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Review

Advances in Hypothermic and Normothermic Perfusion in Kidney Transplantation

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Abstract: Hypothermic and normothermic machine perfusion in kidney transplantation are purported to exert a beneficial effect on post-transplant outcomes compared to the traditionally used method of static cold storage. Kidney perfusion techniques provide a window for organ reconditioning and quality assessment. However, how best to deliver these preservation methods or improve organ quality has not yet been conclusively defined. This review summarises the promising advances in machine perfusion science in recent years, which have the potential to further improve early graft function and prolong graft survival.

Keywords: machine perfusion; kidney; HMP; NMP; regenerative medicine; biotechnology

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1. Introduction

Kidney transplantation is the most economical [1] and effective [2–5] therapy for patients with end-stage renal disease (ESRD). However, there is a worldwide shortage of suitable kidneys for transplantation [6]. Over the next decade, the incidence of chronic kidney disease (CKD) and ESRD is expected to increase considerably, with CKD due to become the fifth leading cause of death by 2040 [7]. Strategies that increase the number of kidneys available for transplantation or improve transplant success rates and outcomes are likely to have a considerable effect on global health.

Machine perfusion technologies have emerged as an important tool in tackling critical problems intrinsic to transplantation, such as ischaemia reperfusion injury (IRI) [7–9], poor post-transplant graft function [10–12] and reduced graft survival [10]. Understanding how machine perfusion ameliorates these problems and optimising these methods will likely further improve patient outcomes. Although the central goal of this research (i.e., increasing the availability and quality of transplant kidneys) is uniform, the means by which this could potentially be achieved differs. Optimisation of machine perfusion technologies may improve kidney transplantation in three ways:

- Improvement of transplant outcomes through delivery of therapeutic agents to repair and regenerate kidneys.
- Reduction in the number of discarded kidneys by developing robust techniques of organ assessment.
- Reduction in ischaemic injury during the preservation interval to improve the 'shelf life' of donated kidneys and increase the number available for transplant.

This review focuses on the current understanding of the biological factors that negatively affect kidney quality and the physiological effects of hypothermic and normothermic techniques. Recent advances that further our understanding of how we can adapt machine perfusion to improve outcomes or assess kidney quality will also be discussed.

2. Why Do We Need Organ Preservation? What Are the Factors Diminishing Kidney Quality?

Modifiable factors that have a key impact on pre-transplant kidney quality are the periods of ischaemia that occur prior to transplant and the reperfusion injury that occurs following transplant. These are illustrated in Figure 1 and described below.

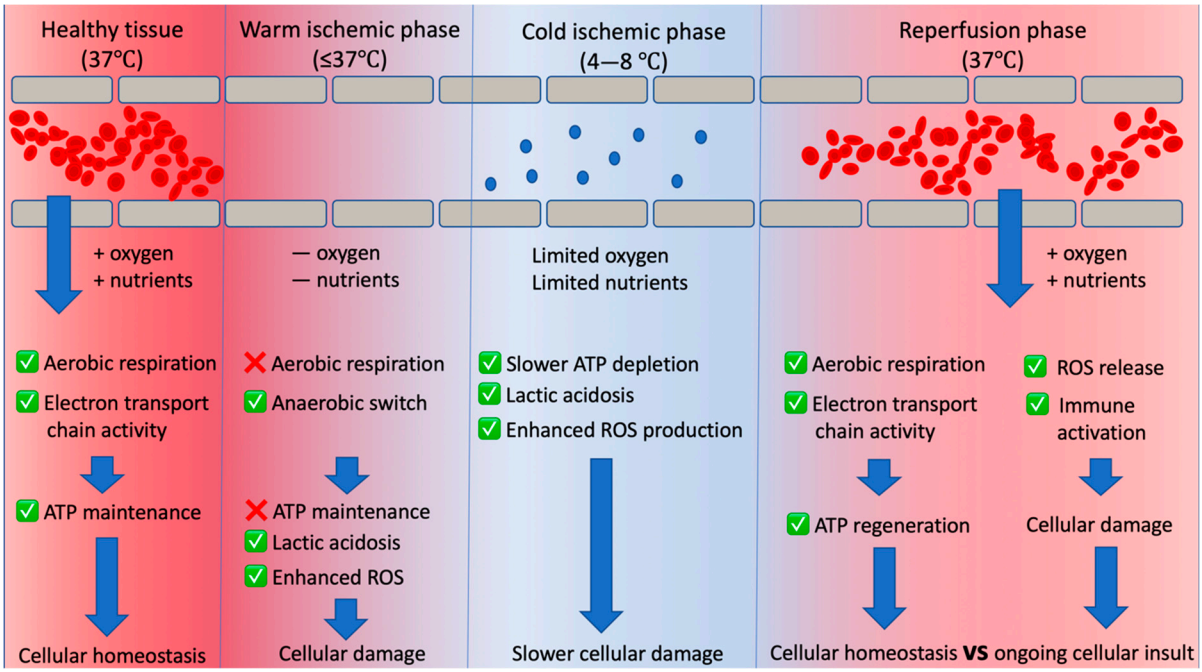

Figure 1. Overview of the changing graft tissue environment between organ donation and implantation. In health, homeostatic mechanisms ensure sufficient oxygen and nutrients are delivered to the renal tissue, resulting in balance between adenosine triphosphate (ATP) usage and regeneration. After donation, cessation of blood flow halts oxygen and nutrient supply (causing warm ischaemia). This causes an anaerobic switch that results in ATP depletion and accumulation of harmful metabolic by-products such as reactive oxygen species (ROS) and lactic acid. Cold ischaemia (chilling the organ) is deliberately implemented to slow the ATP depletion and damage that would occur under warm ischaemia. Restoration of blood flow drives ATP regeneration, but leads to another insult (ischaemia reperfusion injury) which occurs as a consequence of deleterious processes initiated by warm and cold ischaemia.

3. Warm Ischaemia

Preservation techniques maintain organ viability from the time of retrieval until transplantation. These techniques are required to counteract the destructive processes initiated during warm ischaemia. In general, warm ischaemia arises prior to donation [13] and results in impaired delivery of oxygen and metabolic substrates [14,15]. This drives an anaerobic shift [14] and crucially ATP depletion [16], which results in widespread deterioration of tissue structure [17–22]. The warm ischemic injury incurred also stimulates damaging inflammatory responses [23].

The warm ischaemic time (WIT) is associated with increased incidences of delayed graft function (DGF) [24] and therefore, the initial role of kidney preservation is to reduce ATP depletion, cell swelling and hypoxic injury. This is achieved by rapidly flushing the kidney at procurement with a cold preservation solution to slow metabolism and requirements for ATP.

4. Cold Ischaemia

Although effective in reducing metabolism, ongoing depletion of ATP leads to cold ischaemic damage. The cold ischaemic time (CIT) is an independent risk factor for the development of DGF [25]. The mechanisms of damage conferred under conditions of cold ischaemia have been described elsewhere [26].

5. Current Kidney Preservation Methods, Their Advantages and Limitations

5.1. Static Cold Storage

Static cold storage (SCS) is a simple and economical method of kidney preservation. Kidneys are placed in a bag of preservation solution and packed in wet ice, lowering the temperature to around 4 °C. At this temperature, enzymatic activity is reduced by approximately 58% [27]. Different preservations solutions are available, but University of Wisconsin (UW) solution is deemed the gold standard [27]. An overview of solutions used in SCS and the perfusion technologies described below is given in Table 1.

Table 1. Constituents of kidney preservation solutions in clinical use.

	SCS Fluids		**HMP Fluids**	**NMP Fluids**	
	University of Wisconsin (UW) solution	*Custodial-N solution*	*UW Machine perfusion solution (UWPS)*	*Hosgood protocol* [28]	*Minor protocol* [29]
Base fluid	Water	Water	Water	Ringer's solution	Steen solution Ringer's solution
Volume expanders/osmotic agents	Hydroxyethyl starch Raffinose pentahydrate	Mannitol	Hydroxyethyl starch Mannitol (USP) Magnesium gluconate Sodium gluconate	Mannitol	Calcium gluconate
Oxygen carriers	-	-	-	1 unit red blood cells (group O)	-
Drugs	Allopurinol Magnesium sulphate heptahydrate Lactobionic acid	Deferoxamine		Dexamethasone Heparin Prostacyclin Insulin	Ampicillin
Antioxidants	Glutathione	Tryptophan	Glutathione		
Metabolic support	Adenosine	Potassium hydrogen 2-ketoglutarate Sucrose Aspartate Arginine Alanine Glycine	Glucose, beta D (+) Ribose	Glucose, beta D (+) Synthamin 17 Cernevit multivitamins	-
Individual electrolyte additives	-	Magnesium chloride Calcium chloride Potassium chloride Sodium chloride	Calcium chloride	-	-
Buffering agents	Potassium dihydrogen phosphate	Histidine Histidine · HCI	HEPES (free acid) Potassium phosphate (monobasic)	Sodium bicarbonate	Sodium bicarbonate
pH adjustment	Sodium hydroxide/hydrochloric acid Potassium hydroxide	-	Sodium hydroxide	-	-

5.2. Hypothermic Machine Perfusion

Hypothermic machine perfusion (HMP) involves the circulation of cold preservation fluid through the kidney using a mechanical pump. With the exception of several recent clinical trials, clinical HMP does not utilise active oxygenation. The limited metabolic support provided in the perfusion fluid was thought to be sufficient to meet the residual aerobic requirements under hypothermia [18].

ROS are a primary driver of reperfusion injury [30], and commonly used preservation fluids contain potent antioxidants, such as glutathione, to combat ROS activity during preservation. However, extended CIT is associated with marked perfusate glutathione depletion [31].

Machine perfusion solutions provide low-level metabolic support and antioxidant protection throughout perfusion. A key difference between HMP and SCS is the provision of fluid flow, which facilitates nutrient supply, waste removal and a limited amount of tissue reoxygenation with the dissolved oxygen present in the perfusate.

There are several commercially available HMP devices. The Organ Assist Kidney Assist, Waters RM3 and Organ Recovery Systems Lifeport are pressure-controlled systems designed to limit mechanical damage to the kidney during perfusion. Several new devices have been trialled, such as the AirDrive system which includes an oxygenator [32]. A new two-pump perfusion device which circulates fluid through the kidney and also in the organ reservoir has been used to deliver clinical HMP [33,34].

A number of randomised controlled trials and a meta-analysis have shown superiority of HMP over SCS techniques in improving early and longer-term graft function; however, despite this evidence HMP has not gained wide acceptance in some countries.

The evidence base supporting the use of HMP for all deceased donor kidneys is growing, with benefits recently reported in the UK [35,36], France [37], Poland [38] and Brazil [39,40]. HMP can also improve renal function when the CIT is extended [35].

In extend criteria donor (ECD) kidneys, use of HMP enhances 1-year graft survival [41]. However, HMP has not shown a convincing benefit in prolonging longer-term graft survival [42]. The Netherlands is the first country to introduce HMP for all deceased donor kidneys as standard practice [43]. Other countries use HMP specifically for donation after circulatory death (DCD) kidneys, but this practice is not uniform.

5.3. Normothermic Machine Perfusion

Normothermic machine perfusion (NMP) is a relatively new technique of preservation in kidney transplantation. It is currently used in combination with hypothermic preservation strategies as a form of end graft reconditioning. During NMP, kidneys are perfused at near-physiological temperatures and pressures allowing cellular metabolism and function to be restored. In the 1980s, interest in NMP using oxygenated blood-based perfusion solutions started to emerge, and demonstrated that short intervals or an end period of NMP could replenish cellular ATP [44].

The first case of NMP in clinical practice was published in 2011 [45]. The recipient received a kidney from an ECD donor that had been rejected by five other transplant centres in the UK. The kidney underwent NMP for a short interval immediately before transplantation. The recipient did not require dialysis post-transplant and 10 years post-transplant has normal kidney function (personal communication). Subsequently a series of NMP in ECD kidneys demonstrated a remarkably low rate of DGF (11%) compared to SCS kidneys (37%).

More recently, NMP has been used to rescue kidneys that were deemed unsuitable for transplant due to inadequate in situ perfusion after retrieval. Both kidneys were transplanted successfully with immediate graft function following transplant [46]. Building on this work, the authors developed a scoring system which could be used to assess kidney quality prior to transplant [46,47]. A large multicentre clinical trial assessing the effects of 1 h NMP in DCD kidneys compared to SCS has been completed and is due to report this year [28].

NMP conditions are still being developed and have recently been used to counteract 'rewarming injury' which occurs during the warm reperfusion of cold stored grafts. In 2015, the Minor group demonstrated that gradual rewarming (controlled oxygenated rewarming (COR)) of cold stored kidney grafts using machine perfusion improve creatinine clearance and reduces apoptotic signalling when compared to cold stored controls [48].

Kidneys are rewarmed (8–35 °C) over a 1.5 h period to allow metabolic adaptation to the changing thermal environment. Building on this work, the same group trialled their method in the clinical setting, reporting immediate graft function and acceptable levels of creatinine clearance within 1 week of transplantation [29].

More recently, a porcine auto-transplantation model demonstrated that while 8h of NMP improves renal function compared to SCS, a similar improvement in renal function is observed when cold-stored kidneys are subjected to 2 h of COR [49]. The authors speculate that this may be a useful application given the current requirement for hypothermic storage in the logistics of organ transport.

The perfusates used in clinical NMP have been defined in Table 1. The Hosgood et al. protocol provides a more physiological environment, with multiple metabolic substrates and red cells as an oxygen carrier. This contrasts with the Minor protocol, which utilises an acellular perfusate based on Steen solution [29,50].

Adapted cardiac bypass technologies or other perfusion set-ups can be used for NMP. The Kidney Assist, a pressure-controlled system, is the only CE-marked device on the market.

There are some limitations of NMP compared to HMP. These include a more complicated, expensive perfusion circuit and the requirement of personnel for continuous monitoring of the kidney. In a recent publication, RNA sequencing of kidney tissue before and after NMP demonstrated the upregulation of genes associated with oxidative phosphorylation but also inflammatory pathways [51]. Modulation of NMP conditions by incorporating a cytokine filter into the circuit removed the inflammatory cytokines from the perfusate and reduced the inflammatory gene expression.

There is international interest in the development and clinical deployment of NMP [52] and it is the subject of other current clinical trials. The feasibility and safety of normothermic ex vivo kidney perfusion (NEVKP) trial will recruit 25 patients who receive a kidney after 1–10 h of NMP, and assess the device failure rate alongside standard measures of outcome such as DGF, graft failure and patient survival (Clinicaltrials.gov ID: NCT03136848). Perfusion at subnormothermic (20–32 °C) temperatures is also being explored [53]. A new clinical trial is due to start called 'Oxygenated machine preservation in kidney transplantation' (SNOPO; Clinicaltrials.gov ID: NCT04540640), which will address the safety of subnormothermic machine perfusion on transplant kidneys. This is an explorative trial that will also assess the rate of graft discard and assess graft function.

An overview of the active trials investigating both HMP and NMP is given in Table 2.

Alongside this, the more physiological environment generated by NMP permits the use of therapeutic agents to mediate cellular physiology ex vivo and in recent years there have been numerous developments in potential additives to the NMP perfusate which may confer clinical benefit. These are detailed below.

Table 2. Recent clinical trials optimising pretransplant kidney storage and machine perfusion protocols.

NMP Clinical Trials				
NCT Number	**Title**	**Primary Outcome Measure**	**Start Date**	**Completion Date**
NCT05031052	Normothermic machine perfusion (NMP) vs Static Cold Storage (SCS) in Human Kidney transplantation	Kidney function at 6 months post-transplant (eGFR)	August 2021	December 2025
NCT04882254	Normothermic Machine Perfusion: An Additional Value for Kidney Transplant Outcomes?	Number of patients with immediate graft function within three months post-transplant	May 2021	February 2023
NCT03136848	The Feasibility and Safety of Normothermic ex Vivo Kidney Perfusion	1. The ratio of actual/eligible kidney grafts subjected to study intervention at three months after enrolment or up to 4 years whichever is earlier 2. The rate of kidney discard or graft failure attributable to the study intervention from the date of first actual intervention to the date the last participant completes the study follow up period of 3 months post-intervention.	December 2016	April 2019
NCT04693325	PROlonged Ex-vivo Normothermic Machine PERfusion for Kidney Regeneration	Glomerular filtration rate (GFR) at: 6 months post-transplantation	February 2021	July 2022
NCT02525510	Deceased Organ Donor Interventions to Protect Kidney Graft Function	Delayed Graft Function incidence within 1 week of transplantation	August 2017	March 2022
ISRCTN15821205	Ex Vivo Normothermic machine perfusion Trial	Delayed Graft Function incidence within 1 week of transplantation	January 2017	-
HMP Clinical trials				
NCT Number	**Title**	**Primary outcome measure**	**Start date**	**Completion Date**
NCT04619732	Real-time Monitoring of Kidney Grafts on Hypothermic Machine Perfusion	Post-operative recovery of kidney function within: 30 days of transplant	June 2021	December 2021
NCT03378817	Hypothermic Oxygenated Machine Perfusion of Extended Criteria Kidney Allografts from Brain Death Donors	Delayed Graft Function incidence within 1 week of transplantation	December 2017	March 2020
NCT03031067	Hypothermic Oxygenated Perfusion Versus Static Cold Storage for Marginal Graft	Graft function at 3 months post-transplantation	October 2016	February 2018
NCT04359173	Propensity Score Matched Comparison of HMP vs. SCS in Kidney Transplantation	Delayed Graft Function incidence within 1 week of transplantation	August 2015	March 2020
NCT02055950	Pulsed Perfusion for Marginal Kidneys	1. Glomerular filtration rate (GFR) at 6 months post-transplant 2. Renal resistance at 6 hours after pulsatile machine perfusion	July 2013	August 2018
NCT03837197	Clinical Trial of New Hypothermic Oxygenated Perfusion System Versus Static Cold Storage	Delayed Graft Function incidence within 0–30 days of transplantation	December 2018	December 2021
NCT02876692	Prediction and Management of Delayed Graft Function Based on Donor Criteria and LifePort Platform	1. Delayed Graft Function incidence within 1 week of transplantation 2. Transplant nephrectomy at 1 year	January 2016	December 2019

Table 2. *Cont.*

NMP Clinical Trials				
NCT Number	**Title**	**Primary Outcome Measure**	**Start Date**	**Completion Date**
NCT02652520	Evaluation of a Marine OXYgen Carrier: HEMO2Life for hypOthermic Kidney Graft Preservation, Before Transplantation (OXYOP)	Charting within three months of transplant: 1. HEMO2Life adverse effects 2. Graft safety 3. Recipient safety (any adverse event)	March 2016	February 2018
NCT03773211	Renaparin in Kidney Transplantation	Adverse events within 30 days	February 2019	1 April 2020
NCT03024229	Metabolomics in Assessing the Quality of Kidney Transplants Retained on a LifePort Perfusion Machine	Immediate graft function (IGF) (i.e. the absence of a requirement for dialysis) within 7 days post-transplant	March 2017	January 2020
NCT01848249	Deceased Donor Biomarkers and Recipient Outcomes	1. Delayed Graft Function incidence within 1 week of transplantation. 2. Death-Censored Graft Failure within 4 years post-transplant.	May 2010	March 2020

6. What Are the Advances in HMP?

6.1. Active Perfusate Oxygenation under HMP

A key focus in the optimisation of HMP over recent years has been the addition of oxygen. Aerobic metabolism, through facilitation of the electron transport chain, generates more ATP than anaerobic glycolysis, which, alongside compounding the injury caused by ATP depletion, also results in deleterious tissue acidification through the enhanced production of lactic acid [54].

Early studies using murine models demonstrated potential benefits of actively oxygenating the perfusate, with perfusate oxygen consumption during HMP appearing to be linked to improvements in post-transplant glomerular filtration rate (GFR) [55]. This was supported by a porcine kidney ex vivo reperfusion model, in which 21 h of HMP combined with provision of 100% oxygen resulted in improved blood flow and creatinine clearance, when compared with non-oxygenated HMP. In addition, non-oxygenated HMP resulted in evidence of greater tubular damage [56].

The Consortium for Organ Preservation in Europe (COPE) recently reported the COMPARE trial, a randomised controlled phase 3 trial comparing the effects of continuous HMP with oxygen, with HMP without oxygen in pairs of DCD kidneys using the Kidney Assist Device. A small improvement in estimated GFR (eGFR) and a lower incidence of graft failure was observed when compared to standard HMP (i.e., 3% vs. 10%, $p = 0.028$) at 1 year when sensitivity analysis was applied, but there was no significant difference in graft function, the primary outcome measure if both pairs from the same donor were functioning at 1 year [57]. Shorter periods of oxygenated HMP may also have some benefit. In a porcine model, active perfusate oxygenation was found to promote enhanced restoration of aerobic metabolism and also increase endpoint cortical ATP concentrations [58]. This latter finding was repeated by another group, who documented no effect of perfusate oxygenation on graft function, but noted increased endpoint ATP concentrations as a consequence of oxygenation [59].

There are concerns that such high-level oxygenation could be surplus to requirements and actually be deleterious to the preserved tissues through enhanced ROS activity [60].

Using an ex vivo ischaemia reperfusion model, it has been demonstrated that continuous oxygenation under HMP results in superior early graft recovery when compared to SCS, various transient oxygenation strategies and also end ischaemic NMP strategies [61].

Extending this into an auto-transplantation model, the same group compared different oxygenation strategies. The aim of these studies was to identify the minimal oxygenation strategy that provides a therapeutic effect, but avoids the potential for unnecessary oxidative stress. Oxygenation with a concentration of 30% or 90% resulted in no significant

differences in functional outcomes; however, kidneys perfused with higher concentrations of oxygen had reduced perfusate lactate concentration [62].

The same group also examined methods of oxygenation. Kidneys received 22 h HMP with or without a 2 h oxygenation period at the beginning or end of perfusion [63]. In the first 4 days after transplant, kidneys that had received 22 h continuous oxygenation and 2 h oxygenation at the start of HMP had improved graft function compared to those without oxygenation or with HMP with 2 h of end oxygenation. While end ischaemic oxygenation may offer benefit, initial oxygen uploading appears the most promising transient oxygenation strategy. This may be delivered in the clinical setting using currently approved devices. The Waters and Kidney Assist Devices include the capacity for an oxygenator; the Organ Recovery Systems Lifeport is also capable of oxygenation through bubble and surface oxygenation methods [64].

In clinical kidney transplantation, the COPE consortium recently published the results of the HOPE trial. The effects of end ischaemic oxygenated HMP was trialled in ECD kidneys and compared to ECD kidneys undergoing SCS. No significant difference in DGF incidence was observed between group [65].

In contrast, a study published in letter format to the *Journal of Clinical and Translational Research* proposed end ischaemic oxygenation to be particularly therapeutic in renal HMP [66].

Other studies examining the effects of oxygenated HMP using these different strategies are also being carried out (Clinicaltrials.gov ID: NCT03837197).

6.2. Modification of Perfusate under HMP

M101 is an extracellular haemoglobin with a high oxygen-carrying capability. Porcine kidneys perfused with fluids supplemented with M101 had reduced vascular resistance during perfusion and less fibrosis after reperfusion [67].

M101 was assessed in a clinical study including 60 pairs of donation after brain death (DBD) kidneys. The OXYPOP study added M101 during HMP and SCS and found no adverse outcomes. There was some indication of improved early graft function [68].

Perfusate supplementation with Vectisol® is a recent advance that shows preclinical potential. Vectisol is a revesterol–cyclodextin conjugate which increases the solubility of the potent antioxidant resveratrol. When compared to porcine kidneys stored using standard HMP protocols, supplementing HMP perfusate kidney perfusion solution 1 (KPS-1) with 1.56 g Vectisol reduced the levels of oxidative stress and apoptosis in a porcine auto-transplantation model. In addition, Vectisol reduced total plasma levels of superoxide dismutase and lowered plasma creatinine levels at 3 months post-transplant compared to standard HMP [69].

Another recent development in perfusate optimisation includes perfusate supplementation with macromolecular heparin. Reperfusion results in rapid degradation of the endothelial glycocalyx. Using immunofluorescence analysis and confocal microscopy, it has been shown that heparin administered during HMP binds to the vascular endothelium lining the perfused vasculature and, simultaneously, perfusate concentrations of macromolecular heparin are depleted [70]. Using a paired porcine perfusion model with an ex vivo reperfusion model, the same group demonstrated that administration of 50 mg macromolecular heparin resulted in lower renal resistance and faster reductions in serum creatinine than kidneys that did not receive heparin administration. Additionally, heparinised kidneys had lower levels of the biomarker neutrophil gelatinase-associated lipocalin (NGAL) [71].

The 'Renaparin® in kidney transplantation' placebo-controlled trial is currently underway, in which kidney transplant recipients will receive a kidney stored using conventional HMP or HMP with the addition of Renaparin, a heparin analogue. This study will assess the incidence of DGF and adverse events within the first 30 post-operative days (Clinicaltrials.gov ID: NCT03773211).

6.3. Measures of Graft Quality during HMP

In recent years, there has been considerable focus on non-invasive methods through which kidney quality can be assessed during HMP. The most readily available indices on kidney quality come from macroscopic inspection: larger kidneys with poor perfusion at procurement are more likely to go on to experience impaired graft function post-transplant [72]. However, the perfusion period offers more ability to assess organ quality.

An assessment that can be performed in real time is the measurement of perfusion parameters. Renal resistance may have some value in kidney assessment. A renal resistance of <1 mmHg within the first hour of perfusion was associated with reduction in the incidence of DGF and primary non-function (PNF) and also improved rate of decrease in post-operative serum creatinine [73].

The value of using renal resistance to predict transplant outcomes was also demonstrated recently in an oxygenated HMP study. The percentage fall in renal resistance was found to be inversely correlated to serum creatinine on day 1 and 2 after transplant, indicating a functional link between an individual kidney's response to perfusion during storage and graft function [65]. Patients who receive a kidney that exhibited high resistance during the first hour of HMP have higher post-operative serum creatinine at 1 year and a higher incidence of DGF immediately post-transplant [74].

Additionally, a high endpoint renal resistance was reported again to be a strong risk factor for the development of DGF [75] and a retrospective analysis has recently linked high renal resistance with graft failure [76]. Kidneys with resistance readings >0.19 mmHg/mL/min have a higher risk of acute rejection and inferior one-year graft survival compared to kidneys with an endpoint resistance of <0.19 mmHg/min [77].

Nonetheless, the utility of renal resistance in prediction of PNF or long-term kidney graft outcomes appears to be limited [78].

Measuring intracellular ATP using magnetic resonance imaging (MRI) is another strategy which could be used to assess kidney quality. MRI is a non-destructive technique which uses the unique magnetic profile of compounds containing MRI sensitive nuclei to produce images of the biological vessel they inhabit. With a protocol that specifically targets the ^{31}P isotope of phosphorous, a Swiss study showed the deleterious effects of warm ischaemia on intracellular ATP concentrations and also enhanced ATP resynthesis during oxygenated perfusion. ATP levels were linked with the level of damage observed upon tissue examination [79].

Alternatively, the use of optical coherence tomography has been reported as another non-invasive technique which can be used to define macroscopic structural changes in the shallow cortex of pre-transplant human kidneys. Larger proximal tubule diameters had predictive potential for DGF development, and shorter distances between separate proximal tubule lumens was also indicative of DGF [80].

Monitoring of ongoing metabolism during organ preservation has been proposed as a useful method to assess kidney quality [81]. However, logistically, this requires rapid analysis and the use of perfusate samples.

Rapid sampling microdialysis has been applied during kidney perfusion, permitting a continual acquisition of tissue-derived analyte into a detection system [82]. While this approach does not offer comprehensive metabolic profiling, it does allow for continuous profiling of metabolites of interest, such as lactate, and may have a role in defining kidney quality before transplant. This technology will be applied in the upcoming REMOHYMAP trial (Real-time monitoring of kidney grafts on hypothermic machine perfusion) (Clinicaltrials.gov ID: NCT04619732).

Another trial, 'Metabolomics in assessing the quality of kidney transplants retained on a LifePort® perfusion machine' (RENALIFE), will utilise nuclear magnetic resonance (NMR) spectroscopy to define metabolic changes in the perfusate and determine whether these are linked with graft function post-transplant (Clinicaltrials.gov ID: NCT03024229).

A similar study, the ^{13} Champion clinical study, will also utilise NMR to chart changing perfusate metabolic profiles prior to transplantation, with a perfusate modification enabling

tracing of glucose metabolism (i.e., substitution of conventional naturally occurring glucose for glucose that has been carbon-13 enriched). This study will report the results in 2022 [83].

A complementary indicator of graft quality that may be used alongside measures of renal resistance is the detection of damage-associated biomarkers in the perfusate, of which many have been proposed and studied [84,85].

A recent meta-analysis assessed the published literature linking potential perfusate biomarkers with post-transplant outcomes. This study found that assessment of glutathione S transferase offered the best predictive potential for DGF and PNF [86].

7. What Are the Advances in NMP?

For logistical reasons, NMP is used in combination with hypothermic techniques of preservation. Therefore, kidneys are subject to periods of cold ischaemia. Furthermore, after NMP, it is necessary to flush the kidney with cold preservation solution to remove the red cell-based perfusate and cool the kidney in preparation for transplantation. Anastomosis of the renal vessels can take up to 60 min and cooling helps to prevent warm ischaemic injury.

One approach to avoid ischaemic injury is to continually perfuse the kidney using NMP conditions. A single-case report demonstrated use of the Kidney Assist apparatus to perfuse a kidney from a DBD donor from the time of retrieval until transplantation. Although logistically and technically difficult, the authors demonstrated proof of principle that it is possible to avoid ischaemic injury using this approach [87].

A more practical approach is normothermic regional perfusion (NRP), in which kidneys are perfused in situ within the donor's body at the time of retrieval. NRP reduces the incidence of PNF in kidneys from DCD donors compared to in situ cooling [88]. Porcine models have demonstrated that 4 h of NRP is an optimal timeframe to limit kidney injury [89].

7.1. Modification of the Normothermic Perfusion Conditions

NMP may utilise red cells as oxygen carriers [50]; however, with the potential for haemolysis to occur, alternatives are desirable.

Haemoglobin-based oxygen carriers (HBOCs) may be a suitable alternative. Using 14 discarded human kidneys, a 2019 study demonstrated that the use of perfusate supplemented with HBOC (n = 7) exerted no significant differences in oxygen consumption or endpoint tissue ATP levels when compared to perfusate supplemented with packed red cells (n = 7) [90]. In addition, no differences in renal resistance or tissue histology were noted. This indicates that a cellular perfusate may not be required for optimal delivery of NMP.

NMP delivered with controlled rewarming and an acellular perfusate was found to improve creatinine clearance and reduce expression markers of innate immune activation when compared to SCS stored kidneys [91]. However, no benefit was gained by addition of red cells, indicating that they may not be necessary for NMP.

An alternative to supporting oxygen requirements is to suppress oxygen requirements by administering hydrogen sulphide (H_2S) during NMP. Hydrogen sulphide inhibits the activity of complex IV of the mitochondrial electron transport chain. A study investigating the effects of H_2S supplementation during perfusion demonstrated a 61% decrease in oxygen consumption; however, this suppression was quickly reversed after stopping H_2S administration [92]. This suggests that continuous delivery of hydrogen sulphide may be required for longer preservation periods. Nonetheless, no differences in damage indicators were found between H_2S and control kidneys.

7.2. Regenerative Therapies

NMP can also be used to administer regenerative therapies to the kidney. Mesenchymal stem cells (MSCs) have the capability to repair cellular damage and immunomod-

ulation properties to reduce the immune response. To date, large animal models have demonstrated the safety and feasibility of introducing MSCs in this way [93].

Factors limiting the application of MSCs during NMP include observations that the infused cells localise within glomerular lumen and may not confer protection to post-glomerular structures such as the proximal tubules [94]. Additionally, high doses of MSCs (i.e., 10^7) appear to be required as cells can adhere to the filters and tubing of the NMP circuit.

Nonetheless, when trialled in pairs of non-transplanted human kidneys, the infusion of multipotent adult progenitor cells (MAPCs) resulted in decreased injury markers and improvements in both cortical microcirculation and urine output when compared to controls [95]. In a similar study, infusion of MSCs resulted in downregulation of inflammatory cytokines, improvements to tissue ATP concentrations and evidence of enhanced cellular repair [96].

7.3. Therapeutic Agents

A different approach may be to add compounds that target specific immune processes. An Australian study documented the use of a small animal model to determine the efficacy of three drugs added to the NMP perfusate. Of a CD47 receptor agonist (αCD47ab), soluble complement receptor 1 and recombinant thrombomodulin, the antibody therapy proved the most effective in ameliorating IRI. When this was taken forward to a porcine NMP auto-transplant model, the antibody was shown to significantly improve perfusion parameters when compared to controls [97].

Cold ischaemia causes fibrinogen to accumulate in the proximal tubule epithelium [98]. Upon reperfusion, this is released and causes red cell aggregates that plug the renal vasculature. Kidneys can be treated during NMP with the addition of plasminogen and plasminogen activator to clear the microcirculation and improve function [98].

An exciting development recently applied to the kidney is the use of NMP to deliver lentiviral vectors containing constructs that silence expression of major histocompatibility complex (MHC) I and II in rats [99]. This approach has the potential to mask human leucocyte antigen (HLA) differences between donor and recipient and reduce the risk of acute rejection. This study reported no evidence of damage to the kidney; however, there was a significant increase in the levels of inflammatory cytokines detected.

Restoring cellular processing during NMP after ischaemic injury inevitably leads to the upregulation of pro-inflammatory cytokines. The perfusion conditions can be modified by including a cytosorb filter in the NMP circuit to remove the circulating cytokines, which reduces the inflammatory gene expression within the kidney [51].

7.4. Modifying the Duration of NMP

At present, reports of NMP in clinical kidney transplantation use short durations of NMP (1–2 h). This is for logistical reasons and from evidence in experimental work demonstrating that cellular ATP is restored within this timeframe.

A recent study in the Netherlands reported the effect on recipients over the age of 65 of receiving a kidney from an ECD following 2 h of NMP [100]. The follow up included 11 patients who received a kidney stored by NMP and a historical cohort of 53 patients who received a kidney stored by HMP or SCS. This study found no differences in graft outcomes within the 1 year follow up, indicating that within this timeframe a 2 h preservation time is safe.

The PROlonged ex vivo normothermic PERfusion for kidney regeneration (PROPER) clinical trial will evaluate the effects of extending NMP duration on patient outcomes (Clinicaltrials.gov ID: NCT04693325). An initial cohort will examine the effects of 1 h NMP, followed by 3 h and then 6 h, with patient outcomes determining progression to each successive timepoint. The primary endpoint is eGFR at 6 months following transplantation

A recent study has reported 24 h NMP with discarded human kidneys [101]. The longer perfusion times were accredited to the use of a perfusion circuit which permits urine

recirculation. Conventionally, the urine produced during perfusion is isolated and the volume replaced with a suitable fluid, such as Ringer's lactate. In this study, the researchers found use of urine recirculation led to a more stable environment with normal electrolyte and acid–base balance compared to kidneys that did not have urine recirculation.

7.5. Assessment of Graft Quality during NMP

NMP offers enhanced capacity to monitor organ quality, with physiological temperatures allowing measures of blood flow, urine production and macroscopic changes detectable with visual inspection [102]. NMP technology has been used to assess kidneys rejected for clinical use and allowed successful transplantation [46].

Transcriptomic signatures measured in samples of kidney tissue after NMP may be able to predict graft outcome. A bioinformatic analysis performed by researchers at Wuhan University Hospital has identified a set of five genes, the level of transcription of which in post-implantation biopsies was predictive for DGF [103]. The authors postulate their genomic model could have value when used in conjunction with genomic data acquired from kidneys undergoing NMP.

This hypothesis has been proposed previously: an apparent DGF-linked gene signature was identified in NMP reconditioned kidneys. In this study, upregulation of components of inflammatory pathways, such as tumour necrosis factor α (TNF-α) and nuclear factor kappa B (NFκB), were linked with extended DGF durations, whereas enhancements of oxidative phosphorylation genes had the opposite effect [51]. This infers observation of the renal transcriptome during NMP may indicate post-transplant outcomes.

The use of final resistance values may also be useful for predicting poor outcomes after NMP [104]. This study reported that between 59% and 70% of transplanted kidneys that had a postoperative GFR of <30 ml/min at 6 months could be predicted by an endpoint resistance of 0.3 mmHg/ml/min during regional NMP.

However, there may be limitations to the use of short NMP durations to predict transplant outcomes [105] and extended durations may prove more prognostic.

8. Summary

Developments in hypothermic and normothermic machine perfusion technologies show potential in clinical kidney transplantation. HMP has been more widely adopted as the primary method of hypothermic preservation and modification of HMP protocols to include oxygen supplementation may or may not be beneficial. Other translational developments in the field of HMP include passive indications of graft quality through observation of perfusion parameters and more specific indications, such as ongoing metabolic processes or biomarker release.

NMP also has the potential to improve kidney transplant outcomes, with better measures of kidney assessment. Additionally, NMP shows great promise in the delivery of therapeutics which can modify the graft during perfusion. While these are currently in experimental stages, it is not inconceivable that advances similar to those discussed in this review could enhance graft compatibility, quality and survival. The advances described here are an indication that the full clinical potential of different machine perfusion strategies as a whole have not yet been realised.

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Abbreviations

ATP	adenosine triphosphate
CIT	cold ischaemic time
CKD	chronic kidney disease
COR	controlled oxygenated rewarming
DBD	donation after brain death
DCD	donation after circulatory death
DGF	delayed graft function
ECD	extended criteria donor
eGFR	estimated glomerular filtration rate
ESRD	end-stage renal disease
GFR	glomerular filtration rate
HBOC	haemoglobin-based oxygen carrier
HLA	human leucocyte antigen
HMP	hypothermic machine perfusion
IRI	ischaemia reperfusion injury
KPS-1	kidney perfusion solution 1
MAPCs	multipotent adult progenitor cells
MHC	major histocompatibility complex
MRI	magnetic resonance imaging
MSCs	mesenchymal stem cells
NEVKP	normothermic ex vivo kidney perfusion
NFκB	nuclear factor kappa B
NGAL	neutrophil gelatinase-associated lipocalin
NMP	normothermic machine perfusion
NMR	nuclear magnetic resonance
NRP	normothermic regional perfusion
PNF	primary non-function
ROS	reactive oxygen species
SCS	static cold storage
TNF-α	tumour necrosis factor α
UK	United Kingdom
US	United States
WIT	warm ischaemic time

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