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# Opportunities for Therapeutic Intervention During Machine Perfusion

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## Abstract

**Purpose of review—**There is a vast discrepancy between the number of patients waiting for organ transplantation and the available donor organs. *Ex vivo* machine perfusion (MP) has emerged in an effort to expand the donor pool, by improving organ preservation, providing diagnostic information, and more recently, acting as a platform for organ improvement. This article reviews the current status of MP with a focus on its role in organ preconditioning and therapeutic interventions prior to transplantation.

**Recent findings—**MP has allowed longer organ preservation compared to conventional static cold storage and allowed the use of organs that might otherwise have been discarded. Moreover, experimental studies have investigated the role of MP in reducing ischemia reperfusion injury of lungs, kidneys and livers by applying mesenchymal stem cells (MSCs), anti-inflammatory agents, cytotopic anticoagulants, and defatting cocktails.

**Summary—**MP has opened a new era in the field of organ transplantation and tissue medication.

### Keywords

ex-situ machine perfusion; transplantation; preservation; ischemia reperfusion injury

## INTRODUCTION

There are currently 120,000 patients on the wait list for a lifesaving organ transplant in the United Network, but only about 27,000 organs are transplanted a year (https://optn.transplant.hrsa.gov/). At the same time, donor organs are discarded with an average rate of 17.27 for poor quality, having been deemed non-transplantable (http://www.aopo.org/). As the donor population ages and the prevalence of obesity and diabetes increases, donor

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Compliance with Ethical Guidelines

**Conflict of Interest**

Negin Karimian and Heidi Yeh declare no conflict of interest.

**Human and Animal Rights and Informed Consent**

This article does not contain any studies with human or animal subjects performed by any of the authors.

organ quality is likely to further decrease in the coming years[1]. Thus, machine perfusion (MP) has attracted increasing attention, over the past decade, as an alternative or adjuvant to conventional static cold storage (SCS), both to provide superior preservation [2] and to provide data to more accurately evaluate marginal organs [3–6]. This paper will briefly review the preservation and diagnostic advantages of MP, but will focus on the potential for actually improving organ quality after procurement (summarized in table 1).

## Improved Preservation

The initial concept of MP started in the 1960's as non-oxygenated perfusion of organs at 0–10° C, or hypothermic machine perfusion (HMP) (7), and is thought to improve on SCS via multiple pathways. During SCS, the graft suffers from ischemia, which results in accumulation of reactive oxygen species (ROS) causing extra damage to the organ upon reperfusion [7, 8], and simple HMP may help wash out some these waste products. Studies show that HMP of intestine results in less inflammation and ulceration compared to SCS [9]. Twelve hour HMP of pig donor hearts has shown better preservation of myocardial fibers, less mitochondrial damage and less ATP depletion compared to the same duration of SCS [10]. In addition, the no-flow state of SCS may disrupt nitric oxide (NO) homeostasis in the microcirculation, by removing the constant flow normally seen by endothelial cells [11]. Non-oxygenated HMP has been shown to protect NO signaling pathways both in the renal cortex and the renal artery after transplantation [12]. Non-oxygenated HMP of kidney grafts from donors after cardiac death (DCD) is now widely used clinically and has also been used in a clinical trial transplanting marginal liver grafts turned down for transplant in the originating Donation Service Area with improved results compared to historic controls [13].

However, even at 0–10° C, there is low level metabolic activity that may be better supported with oxygenation, which has been used in other studies [14]. Rat and pigs receiving kidneys treated with oxygenated HMP show faster return to normal serum creatinine post-transplantation compared to SCS [15, 16]. Similarly, pig and rat livers actually replenish tissue adenosine triphosphate (ATP) during oxygenated HMP, resulting in improved preservation of the bile ducts [17, 18]. Oxygenated HMP has also been used in a clinical trial transplanting DCD livers, with good results [19, 20].

## Viability Assessment

At 0–10° C, minimal metabolic activity means that even oxygenated HMP provides limited functional information, which could be important in making clinical decisions to use marginal organs or not. Both subnormothermic (SNMP) at room temperature (~21° C), and normothermic (NMP) at 37° C can provide such data. For example, kidneys initially declined for transplantation because of poor flush have been placed on NMP and based on reaching defined thresholds for macroscopic appearance, renal blood flow, and urine production, both were subsequently transplanted into patients. One patient had immediate graft function and the other required transient dialysis, but both were off dialysis with Cr < 2 mg/dL 3 months post transplantation [21]. Similarly, a liver declined universally for poor flush was accepted for research, and because of excellent bile production and lactate clearance on 26 hours of NMP, subsequently transplanted into a patient, who went on to have a fairly unremarkable recovery [22]. The largest clinical experience using NMP

parameters to determine transplantability of otherwise discarded organs is in lung transplantation. Lungs declined for poor FiO2 or radiographic infiltrates were placed on NMP combined with airway ventilation. Decisions to transplant or not were based on oxygenation, hemodynamic and respiratory parameters, and macroscopic appearance [23].

More detailed organ assessments have been proposed, but not yet well-validated by clinical post-transplantation outcomes. It has been suggested that measurement of urinary biomarkers such as endothelin-1 (ET-1), neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) during NMP of discarded human kidneys in combination with perfusion parameters such as renal arterial flows and serum creatinine levels could be valuable in selecting which marginal kidneys are suitable for transplantation [4]. A variety of criteria have been suggested for assessment of human livers during MP, including the cumulative amount and the rate of bile production during NMP [3] or energy charge during SNMP [24]. In an experimental study on NMP of porcine DCD hearts, one group found that stroke work, ejection fraction and minimum rate of diastolic pressure change were more reliable predictors of organ viability and function than coronary vascular resistance (CVR), myocardial oxygen consumption ($MVO_2$) and venoarterial lactate difference [25]. Barlow *et al.* were the first to show the feasibility of *ex-situ* NMP to assess the viability and function of declined human pancreas for transplantation based on pancreas blood flow, exocrine function indicated by insulin secretion and endocrine function indicated by lipase and amylase secretion [6].

## MACHINE PERFUSION: A Platform for Organ Treatment

Most interesting from these detailed studies is the suggestion that simple SNMP or NMP can actually improve organ quality, in addition to preventing the slow decay that occurs during SCS and providing functional information. Of course, most studies have compared the outcomes of MP to equal SCS time, rather than to minimal or no cold ischemia time, due to the impossibility of immediate implantation in clinical transplantation; it is therefore difficult to assess whether changes during the period of MP represent an actual improvement in the baseline quality of the organ or simply an indication of the resuscitation that would otherwise occur after transplantation and reperfusion. However, it is likely that stresses on the organ are less on MP, where physiologic support is provided in a controlled manner, whereas clinical reperfusion is associated both with the normal demands placed on the organ by the recipient corpus, as well as any additional hemodynamic or metabolic abnormalities attendant with end stage organ failure.

For example, metabolomics profiling of discarded human livers placed on SNMP show improved energy cofactors and reversal of ischemia-induced disturbances in lactate metabolism over the 3 hours on pump [26]. Small series of clinical transplantation of suboptimal liver grafts after resuscitation by NMP resulting in better than expected outcomes compared to historic experience are also suggestive [27, 28]. Similarly, an experimental study of NMP on DCD rat hearts demonstrated beneficial effects on recovery of electrical activity and contractility of the myocardial tissue [29]. Controlled oxygenated rewarming (COR) technique combines 30 minutes HMP, 30 minutes slow warming to 20° C, and then 30 minutes of SNMP, and has been applied after similar periods of SCS to both

porcine livers and kidneys procured after cardiac death [5, 30]. In simulated reperfusion models, COR kidneys showed better creatinine clearance, decreased expression of inflammatory molecules, decrease endothelial cell activation, and improved tubular cell integrity compared to untreated kidneys. COR livers similarly had increased bile production, decreased histologic injury and caspase-3 activation, decreased lipid oxidation products, and decreased inflammatory gene expression. In a clinical trial of COR, 6 livers transplanted after COR resulted in about half the level of serum transaminases in the recipients and better graft and patient survival compared to historic controls, although none of these trends was statistically significant [31].

More exciting than the resuscitation of organs by simple MP, at any temperature, is the potential to apply specific therapeutics to improve organ quality during MP, especially NMP. The main advantage of treating organs during MP rather than in the donor or in the recipient is the ability to target different organs with different therapies and avoid systemic side effects. In addition, interventions to decrease immunogenicity or prevent recurrent disease could also be carried out during MP, to decrease the need for immunosuppression or improve long-term outcomes separate from the physiologic quality of the organ at the time of transplantation. It is possible that in the case of genetic defects, organs could be removed prior to end stage organ damage setting in, undergo ex vivo gene therapy during MP, and then be auto-transplanted back into the recipient, obviating the need for lifelong immunosuppression.

## Lung

In lung transplantation, this can be as simple as adding anti-coagulants and thrombolytics to the perfusate to rescue an organ that was initially untransplantable because of pulmonary embolus and then transplant the lungs after lysing or surgically removing the clot [32]. Lungs can also be “dried out” during NMP, allowing transplantation of lungs that would normally be declined for pulmonary edema [33]. Airway manipulation has also shown promise in improving lung function. Airway pressure release ventilation (APRV) during NMP of porcine DCD lungs improves alveolar recruitment, improved oxygenation, improved hemodynamics and attenuation of barotrauma. Lungs treated with this technique had significantly higher compliance and reduced edema when compared to lungs receiving conventional ventilation during NMP [34]. Another reason for declining donor lungs is infection and pneumonia. Twelve hour NMP with high dose antibiotics not only decreased bacterial counts in the bronchoalveolar lavage, but also improved pulmonary oxygenation and compliance and reduced pulmonary vascular resistance to a degree not seen with simple HMP. This corresponded to decreased levels of endotoxin and other inflammatory proteins in the perfusate [35]. We can see that interventions to rescue untransplantable organs MP are likely to be customized to the issues of the individual donor.

On the other hand, graft injury post-procurement is common to all organs; one universal cause of graft dysfunction post-transplantation is ischemia reperfusion injury (IRI), and marginal organs will be even more vulnerable to IRI. The adenosine A2A receptor downregulates inflammation, including suppressing CD4+ and CD8+ T cells [36] and increases endothelial cell nitric oxide [37]. The addition of adenosine A2A receptor agonists

during NMP of DCD porcine lungs resulted in significantly higher $PaO_2/FIO_2$ ratios of > 400 mm Hg after transplantation compared to adding the agonist vehicle alone [38]. Notably, addition of the agonist at reperfusion showed little advantage over adding it during NMP alone, and the improvement in oxygenation post-transplant was far greater than that seen pre-transplant, suggesting that pre-treatment during NMP yielded post-transplant benefit not seen during NMP. β2-adrenoreceptors are another potential target NMP for decreasing IRI, as β2-adrenoreceptors are distributed throughout the lung and mediate relaxation of pulmonary artery. Moreover, β2-adrenoreceptor agonists enhance alveolar fluid clearance, and mitigate acid induced lung injury in animal models [39]. In a canine model of DCD lung donation, β2-adrenoreceptor agonists in the airspace during NMP of lungs resulted in higher lung ATP levels, lower airway pressures, higher pulmonary compliance, lower pulmonary vascular resistance, and less pulmonary edema [40].

Mesenchymal stem cells (MSCs) are multi-potent, self-renewing cells that could proliferate and differentiate into many different cell lines. The therapeutic application of MSCs has been studied in reducing the IRI after organ transplantation, although their mechanism of action is still unclear [41, 42]. Twelve hour NMP of DCD porcine lungs with intravascular delivery of MSCs has shown increased levels of vascular endothelial growth factor (VEGF) in lungs and reduced levels of interleukin-8 (IL-8), which is a pro-inflammatory cytokine associated with reperfusion injury [43].

Finally, one group has also treated lungs with adenovirus carrying the gene for the anti-inflammatory cytokine IL-10 and noted decreased expression of inflammatory cytokines such as IL-1, TNF-alpha, and IL-6, as well as attenuation of the allo-immune response post-transplant [44]. Long term expression of other anti-inflammatory or immunoregulatory molecules could be another step forward in the ever present journey towards tolerance, or transplantation without the systemic immunosuppression that carries a multitude of side effects, in addition to the risk of infection and malignancy.

## Kidney

The potential benefits of MSCs in reducing reperfusion injury have also been studied in kidneys. The administration of luciferase-lacZ reporter transgenic MSCs to DCD rat kidneys during 60 minutes of SNMP improved post-transplant survival from 50% in kidneys undergoing SNMP without MSCs to 75% post-transplantation survival. MSC treated kidney recipients had better renal function with improved renal function compared to 50% survival rate of the rats that received a kidney without MSC treatment [45]. Interestingly, lacZ staining was visible for only 24h along the renal tubules and then was undetectable, suggesting an early effect, not requiring the persistence of MSCs. No tumor formation as a result of MSC treatment was found in recipients followed out to 3 months.

It has been shown that IRI activates many signaling pathways and upregulates many genes that eventually lead to complement cascade activation and therefore cell apoptosis and death [46]. In one study, mouse kidneys were flushed with and preserved in a solution containing a cocktail small interference RNA (siRNA) targeting complement 3, RelB, and Fas expression. Recipients of the kidneys treated with siRNA had longer survival, lower creatinines, and lower levels of pro-inflammatory cytokine expression, while the donor

kidneys had less cell apoptosis, compared to the recipients of the kidneys without siRNA pretreatment [47]. Although this did not require MP per se, it demonstrates the promise of delivering siRNA to organs ex situ and altering gene expression to improve post-transplant outcomes.

One of the complications after transplantation of marginal kidneys is the risk of microvascular thrombosis as a result of IRI mediated endothelial cell injury, platelet activation, and edema [48–50]. Although systemic anti-coagulants such as heparin can be used during organ transplantation to prevent such complications, this is not always effective and systemic heparin carries the risk of bleeding, as well. Thrombalexin is a novel cell binding thrombin inhibitor and pre-treatment of porcine kidneys during HMP resulted in tethering of thrombalexin to the kidney endothelial cells. This was associated with improved perfusion, decreased lactate levels, and decreased fibirin generation compared to kidneys receiving simple perfusate or perfusate containing an inactive form of thrombalexin. [51]. Again, MP provides the luxury of local treatment of the organ and avoiding systemic side effects.

## Liver

Currently, cirrhosis secondary to chronic hepatitis C infection (HCV) is one of the most common indications for liver transplantation [52]. The inevitable reinfection of the donor liver by HCV and the more rapid course of HCV to end stage liver disease has significantly limited post-transplantation outcomes [53]. One group has utilized NMP to deliver an RNA that sequesters and inactivates miR-122, which is required for HCV to establish infection in the hepatocyte, to porcine livers. In this proof of concept study, they showed efficient delivery of the therapeutic RNA, inactivation of miRNA-122, and decreased HCV infection post-transplant [54]. With the advent of highly effective HCV treatment with low side effect profiles in the past 2 years, pre-treatment of livers against HCV re-infection may not be a critical player in our anti-HCV armamentarium, but the ability to introduce RNA into donor livers on MP and influence post-transplantation gene expression opens up a wide array of potential therapeutics, including those mentioned in previous sections.

Another problem peculiar to liver transplantation is the need for size matching, which is not so critical in kidney transplantation. This is mitigated on the one hand by a relatively small livers' ability to regenerate and grow to the necessary size, and an anatomy conducive to reducing the size of a relatively large organ [55]. Graft size reduction, whether by anatomic splitting into to two usable grafts or simple removal of excess parenchyma without regard to the usability of what is removed, is technically complex and frequently performed post-procurement at the recipient institution. This can add to the cold ischemia time and contribute to the higher complication rate of transplanting reduced size livers compared to whole liver grafts. NMP has been used in a pig model of liver transplantation, simulating *in-vivo* liver resection and improving preservation. This strategy resulted in better post-transplantation outcomes when compared with the outcomes of livers that underwent reduction in SCS [56]. There have been reports of hepatocyte replication and biliary epithelial cell regeneration being detected during NMP [57, 58], and one can imagine also growing livers during NMP to either jumpstart the growth of a small liver prior to

transplantation or a longer-term perfusion actually increasing the size of a small segment prior to transplantation.

As in other organs, IRI is a problem, sometimes resulting in primary non-function (PNF) or early allograft dysfunction (EAD), the former of which requires immediate transplantation and the latter of which is associated with poorer long term outcomes. In a pig model of liver transplantation, supplementation of SNMP perfusate with the vasodilators and anti-inflammatory agents prostaglandin E1, acetylcysteine, carbon monoxide and sevoflurane resulted in lower AST, IL-6, TNF-α, and galactosidase levels and increase IL-10 levels during perfusion. Post-transplantation, animals receiving treated livers had lower ALT and AST peaks and lower bilirubin levels, as well as lower hyaluronic acid levels, a marker of improved endothelial function [59].

Steatotic livers are especially sensitive to IRI and transplantation of livers with >30% macrosteatosis is associated with high rates of PNF after transplantation [60, 61]. Even moderately steatotic livers are associated with increased acute kidney injury [62] and EAD [63]. Interestingly, even HMP of steatotic rat livers improves bile production, liver ATP and liver oxygen consumption in a simulated reperfusion model, when compared to steatotic livers preserved by SCS [64]. Oxygenated SNMP of rat steatotic livers improves ALT release, ATP, bile production, lipid peroxidation and tissue glutathione compared to SCS preserved steatotic rat livers [65]. In spite of improvements of steatotic liver parameters on MP [64, 65], in depth metabolomics profiling of declined human livers during oxygenated SNMP revealed that steatotic livers still replenish their ATP and uptake oxygen less than even DCD livers. Moreover, steatotic livers have significantly more arterial resistance [26]. Thus, even improvements on simple MP likely leaves these steatotic livers still untransplantable.

Strategies to reduce the fat content of steatotic livers may further improve these livers. A defatting cocktail containing forskolin, GW7647, scoparone, hypericin, visfatin, GW501516, and L-carnitine aimed at promoting lipid droplet breakdown and free fatty acid oxidation has been effective in a rat hepatocyte system. Treatment of steatotic rat hepatocytes with the defatting cocktail decreased intracellular triglycerides by approximately 57%, increased ATP content, decreased oxidative stress as measured by the lower oxidized to reduced glutathione ratios, improved viability, increased bile canalicular transport, and increased urea secretion [66]. A slightly different cocktail was used in a SNMP system with steatotic rat livers, but there were not similar decreases in fat content, although the droplets decreased in size [67]. SNMP may not be sufficient for significant mobilization of triglycerides to occur, and NMP is likely required for efficient defatting of whole livers, as in cultured hepatocytes. The ability to transplant steatotic livers could significantly increase the donor pool, as obesity and fatty liver disease are both on the rise, with the latter likely to become the most common indication for liver transplantation in the next 10 years [68].

## CONCLUSION

Machine perfusion offers the potential to vastly expand the donor pool by improving the quality of procured organs via therapeutic interventions during *ex vivo* perfusion. Dynamic,

functional data provided during machine perfusion could also increase the use of marginal organs that are currently discarded based on static donor parameters. Improved preservation of currently used organs would allow longer transport of organs that are currently shipped by static cold storage, encouraging the use of marginal organs with long times between procurement and transplantation, whether for allocation, transportation, or recipient issues. Machine perfusion also offers the possibility to improve outcomes of currently used organs, decreasing complications, hospital length of stay, and ultimately, the need for intense, long term immunosuppression. We are able to treat infections, reduce ischemia reperfusion injury by preconditioning donor organs with MSCs or anti-inflammatory agents, and improve the quality of steatotic livers by applying defatting agents. A summary of potential interventions during machine perfusion is presented in Figure-1.

There are still many opportunities to expand the role of machine perfusion in improving organs for transplantation and improving outcomes after transplantation. Extending the viable donor organ preservation time could play a key role to overcome geographical limitations and improved global organ sharing. Tolerance induction could be pursued by delivering tolerogenic molecules via machine perfusion. Most importantly, all of these therapies can be studied in depth during diagnostic simulated reperfusions using the same techniques as for therapeutic perfusions.

## Abbreviations

| | |
|---|---|
| **OPTN** | Organ Procurement and Transplantation Network |
| **UNOS** | United Network for Organ Sharing |
| **COP** | Conditions of Participation |
| **MELD** | Model for End-Stage Liver Disease |
| **ECD** | Extended Criteria Donor |
| **DCD** | Donation after Circulatory Death |
| **SCS** | Static Cold Storage |
| **MP** | Machine Perfusion |
| **HMP** | Hypothermic machine Perfusion |
| **SNMP** | Subnormothermic Machine Perfusion |
| **NMP** | Normothermic Machine Perfusion |
| **NRP** | Normothermic Regional Perfusion |
| **COR** | Controlled Oxygenated Rewarming |
| **ALT** | Alanine Aminotransferase |
| **AST** | Aspartate Aminotransferase |

| | |
|---|---|
| **PNF** | Primary Non-function |
| **EAD** | Early Allograft Dysfunction |
| **HCV** | Hepatitis C |
| **IRI** | Ischemia Reperfusion Injury |
| **ROS** | Reactive Oxygen Species |
| **NO** | Nitric Oxide |
| **ATP** | Adenosine triphosphate |
| **cAMP** | Cyclic adenosine monophosphate |
| **CVR** | Coronary Vascular Resistance |
| **$MVO_2$** | Myocardial Oxygen Consumption |
| **PaO2/FiO2** | Partial Pressure of Oxygen/Fraction of Inspired Oxygen |
| **CFTR** | Cystic Fibrosis Transmembrane Conductance Regulator |
| **APRV** | Airway Pressure Release Ventilation |
| **IL** | Interleukin |
| **TNF-α** | Tumor Necrosis Factor- α |
| **VEGF** | Vascular Endothelial Growth Factor |
| **ET-1** | Endothelin-1 |
| **KIM-1** | Kidney Injury Molecule-1 |
| **NGAL** | Neutrophil Gelatinase-Associated Lipocalin |
| **MSCs** | Mesenchymal Stem Cells |
| **TG** | Triglyceride |
| **FFA** | Free Fatty Acids |

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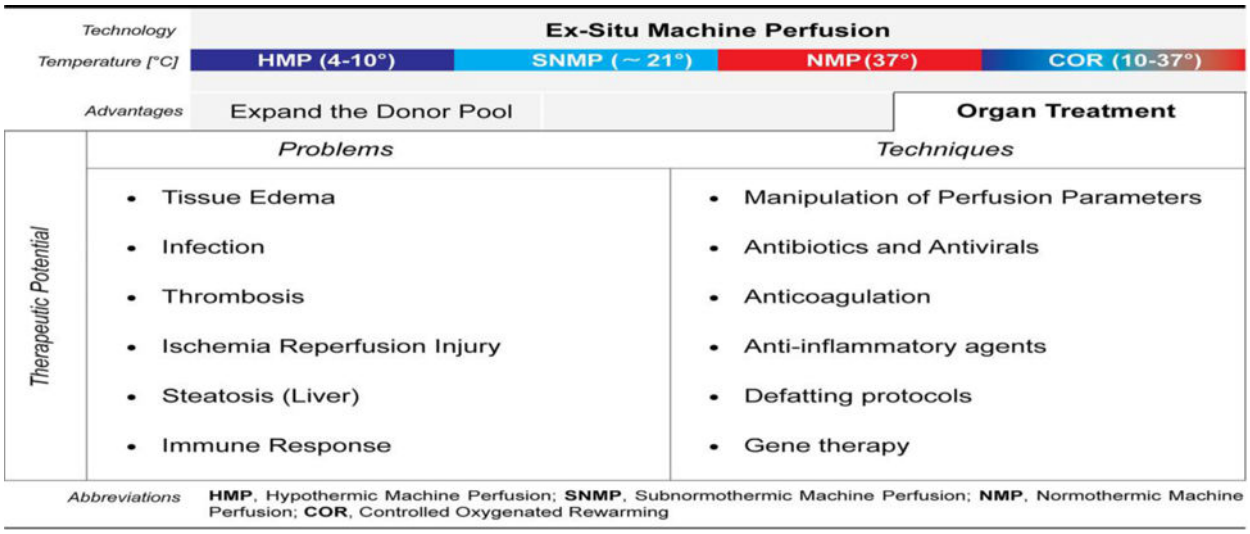


**Figure 1.**