

REVIEW

Cell therapy during machine perfusion

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SUMMARY

There has been increasing use of organs from extended criteria or donation after circulatory death donors to meet the demands of the transplant waiting list. Over the past decade, there has been considerable progress in technologies to preserve organs prior to transplantation to improve the function of these marginal organs. This has led to the development of normothermic machine perfusion, whereby an organ is perfused with warmed, oxygenated blood and nutrients to resume normal physiological function in an isolated *ex-vivo* platform. With this advance in preservation comes significant opportunities to recondition, repair and regenerate organs prior to transplantation using cellular therapies. This review aims to discuss the possibilities of machine perfusion technology; highlighting the potential for organ-directed reconditioning and the future avenues for investigation in this field.

Transplant International 2021; 34: 49–58

Key words

cell therapy, machine perfusion, preservation, regenerative medicine, transplant

Received: 28 April 2020; Revision requested: 3 June 2020; Accepted: 28 October 2020; Published online: 24 November 2020

Organ preservation technology

Numerous methods of organ preservation have been developed in an attempt to optimize the condition of donor organs prior to transplantation. Conventionally, organs after procurement are flushed with cold preservation solution to rapidly cool and minimize cellular metabolism before transportation on ice to the implanting centre. As the reliance on extended criteria (ECD) and donation after circulatory death (DCD) donors has increased, the focus on improving organ preservation has been revisited. Normothermic machine perfusion has emerged as a novel alternative preservation strategy.

Normothermic machine perfusion

Over recent years, normothermic machine perfusion (NMP) has progressed from an experimental technology to a clinical standard with a number of commercially available devices being adopted into practice for heart, liver and lung transplantation [1]. Nasralla *et al.* reported

recently in *Nature* the first international, multicentre, randomized controlled trial (RCT) of 220 liver transplants investigating NMP as a method for liver preservation. This study demonstrated NMP had significant benefits, compared with static cold storage (SCS), that would both improve liver transplant outcomes and therefore reduce waiting list mortality [2]. Authors reported increased graft utilisation and demonstrated that NMP enables the objective assessment of organ viability prior to transplantation. This exciting, promising study has confirmed NMP as a viable, realistic technology with wider implications for its translation into other solid organ types and *ex-vivo* organ reconditioning as a whole.

The technique required for kidney NMP was first described in 2008 [3]. In this system, a paediatric cardiopulmonary bypass machine and membrane oxygenator is used to provide an *ex-vivo* kidney with oxygenated red blood cells suspended in crystalloid at 37 °C, Fig. 1. This system was first reported as a method of kidney preservation in 2011 [4] and the first single centre clinical trial described a significant

reduction in the rate of DGF from marginal kidneys (5.6% vs. 36%) when compared with historic matched SCS controls [5]. A case series describes how this novel technology has been used to expand the donor pool by facilitating assessment of kidneys for transplant that would have otherwise been discarded [6]. A national multicentre phase 3 randomized controlled trial, scheduled to report in 2021, may validate these promising findings [7].

Organ-directed reconditioning

Normothermic machine perfusion facilitates restoration of cellular metabolism, effectively reviving the organ *ex-vivo* to resume its normal physiological functions [8]. NMP provides a unique opportunity to deliver organ-directed, reconditioning therapies. By establishing an isolated *ex-vivo* platform with a metabolically active organ, therapies targeting ischaemia-reperfusion injury can be delivered directly to the organ and limit systemic recipient exposure [9]. Therapies that have been investigated in the NMP setting include erythropoietin, hydrogen sulphide, carbon monoxide and argon gases, antibiotics and streptokinase [8,10–14]. Furthermore, where NMP could prove to be transformative is the delivery of cell therapies direct to the target site reducing the risk of undesired side effects.

Two cell therapies have garnered the most attention as potential NMP reconditioning agents—mesenchymal stromal cells (MSCs) and multipotent adult progenitor cells (MAPCs).

Mesenchymal stromal cells

Mesenchymal stromal cells are a population of adult, adherent, multipotent, stromal cells of mesodermal origin. They were first identified in 1974 by Friedenstein *et al.* [15], when isolated from bone marrow. There are now a number of different sources, including adipose tissue and umbilical cord blood. Given the variety of sources and isolation protocols, the International Society for Cellular Therapy formulated a minimal criterion for defining MSCs in 2006 [16], Table 1. The exact role of MSCs in normal human physiology has been debated; however, Sachetti *et al.* were able to characterize a population of self-renewing CD146+ cells in the subendothelial layer of bone marrow sinusoids which behaved much like MSCs in culture. The niche that this cell population established *in vivo* represented a subpopulation of pericytes [17]. As pericytes are ubiquitous throughout the vasculature of the body, a putative role for MSCs as regulators of immune homeostasis within the perivascular space has been hypothesized [18].

Mesenchymal stromal cells possess many desirable characteristics for cell therapy in solid organ transplantation [19], Fig. 2. The cells induce these effects through soluble mediators in their secretome and via direct cell-to-cell contact, interacting with the recipient's immune response and repair pathways [20]. This results in decreased antigen presentation, an anti-inflammatory environment and protolerogenic immune profile [19].

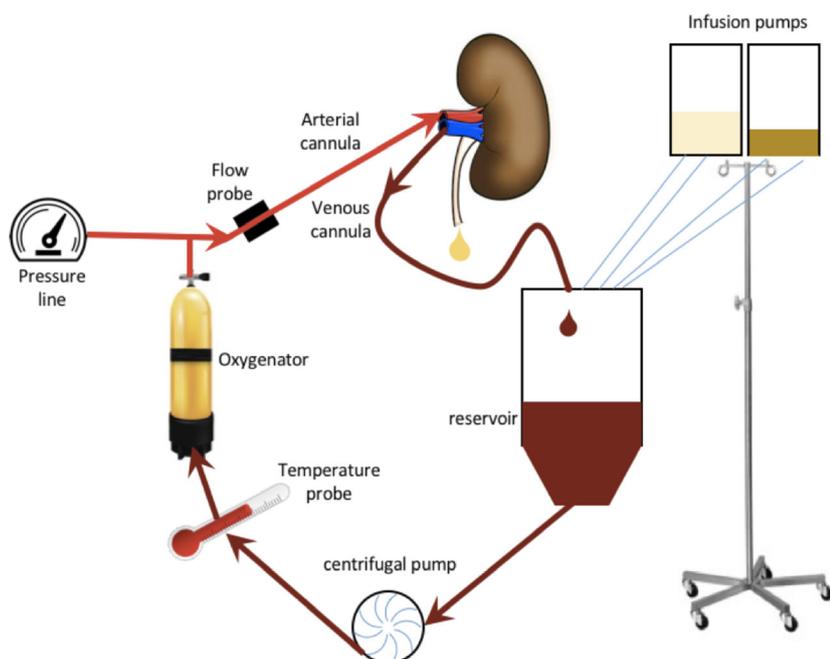


Figure 1 Schematic diagram of kidney normothermic machine perfusion system. Arrows indicate direction of blood flow. The renal artery and vein are cannulated. Urine is collected for measurement and analysis. Real-time recording of renal physiology and samples of blood perfusate and urine can be sampled to monitor reconditioning.

Table 1. Summary of ISCT criteria to identify mesenchymal stromal cell.

(1) Adherence—they must be plastic adherent in standard culture conditions		
(2) Phenotype	Positive (>95%+)	Negative (<2%+)
	CD105	CD45
	CD73	CD34
	CD90	CD14 or CD11b
		CD79alpha or CD19
		HLA-DR
(3) In vitro differentiation potential: osteoblasts, adipocytes, chondroblasts under standard in vitro differentiating conditions		

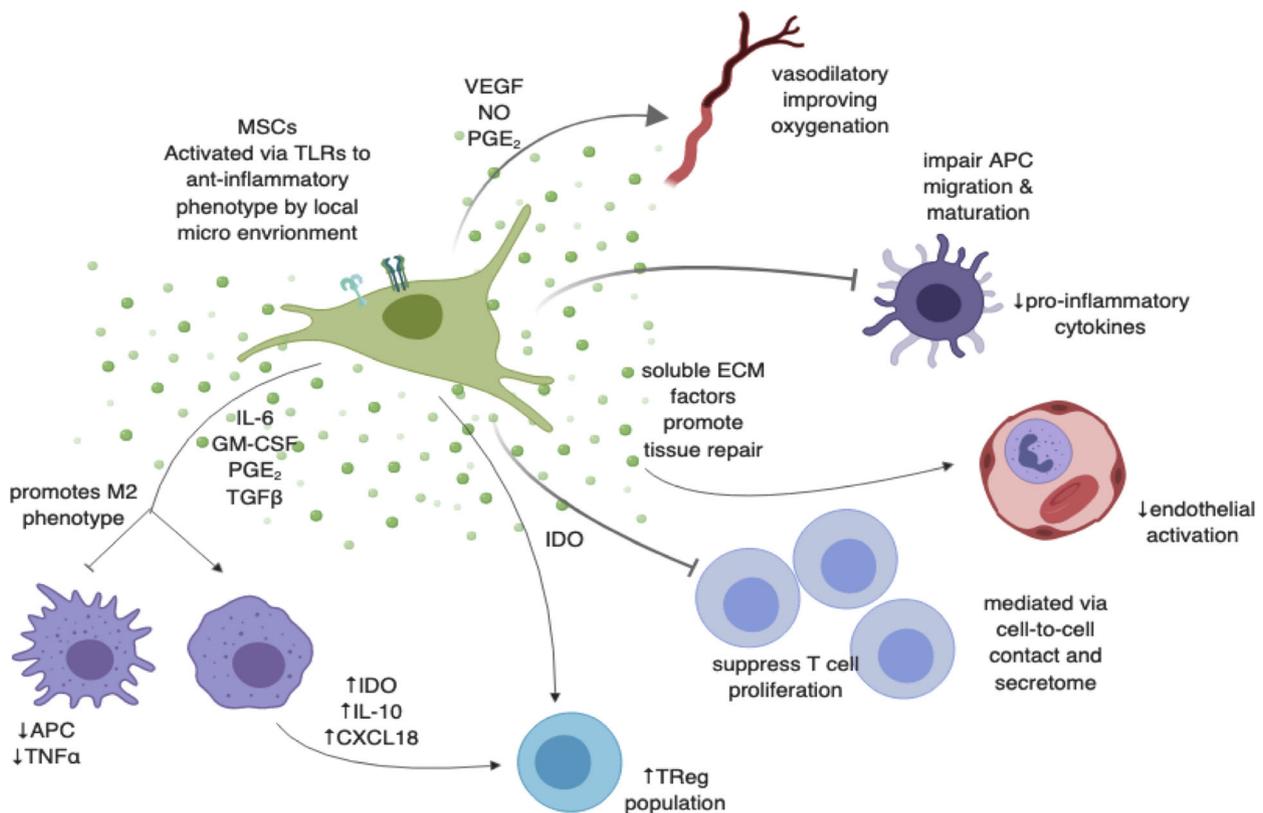
Multipotent adult progenitor cells

Multipotent adult progenitor cells are inherently very similar to MSCs. In 2002, MAPCs were first isolated from mouse bone marrow and this technique has subsequently been replicated for human MAPCs [21]. MAPCs possess very similar properties to MSCs and are essentially a product of different culture and expansion protocols [22]. These protocols are now subject to patent by Athersys in their product MultiStem™.

MAPCs represent a very attractive ‘off-the-shelf’ cell therapy due to the lack of MHC Class II or costimulatory molecules (CD80, CD86 and CD40) leading to a degree of immune privilege [23]. MAPCs release anti-inflammatory, immunomodulatory and protolerogenic cytokines limiting infiltrating pathogenic immune cells and diminishing T-cell proliferation [24]. MAPCs can also redress the balance between pathogenic and protective cytokine production.

Multipotent adult progenitor cells and MSCs have both demonstrated in numerous studies a profound ability to reduce ischaemia reperfusion injury and the inflammatory response associated with solid organ transplantation [25–27]. This effect is predominantly mediated through a paracrine process whereby soluble mediators in the cells secretome aid in the restoration of homeostasis, decrease immune activation and promote repair. As the damage from ischaemia-reperfusion injury is typically at the endothelial/microcirculation interface and mediated by circulating chemokines/cytokines, harnessing the modulatory effects of the MSC/MAPC secretome during NMP should be effective.

To date, there have been no clinical transplants with cell therapy during NMP, but there have been a number

**Figure 2** Immunomodulatory role of mesenchymal stromal cells in solid organ transplantation setting.

of studies in preclinical models using discarded human organs and in large animal models.

Cell therapy in kidney machine perfusion

The feasibility of administering MSCs to kidneys during NMP has been investigated in a number of porcine models. Labelled, human, adipose-derived MSCs have been delivered to porcine kidneys during NMP to investigate their fate [28]. This study delivered increasing doses of MSCs (0 , 10^5 , 10^6 or 10^7) to single porcine kidneys via the arterial sample port and continued perfusion for 7 h. The authors demonstrated that during NMP, MSCs remained intact, with a large proportion becoming resident in the kidney. They were predominantly localized to the lumen of the glomerular capillaries, presumably taking the path of least resistance before becoming lodged within the microvasculature. The human cells did not appear to migrate into the parenchyma of the pig kidney. The number of circulating MSCs in the perfusate also decreased during NMP indicating the cells were either becoming resident in the kidney or being lysed by the process. This potentially could be due to exposure of cells to a centrifugal pump with associated high perfusion pressure to which they are poorly adapted due to their adherent nature in culture.

The distribution of MSC within the kidney was similar to that observed in a study investigating intra-arterial delivery of porcine MSCs in an *in vivo* model of ischaemia reperfusion [28]. In this study, MSCs were found both within glomerular capillary networks and within peritubular capillaries. This localization was independent of cell viability suggesting a passive retention mechanism as opposed to active homing. The long-term residence of the cells delivered in this localized manner also revealed the cells did not persist beyond 14 days.

A more recent study by the same group compared the immunomodulatory effect of different MSC sources during kidney NMP—adipose derived (A-MSC) vs bone marrow derived (BM-MSC) [29]. These were human cells delivered to a porcine kidney. They were unable to discern specific differences between the two MSC sources. Neither MSC treatment improved renal function parameters such as creatinine clearance, fractional excretion of sodium or urine output. However, there was a reduction in injury biomarkers: N-acetyl- β -D glucosaminidase (NAG), lactate dehydrogenase (LDH), neutrophil gelatinase-associated lipocalin (NGAL) and endothelin-1 in the perfusate. The cytokine response

was paradoxical; despite the reduction in injury biomarkers, there was a significant increase in perfusate IL-6 and IL-8—both traditionally proinflammatory cytokines. Understanding this phenomenon is difficult due to the possible xeno-interaction, but the data support the potential for cellular reconditioning during NMP.

The effect of kidney NMP perfusion fluid constituents on the viability of MSC has been investigated. This study stimulated thawed and fresh MSC with perfusate and demonstrated MSC behaviour can be adversely affected by the conditions of the perfusion fluid [30]. Data showed that thawed MSCs have reduced viability in perfusion fluid, with reduced adherence to endothelial cells when compared with fresh MSCs. These effects were mediated by reactive oxygen species (ROS) formed during the thawing process. During kidney NMP, there is a significant burden of ROS production which could potentially damage mitochondria further. As the thawing process is inevitable, adjustments to perfusion fluid may be required to improve MSC survival. However, the study also demonstrated that MSC proliferation and their secretory profile were unaffected by culture with perfusion fluid suggesting that, although their adherence and viability are reduced, MSCs could maintain their therapeutic effects in NMP.

Our group described the first successful evidence of reconditioning in human kidneys with MAPC therapy during NMP [31]. This study utilized pairs of discarded human kidneys that were perfused simultaneously for 7 h. After 1 h of perfusion, kidneys were randomized to receive 50×10^6 MAPCs delivered via the renal arterial cannula or to vehicle-treated control. The paired analysis revealed that kidneys treated with MAPC therapy had increased urine output and decreased production of kidney injury biomarker NGAL. These two findings were consistent with evidence of potential reconditioning as clinical studies on NMP have demonstrated that kidneys which produce more urine and have lower levels of NGAL have better post-transplant outcomes [6]. This reconditioning seems to be mediated through changes in circulating cytokines and immune mediators towards an anti-inflammatory profile (decreased IL-1 β , increased IL-10, increased indoleamine-2, 3 dioxygenase activity). This profile was less likely to induce neutrophil chemotaxis and endothelial cell activation. MAPC therapy resulted in improved microcirculation and perfusion of the kidneys when assessed using contrast-enhanced ultrasound during NMP. MAPCs were found within the glomerular capillaries and peritubular capillaries throughout the kidney with evidence of cells crossing the vascular endothelium to reside in the

perivascular space. The study was limited in its ability to infer what impact the MAPC reconditioning might have upon reperfusion in the recipient. However, it was able to demonstrate that it is feasible to use a cryopreserved, 'off-the-shelf' cell therapy product within the time constraints of a deceased donor transplant setting to achieve reconditioning of marginal kidneys, Fig. 3.

One of the most interesting studies to date investigating MSC in kidney NMP was actually performed at subnormothermic temperatures. This study utilized an acellular perfusate in a system termed 'exsanguinous metabolic support' that restores oxidative metabolism at 32 °C and investigated human MSCs in DCD discarded kidney pairs [32]. Initially, the authors undertook a dose escalation study to establish the maximal tolerated dose of cells as determined by the oxygen consumption and renal physiology, and this was deemed to be 1×10^8 cells. Five pairs of kidneys were then perfused with this optimal dose for 24 h; one kidney was an untreated control and the other received the MSCs via infusion in the renal artery. The authors reported that kidneys treated with MSCs demonstrated increased ATP synthesis, normalization of the cytoskeleton and

increased mitosis in the renal epithelium indicating a degree of regeneration. There was also a significant increased production of growth factors that are associated with regenerative pathways after ischaemic insults; epidermal growth factor (EGF), fibroblast growth factor (FGF-2) and transforming growth factor alpha (TGF- α). It is unclear if these factors were produced by the MSCs or the kidney itself. Overall, the MSC-treated kidneys had a generally reduced inflammatory state with specific reductions in multiple cytokines and chemokines. It is possible that this more marked improvement in the MSC-treated kidney than seen in previous MSC studies might be due to the prolonged perfusion time (24 h)—giving the cells more time to effect change within the kidneys. Interestingly, in this subnormothermic acellular perfusate model there was no migration of MSCs into the parenchyma and the authors were able to recover >95% of infused cells. The fact there was such significant benefit achieved without cell residency suggests that the cells are able to recondition effectively using predominantly paracrine mechanisms in this system 66. It may also be that normothermic temperatures are required for MSCs to diapedese and migrate through

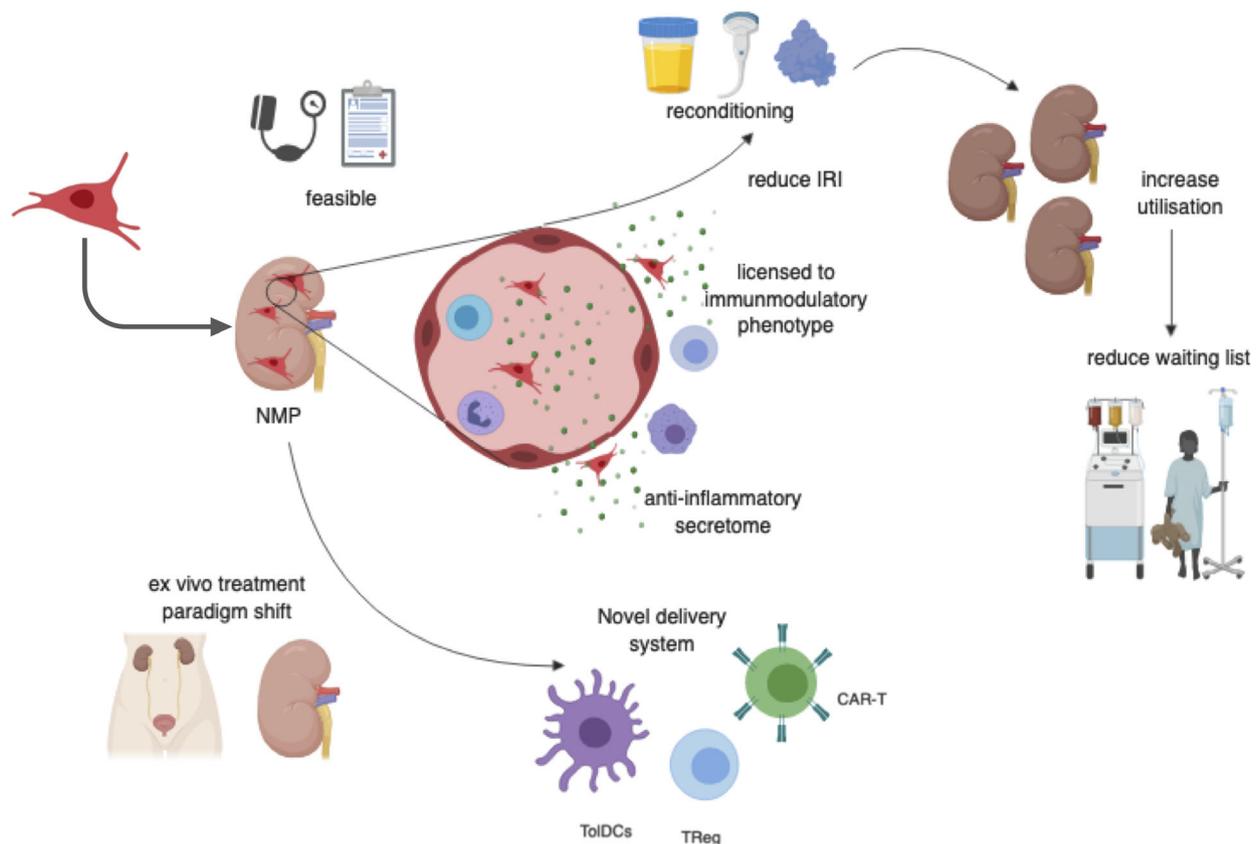


Figure 3 Proposed benefit of cellular therapies in normothermic machine perfusion of kidneys for transplantation.

the vascular endothelium. This is the only study to demonstrate possible epithelial cell regeneration during machine perfusion. When considering marginal kidneys with a high incidence of acute tubular necrosis and subsequent delayed graft function, the ability to promote epithelial cell regeneration is a very attractive concept for improving graft survival and increasing utilization. The authors of the study have also been able to demonstrate that stable kidney perfusion for up to 3 days using acellular machine perfusion, thus providing a much longer window of opportunity for repair and regeneration with cell therapies.

These early studies of cell therapy in kidney NMP have highlighted the advantage of the paired kidney model, whereby an organ that has undergone the same ischaemic/inflammatory insult as the sister kidney is used as control to measure the impact of the therapy. This is important to take into account, as MSCs/MAPCs are licensed by their microenvironment to effect subsequent reconditioning unlike a simple pharmacological agent. The mechanistic effects described may inform studies of cell therapy in NMP of other organs. These studies have also demonstrated that it is important to investigate within species, for example human cells in a human discard model, in order to fully evaluate the potential for reconditioning. Future studies should concentrate their efforts on this approach.

Cell therapy in liver machine perfusion

Studies of MAPC therapy in liver NMP have also recently been reported. Laing *et al.* utilized human discarded livers ($n = 6$) and human MAPCs that were delivered into the either the right portal vein (PV) or right hepatic artery (HA) via a catheter placed over a guide-wire during back-benching [33]. They were able to demonstrate the MAPC cell therapy is feasible during liver NMP and described a novel technique to secure infusion. The MAPCs were fluorescently labelled prior to perfusion and could be tracked within the liver. No MAPCs were found in the left lobe of the liver, indicating that the cells take up residence on a first pass and do not continually circulate, unlike in kidney NMP. Interestingly, the infusion site determined where the cells resided—if cells were infused via the PV the MAPCs tended to arrest in the sinusoidal channels, whereas if delivered via the HA, the cells seemed to home and take up residence in the parenchyma by migrating through the endothelium. The authors also found a change to the cytokine profile of the MAPC-treated livers and proteomics analysis revealed that MAPCs likely secrete

cytokine, chemokines and growth factors that regulate and interact with a number of IRI cytoprotective pathways. These are very promising results, but it is more difficult to tease out the objective effect of MAPC therapy in liver perfusion when there is no paired control. Here, the authors compared cytokine profiles to a historical control cohort of discarded livers and clinically transplanted livers to investigate the impact of MAPC therapy. Overall, this represented a heterogeneous cohort of donors and the timing of cell infusion differed between treatment groups making it more difficult to draw definitive conclusions on the MAPC ability to recondition a liver in the NMP setting.

MAPCs have also been used in a clinical liver transplant study [34]. MAPCs were infused into the donor liver intraoperatively via the portal vein prior to reperfusion and a secondary dose administered via systemic intravenous infusion on day 2. There were no reported adverse effects of MAPC administration. Interestingly, the recipient's leucocyte population was profiled and revealed a marked increase in regulatory T cells on day 4 post-transplant. Associated with this was a downregulation of MHC class II expression by CD14⁺ monocytes thought to be associated with diminished immune activation.

It is clear that there is real potential for immunomodulation and reconditioning with MAPC therapy in liver NMP and to take this forward, further studies including a larger cohort of HA-delivered MAPC therapy in NMP, with adequate controls, will be interesting to evaluate these preliminary findings further. This could also investigate the potential underlying mechanism for inducing a tolerogenic immune profile in recipients.

Cell therapy in lung machine perfusion

In *ex-vivo* lung perfusion (EVLV), there have been a number of studies investigating both MSC and MAPC therapy. Lung perfusion commonly requires only an acellular perfusate, and oxygenation is provided via mechanical ventilation. A porcine EVLV pilot study demonstrated that an intravascular infusion of 150×10^6 umbilical cord-derived MSCs resulted in a decrease in circulating IL-8, increased VEGF production and diminished neutrophil chemotaxis [35]. It has previously been demonstrated that a proinflammatory EVLV perfusate cytokine burden correlates with poorer lung transplant outcomes [36]. If cell therapy can modulate the cytokines in the perfusate resulting in a less inflammatory organ, this may result in better post-transplant function and graft survival.

In 2014, a human discard lung EVLP study investigated the use of human BM-MSCs during 4 h of perfusion and demonstrated a reduction in alveolar fluid retention and improved lung function; however, there was no difference in cytokine profiles as seen in the previous study [37].

A study investigating MAPC therapy in pig lung EVLP reported interesting findings when cells were delivered intrabronchially [38]. There was no functional improvement with MAPC EVLP therapy; however, there was decreased neutrophilia on bronchoalveolar lavage (BAL) and a significant decrease in proinflammatory cytokines TNF- α , IL-1 β and IFN- γ . A similar human discard lung EVLP model has also been used for investigating MAPC therapy. In this series of four lungs, the left lower lobe received MAPCs intrabronchially and the right lower lobe was used as a vehicle-treated control for comparison [39]. This demonstrated the MAPC-treated lobe had a significant reduction in histological markers of ischaemic damage and BAL fluid had a reduced number of macrophages, neutrophils and eosinophils. The authors postulated that if transplanted, this reconditioning effect could result in a reduction in primary graft dysfunction, a major hurdle in utilizing marginal organs in lung transplantation.

In lung perfusion, there has also been some success investigating derivatives of MSC therapy such as extracellular vesicles (EVs) or conditioned media. These are potentially attractive options as it harnesses the effects without the need for the cells themselves. EVs are reported to be immunoprivileged; contain mRNA, miRNA and growth factors potentially capable of mediating reparative processes required during NMP [40]. A discard human lung study compared EVs derived from human MSCs (EV-MSC) with EVs derived from human fibroblasts [41]. The EV-MSC-treated lungs had improved alveolar fluid clearance and decreased weight gain during EVLP in a dose-dependent manner. This was similar to the group's previous findings when using whole cells during EVLP [37]. Investigating the reconditioning potential of cell therapy derivatives or other methods of delivering the MSCs active secretome may 'de-risk' the use of MSCs and MAPC, where there are naturally concerns over the fate of these cells if they are intentionally implanted in immunosuppressed recipients.

Other cell therapies with potential for investigation in NMP

Mesenchymal stromal cells or MAPCs are not the only cell therapy that may have advantageous actions in

transplantation. A number of other cell types have previously been investigated for systemic delivery in solid organ transplantation, and their benefits could be coupled with NMP delivery in the future. These include T regulatory cells (Tregs) and human amniotic epithelial cells (hAECs).

The ONE Study and TRACT trial investigated the safety, feasibility and therapeutic effects of administering isolated and expanded polyclonal patient-derived autologous Treg cells to kidney transplant recipients [42,43]. This was also carried out in the context of liver transplants in the ThrIL trial [44]. No adverse effects were observed as a result of Treg infusion, although methods of successfully and reproducibly manufacturing Tregs are yet to be fully optimized. It is unclear how Tregs in a leucocyte-depleted NMP system would mediate their immunomodulatory effect; however, if the cells can engraft and remain resident in the kidney throughout transplantation and reperfusion there may be benefit. Unlike with MAPCs and MSCs, the intention with Tregs is to facilitate local delivery and not repair the organ during NMP *per se*. The hypothesis being that tolerance induction would be more effectively facilitated than during systemic delivery. Proof of concept in a preclinical model remains to be established.

Human amniotic epithelial cells (hAECs) are derived from placental tissue and are reported to possess the capacity to prevent injury and help in the repair of lung damage through the modulation of inflammatory environments [45]. hAECs are currently being investigated as a therapeutic option during EVLP with results from *in vitro* studies demonstrating reduced inflammatory cytokine production and endothelium activation [46,47]. A dose escalation study has shown no long-term adverse effects from hAEC administration [48].

Future directions

The main hurdle to regulatory approval of cell therapy in machine perfusion is the knowledge gap around the fate of cells following transplantation. If delivered systemically *in vivo*, MSC/MAPCs are only present transiently for approximately 72 h [49]. However, if cells are delivered through NMP would this increase their ability to engraft and remain resident in the target organ for longer? In an immunosuppressed population, there remains a real concern over malignant transformation and sensitization (anti-HLA antibodies for instance)—clarification in animal NMP and long-term follow-up transplant models will be required to bridge

the gap. These experiments would not only answer the safety questions, but also enable a better understanding of the durability of reconditioning achieved during NMP. Long-term follow-up transplant studies will also provide better understanding of the potential for anti-HLA antibody production against therapeutic cells.

Reassuringly, MSCs/MAPCs have been used in a number of phase I/II clinical studies as a systemic therapy in kidney transplantation [50–57]. However, this success has not translated into widespread clinical use and there are important lessons for investigators transitioning cell therapy in NMP. Decisions regarding the cell source and whether it is an autologous or allogeneic therapy impact on ease of use. Currently, many cell therapies are manufactured in small batches by academic institutions. For a cell therapy to meet the requirements of international deceased donor transplant programs an off-the-shelf, nonimmunogenic, allogeneic product will be necessary. The ideal product would also require no preinfusion culture or manufacture steps, as this would again limit its use to specialist institutions. The cells also need to be amenable to scaled-up manufacture.

The timing of MSC infusion relative to the organ transplant plays an important role in the potency of the cell's immunomodulatory effect [58–63]. Many studies have concluded that cell delivery prior to transplant may be best, NMP facilitates this. But future studies may need to investigate a combined approach whereby, a secondary dose is delivered following the transplant to further promote tolerance. Understanding the cell's mechanism of action in this setting will be important for deriving the correct clinical end-points for investigation.

Looking to the future of organ transplantation, machine perfusion may have a valuable role to play in organ regeneration by providing the optimal conditions for bioengineering. A recent paper demonstrated we can now preserve liver grafts for up to 1 week using machine perfusion [64]. This extended time-frame may facilitate opportunities for other stem cell therapies that require an increased therapeutic window

to result in repair and regeneration of marginal organs. This potential has been realized in a pig model of lung transplantation, whereby EVLP was used to optimally decellularize and repopulated the scaffold with targeted autologous cells. The lungs were subsequently successfully transplanted back into pigs resulting in good function and no evidence of rejection [65].

Conclusion

The marriage of cellular therapy and solid organ transplantation to modulate the recipient's immune response has been keenly investigated in many settings over the past decade. However, there have been numerous barriers preventing the translation of this therapy into widespread use. The advent of normothermic machine perfusion provides a solution to many of these problems. Preliminary studies investigating this avenue with MSCs/MAPCs have demonstrated promise; successfully targeting cells to the organ with no adverse effects and evidence of reconditioning. As machine perfusion and cell therapy technology advance, so do the possibilities for synergistic organ treatments.

Funding

This study was supported by Kidney Research UK, the National Institute for Health Research (NIHR) Newcastle Biomedical Research Centre and the NIHR Blood and Transplant Research Unit in Organ Donation and Transplantation at the University of Cambridge, in collaboration with Newcastle University and in partnership with National Health Service Blood and Transplant (NHSBT). The views expressed are those of the authors and not necessarily those of the National Health Services.

Conflict of interest

The authors have declared no competing interests.

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