Hypothermic and Normothermic Perfusion in Kidney Transplantation

Subjects: Transplantation

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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.

machine perfusion kidney HMP NMP regenerative medicine biotechnology

1. Introduction

Kidney transplantation is the most economical [1] and effective [2][3][4][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][8][9], poor post-transplant graft function [10][11][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.

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.

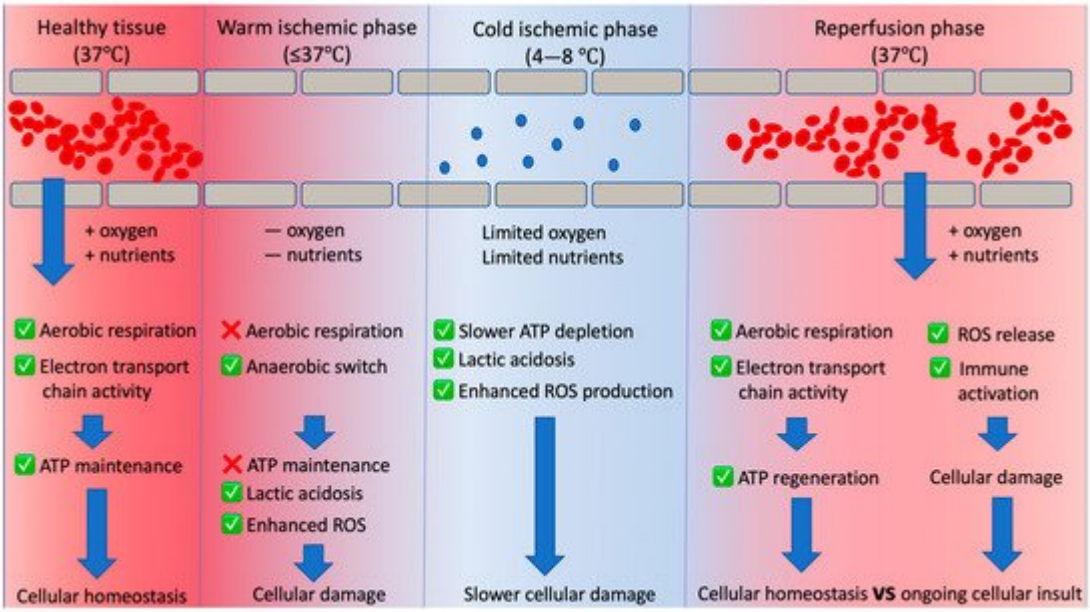

Figure 1. Overview of the changing graft tissue environment between organ donation and implantation. In health, homeostatic mechanisms ensure sufficient oxygen and nutrients are delivered to the renal tissue, resulting in balance between adenosine triphosphate (ATP) usage and regeneration. After donation, 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][18][19][20][21][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

	SCS Fluids		HMP Fluids	NMP Fluids	
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 · HCl	HEPES (free acid) Potassium phosphate (monobasic)	Sodium bicarbonate	Sodium bicarbonate
pH adjustment	Sodium hydroxide/hydrochloric acid Potassium hydroxide	-	Sodium hydroxide	-	-

/ 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**.

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	Number of patients with immediate graft function within	May 2021	February 2023

NMP Clinical Trials				
NCT Number	**Title**	**Primary Outcome Measure**	**Start Date**	**Completion Date**
	Additional Value for Kidney Transplant Outcomes?	three months post-transplant		
NCT03136848	The Feasibility and Safety of Normothermic ex Vivo Kidney Perfusion	• The ratio of actual/eligible kidney grafts subjected to study intervention at three months after enrolment or up to 4 years whichever is earlier • 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

NMP Clinical Trials

NCT Number	Title	Primary Outcome Measure	Start Date	Completion Date
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	• Glomerular filtration rate (GFR) at 6 months post-transplant • 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	• Delayed Graft Function incidence within 1 week of transplantation • Transplant nephrectomy at 1 year	January 2016	December 2019
NCT02652520	Evaluation of a Marine OXYgen Carrier: HEMO2Life for hypOthermic Kidney	Charting within three months of transplant: • HEMO2Life adverse effects	March 2016	February 2018

NMP Clinical Trials

NCT Number	Title	Primary Outcome Measure	Start Date	Completion Date
	Graft Preservation, Before Transplantation (OXYOP)	• Graft safety • Recipient safety (any adverse event)		
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	• Delayed Graft Function incidence within 1 week of transplantation. • Death-Censored Graft Failure within 4 years post-transplant.	May 2010	March 2020

P.K. Comparison of Mortality in All Patients on Dialysis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant. N. Engl. J. Med. 1999, 341, 1725–1730.

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