General Introduction and Aims of this Thesis
Orthotopic liver transplantation is the only curative treatment for patients with end-stage liver disease. Unfortunately, globally an immense gap exists between the demand and availability of donor livers for transplantation. This has led to the establishment of strict recipient criteria for transplantation, though still, many transplant candidates die awaiting a donor liver or are removed from the waiting list as they become too ill for transplantation. Of all patients listed for a liver transplant in the Eurotransplant region in 2017, 16% of patients deceased, nearly 4% became unfit to transplant and only 63% were actually transplanted. For this reason, efforts to expand the donor liver pool are crucial. Aside from public campaigns aimed at increasing the number of registered donors, it is important to optimize the use of organs within the existing pool of donor livers. The present thesis focuses on the latter.

By loosening the criteria for liver donation, many more donor livers have become available for transplantation. Such livers are referred to as extended criteria donor (ECD) livers and include livers that are donated after circulatory death (DCD). Compared to donation after brain death (DBD), DCD livers inherently undergo a period of warm ischemia in which the organs are metabolically active but without circulation and provision of oxygen. This causes additional injury to the liver and results in higher complication rates after transplantation. Nevertheless, the number of DCD liver transplantations is rising, with 40% of deceased donor liver transplants coming from DCD donors in the Netherlands in 2017.

The most feared complication after DCD liver transplantation is the development of non-anastomotic strictures (NAS) of the biliary tree, also referred to as ischemic-type biliary lesions (ITBL). These strictures occur in 13 - 35% of DCD livers, compared to only 1 – 24% in DBD livers. The development of NAS is multi-factorial, with the main causes including ischemia-related injury, immune-mediated injury, bile-salt toxicity and a lack of regenerative capacity of the biliary tree.

Classically, surgeons could only base their decision to transplant a liver on donor characteristics, imaging results, macroscopic appearance of the liver and in some countries, also frozen histology sections of the liver. Machine perfusion, a technique that was re-introduced relatively recently after its initial publication in 1960s and 70s, is a technique in which donor livers are perfused ex situ, offering the possibility to objectively assess organ viability and allowing for the selection of livers that are suitable for transplantation.

At 37°C, normothermic machine perfusion (NMP) renders the liver metabolically active, providing the possibility to objectively assess organ viability and allowing for the selection of livers that are suitable for transplantation. NMP of human livers was first performed using a perfusion solution based on human blood products, including packed red blood cells (RBC) and fresh frozen plasma (FFP). The aim of chapter 3 is to describe the procedure of NMP using the Liver Assist
(Organ Assist), which is further illustrated in the online video with this article, and share hepatocellular function and injury data that was obtained during NMP of human donor livers that were declined for transplantation.\(^{14}\)

Due to the scarcity, logistical challenges and potential risk of transmitting infections, the aim of chapter 4 was to find an alternative for the use of human blood products during NMP. In this chapter, we tested the feasibility and compared the efficacy of first replacing RBCs with an acellular hemoglobin-based oxygen carrier (HBOC-201, product name Hemopure), and as a second step replacing FFPs with gelofusine, a widely used gelatin-based colloid volume expander, and other nutrients.\(^{15}\)

Hypothermic machine perfusion (HMP), performed at 10-12°C, has been shown to resuscitate mitochondria, replete adenosine triphosphate (ATP) levels and ameliorate ischemia-reperfusion injury after transplantation.\(^{16}\) Especially in the case of DCD livers, oxygenated HMP prior to transplantation has been hypothesized to reduce the development of NAS. HMP can be performed using single perfusion through the portal vein, or dual through both the portal vein and hepatic artery. In particular dual hypothermic oxygenated machine perfusion (DHOPE) is expected to be most efficient as bile duct epithelial cells (cholangiocytes) are mostly dependent on arterial oxygenation.

A very important property of HBOC-201, compared to RBCs, is that it can be used at different temperatures. This allows for the possibility to first perfuse at lower temperatures using DHOPE, followed by a period of controlled oxygenated rewarming (COR) and lastly by NMP for viability assessment. The aim of the next study was therefore to test the feasibility and safety of performing machine perfusion at different temperatures using a HBOC-201-based perfusion solution. Chapter 5 describes the results of a study on the first 7 human donor livers that were declined for transplantation nationally, of which 5, after being deemed viable in the NMP phase of the DHOPE-COR-NMP protocol, were transplanted.\(^{17}\)

Several viability criteria have been established to assess hepatocellular injury of livers during NMP. These criteria include bile production, lactate clearance, pH buffering capacity, glucose metabolism, flows and macroscopic appearance of the liver. Criteria regarding bile duct viability, however, were lacking despite evidence that histological biliary injury prior to transplantation is a strong predictor for the later development of NAS. Therefore, the aim of chapter 6 was to establish biomarkers of biliary viability during NMP by determining the value of biliary biomarkers in predicting the presence of histological biliary injury.\(^{18}\) The assessed biomarkers were based on both cholangiocyte injury and function and included biliary pH, bicarbonate (secreted by cholangiocytes), glucose and the perfusate/bile glucose ratio (glucose is resorbed from bile by cholangiocytes), as well as biliary lactate dehydrogenase (LDH), reflecting biliary injury.

In order to establish other useful criteria during machine perfusion, we examined the release of microRNAs in NMP. MicroRNAs are small, non-coding RNAs that have emerged as sensitive, specific and stable markers for cell
function, stress and injury. The aim of chapter 7 was to determine the value of hepatocyte-derived miRNA-122 and cholangiocyte-derived miRNA-222 in both perfusate and bile in predicting conventional hepato-cholangiocellular injury and function parameters during NMP.

Despite the emergence of machine perfusion as an alternative preservation technique, static cold storage (SCS), in which donor livers are transported on melting ice, remains the gold standard for liver preservation. The two most frequently used preservation solutions for static cold storage in clinical practice are University of Wisconsin (UW) solution and Histidine-tryptophan-ketoglutarate (HTK) solution. In the literature, conflicting results have been published regarding these preservation solutions’ ability to preserve the liver and biliary tree. Furthermore, polyethylene glycols (PEGs) are non-immunogenic, non-toxic, water soluble and FDA-approved compounds with high flexibility, hydrophilicity, protein-rejecting properties and a greater hydrodynamic volume. PEGs have been shown to protect against ischemia reperfusion injury of different organs and we hypothesized that they would also play a significant role in protecting the biliary tree. Therefore, in chapter 8, human extrahepatic bile duct segments were preserved in various preservation solutions with added PEGs in order to determine the optimal preservation solution for protecting the bile ducts.

Lastly, machine perfusion has the potential to resuscitate donor livers. Injury to the peribiliary glands (PBG), which are niches of cholangiocyte progenitor cells embedded in the bile duct wall from which cholangiocytes regenerate, plays a role in the development of NAS. PBG, however, have only recently gained interest and have not been well described. Therefore, our aim was to develop a human ex vivo model to study human PBG in depth. Chapter 9 describes the establishment of a novel technique, called precision-cut bile duct slices (PCBDS), and shows that progenitor cells in the PBG differentiate into mature cholangiocytes after severe biliary injury. This technique involves the in vitro culturing of human bile duct slices and circumvents the use of laboratory animals.

In chapter 10, the chapters of the present thesis are summarized and discussed, followed by future perspectives to build on the present research. Lastly, this thesis concludes with a Dutch summary in chapter 11.
REFERENCES


