Open Access

OBM Transplantation

LIDSEN Publishing Inc.

Review

Current and Future Applications of Machine Perfusion and Other Dynamic Preservation Strategies in Liver Transplantation

Yue Qiu [1, †], Yinqian Kang [2, †], Hao Liu [3, †], Ibtesam Hilmi [1, †, *]

1. Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, USA; E-Mails: qiuy@upmc.edu; hilmiia@upmc.edu
2. Department of Anesthesiology, University of Iowa Hospital and Clinics, Iowa City, IA, USA; E-Mail: yinqian-kang@uiowa.edu
3. Department of Surgery and J.C. Walter Jr. Transplant Center, Houston Methodist Hospital, TX, USA; E-Mail: hliu3@houstonmethodist.org

† These authors contributed equally to this work.

* **Correspondence:** Ibtesam Hilmi; E-Mail: hilmiia@upmc.edu

Academic Editor: Yasuhiko Sugawara

Special Issue: The Current Role and Future Applications of Machine Perfusion in Liver Transplantation

OBM Transplantation
2024, volume 8, issue 3
doi:10.21926/obm.transplant.2403224

Received: May 12, 2024
Accepted: August 28, 2024
Published: August 30, 2024

Abstract

Machine perfusion (MP) techniques, which simulate physiological conditions to allow for the assessment and preservation of organ viability, are currently applied in various solid organ transplantation fields. Owing to the growing demand for liver transplants and the scarcity of available donor livers, MP offers a practical solution for recovering high-risk grafts and increasing the number of potentially usable donor organs. Furthermore, testing and administering novel therapies to allografts may also become advantageous. Therefore, it has become essential to examine the role of MP in liver transplantation (LT), identify the challenges in its application, and determine future research directions in this field. This review summarizes the findings from clinical trials on hypothermic MP, normothermic MP (NMP),

explores novel dynamic preservation approaches, such as normothermic regional perfusion, ischemia-free transplantation, combinations of MP techniques, and long-term NMP, addresses the obstacles to standardizing MP protocols, and highlights the critical role of clinical trials in validating various aspects of the perfusion process.

Keywords
Machine perfusion; liver transplantation; hypothermic machine perfusion; normothermic machine perfusion; normothermic regional perfusion

1. Introduction

Liver transplantation is one of the most successful forms of solid organ transplantation and continues to be the only curative treatment for end-stage liver disease. Developed as a standard medical practice in the mid-1980s, liver transplantation has been remarkably effective for the treatment of end-stage liver disease, bestowing a quality of life that nearly mirrors normalcy upon individuals who would otherwise face imminent mortality [1]. Consequently, the need for liver transplantation has increased globally.

Approaches to minimize the disparity between the supply and demand for liver grafts have included the utilization of what was once considered non-utilizable as "marginal" or "extended criteria" liver grafts, along with livers obtained from donation after cardiac death (DCD) [2]. However, these grafts are underutilized because they are associated with increased risks of post-transplant primary graft failure. Moreover, these grafts are more susceptible to ischemia–reperfusion injuries and are associated with an increased risk of biliary system complications [3].

Static cold storage (SCS) has been the gold standard for graft preservation [4]. However, the technique for the procurement of organs from DCD donors typically involves discontinuing all life-support measures. The donors experience hemodynamic instability and hypoxia during this period until circulatory arrest is confirmed. Following this event, a mandatory 5-minute period of waiting is required prior to organ procurement, which further compromises the quality of the graft [5]. The interval from the withdrawal of life-sustaining therapy to the introduction of a cold preservation solution is called warm ischemia [6]. Although SCS reduces metabolic activity, anaerobic processes still persist, leading to the depletion of adenosine triphosphate and the build-up of reactive oxygen species [6]. SCS offers significant benefits, such as ease of use and affordability, and is suitable for the procurement of low-risk organs. However, SCS has four main limitations: it does not reverse ongoing organ damage, it may cause additional harm to the organ during storage, organ viability cannot be evaluated during storage, and the storage duration is restricted [6]. These issues become more critical in cases of livers obtained from high-risk donors, which comprise an increasing proportion of liver donors. Severe ischemia–reperfusion-related injuries pose significant challenges in fulfilling the need for critical transplantation [7]. Machine perfusion (MP) is being adopted with greater frequency to address the constraints of SCS. This technique expands the pool of usable higher-risk donor organs and can reduce the cold ischemia time, which is particularly beneficial for obtaining marginal organs that are more prone to damage caused by ischemia and reperfusion.

Two primary MP methods are currently in use: hypothermic MP (HMP), which slows cellular metabolism using cold temperatures while flushing out metabolites and toxins; and normothermic MP (NMP), which maintains cellular functions at normal body temperatures. NMP allows the assessment of organ viability under near-physiological conditions. Innovative variations of MP, such as normothermic regional perfusion (NRP), ischemia-free liver transplant, sub-normothermic MP, and long-term NMP, have also been introduced recently.

This review article examined MP as an innovative advancement in the field of liver transplantation, reviewing the possible solutions to the limited availability of organs and improving the viability of donated livers.

2. Hypothermic Machine Perfusion (HMP)

HMP refers to various mechanical perfusion techniques that are based on the use of cold perfusion. Hypothermic oxygenated perfusion (HOPE) involves delivering the perfusate through the portal vein; while dual HOPE (D-HOPE) delivers perfusate through both the portal vein and the hepatic artery [8].

The first prospective trial on ex-situ HMP was published in 2010, which reported a significant reduction in patients' post-operative liver transaminase and serum bilirubin levels [9]. Another milestone in HOPE was achieved after a study showed that liver grafts obtained from DCD have comparable postoperative outcomes to matched liver grafts from brain death (DBD) [10]. A further study including a larger cohort of the same patient groups substantiated these findings [11].

HOPE and SCS have been compared in randomized controlled trials (RCTs). The trials demonstrated that the use of HOPE-treated DCD allografts reduced non-anastomotic biliary strictures, the incidence of post-reperfusion syndrome, early allograft injury, graft failure [12, 13], and liver-related complications [12]. Additionally, HOPE was found to improve long-term graft survival and reduce late-onset morbidity [14].

These previous studies demonstrated that HOPE enhances graft survival and lowers the rate of complications (Table 1). The protective effects of this method may facilitate increased use of marginal organs, thereby addressing the growing need for donor organs.

Table 1 Overview of published randomized controlled trials on the use of hypothermic machine perfusion in liver transplantation.

Authors	Study design	Intervention	Perfusion characteristics	Endpoint	Outcome	Comments
Guarrera et al. [9]	Prospective trial	HMP vs. CS		Mean incidences of PNF, EAD, and patient and graft survival at 1 month and 1 year.	EAD: 5% (HMP) vs. 25% (CS). The HMP group had significantly lower serum injury markers. Mean hospital stay: 10.9 ± 4.7 days (HMP) vs. 15.3 ± 4.9 days CS) (p = 0.006).	HMP safely and reliably preserved donor livers.
Dutkowski et al. [10]	Cohort study	HOPE of DCD (n = 8) vs. DBD (n = 8)	perfusate cooled (10°C) and oxygenated (pO_2 60 kPa) Device: ECOPS device (Organ Assistò). Perfusion pressure: <3 mmHg, Flow rates: 100 to 150 ml/min (0.13 ml/min/g liver).	Liver enzyme levels, kidney function, and hospital and ICU stay lengths	HOPE of DCD grafts showed comparable or better outcomes, including liver enzyme levels, kidney function, ICU and hospital stay, than matched DBD grafts.	The first report on HOPE of DCD liver grafts and subsequent transplantation compared with DBD graft.
Dutkowski et al. [11]	Cohort study	HOPE of DCD (n = 25) vs. SCS of DCD (n = 50)	Perfusate: Recirculated UW gluconate solution (KPS-1) Flow rate: 120–180 mL/min Oxygenation: pO_2 80–100 kPa Temperature: 10°C Device: Organ Assist	Graft injury, graft failure	Peak ALT level (1239 vs. 2065 U/L, p = 0.02); biliary complications (20% vs. 46%, p = 0.042), intrahepatic cholangiopathy (0% vs. 22%, p = 0.015); 1-year graft survival (90% vs. 69%, p = 0.035); graft failure (0% vs. 18%)	Higher-risk DCD liver grafts preservation might benefit from HOPE
Van Rijn et al. [15]	Multicenter RCT (DHOPE-DCD ClinicalTrials.gov number, NCT02584283)	HMP (n = 78) vs. SCS (n = 78)	HA perfusion pressure: 25 mm Hg PV perfusion pressure: 5 mm Hg Perfusate temperature: 10°C	Primary outcome: incidence of non-anastomotic biliary strictures within 6 months after transplantation. Secondary endpoints: other graft-related and general complications.	Non-anastomotic biliary strictures: 6% vs. 18% (risk ratio, 0.36; 95% CI, 0.14 to 0.94; p = 0.03). Post-reperfusion syndrome: 12% vs. 27% (risk ratio, 0.43; 95% CI, 0.20 to 0.91). Early allograft dysfunction: 26% vs. 40% (risk ratio, 0.61; 95% CI, 0.39 to 0.96).	HOPE is associated with fewer non-anastomotic biliary strictures following DCD graft LT compared with SCS.
Czigany et al. [12]	Multicenter RCT	HOPE ECD (n = 23) vs. DBD (n = 23)	Perfusate:	Primary outcome: peak ALT levels within 7 days following LT. Secondary endpoints: EAD,	Serum peak ALT (418 [IQR: 221-828] vs. 796 [IQR: 477–1195] IU/L p = 0.030), 90-day complications (44% vs. 74%, p = 0.036), CD	HOPE reduces EAD and enhances outcomes in ECD-DBD LT.

			3 to 4 L of oxygenized (pO_2 of 60–80 kPa) and cooled (10°C) re-circulating UW-MPS solution. End-ischemic HOPE was applied through the PV for at least 1 h in a pressure-controlled system (2–3 mm Hg)	postoperative complications, CCI, ICU and hospital length of stay,	grade ≥3 (32 [IQR: 12–56] vs. 52 [IQR: 35–98]) CCI, p = 0.021], ICU and hospital stays (5 [IQR: 4–8] vs. 8 [IQR: 5–18] days, p = 0.045; 20 [IQR: 16–27] vs. 36 [IQR: 23–62] days, p = 0.002). EAD (17% vs. 35%; p = 0.314).	
Ravaioli et al. [16]	RCT	HOPE (n = 55) vs. SCS (n = 55)	Device: Vitasmart (Bridge to Life, DG, USA)	Primary outcome: incidence of EAD.	HOPE group: Lower EAD rate (13% vs. 35%, p = 0.007) and higher graft survival rate (p = 0.03, log-rank test) one year after liver transplantation; Lower re-transplantation (0% vs. 11%, p = 0.03, NNT of nine), lower re-admission at 6 months (20% vs. 38%, p = 0.04), lower cardiovascular complications (three vs. 11 cases, p = 0.04)	HOPE of ECD grafts is linked to lower dysfunction rates and longer graft survival than SCS.
Grat et al. [13]	RCT (ClinicalTrials.gov, NCT04812054)	(HOPE (n = 26) vs. SCS (n = 78)	At least 2 h of HOPE through the PV (continuous flow: pressure 3–5 mm Hg) and HA (pulsatile flow: 30 mm Hg/20 mm Hg) Perfusate: UW solution. Perfusate partial oxygen pressure of ≥450 mm Hg. Temperature: 12°C Device: Organ Assist, now XVIVO)	Primary outcome: MEAF score. Secondary outcome: 90-day morbidity, liver allograft-related complications	Mean MEAF: 4.94 vs. 5.49 in the HOPE and SCS groups, respectively. (p = 0.24). MEAF >8: 3.8% (1/26) vs. 15.4% (12/78) in the HOPE and SCS groups, respectively (p = 0.18). Median CCI: 20.9 vs. 21.8) in the HOPE and SCS groups, respectively (p = 0.19).	Routine use of HOPE of DBD in LT does not seem to be justified as its clinical benefits are limited to high-risk donors.
Schlegel et al. [17]	Multicenter RCT	HOPE (n = 88) vs. SCS (n = 89)	Perfusate: 3 L of oxygenated (70–110 kPa), cooled (8-12°C) re-circulating Belzer MPS (Bridge to Life Ltd.) Flow rate: 150-300 ml/min	Primary outcome: the occurrence of at least one post-transplant complication per patient (CD grade >III) within 1-	The number of patients with at least one CD grade >III complication: 44/85 (51.8%) vs. 46/85 (54.1%) for the HOPE and SCS groups, respectively (odds ratio: 0.91; 95% CI, 0.50–	The number of patients with at least one CD grade >III complication was

			Pressure: 3 mmHg, Perfusate The minimum perfusion duration: 1 h	year after LT. Secondary outcomes: biliary complications; CCI, laboratory parameters, hospital and ICU Length of stay, graft and patient survival.	1.66; $p = 0.76$). Less liver-related CD grade >IIb complications in the HOPE group (risk ratio, 0.26; 95% CI, 0.07–0.77; $p = 0.027$) after A post hoc analysis. Secondary endpoints were similar between groups.	not significantly affected by HOPE, compared to SCS
Czigany et al. [14]	RCT (HOPE-ECD-DBD trial (clinicaltrials.gov: NCT031 24641)	HOPE ECD (n = 23) vs. DBD (n = 23)	Median dynamic preservation time of 145 minutes (101–203 minutes) Device: LiverAssist; XVIVO Perfusion AB, Göteborg, Sweden)	Late-onset complications, long-term graft survival, and patient survival.	HOPE group: Median reduction of 23 CCI points ($p = 0.003$), fewer major complications (CD grade ≥3, 43% vs. 85% [$p = 0.009$]), less primary graft loss (HOPE, n = 3 vs. SCS, n = 10) SCS group: Significantly lower overall graft survival ($p = 0.029$) and adverse 1-, 3-, and 5-year survival probabilities (HOPE 0.913, 0.869, and 0.869 vs. SCS 0.783, 0.606, 0.519, respectively)	HOPE reduced late-onset morbidity and enhanced long-term graft survival. These are clinical data that support using HOPE more widely in LT.
Panayotova et al. [18]	RCT (NCT03484455)	HMP-O 2 (n = 90) vs. SCS (n = 89)	HA and PV perfusion: continuous, non-pulsatile, flow-controlled (target flow 0.66 mL/g liver/minute adjusted by graft weight)	Primary endpoint: Incidence of EAD	EAD 11.1% HMP-O2 (n = 7) and 16.4% SCS (n = 12).	HOPE shows reduced primary non-function and early allograft dysfunction rates, along with fewer biliary strictures.

Abbreviations: ALT, alanine aminotransferase; DBD, donation after brain death; DCD, donation after circulatory death; CCI, comprehensive complication index; CD, Clavien–Dindo; CI, confidence interval; CS, cold storage; EAD, early allograft dysfunction; EASE, early allograft failure simplified estimation; ECD, extended criteria; HA, hepatic artery; HOPE, hypothermic oxygenated perfusion; HMP, hypothermic machine perfusion; ICU, intensive care unit; LT: liver transplantation; MEAF, model for early allograft function; PV, portal vein; SCS, static cold storage; RCT, randomized controlled trial; UW: University of Wisconsin.

3. Normothermic Machine Perfusion (NMP)

NMP restores cell metabolism and maintains the physiological state of the liver by keeping the graft at a normal temperature and supplying sufficient oxygen and nutrients throughout the entire liver preservation process [19]. The main components of NMP devices are a blood reservoir, heat exchanger, oxygenator, and one or two pumps for the portal venous and hepatic artery [19, 20].

The creation of a perfusion chamber in 1935 for the preservation of organs at room temperature marked the beginning of NMP's history [21]. Ravikumar et al. investigated liver transplantation utilizing NMP-preserved livers in a phase I trial in 2016, showing that this approach is safe and feasible [22]. Subsequently, a phase III multicenter RCT wherein NMP and SCS were compared indicated that NMP is associated with a lower incidence of graft injury, organ loss, and longer mean preservation time [23]. In 2019, Ceresa et al. conducted a study of 31 liver transplants to assess NMP after cold storage. The results indicated a 94% graft survival and 13% incidence of early allograft dysfunction within 30 days. The graft and patient survival rates at 12 months were 84% and 90%, respectively [24]. In a study by Ghinolfi et al., 20 liver transplant recipients who received livers from donors aged 70 years or older were divided into NMP and cold storage (CS) groups for evaluation. At 6 months, both groups showed similar survival rates and biopsy results. No major histological advantages of NMP were noted [25]. Bral et al. compared NMP with SCS for liver transplantation and found no difference in short- or mid-term graft survival; however, the NMP group had a longer hospital stay than the SCS group [26].

The primary justification for NMP is its ability to measure objective graft function parameters and identify marginal organs that would otherwise be eliminated as transplantable. NMP is used to assess the graft survival rate by assessing liver and bile duct function parameters, liver injury parameters, and hemodynamic analysis [27]. The survival rate test offers quantifiable predict of liver function following liver transplantation [28].

The viability of high-risk donor livers that underwent NMP prior to transplantation was investigated by Mergental et al. The results showed that despite initial preservation by static refrigeration, the transplanted livers functioned well immediately post-transplant, with normal liver function test results recorded over an average follow-up period of 7 months. This pilot study demonstrated that after viability assessment using NMP, livers previously deemed unsuitable for transplantation could be successfully transplanted to low-risk recipients without compromising safety [29]. Mergental et al. further conducted a single-center phase II trial evaluating high-risk livers previously deemed “non-transplantable” using NMP and found the 1-year patient and graft survival rates to be 100% and 86%, respectively; the 5-year patient and graft survival rates were 82% and 72%, respectively, based on long-term follow-up of the NMP group [30]. Furthermore, Quintini et al. found that of 21 livers that were declined for transplant, 15 (71.5%) were deemed transplantable after assessment using NMP. There were no instances of primary non-function post-transplant; however, seven livers showed early dysfunction that recovered quickly [31]. Although there are no definitive criteria for pre-transplant assessment of liver function using NMP, the metrics commonly used included perfusate lactate clearance, pH stability without the need for bicarbonate supplements, stable hemodynamics in the hepatic artery and portal vein; liver transaminase levels, and serum glucose levels [32]. By employing these techniques for pre-transplant viability assessment, the hazards of using marginal donor livers can be minimized, the probability of organ

rejection and primary non-function after transplant can be decreased, and more organs can be made available for patients in need.

A primary limitation of NMP is that organs often undergo SCS before NMP is performed at the transplant center, owing to logistical issues. Notably, a portable NMP device was employed to avoid using SCS in a recently published RCT. The use of the portable NMP device also allowed the use of livers from DCD [33]. Another drawback of NMP is its high cost compared to HMP/HOPE or SCS; however, it should be noted that its cost-effectiveness was demonstrated by fewer post-transplant complications and increases in the donor pool. A summary of published studies on NMP LT is presented in Table 2.

Table 2 Overview of published studies on the use of NMP in liver transplantation.

Trial	Type	Intervention	Perfusion characteristics	Perfusate	MP time	Endpoint	Outcome	Conclusion/Comments
Ravikumar, et al. [22]	Phase 1 (First-in-Man) clinical trial	NMP (n = 20) 1:2 to SCS (n = 40)	Hepatic artery flow (280 ± 120 ml/min) Portal vein flow (1.11 ± 0.2 L/min) HA: 60–75 mmHg	3 units of cross-matched pRBC + 1 unit of Gelofusine® (B Braun)	9.3 h (3.5–18.5 h)	Primary endpoint: 30-day graft survival	Similar 30-day graft survival between groups (NMP, 100% vs. SCS, 97.5%; *p* = 1.00).	This first report of LT using NMP-preserved livers.
Selzner et al. [34]	single-arm, nonblinded pilot study	NMP (n = 10) SCS (n = 30)	HA: 0.3 L/min (0.2–0.4) PV: 1.25 L/min (1.2–1.3)	3 units PRBC + Steen solution	8 h (5.7–9.7 h)	Lactate, bile production, ALT/AST, ICU stay, hospital stay, complications	No significant difference in graft function, hospital/ICU stay, or complications	NMP is safe and results in comparable outcomes to CS
Bral et al. [26]	Single-center study	NMP (n = 10) vs. SCS (n = 30)	Not reported	500 mL of Gelofusine (B Braun, Melsungen, Germany) + 3 units of type O-pRBC.	11.5 h (3.3–22.5 h)	Primary outcome: 30-day post-transplant graft survival.	30-day graft survival 90% (NMP) vs. 100% (SCS) (*p* = 0.08); 6-month graft survival NMP: 8/10 (80%) vs. SCS: 30/30 (100%) (*p* = 0.01); longer ICU and hospital stay in the NMP group	NMP has potential technical risks, larger randomized study needed.
Nasralla et, al. [23]	Phase III multicenter RCT	NMP (n = 121) vs. SCS (n = 101)	HA ≈ 0.28 L/min PV ≈ 1.1 L/min	Gelofusine® (B Braun) + 3-unit donor-matched pRBC	9.1 h (6.2–11.8 h)	Primary outcome: peak AST during the first 7	The NMP group showed a 49.4% reduction in peak	>50% fewer discarded organs in the NMP group.

					days after transplantation	AST level during the first 7 days after transplant compared to the SCS group	
Ceresa et al. [24]	Multicenter prospective study	31 livers (23 DBD and 8 DCD) transplanted (pSCS-NMP)	HA: 0.44 L/min (0.29–0.59) PV: 1.08 L/min (0.96–1.2) HA: 67 mmHg (64–70)	Gelofusine® (B Braun) + unspecified blood products	14 h 10 min +/- 4 h 46 min	The primary endpoint: 30-day graft survival Secondary endpoints: peak AST, EAD, PRS, adverse events, ICU and hospital stay, biliary complications, and 12-month graft survival.	30-day graft survival rate: 94%. EAD: 13% within 30 days; PRS in 3 (10%) livers; 12-month graft survival was 84%, and patient survival was 90%.	pSCS-NMP is feasible and safe
Jessem et, al. [35]	retrospective analysis	NMP (n = 12) vs.SCS (n = 27)				Peak AST, INR, ALP, bilirubin. AST, INR, ALP, bilirubin at 7 days. Length of ICU stay, rate of rejection, and graft survival at one year	significantly lower peak AST within 7 days in NMP group. ALP and total bilirubin levels, ICU stays, acute rejection rates, and one-year survival of grafts and recipients were comparable across the groups.	Reduced IRI in NMP recipients resulted from both inflammation inhibition and enhanced graft regeneration.
Ghinolfi et, al. [25]	RCT	NMP (n = 10) vs. SCS (n = 10)		Blood-based perfusate	250 (195–282) min	Survival of graft and patient 6 months after LT Secondary endpoint: IRI, biliary	No significant difference in the survival of grafts and patients; Histological evidence of reduced	NMP of older liver grafts shows histological signs of reduced IRI, but its

						complications, evaluation of liver and bile duct biopsies	ischemia/reperfusion injury in NMP liver grafts.	clinical advantage is yet to be proven.
Mergental et al. [28]	VITTAL clinical trial (ClinicalTrials.gov number NCT02740608) (Prospective, non-randomized, adaptive phase 2 trial in a large single center)	NMP (n = 22) vs. SCS (n = 44)			DBD 629 (509–700) min DCD 549 (424–780) min Overall 587 (450-705) min	Primary: feasibility of NMP for the discarded organ recovery and achievement of successful LT Secondary: LFTs, 90-day graft survival, hospital stay, vascular complications, biliary strictures with MRCP at 6 months	Survival rates for patients and grafts are comparable at 12 months; Higher rate of EAD and non-anastomotic biliary strictures in NMP group. No differences in other outcome metrics.	Many discarded livers can be successfully recovered for transplantation using NMP.
Markmann et al. [33]	RCT	Portable NMP (n = 153) vs. ICS (n = 147)	HA: 0.7 ± 0.2 L/min PV: 1.3 ± 0.1 L/min HA: 70.6 ± 16.2 mmHg PV: 5.4 ± 2.3 mmHg	Warm, oxygenated and nutrient-enriched, blood-based perfusate	276.6 min ± 117.4 min	Primary effectiveness endpoint: incidence of EAD. Primary safety endpoint: severe adverse events related to liver grafts within 30 days post-LT	EAD: 27/150 (18%) in the NMP group vs. 44/141 (31%) in the ICS group; $p = 0.01$). Moderate to severe lobular inflammation: 9/150 (6%) vs. 18/141 (13%) ($p = 0.004$). Use of DCD livers (28/55 (51%) vs. 13/51 (26%) ($p = 0.007$) IBC 6 months (1.3% vs. 8.5%; $p = 0.02$)	NMP preservation of deceased donor livers reduces the incidence if both post-transplant EAD and IBC. NMP is associated with superior post-transplant outcomes and increased donor liver use.

					and 12 months (2.6% vs. 9.9%; $p = 0.02$)	
Chapman et al [36]	RCT	NMP (n = 133) vs. SCS (n = 130)	NMP: 553.8 ± 115.9 minutes (135 ± 35.7 minutes cold and 356 ± 106 minutes normothermic preservation)	Primary outcome: EAD incidence. Secondary outcomes: graft and patient survival, postreperfusion syndrome, liver and kidney function, IRI, biliary complications, organ use, health economics, and safety	EAD 20.6% (NMP) vs. 23.7% (SCS) Reduced postreperfusion syndrome in the NMP group (5.9% vs 14.6%)	NMP did not reduce EAD; higher-risk donor livers appeared to benefit more than lower-risk donor livers.

Abbreviations: ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine aminotransferase; CD, Clavien–Dindo; CI, confidence interval; CS, cold storage; DBD, donation after brain death; DCD, donation after circulatory death; EAD, early allograft dysfunction; ECD, extended criteria; HA, hepatic artery; HOPE, hypothermic oxygenated perfusion; IBC, ischemic biliary complications; ICS, ischemic cold storage; ICS, ischemic cold storage; ICU, intensive care unit; INR, international normalized ratio; IRI, ischemia reperfusion; IRI: Ischemia/reperfusion injury; IU, international unit; LFT, liver function test; MRCP, magnetic resonance cholangiopancreatography; NMP, normothermic machine perfusion; POD, postoperative day; pRBC: packed red blood cell; PRS, postreperfusion syndrome; pSCS-NMP: post-static cold storage normothermic machine perfusion; PV, portal vein; RCT, randomized controlled trial; SCS, static cold storage.

4. Normothermic Regional Perfusion (NRP)

NRP is used in potentially transplantable organs as a way of in situ perfusion after circulatory arrest [37, 38]. It has been used increasingly to reduce biliary complications and graft failure post-transplantation. Unlike other MP technologies (HOPE and NMP) that can be applied in DBD and DCD liver allograft management, NRP can only be used in DCD liver allograft management.

After circulatory arrest and a hands-off period that can range from 5 to 20 minutes depending on local regulations, extracorporeal membrane oxygenation is initiated with cannulation of the femoral artery or aorta and with venous return from the femoral vein or inferior vena cava [39]. It is also important to distinguish between abdominal normothermic regional perfusion (A-NRP) and thoracoabdominal normothermic regional perfusion (TA-NRP). A-NRP specifically targets the abdominal organs by providing in situ perfusion through the infrarenal aorta and IVC, or the femoral artery and vein, without initiating cardiac activity by applying supraceliac aortic cross-clamp/occlusion balloon. In contrast, TA-NRP performs in situ perfusion of both thoracic and abdominal organs via the aortic arch and right atrium and includes the restoration of cardiac activity, with the aortic arch vessels clamped to prevent brain perfusion [40]. Initial studies show promising outcomes for DCD livers obtained through TA-NRP [41]. However, ethical concerns about restarting cardiac activity during the process have led to a temporary pause of TA-NRP in some organ procurement organizations, pending further ethical review [42]. Conversely, A-NRP faces fewer ethical concerns because it does not reinitiate cardiac activity, and perfusion is confined regionally to only the abdominal organ.

Though an RCT on NRP has not been published yet, retrospective data have demonstrated that NRP transplantation produces better results than SCS transplantation. Overall, preliminary data showed that NRP can enhance the results of DCD organ transplantation through the mitigation of early allograft dysfunction, enhancement of graft survival, reduction of biliary problems, and mitigation of retransplantation risk (Table 3). Owing to positive clinical outcomes, France and other European nations now require NRP for all DCD donations [39, 43].

Table 3 Overview of recent studies on normothermic regional perfusion.

Authors	Type	Intervention	Perfusion characteristics	MP time	Results	Conclusion/Comments
Hesshemier et al. [37]	Observational cohort study of cDCD liver transplants	A-NRP (n = 545) vs. SRR (n = 258)	pump flow 2.2–2.4 L/min/m	A-NRP duration 111 [81–126] min	Overall biliary complications (OR, 0.300; 95% CI, 0.197–0.459; $p < 0.001$), ischemic-type biliary lesions (OR, 0.112; 95% CI, 0.042–0.299; $p < 0.001$), graft loss (HR 0.371; 95% CI, 0.267–0.516; $p < 0.001$), and patient death (HR, 0.540; 95% CI, 0.373–0.781; $p = 0.001$)	A-NRP lessened traditional limitations of cDCD LT.
Hesshemier et al. [44]	Observational cohort study	A-NRP (n = 95) vs. SRR and cold preservative solution (n = 117)	Pump flow is maintained >1.7 L/min/m^2, temperature 37°C, PaO_2 100-150 mmHg, and hematocrit >20%.	Post-mortem NRP 120 [79–136] min	Overall biliary complications (OR, 0.14; 95% CI, 0.06–0.35; $p < 0.001$), ischemic-type biliary lesions (OR, 0.11; 95% CI, 0.02–0.57; $p = 0.008$), and graft loss (HR, 0.39; 95% CI, 0.20–0.78; $p = 0.008$)	In cDCD LT, postmortem NRP reduces graft loss, ischemic-type biliary lesions, and postoperative biliary complications, it also permits LT from cDCD donors of advanced age
Watson et al. [45]	Retrospective analysis	NRP-DCD donors (n = 43) vs. standard DCD donors (n = 187)	Abdominal flow = 2.5–3 L/min Thoracoabdominal flow = 4–6 L/min	284 (122–530 min)	EAD: NRP, 12% vs. standard DCD group, 32% ($p = 0.0076$). Ischemic cholangiopathy: 0% vs 27% ($p < 0.0001$).	NRP as part of recovery after DCD transplant leads to superior hepatic outcomes.
Gaurav et al. [46]	Single center, retrospective analysis of data collected prospectively	NRP (n = 69) vs. NMP (n = 67) vs. SCS (n = 97).	NRP: 2.5 to 3 L/min	NRP duration 133 (121–143) min; NMP duration 460 (330–569) min fWIT NRP 19 (15–24) min, fWIT NMP 15 (12–18) min	6-month survival: NRP, 94% vs. NMP, 90% vs. SCS, 87%. Three-year survival: NRP, 90% and 76% for both SCS and NMP.	In NRP group, both A-NRP and TA-NRP are applied. NRP and NMP result in improved early liver function compared to SCS, with NRP providing better preservation of the biliary system.

Rodriguez et al. [47]	Observational cohort study	NRP (n = 39) and DBD (n = 78)		90–120 minutes	Postoperative mortality: NRP, 5.1% vs. DBD, 8.9% (p = 0.442). Post-biliary complications: NRP, 12% vs. DBD, 5% (p = 0.104).	NPR converts suboptimal DCD liver grafts into organs comparable to DBDs
Barbier et al. [48]	Retrospective, multicenter study	A-NRP-cDCD donors (n = 156)	A-NRP pump flow: 2 and 3.5 L/min NRP lasted between 1 and 4 h	Mean NRP duration was 179 (±43) min; Mean fWIT 22 min	Three patients (1.9%) showed graft non-function, whereas 30 (19.2%) showed EAD, with an average MEAF score of 7.3 (±1.7). NRP lasted and average of 179 (±43) minutes and had with no effect on EAD rates.	A-NRP duration in cDCD donors appears to have no effect on liver graft function and biliary outcomes post-transplantation.
Savier et al. [49]	Multicenter retrospective study	A-NRP of DCD grafts (n = 50) vs. DBD grafts (n = 100)		A-NRP median duration 190 (151–223) min; median fWIT 22 (20–26.8) min	Two-year graft survival: DCD grafts, 88% vs. DBD grafts, 85% (p = 0.91). Biliary complications: 16% vs. 17%, (p = 0.94)	cDCD LT following postmortem NRP is safe and effective in selected recipients, offering comparable graft and patient survival, and similar risks of biliary complications and EAD as DBD LT.
Ruiz, Valdivieso et al. [50]	Single center retrospective study with a 1:2 case-matching ratio	A-NRP in cDCD LT (n = 100) vs DBD LT (n = 200)	Pump flow 1.7 L/min, perfusion pressure > 60 mmHg	121 (118-128) min; median fWIT was 10 (8.5–12.2) min	Perioperative ALT levels and EAD were similar between the DBD and cDCD transplant groups. Overall graft survival for the cDCD group 1-year and 3-year were 99% and 93%, respectively, compared to 92% and 87% for the DBD group, respectively (p = 0.04).	A-NRP in cDCD LT shows similar outcomes as those obtained with DBD LT.
Viguera et al. [51]	Multicenter cohort study	cDCD with A-NRP vs. DBD			Number of RBC units transfused in the DBD vs. cDCD groups: 4.7 (0.2) vs. 5.5 (0.4) (p = 0.11). Similar graft and patient survival rates were observed between the cDCD and DBD groups.	cDCD with A-NRP did not require more RBC transfusions and can be safely expanded in use
Mohkam et al. [52]	International observational study	In situ A-NRP vs. ex situ NMP		Total dynamic perfusion time 184 min (NRP) vs. 525 min (NMP)	Incidence rates for non-anastomotic biliary strictures were 1.5% vs. 2.9% (p > 0.99), EAD at 20.6% vs. 8.8% (p = 0.13), and 30-day graft loss at 4.4% vs. 8.8% (p = 0.40). Peak post-	Both A- NRP and NMP produced comparable results and met the expected standards for DBD livers.

				Total ex situ preservation times 516 min (NRP) vs. 651 min (NMP)	transplant AST levels were 872 vs. 344 IU/L ($p < 0.001$).	
Muller et al. [53]	Retrospective, comparative, cohort study	NRP (n = 132) vs. HOPE (n = 93) in LT from cDCD.	Minimal flow 1.7 L/min	NRP duration 184 (159–207) min; HOPE duration 132 (105–165) min	One-year tumor-death censored graft survival was 93% for NRP versus 86% for HOPE ($p = 0.125$), and patient survival was 95% for NRP versus 93% for HOPE ($p = 0.482$).	NRP and HOPE methods in cDCD LT achieve recipient and graft survival rates above 85%, similar to DBD LT outcomes.
Croome et al. [40]	Case series report	14 A-NPR donations result in 11 LT		Median A-NRP time 68 min	No case of post-reperfusion syndrome or PNF, and all livers functioned well without IC at the latest follow-up.	This report shared U.S experience of implement a portable A-NRP program, that has produced good short-term results.
Brubaker et al. [38]	Retrospective, observational cohort study	NRP (TA NRP,79, and A-NRP 27) vs SRR (n = 136)	Pump flow ranged from 2.0 to 5.0 L/min, varying by whether A-NRP or TA-NRP was used.	Perfusion duration per transplant center and OPO practice guidelines.	PNF only in SRR group (n = 2), EAD: SRR, 56.1 vs NRP, 36.4% ($p = 0.007$); biliary anastomotic stricture: SRR, 22.4% vs NRP,6.7% ($p = 0.001$); IC: SRR 9% vs NRP, 0% ($p = 0.002$); comparable patient and graft survival in both groups	The practicality of Adopting A-NRP and TA-NRP across various US transplant centers indicate the feasibility of expanding NRP use to enhance organ availability and reduce waitlist mortality.

Abbreviations: A-NRP, abdominal normothermic regional perfusion; ALT, alanine aminotransferase; cDCD, controlled donation after circulatory death; CI, confidence interval; CS, cold storage; DBD, donation after brain death; DCD, donation after circulatory death; EAD, early allograft dysfunction; ECD, extended criteria; fWIT, functional warm ischemia time; HOPE, hypothermic oxygenated perfusion; IC, ischemic cholangiopathy; LT: liver transplantation; NRP, normothermic regional perfusion; OPO, organ procurement organization; PNF, primary nonfunction; RBC, red blood cell; SCS, static cold storage; SRR, standard rapid recovery; TA-NRP, thoracoabdominal normothermic regional perfusion NMP, normothermic machine perfusion.

5. Combination of Machine Perfusion Techniques

The combination of HOPE with controlled oxygenated rewarming (COR) and NMP could leverage their benefits. This is because HOPE reduces ischemia–reperfusion injury, COR eases the transition from cold to warm states, minimizing organ damage, and NMP at 37°C allows for functional assessment while mitigating ischemic injury. De Vries et al. introduced a protocol in which D-HOPE, COR, and NMP were combined with a new hemoglobin-based oxygen carrier. Based on specific parameters (perfusate pH, lactate levels, bile production, and biliary pH), 5 of 7 livers that were previously declined were transplanted, and all showed a 100% graft survival rate at 3 months [54]. Van Leeuwen et al. studied 16 DCD livers subjected to a sequence of D-HOPE-COR-NMPs after SCS during transportation; consequently, 11 were deemed suitable for transplantation [55]. In a prospective observational cohort study conducted by the same group, the authors noted that ex situ machine perfusion employing sequential D-HOPE-NMP for the resuscitation and viability assessment of high-risk donor livers achieved excellent transplant outcomes [56].

Many centers in Italy have implemented in situ abdominal NRP followed by ex situ MP of DCD organs. Despite the significant hurdle presented by prolonged warm ischemia time, the Italian centers reported good outcomes of DCD live transplantation using this approach [57, 58].

Patrono et al. compared abdominal NMP plus D-HOPE in controlled DCD livers versus DBD livers and found similar early outcomes between groups. Biliary complication rates were 15% for DCD and 22% for DBD, with comparable ischemic cholangiopathy incidences (DCD, 5%; DBD, 2%). One-year patient survival was 100% for DCD and 95% for DBD, while graft survival rates were 90% and 95%, respectively. Furthermore, the combination of abdominal NRP and D-HOPE in DCD liver with a prolonged warm ischemia time resulted in outcomes comparable to those of DBD [59].

6. Novel Approaches

6.1 Ischemia-Free Liver Transplant

The complex nature of ischemia–reperfusion injury makes it challenging to advance new scientific concepts in its management. Various MP techniques, such as HMP, HOPE, and NMP, have been introduced to enhance organ preservation in clinical settings, with studies confirming their safety and effectiveness. However, these methods still involve a period of ischemia during the procurement and implantation of the allograft because they are implemented following a cold storage period. Ischemia-free liver transplantation is an innovative technique that maintains continuous blood supply under continuous in-situ NMP during the procurement and transplantation process, thus reducing post-reperfusion syndrome upon allograft revascularization [60]. In 2018, He et al. described an MP method that initiates blood flow in the donor liver before circulatory arrest. This technique involves the cannulation of the common bile duct, infrahepatic inferior vena cava, portal vein, and hepatic artery to establish an in-situ NMP circuit, while harvesting the liver. During transplantation, the liver was connected while still being perfused by the machine, ensuring continuous oxygenated blood flow and preventing ischemia and reperfusion injury [61]. An RCT showed that compared to traditional methods, this technique reduces complications from ischemia-reperfusion injury in liver transplant recipients. This approach could advance transplantation

practices, improve outcomes, increase organ utilization, and offer insights into how organ injury affects alloimmunity [62].

6.2 Long-Term Normothermic Machine Perfusion (NMP)

Current MP technology allows for short-term ex-situ preservation and viability assessment of the liver before transplantation. The field of long-term normothermic perfusion is growing and offers significant prospects for evaluating, recovering, and improving organ function. Clavien et al. described a case wherein a human liver graft was successfully transplanted after being kept for 3 days by utilizing ex situ NMP [63]. After initial experiments with partial swine livers, Mueller et al. applied the protocol to 21 partial human livers and achieved a perfusion duration goal of 1 week. The liver sections demonstrated stable perfusion and normal function, while maintaining their structural integrity for up to 1 week [64]. Lau et al. focused on creating a long-term ex-situ perfusion model and achieved a median viability of 125 hours and a median survival of 165 hours during ex-situ perfusion. This study demonstrated the feasibility of long-term ex-situ liver perfusion and perfusing livers using a standardized approach [65].

6.3 Extracorporeal Liver Perfusion (ECLP)

Beyond its application in liver transplantation, MP potentially can assist liver metabolism for patients with liver failure. A circuit that circulates the patient's blood through an entire liver—which could be human or animal—is used in ECLP [66-68]. An MP circuit with a healthy liver connected to the patient's circulation would make this feasible. In situations where spontaneous recovery might occur, for example in those with acetaminophen toxicity, treatment utilizing daily ECLP via an isolated genetically modified pig liver could offer a vital support. This approach would eliminate the need for extensive immunosuppressive treatments or a liver transplant. Preclinical investigations shown ECLP using pig livers can potentially preserve injured human livers for 1 week [69]. Although the numbers were small, using ECLP with pig livers in ALF patients showed a survival benefit [70-72]. Research indicates this method is safe and practical for bridging patients to liver transplantation [73].

7. Current Challenges

The growing interest in MP owing to its beneficial effects on organ quality and recipient outcomes has not yet been translated into the clinical practice of liver transplantation. The factors determining the wider adoption of specific technologies in clinical practice remain unclear. For example, the benefits of MP for low-risk liver grafts, which already yield favorable short and long-term outcomes with SCS, are not well-studied [74]. It is unsure at what threshold of risk should MP be involved. Even though there are many RCTs already published or ongoing, more information about allograft function, liver use rate, recipients' outcomes, and cost-effectiveness should be evaluated for clinical applications and impact. Furthermore, it should be mentioned that most currently available evidence is restricted to 1-year follow-up, which seems to be one of the primary drawbacks in the current literature [75]. Consensus and RCTs are critical for validating aspects of MP, such as infusion pressure, solution components, perfusion duration, and biological indicators for organ quality assessment.

Criticism has been raised that the aspartate transaminase/alanine aminotransferase level is inconsistent with transplant outcomes, especially in DCD liver grafts [76]. Hospital and intensive care unit stay durations may differ significantly between centers owing to varying discharge policies and facility availability, potentially weakening their reliability as endpoints [75, 77]. Therefore, clinically relevant endpoints and future trial guidelines need to be established [78].

To fully comprehend how MP influences the utilization of donor livers and identify areas for improvement, it is vital to understand the reasons behind liver discard [79]. Establishing clear criteria for donor, liver, and recipient risk factors are necessary for routine applications of MP. Creating simpler device registration policies and innovative financial models is essential to facilitate the adoption of MP globally. Moreover, the significant variations in the costs and affordability of new technologies will undoubtedly impact their broader use [79, 80].

8. Future Applications

Enhancing liver preservation, organ usage, functional assessment, and outcomes are areas where MP has shown promise. Future perspectives in MP include ex vivo liver repair and potential personalized organ preservation [81]. Recondition steatotic grafts is a promising application of MP in liver transplantation, given the impact of steatosis on graft viability [82]. Nagrath et al. conducted a preclinical study using a “defatting cocktail” on steatotic rat livers during NMP, which significantly lowered intracellular lipid levels by 50% after 3 hours [83]. MP could also serve as an effective method for removing viral infections from donated livers, including hepatitis C virus infections [84]. Furthermore, with long-term MP, previously wasted livers could be treated with stem cells, organoids, senolytics, or compounds that target the mitochondria and downstream signaling to modulate repair mechanisms and regeneration [85, 86].

9. Conclusion

In this review, we summarize findings from clinical trials on MP techniques, explore emerging MP approaches, address obstacles to standardizing MP protocols, and highlight the critical role of clinical trials in validating various aspects of the perfusion process. Furthermore, we discuss the potential of cutting-edge MP techniques in preserving and enhancing graft quality.

Abbreviations

CD	Clavien–Dindo
cDCD	controlled donation after circulatory death
CI	confidence interval
CS	cold storage
DBD	donation after brain death
DCD	donation after circulatory death
EAD	early allograft dysfunction
EASE	early allograft failure simplified estimation
ECD	extended criteria
ECLP	extracorporeal liver perfusion
HMP	hypothermic machine perfusion

HOPE	hypothermic oxygenated perfusion
LT	liver transplantation
MEAF	model for early allograft function
NMP	normothermic machine perfusion
RCT	randomized controlled trial
SCS	static cold storage

Author Contributions

Yue Qiu MD: writing – original draft. Yinqian Kang MD: review and editing. Hao Liu MD: review and editing. Ibtesam Hilmi, MBCHB, FRCA: Conceptualization, review, and editing. All authors have read and approved the published version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

References

1. Meirelles Júnior RF, Salvalaggio P, Rezende MB, Evangelista AS, Guardia BD, Matielo CE, et al. Liver transplantation: History, outcomes and perspectives. Einstein. 2015; 13: 149-152.
2. Malkawi D, Savsani K, Alfonso A, Lee SD, James N, Sarkar D, et al. The role of normothermic machine perfusion in extended criteria donor grafts: A new direction in liver graft assessment and preservation. Livers. 2023; 3: 709-726.
3. Czigany Z, Lurje I, Schmelzle M, Schöning W, Öllinger R, Raschzok N, et al. Ischemia-reperfusion injury in marginal liver grafts and the role of hypothermic machine perfusion: Molecular mechanisms and clinical implications. J Clin Med. 2020; 9: 846.
4. McAnulty JF. Hypothermic organ preservation by static storage methods: Current status and a view to the future. Cryobiology. 2010; 60: S13-S19.
5. Manara AR, Murphy PG, O'Callaghan G. Donation after circulatory death. Br J Anaesth. 2012; 108: i108-i121.
6. Jing L, Yao L, Zhao M, Peng LP, Liu M. Organ preservation: From the past to the future. Acta Pharmacol Sin. 2018; 39: 845-857.
7. Ozgur OS, Namsrai BE, Pruett TL, Bischof JC, Toner M, Finger EB, et al. Current practice and novel approaches in organ preservation. Front Transplant. 2023; 2: 1156845.
8. Monbaliu D, Brassil J. Machine perfusion of the liver: Past, present and future. Curr Opin Organ Transplant. 2010; 15: 160-166.
9. Guarrera JV, Henry SD, Samstein B, Odeh-Ramadan R, Kinkhabwala M, Goldstein MJ, et al. Hypothermic machine preservation in human liver transplantation: The first clinical series. Am J Transplant. 2010; 10: 372-381.
10. Dutkowski P, Schlegel A, de Oliveira M, Müllhaupt B, Neff F, Clavien PA. HOPE for human liver grafts obtained from donors after cardiac death. J Hepatol. 2014; 60: 765-772.
11. Dutkowski P, Polak WG, Muiesan P, Schlegel A, Verhoeven CJ, Scalera I, et al. First comparison of hypothermic oxygenated PErfusion versus static cold storage of human donation after cardiac death liver transplants: An international-matched case analysis. Ann Surg. 2015; 262: 764-771.

12. Czigany Z, Pratschke J, Froněk J, Guba M, Schöning W, Raptis DA, et al. Hypothermic oxygenated machine perfusion reduces early allograft injury and improves post-transplant outcomes in extended criteria donation liver transplantation from donation after brain death: Results from a multicenter randomized controlled trial (HOPE ECD-DBD). Ann Surg. 2021; 274: 705-712.
13. Grąt M, Morawski M, Zhylko A, Rykowski P, Krasnodębski M, Wyporski A, et al. Routine end-ischemic hypothermic oxygenated machine perfusion in liver transplantation from donors after brain death: A randomized controlled trial. Ann Surg. 2023; 278: 662-668.
14. Czigany Z, Uluk D, Pavicevic S, Lurje I, Froněk J, Keller T, et al. Improved outcomes after hypothermic oxygenated machine perfusion in liver transplantation-Long-term follow-up of a multicenter randomized controlled trial. Hepatol Commun. 2024; 8: e0376.
15. van Rijn R, Schurink IJ, de Vries Y, van den Berg AP, Cortes Cerisuelo M, Darwish Murad S, et al. Hypothermic machine perfusion in liver transplantation-A randomized trial. N Engl J Med. 2021; 384: 1391-1401.
16. Ravaioli M, Germinario G, Dajti G, Sessa M, Vasuri F, Siniscalchi A, et al. Hypothermic oxygenated perfusion in extended criteria donor liver transplantation-A randomized clinical trial. Am J Transplant. 2022; 22: 2401-2408.
17. Schlegel A, Mueller M, Muller X, Eden J, Panconesi R, von Felten S, et al. A multicenter randomized-controlled trial of hypothermic oxygenated perfusion (HOPE) for human liver grafts before transplantation. J Hepatol. 2023; 78: 783-793.
18. Panayotova GG, Lunsford KE, Quillin III RC, Rana A, Agopian VG, Lee-Riddle GS, et al. Portable hypothermic oxygenated machine perfusion for organ preservation in liver transplantation: A randomized, open-label, clinical trial. Hepatology. 2024; 79: 1033-1047.
19. Ceresa CD, Nasralla D, Jassem W. Normothermic machine preservation of the liver: State of the art. Curr Transplant Rep. 2018; 5: 104-110.
20. Shen C, Cheng H, Zong T, Zhu H. The role of normothermic machine perfusion (NMP) in the preservation of ex-vivo liver before transplantation: A review. Front Bioeng Biotechnol. 2023; 11: 1072937.
21. Lindbergh CA. An apparatus for the culture of whole organs. J Exp Med. 1935; 62: 409-431.
22. Ravikumar R, Jassem W, Mergental H, Heaton N, Mirza D, Perera MT, et al. Liver transplantation after ex vivo normothermic machine preservation: A Phase 1 (first-in-man) clinical trial. Am J Transplant. 2016; 16: 1779-1787.
23. Nasralla D, Coussios CC, Mergental H, Akhtar MZ, Butler AJ, Ceresa CD, et al. A randomized trial of normothermic preservation in liver transplantation. Nature. 2018; 557: 50-56.
24. Ceresa CD, Nasralla D, Watson CJ, Butler AJ, Coussios CC, Crick K, et al. Transient cold storage prior to normothermic liver perfusion may facilitate adoption of a novel technology. Liver Transplant. 2019; 25: 1503-1513.
25. Ghinolfi D, Rreka E, De Tata V, Franzini M, Pezzati D, Fierabracci V, et al. Pilot, Open, randomized, prospective trial for normothermic machine perfusion evaluation in liver transplantation from older donors. Liver Transplant. 2019; 25: 436-449.
26. Bral M, Gala-Lopez B, Bigam D, Kneteman N, Malcolm A, Livingstone S, et al. Preliminary single-center Canadian experience of human normothermic ex vivo liver perfusion: Results of a clinical trial. Am J Transplant. 2017; 17: 1071-1080.
27. Li J, Lu H, Zhang J, Li Y, Zhao Q. Comprehensive approach to assessment of liver viability during normothermic machine perfusion. J Clin Transl Hepatol. 2023; 11: 466-479.

28. Mergental H, Laing RW, Kirkham AJ, Perera MT, Boteon YL, Attard J, et al. Transplantation of discarded livers following viability testing with normothermic machine perfusion. Nat Commun. 2020; 11: 2939.
29. Mergental H, Perera MT, Laing RW, Muiesan P, Isaac JR, Smith A, et al. Transplantation of declined liver allografts following normothermic ex-situ evaluation. Am J Transplant. 2016; 16: 3235-3245.
30. Mergental H, Laing RW, Kirkham AJ, Clarke G, Boteon YL, Barton D, et al. Discarded livers tested by normothermic machine perfusion in the VITTAL trial: Secondary end points and 5-year outcomes. Liver Transplant. 2024; 30: 30-45.
31. Quintini C, Del Prete L, Simioni A, Del Angel L, Diago Uso T, D'Amico G, et al. Transplantation of declined livers after normothermic perfusion. Surgery. 2022; 171: 747-756.
32. Watson CJ, Kosmoliaptsis V, Pley C, Randle L, Fear C, Crick K, et al. Observations on the ex situ perfusion of livers for transplantation. Am J Transplant. 2018; 18: 2005-2020.
33. Markmann JF, Abouljoud MS, Ghobrial RM, Bhati CS, Pelletier SJ, Lu AD, et al. Impact of portable normothermic blood-based machine perfusion on outcomes of liver transplant: The OCS liver PROTECT randomized clinical trial. JAMA Surg. 2022; 157: 189-198.
34. Selzner M, Goldaracena N, Echeverri J, Kaths JM, Linares I, Selzner N, et al. Normothermic ex vivo liver perfusion using Steen solution as perfusate for human liver transplantation: First North American results. Liver Transplant. 2016; 22: 1501-1508.
35. Jassem W, Xystrakis E, Ghnewa YG, Yuksel M, Pop O, Martinez-Llordella M, et al. Normothermic machine perfusion (NMP) inhibits proinflammatory responses in the liver and promotes regeneration. Hepatology. 2019; 70: 682-695.
36. Chapman WC, Barbas AS, D'Alessandro AM, Vianna R, Kubal CA, Abt P, et al. Normothermic machine perfusion of donor livers for transplantation in the United States: A randomized controlled trial. Ann Surg. 2023; 278: e912-e921.
37. Hessheimer AJ, de la Rosa G, Gastaca M, Ruíz P, Otero A, Gómez M, et al. Abdominal normothermic regional perfusion in controlled donation after circulatory determination of death liver transplantation: Outcomes and risk factors for graft loss. Am J Transplant. 2022; 22: 1169-1181.
38. Brubaker AL, Sellers MT, Abt PL, Croome KP, Merani S, Wall A, et al. US liver transplant outcomes after normothermic regional perfusion vs standard super rapid recovery. JAMA Surg. 2024; 159: 677-685.
39. Eden J, Sousa Da Silva R, Cortes-Cerisuelo M, Croome K, De Carlis R, Hessheimer AJ, et al. Utilization of livers donated after circulatory death for transplantation-An international comparison. J Hepatol. 2023; 78: 1007-1016.
40. Croome KP, Brown TE, Mabrey RL, Sonnenwald SL, Burns JM, Mao SA, et al. Development of a portable abdominal normothermic regional perfusion (A-NRP) program in the United States. Liver Transplant. 2023; 29: 1282-1291.
41. Croome KP, Mao S, Taner CB. The current landscape of liver transplantation after ex situ machine perfusion and normothermic regional perfusion in the United States. Liver Transplant. 2022; 28: 1108-1112.
42. Entwistle JW, Drake DH, Fenton KN, Smith MA, Sade RM, Backhus L, et al. Normothermic regional perfusion: Ethical issues in thoracic organ donation. J Thorac Cardiovasc Surg. 2022; 164: 147-154.

43. Antoine C, Jasseron C, Dondero F, Savier E, French National Steering Committee of Donors After Circulatory Death. Liver transplantation from controlled donors after circulatory death using normothermic regional perfusion: An initial French experience. Liver Transplant. 2020; 26: 1516-1521.
44. Hessheimer AJ, Coll E, Torres F, Ruíz P, Gastaca M, Rivas JI, et al. Normothermic regional perfusion vs. super-rapid recovery in controlled donation after circulatory death liver transplantation. J Hepatol. 2019; 70: 658-665.
45. Watson CJ, Hunt F, Messer S, Currie I, Large S, Sutherland A, et al. In situ normothermic perfusion of livers in controlled circulatory death donation may prevent ischemic cholangiopathy and improve graft survival. Am J Transplant. 2019; 19: 1745-1758.
46. Gaurav R, Butler AJ, Kosmoliaptsis V, Mumford L, Fear C, Swift L, et al. Liver Transplantation Outcomes from Controlled Circulatory Death Donors: SCS vs in situ NRP vs ex situ NMP. Ann Surg. 2022; 275: 1156-1164.
47. Rodriguez RP, Perez BS, Daga JA, Diaz FJ, Aguilar JL, Muñoz MA, et al. Outcome of liver transplants using donors after cardiac death with normothermic regional perfusion. Transplant Proc. 2022; 54: 37-40.
48. Barbier L, Guillem T, Savier E, Scatton O, Dondero F, Si Larbi AG, et al. Impact of the duration of normothermic regional perfusion on the results of liver transplant from controlled circulatory death donors: A retrospective, multicentric study. Clin Transplant. 2022; 36: e14536.
49. Savier E, Lim C, Rayar M, Orlando F, Boudjema K, Mohkam K, et al. Favorable outcomes of liver transplantation from controlled circulatory death donors using normothermic regional perfusion compared to brain death donors. Transplantation. 2020; 104: 1943-1951.
50. Ruiz P, Valdivieso A, Palomares I, Prieto M, Ventoso A, Salvador P, et al. Similar results in liver transplantation from controlled donation after circulatory death donors with normothermic regional perfusion and donation after brain death donors: A case-matched single-center study. Liver Transplant. 2021; 27: 1747-1757.
51. Viguera L, Blasi A, Reverter E, Arjona B, Caballero M, Chocron I, et al. Liver transplant with controlled donors after circulatory death with normothermic regional perfusion and brain dead donors: A multicenter cohort study of transfusion, one-year graft survival and mortality. Int J Surg. 2021; 96: 106169.
52. Mohkam K, Nasralla D, Mergental H, Muller X, Butler A, Jassem W, et al. In situ normothermic regional perfusion versus ex situ normothermic machine perfusion in liver transplantation from donation after circulatory death. Liver Transplant. 2022; 28: 1716-1725.
53. Muller X, Mohkam K, Mueller M, Schlegel A, Dondero F, Sepulveda A, et al. Hypothermic oxygenated perfusion versus normothermic regional perfusion in liver transplantation from controlled donation after circulatory death: First international comparative study. Ann Surg. 2020; 272: 751-758.
54. de Vries Y, Matton AP, Nijsten MW, Werner MJ, van den Berg AP, de Boer MT, et al. Pretransplant sequential hypo- and normothermic machine perfusion of suboptimal livers donated after circulatory death using a hemoglobin-based oxygen carrier perfusion solution. Am J Transplant. 2019; 19: 1202-1211.
55. van Leeuwen OB, de Vries Y, Fujiyoshi M, Nijsten MW, Ubbink R, Pelgrim GJ, et al. Transplantation of high-risk donor livers after ex situ resuscitation and assessment using

combined hypo- and normothermic machine perfusion: A prospective clinical trial. Ann Surg. 2019; 270: 906-914.
56. van Leeuwen OB, Bodewes SB, Lantinga VA, Haring MP, Thorne AM, Brüggenwirth IM, et al. Sequential hypothermic and normothermic machine perfusion enables safe transplantation of high-risk donor livers. Am J Transplant. 2022; 22: 1658-1670.
57. De Carlis R, Schlegel A, Frassoni S, Olivieri T, Ravaioli M, Camagni S, et al. How to preserve liver grafts from circulatory death with long warm ischemia? A retrospective Italian cohort study with normothermic regional perfusion and hypothermic oxygenated perfusion. Transplantation. 2021; 105: 2385-2396.
58. Ghinolfi D, Dondossola D, Rreka E, Lonati C, Pezzati D, Cacciatoinsilla A, et al. Sequential use of normothermic regional and ex situ machine perfusion in donation after circulatory death liver transplant. Liver Transplant. 2021; 27: 385-402.
59. Patrono D, Zanierato M, Vergano M, Magaton C, Diale E, Rizza G, et al. Normothermic regional perfusion and hypothermic oxygenated machine perfusion for livers donated after controlled circulatory death with prolonged warm ischemia time: A matched comparison with livers from brain-dead donors. Transpl Int. 2022; 35: 10390.
60. Guo Z, Zhao Q, Huang S, Huang C, Wang D, Yang L, et al. Ischaemia-free liver transplantation in humans: A first-in-human trial. Lancet Reg Health West Pac. 2021; 16: 100260.
61. He X, Guo Z, Zhao Q, Ju W, Wang D, Wu L, et al. The first case of ischemia-free organ transplantation in humans: A proof of concept. Am J Transplant. 2018; 18: 737-744.
62. Guo Z, Zhao Q, Jia Z, Huang C, Wang D, Ju W, et al. A randomized-controlled trial of ischemia-free liver transplantation for end-stage liver disease. J Hepatol. 2023; 79: 394-402.
63. Clavien PA, Dutkowski P, Mueller M, Eshmuminov D, Bautista Borrego L, Weber A, et al. Transplantation of a human liver following 3 days of ex situ normothermic preservation. Nat Biotechnol. 2022; 40: 1610-1616.
64. Mueller M, Hefti M, Eshmuminov D, Schuler MJ, Sousa Da Silva RX, Petrowsky H, et al. Long-term normothermic machine preservation of partial livers: First experience with 21 human hemi-livers. Ann Surg. 2021; 274: 836-842.
65. Lau NS, Ly M, Dennis C, Jacques A, Cabanes-Creus M, Toomath S, et al. Long-term ex situ normothermic perfusion of human split livers for more than 1 week. Nat Commun. 2023; 14: 4755.
66. Lamm V, Ekser B, Vagefi PA, Cooper DK. Bridging to allotransplantation-is pig liver xenotransplantation the best option? Transplantation. 2022; 106: 26-36.
67. Wu WK, Tumen A, Stokes JW, Ukita R, Hozain A, Pinezich M, et al. Cross-circulation for extracorporeal liver support in a swine model. ASAIO J. 2022; 68: 561-570.
68. Wu WK, Ukita R, Patel YJ, Cortelli M, Trinh VQ, Ziogas IA, et al. Xenogeneic cross-circulation for physiological support and recovery of ex vivo human livers. Hepatology. 2023; 78: 820-834.
69. Eshmuminov D, Becker D, Bautista Borrego L, Hefti M, Schuler MJ, Hagedorn C, et al. An integrated perfusion machine preserves injured human livers for 1 week. Nat Biotechnol. 2020; 38: 189-198.
70. Horslen SP, Hammel JM, Fristoe LW, Kangas JA, Collier DS, Sudan DL, et al. Extracorporeal liver perfusion using human and pig livers for acute liver failure. Transplantation. 2000; 70: 1472-1478.

71. Chari RS, Collins BH, Magee JC, DiMaio JM, Kirk AD, Harland RC, et al. Brief report: Treatment of hepatic failure with ex vivo pig-liver perfusion followed by liver transplantation. N Engl J Med. 1994; 331: 234-237.
72. Cooper DK, Wijkstrom M, Hariharan S, Chan JL, Singh A, Horvath K, et al. Selection of patients for initial clinical trials of solid organ xenotransplantation. Transplantation. 2017; 101: 1551-1558.
73. Levy MF, Crippin J, Sutton S, Netto G, McCormack J, Curiel T, et al. Liver allotransplantation after extracorporeal hepatic support with transgenic (hCD55/hCD59) porcine livers: Clinical results and lack of pig-to-human transmission of the porcine endogenous retrovirus. Transplantation. 2000; 69: 272-280.
74. DeOliveira ML, Jassem W, Valente R, Khorsandi SE, Santori G, Prachalias A, et al. Biliary complications after liver transplantation using grafts from donors after cardiac death: Results from a matched control study in a single large volume center. Ann Surg. 2011; 254: 716-723.
75. Parente A, Sun K, Dutkowski P, Shapiro AJ, Schlegel A. Routine utilization of machine perfusion in liver transplantation: Ready for prime time? World J Gastroenterol. 2024; 30: 1488-1493.
76. Rosen HR, Martin P, Goss J, Donovan J, Melinek J, Rudich S, et al. Significance of early aminotransferase elevation after liver transplantation. Transplantation. 1998; 65: 68-72.
77. Wehrle CJ, Jiao C, Sun K, Zhang M, Fairchild RL, Miller C, et al. Machine perfusion in liver transplantation: Recent advances and coming challenges. Curr Opin Organ Transplant. 2024; 29: 228-238.
78. Flores Carvalho M, Boteon YL, Guarrera JV, Modi PR, Lladó L, Lurje G, et al. Obstacles to implement machine perfusion technology in routine clinical practice of transplantation: Why are we not there yet? Hepatology. 2024; 79: 713-730.
79. De Goeij FH, De Meijer V, Mergental H, Guarrera JV, Asthana S, Ghinolfi D, et al. Challenges with the implementation of machine perfusion in clinical liver transplantation. Transplantation. 2024; 108: 1296-1307.
80. Croome KP. Introducing machine perfusion into routine clinical practice for liver transplantation in the United States: The moment has finally come. J Clin Med. 2023; 12: 909.
81. Lascaris B, de Meijer VE, Porte RJ. Normothermic liver machine perfusion as a dynamic platform for regenerative purposes: What does the future have in store for us? J Hepatol. 2022; 77: 825-836.
82. Boteon YL, Boteon AP, Attard J, Mergental H, Mirza DF, Bhogal RH, et al. Ex situ machine perfusion as a tool to recondition steatotic donor livers: Troublesome features of fatty livers and the role of defatting therapies. A systematic review. Am J Transplant. 2018; 18: 2384-2399.
83. Nagrath D, Xu H, Tanimura Y, Zuo R, Berthiaume F, Avila M, et al. Metabolic preconditioning of donor organs: Defatting fatty livers by normothermic perfusion ex vivo. Metab Eng. 2009; 11: 274-283.
84. Goldaracena N, Spetzler VN, Echeverri J, Kaths JM, Cherepanov V, Persson R, et al. Inducing hepatitis C virus resistance after pig liver transplantation-A proof of concept of liver graft modification using warm ex vivo perfusion. Am J Transplant. 2017; 17: 970-978.
85. Schlegel A, Mergental H, Fondevila C, Porte RJ, Friend PJ, Dutkowski P. Machine perfusion of the liver and bioengineering. J Hepatol. 2023; 78: 1181-1198.

86. Luijmes SH, Verstegen MM, Hoogduijn MJ, Seghers L, Minnee RC, Mahtab EA, et al. The current status of stem cell-based therapies during ex vivo graft perfusion: An integrated review of four organs. Am J Transplant. 2022; 22: 2723-2739.