Strategies to improve donation after circulatory death kidneys for transplantation

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CHAPTER 10

General discussion and future perspectives

GENERAL DISCUSSION

Organ transplantation is the optimal, and often only available treatment for patients with end stage organ disease and terminal failure. The demand of suitable organs for donation exceeds the number of available donor organs by far. 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.[1] This implies that only patients meeting rather strict criteria are allowed to receive a donor organ. In spite of this, waiting lists are still increasing. The number of patients registered on the kidney waiting list in the USA doubled between 2003 and 2013 up to 100,000 with a median waiting time of 4-5 years, resulting in an annual death of approximately 5,000 patients.[2] Similar figures are seen in other countries. Although these numbers demonstrate the severity of the problem, this appears to be the tip of the iceberg since only patients on the waiting lists are included and not even the population that never reached registration on a waiting list. To solve this immense problem and reduce the severe shortage, the number of available donor organs needs to increase considerably. In this thesis, three different approaches are described that could be helpful to increase the number of transplantable deceased donor organs.

The use of unexpected donation after circulatory death (uDCD) donors to increase the number of organs for transplantation

Several transplant centres, including the Maastricht University Medical Center (MUMC) in The Netherlands, have reported successful donation- and subsequent transplantation of kidneys and lungs derived from uDCD donors.[3-24] These successes in combination with the severity of organ shortage led to the development of a Dutch regional protocol for uDCD donation in the eastern part of The Netherlands. **Chapter 2** describes the implementation of this regional uDCD protocol. Prior to the start of this multicenter protocol, the potential was calculated based on local data from out-of-hospital cardiac arrest (OHCA) patients that entered the emergency department within the age range of 18 – 65 years. Extrapolation of this data, in combination with 35-years of experience with this type of organ donors in the MUMC[22], resulted in an estimation of an additional potential 40 lung and 28 kidney transplants. This estimated number was unfortunately never achieved. The multidisciplinary protocol was successfully implemented since only 3 out of 553 announcements were missed by the teams. Nevertheless, no actual donation and subsequent transplantation of neither lungs nor kidneys were performed. The most plausible reasons why no uDCD donors were

included are the very conservative donor inclusion criteria intended to minimize the risk of transplant failure. The perceived risk was due to the fact that data supporting the safe use of kidneys and lungs from uDCD donors were lacking at the time when the program was initiated.[10,11] A recent publication including a large Spanish cohort consisted of 517 kidney transplants from 288 uDCD donors identified risk factors with an impact on post-transplant results.[10] Primary non function (PNF) of kidneys is an important challenge in uDCD-derived kidney transplantation and risk factors identified for PNF in this cohort are donor age >60 years, *in-situ* cooling with subsequent static cold storage preservation instead of hypothermic or normothermic regional perfusion (HRP/NRP), and WIT greater than 130 minutes. In addition, delayed graft function (DGF) was also associated with the type of *in-situ* preservation method, with better results observed after NRP. Although high incidences of DGF (76%) were reported, this did not affect death-censored graft survival at one year. Another study comparing Spanish uDCD kidneys (n=774) with SCD and ECD-DBD donors found that PNF was the main cause of graft loss in uDCD kidneys and in addition identified pulmonary embolism as cause of death, extra-hospital CPR >75 minutes and in-hospital CPR >50 minutes as important risk factors.[25] However, long-term outcomes demonstrated excellent results with uDCD kidneys, that were even superior to those with ECD-DBD kidneys. This finding emphasizes that uDCD kidneys can be a valuable source of donor organs to increase the current donor pool. However, to optimize the chance of success all identified risk factors of PNF and graft loss in this donor population must be considered. Prior to initiation of the Dutch uDCD protocol in 2014 as described in this thesis, these factors had not been established yet, explaining why on hindsight the in- and exclusion criteria were probably too risk avoiding, however, also now appear to justify the choice of NRP and/or hypothermic machine perfusion.

Another factor that lead to the disappointing lack of inclusion was the low family consent for donation. Obtaining consent for organ donation has been a significant bottleneck in the Netherlands illustrated by the high refusal rate of 60%.[26] Lack of family consent during this project resulted in the termination of twenty uDCD procedures. Culture, legislation and public opinion play an important role in the delicate matter of consent for organ donation. In July 2020, The Netherlands changed from an opt-in towards an opt-out system. Time will tell if this switch will enhance consent rates for organ donation or not. Despite the disappointing results in terms of actual donation and transplantation, the implementation of the protocol designed for uDCD donation provided valuable information as regards

contraindications that are never visible in EMS databases. Our uDCD experience, together with the experiences in New York City[27,28], are the only published 'negative' results of uDCD protocols that were implemented, and may be helpful for other countries and hospitals that intend to start a similar program.

In **Chapter 3** an analysis of the potential of uDCD donation in regions with different demographic characteristics was made. For this purpose, out-of-hospital cardiac arrest (OHCA) numbers from the uDCD project and data from resuscitation databases from different parts of The Netherlands were used. Only patients that were transferred to the Emergency Room (ER) within the age criteria of 18 – 65 years were considered true potentials. Between 22 - 40% of these patients died in the ER and are therefore potential eligible donors. Correction for witnessed arrest, an important inclusion criterium for uDCD donors, decreased the potential to 16 - 22%. However, in terms of all resuscitations started by EMS, the potential appeared to be only 5- 9%. Although comparable percentages were found between different geographical areas, it is important to consider the respective population densities. In urban areas, with a higher population density, the actual number is higher and therefore the chance to effectuate a potential donor will also increase. An accessory benefit in urban areas is shorter warm ischemic times because the distance from the site of the incident to the ER is shorter than in rural areas. Not only the population density plays a role but also the size of the area. When comparing the Dutch situation with published results from successful uDCD programs in Paris, Barcelona and Sint Petersburg, these demographic effects can be clearly seen. The latter cities have a large number of inhabitants (between 1.6 – 5 million) and cover a wider area (between 100 – 1439 km^2). However, despite that these cities satisfy the geographical and demographic criteria, the actual number of effectuated donors remains low with an average of 2.2 – 7.3 donors pmp per year.

Chapter 2 and 3 illustrate the complexity of a uDCD donor program. The most important message of these chapters is to not rely solely on the number of patients that die in the ER when implementing a uDCD program. Important variables to consider are (i) the chosen inclusion criteria, (ii) demographics of the region, (iii) national legislation, and (iv) cultural constraints as regards organ donation. Implementing and subsequently using uDCD protocols in the Emergency Department requires perseverance and dedication from a large multi-disciplinary team.

Future perspectives of uDCD donation

Despite the lack of success in terms of actual donations in our program, and the potential hurdles concerning implementation of uDCD donation, this type of donation remains a realistic opportunity to close the gap between demand and actual number of donor organs retrieved. Especially, since OHCA affects approximately 350,000 Europeans per annum and the survival rate is very low at only 10%.[29,30] In **Chapter 3** it was concluded that a densely populated area in combination with a minimal number of 500,000 inhabitants is a prerequisite for a successful uDCD program, at least in the current Dutch situation. However, there are alternative approaches that can be considered to enable uDCD donation. One possibility is the use of DCD Maastricht Category 1 donors ('found dead on arrival') in addition to DCD Maastricht Category 2a donors ('out of hospital cardiac arrest) as described in **Chapters 2** and **3**. DCD I donors are declared dead in an out-of-hospital setting and transferred to the ER with the sole purpose of organ donation. The potential of DCD 1 donors is clearly shown by the Spanish organ donation numbers in which 80% of the utilized uDCD donors are DCD 1 donors.[31] The exact potential of DCD1 donors in The Netherlands could not be assessed in **Chapter 3** because the databases used did not include the number of unsuccessful and terminated on-site resuscitation attempts. However, in The Netherlands approximately 35% of resuscitation procedures are terminated on-site of the collapse when the procedure appears to be unsuccessful, and this population could be eligible for DCD 1 donation.[32,33] Changing pre-hospital protocols and transporting every OHCA patient to the ER, should increase the Dutch uDCD potential. This policy would, however, require major adaptations of our current EMS system. Furthermore, it might result in a significant financial and logistic burden as shifting a significant part of the donor recognition towards the ambulance services.

The use of extracorporeal membrane oxygenation (ECMO) assisted cardio-pulmonary resuscitation (E-CPR) could also open new possibilities for uDCD organ donation. E-CPR is a commonly used therapy for refractory cardiac arrest and has been shown a successful rescue therapy for in-hospital cardiac arrests. The results for E-CPR in an OHCA setting are also encouraging and become increasingly available on EDs.[34,35] E-CPR is a temporary replacement therapy for both cardiac and respiratory support, offering oxygenated perfusion of all vital organs, while the heart recovers or before life-saving interventions are applied. If E-CPR would become standard of care for OHCA patients, it could support a novel source of potential organ donors since it provides essential oxygenation to the

organs until actual organ retrieval. There are, however, ethical considerations to be considered when applying E-CPR, especially in combination with uDCD protocols. This includes standardized guidelines for the termination of resuscitation, which resuscitation method is to be used during transport to the hospital, and whether to start with E-CPR as a lifesaving therapy or purely aiming to preserve potential donor organs. Although the possible utilization of DCD1 donors, especially in combination with the use of E-CPR, could offer advantages in terms of the number of transplantable organs, it needs to be carefully scrutinized before initiation. On the other hand, and keeping the tremendous organ scarcity in mind, every possible option to retrieve an organ for transplantation needs to be carefully considered.

The effect of oxygen during machine perfusion of donor kidneys

Oxygenation during hypothermic machine perfusion

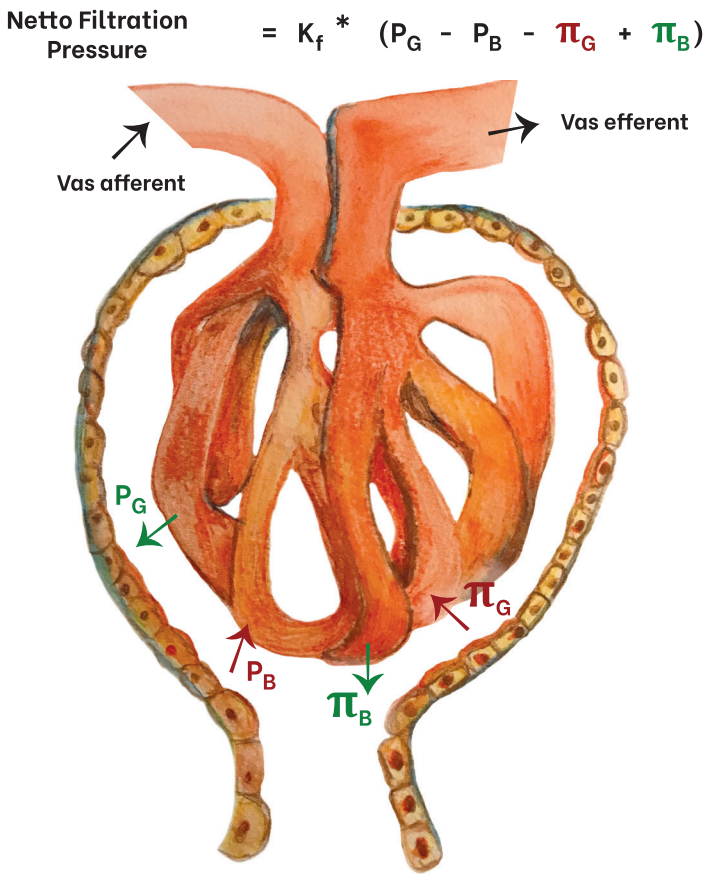
One of the major key points in donation and transplantation is the need for oxygen for all basic biological processes that support life and have been explained in the introduction of this thesis. Considering the ongoing albeit low oxidative phosphorylation during hypothermic temperatures, it is remarkable that during preservation of organs for transplantation, the addition of oxygen has been absent until recently. In The Netherlands, hypothermic machine perfusion (HMP) of kidneys is nowadays the clinical standard. Pre-clinical data support the addition of oxygen during HMP of both kidneys and livers[36–42] and although it has been technically possible to add oxygen during HMP, the results of the international multi-center COMPARE trial by the COPE consortium comparing the effect of standard HMP with oxygenated HMP were awaited. The recently reported results from this trial showed that oxygenation at cold temperatures was beneficial in older DCD donor kidneys and have led to the start of oxygenated HMP of this donor type in The Netherlands. The exact mechanism how oxygen is effective and which amount is needed is not unraveled yet. In **Chapter 5** we addressed this clinically relevant question using a slaughterhouse kidney perfusion model as described in detail in **Chapter 4**. This donor model has proven its efficiency to assess kidney quality, which depends on factors such as warm ischemia time and preservation method. Since we did not have any data of effect of oxygen on kidney function, the model was used to test the impact of different partial pressures during HMP. **Chapter 5** supports that the addition of oxygen during HMP is beneficial which is reflected by an improved energy status and lower levels of ASAT. The addition of oxygen during short- and long-term (1-24 hours) HMP results in a significant increase in ATP content in both rat and porcine kidneys as

demonstrated by our own group and others.[36,39,41,43] ATP production and oxygen consumption during HMP are strong indicators of ongoing aerobic respiration during hypothermia. Chouchani et al. have shown that in the absence of oxygen, succinate plays a leading role in ischemia and subsequent ischemia reperfusion injury (IRI). It accumulates after mitochondrial Complex 2 during ischemia, when it is rapidly re-oxidized after the reintroduction of blood flow, producing ROS through reverse electron transport (RET) in the mitochondrial complex 1.[44] To what extent succinate can serve as true marker of ischemic injury has remained unclear. Darius et al. found significantly decreased succinate levels in the presence of increased oxygen concentrations.[39] Similar changes were shown for other major central metabolites such as formate and acetate. However, Patel et al. did not see significant differences in succinate levels in the perfusate at the end of 18 hours of HMP with 21 or 95% oxygen. Tissue levels of succinate even show opposite values with significantly higher succinate levels in the kidneys when oxygenated with 95% oxygen during HMP. Although it is unclear whether succinate is the pivotal factor in the electron chain targeted by oxygen, it has been confirmed that addition of oxygen during HMP of livers and kidneys results in reduced levels of ROS.[41,43,45,46] Two multicenter randomized controlled trials[47,48] on the addition of oxygen during HMP of kidneys were performed by the COPE consortium. The recent publication on the COPE-COMPARE study showed a significant lower graft failure and a lower incidence of acute rejection in DCD-derived kidneys of elderly donors (>50years) that were preserved with oxygenated HMP compared to the non-oxygenated kidneys. At 12 months follow-up, estimated GFR was not significantly different (p=0.062). However, 24 hours creatinine clearance was significantly improved in the oxygenated HMP group.[49] Although it is likely that not only the older DCD kidneys may benefit from active oxygenation during HMP, more clinical data are needed to confirm this assumption. As in kidney preservation, the benefit of oxygenated hypothermic machine perfusion of livers has become evident as well. Clinical studies demonstrated improved outcomes in DCD-derived liver transplants treated with short-term (Dual) Hypothermic Oxygenated Perfusion (DHOPE/HOPE) versus non-treated DCD-derived liver grafts and comparable to DBD-derived livers.[50–54] Although the exact biological mechanisms on oxygenated machine perfusion are not yet fully understood, there is emerging evidence of its beneficial impact on transplantation outcomes. For the liver, we now need to await the results from both multi-center randomized controlled liver trials liver[55,56] before the oxygen addition using the HOPE concept can be implemented in daily clinical practice.

Oxygenation during normothermic machine perfusion

Although kidneys only account for less than 0.5% of our total body weight, they consume about 10% of all oxygen.[57] This phenomenon is in 99% attributed to active sodium reabsorption by the tubuli.[57–59] Sodium, and other electrolytes are only reabsorbed after filtrated in the glomeruli. As illustrated in figure 1, the Net Filtration rate depends on the filtration constant (K_f) multiplied by the net sum of hydrostatic and colloid osmotic pressures (COP's). In the glomerular capillaries the hydrostatic pressure in the glomerular capillaries ($P_{G)}$ is counteracted by the colloid osmotic pressure (ϖ_G), In Bowmans capsules the same opposing forces exist hydrostatic and colloid osmotic pressure in the Bowmans capsule (P_B and ϖ_B).

Figure 1. Nett filtration pressure (NFP) within the glomerulus depends on opposing forces.



In the capillaries hydrostatic pressure P_g is counteracted by the colloid osmotic pressure ϖ_G. In Bowman's space, the hydrostatic pressure P_B can either be counteracted or enhanced by the colloid osmotic pressure ϖ_B.

It is important to bear in mind that during normothermic perfusion (NMP) these parameters affecting GFR can be very different from the physiological situation since blood is replaced by artificial solutions with different COP's and perfusion pressures are imposed on the renal tissue by a pressure controlled pump system.

In most cases, lower hydrostatic pressures (P_G) are applied (70-95 mmHg) but also COP from perfusion solutions are often lower. A prerequisite for an optimal GFR is an intact glomerular barrier. Ischemic injury prior to donation however leads to damage to the glomeruli and may result in leakage of proteins. Proteinuria is reported to be present in approximately 50% of all donated kidneys.[60] Therefore, the COP in the Bowman's capsule (ϖ_B) is not negligible during NMP of donor kidneys and is essential to bear in mind when comparing different perfusion fluids.

In **Chapter 6**, the use of NMP in porcine kidneys and a possible alternative for a blood-based solution, AQIX® RS-I is evaluated. The AQIX solution is based on intercellular conditions and is in general used for storage of tissue. To be suitable as normothermic perfusion fluid it needs colloids and possibly an oxygen carrier. To increase the COP the AQIX solution was supplemented with bovine serum albumin (BSA) or dextran40. The perfusions were performed with and without the addition of red blood cells (RBC) as oxygen carriers. A priori, the hypothesis was that the use of supraphysiological oxygen tension (>60 kPa, 900 mmHg) would provide enough oxygen to kidneys during NMP. This was found, however, not to be the case, as reflected by significantly lower oxygen consumption rates in the acellular groups, that subsequently resulted in impaired fractional sodium excretion levels. In terms of creatinine clearance, total sodium reabsorption, ROS production and ATP production of kidneys that were perfused with standard autologous blood perfusion solution as previously described in **Chapter 4** performed better when compared to the RBC supplemented AQIX groups. The blood-based perfused kidneys had higher ATP levels and lower ROS markers which is indicative of an improved mitochondrial quality. The superior results in blood-based perfused kidneys does not only underpin the importance of RBCs in the perfusate, but also the need for plasma components, including electrolytes and proteins. It is likely that other oxygen carriers can be used as well. A major advantage of synthetic oxygen carriers is that they can be used during hypothermic and sub-normothermic perfusion (sNMP), in contrast to when RBCs are included. Publications available on liver perfusion with a bovine derived hemoglobin-based oxygen carrier (HBOC-201) suggest its usefulness.[61–64] The use of this alternative oxygen carrier for kidney NMP is limited to one recent publication in which

discarded human kidneys were perfused for six hours with Williams Medium E with RBC or HBOC-201 at a mean arterial pressure of 70 mmHg.[65] Kidneys perfused with HBOC-201 showed similar results in terms of renal function and energy status and it seems that renal integrity was maintained based on histological appearance. Interestingly, renal oxygen consumption rates during NMP presented in this study are comparable (0,5 – 3,5 mlO_2/min/gram) to renal oxygen consumption rates that were found during perfusion of porcine kidneys in this thesis (**Chapters 4-9**).

The results of the NMP experiments performed in **Chapters 4-9** all indicate that NMP is a valuable tool to access organ function. They also show that a better understanding of "on pump" renal physiology is necessary. In the machine perfusion literature, especially in the NMP literature, the term physiological is often used. However, *ex vivo* isolated organ perfusion is not a physiological situation for many reasons: i) the organs do not have interactions with other organs, ii) there is no humoral or iii) neural regulation, iv) the use of artificial perfusion solutions, and v) the existence of a pathophysiological situation since organs are already damaged due to the process of dying and during retrieval. Therefore, it is important to perform research that will help to redefine "physiology" to "on-pump physiology". This information is mandatory to understand the metabolic demands of kidneys during NMP and define which components are necessary for artificial alternative perfusion solutions.

Machine perfusion as a platform for the addition of pharmaceuticals and to decrease ischemia reperfusion injury

Although the optimal "on pump physiology" has not been determined yet, NMP and HMP can provide useful platforms for the addition of pharmaceuticals to treat organs prior to transplantation.

In **Chapter 7**, HMP was used to study the effect of SUL-138, a 6-hydroxychromanol, that has been shown to be protective against ischemia of renal cells during hypothermic *in vitro* experiments.[66] It is well established that mitochondria play an essential role in the pathogenesis of IRI[67,68], and oxygen deprivation leads to mitochondrial uncoupling and subsequent ROS production. In vitro, 6-hydroxychromanols are able to improve mitochondrial oxidative phosphorylation.[66] The SUL-138 compound is considered to maintain mitochondrial integrity and could be an interesting strategy to protect against renal IRI. To test this hypothesis, the addition of SUL-138 was studied during the first flush-out and during 24 hours of HMP as regards its effect on kidney quality parameters such as

creatinine clearance, fractional sodium reabsorption, oxygen consumption and ATP production. No significant differences between the groups were found in terms of quality during normothermic reperfusion. The only significant difference in favour of the SUL-138 group was the lower release of ASAT indicating less mitochondrial damage. In **Chapter 5**, we also found significantly lower ASAT levels found in kidneys that had been exposed to 100% oxygen during HMP compared to all other groups. In these kidneys higher ATP levels were seen after 24 hours oxygenated preservation. With SUL-138, mitochondrial integrity has been only demonstrated through the ASAT levels, but could not be confirmed by higher ATP levels. A possible explanation may be the lack of oxygen during HMP in this experiment. ATP levels found after oxygenated HMP in **Chapters 5** and **6** are in fact 4-6 times higher compared to non-oxygenated HMP kidneys. Even when there is better mitochondrial protection due to SUL-138, ATP levels are likely to stay low since oxygen is needed to support mitochondrial ATP production. A recent publication by Garonzik-Wang has shown that the mitochondrial membrane potential (MMP) independently predicts DGF in a cohort of deceased donor kidneys and might be a promising marker for tissue health.[69] Unfortunately, technical issues resulted in failed MMP measurements in our SUL-138 study, but it would be worthwhile to consider the use of 6-hydroxychromanols in combination with oxygenated perfusion.

In **Chapter 8** the addition of biguanide metformin, an antihyperglycemic drug normally used for patient with Diabetes Mellitus type 2, was assessed as regards its potential protective effect on IRI. Metformin mildly decreases cellular respiration at the level of mitochondrial Complex 1 and decreases ROS production by inhibition of RET .[70] These specific mitochondrial modes of action may attenuate IR and could be interesting in the setting of organ transplantation. In **Chapter 8** the potential of metformin was investigated as pre-conditioning agent or as post-conditioning agent during NMP, in both a rat and porcine model. Pre-conditioning resulted in slight beneficial effects such as reduced cellular breakdown. Low and high-dose post-conditioning resulted in improved kidney gene expression levels of adhesion molecules (eNOS and VCAM-1), and improved histological findings (necrosis and vacuolation), respectively. However, the pre- and/or post-conditioning effects of metformin in both models remained minor and no obvious regimen of metformin or administration time resulted in (significant) beneficial renal effects. The use of metformin as a therapeutic drug against IRI appears therefore to be limited.

A possible side effect of metformin induced reduction of Complex 1 is studied in **Chapter 9**. Especially, in patients with impaired renal function metformin

levels can accumulate and lead to a severe and uncommon clinical condition called metformin-associated lactic acidosis (MALA). For this reason, metformin is contraindicated for diabetics with a known renal impaired function.[71] Since metformin is actively secreted by the proximal tubules in combination with mild inhibition of mitochondria by metformin, the hypothesis is that elevated concentrations as a result of renal impairment, results in inhibition of elimination of metformin leading to MALA. We found that increasing metformin dosages lead to higher metformin levels in both perfusate and tissues whilst almost no effect on renal metabolic markers were observed. Metformin excretion was indeed reduced under increasing concentrations; however this was not due to a self-inhibitory effect but can be explained by saturation of the organic cation transporters. Fortunately, even though metformin doses used during NMP are relatively high to the extent of being toxic *in vivo*, no increased levels of cellular (LDH) and mitochondrial damage markers (ASAT) were found, as described in **Chapter 8.** Therefore, metformin may be safely used during *ex vivo* kidney perfusion as a strategy to influence Complex I activity, possibly in combination with other drugs influencing mitochondria.

Future perspectives of machine perfusion

Machine perfusion has gained interest in the last 10 years. It will be an important technical modality to achieve clinical advancements in the field of organ transplantation and beyond.

Future perspectives of machine perfusion in transplantation

Better understanding of the pathophysiology of organ donors and their respective grafts-to-be is necessary to improve donor organ quality and viability. More in-depth insight in biological processes in addition to functional assessment is required. A multi-omics approach can be helpful in providing information on molecular profiling and pathways in biological systems that play an essential role during preservation of organs from different donor types. Metabolomic analyses of both kidney tissue and perfusate samples taken during preservation have already shown to be able to discriminate between preservation strategies and also between immediate and delayed graft functioning kidneys.[36,72,73] Furthermore, proteomic analysis also provided the first evidence of different renal proteomes dependent on the origin of the donor.[74] We are currently performing experiments to assess the degradome of kidneys in order to understand how renal fibrosis develops, with the aim to decrease its formation with the help of machine perfusion.

To further decrease organ shortage, more organs are needed and therefore, there is an absolute need for optimization of machine perfusion protocols that can predict organ function in the recipient. Currently, viability assessment of kidneys is based on rather general criteria such as urine production and blood flow. Both criteria are dependent on physical characteristics such as hydrostatic pressure, colloid osmotic pressure, and viscosity of the perfusion solution. The usefulness of blood flow and urine production is therefore limited when comparing different perfusion strategies since these parameters are dependent on the perfusion protocol and solution.

Important questions such as how blood flow is distributed through the kidney during machine perfusion or whether the autoregulation of the kidney is present during NMP are still unknown. Real-time magnetic resonance imaging (MRI) experiments of kidneys after procurement and prior to transplantation are currently performed by our research group to gain knowledge regarding these physiological phenomena.

Real-time viability assessment is an important focus point regarding machine perfusion technology. The discard rate of DCD livers in the United Kingdom is reported to be approximately 30%[75] and in the United States 3,159 retrieved kidneys were discarded in 2015 which is 19.2% of all kidneys that were offered.[76] Medical contraindications such as anatomical abnormalities, infectious diseases or cancer are valid reasons for discard. However, most organs are discarded because clinicians are uncertain about the organ quality and the risk of lack of life-sustaining post-operative function in the recipient. An organ quality test prior to transplantation will result in a considerable increase in transplantable organs and prevention of primary non function. The first pre-clinical and clinical examples of real-time viability assessment published by the Dutkowski group is e.g. the use of fluorescence spectroscopy to determine circulating mitochondrial flavin mononucleotide (FMN) in livers.

Our collaborative work with the University of Aarhus also focuses on real-time viability assessment of kidneys during NMP with the help of nanoparticles. These biodegradable nanoparticles added to the perfusion solution will act as sensors for cell vitality. Near infrared fluorescent imaging (NIRF) will be used real-time to measure the potential of renal cells to take up these particles via energy depending processes, which is only possible by vital cells. Pilot experiments have

demonstrated feasibility in both rat and porcine kidneys. Differences in fluorescent signals were found between fresh and 20 hours cold ischemic stored kidneys.

Future perspectives of machine perfusion beyond transplantation

Application of machine perfusion as a therapeutic platform for cancer therapy is a possible new development. Using ex-vivo treatment would not only decrease side-effects for the patient but also provides the opportunity of applying higher doses that would be detrimental for the rest of the 'healthy' body parts of the patient. In addition, machine perfusion can also be applied in safety or toxicology experiments with novel drug interventions to exclude severe side effects for the patient when systemically applied. However, before these application strategies can become a reality it is of utmost importance to know how to maintain and support the viability of the organ.

Another new development is the use of machine perfusion technology in the field of tissue engineering.[77] New tissues, organoids and organs are engineered with mostly autologous cells. These processes include decellularization when using biological scaffolds, and always includes recellularization for repopulating the biological or synthetic scaffolds. In large organs, decellularization is difficult and perfusing via the vessels offers the best route to reach all cells.[77] Machine perfusion technology developed for organ transplantation is very suitable for decellularization protocols, especially since low flow and pressures can be used. For recellularization oxygenated machine perfusion offers a good platform. Unfortunately, reseeding protocols are still in its infancy and more research is necessary to find the right cell types that can reseed the extracellular matrix of organs and tissues to regain full organ function. However, if successful, 'building new organs' using autologous cells in combination with a biological inert scaffold could be an important solution to minimize the shortage of donor organs.

This thesis has addressed several different strategies to increase the number and quality of transplantable organs. Undoubtedly, machine perfusion will play a pivotal role in the future of organ transplantation. Therefore, we need to thoroughly invest in dedicated and high-quality research of essential biological mechanisms that play a crucial role during *ex vivo* organ perfusion. Learning to better perfectly understand how organs function in a non-physiological environment will not only take machine perfusion to the next level but also help our insight in normal *in vivo* biology and widen our horizon towards a broader range of applications that will benefit our patients.

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