INTRODUCTION

One initiative to reduce the number of patients waiting for a kidney transplant has been the use of kidneys from extended criteria donors (ECD) and donation after circulatory death (DCD) donors. Increasing age, co-morbidities, and ischemic injury in these kidneys result in higher rates of early graft dysfunction compared to kidneys from younger, standard criteria donors (SCD).\(^1\) Delayed graft function (DGF) complicates recovery, increases the likelihood of acute rejection and can reduce graft survival. Hypothermic preservation techniques have been pivotal in the success of organ transplantation.\(^2\) Kidneys can be stored on ice or perfused with cold preservation solution and safely transported between donor and recipient centers. Nonetheless, in an anaerobic environment the condition...
of the kidney gradually deteriorates.\(^2\) This has brought about a change in thinking in recent years. Preserving an organ at a normothermic temperature can restore cellular metabolism and replenish adenosine triphosphate (ATP) synthesis. If the optimal conditions are applied, this may prevent deterioration of the organ and promote recovery. Furthermore, by restoring function normothermic machine perfusion (NMP) also provides an opportunity to assess organ quality pre-transplantation and to serve as a platform for the delivery of pretransplant therapies to promote recovery.\(^3\) However, there is no clear consensus on the best NMP strategy for the kidney. Figure 1 details the different published components used for NMP.

There are only a handful of clinical reports but there is an increasing volume of experimental work exploring a variety of different approaches that may lead to more widespread application. This review will examine the different protocols and practices used for kidney NMP (Table 1) and provide some insight into the mechanistic effects and future consideration of NMP. This will aid the development and translation of NMP technologies for the kidney into clinical transplantation.

### 2 | NORMOTHERMIC MACHINE PERFUSION SYSTEMS

At present the Kidney Assist™ Device made by Organ Assist based in the Netherlands is the only commercially available, CE marked, NMP system for the kidney. Other reported systems are in-house adaptations that use current cardiopulmonary bypass technology or custom-made perfusion devices using centrifugal or roller pumps. The UK-based company OrganOx has designed a prototype portable system for maintaining a kidney under NMP conditions for prolonged periods but at the time of writing this has not been marketed.\(^4\)

### 3 | NORMOTHERMIC MACHINE PERFUSION PROTOCOLS

#### 3.1 | Oxygen carriers

Red blood cell (RBC)-based solutions are the most commonly used for NMP. Compatible units of banked RBCs are readily available and relatively inexpensive. In addition to their efficient oxygen carrying capacity the flow of red cells through the vasculature reduces shear stress and promotes normal endothelial cell function. Nonetheless, there are a number of limitations. The transfusion of older stored RBCs is known to increase the amount of nontransferrin bound iron.\(^5\) This produces acute tissue iron deposition and initiates an inflammatory response. Hemolysis occurs due to an increase in unconjugated bilirubin levels and contact with the artificial surfaces of the perfusion circuit.\(^6\) There are also time-dependent metabolic and biochemical alterations including diminished intracellular ATP levels, diminished 2,3-Diphosphoglycerate (2,3-DPG) levels, and a loss of bioactive nitric oxide (NO) derivatives. Functionally, longer-banked RBCs are less deformable and adhere excessively to the vascular endothelium.\(^7\) Furthermore, the high doses of heparin that are added to prevent thrombosis can, paradoxically, cause increased RBC aggregation.

Whole blood has been used in NMP systems as a surrogate for revascularization to assess function and viability. However, its use is not considered ideal for NMP. The presence of leukocytes and platelets heightens the ischemia reperfusion injury response, with increased neutrophil infiltration, the release of reactive oxygen species (ROS) and endothelial and tubular damage.\(^8\)

Autologous whole blood with the leukocytes and platelets removed but retaining the plasma component has also been used in the development of NMP techniques.\(^9\) Plasma contains albumin and globulins to maintain osmotic pressure, electrolytes to help maintain blood pH and immunoglobulins to fight infection and therefore beneficial for NMP conditions.\(^10\) However, it also...
### TABLE 1  Main components of perfusates used in clinical and experimental NMP studies. Includes human and porcine models

| Protocol | Modela | Oxygen carrier | Priming solution | Fluid replacement | Colloid | Nutrient/ supplementation | Anticoagulant | Protective additives | Antibiotics |
|----------|--------|----------------|------------------|------------------|--------|---------------------------|---------------|----------------------|-------------|
| Hosgood et al17 | Human clinical | RBC 1 unit | Ringer’s solution b | Ringer’s solution b | Glucose 5%, insulin 100 IU, synthamin 17 | Heparin b | Mannitol 10%, dextamethasone 8 mg, epoprostenol sodium (Flolan) 0.5 mg, sodium bicarbonate 8.4%, multivitamins (cernevit) b |
| Minor et al16 | Human clinical | STEEN solution 1L, Ringer’s solution 1 L | STEEN solution c | Calcium gluconate 7 ml, sodium bicarbonate 8.4% 16 ml | Ampicillin 1 g |
| Kabagambe et al47 | Human | RBC 250 ml | Plasma Lyte A 250 ml | Plasma Lyte A b | TPN baxter, b clinimix, b insulin 100 IU | Heparin 2000 IU | Multivitamins 5 ml, sodium bicarbonate 8.4% 26 ml/L |
| Weissenbacher et al4 | Human | RBC 1 unit | 5% human albumin 250 ml | Ringer’s solution/ urine recirculation b | TPN nutriflex b | Mannitol 10% 10 ml, epoprostenol sodium 4 µg/h, calcium gluconate 10% 10 ml, sodium bicarbonate 8.4% 5–15 ml | Cefuroxime 750 mg |
| Aburawi et al12 | Human | RBC/ hemopure 500 ml | Williams media E 1500 ml | Ringer’s solution b | Dextrose, b insulin 5 U/L | Heparin 1000 U/L | Dextamethasone 8 mg/L, sodium bicarbonate 8.4% b |
| Pool et al27 | Porcine | RBC 350 ml | Williams media E 500 ml | Albumin (bovine serum albumin) 40 g | Heparin b | Amoxicillin-Clavulanate 1000 mg/200 mg |

(Continues)
| Protocol | Model \(^a\) | Oxygen carrier | Priming solution | Fluid replacement | Colloid | Nutrient/supplementation | Anticoagulant | Protective additives | Antibiotics |
|----------|------------|----------------|------------------|-------------------|--------|------------------------|---------------|---------------------|-------------|
| Urbanellis et al\(^{22,23}\); Kaths et al\(^{23,31}\) | Porcine | RBC 125 ml | STEEN solution | 150 ml, Ringer’s solution, 200 ml, Double reverse osmosis water, 27 ml | STEEN solution\(^b\) | Amino acids 1 ml/h, glucose 1 ml/h, insulin 5 IU/h | Heparin 1000 IU | Verapamil 0.25 mg/h, calcium gluconate 10% 100 mg/ml, sodium bicarbonate 8.4% 8 ml |
| Blum et al\(^{20}\) | Porcine | RBC 202 ml | Ringer’s solution | 282 ml, water 44 ml, Krebs-Henseleit Buffer 322 ml | Albumin (bovine serum albumin) 22.5 g | Amino acids 0.05 g/h, insulin 5 U/h | Heparin 2000 IU +500 IU/h | Dexamethasone 10 mg, verapamil 0.25 mg/h, calcium gluconate 10% 3 ml, sodium bicarbonate 8.4% \(^b\) | Ampicillin 1 g, Cefotaxime 1 g |
| Urucuyo et al\(^{21}\) | Porcine | Whole blood/ STEEN solution\(^b\) | | | | | | Methylprednisolone 50 mg, multivitamins 0.25 mg/h, 10% calcium gluconate (whole blood only) 20 ml, verapamil 10 mg+1 mg/h or nitroprusside 25 mg/h or vasodilator cocktail, sodium bicarbonate 8.4% 20 ml |

\(^a\)Model; “human clinical” refers to kidneys that were transplanted and “human” refers to human experimental (nontransplanted kidneys).  
\(^b\)Volumes/concentration not indicated in protocol.  
\(^c\)Colloid included as part of priming solution.
contains fibrinogen, which may contribute to the development of microvascular thrombi.

Substituting RBCs with an artificial oxygen carrier is an alternative for NMP. Hemopure (haemoglobin glutamer-250 [bovine]; HBOC-201, Haemoglobin Oxygen Therapeutics LLC) is a polymerized bovine hemoglobin-based oxygen carrier (HBOC) of low immunogenicity. It has an oxygen carrying capacity similar to that of human hemoglobin at normothermic temperatures and releases oxygen to tissues more readily than corpuscular hemoglobin. Hemopure has been used for NMP in a series of experimental porcine and human kidneys that demonstrated equivalence in comparison to RBCs. Nonetheless, Hemopure has not been widely tested and difficulties in supply limit its use. Furthermore, the hemoglobin contained in Hemopure has a tendency to become oxidized to the ferric (Fe$^{3+}$) form thus generating methemoglobin, which is toxic and increases oxidative stress.

Pyridoxylated bovine hemoglobin is another alternative oxygen carrier reported by Brasile et al. Their Exsanguinous Metabolic Support (EMS) solution (Breonics) is made up of a highly enriched tissue culture-like medium containing essential and nonessential amino acids, lipids and carbohydrates supplemented with bovine hemoglobin.

### 3.2 | Acellular solutions

A number of experimental studies have used acellular-based mediums that have the capacity to carry oxygen. Lifor is an artificial preservation medium containing a nonprotein oxygen carrier that can be used at room temperature. Aqix RS-I is a preservation medium formulated for use in tissue, organ, and cellular preservation. It is designed to maintain all aspects of cellular function across a range of temperatures (4–37.4°C) by providing natural colloidal buffering to prevent edema. STEEN solution is a preservation solution that contains a high concentration of albumin and dextran to create a high osmotic pressure. STEEN solution is not naturally formulated for the kidney and dilution is required to prevent cellular damage and diffuse vacuolation of the tubular cells.

### 3.3 | Supplements

A number of essential ingredients are needed to provide volume to the perfusate and metabolic support during NMP (Table 1). In brief, the protocols list the following ingredients: crystallloid- or colloid-based solution with or without supplementation of albumin to maintain volume and prevent cellular edema, mannitol to increase osmolality and enhance renal blood flow, vasodilators such as epoprostenol sodium, a synthetic prostacyclin, verapamil, a calcium channel blocking agent, or sodium nitroprusside, and corticosteroids to reduce inflammation.

Basic nutrient preparations such as glucose and amino acids can be administered to support metabolism. Insulin is normally added to the parenteral solution to promote the absorption of glucose (Table 1). The administration of multivitamins or vitamin C may also have some beneficial effects to reduce oxidative stress during NMP.

These preparations are based on the experiences of cardio-pulmonary bypass technology without being thoroughly tested in kidney NMP models. Therefore, the true metabolic requirements of kidney NMP are unknown. The nutritional and metabolic demand may be different for kidneys from different donor types and for more prolonged periods of perfusion. Colloids such as albumin may be required to prevent cellular edema and substituting glucose with a lipid may be a more efficient source of energy.

### 3.4 | Antibiotics

Bacterial or fungal contamination of the preservation solution used to store deceased donor organs is common due to circumstances arising in the donor or during the retrieval process. A recent clinical study found that 56% of kidneys undergoing NMP had positive bacterial growth of Staphylococcal origin. None of the NMP perfusate-cultured organisms were implicated in episodes of infection in the recipients. Broad-spectrum antibiotics such as Cefuroxime or Augmentin can be added during NMP. However, the risk of nephrotoxicity or antibiotic resistance must be kept in mind.

### 4 | NORMOTHERMIC MACHINE PERFUSION STRATEGIES

Most NMP protocols combine hypothermic and normothermic techniques to provide a more practical logistical approach. Our group established a protocol of a 1 h end period of NMP using a RBC-based solution (end-ischemic approach). The protocol was based on experimental work in porcine kidneys, which demonstrated that 1–2 h of NMP was sufficient to replenish ATP and upregulate protective mechanisms after a period of hypothermic preservation. The results were encouraging as 1 h NMP was associated with a significant reduction in the rate of DGF (5.6%) compared to a historical control group of kidneys undergoing static cold storage alone (36.2%).

We also demonstrated that after NMP, kidneys could be safely transitioned back into hypothermic storage for a substantial period of time before transplantation.

In contrast, other studies have found no benefit in a short end period of NMP compared to static cold storage. Darius et al recently used a porcine autotransplant model to assess a 2 h NMP period with a RBC-based solution and Kaths et al investigated 1 h of NMP with a RBC and STEEN solution preparation. The discrepancy between the clinical and experimental studies maybe attributed to the added warm ischemia used in the experimental studies or differences in perfusion conditions. In the clinical series the majority of kidneys were from ECD donors.

The controlled rewarming strategy reported by Minor et al with an acellular solution is a promising new technique of end NMP (discussed in the next section).
In a series of studies using a porcine autotransplant model the Toronto group investigated NMP and the combination of different hypothermic and normothermic durations. Their protocol used a RBC and STEEN solution-based perfusate. Sole use of NMP for the entire preservation period was the most favorable for early graft function. Moreover, in combination with hypothermic preservation, longer durations of NMP (8 h) gave better results than 1 h NMP. This evidence suggests that longer periods of NMP may be necessary to promote recovery after warm and cold ischemic injury. Therefore, different NMP protocols may be necessary for DCD, ECD, and ECD/DDC kidneys.

The feasibility of NMP for 24 h has been reported in several non-transplanted human kidney studies. Weissenbacher et al developed NMP (at 37.4°C) for 24 h using a RBC-based solution. Rather than replacing urine output with a crystalloid solution the urine was recirculated. This maintained a constant perfusate volume, maintained better homeostasis, and avoided any electrolyte imbalance caused by excessive urine production.

5 | NORMOTHERMIC MACHINE PERFUSION CONDITIONS

5.1 | Temperature

Hypothermic preservation is based on the principle that at 4°C the rate of metabolism is approximately 10% of the level at normal physiological temperature. This slows the depletion of ATP and also inhibits the degrading processes (phospholipid hydrolysis). However, during static cold storage the gradual depletion of ATP due to the inhibition of oxidative metabolism induces a shift to adenosine monophosphate (AMP) as the predominant nucleotide. This increases levels of adenosine, inosine, and hypoxanthine leading to the formation of lactic acid within the cell. This, in turn, lowers the intracellular pH causing lysosomal instability with the activation of lytic enzymes. The depletion of ATP also reduces a large number of cellular processes. Inactivation of Na⁺/K⁺ ATPase pumps allows the accumulation of calcium, sodium, and water within the cell causing cellular swelling. Fatty acids, lysophospholipids, and diacylglycerol also accumulate within the cell. The binding of transition metals such as iron to their carrier proteins (transferrin, ferritin) is inhibited, which increases the intracellular concentration of free iron. This acts as a strong catalyst for the generation of oxygen free radicals. Furthermore, opening of mitochondrial permeability transition pores (MPTP) results in a reduced metabolic energy status of the cell. Of note, HMP supports a higher level of ATP synthesis than static cold storage and this can be supported further with the addition of oxygen. This may prevent some of the damaging effects of cold ischemia.

It is proposed that NMP at physiological temperatures (36–37°C) can replenish ATP and prevent ischemic injury. However, there is a risk that, due to the high metabolic demand, the conditions could accelerate the breakdown of ATP and cause reversion to anaerobic glycolysis. To reduce the metabolic rate but maintain aerobic metabolism and viability, NMP can be performed using subnormothermic temperatures (21–32°C). However, one consideration is that the oxygen carrying capacity of RBCs is reduced at lower temperatures and may potentially cause kidney injury. Encouragingly, one study found that the outcome of using RBCs at 22°C for 4 h was similar to NMP with Hemopure at 22°C. There was no comparison to NMP at 37°C but both conditions were superior to cold storage.

For more prolonged periods (24 h), the acellular approach using STEEN solution at 21°C supported a lower and more stable vascular resistance compared to blood-based solutions at 37°C. The work by Brásile et al also advocated the use of a subnormothermic temperature of 32°C using their EMS medium.

The gradual transition from cold to warm, described by Minor et al, is also a promising strategy. The protocol involves a 90-min controlled phase of rewarming before NMP at 35°C using STEEN solution. This could protect against mitochondrial and cellular injury by reducing the levels of damage-associated molecular patterns (toll-like receptor 4 and high mobility group box 1 protein) during reperfusion. Furthermore, it could more efficiently optimize ATP and oxygen consumption levels during reperfusion compared to the immediate transition from 4°C.

5.2 | Oxygenation

The majority of reported NMP systems use high levels of oxygen, either 100% or a 95% oxygen/5% carbon dioxide (carbogen) balance to maintain pH delivered through a membrane oxygenator. Typically, the partial pressures of oxygen are supraphysiological (70–75 kPa). The delivery of 100% oxygen during NMP with an acellular-based perfusate is capable of maintaining the PO₂ above 500 mm Hg (66.67 kPa) across a range of temperatures. The NMP conditions described by Brásile and Weissenbacher used a PO₂ that was closer to physiological levels (26 kPa). There is no clear evidence to suggest the optimal oxygen concentration. However, due to the kidney's high metabolic demand and the unique regulation of renal metabolism, a range of concentrations appear to be tolerated without adverse consequences.

6 | MECHANISMS OF ACTION

There is little information on the mechanistic effects of NMP. From experimental evidence we have shown that inflammatory cytokines (IL-6, IL-8) and heat shock proteins (HSPs) are upregulated during a 1 h period of NMP. There is an assumption that these processes help to condition the kidney in preparation for reperfusion. A recent analysis of the transcriptional changes in pairs of human kidneys rejected for transplantation showed some support for this theory. A 1 h period of NMP activated protective stress responses and promoted cell survival and proliferation. This suggests that even a short period of NMP after cold ischemia can promote recovery.
during reperfusion. However, a short duration of NMP is unlikely to demonstrate the effect of repair.

The release of inflammatory cytokines and chemokines into the perfusate during NMP warrants further investigation. This may be related to the type of donor, previous ischemic injury, or caused by the perfusion conditions themselves. The passage of donor leukocytes from the interstitial compartment of the kidney into the circulation may also be a source of inflammation. The incorporation of specialist filters into the circuit could reduce circulating leukocytes and cytokines to reduce inflammation.41,42

Brasile et al demonstrated that a 24 h period of NMP could facilitate recovery of a kidney and upregulate repair processes after ischemic injury. NMP conditions could be enhanced further by the addition of mesenchymal stromal cells.33 The delivery of cellular therapies during NMP is a topical area of research but beyond the scope of this review.

7 | VIABILITY ASSESSMENT

One particular benefit of NMP is that it may allow an assessment of the quality of a kidney prior to transplantation. We have formulated a basic scoring system (1 best, 5 lowest quality) using the macroscopic appearance, renal blood flow, and urine production during 1 h of NMP. It can be calculated in real time and has some association with early graft function.43 Kaths et al proposed that rising levels of lactate and aspartate aminotransferase during NMP could predict reduced function after transplantation.44 These functional and biological parameters are of use but they have not been validated in large cohorts and have not demonstrated the ability to determine primary nonfunction. With the reduced metabolic activity using NMP at subnormothermic temperatures, it may not be possible to make a full functional assessment of the kidney. The pattern of perfusion parameters (flow and resistance) and the measurement of biomarkers such as neutrophil gelatinase lipocalin, liver fatty acid binding protein and inflammatory cytokines (IL-6, IL-8, IL-18) could be helpful in determining the level of injury and in predicting outcome in these cases and for more prolonged periods of NMP. Recently reported new biomarkers, such as extracellular vesicles, microRNAs, and nanoparticles derived from blood/urine or imaging technologies to assess the microcirculation could be applied to assess viability during NMP in the future.45,46

8 | CLINICAL APPLICATION

There is a limited amount of evidence for NMP in clinical kidney transplantation with only one case series and several individual cases reported in the literature. Our group are trialling a 1 h end-ischemic period of NMP after static cold storage in a large multicenter UK trial of DCD kidneys (ISRCTN15821205) with completion expected in 2021.17

Most recently Minor et al used the Kidney Assist™ device and the controlled rewarming approach with STEEN solution. Using this protocol, an ECD kidney was successfully transplanted with immediate graft function.22

There is one report of using NMP technology to avoid any exposure to cold ischemia.47 He et al adapted the Kidney Assist™ device to perfuse a DCD kidney by cannulating the infrarenal abdominal aorta and suprarenal inferior renal cava and transferring to the Kidney Assist™ device while maintaining circulation. The kidney was perfused for 110 min with a RBC-based solution before implantation into the recipient. Circulation was maintained throughout and the recipient had immediate graft function.

Two other groups, one in the Netherlands and the other in Toronto, Canada have started clinical programs of NMP using longer periods of NMP (2–6 h). Results are expected later this year.

9 | SUMMARY AND CONCLUSION

There is an increasing amount of evidence from experimental work in support of using NMP technology in kidney transplantation. Nonetheless, the application of NMP into clinical practice has been limited.

Despite the potential benefit in the application of NMP techniques, logistical requirements are at the forefront. Prolonged periods of NMP, or indeed complete avoidance of cold ischemia, may well be the best conditions in which to preserve a kidney. However, the practicalities of applying these should not be under-estimated.

NMP in kidney transplantation lags behind the development and application of NMP in the liver, lung, and heart. The lack of industry support and the continued development of kidney HMP strategies may be significant factors. Kidneys can tolerate substantial cold ischemic injury and the failsafe use of dialysis in DGF may also reduce the priority of NMP technologies. Nonetheless, in order to significantly increase transplant rates, we need to extend the boundaries of marginal kidney utilization. In particular, the uncontrolled DCD donor pool remains a largely untapped resource. The development of NMP programmes may be essential in delivering this idea by providing an accurate means of organ assessment and a platform for promoting the repair of suboptimal organs. Future clinical trials need to assess the effects of different durations of NMP, NMP conditions and their impact on early and longer-term graft function. Future clinical trials should also include mechanistic analysis, which will be crucial for further development and refinement of techniques.

Ultimately, NMP strategies require significant financial resources and will only be introduced more widely if they can be shown to have significant benefits over current hypothermic techniques.

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**DISCLOSURE**

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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