregation as a major drawback during wash-out is presented and a strategy is defined for preventing it.

After understanding liver anatomy and physiology and having defined a strategy to optimize organ procurement, in the third section of this thesis we describe the development of the Groningen Portable Hypothermic Liver Perfusion System. For this purpose, an extensive literature review is presented in chapter 6 addressing the major concepts and criteria in HMP, including the type of preservation solution, the characteristics of perfusion dynamics and the necessity of additional oxygen. As a result of this review, the list of requirements for a HMP system is defined. Subsequently, chapters
7 and 8 deal with separate components of the HMP system (i.e. oxygenator, pumps and sensors), and the integration of these components in the first prototype. Chapter 9, finally, presents the tests that were performed with the developed prototype to judge if it complies with the list of requirements defined in chapter 6.

After the tests, we can conclude that a HMP system has been developed according to a state-of-the-art list of requirements. The system should be capable of substantially contributing to the necessary decrease of the persistent organ donor shortage by improving quality of preservation which enables inclusion of NHBDs in the donor pool.

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