Improving liver preservation
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Chapter I

Introduction
The first steps towards clinical organ transplantation were already made in the early 1940s. In these days the interest and study of kidney diseases resulted in the development of the artificial kidney and the Kolff-Brigham device for haemodialysis. This was the first major step towards innovative developments in medical devices and created an interest in innovation of medical technologies and approaches to treat patients with renal failure. Following these artificial methods to overcome organ failure organ replacement was considered a more permanent therapy in patients with end stage organ disease. In the early 1950s, two groups simultaneously initiated clinical kidney transplantation using living donors. In 1952 in Paris, Michon, Küss and Hamburger performed the first living-related kidney transplantation. The graft survived 22 days before it was rejected. In 1954 Murray and Hume transplanted a kidney from the identical twin brother of a patient suffering from polycystic kidney disease. The recipient survived for eight years. Remarkably, these kidney transplantations were 22 years after the first reported skin transplantation which is in fact more difficult to perform. Skin transplants, performed during World War II in burn patients, resulted in a new important breakthrough as well since Gibson and Medawar recognized that a second tissue allograft from the same donor resulted in accelerated rejection. They described this phenomenon in 1942 and concluded that this was due to an allergic or immunologic process. In the early years in solid organ transplantation these experiences in skin transplantations were used to predict survival of the transplanted kidney, simply by watching the viability of a skin graft that was transplanted simultaneously. With the introduction of immunosuppressive drugs in 1959 renal allograft transplantation soon followed. In 1961, the first unrelated kidney was transplanted using azathioprine as immunosuppressant. Although the transplants survived for one month now, the side effects were tremendous since the dosage of azathioprine, which was previously used in dogs, proved to be toxic in humans. From 1965 on the 1-year survival rates for renal allografts from living donors improved and reached 80% and 65% for cadaveric donors.

In 1967, the initial experience in kidney transplantation was followed by the first human-to-human successful liver transplantation by Dr. Starzl. New developments in immunosuppressive drugs, improvements in surgical techniques and refinements in preservation methods resulted in improved survival rates. Orthotopic liver transplantation has nowadays become an effective treatment for patients with end-stage liver disease. Despite new developments in drugs and techniques, however, the waiting list for liver transplantation is long and increasing. In 2000 there were 1174 liver donors in the Eurotransplant region. By the end of 2000 the number of patients awaiting a liver transplant was 765. In the following years the amount of liver donors rose to 1361 while the number of patients on the Eurotransplant waiting list increased to a disproportional total of 2066. To date, the waiting lists are still increasing, and transplant results have became even better and are more encouraging. In a single center study with 920 transplantations, for example, one year liver graft and patient survival were reported to...
reach 78% and 85%, respectively. At five years, 66% graft and 75% patient survival were observed for patients transplanted between 1984 and 2002. The success of a liver transplantation and the number of waiting patients is largely dependent on several variables. Factors contributing to successful liver transplantation can roughly be divided into the recipient diagnosis and severity of the disease which are usually liver cirrhosis, hepatitis C, primary sclerosing cholangitis, biliary atresia, primary biliary cirrhosis and hepatocellular carcinoma. Other pertinent factors are donor related: medical history of the donor, duration of circulatory arrest; or are procurement and preservation related: warm and cold ischemia times and method of organ preservation. Reperfusion injury of the graft is then a final crucial factor in the entire donation-transplantation cascade. As outcomes after transplantation improve, the demand for organs continues to exceed the supply. Decreasing the transplant waiting lists has been attempted in several ways. Some examples are worth mentioning just to indicate which ways have been explored to better approach the required number of donor livers. One of them is increasing donor awareness with better information of the general population or better informing relatives of the deceased. The allocation system reviewing both liver disease and markers addressing the severity of the disease is another good example. Allocation models have been reviewed and better ways to allocate donor livers have been developed. In the early years when liver transplantation became a feasible treatment method, livers were assigned based on the ‘sickest-first’ principle with some attention for the time spent on the waiting list. Newer methods include the use of the Child-Turcotte-Pugh (CTP) scores, the Model for End-stage Liver Diseases (MELD) and the Pediatric End-stage Liver Diseases (PELD) scores. In the Child-Turcotte-Pugh scoring system the severity of the disease is an important item that is scored. The so-called Status 1 represents a category with the highest priority for a liver transplantation, e.g. fulminant hepatic failure, primary-non-function after liver transplantation and hepatic artery thrombosis within seven days post transplantation. Other items are, bilirubin, albumin and the International Normalized Ratio. More subjective measures are, absent, mild or severe ascites and encephalopathy. The MELD/PELD score use primarily objective parameters like serum bilirubin, creatinine, INR, growth failure, age and glomerular filtration rate. Besides the search for an effective allocation system, new methods to increase the donation pool are being explored as well, such as, the debate whether to increase the maximum donor age for liver transplantation beyond 70 years. Until now, inclusion criteria have remained to be a donor age between one month and 70 years, a known identity of the deceased and an established cause of death. Exclusion criteria are, refractory sepsis, malignancy or positive serology for HIV. In liver donation the number of potential donors that fit these criteria were previously exclusively brain-dead donors with an intact circulation. Nowadays, also non-heart-beating category III donors (Table 1), domino transplantation and (living)split liver donation are used as well, and have slightly increased the donor pool.
Table 1: Maastricht classification of Non-Heart-Beating donors.

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>Dead on arrival</td>
</tr>
<tr>
<td>II</td>
<td>Unsuccessful resuscitation</td>
</tr>
<tr>
<td>III</td>
<td>Awaiting cardiac arrest</td>
</tr>
<tr>
<td>IV</td>
<td>Cardiac arrest while brain-dead</td>
</tr>
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</table>

Domino transplantation is a transplantation technique in which the explanted livers from liver transplantation patients suffering from, for example amyloidotic polyneuropathy, oxalosis and possibly familial hypercholesterolemia\textsuperscript{86,87} are used as liver donors for another patient. The second technique resulting in expansion of the donor pool is the split liver technique\textsuperscript{3,88}. Liver lobes derived from healthy individuals are split along anatomic divisions and the segmental liver graft is transplanted. Success largely depends on the regenerating capacity of the liver. Enlarging the donor pool with marginal or extended criteria donors\textsuperscript{11,12}, older donors and possibly inclusion of non-heart-beating donor categories II or IV remains an important topic in organ donation and transplantation in general\textsuperscript{13}.

The inclusion of additional categories appear to be dependant on new and better preservation techniques requiring a better understanding of ischemia-reperfusion injury. Further developments can be achieved knowing which additives to existing preservation solutions could improve reperfusion results and by knowing which components do have a beneficial effect during the initial procurement operation and the following cold static preservation period. Static cold storage was originally chosen as a preservation method since it constitutes an easy procedure followed by good transplant results. Skilled personnel is not required and running costs remain low. Cold-storage, thus, became the standard method to preserve donor organs in most centres. Machine preservation, however, was the technique initially used by Belzer to preserve donor kidneys. Although it proved to be effective skilled personnel and complex equipment was required. New developments in medical devices have now become available and the understanding of ischemia-reperfusion injury is greatly improved. Machine preservation is now regaining interest in both kidney and liver transplantation centers as it better monitors the graft, improves washout of injurious waste products and eventually allows pharmaceutical intervention during preservation.
From Organ Donation to Transplantation

In the sequence from donation until reperfusion at time of transplantation, four important periods can be distinguished. These four time periods include: donor management and organ procurement, washout and preservation, rewarming and reperfusion during actual transplantation. Each period has its own characteristics resulting in injury of the graft in a specific manner. In this thesis we will address organ procurement and preservation of the liver as well as some potential improvements in clinical liver preservation. To allow a comprehensive analysis of improvements in preservation understanding of all four time periods is necessary to properly evaluate the importance of preservation in our experiments and clinical practice.

During the procurement phase the donor organ is retrieved and donor blood is flushed out of the microvasculature. Very important is the warm-ischaemia time which is defined as the time between clamping of the afferent vasculature of the donor organ and the initiation of vascular perfusion with preservation solution. Effective organ preservation starts with an effective blood wash-out of blood from the donor organ, to minimize the duration of warm ischemia and prepare the graft for the preservation period. Already in the 1980s a thorough blood wash-out was thought to be mandatory to improve organ outcome after transplantation\textsuperscript{14,15}. Perfusion of abdominal organs is usually performed via the aorta, using University of Wisconsin organ preservation solution or histidine-tryptophane-ketoglutarate solution. The time needed to cannulate the aorta and, in case of liver procurement the portal vein, is a period in which the organ becomes ischaemic and should be kept as short as possible\textsuperscript{12,13,16,17}. One way to rapidly decrease catabolic processes during stasis of warm blood is to cool-down the organ quickly by vascular perfusion with ice-cold preservation solution and by surface cooling with ice-cold saline. The donation phase involves three donor related risk factors that, apart from the warm ischemia time, are important in clinical transplantation as well. These three factors are the degree of liver steatosis, duration of hospital stay and donor age\textsuperscript{4,18,19}.

The preservation time is the time period that bridges between procurement, reperfusion and rewarming during transplantation. During preservation the organ is cold stored to allow allocation and transportation to the recipient in the transplant center. In our studies we defined preservation time as the period between completion of the procurement operation until initial reperfusion in the recipient. Hypothermia of 0 to 4 °C minimizes cellular injury and decreases metabolism during procurement and preservation, however, on the other hand increases cold-induced injury. During the preservation period a decrease in cell homeostasis occurs with a loss in integrity of the cytoskeleton and mitochondrial function. Consequently, cellular structures are affected and the generation of ATP and energy is low. Cell homeostasis is insufficient and cellular structure and functions are compromised. The preservation time has been found to be...
an important variable in organ outcome after transplantation. Extended preservation times beyond 12 hours have been shown to result in higher primary dysfunction (PDF) rates\textsuperscript{18,20,21}. During cold preservation the use of an effective preservation solution is indicated to minimize cold-induced injury and counteract the negative effects of hypothermia. In general, preservation solutions are used during procurement, when donor blood is flushed out of the liver, and during preservation, while the graft is allocated and transported to the transplant center. The development of the University of Wisconsin cold-storage solution (UW-CSS) in 1986 improved organ preservation and resulted in a better understanding in preservation related injury\textsuperscript{3,22,23}. Since the introduction of UW solution in 1986, a number of new organ preservation solutions or ‘UW look-a-likes’ have been developed. These solutions, however, have not resulted in significant contributions to a better and longer preservation of donor organs\textsuperscript{24-26}.

Immediately prior to reperfusion and during implantation, the organ suffers from warm ischemia when the vascular anastomosis are completed. In this third period the graft consequently re-warms due to the recipients’ body temperature. This second warm ischemia time has been shown to be an important phase during transplantation\textsuperscript{20,27-29}. This period is defined as the time needed to complete the vascular anastomosis between the donor artery and vein(s) and the vasculature of the recipient\textsuperscript{18,29}. During this period of approximately 30 to 60 minutes, the liver is resting in the abdominal cavity and as a result of the recipients’ body temperature tissue injury is enhanced and function decreases at a faster rate than observed when the organ is in cold-storage.

The last and most important phase in organ transplantation and ischemia-reperfusion injury of the graft is perfusion with recipient blood. Initial function and viability of the transplanted liver can be assessed after this final phase. Important factors for the reperfusion phase are re-oxygenation of the tissue, activation of Kupffer cells\textsuperscript{27,30,31}, alteration in endothelial cell structure\textsuperscript{27,30,32-34}, widening of endothelial fenestrations\textsuperscript{30,31,33} and influx of neutrophils\textsuperscript{31,34,35}. A frequently described mechanism resulting in cellular injury is the generation of reactive oxygen species\textsuperscript{36}. After a hypoxic period it appears to be logical that it is beneficial when oxygenation of the tissue is restored. Re-oxygenation, however, can be injurious and can lead to excessive production of these reactive oxygen species (ROS). In the cold xanthine dehydrogenase can be converted to xanthine oxidase\textsuperscript{37,38} which upon reperfusion and re-oxygenation generates toxic ROS. This is the so-called oxygen paradox\textsuperscript{39}. This results in injury of endothelial cells first, followed by the hepatocytes\textsuperscript{40-42}. The mechanism of cellular death differs between the sinusoidal endothelial cells (SECs) and hepatocytes. While SECs are 50 times more often prone to apoptosis than hepatocytes\textsuperscript{43}, the latter show more necrosis despite that necrosis occurs in a later stage of cold ischemia and reperfusion injury\textsuperscript{43}. Injury of the hepatic cells results in primary dysfynction (PDF), possibly, without regaining any function at all. PDF is normally used as the overall definition for poor liver function following the early postoperative period, however, the definition of
PDF differs between authors\textsuperscript{4,20}. Some transplant centers use the definition PDF or primary non function (PNF) when the graft does not support life within the first seven post operative days, others used a range of up to two weeks with the addition that ‘initial poor function’ was observed just after liver transplantation\textsuperscript{18}. PDF or PNF of the transplanted graft is an important complication after transplantation and an early retransplantation in the first postoperative week is often indicated. The incidence of PDF is remarkably similar between transplantation centers and between 2.7 and 7.9\%\textsuperscript{4,20}. Some other factors contributing to pre-preservation injury and PDF are brain death of the donor, preexisting diseases of the graft and injury occurring during organ retrieval, preservation and reperfusion\textsuperscript{18,19,44,45}. After transplantation, liver function depends on vascular perfusion, i.e. the occurrence of endothelial cell injury, bleb formation causing no reflow, and the occurrence of acute or chronic rejection. The multifactorial origin of PDF\textsuperscript{19} can be diagnosed when reperfusion is impaired and has resulted in several diagnostic criteria. Hypoglycemia, coagulopathy, absent bile production, an increase in serum transaminases, coma, kidney failure and cardiogenic shock may all be present.

Until now we have discussed four important time periods in donation and transplantation, including procurement, preservation, rewarming and reperfusion. These time periods are all crucially important as they are subjected to ischemia and as they all have their influence on viability and outcome of the graft after transplantation. Studies aiming at improving preservation should, thus, take all time periods into account to allow a sound analysis of the data.
Cold-storage preservation

To date, the clinically most popular and applied technique in organ preservation is static cold-storage (CS) which was first introduced in kidney transplantation by Collins et al\textsuperscript{46} in the late sixties. To allow CS preservation, the donor organ is flushed in-situ to remove donor blood and cool down the core temperature of the organ with a cold preservation solution. The graft is subsequently stored on melting ice in a sterile bag filled with preservation solution. To successfully transplant the organ a number of prerequisites are defined for effective preservation and to prevent a decrease of its viability in the cold and without any circulation. To minimize preservation related injury two principles should be distinguished. First, the core temperature of the organ is lowered to benefit from the \textit{temperature effect}. During storage the temperature is lowered to 0-4 °C to reduce metabolism and generation of catabolic enzymes\textsuperscript{47,48}. Next, to protect the organ during preservation it is perfused with a preservation solution, the \textit{solution effect}. The solution effect depends on the components of the preservation solution that counteract the negative sequelae of hypothermia. Important components of a preservation solution are directed towards the physical and the biochemical environment in which the solution is used. Components affecting the physical environment counteract edema formation. Other agents act on the biochemical environment and minimize potentially harmful substances like reactive oxygen species\textsuperscript{33}.

Since the introduction of the static cold-storage technique several solutions have been tested. Nevertheless, preservation times have remained rather short and the donor pool limited. The UW-solution allows preservation of donor livers for merely 12 to 18 hours in the clinical setting and 48 hours in laboratory experiments\textsuperscript{54-56}. One of the cellular mechanisms that limits duration of cold-storage, is the occurrence of swelling of endothelial cells and of small disruptions in the sinusoidal lining cells\textsuperscript{27,33}. This injury in the liver microcirculation is reflected by loss of sinusoidal lining, rounding of sinusoidal lining cells and irreversible detachment from hepatocytes\textsuperscript{33}. Another important factor is maintenance of mitochondrial function. Better mitochondrial oxidative phosphorylation and, consequently, higher cellular ATP levels are an improvement for preserved organs\textsuperscript{49}. Mitochondrial dysfunction after ischemia-reperfusion injury can be prevented by a rapid decrease in core temperature during procurement, resulting in a short first warm-ischemia-time. In addition to age and the condition of the vascular system, cerebral injury leading to brain death has been recognized as a crucial factor that determines the outcome after ischemic injury and transplantation. Both, heart-beating and non-heart-beating donors are well known examples\textsuperscript{50}. The onset of brain death results in a massive release of catacholamines contributing to a systemic alteration of the immune system enhancing ischemia-reperfusion injury and allorecognition\textsuperscript{45,51-53}. An increased occurrence of delayed graft function and transplant dysfunction are seen due to a number of specific detrimental mechanisms that have been initiated before the preservation period has started.
Hypothermic Machine Perfusion Preservation

Although the majority of the transplant centers nowadays uses the cold-storage technique as standard preservation method, preservation started originally with continuous hypothermic machine preservation (HMP) in the early 1960s. To date, only a small number of transplant centers continue to use the HMP technique in kidney transplantation. Due to the more complex nature of this technique versus cold-storage, initially with Collins solution and later with the University of Wisconsin organ preservation solution, this difficult and cumbersome technique was thought to be redundant. Results of machine preservation, however, have always been very good and many times found superior to cold-storage. With the persistent shortage of donors, older and more marginal donors, in the past years HMP has regained interest not only in kidney transplantation, but also for liver transplantation as it offers a number of advantages which would be beneficial in the less viable organ.

To allow re-introduction of machine perfusion in clinical practice in kidney transplantation new and user-friendly techniques are a prerequisite. Before HMP can be used in liver preservation a number of unanswered questions have to be clarified as well: The question concerning the best route of perfusion, which perfusion pressure(s) are required and which components are to be included in the perfusion solution. The vascular anatomy of the liver is important when the choice is made whether perfusion should use the portal vein, hepatic artery or both vessels and also to determine which ratio should be used between portal vein and hepatic artery perfusion. The vascular anatomy of the liver comprises two afferent vessels, i.e. the hepatic artery that is normally perfused at 120/80 mmHg and the portal vein perfused at 8 to 12 mmHg. The portal vein receives blood mainly from mesenteric and splenic veins, but the pancreatic and omental veins drain on the portal vein as well. Portal vein, hepatic artery together with the bile duct are called the portal triad. From the portal triad blood flows through the sinusoids which are the small capillaries of the liver in which the exchange of toxic substances, nutrients and coagulation factors between blood and hepatocytes takes place. The arterial blood supply, which contributes 20 to 33% to the total hepatic perfusion, enters the sinusoids and the peribiliary capillary plexus. The tissue surrounding the portal triad consists of periportal parenchyma with physiological fibrosis, capillaries and bile canaliculi. The acinar zone one is a functional zone instead of an anatomically defined area containing hepatocytes in close proximity to the portal triads. Tissue in between zone one and the central vein is situated in the functional acinar zone two and tissue surrounding the central vein is called the acinar zone three. An interesting question in HMP preservation is where blood from the hepatic artery meets the blood from the portal vein. It is known that some arterial venous shunting exists but it remains unknown to which extent there is portal venous shunting.
In the late 1960s Slapak et al\textsuperscript{62}, Brettschneider et al\textsuperscript{63} and in 1970 Belzer et al\textsuperscript{64}, experimented with continuous hypothermic machine perfusion of the liver in an experimental setting. Their results were comparable or even better than livers preserved using the static CS technique, however, this technique never gained clinical acceptance. Now, forty years after it was first used in kidneys (Figure 1), continuous hypothermic machine perfusion is revisited by many groups. With HMP, the use of non-heart-beating, older and marginal kidney donor is now feasible\textsuperscript{65-68}. Due to the success in increasing the kidney donor pool and prolonging storage times, continuous perfusion preservation has now regained interest by liver teams as well. Kidney perfusion preservation was developed in the 1960s. The solution in this first machine preservation system was a plasma based solution which was completely different from the preservation solutions that are nowadays applied\textsuperscript{58}. Moreover, the first preliminary reports concerning the development of UW-MP were published in the early 1980s, 15 years after the first attempts to machine perfuse the kidney\textsuperscript{69}. Mean kidney perfusion pressures were originally set at 60 mmHg with the plasma based solution. Despite the fact that in the 1980s the perfusion solution improved and that the plasma based solution became obsolete, perfusion pressures were never changed. The newer preservatives with different rheological properties than the plasma based solution, however, did not result in a re-definition of perfusion pressures. Despite the successful application of HMP in kidneys\textsuperscript{70}, clinical liver perfusion has not been practiced yet. The first successful experiments using HMP for the liver were already documented in 1986 by D’Alessandro et al\textsuperscript{66} and Pienaar et al\textsuperscript{71}. Both managed to preserve and transplant good quality canine livers after 72 hours in a HMP dog model and demonstrated that machine preservation of the liver is feasible.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image1.png}
\caption{Dr. Belzer standing next to his first ‘transportable’ Hypothermic Machine Perfusion system used for kidney preservation.}
\end{figure}
Previously it has been postulated that the occurrence of PDF or PNF might decrease when livers are preserved using machine preservation\textsuperscript{21,72}. Also, a considerable morbidity after orthotopic liver transplantation is due to the development of Ischemic-Type-Biliary-Lesions (ITBL). Although the pathogenesis is unclear, it has been shown that effective perfusion with preservation solution via the hepatic artery minimizes the development of ITBL\textsuperscript{73}. To improve preservation of the liver with prolonged cold ischemia times and a decrease in ITBL rate with complete perfusion of the liver we invested in the concept of HMP preservation based upon perfusion via both the portal vein and hepatic artery. During liver procurement improvement has been described using arterial perfusion in vivo, higher initial perfusion pressures during the back-table procedure or better perfusion solutions\textsuperscript{73,74}. During preservation, improvement in liver viability could be achieved using dynamic preservation techniques instead of standard static cold-storage.

In our experiments we decided to continue to use hypothermia as it is still a basic principle in organ preservation. Although interesting, we did not consider the use of normothermia or the concept of hibernation at this point in time, since these concepts demand a completely different approach in preservation and the use of a preservation solution that has not been defined yet. The UW-MP organ preservation solution has been used successfully in cold kidney machine preservation and is nowadays the best solution available for hypothermic kidney preservation\textsuperscript{69,75}. The use of hypothermia demands protection of cellular integrity. Thus, the dynamic characteristics of HMP are very important. Based on existing parameters we could not come to a conclusive statement about the optimal perfusion characteristics, since in the literature either perfusion pressure or perfusion flow has been reported instead of both. Reviewing the literature concerning HMP we concluded that perfusion is best performed via both the hepatic artery and the portal vein to achieve homogeneity of the microcirculation\textsuperscript{76,77}. Also, pulsatile perfusion offers a better distribution throughout the tissue compared to continuous perfusion and results in lower vascular resistance compared to a continuous flow\textsuperscript{78,79}. So, perfusion of the liver in a physiological manner, i.e. pulsatile arterial perfusion and continuous portal venous perfusion, offered the best options for successful machine preservation of the liver. In addition, HMP allows continuous wash-out of potentially hazardous waste products and a continuous supply of oxygen to the perfused tissue. Oxygenation of the preservation solution has been used for HMP of the kidney, however, the ideal partial oxygen tension during HMP of the liver is scarcely documented\textsuperscript{80-83}. Although oxygenation may be beneficial during preservation, it could be harmful as well, since it is a potential source for toxic reactive oxygen species\textsuperscript{84}. The combination of perfusion with oxygen could enhance formation of ROS even more.
Conclusion and Specific Aim

With the development of the concept of the UW cold-storage solution and its modifications several authors have stated that the limits in preservation using static cold-storage have been reached. Organ preservation by cold-storage, even though some extra beneficial components can still be added to newer solutions, merely slows and ameliorates extracorporeal ischemic and hypoxic damage, rather than reverse it. An improvement in donor preparation or preservation of the liver is, thus, necessary to expand the donor pool. To further improve organ viability conventional cold-storage could be reverted to continuous machine perfusion, either at hypothermic or normothermic temperatures, to reduce catabolism and support anabolic metabolism. Machine preservation could increase liver viability and consequently the donation pool. The possible inclusion of older donors, marginal donors with more co-morbidity, extended criteria donors or possibly non-heart-beating donors could result in more viable donor livers and a decrease of the number of patients on the waiting lists.

According to the Maastricht classification NHB-cat III patients, patients awaiting cardiac arrest are donors that are now considered to be suitable for transplantation. Improvements in preservation could allow inclusion of other NHB categories as well.

Inclusion of more donor categories is not the single benefit of machine preservation. Several other aspects in preservation could be improved using this technique. A prolongation of preservation time without loss of viability for example, could result in larger geographical areas cooperating to find the optimal donor-recipient combination. Also, it has already been shown that during a ‘back-table’ procedure an additional UW flush via the hepatic artery improves liver outcome after transplantation. Liver HMP perfuses the hepatic artery as well and, in addition to a single flush, allows continuous arterial perfusion throughout the entire preservation period. Continuous perfusion of the hepatic artery and thus the peribiliary capillaryplexus is expected to result in a potential decrease in arterial complications and Ischemic-Type-Biliary-Lesions. Finally, continuous perfusion compared to CS allows the use of biochemical markers and interventions with beneficial agents. So the use of continuous machine perfusion, known to be a better preservation method for kidneys, might be a better mode to overcome the gap between liver procurement and transplantation.

In this thesis we will address a number of items that concern the technical components necessary for a new HMP technique to preserve livers. Although it is tempting to copy the existing technique for kidney preservation, HMP of the kidney still uses components that never have been studied or were not updated since their first use in the 1960s. Furthermore, since kidneys are perfused via one afferent blood vessel, successful kidney HMP can not simply be translated as a safe and effective technique for HMP of the liver. In our studies we have applied basic principles in hypothermic
organ preservation compatible with human liver preservation. To reach our goals we have collaborated intensively with biomedical engineers and defined the conditions for effective HMP. Next, the components that met our conditions in the actual HMP design were defined. As dynamic preservation allows a better assessment of liver viability than static cold storage we have also studied pathophysiological mechanisms affecting function or viability during preservation. A better insight in relevant mechanisms of injury and repair will enhance appropriate interventional strategies to improve liver viability, increase immediate function and outcome after liver transplantation.
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