CHAPTER 1

General introduction and aim

INTRODUCTION

The issue: donor organ shortage

At the end of 2018, a total of 14.129 patients had been registered on the Eurotransplant waiting list for a solid organ transplant, with 76% of these patients waiting for a kidney.[1] In the United States, 124.152 patients are currently waiting for an organ with 83% waiting for a kidney transplant.[2] These numbers emphasize the imperative to increase the numbers of transplantable organs. Currently, only patients that are suffering from end-stage organ failure and meeting strict criteria are placed on the waiting list for transplantation. However, the number of patients dying from end-stage organ diseases is approximately 15 times higher than the number of new patients that are added to the waiting list.[3] The World Health Organization has calculated that only 10% of the total organ demand is met at this point in time[4]. In addition, an estimated 35% of all annual deaths in the United States could be prevented or at least delayed by a transplanted organ, if all waiting list restrictions would be removed.[5] Thus, waiting lists represent only a small proportion of a much bigger problem we have been facing in the past decades: the persistent donor organ shortage in all countries in the world that prevent us from helping to cure patients with end stage organ failure.

Milestones in the beginning of organ transplantation

The idea of transplanting organs and tissues has been of interest to mankind for a long time, and intriguing descriptions exist in mythologic, religious and historical literature.[6] In clinical practice, the first successful transplants of non-visceral tissue, such as skin are reported in the beginning of the 19th century.[7] For solid organ transplantation, the work of the French surgeon Alexis Carrel, who perfected vascular anastomoses techniques in the beginning of the 20th century, was fundamental for the field of transplantation.[8] He was also the first to perform a kidney auto-reimplantation in a dog.[9] Although surgical successes were booked, immunological phenomena were not yet understood and all human kidney transplants performed between 1936 – 1952 resulted in rejection of the grafts.[10] The first successful kidney transplantation was reported in 1954 between two identical twin brothers where genetic similarity prevented rejection in the absence of clinically effective immunosuppressive medication.[11] Drs. Murray and Merril where the two lead surgeons of this first successful kidney transplantation, but were also the first to succeed in a kidney transplant in 1962 that was retrieved from a deceased donor.[12] The important findings of Drs. Thomas Starzl and Roy Calne at the beginning of the 1960s, that azathioprine

in combination with prednisolone was able to reverse renal graft rejection and induce host tolerance, opened the door towards clinical implementation and more successful outcomes when using organs from both deceased and living donors.[13] However, long-term outcomes remained poor (18 – 30% 1-year patient survival)[10] until the discovery of cyclosporine by the Swiss physician Borel in 1977 and its subsequent approval by the Food and Drug Administration in 1983.[14,15] From then on with many more developments in immunosuppression including the use of induction therapy and replacing cyclosporine with tacrolimus in the 90s and onwards have propelled results in transplantation to remarkable high levels. The multidrug immunosuppressive regimens that became then possible resulted in increased 1-year graft survival to rates of 90% in kidney transplantation.[16]

Organ donor types

History

Due to the lack of effective immunosuppression, the first kidney donors were closely related living family members, but immunological discoveries soon resulted in the usage of deceased donors. These first deceased donors were patients with severe brain injury taken to the operating theatre for organ retrieval after cessation of treatment due to medical futility and ventilator switch off that resulted in a cardio-respiratory and circulatory arrest: the non-heart-beating (NHB) or cardiac-death donors.[17] Technically, these donors could not be referred to as brain dead since they the patient's death had been declared by absence of respiration and heartbeat, as in those days the so called 'brain death criteria' had not been established. The first mention of brain death or coma dépassé, was reported in 1959 in Louvain, Belgium.[18] The first donation from a brain dead patient also took place in Louvain in 1963, which implied that mechanical ventilation was continued up to the moment of cross-clamping of the aorta followed by organ retrieval and the first heart-beating (HB), brain-dead donor was a fact.[19] As consensus was lacking on the exact definition and diagnosis of brain death, in 1968 the new standard concerning "A definition of irreversible coma" was developed by an Ad Hoc Committee at Harvard Medical Scool.[20] Brain death was defined as the irreversible and total loss of brain function as a result of absent blood circulation in the brain.[17] This implies that the center of respiration is irreparably damaged and ventilator treatment is necessary to support circulation. It also meant that the standing diagnosis of death previously exclusively based on the cessation of cardiopulmonary function and cardiac death, now related to the lack of cerebral perfusion as the overarching criterium for the death of a human being. In the

early days of transplantation, brain dead donors were called heart-beating (HB) donors.[21] Currently, the term has switched to donation after brain death (DBD) to address this organ donor type.

In the 1990s, the increasing demand for organs retrieved from deceased donors relative to its supply resulted in programs in which organs were rapidly procured after elective withdrawal of life support in patients with an infaust prognosis. In 1995 the term Non-Heart-Beating Donor (NHBD) was used to describe these type of organ donors which deceased after a circulatory arrest. Although the first deceased donors were in fact NHBDs, from the mid '90s this organ donor type was referred to as NHBDs. The increasing numbers and experience in NHBD resulted in the need for subcategories that could distinguish different end-of-life situations. In 1995 the Maastricht classification was introduced (Table 1).[22]

Table 1. The Maastricht classification of NHBD

Category I	Dead on arrival at hospital
Category II	Death after unsuccessful resuscitation
Category III	Awaiting cardiac arrest
Category IV	Cardiac arrest while brain dead

In 2013, the international conference on organ donation after circulatory death, was held to clarify terminology in the growing field of deceased organ donation and from this moment NHBD was officially changed into donation after circulatory death (DCD) and the Maastricht subcategories were extended (Table 2).[21]

DCD donors were classified into five different subcategories (I-V), and these were additionally subdivided into controlled/expected and uncontrolled/unexpected based on the predictability of death (Table 2). Controlled/uncontrolled and expected/unexpected are definitions that are still both used in protocols and literature. In this thesis the classification expected and unexpected will be used.

Table 2.[21] The modified Maastricht classification of DCD

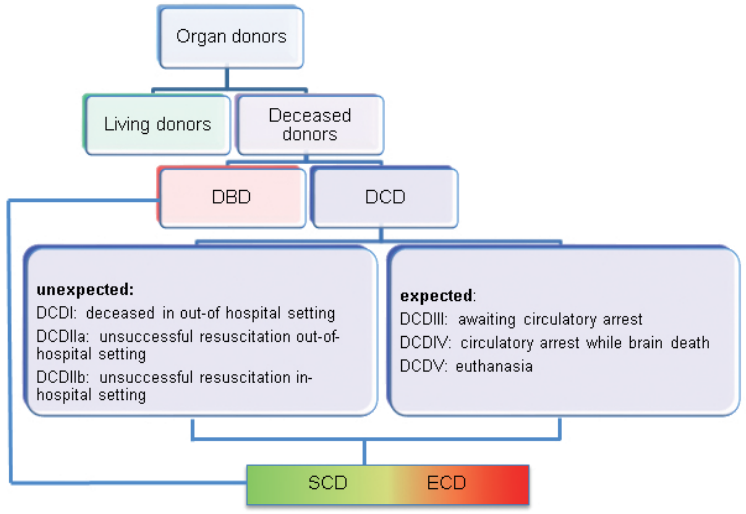
Category I **Uncontrolled**	*Found dead* I a. Out-of-hospital I b. In-hospital	Sudden unexpected CA without any attempt of resuscitation by a life-medical team; WIT to be considered according to National life-recommendations in place; reference to in- or out-of-hospital life-(IH-OH) setting
Category II **Uncontrolled**	*Witnessed cardiac arrest* II a. Out-of-hospital II b. In-hospital	Sudden unexpected irreversible CA with unsuccessful resuscitation life-by a life-medical team; reference to in- or out-of-hospital (IH-)H) life-setting
Category III **Controlled**	*Withdrawal of life-sustaining therapy*	Planned withdrawal of life-sustaining therapy*; expected CA
Category IV **Uncontrolled** **Controlled**	*Circulatory arrest while life-brain dead*	Sudden CA after brain death diagnosis during donor life-management but prior to planned organ recovery
Category V** **Controlled**	*Medical assisted CA*	Expected CA as a result of euthanasia

CA, circulatory arrest; WIT, warm ischemic time. * This category mainly refers to the decision to withdraw life-sustaining therapies. ** Legislation in some countries allows euthanasia (medically assisted CA) and subsequent organ donation described as the fifth (V) category.

Present

Nowadays, organs are retrieved from three types of donors to be used for transplantation (Figure 1): living donors, donation after circulatory death (DCD) donors and donation after brain death (DBD) donors. The DCD donors are categorized according to the five modified Maastricht classification (Table 2). In addition to DBD and DCD, organ donors are classified into two groups based on variable organ quality: standard criteria donors (SCD) or expanded criteria donors (ECD). ECD donors are defined as aged ≥60 years or between 50 to 59 years with at least two of the following three criteria: cerebrovascular accident as cause of death, pre-existing history of systemic hypertension, and terminal serum creatinine of >1,5 mg/dl. [23]

Figure 1. Categories of organ donors: DBD, donation after brain death; DCD, donation after circulatory death; SCD, standard criteria donor; ECD, expanded criteria donor.



Distribution of donor type utilization

Currently, most organs worldwide are derived from DBD donors (83% in 2015).[24,25] However, the availability of DBD donors is decreasing due to improved road safety and better neurosurgical techniques,[26] which forced the transplant community to accept organs from other organ donor types to reduce the persistent global organ shortage. This development has resulted in the increased use of organs from ECD and DCD donors. The utilization rate of DCD donors has significantly increased in several countries in Europe (Austria: 2,2%, Belgium:25,5%), in the USA (18,3%) and Australia (25,2%) during the past 12 years.[27,28] Whether DCD donors are used in a particular country and if so which type of DCD donor, will depend on many factors including legislative and ethical obstacles, the organizational donation structure, but also on how end-of-life care and ambulance services are organized.[29] For example, The Netherlands retrieved 58,4% of its organs from DCD III donors[30], while Spain has used DCD I and II donations since the 1980s and just recently started in 2012 with the utilization of DCD III donors[31], whereas Germany only uses DBD donors.[27] Despite all these efforts in strategies in terms of organ donor type, it has not resulted in shorter waiting lists, globally. In the Eurotransplant region waiting lists have been comparable in the last 20 years, while in the USA the kidney waiting list has doubled between 2003 and 2013 due to several factors such as the growing incidence of end-stage organ disease and comparable numbers of available donors.[1,32]

Quality and injury differences between donor types

There are clear differences in quality and injury profiles between the three organ donor types. In general, living donor-derived organs have superior quality, followed by medium graft quality in DBD and suboptimal graft quality in DCD organs. These differences can be attributed to the nature of the pre-existing injury, the retrieval process and how organs are preserved before implantation in the recipient.

First, living donors are healthy individuals with excellent renal function enabling the retrieval from one kidney. In contrast, deceased donors have experienced injuries that resulted in death and kidneys suffer from the events taking place in the last hours or days in the intensive care unit prior to death. The nature of these injuries is very different for DBD and DCD donors. DBD donors experience hemodynamic instability, a cytokine storm and hormonal changes due to cerebral injury followed by brain death underlying the damage found in DBD-derived organs.[33,34] Although DCD-III donors which are the most commonly used donors, also suffer from severe brain injury, the typical DBD related injuries are less pronounced.

Second, warm ischemia because of the cessation of blood flow during retrieval of the organs is an important impeller of injury. In living donors, the retrieval procedure is quick resulting in warm ischemia times of 2-5 minutes. In DBD donors, the organ retrieval procedure takes place without circulatory arrest until the systemic cold flush is started. The systemic flush in combination with topical cooling result in lower temperatures reducing the true warm ischemic injury. However the retrieval time is a major factor influencing short and long-term organ function.[35–37] Warm ischemia is a major issue in DCD donors. Many different warm ischemic periods are distinguished during DCD donation procedures. In case of DCD II donors, circulatory arrest results in asystole and a period of absolute WIT (aWIT), due to the total cessation of blood flow. After start of the resuscitation, functional WIT (fWIT), a period of low flow is present. If resuscitation is deemed unsuccessful, the patient will be declared dead and a mandatory period of no-touch will follow to secure brain death. The combination of absolute, functional WIT and the no-touch period, up to the moment of cold organ preservation, results in the total WIT.[21] DCD III donors partly experience similar periods of warm ischemia. The DCD donor procedure starts with withdrawal of life-sustaining therapy (WSLT). This will lead to the withdrawal or agonal phase ending with asystole. During the withdrawal phase fWIT is calculated as time between the

drop of systolic blood pressure below a threshold of 50 or 60 mmHg until asystole.[21] After asystole, as with other DCD II, the no-touch period extents the warm ischemic time referred to as the asystolic phase or donor WIT (dWIT).

The total WIT in a DCD III donor is defined as the time of start of WSLT up to cold organ preservation which includes the organ retrieval time period.[21] A commonly used term used in literature describing effects of pre-transplant ischemic periods is the first warm ischemic period. The first WIT includes all ischemic episodes in the donor until the time of cold preservation. The first WIT is generally short in living and DBD donors (<10 minutes) because of an intact circulation resulting in organ perfusion until the moment of recovery. Significant longer and variable first WIT are reported in DCD donors, generally the longest in unexpected DCD donors compared to expected DCD donors. Median first WIT (DWIT) is 17 minutes in a large cohort of almost 1100 expected DCD donors within the Eurotransplant region.[35] DWIT up to over 1 hour are reported in this study, however, 91% of these donors experienced a DWIT<30 minutes. In unexpected DCD donors WIT's are longer and reported in a different way. Two large cohorts including kidney donation from unexpected DCD donors in France and Spain report an aWIT with a mean time of 9.9±6.6 minutes and median (interquartile range) of 10 (5-15) minutes, and fWIT of 135.5±15 minutes and a median (interquartile range) total WIT of 130 (116 – 141) minutes, respectively. These difference in nomenclature and definitions of warm ischemic phases makes it difficult to compare but WIT, in general, is an important risk factor for adverse long-term graft and patient survival.[35,38,39]

Third, cold ischemia time (CIT) occurs during preservation of the organ to allow transportation and preparatory procedures prior to implantation of the organ in the recipient. In living donor transplantation, donation and implantation procedures almost exclusively take place in the same hospital resulting in short preservation time up to several hours. DBD and DCD organs are allocated to recipients on the waiting lists and need to be transported often towards different hospitals elsewhere in the country and sometimes even abroad. For example, median CIT time for kidneys from deceased donors in the Eurotransplant region is 15 hours.[40] Longer cold ischemic preservation times are associated with increased risk of graft failure, delayed graft function and mortality.[41,42]

Possible solutions for organ shortage

The challenge that we are facing today is to use more complex donors without compromising transplant outcomes. We are aiming for immediate function and

long-term graft and patient survival and low numbers of primary non function (PNF) or delayed graft function (DGF). There are several strategies to be considered that may be helpful to expand the current donor pool.

The utilization of new donor sources of (suboptimal) organ donors, such as unexpected DCD I and II donors could be considered. France and Spain have extensive experience with these type of donors which require a well-orchestrated collaboration between emergency services and transplant services [25,43] An Out of Hospital Circulatory Arrest (OHCA) is a common clinical condition that affects approximately 350.000 persons a year in Europe.[44] A prospective analysis from 27 European countries, with an estimated population of 174 million, confirmed almost 11.000 OHCA within one month. In 66% of these cases, cardiopulmonary resuscitation (CPR) was started by emergency medical services (EMS) or public bystanders. After a 30-days follow-up only 10.3% of the population, in which CPR was performed, was still alive.[45] The primary objective for clinicians is obviously patient survival but organ donation is a positive secondary objective in case of unsuccessful resuscitation.[44] In most patients with OHCA, death will occur following unsuccessful CPR and no return of spontaneous circulation (ROSC). Therefore, the potential of this organ donor type is substantial when considering the number of deaths due to OHCA. In **Chapters 2 and 3** we are exploring the potential utilization of unexpected DCD II a donors (uDCD) for the Dutch situation.

Other strategies to expand the current donor pool are;

A) The use of more organs from suboptimal organ donors (DCD – ECD), maximizing the utilization rate of organs per available donor.
B) Increasing the quality of transplanted allografts by conditioning strategies resulting in increased graft survival.
C) The use of initially discarded organs from all organ donor types by pre-transplant conditioning strategies.

In this thesis, the focus will be on DCD donation and how to increase the number of suitable organs by the potential use of DCD IIa donors and to increase the quality of DCD-retrieved kidneys by using improved organ preservation techniques.

Organ preservation

Background organ preservation

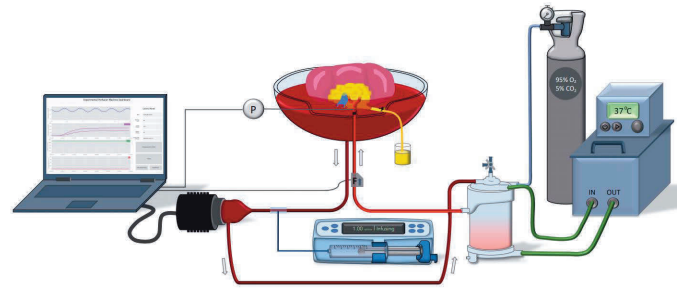
Organ preservation is a fundamental and essential part of the transplantation procedure by bridging the period between retrieval from the donor until implantation in the recipient. Since the 1970s, the standard preservation method has been static cold storage (SCS), where organs are flushed with a cold preservation solution to remove the blood and are submerged in cold preservation fluid to be boxed and transported on melting ice. Hypothermia slows down cellular metabolism and enzymes that are responsible for the degradation rate of essential components required for organ viability.[46] Recently, our group showed that the metabolic rate, expressed by oxygen consumption, indeed follows the proposed Q_{10} temperature coefficient, which is the measurement for the rate change of biological or chemical systems due to a temperature shift of 10° Celsius. [47] This implies that during SCS at a temperature of 5° Celsius, metabolism is reduced to approximately 15% compared to normothermia (37° Celsius). This results in a decline of 85% in terms of nutritional and oxygen requirements and allows the use of SCS as simple form of preservation, which has proven its usefulness during the past decades with good transplant outcomes. However as indicated before, with increasing SCS preservation time the quality of the graft is declining and therefore other preservation modalities are developed especially for high-risk organs such as DCD.

The use of dynamic organ preservation techniques, such as continuous machine perfusion, has (re)entered the field of donation and transplantation. Machine perfusion technology offers a more sophisticated preservation method in which organs are connected to an extracorporeal circulation using a preservation solution that is continuously pumped through the vasculature of the organs (Figure 2). The concept of machine perfusion is to support the remaining metabolism, keep the vessels open and remove waste products while regulating the temperature of the organ.

The first publication describing the idea of machine perfusion can be traced back to the beginning of the 20th century by Alexis Carrel in 1912, with others to follow in the 1950s to1970s.[48–52] Due to the selection of excellent quality DBD donor organs in the early days of the clinical introduction of organ transplantation and the discovery of SCS solutions to preserve donor kidneys, the more complex technology of machine perfusion was thought to be superfluous and was abandoned in most transplant centres.

However, in our era today, with most centres accepting older and more marginal donor organs there is a renewed interest for machine perfusion.[53–55]

Figure 2. Machine perfusion set-up.



Ischemia, ischemia-reperfusion injury and oxygen

To understand why preservation techniques shifted from SCS towards machine perfusion strategies, some basic understanding of cellular biology is necessary. During normal aerobic situation, all mammalian cells require a constant supply of oxygen and nutrients to support metabolism. The necessity of oxygen for most organisms originates from an era 4 billion years ago when the atmosphere of the earth contained poisonous fumes and limited oxygen. Despite the toxic environment, bacterial organisms already existed and participated in one of the most important metabolic developments for future life to come in this Archeïcum: the *photosynthesis.* Cyanophyta used energy from light to remove electrons from the abundantly available water molecules, thereby converting CO_2 into carbohydrates and producing oxygen. It took another 2 billion years before the atmosphere contained 21% oxygen, which was necessary for the next step in the evolution.[56] The development of the eukaryote, i.e. cells that contain a membrane enclosed nucleus and organelles was to follow. Although there is still some debate about the eukaryotic cell and the evolution of the mitochondria, current genomic data have proven that mitochondria were once free-living ancestral α-proteobacteria, that were engulfed by our ancestral eukaryotes.[57] These proteobacteria were able to use oxygen, and produce energy from the oxygenation of nutritional molecules. Once taken up by eukaryotes, these processes continued within these cells, thereby changing their anaerobic state ("living without air") towards an aerobic state ("living in the presence of air").[58] This was the early beginning of cellular respiration and a more complex form of life. Cellular respiration is a combination of metabolic reactions that convert biochemical energy from food molecules into

energy to be utilized by cells. To generate energy from nutrients, three stages are completed, that will finally lead to producing energy in the form of adenosine triphosphate (ATP). *Stage 1* is the enzymatic breakdown of food molecules into small molecules, monomers, that can be used by cells. *Stage 2* is the gradual oxidation of these small molecules into acetyl CoA (and a limited production of ATP). *Stage 3* is the complete oxidation of acetyl CoA, within the mitochondria, to H_2O and CO_2, accompanied by the production of large amounts of ATP. This entire process results in a net yield of 30 – 32 ATP molecules.[59] In the absence of oxygen, stage 3 will not occur, and lactate is formed, changing the net yield into only 2 ATP molecules, which is insufficient for high-energy demanding aerobic cells. During organ donation and transplantation there are several stages in which organs do not have access to oxygen and nutrients due to lack of blood supply anymore leading to ischemic periods, forcing cells to shift towards anaerobic metabolism. Decreased ATP production in combination with increased lactate formation results in intracellular acidosis and the combination of both result in reactive oxygen species (ROS) formation and injury to cells. The real problem, however, occurs at time of reperfusion of ischemic tissue, which is called ischemia-reperfusion injury (IRI). It is known that most of the damage of IRI happens due to an early burst of ROS produced by mitochondria[60] in combination with reduced antioxidant capacity. This, subsequently results in injury to cell membranes, the cytoskeleton and DNA, as well as in the induction of apoptosis and necrosis pathways but also upregulation of inflammatory cytokines and activation of the immune system.[61] IRI is a highly complicated, multifactorial pathophysiological condition that is known to result in kidney dysfunction, acute rejection and reduced graft survival.[61] Therefore, one of the key aspects to increase organ quality is by focusing on reducing ischemia by supporting aerobic metabolism. Machine perfusion offers a platform to diminish IRI as it provides metabolic support during the ischemic phases of the donation and transplantation procedure.

Machine perfusion strategies

Machine perfusion can be used at different stages in the donation and transplantation process. The donation and transplantation setting can be divided into three different phases: 1) donor management and organ retrieval, 2) organ flush-out and preservation, and 3) implantation and reperfusion.

Phase 1: donor management and organ retrieval

The standard abdominal organ retrieval approach is a flush-out with cold preservation solution through the aorta after introducing a cannula in the distal

aorta or common iliac artery, with the aim to cool down the organs and reduce metabolic activity. An alternative approach applicable in DCD donation is the use of oxygenated regional perfusion (hypothermic or normothermic regional perfusion; HRP or NRP) after the circulation has stopped. This will not only reduce the dWIT but also provide retrieval surgeons with a time window to inspect organ appearance and function. It also reduces the time pressure which may result in less organ damage and higher organ utilization rates with good outcomes.[62–64]

Phase 2: organ flush-out and preservation

In terms of machine perfusion as preservation strategy, different temperature settings are described. In the (pre)clinical setting the use of hypothermic (0 -12˚C), subnormothermic (±20˚C), controlled oxygenated rewarming (COR, rewarming from 5 - 25˚C) and normothermic (35 - 38˚C) machine perfusion (NMP) are all tested for hearts, livers, lungs and kidneys.[65–73] Both short-term in-house reconditioning (at the end of the preservation phase) as prolonged periods during the total preservation phase are reported to support organ quality and diminish the negative effects of warm and cold ischemia.

Clinically most used is continuous hypothermic machine perfusion (HMP) of kidneys, where kidneys are immediately attached to the perfusion machine after retrieval and perfused up to time of transplantation in the recipient. Some devices offer the possibility of oxygenated perfusion to support remaining metabolic activity. Recently a multicenter randomized controlled trial[74] has been performed to assess the effectivity of addition of oxygen during HMP in DCD-kidneys of elderly donors (>50 y) showing improved graft survival in oxygenated kidneys compared to the control.[75]

In-house reconditioning or end-ischemic reconditioning after transport (either SCS or HMP) is another opportunity to use machine perfusion to improve organs prior to transplantation. The rationale is to improve the energy status of a donor organ by providing a short period of oxygenated perfusion just prior to implantation. This technique is mostly used for livers in hypothermic conditions and is currently subject of several clinical trials.[68,76–80] In kidneys this strategy is less applied probably due to the availability of portable HMP devices. [81,82] The first results of end-ischemic hypothermic reconditioning for kidneys are promising with decreased PNF and reduction of DGF.[81] NMP after SCS or HMP has gained considerable interest since this technique offers the potential to test function given the full metabolic activity under normothermic conditions[83–98] Clinical potential has already been proven by the Nicholson-Hosgood group and a

multicenter randomized controlled trial is ongoing to assess the efficacy of 1-hour normothermic reconditioning after SCS.[90] Currently our center is involved in a multicenter trial to investigate prolonged NMP beyond 1 hour (Proper study). More experimental approaches such as Subnormothermic machine perfusion or COR are primarily studied in the preclinical setting.[65,99–103] However, there is only one case study describing the protocol in the clinical situation.[67] The concept of Normothermic machine perfusion as long-term preservation technique eliminating the cold preservation has been widely studied by Friend and colleagues and recently shown to be effective for livers in an RCT.[104] Although preclinical work shows its applicability in kidneys with good results[55,105–110] it has less potential in kidneys given the option of oxygenated HMP.

Phase 3: Transplantation with reperfusion

Machine perfusion during implantation of the graft in the recipient is still in its infancy but potential has been shown in both a liver and kidney transplantation in DBD donors.[111,112] The concept is to remain blood circulation intact during the whole procedure of donation and transplantation, thereby avoiding all detrimental consequences of warm-and cold ischemia and subsequent IRI. It is questionable if the complex logistical and technical challenges in Ischemia-free donation and transplantation will result in substantially improved transplantation outcome.

Development of a model to study ischemia-reperfusion injury

Despite the clinical use of (first generation) machine perfusion at a global scale, there are still many questions unanswered that could further improve efficacy and use of MP. Basic questions such as the optimal duration and timing of the intervention require answering but also more complex questions about the biological underlying mechanisms need consideration. To answer some of these questions we developed a porcine kidney perfusion model using organs obtained from a commercial as described in **Chapter 4.** The use of slaughterhouse waste material not only provides kidneys to address different research questions but also avoids the use of laboratory animals.

Hypothermic machine perfusion

The addition of oxygen during hypothermic machine perfusion

Multicenter randomized controlled trials have already proven the effectiveness of HMP in terms of a significant lower incidence of DGF in deceased donors (DBD and DCD).[113,114] The beneficial effect of HMP remained in kidneys from DBD donors and

even more evident in ECD donors resulting in improved 3 year graft survival but was not evident in DCD donors.[114–117] It was postulated that the addition of oxygen during HMP would support the ongoing cellular respiration (15% compared to normothermia). Especially for DCD donor organs the need for oxygen may be high since these organs experienced a period of severe hypoxia during warm ischemia resulting mitochondrial dysfunction leading to decreased long-term patient and kidney survival.[38,61] In **Chapter 5** the addition of different oxygen concentrations during long-term hypothermic machine perfusion of porcine DCD kidneys derived from the slaughterhouse is investigated.

Normothermic machine perfusion

Substitute for blood, AQIX® RS-I

Normothermic machine perfusion requires full metabolic support in terms of nutrient supply and oxygen delivery. The first clinical experiences with NMP of livers[118,119], lungs[71] and kidneys[89,90] are already published and resulted in an expansion of the organ pool. Currently, blood-based solutions are used for clinical NMP of kidneys. However, alternative solutions are warranted, especially with the increasing numbers of NMP. The complexity of normothermic perfusion lies in the high (100%) nutritional and oxygen demand and therefore it is of utmost importance that alternative perfusion solutions offer all necessary components for metabolic support. In **Chapter 6** we compared the colloid enriched AQIX® RS-I solution with or without red blood cells (RBC) to a blood-based perfusion solution in the model described in chapter 4.

Potential protective agents against ischemia-reperfusion injury

Ex-situ machine perfusion offers the opportunity to treat isolated organs. Organ-specific treatments can be provided during preservation or during a reconditioning protocol prior to transplantation. A benefit of using this platform is that therapeutics are directly delivered to the target organ circumventing side effects that can occur during systemic delivery.[120] Examples of such treatments for kidneys are stem cell therapy during NMP as regenerative therapy,[86,121] gene delivery [122] and the delivery of drug loaded nanoparticles.[123] In this thesis we used machine perfusion as a platform to provide pharmaceuticals to kidneys, with the aim to decrease ischemia-reperfusion injury (IRI). In chapter **7, 8 and 9** we investigated potential mitochondrial protective agents during different phases of a transplantation setting. In the final chapter of this thesis, all results obtained brought into perspective and future developments are discussed.

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PART A

Unexpected donation after circulatory death (uDCD) donors