CHAPTER 1

General Introduction and Aims of this Thesis

Transplantation is the transfer of human tissues or organs from a donor to a recipient with the aim of restoring essential functions where no alternative of comparable effectiveness exists (World Health Organization). Ever since the first successful human organ transplant in 1954, the field of organ transplantation has been greatly developing. New surgical techniques and the introduction of post-operative immunosuppression regimens have greatly improved patient outcome. Nowadays, organ transplantation remains the only life-saving treatment for patients with end-stage organ failure, conferring immense benefit to hundreds of thousands of patients each year. The increasing success of organ transplantation has, however, ironically become one of the biggest challenges the transplant community is facing to date. According to recent data of United Network for Organ Sharing (UNOS), over 114 000 patients are waitlisted for organ transplantation, while only 13 000 organ donors were able to donate in 2018. The worldwide discrepancy between supply and demand of suitable organs for transplantation, has resulted in high waiting list mortality. In fact, every candidate who is accepted for organ transplantation has a 10-30% chance of dying on the waitlist, depending on the organ (1). In an attempt to minimize organ scarcity, the criteria for organ donation are progressively being extended. These so called 'extended criteria donor' (ECD) organs once thought to be too high-risk for transplantation include grafts of elderly donors, donors with a higher body mass index, or grafts that are donated after circulatory death (DCD) (2–4). These suboptimal grafts are, however, more prone to preservation and reperfusion related injury and are associated with in inferior transplant outcomes. For example, liver transplantation of DCD grafts is associated with higher incidence of primary graft non-function, early graft dysfunction, and increased rates of severe biliary tract complications after transplantation, compared to transplantation of organs from donation after brain death (DBD) donors (5, 6). Thus, to successfully utilize ECD grafts and to achieve satisfactory post-transplant outcomes, innovative methods to better preserve and even improve organ viability prior to reperfusion are needed.

The current gold standard in organ preservation is based on cooling the organ with a cold preservation solution and storing it in a box of ice until transplantation, referred to as static cold storage. Hypothermia has long been the cornerstone in preservation as it successfully lowers the metabolic rate of mammalian cells, thereby lowering the demand for oxygen (7). This creates a limited time window wherein organs can be kept outside of the body. The maximum cold ischemia time a graft can tolerate, however, greatly depends on the tissue type and graft quality; i.e. a maximum of 10-12 hours for livers and ideally no longer than 4-6 hours for muscle-containing grafts such as extremities (8, 9). However, cold ischemia itself is also harmful to the organ as it causes cellular damage. During cold ischemia, the lack of oxygen supply causes cells to switch to anaerobic cellular respiration causing adenosine triphosphate (ATP) depletion and a

decrease in cellular pH. As a consequence, ATPase-dependent ion transport mechanisms are disrupted, contributing to mitochondrial dysfunction (calcium overload), cellular swelling and cell membrane perturbations, resulting in ischemic cell death (10). Moreover, ischemic injury is further exacerbated upon reperfusion with the formation of reactive oxygen species and proinflammatory cytokines. Endothelial cells lining the vasculature are especially challenged during cold ischemia. The endothelium plays a key role in the control of vascular tone and hemostasis. Upon reperfusion, damaged endothelial cells initiate a variety of unwanted events including blood coagulation and inflammation, while repressing production of the vasodilator nitric oxide, resulting in poor tissue perfusion, prolonged hypoxia, cell death and even immune activation (11, 12).

Machine perfusion is gaining increasing (renewed) attention as an alternative method of organ preservation as it hold many advantages over static cold preservation. Machine perfusion is the technique by which a perfusion solution (cellular or acellular and non-oxygenated or oxygenated) is pumped through the vasculature of the donor organ *ex situ* by a mechanical device. The dynamic nature of machine perfusion provides many advantages over static cold storage (SCS), as it has the opportunity to provide essential nutrients, "wash out" toxins and waste products, resuscitate the organ, and assess its viability prior to transplantation. In the clinical setting, machine perfusion is increasingly explored as an alternative method of preservation of marginal donor grafts (13–15).

Moreover, animal studies show promising results with the implementation of the technique of machine perfusion in a new area of transplantation; vascularized composite allotransplantation, such as limbs (16).

The aim of this thesis is to study the effects of oxygenated machine perfusion on both donor livers and limbs in more detail, in part A and B respectively. The main focus is on the effects of machine perfusion on endothelial activation and function, and study the effects of new perfusion solutions on graft function both *ex situ* and *in vivo* (after transplantation).

PART A: OXYGENATED MACHINE PERFUSION AND TRANSPLANTATION OF HUMAN LIVERS

In **Chapter 2**, we aimed to discuss the role of machine perfusion as an alternative method of DCD liver preservation in more detail. In this chapter, the different modalities and technical aspects of liver machine perfusion that have emerge as clinically relevant are discussed. Temperature is an important discriminating factor between the different

types of machine perfusion. Three main temperature ranges at which machine perfusion of livers is often preformed are hypothermic (0–12°C), subnormothermic (25–34°C), or normothermic machine perfusion (35–38°C) (17).

Hypothermic machine perfusion (HMP) of donor livers has shown to increase ATP levels up to 15-fold prior to reperfusion (18). Previous studies have shown that ATP levels prior transplantation strongly correlate with graft function upon reperfusion (19). Furthermore, a short period of end-ischemic HMP offers better preservation of the hepatobiliary excretory function and peribiliary vascular plexus upon re-oxygenation of the liver, compared to liver grafts only preserved by SCS (18, 20, 21). However, the effect of end-ischemic HMP on vascular endothelial cells remains largely unexplored. In **Chapter 3**, we aimed to study the effect of end-ischemic oxygenated HMP on endothelial cell function of extended criteria donor livers.

Recently, end-ischemic oxygenated HMP of donor livers has been introduced into clinical practice as an alternative method of organ preservation. Orthotopic liver transplantation of SCS-preserved livers is often accompanied by acute hyperkalemia during reperfusion. When untreated, hyperkalemia may cause life-threatening arrhythmias and anesthesiologists therefore often take preventive measures to counteract this expected rise in serum potassium levels. However, during our first clinical series of dual end-ischemic oxygenated HMP we noted that *in vivo* graft reperfusion resulted in hypokalemia, instead of hyperkalemia, in three out of ten recipients (22). Therefore, in **Chapter 4**, we aimed to determine to the effect of dual end-ischemic oxygenated HMP on potassium and sodium shifts in human donor livers during machine perfusion and subsequent warm reperfusion in both a preclinical *ex situ* reperfusion model as well as in patients.

Normothermic machine perfusion (NMP) is a technique human donor livers are perfused *ex situ* at physiological temperature. During NMP, the graft is functioning at full metabolic pace, allowing for both resuscitation and viability testing prior to transplantation. During NMP, adequate oxygen delivery is an absolute must. In **Chapter 5**, we aimed to develop a machine perfusion solution which allows for optimal oxygen delivery, without the need of human blood products. Hemoglobin-based oxygen carriers (HBOCs) are an interesting substitute for packed red blood cells (RBC). However, concerns have been raised about the potential nitric oxide scavenging properties of HBOCs (23). The aim of **Chapter 6** was, therefore, to study the effect of polymerized bovine HBOC-201 on liver endothelial cell function during *ex situ* NMP of donor livers.

Vascular endothelial cells are the first interface between donor and recipient (24). In organ transplantation, this dynamic layer of cells plays a key role in initiating ischemia/reperfusion injury related damage, which makes the endothelium an interesting therapeutic target (24, 25). Recombinant human soluble thrombomodulin (ART-123) is a novel drug composed of the active, extracellular domain of thrombomodulin. Thrombomodulin is a transmembrane glycoprotein ubiquitously expressed on vascular endothelial cells, and it is known to play a key role in both coagulation and inflammation (26). In previous animal studies, it has been shown that ART-123 has important organ protective effects as well as cytoprotective effects on endothelial cells (27, 28). However, for safe application of ART-123 in transplant recipients, the anticoagulant and profibrinolytic effects of ART-123 first have to be investigated *in vitro*. This is especially important in the transplant population as the hemostatic system of patients with end-stage liver disease substantially differs from healthy individuals (29). Therefore, in **Chapter 7**, we aimed to study the in vitro effects of ART-123 on coagulation and fibrinolysis in plasma samples taken from patients during and after liver transplantation.

PART B: OXYGENATED MACHINE PERFUSION AND TRANSPLANTATION OF LIMBS

The idea of replacing diseased or damaged body parts prevailed for millennia (30). As early as in the third century, the idea of complex transplants were envisioned in miracle tales. The 'Miracle of the Black Leg' describes the story of two sainted doctors, Cosmas and Damian, who amputated the diseased leg of a verger and 'successfully' replaced it with the leg of a recently died man (31). Complex tissue reconstructions, such as limb transplantation, have long been considered pure experimental and controversial procedures. Decades of continued successes and advances in organ transplantation have, however, enabled complex tissue reconstruction to expand as a new field of transplantation that holds great promise (32). Vascularized composite allotransplantation (VCA) is an emerging area of reconstructive transplantation that focusses on the reconstruction of severe tissue defects not amendable to conventional reconstruction. The term VCA is used as an umbrella term for vascularized grafts comprised of different tissue types such as skin, muscle, nerve, vessels and bone (e.g., hand, face, penis et cetera). In 2014, VCA grafts were added under the definition of 'organs' in the Organ Procurement and Transplantation Network Finale Rule. While the field if rapidly growing, broad application of VCA is constrained by the very limited cold ischemia time that is tolerated by VCA grafts (32). In **chapter 8**, we aimed to discuss the latest advances in organ preservation, machine perfusion and cryobiology (subzero temperatures) and lay out a vision of how advancements in solid organ preservation

can help to overcome practical hurdles in VCA. In **chapter 9**, we aimed to develop a protocol for 6 hours of subnormothermic machine perfusion of VCA grafts. We have compared different perfusion solutions and aimed to validate the most optimal protocol in a heterotopic transplant model.

PART C: ADDENDUM

In **chapter 10** the details of a new subzero non-freezing protocol that hasve been developed for extended preservation of VCA grafts are described. Part of the subzero non-freezing protocol is the technique of machine perfusion as developed and studied in chapter 9. The results of all chapters are summarized and discussed in **chapter 11**, followed by a discussion and future perspectives. This section is concluded by a Dutch summary of this thesis in **chapter 12**.

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