Minireview

Bioengineering approaches to organ preservation *ex vivo*

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Impact statement

Over the past several decades, *ex vivo* perfusion has emerged as a promising technology for the assessment, preservation, and recovery of donor organs. Many exciting pre-clinical findings have now been translated to clinical use, and successful transplantation following *ex vivo* perfusion has been achieved for heart, lung, and liver. While machine perfusion provides distinct advantages over traditional cold preservation, many challenges remain, including that of long-term (multi-day) *ex vivo* support. Here, we provide an overview of the current status of *ex vivo* machine perfusion in the pre-clinical and clinical setting and share our perspective on the future direction of the field.

Abstract

The advent of successful solid organ transplantation is undoubtedly among the most significant medical achievements of the 20th century. Despite advances in the field of transplantation since its inception over 50 years ago, our approach to donor organ preservation outside of the body remains unchanged. Recently, attempts have been made to replace static cold storage with more sophisticated *ex vivo* machine perfusion. Rather than cooling the organ on ice to slow metabolic processes, machine perfusion aims to support normal metabolic function in a near-physiologic environment and to provide a platform on which the organ can be evaluated, preserved, and recovered. *Ex vivo* machine perfusion devices have demonstrated early success with respect to transplant outcomes in heart, lung, and liver, with perfusion times limited to several hours. The continued development of more advanced perfusion systems is likely to extend the duration of *ex vivo* organ support to days or even weeks, and enable recovery of initially unsuitable donor organs. In this review, we discuss recent clinical and pre-clinical studies, state-of-the-art organ preservation technologies, existing limitations, and a perspective on future developments.

Keywords: Lung, liver, heart, perfusion, normothermic, organ donation

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Introduction

Organ transplantation remains a life-saving therapy for patients suffering end-stage organ failure. However, the full potential of this therapy has yet to be realized due to the limited availability of donor organs suitable for transplant. Currently, thousands of patients await transplantation of heart, lung, and liver. Many of these patients will never receive the organ they need, and will either die on the waitlist or be removed due to illness, presumably to die soon after (Table 1).[1–3] While patients wait months or even years for a transplant, potential life-saving donor organs are discarded every day due to (i) inability to effectively assess organ viability and (ii) poor function as a result of injury or disease. The possibility of assessing, preserving, and recovering these organs *ex vivo* prior to transplantation could dramatically increase the number of available organs.

Successful *ex vivo* perfusion was first reported in 1935 when Alexis Carrel and Charles Lindbergh[4] perfused cat thyroid glands and ovaries for more than 20 days, achieving what they called "culture of whole organs." At the time, this advance was principally recognized for its ability to aid in understanding organ physiology, and not as a means of preserving organs, as solid organ transplantation was not yet a reality. By the latter half of the 20th century, however, transplantation of heart, lung, and liver had been achieved, and clinicians began to search for viable *ex vivo* organ preservation methods. In the decades that followed, immunosuppressive drug technology advanced dramatically leading to decreased immunologic rejection and to significant growth in the number of organ transplants. Improved immunosuppression has in turn led to more liberal transplantation criteria. This milestone in organ transplant history did not occur without consequences: with an increased

Table 1. Heart[1], lung[3], and liver[2] transplantation statistics.

					Transplant outcomes	
	Patients added to waitlist	**Median wait time**	**Waitlist mortality (%)**	**Total # of transplants**	**1-year survival (%)**	**5-year survival (%)**
Heart	3521	9.4 months	9.7	3,209	90.1	78.3
Lung	2692	2.5 months	15.1	2,345	85.0	55.5
Liver	11,340	11.3 months	5.5	7,841	88.0	81.2

number of patients qualifying for transplant, donor organ shortage began to manifest itself as a critical problem facing the field.[5]

The traditional method of static cold storage for transportation from donor to recipient, although widely used, remains fraught with inadequacies including prolonged ischemia and reperfusion injury which occurs upon transplantation.[6] Complications associated with increased ischemic time hamper dissemination of donor organs, as transport of organs from densely populated regions to recipients in remote hospitals necessitates longer preservation. Reperfusion injury leads to increased rates of primary graft dysfunction and failure.[7–9] Expanded recipient transplant criteria as well as shortcomings of static cold storage (SCS) spurred efforts toward donor pool expansion and improved preservation strategies including through use of (i) extended donor criteria, (ii) marginal quality organs, (iii) donation after circulatory death (DCD) grafts, and (iv) *ex vivo* machine perfusion (MP).[10,11] Of these strategies, *ex vivo* MP appears to have the greatest potential, as it can be used to support and recondition organs that fall into categories (i) to (iii). Additionally, MP can decrease ischemia–reperfusion injury, leading to better transplant outcomes.

MP platforms maintain the organ in a physiological environment by circulation of an oxygen-rich perfusate through the organ at either normothermia or hypothermia. *Ex vivo* MP has the potential to (i) enable functional assessment of the organ and prediction of post-transplant outcomes,[12–15] (ii) decrease ischemic time and therefore attenuate reperfusion injury,[16–19] and (iii) allow for recovery of organs initially unsuitable for transplant.[20,21]

SCS and ischemic reperfusion injury

Ischemic reperfusion injury (IRI) refers to the combined detrimental effects of (i) ischemic period encountered during organ procurement and preservation and (ii) reperfusion injury incurred upon transplantation, and is the primary factor contributing to post-transplant organ dysfunction and failure.[6] Furthermore, the severity of graft injury during preservation has been linked to the severity of IRI in recipients,[7] and in several organs, the duration of the ischemic period has been directly correlated to transplant outcomes.[22]

Typically, organ retrieval from the donor begins with termination of blood supply, followed by flush cooling *in situ* (using an organ-specific solution), explant, and preservation on ice.[7] This practice, termed SCS, remains the gold standard for organ preservation, despite several detrimental effects of low temperature on molecular, cellular, and tissue properties. Cooling the organ to ~4°C slows cellular metabolism, thereby reducing oxygen demand allowing the organ to survive in hypoxic environment.[23] For every 10°C drop in temperature, aerobic metabolism is slowed 1.5 to 2-fold, but anaerobic metabolism and other cellular processes can continue at temperatures as low as 1°C.[24,25]

In the ischemic environment, a combination of oxidative stress, inflammatory signaling, and structural changes contribute to declining organ function. Diminished ATP supply leads to acidosis, and malfunction of the sodium–potassium membrane pump disrupts electrochemical equilibrium and membrane integrity.[6,25,26] Cellular edema ensues which then triggers an influx of free calcium as well as an inflammatory response, ultimately leading to cell death via apoptosis or necrosis. Additionally, cytoskeletal changes can lead to dislocation of endothelial cells within the graft.[6,7] The lack of perfusion during SCS prevents clearance of cellular waste products, contributing to their build-up in the donor organ. At the same time, in the absence of oxygen, the functions of certain enzymes critical for the breakdown of metabolic byproducts and debris are fundamentally altered. For example, xanthine dehydrogenase is converted to xanthine oxidase which generates free radicals in the recipient upon reperfusion.[6] The build-up of metabolic waste products followed by release of free radicals precipitates microvascular and parenchymal cell injury and death[7] in the donor organ and in the recipient following vascular anastomosis.

While the IRI cascade occurs in all donor grafts, its effects are exacerbated in grafts donated following circulatory death (DCD) due to the period of warm ischemia that occurs prior to vascular clamp and cold flush in the donor.[6,23] During this brief phase, normothermic circulation continues, allowing cellular metabolism to proceed while simultaneously diminishing ATP supply. Because of this unavoidable warm ischemic period and its deleterious effects on graft function, surgeons have traditionally avoided the use of DCD organs for transplantation.[27] However, if properly procured and preserved, DCD grafts could help combat the current disparities in donor organ availability. *Ex vivo* MP represents a possible means of functionally assessing graft viability, extending preservation time with decreased ischemic period, and reconditioning marginal donor organs, including those procured from DCD donors.

Current use of *ex vivo* MP

Organs are assessed *in situ* by the transplant team and are either (i) accepted for transplant and transported to the

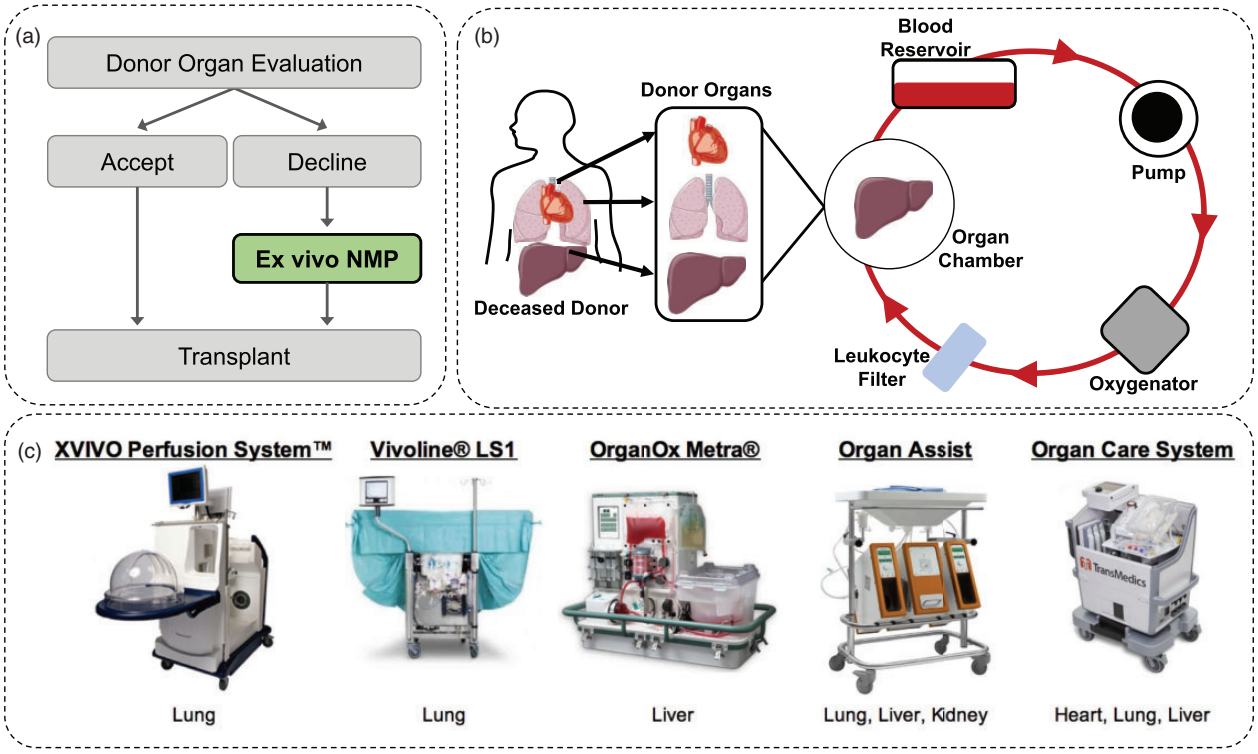

Figure 1. Current *ex vivo* machine perfusion systems (a) Donor organ path-to-transplant. Donor organs are evaluated and either (i) accepted for transplantation, or (ii) initially declined and placed on *ex vivo* normothermic machine perfusion (NMP) for assessment, preservation, and recovery; (b) Schematic showing standard *ex vivo* machine perfusion circuit; (c) Commercially available perfusion platforms and respective supported organs. (A color version of this figure is available in the online journal.)

recipient hospital, or (ii) declined for transplant and discarded. *Ex vivo* MP aims to modify this simple accept/decline dichotomy by providing an avenue through which initially rejected organs can be recovered for eventual transplantation (Figure 1(a)). The typical *ex vivo* MP circuit contains a humidified organ chamber, perfusion solution, reservoir for perfusion solution collection, oxygenator, and optional leukocyte filter (included when whole blood is used as the perfusion solution) connected via sterile tubing (Figure 1(b)).[16,26,28,29] This basic configuration has been adapted by several companies to develop the existing commercial *ex vivo* perfusion platforms, including TransMedics organ care system (OCS), OrganOx metra, XVIVO perfusion system (XPS), Organ assist, and vivoline LS1 (Figure 1(c)).[6,23] These devices have been employed in a variety of pre-clinical and clinical studies across multiple organs and have demonstrated an ability to successfully evaluate and preserve organs *ex vivo*, with some studies focused on recovery of injured grafts.

Functional assessment

At its early stages, *ex vivo* MP was viewed primarily as a means of assessing organ function prior to transplantation. When considering an organ for transplant, many factors are weighed including donor cause of death, mechanism of death (circulatory death vs. brain death), donor age, extraction time, and graft injury/disease.[30] Often times, surgery teams are unable to effectively gauge organ quality, and due to the risk associated with transplanting an unsuitable graft, they are inclined to err on the side of rejection. This "better safe than sorry" mentality results in a significant number of organs being discarded despite the fact that they may have been usable. Particularly challenging to evaluate are DCD organs because the window of time in which the procurement team can assess function is insufficient to accurately determine organ quality.[31] As a result, these grafts are frequently rejected. *Ex vivo* MP can address this issue by serving as a platform for evaluating the functionality of donor organs following explant. Normothermic MP (NMP) provides a near-physiologic environment for assessing overall graft function, metabolic demand, and viability, which may afford more accurate predictions of primary graft failure and post-transplant outcomes.

Heart

Heart grafts recovered from DCD donors present unique challenges including inflammation, high levels of cardiomyocyte necrosis,[32] and potential contractile dysfunction due to reactive oxygen species-mediated myofilament protein modifications.[33] Utilization of DCD heart grafts could increase the number of heart transplants by 11–15%[34]; however, unavoidable ischemic periods in DCD hearts correlate with primary graft dysfunction and failure.[9] Despite these notable obstacles, successful transplantation of DCD hearts with limited graft ischemic time has been shown both in animal models[35–37] and in humans.[9,38–40] This suggests that transplantation of certain DCD hearts is feasible, though grafts need to be evaluated on a case-by-case basis.

Ex vivo MP can provide a platform suitable for assessment of heart grafts, including those procured from DCD donors. Currently, four hospitals transplant DCD hearts following evaluation and support on the OCS *ex vivo* platform (Sydney, Australia; Papworth, UK; Harefield, UK; Manchester, UK).[9] Expansion of this practice would enable more widespread use of DCD grafts, thereby combatting the donor organ shortage.

By extending the period during which surgeons can evaluate graft function, *ex vivo* MP enables the use of "questionable" grafts, including those from DCD donors. Parameters of interest for heart assessment include aortic pressure, flow rate, and hemodynamic profile. As research into NMP of the heart has expanded, groups have begun to establish benchmarks that can be assessed *ex vivo* to predict graft outcome post-transplant. For example, elevated lactate levels (>5 mmol/L) have been shown to predict graft failure in multiple studies, including in a porcine model of DCD.[14,41] Additionally, increased lactate levels in the venous compared to the arterial side can be indicative of ischemic injury or other damage.[9,42,43] Based on this preclinical evidence, three hearts were discarded due to elevated lactate levels in the recent OCS PROCEED II clinical trial (Table 2).[26] Lactate level is a practical benchmark, as it can easily be monitored in real-time using blood analysis systems.

In addition to blood analysis values and perfusion parameters, *ex vivo* MP also allows for more sophisticated non-invasive assessments. Ghodsizad *et al*.[49] performed a coronary angiography on a heart supported on the OCS platform, facilitating the assessment of the donor organ by allowing visualization of the coronary arteries. This practice confirmed the presence of a minor sclerosis and showed, for the first time, that angiography can be performed on an *ex vivo* machine-perfused heart. Recently, functional echocardiographic assessment was achieved on an isolated beating porcine heart during left ventricular loading,[50] and showed results comparable to those obtained with standard transesophageal and transthoracic echocardiogram. Similarly, Hassanein *et al*.[51] showed that contrast echocardiography could be used to assess coronary perfusion in a heart supported using the OCS. Combined analysis of perfusate properties and *ex vivo* imaging could enhance organ assessment and predictive capability for hearts, particularly in DCD heart grafts.

Lung

The opportunity to evaluate organs *ex vivo* prior to transplantation is particularly attractive in the case of lung, where less than 20% of donated organs are transplanted.[52] In addition to the classical challenges surrounding transplant of DCD grafts, donation following brain death (DBD) presents a major obstacle in the form of a "cytokine storm" encountered in the lungs.[31] Due to complex tissue architecture and the possibility of airway and alveolar collapse, lung transplantation is an extremely delicate procedure. Surgeons typically avoid transplantation of borderline quality lungs. *Ex vivo* lung perfusion (EVLP) was initially introduced as a means of extending the timeline during which grafts can be assessed, with the goal of accurately gauging the functional capability of these marginal quality organs.[53]

In 2001, Steen *et al*.[53] reported the first use of EVLP for assessment of lungs harvested from a non-heart-beating donor. Following evaluation of blood gases, oxygenation, and vascular function, the lungs were successfully transplanted into the recipient. The same group used EVLP to assess explanted porcine lung function prior to transplantation of the left lung into a porcine recipient. A right pneumonectomy was performed to demonstrate that the transplanted lung was sufficient for normal function during the 24-h of post-operative observation.[54] Following Steen's pioneering work, other groups began to perform similar short-term (1–2 h) EVLP studies for graft assessment of donated organs following circulatory death in humans and large animal models.[12,55–57] Together, these data showed that normothermic EVLP could be used as a platform for lung evaluation following circulatory death.

The evaluation of lung function includes: (i) hemodynamic stability (pulmonary artery pressure, flow, pulmonary vascular resistance); (ii) perfusate hemogas and biochemical properties (arterial blood gas (ABG) values, lactate levels, pH); (iii) gross organ anatomy (weight, consolidation, edema); (iv) ventilation (airway pressure, dynamic compliance); and (v) oxygenation capacity (PaO_2/FiO_2, Δp_{O2}, Δp_{CO2}).[10,28] While challenging to evaluate in the donor, these parameters can be easily monitored in real-time in *ex vivo* perfused lungs.

Supporting lungs by a near-physiologic environment also facilitates bronchoscopic and radiologic visualization similar to those performed *in vivo*.[28,58,59] Bronchoalveolar lavage (BAL) fluid and tissue biopsies can be obtained for microbiological, metabolic, and molecular analysis,[16,23,28,31] which could provide mechanistic insight into *ex vivo* organ physiology and recovery. Innovative functional "challenges" which include changing ventilator settings and monitoring hemogas, or delivery of nitric oxide and measurement of the effects on pulmonary vascular resistance have also been explored as a means by which to measure oxygenation and endothelium responsiveness, respectively.[53,58,60]

Attempts have been made to identify biomarkers predictive of post-transplant graft outcome in both large-animal models and human lungs on EVLP. For example, increased levels of glucose and pyruvate/lactate ratios were shown to lead to pulmonary edema and poor lung function.[61,62] Changes in inflammatory gene expression and circulating cytokine levels have also been studied during *ex vivo* MP,[31,58] with microarray analysis showing that EVLP recovered lungs exhibit down-regulation of inflammatory genes with a simultaneous upregulation in cell signaling genes.[63] Although a variety of possible markers have been studied during *ex vivo* MP, a clear consensus on the predictive capability of these markers has yet to be agreed upon. As we gain insight into the mechanisms underlying *ex vivo* organ recovery, more specific criteria capable of predicting transplant outcomes will be defined.

Table 2. Recent clinical studies of normothermic machine perfusion followed by transplantation into recipient.

	Authors	Tenderich *et al.*[44]	McCurry *et al.*[45]	Ardehali *et al.*[48]	García Saez *et al.*[43]	Koerner *et al.* [49]
Heart	Title of paper	Prospective multi-center European trial to evaluate the safety and performance of the organ care system for heart transplants (PROTECT)	Prospective multi-center safety and effectiveness evaluation of the organ care system device for cardiac use (PROCEED)	*Ex-vivo* perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicenter, randomized non-inferiority trial	Evaluation of the organ care system in heart transplantation with an adverse donor/recipient profile	Normothermic *ex vivo* allograft blood perfusion in clinical heart transplantation
	Name of trial	PROTECT I (Europe)	PROCEED I (US)	PROCEED II (US)	NA	NA
	Number of subjects	$n = 20$	$n = 13$	$n = 130$	$n = 26$	$n = 159$
	Experimental group	OCS ($n = 20$)	OCS ($n = 13$)	OCS ($n = 67$)	OCS ($n = 26$)	OCS ($n = 29$)
	Perfusion time	222 ± 54 min	NR	210 min	285 ± 92 min	NR
	Control group	NR	NR	SCS ($n = 63$)	NR	SCS ($n = 130$)
	Endpoint(s)	Primary endpoint: 7-day survival, Secondary endpoint: 30-day patient and graft survival	Primary endpoint: 7-day survival, Secondary endpoint: 30-day patient and graft survival	Primary endpoint: 30-day patient and graft survival, Secondary endpoint: incidence of cardiac SAE, incidence of rejection and ICU duration	One-month survival, ICU stay	Primary endpoint: 30-day, 1- and 2-year survival, Secondary endpoints: allograft failure, noncardiac complications, length of hospital stay
	Results	All 20 patients met 7 and 30-day survival endpoints, 5 patients experienced SAE, 19 of 20 weaned off bypass on first attempt	11 of 13 recipients reached 7- and 30-day endpoints, two cases of PGF and five SAEs occurred	94% OCS group and 97% SCS group met 30-day endpoint, 13% (8) OCS patients and 14% (9) patients in SCS group experienced SAE	100% survival recorded at one month, 96% survival recorded at follow up day 257 ± 116 days, median ICU stay was 6 days	30 day, 1-year, and 2-year survival were higher in NMP group were 96%, 89%, and 89% vs. 95%, 81%, and 79% in SCS. Primary graft failure and acute rejection were deceased in NMP group (6.89% PGD, 17.2% AR) vs. SCS (15.3% PGD, 23% AR).

	Authors	Cypel *et al.*[82]	Warnecke *et al.* [85]	Fisher *et al.*[84]	Sage *et al.*[47]	Cypel *et al.*[46]	Slama *et al.*[83]
Lung	Title of paper	Normothermic *ex vivo* lung perfusion in clinical lung transplantation	Normothermic *ex-vivo* preservation with the portable Organ Care System Lung device for bilateral lung transplantation (INSPIRE): a randomized, open-label, non-inferiority, phase 3 study.	An observational study of donor *ex vivo* lung perfusion in UK lung transplantation: DEVELOP-UK	Lung transplantation from initially rejected donors after lung reconditioning: the French experience	Experience with the first 50 *ex vivo* lung perfusions in clinical transplantation	Standard donor lung procurement with normothermic *ex vivo* lung perfusion: A prospective randomized clinical trial
	Name of trial	HELP	INSPIRE	DEVELOP-UK	NA	NA	NA
	Number of subjects	$n = 136$	$n = 320$	$n = 202$	$n = 112$	$n = 317$	$n = 76$
	Experimental group	XVIVO ($n = 20$)	OCS ($n = 151$)	Vivoline ($n = 18$)	XVIVO ($n = 31$)	XVIVO ($n = 50$)	Non-commercial ($n = 35$)
	Perfusion time	4 h	Up to 330 min	NR	243 min (124–460)	4–6 h	4 h
	Control group	SCS ($n = 116$)	SCS ($n = 169$)	SCS ($n = 184$)	SCS ($n = 81$)	SCS ($n = 267$)	SCS ($n = 41$)
	Endpoint(s)	Primary endpoint: primary graft dysfunction 72 h	Primary endpoints: mean number of lung SAEs	Primary endpoint:12-month survival	Primary endpoint: PGD3, Secondary	Primary endpoint: incidence of	Primary endpoint: PGD, PaO2/FiO2 at 24 h

(continued)

Table 2. Continued

	Authors	Cypel *et al*.[82]	Warnecke *et al*. [85]	Fisher *et al*.[84]	Sage *et al*.[47]	Cypel *et al*.[46]	Slama *et al*.[83]
		after transplant, Secondary endpoints: 30-day mortality, bronchial complications, duration of mechanical ventilation, length of stay in ICU	within 30 days, 72-h absence of PGD3, 30-day survival		endpoints: length of intubation, ICU stay, length of hospital stay, 30-day mortality	primary graft dysfunction grade 3 at 72 h	post-transplant, Secondary endpoints: need for prolonged ECMO, ICU and hospital stay, 30-day survival
	Results	PGD was 15% in EVLP group and 30% in the control group. No differences in secondary endpoints, and no SAE attributable to EVLP	Patient survival at 30 days was 95.7% in OCS group and 100% in SCS group and at 12 months was 89.4% OCS vs. 88.1% SCS, incidence of PGD3 was 17.7% in OCS group vs. 29.7% of SCS	Higher rate of very early PGD3 in the EVLP group compared to the control but no difference at 72 h, no significant difference in 12-month survival	Incidence of PGD3, duration of intubation, ICU and hospital stay, and 30-day mortality were not statistically different between EVLP and control groups.	PGF, 30-day mortality, and 1-year survival did not significantly differ across groups.	Incidence of PGD and need for post-operative ECMO were decreased in EVLP group compared to SCS. ICU stay, hospital stay, and 30-day survival were similar between groups.

	Authors	Nasralla *et al*.[93]	Bral *et al*.[94]	Ravikumar *et al*. [97]	Selzner *et al*.[96]
Liver	Title of paper	A randomized trial of normothermic preservation in liver transplantation	Preliminary single-center Canadian experience of human normothermic *ex vivo* liver perfusion: results of a clinical trial	Liver transplantation after *ex vivo* normothermic machine preservation: a phase 1 (first-in-man) clinical trial	Normothermic *ex vivo* liver perfusion using steen solution as perfusate for human liver transplantation: First North American results
	Name of trial	COPE-WP2	NA	NA	NA
	Number of subjects	$n = 220$	$n = 39$	$n = 60$	$n = 40$
	Experimental group	OrganOx metra ($n = 121$)	OrganOx metra ($n = 9$)	OrganOx metra ($n = 20$)	OrganOx metra ($n = 10$)
	Perfusion Time	9 h 17 min	11.5 h (3.3–22.5)	9.3 h (3.5–18.5)	480 min (340–580)
	Control Group	SCS ($n = 101$)	SCS ($n = 30$)	SCS ($n = 40$)	SCS ($n = 30$)
	Endpoint(s)	Primary endpoint: peak AST during the first 7 days post-transplant, early allograft dysfunction	Primary endpoint: 30-day graft survival, ICU stay, hospital stay, Secondary endpoints: 30-day survival, peak AST in first 7 days, EAD in 7 days, liver biochemistry, major complications, patient and graft survival at 6 months, biliary complications at 6 months	Primary endpoint; 30-day graft survival, Secondary endpoints: liver biochemistry, patient and graft survival, graft function at 6 months	Graft function, duration of ICU and hospital stay, major complications
	Results	Peak AST was reduced by 49.4% in NMP compared to SCS, early allograft dysfunction was less likely in NMP than in SCS	No difference in graft survival, ICU and hospital stay were longer in the NMP group	100% 30-day survival reported in NMP group vs. 97.5% survival in SCS group, lower level of peak AST in NMP group compared to SCS, 3 (15%) NMP patients experienced early allograft dysfunction vs. 9 (23%) in the control group	No difference in graft function on day 7 and no difference in ICU stay found between groups, 1 (10%) major complication reported in NEVLP group vs. 7 (23%) in CS group, no graft loss or patient death reported at 3 months

Liver

A major limitation in the traditional SCS method of liver preservation is the extreme difficulty of predicting the post-transplant function. Current methods for evaluating donor livers lack objectivity, relying primarily on gross visual inspection and donor history.[6,30] In 2016, only 78% of patients on the waitlist received liver transplants, while over 30% of eligible donor livers were declined, frequently based on subjective evaluations.[2,52] There remains a critical need for objective viability assessment of liver grafts to ensure that usable grafts are not discarded.

Ex vivo MP of the liver has been widely explored as a tool for viability assessment,[15,64,65] and several key markers for predicting transplant outcome have been identified. For example, based on *ex vivo* liver perfusion data, Mergental *et al*.[66] developed a set of viability criteria including circulating lactate level of <2.5 mmol/L, bile production, acid/base homeostasis, pressure/flow parameters, and homogenous perfusion with "soft" parenchymal consistency. Perfusion parameters and perfusate properties are easily monitored and provide insight into graft functionality. For example, perfusate pH levels can be used to assess liver injury and function.

Because the liver metabolizes ammonia to maintain physiologic pH levels, acidosis is indicative of metabolic disruption.[30] Elevated levels of bicarbonate[15] and factor V[67] have been shown to indicate graft viability and metabolic and synthetic function in the *ex vivo* liver. Real-time measurement of hepatocellular biomarkers including aspartate transaminase (AST), alanine transaminase (ALT), glutamate dehydrogenase (GLDH), and beta-galactosidase enzymes can also be used to assess cellular injury and death.[6]

As is the case for ex vivo heart perfusion, circulating lactate levels and lactate clearance have predictive potential in NMP of the liver, and are widely accepted as parameters for liver evaluation.[30] The liver is comprised of hepatic lobules, which are further divided into three zones, each with distinct metabolic capabilities critical to liver function. Notably, lactate clearance is representative of only zone 1 hepatocyte function and provides little insight into function of hepatocytes in zones 2 and 3. Perfusate glucose level has been proposed as a means of assessing zone 3 function, as glycogen breakdown in zone 3 should lead to decreased circulating glucose value. Similarly, acidosis may indicate failure of zones 1 and 2 hepatocytes to metabolize ammonia during urea synthesis.[30] Accurate measurement of hepatocellular function *ex vivo* may enable prediction of primary non-function of the liver.

Collection and evaluation of bile produced during NMP can provide valuable insight into graft function. Conflicting results have been presented concerning the predictive capability of total bile production. Sutton *et al.*[15] found that >30 g bile produced in 6 h of NMP coincides with lower perfusate transaminase and potassium levels and decreased venous congestion and cell necrosis as indicated histologically. However, others have shown no correlation between bile production and organ outcome.[65,68] These conflicting findings suggest that the prognostic capacity of bile may be more nuanced than previously thought, and that bile content and specific properties are possibly more significant than total bile production. Bile duct cholangiocytes are responsible for modifying bile content through resorption and secretion of bicarbonate, water, glucose, amino acids, and bile salts. Therefore, monitoring bile content throughout NMP can be used to gauge cholangiocyte resorptive and secretory capabilities.[30] Consideration must also be given to markers of cholangiopathy, which can have a detrimental effect on graft function and recipient stability.[29] Production of alkaline bile (pH > 7.5) has been proposed as a marker of cholangiocyte function and corresponds to a decreased likelihood of intrahepatic cholangiopathy post-transplantation.[30]

Preservation with decreased ischemic time

Ex vivo perfusion has enabled accurate assessment of donor organs with the capability of predicting recipient post-transplant outcomes in some cases. These viability tests can be conducted relatively quickly (within 1–2 h). The possibility of extending NMP beyond this time could enable long distance transport of organs, decreased ischemic preservation time, and possible reconditioning of marginal grafts. Ischemic preservation time has been shown to correspond with transplantation outcomes, including primary graft failure.[29] In the heart and lung, graft ischemic time has been correlated with negative short-term and long-term outcomes including diminished gas exchange capabilities and recipient survival.[22,69] A similar relationship has been demonstrated in liver, where increased cold ischemic time led to higher incidence of primary non-function and initial poor function which ultimately caused increases in graft failure, re-transplant, and patient mortality.[70]

Therefore, the possibility of replacing the ischemic SCS period with a period of normothermic MP has the potential to ameliorate the negative consequences of prolonged ischemia. NMP offers many advantages compared to SCS, including potentially extended preservation times (Table 3). This may enable the transport of donor organs across longer distances, which could increase access to transplantation in remote areas and also allow for matching donor organs with the "ideal" recipient, regardless of where in the world that patient is located. Substantial pre-clinical evidence suggests that NMP is superior to SCS (Figure 2), and clinical studies have thus far indicated that NMP-preserved grafts perform at least as well as SCS grafts post-transplantation (Table 2).

Table 3. Comparison of normothermic machine perfusion (NMP) and static cold storage (SCS).

	NMP	SCS
Preservation time (h)	12+	4–6
Temperature (°C)	37	4
Oxygenation	Normoxia	Hypoxia
Parenchyma preserved	✓	✓
Functional evaluation	✓	–
Therapeutic intervention	✓	–

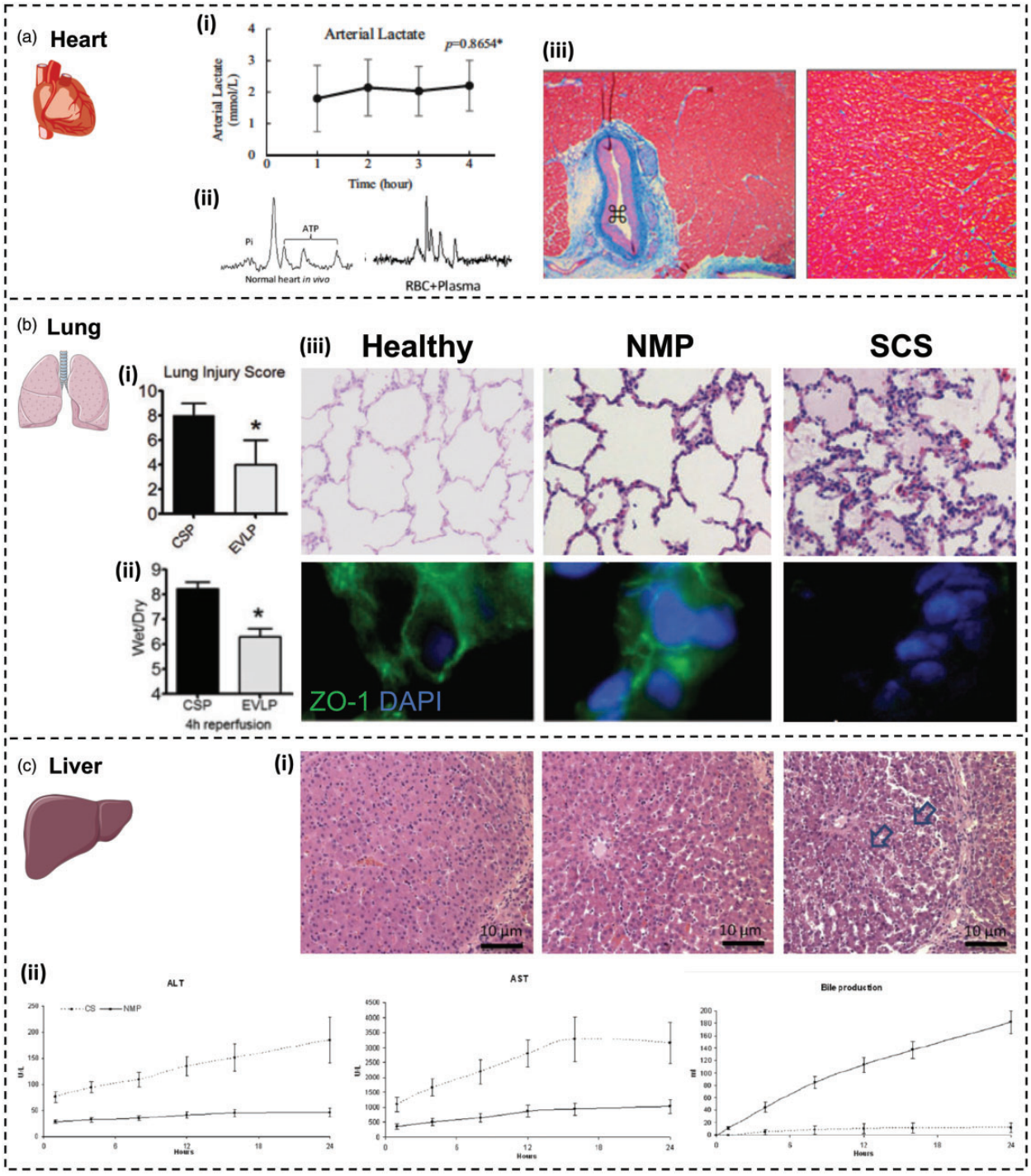

Figure 2. Comparison of *ex vivo* normothermic machine perfusion with traditional static cold storage preservation: Representative data demonstrating benefits of normothermic machine perfusion of (a) Heart, including (i) arterial lactate trends, (ii) magnetic resonance spectra obtained *ex vivo* from heart perfused with red blood cells and plasma compared to normal heart *in vivo*, and (iii) histological characterization with Mason trichrome at 4× (left) and 10× (right) from a heart perfused with red blood cells and plasma *ex vivo*; (b) Lung, including (i) lung injury score, (ii) wet/dry ratio to assess organ edema, (iii) histological comparison of NMP and SCS with H&E (upper panel) and preservation of tight integrity junction (ZO-1) (lower panel); (c) Liver, including (i) histological comparison of NMP and SCS-preserved grafts, (ii) functional measurements of AST and ALT values, and bile production throughout preservation time. SCS: static cold storage; NMP: normothermic machine perfusion; CSP: cold static preservation; RBC: red blood cell; AST: aspartate aminotransferase; ALT: alanine aminotransferase. (A color version of this figure is available in the online journal.)

Heart

Prolonged ischemia in the heart is particularly detrimental to graft function due to the extreme sensitivity of cardiac tissue to hypoxia.[40] Heart grafts can be preserved for a maximum of 4–6 h using SCS[7]; however, increasing cold storage time leads to higher rates of primary graft failure, which is the leading cause of death in recipients.[71] One study of transplant data in the UK found that each

additional hour of ischemic time (>1 h) led to a 25% increased risk of death in the first year.[72]

MP of the heart attempts to remedy this situation by decreasing ischemic preservation time and replacing it with a longer normothermic preservation period. One of the earliest studies investigating NMP in comparison with SCS showed that normothermic blood perfusion of an isolated swine heart enables maintenance of coronary endothelial vasomotor function and decreased myocardial edema and acidosis.[19] In addition to decreasing or potentially eliminating the ischemic preservation period, normothermic MP also aims to prolong total preservation time. Feasibility of NMP for up to 12 h, double the current SCS preservation time limit, has been shown in a clinically relevant large animal model.[19,73]

Over the past decade, clinical investigation into the use of MP for donor heart preservation has commenced and shown promising results. Pre-clinical animal studies have suggested that hypothermic machine perfusion (HMP) is effective at preserving heart grafts[74–77] and could be superior to SCS. Like NMP, HMP supplies oxygen, metabolic substrates, and antioxidants, and can enable clearance of inflammatory molecules, toxins, and other cellular debris. HMP devices for human heart support have been successfully developed and are now being used in pre-clinical and clinical investigations, with at least one report of heart transplantation following HMP with Steen solution (Skane University Hospital, Lund, Sweden).[23] Despite some success, HMP is limited by its inability to provide meaningful functional readouts due to the effects of low temperature on metabolism. Additionally, some evidence suggests HMP leads to impaired diastolic function due to myocardial edema.[26]

Multiple other studies have used the OCS platform to maintain the donor heart in a physiological state (i.e. warm, beating) prior to transplantation. The PROTECT I first-in-man trial conducted in Europe between January 2006 and February 2007 demonstrated the transplantability of hearts preserved on OCS for an average of over 200 min. All 20 recipients met the 30-day survival criteria.[78] This study was followed by the PROCEED I and PROCEED II trials in the United States, which together demonstrated the safety and efficacy of transplanting hearts following OCS perfusion[45,48] (Table 2).

Across multiple studies, outcomes including recipient mortality, primary graft dysfunction, and acute rejection were shown to be equivalent or decreased in the NMP group compared to the group receiving hearts preserved with SCS[43,48](Table 2). Extended *ex vivo* preservation of hearts has not yet been demonstrated clinically, as most trials have reported median perfusion times of ~4 h. Still, there appear to be potential benefits to replacing the traditional ischemic time with either hypothermic or normothermic MP.

Lung

Following the successful establishment of EVLP as a means of short-term *ex vivo* organ support for evaluating lung function, scientists and clinicians began attempts to extend *ex vivo* preservation time. Traditional static (non-ventilated) storage leads to diminished epithelial barrier integrity as well as airway and alveolar collapse, which can contribute to poor outcomes in the recipient.[79] NMP can circumvent these complications through ventilation and perfusion of the donor graft.

In 2008, Keshavjee *et al.* presented the Toronto Protocol for EVLP as the first example of prolonged *ex vivo* lung preservation. Six porcine double-lung blocks and five single human lungs were maintained for a period of 12 h using an acellular perfusate at "low-flow" to prevent hydrostatic edema. Following 12 h of normothermic perfusion and monitoring, the porcine lungs were successfully transplanted, though the recipients were euthanized 4-h post-transplant.[16] The same group then investigated post-transplant outcomes in lungs preserved for 24 h using SCS and in those preserved for 12 h with SCS followed by 12 h of EVLP. They showed that normothermic EVLP was able to attenuate the injury associated with prolonged ischemia and lead to improved outcomes immediately following transplantation in a large animal model[80] (Figure 2).

The first example of clinical use of EVLP was reported in a landmark publication by the Toronto Group in 2011. Of the 23 lungs initially deemed 'high-risk' for transplantation, 20 were transplanted following 4 h of support on EVLP. Primary graft dysfunction 72-h post-transplantation was lower (15%) in the EVLP group, compared to the non-EVLP control (30%). Other outputs including 30-day mortality, bronchial complications, length of hospital stay, and duration of mechanical ventilation were consistent between the two groups, indicating that lungs supported on EVLP perform at least as well as lungs preserved with traditional means following transplantation.[81] In the years that followed, EVLP was employed in several other clinical trials including the HELP, INSPIRE, and DEVELOP-UK studies which again showed comparable outcomes between groups including primary graft failure and short-term survival, highlighting the safety of NMP for lung preservation[81,82,84] (Table 2).

Pre-clinical studies have demonstrated feasibility of long-term (>12 h) perfusion, though the majority of clinical studies report a median perfusion time of 4–6 h (Table 2). Even short-term MP can significantly increase total *ex vivo* graft preservation time when combined with a period of cold ischemia. For example, a retrospective study of the Toronto Lung Transplant Program showed that total (ischemic + EVLP) preservation time greater than 12 h does not negatively affect short term-lung transplantation outcomes when compared with grafts preserved for less than 12 h.[85] These encouraging findings show that the use of EVLP can more than double total *ex vivo* preservation time compared to SCS alone, potentially enabling transport of grafts across longer distances.

Liver

Due to its high metabolic demand, the liver is extremely challenging to preserve *ex vivo*. SCS is sufficient for preserving high-quality donor grafts, but marginal and high-risk livers present additional complications, as they are more

susceptible to ischemic injury and early allograft dysfunction.[6,29] *Ex vivo* liver perfusion can enable dynamic support of the donor liver under hypothermic or normothermic conditions.

Early investigations into the *ex vivo* liver preservation were attempted with HMP due to the success of this technology in renal transplantation.[86] Under hypothermic conditions, cellular processes are slowed 12 to 13-fold,[24] but minimal levels of metabolism are maintained. In this sense, livers supported with HMP are essentially functioning in slow motion, which may prevent further injury or stress on the organ.

In 2010, a Phase I clinical trial was conducted in which 20 human livers were transplanted following 3–7 h of HMP at 4–6°C. The patient cohort receiving the HMP livers showed superior outcomes including decreased early allograft dysfunction, reduced biliary complications, an absence of vascular complications, reduced serum injury markers, and shorter hospital stay compared to those receiving traditional SCS livers.[87] This pilot study demonstrated safety and reliability of NMP for liver preservation, and inspired further investigation into the technology for recovery of extended criteria donor (ECD) livers. The same protocol was used to support 31 marginal livers initially declined by the United Network for Organ Sharing.[86] Following 3–7 h of hypothermic perfusion (with total preservation time <12 h), these ECD livers were successfully transplanted and outcomes were compared to ECD-SCS cases in a matched cohort. The HMP group showed significantly less biliary complications, decreased mean hospital stay, and decreased, albeit non-significant, rates of early allograft dysfunction and one-year mortality. Despite its effectiveness at preserving some liver grafts for several hours, HMP does not provide a physiologically relevant environment in which to evaluate the organ. This lack of an *in vivo* milieu for functional assessment renders HMP inferior to its normothermic counterpart.[15]

As a result, other groups have focused their efforts on the use of normothermic perfusion for preservation of DCD liver grafts. In 2001, Schön *et al*. performed the first mammalian liver transplant using NMP to resuscitate grafts injured by 60 min warm ischemia, as a model for DCD. Swine recipients of the NMP-preserved grafts all survived >7 days post-transplantation, whereas recipients of livers subjected to warm ischemia followed by SCS all developed primary graft dysfunction in 24 h.[88] Other groups have confirmed the superiority of NMP over SCS for preservation of DCD livers exposed to warm ischemic injury in large animal models (Figure 2).[68,89] Prolonged support of porcine donor livers using NMP has also been shown for a period of up to 72 h.[90]

Promising results in animal models raised interest in NMP as an option for clinical liver preservation. The first human studies demonstrated the feasibility of preserving discarded livers using NMP for several hours.[64] Quintini *et al*. at Cleveland Clinic recently reported 86-h *ex vivo* perfusion of a discarded human liver, the longest *ex vivo* maintenance of a human organ to date. Liver viability was maintained throughout the perfusion time, as evidenced by bile production and lactate clearance; however, this study did not show transplantability of the preserved liver.[91] In 2016, success of the first post-NMP liver transplant was reported.[92] Following this initial success, other case studies were reported in small patient cohorts receiving liver grafts preserved with NMP.[66,95,96] One initially declined liver was preserved for 26 h using NMP prior to successful transplantation, representing the longest *ex vivo* perfusion of a human liver followed by transplantation to date.[97]

In addition to these case studies, multiple clinical studies with larger patient cohorts have been conducted in which liver grafts were perfused using the OrganOx metra device for *ex vivo* support prior to transplantation (Table 2). These studies have shown non-inferiority of NMP compared to SCS; however, none of the included livers were considered ECD. Results are pending in several ongoing Phase II trials.

Table 2 summarizes recent clinical studies in which organs are supported by NMP followed by transplantation into patients. This is not meant to be an exhaustive list of all post-NMP transplants, but rather aims to demonstrate the safety and non-inferiority of MP compared to current standard clinical practice across heart, lung, and liver.

The authors acknowledge that there have been several other impactful clinical NMP studies, particularly in lung transplantation. For practical reasons, we limited this section to include studies that transplanted at least 10 NMP lungs and showed a comparison with SCS. In liver and heart transplantation, the exclusion criteria were less stringent, as fewer clinical studies have been conducted in these organs.

While the data presented in Table 2 alone does not suggest that NMP is superior to SCS, it does highlight the safety of NMP and demonstrate that NMP-preserved organs lead to similar transplant outcomes as traditionally stored organs. The authors speculate that MP alone will not dramatically alter transplant outcomes, but rather that the distinct advantages afforded by NMP (Table 3) will enable therapeutic intervention in *ex vivo* organs. These advances will ultimately lead to transplantation of functional well-preserved grafts and improved patient outcomes.

Recovery of damaged organs

From a tissue engineering perspective, the most exciting opportunity presented by *ex vivo* MP is that of recovering and regenerating otherwise unusable organs. Disease, physical trauma, and infection are among the conditions that render an organ unsuitable for transplantation. The damage incurred by many such conditions could be reversed with sufficient time in an appropriate environment. In fact, simply providing oxygen and other metabolic factors via normothermic perfusion may be enough to stimulate endogenous repair mechanisms and begin to recover a damaged graft. Further intervention with organ and injury-specific therapies will enable more timely recovery of grafts supported on *ex vivo* MP. These advanced therapies might include partial or full decellularization followed by repopulation with patient-specific iPSC-derived cell populations, targeted gene therapy, or delivery of therapeutic exosomes, high-dose drugs, or radiation.

Heart

Extended *ex vivo* preservation of the heart could enable advanced therapeutic intervention to recover marginal grafts to a level sufficient for transplantation. The possibility of recovering these damaged grafts on an *ex vivo* platform could greatly expand the donor heart pool. As previously mentioned, resuscitation of DCD hearts using *ex vivo* NMP followed by successful transplantation has been achieved.[98,99] These studies claim to demonstrate recovery of the DCD hearts; however, they did not incorporate any *ex vivo* therapies beyond simply perfusing the organ.

Thus far, there has been limited investigation into therapeutic interventions to recover donor hearts *ex vivo*, though NMP platforms provide immense potential toward these aims. *Ex vivo* heart surgery has been performed in the past for tumor resection, and we speculate that similar procedures could be implemented in machine-perfused hearts. These grafts could be removed from a patient, repaired on NMP while the patient is supported on cardiopulmonary bypass, and auto-transplanted following successful modification, such as tumor resection. Additionally, delivery of stem cells and iPSC-derived extracellular vesicles has shown promise at recovering cardiac tissue *in-vitro* and *in vivo*[100–102] and could also be facilitated using NMP.

Lung

Lungs remain the most under-utilized solid organ, with an 80–85% discard frequency of donor organs.[52] The potential of recovering marginal or even severely injured lungs could dramatically change the outlook for thousands of patients who await lung transplants worldwide. Multiple groups have shown that EVLP can be used to recover initially rejected donor lungs followed by successful transplantation.[13,20,84] Notably, these lungs were all of marginal quality due to poor oxygenation, consolidation, or infiltration, and were not *severely* injured.

Gastric aspiration, the most common severe donor lung injury, occurs in 14% of all donor grafts declined for transplantation.[103] Several groups have attempted to recover aspiration-injured lungs using therapeutic intervention on EVLP.[103–106] Following induction of a gastric aspiration injury, porcine lungs were supported on NMP for 2–6 h with an acellular[103,105,106] or a blood-based perfusate.[104] Multiple studies demonstrated the synergistic effect of bronchoalveolar lavage and surfactant delivery toward recovery of lungs injured by gastric aspiration.[104,106] The Toronto Group was the first to demonstrate transplantability of these EVLP-recovered grafts into recipient swine[106] with promising short-term outcomes, though recipients were sacrificed at 4 h post-transplant.

A variety of other therapies have been effective at recovering marginal grafts on EVLP. These include delivery of vasoconstrictors such as epinephrine,[107] bronchodilators,[108] and high-dose antibiotics[109] used to clear alveolar epithelial fluid, improve ventilation prior to transplantation, and clear infection in human donor lungs, respectively. Additionally, the Toronto Group administered IL-10 gene therapy to injured human lungs during 12 h of EVLP, and observed improved function indicated by arterial oxygen pressure and pulmonary vascular resistance in the treated group compared to the untreated control.[110]

Bioengineering of donor lungs supported on EVLP through regional decellularization and cell replacement has recently been documented by our group. In these studies, human lungs rejected for transplantation were perfused *ex vivo* for up to 6 h, enabling selective removal of the epithelium followed by mesenchymal stem cell and airway epithelial cell delivery and attachment.[111] The major limitation of the EVLP platform is that it is unable to provide systemic clearance of metabolic waste products and cellular debris. This constrains the effectiveness of EVLP to an average perfusion time of 4–6 h (Table 2) with a maximum perfusion time of 12 h before evidence of graft decline, including deterioration of vascular and epithelial barrier integrity and development of edema.[58,111] To enable full recovery of severe lung injury, the timeline must be extended from hours to days.

To address this problem, our group pioneered a swine cross-circulation platform for long-term preservation and recovery of injured lungs.[58] The introduction of an anesthetized porcine host into the perfusion circuit enables systemic clearance of metabolites and other circulating factors. We demonstrated that this system allows for at least 36 h of *ex vivo* lung perfusion with a total *ex vivo* preservation time of up to 56 h. The experiments were terminated following 36 h of cross-circulation due to veterinary protocol constraints, not due to any obvious limitations of the perfusion platform. Therefore, we speculate that this timeline could be further extended to multiple days. During the 36 h of cross-circulation support, we demonstrated feasibility and effectiveness of several interventions including airway lavage, surfactant replacement, localized decellularization, and stem cell delivery. We established the platform could also enable real-time functional assessment through thermography, radiography, and trans-pleural imaging, as well as measurement of metabolic activity, dynamic compliance, and lung weight. We have demonstrated that this platform can significantly extend extracorporeal preservation time compared to existing EVLP systems, and believe that a similar cross-circulation set-up could be used to preserve other organs.

Liver

Steatosis is found in 40% of all donor liver grafts and remains the primary reason donor livers are rejected,[112] as steatotic liver is especially susceptible to damage during cooling and IRI. NMP could help recover steatotic livers by avoiding cooling, reducing ischemic time, and increasing fat metabolism through pharmacological intervention.[6] Pre-clinical studies have shown promise in recovering steatotic livers using NMP in swine and rat models via delivery of defatting agents.[21,113] Recently, one group reported on the use of NMP to solubilize fat in steatotic donor human livers by exposing the grafts to defatting drugs in the perfusate.[112] Due to the ubiquity of steatosis in the donor organ pool, the possibility of recovering these organs on NMP

holds immense potential for expanding access to liver transplantation.

Delivery of therapeutic molecules in the perfusate has been investigated including high-dose antibiotics for treatment of contamination or donor infection, delivery of anti-inflammatory drugs, and stem cell-derived extracellular vesicles.[64,114] Recently, Goldaracena *et al.*[115] investigated the efficacy of administering miravirsen to inhibit miR-122 function and induce hepatitis C (HCV) resistance in porcine livers on NMP. Hepatitis C patients receiving transplants experience acute reinfection upon reperfusion, which is often more severe and leads to cirrhosis of the liver in up to 30% of patients within five years.[115] Therefore, the possibility of inducing HCV resistance in a graft ex vivo is extremely promising for these patients, who make up 22.7% of the waitlist in the United States.[2]

Current limitations and future outlook

In their 1935 publication documenting culture of organs *ex vivo*, Carrel and Lindbergh[4] noted, "although interesting results are obtainable, it has always to be kept in mind that one is dealing with a dying organ." This claim holds true nearly a century later, and the field of *ex vivo* MP remains faced with the same daunting challenge. Despite the many successes of MP systems over the past 20 years, the technology has yet to live up to its envisioned potential of meaningfully expanding the donor organ pool.

Recurrent issues that hinder MP platforms include (i) organ edema, (ii) inability to clear metabolic waste products and cellular debris, and (iii) cell death via apoptosis and necrosis. Unfortunately, these complications generally confine *ex vivo* perfusion to a period of several hours before the organ begins to fail. For example, in our group's experience with human lungs supported on EVLP, we observed evidence of graft decline (airway and parenchymal edema, increased lung weight, reduced tidal volume) beginning at 2 h of perfusion. Techniques to overcome these obstacles, such as using blood-based perfusate with increased oncotic pressure, incorporation of additional circuit filtration components, and lower perfusate flow rates have been studied with some success. However, these modifications have been unable to significantly extend preservation time, and obtaining repeatable results demonstrating the safety and efficacy of extended preservation methods has been challenging.

To significantly increase the donor organ pool, and thereby improve prognosis for patients awaiting transplantation, we need to shift our efforts away from short-term perfusion for incremental organ *recovery*, and focus instead on true *regeneration* of the damaged organ. The current time limitations of *ex vivo* MP preclude administration of advanced therapies for potential graft regeneration. Consequently, the immediate goal of the MP field must be to overcome the aforementioned limitations and extend *ex vivo* preservation time from hours to days. A radical shift in the relevant timeline for preservation could allow for advanced *ex vivo* organ assessment and therapeutic intervention, which will in turn lead to (i) development of specific evaluation criteria, (ii) expansion of the donor organ pool by recovering initially unusable grafts, and (iii) improved patient outcomes including decreased waitlist mortality and long-term survival post-transplantation (Figure 3). The development of these assessment and intervention strategies serves as an exciting avenue for future investigation in the field.

For example, bioengineering organs through decellularization and repopulation with specific cell types have been explored in *ex vivo* MP and bioreactor platforms by our group and others,[58,111,116–118] though the current timeline of *ex vivo* MP does not allow us to effectively assess cell engraftment and function. Extended preservation time could enable development of improved cell-based therapies and allow for cell uptake and engraftment prior to transplantation. By denuding the donor graft and

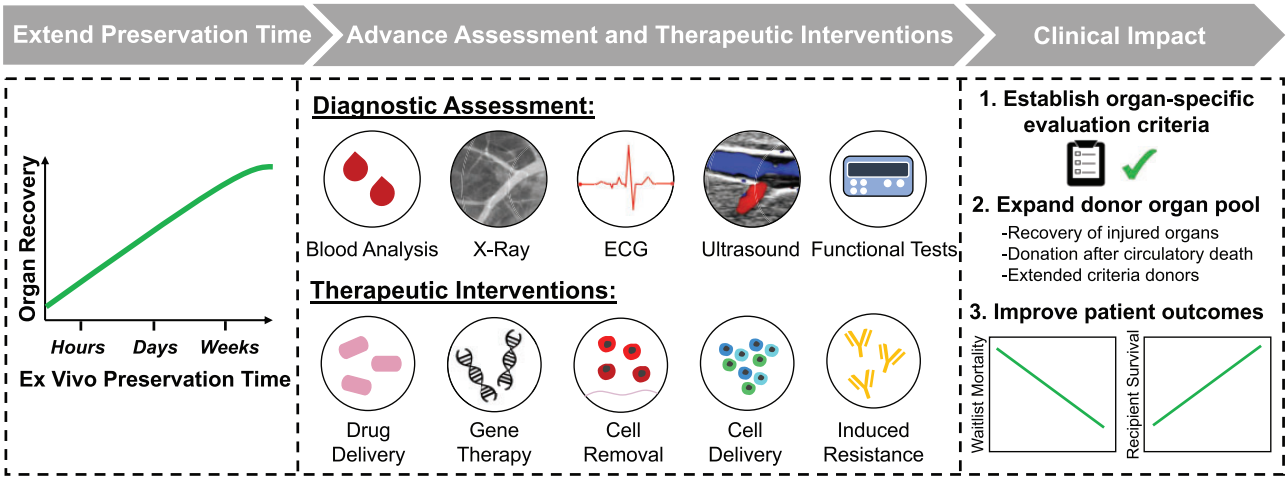

Figure 3. Envisioned developments and applications of *ex vivo* machine perfusion. Extending *ex vivo* preservation time beyond hours to days or weeks could enable extensive diagnostic assessment and advanced therapeutic interventions. Characterization of *ex vivo* grafts could contribute to the development of organ-specific evaluation criteria enabling better prediction of graft failure and patient outcomes. This predictive capability combined with *ex vivo* therapies can expand the donor organ pool by allowing transplant of previously unusable grafts. Together, these developments can contribute to decreased waitlist mortality and graft failure, with increased long-term patient survival. (A color version of this figure is available in the online journal.)

potentially repopulating with patient-derived cell types, we may be able to attenuate recipient immune rejection, obviating the need for high-dose immunosuppressive drugs post-transplantation. This could increase one and five-year survival rates post-transplantation (Table 1),[1–3] as the immunosuppressed patient is particularly susceptible to opportunistic infections which contribute to recipient mortality. It is conceivable that in the future these platforms could even be used to recondition a patient's own malfunctioning organ *ex vivo* while the patient is supported on bypass or extracorporeal membrane oxygenation, altogether eliminating the need for transplant among certain groups.

In addition to providing the time necessary to regenerate damaged grafts, longer preservation times may enable improved mechanistic understanding of organ recovery which could then inform the development of specific metrics for gauging transplantability. While many groups have identified benchmarks for functional assessment and prediction of transplant outcomes, there are no major agreed-upon parameters for determining an organ's fitness for transplant. As the field of MP continues to advance, sensitive organ-specific criteria should be defined to discriminate between suitable and unsuitable grafts *ex vivo*.

Many have speculated that in the future, large organ recovery centers will serve as hubs for preservation and reconditioning of donor grafts using *ex vivo* MP systems. In this model, specific treatment regimens could be administered to "customize" the organ for the recipient prior to transport to the recipient hospital. Additionally, the use of transportable *ex vivo* MP devices presents the opportunity for broad dissemination of donor grafts, enabling the ideal donor–recipient match regardless of distance between the two patients. Although many challenges exist, *ex vivo* MP remains in the vanguard of transplantation research and holds immense potential for expanding the availability of donor organs in the future.

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