Chapter 4

Subnormothermic and Normothermic Ex Vivo Liver Perfusion as a Novel Preservation Technique

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Abstract

Due to the worldwide organ shortage, interest in the use of marginal liver allografts has increased. More widespread use of marginal grafts is limited by graft injury from cold storage and the risk of poor outcomes after transplantation. Warm (subnormothermic and normothermic) ex vivo liver perfusion has emerged as a novel preservation strategy to recover marginal organs and potentially increase the organ pool. Over the last decade, advances in the field have taken warm ex vivo liver perfusion from the laboratory to clinical trials. While most investigation thus far has focused on the rescue of marginal grafts for expansion of the donor pool, warm perfusion (WP) preservation also has great potential to facilitate novel graft interventions prior to transplantation.

Keywords: ex vivo liver perfusion, warm perfusion, machine perfusion, ischemia-reperfusion injury, organ preservation, subnormothermic machine perfusion, normothermic machine perfusion

1. Introduction

Liver transplantation (LT) is the treatment of choice for patients with end-stage liver disease. Since its origin in the 1960s, outcomes after LT have improved dramatically. Advances in surgical technique, anesthetic management, critical care, and immunosuppression have led to consistently safe performance of LT in the modern era.

However, in the last few decades, the number of patients on liver transplant waiting lists (WL) worldwide has increased significantly, greatly exceeding the number of available liver grafts.

This discrepancy between supply and demand has resulted in increasing mortality on the liver transplant WL. The severe organ shortage has triggered interest in increasing the donor pool by expanding donor criteria. These extended criteria organs include grafts donated after cardiocirculatory death (DCD), grafts with higher degrees of steatosis, grafts from elderly donors, and grafts with prolonged cold storage time. Preclinical and clinical experience with extended criteria grafts demonstrates an increased susceptibility to preservation injury during cold static storage and higher rates of graft dysfunction after transplantation [1,2].

Historically, cold static storage has been the preferred method of preservation due to its simplicity, low cost, and acceptable transplant outcomes with good-quality organs. The fundamental principle underlying hypothermic organ preservation is the reduction of cellular metabolism and oxygen demand. This prolongs organ viability by slowing down progression of ischemic injury. While cellular metabolism is significantly reduced at 4°C, ongoing low-level metabolic processes continue and lead to the development of energy debt and depletion of adenosine triphosphate (ATP) stores. ATP depletion results in dysfunction of Na^+/K^+ cell membrane pumps, accumulation of toxic products derived from anaerobic metabolism, mitochondrial injury, and cell swelling. At the time of graft reperfusion, restoration of oxygen supply to dysfunctional mitochondria results in the generation of reactive oxygen species (ROS), leading to cellular damage and activation of pro-inflammatory pathways. Depending on the initial quality of the graft and the duration of cold ischemic injury, the effects of ischemia-reperfusion injury range from minor cellular dysfunction to primary nonfunction of the graft.

While standard criteria donor organs typically have the physiologic reserve to tolerate the injury associated with cold storage preservation, the diminished ability of marginal grafts to tolerate this process has triggered research to improve organ preservation. The shortcomings of cold static storage coupled with advances in organ perfusion technology have resulted in increased interest in warm ex vivo liver perfusion as an alternative to cold static storage. Warm perfusion (WP) preservation can potentially reduce injury from cold ischemia, facilitate a window of graft assessment during the preservation period, and serve as a platform for graft modification before LT.

2. Basic principles of warm liver perfusion

The primary objective of WP preservation is restoration of physiologic conditions and cellular function. The graft is supplied with nutrients and oxygen to restore and maintain cellular metabolism at physiologic or near-physiologic temperature. Simultaneously, toxic products from the cellular milieu are continuously eliminated. Under these conditions, ATP and glycogen reserves can be actively restored. If pro-inflammatory mediators are excluded from the perfusate (cytokines, leukocytes, platelets), reperfusion injury is minimized. The mechanisms underlying the observed benefit of WP have not yet been elucidated, but preclinical data suggest improved preservation of the graft endothelium may be contributory [3,4].

A second important characteristic of WP is the ability to perform an assessment of the graft during the preservation period. Since the organ is metabolically active, its performance can be

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evaluated by vascular flow parameters, injury markers, and functional indicators like bile production and lactate clearance. By assessing graft injury and metabolic function during organ perfusion, transplant physicians and surgeons can accept or decline liver grafts based on performance data, rather than purely clinical history and graft appearance. Perhaps most relevant for future research, the active metabolism during warm ex vivo perfusion also offers the opportunity to apply repair strategies to improve the quality of liver grafts.

3. Technical aspects of warm liver perfusion

Liver perfusion involves two separate inflow vessels (the hepatic artery and portal vein) with different pressures and flow requirements. While the hepatic artery requires high pressure (50–70 mmHg) and moderate flow (300–600 mL/min), the portal venous system has low pressure (3–5 mmHg) with higher flow (600–900 mL/min). Most groups have used continuous flow as opposed to a pulsatile flow in the hepatic artery. So far, there are no data available demonstrating superiority of either system. Clinical experience from left ventricular assist devices suggests that continuous flow devices are simpler to implement and more reliable, with comparable functional results, making it reasonable to assume similar outcomes for warm liver perfusion.

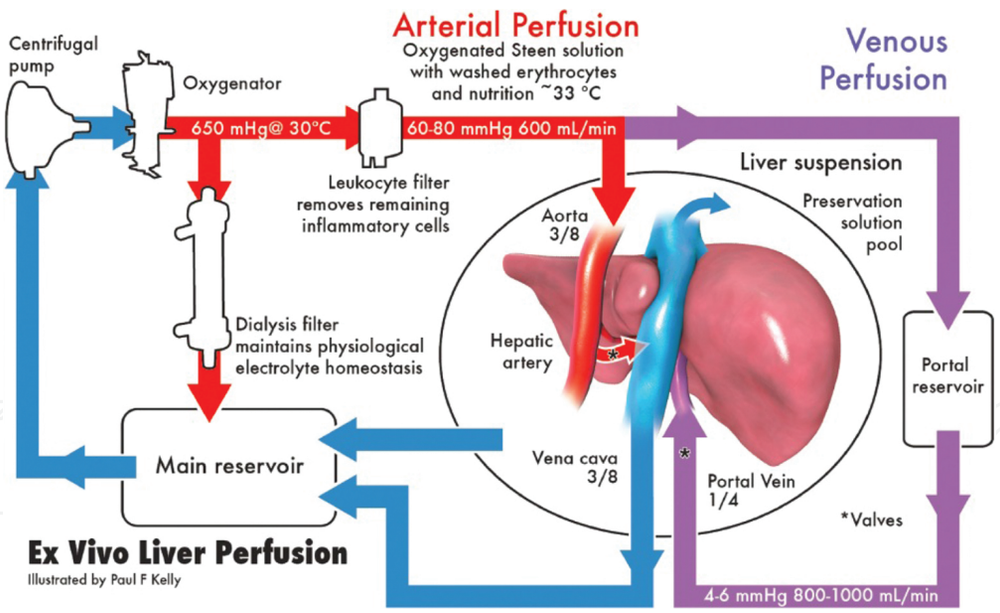

Figure 1. University of Toronto ex vivo liver perfusion system.

Regarding venous drainage, two different systems have been applied. In the simplest system, the venous blood drains directly into the organ basin, from where it is collected and recirculated. Alternatively, the venous blood can be drained through a closed tubing system either by dual vena cava outflow (infrahepatic and suprahepatic cannulas) or single vena cava outflow (**Figure 1**, Toronto perfusion scheme).

Ideally, warm organ perfusion should be initiated immediately after organ retrieval in order to avoid prolonged cold ischemic injury. Preclinical studies have demonstrated improved outcomes if cold storage is minimized prior to WP preservation for DCD grafts [1]. In clinical practice, however, in order to initiate warm perfusion immediately at the time of organ retrieval, a portable perfusion machine that can be transported to the donor hospital is required. Warm perfusion during organ ground transportation adds complexity to the preservation process and requires a safe and reliable system to maintain stability during this period. The cost of system failure is high, as this would lead to graft loss. An alternative strategy that has been employed to circumvent this issue is an initial period of cold storage, followed by transport and delayed start of WP at the transplant center. This strategy may require a modified perfusion solution to compensate for the inflammatory stimulus of the cold storage period.

4. Perfusate alternatives for warm ex vivo liver perfusion

Different perfusates have been explored in preclinical models. Due to its significant metabolism and large size, the liver requires a robust oxygen supply that cannot be provided without the addition of oxygen carriers. This is in contrast to normothermic lung perfusion, in which sufficient oxygen levels can be achieved by ventilation. While some preclinical studies have used whole blood from the donor animal for WP, most studies have used isolated RBCs as the primary oxygen carrier to avoid inflammatory mediators found in whole blood. Alternative cell-free oxygen carriers have been developed and incorporated in WP strategies with success in the preclinical setting [5].

The RBCs or alternative oxygen carriers are typically mixed with a colloid solution to replace the plasma component of whole blood. Examples of such colloid solutions include fresh frozen plasma, albumin-rich Steen solution, or starch-based solutions. Additional perfusate components typically include antibiotics, amino acids, glucose, anticoagulants, and antioxidants.

5. Temperature conditions for warm ex vivo liver perfusion

Two temperature settings have been explored for warm ex vivo liver perfusion. Normothermic perfusion is conducted at 37°C, while subnormothermic ex vivo liver perfusion (SNP) is carried out at lower temperatures (20–34°C). Both approaches have relative pros and cons, which will be highlighted below.

5.1. Subnormothermic ex vivo liver perfusion (SNP)

At the intermediate temperatures used in SNP, graft cellular activity and metabolism are greatly increased over cold storage, facilitating a window of observation of graft function prior to transplantation. The primary theoretical advantage of perfusion under subnormothermic

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conditions is that increased solubility of oxygen at lower temperatures (relative to 37°C) facilitates the use of perfusate solutions without the need for oxygen carriers.

5.1.1. Preclinical studies

Several groups have developed preclinical models in the pig, which is thought to most closely approximate human liver transplantation. Below, we highlight some of the most recent advances from these preclinical studies. In 2013, Minor and colleagues compared the effects of hypothermic perfusion (4°C), SNP (20°C), and controlled oxygenated rewarming, in which perfusion temperature was gradually increased from 4 to 20°C during perfusion [6]. Graft preservation consisted of an initial period of cold storage for 18 hours, followed by 90 minutes of machine perfusion preservation. Graft reperfusion was performed ex vivo with blood-containing perfusate for a period of 4 hours to simulate transplantation. Tissue ATP and energy charge were improved in the controlled rewarming and subnormothermic machine perfusion groups. Aspartate aminotransferase (AST) release and bile production were significantly improved in the controlled rewarming group relative to the other groups. These findings suggest there may be value in gradually increasing perfusion temperature to subnormothermic levels during preservation. In 2014, Knaak and colleagues at the University of Toronto reported a DCD study comparing cold storage versus SNP at 33°C [3]. After 45 minutes of in situ warm ischemia, livers underwent either cold storage for 10 versus 7 hours of cold storage followed by 3 hours of SNP. Grafts were then transplanted, and recipients followed for 7 days post transplant. SNP improved bile duct preservation and function with lower serum alkaline phosphatase (ALP) and bilirubin, lower LDH levels in bile, and the absence of biliary necrosis on histologic examination. Additionally, SNP had beneficial effects on graft endothelium. In 2015, Fontes and colleagues investigated SNP at 21°C using a novel hemoglobin-based oxygen carrier in a standard criteria donor model [5]. Grafts were preserved by SNP versus cold storage for 9 hours, followed by transplantation. Posttransplant survival at 5 days was significantly increased for SNP-preserved grafts (100 % SNP versus 33 % cold storage). SNP recipients demonstrated improved serum markers of cellular injury (AST), alanine aminotransferase (ALT) significantly increased bile production, and significantly decreased ischemia-reperfusion injury by histologic analysis. In recent report, Spetzler and colleagues at the University of Toronto performed a study with the objective of establishing the safety of SNP for standard criteria grafts [4]. In this study, heart-beating donor grafts were preserved by either 3 hours cold storage followed by 3 hours of SNP at 33°C versus 6 hours of cold storage, followed by transplantation. Following transplantation, serum levels of AST, ALP, and hyaluronic acid were lower in the SNP group. Immunohistochemistry demonstrated decreased apoptosis of sinusoidal cells in the SNP group.

5.1.2. Human studies

Thus far, SNP has not been studied in the clinical setting, although studies are likely forthcoming given the preclinical success described above. Human studies have been limited to examining the effects of SNP in discarded allografts. In 2014, Bruinsma and colleagues reported their experience with seven discarded grafts (five DCD, two DBD). SNP was carried out for

3 hours at 21°C using a bloodless perfusate [7]. Observations included increasing oxygen uptake, increased clearance of lactate, increased volume of bile production, and improved ATP content of the liver tissue during the course of perfusion, suggesting improvement in organ function. Histologic analysis demonstrated preservation of hepatocyte morphology and the sinusoidal endothelium.

5.2. Normothermic ex vivo liver perfusion (NMP)

Normothermic perfusion is performed at physiologic body temperature. The advantages of perfusion under normothermic conditions include rapid restoration of normal organ function, the ability to assess organ performance at full metabolic activity, and being a potential platform for organ repair/modification interventions (**Figure 2**).

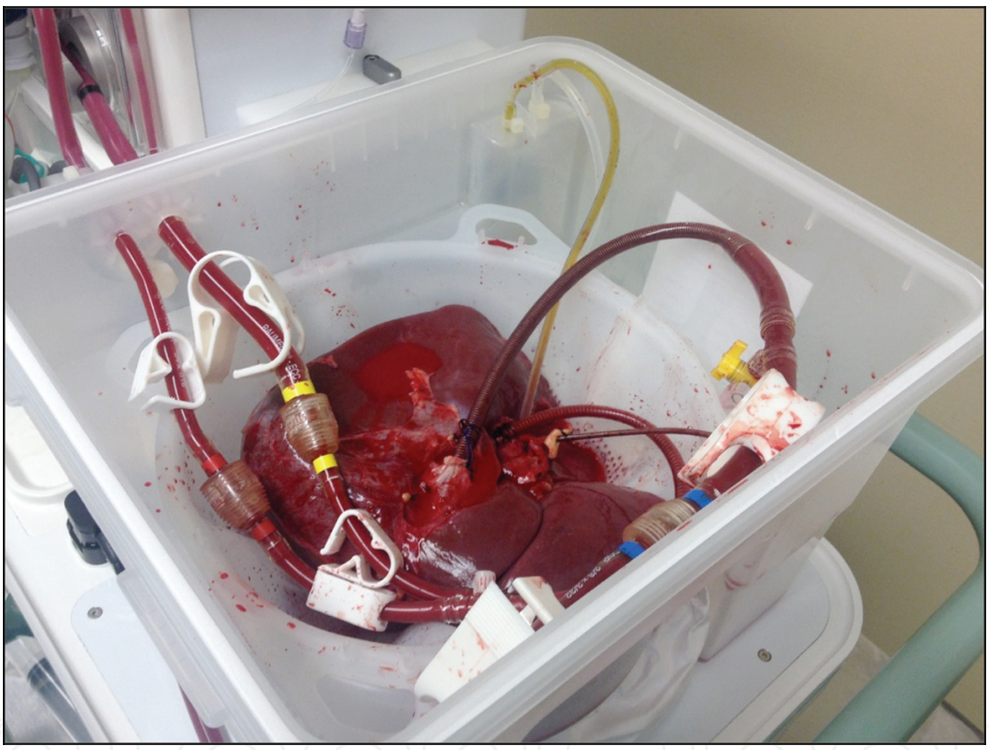

Figure 2. Liver graft connected to the Metra device being actively perfused.

5.2.1. Preclinical studies

Several preclinical studies have examined the effects and mechanisms of NMP, and encouraging results have prompted further investigation in clinical trials. The most clinically relevant preclinical experiments have been performed in porcine models, and below we highlight some of the most important studies in the preclinical setting.

Schon and colleagues reported one of the earliest studies describing the potential benefits of NMP in 2001 [8]. In this study, the effects of NMP were assessed in a DCD model with 1 hour of in situ warm ischemia. Grafts were preserved by cold storage for 4 hours versus NMP for

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4 hours, followed by transplantation. In the cold storage group, all grafts exhibited primary nonfunction and recipient death. In contrast, the NMP grafts demonstrated normal graft function with 100 % recipient survival. Histologic examination demonstrated confluent necrosis in the cold storage group, while the NMP group demonstrated preservation of liver architecture. In 2005, Reddy and colleagues highlighted the detrimental effect of an initial period of cold storage prior to NMP in a DCD model [1]. In this study, grafts were retrieved after 1 hour of in situ warm ischemia and preserved either by 1 hour cold storage then 23 hours NMP or 24 hours of NMP. Markers of cellular injury (AST, ALT), sinusoidal dysfunction (hyaluronic acid), and Kupffer cell injury (β-galactosidase) were significantly higher in the grafts initially preserved with 1 hour of cold storage prior to NMP. These findings suggest that minimizing cold storage time as much as possible may improve preservation. In 2009, Brockmann and colleagues reported a study comparing NMP to cold storage in a DCD model (in situ warm ischemia of 40 versus 60 min) and also assessed the effect of extended preservation times (20 hours) [9]. At extended preservation times (20 hours), there was a significant improvement in recipient survival in the NMP group (86 % versus 27 % for grafts with no warm ischemia, 83 % versus 0 % for grafts with 40 minutes of warm ischemia). For longer warm ischemic times (60 minutes), there were no survivors in either group. An analysis of factors available during NMP that distinguished survivors from non-survivors demonstrated that bile output, base excess, AST, ALT, hyaluronic acid, portal venous pressure, and portal venous resistance were all significantly different between survivors and non-survivors. In 2013, Boehnert and colleagues at the University of Toronto reported results from a DCD study assessing the impact of NMP after an initial period of cold storage [10]. The goal of this study design was to more closely approximate the timeline of events in actual clinical practice. In the study, grafts were subjected to 1 hour of in situ warm ischemia, followed by either 4 hours of cold storage or 4 hours cold storage plus 8 hours NMP using an acellular colloid perfusate. After a period of ex vivo reperfusion to simulate transplantation, NMP-preserved grafts demonstrated lower ALT, higher oxygen extraction, more physiologic biliary composition, and less bile duct necrosis. CT angiography demonstrated superior hepatic artery perfusion in NMP grafts. In 2014, Liu and colleagues investigated the effect of NMP on bile duct preservation [11]. DCD grafts (1 hour in situ warm ischemia) were preserved by 10 hours of cold storage versus NMP. Grafts were reperfused ex vivo for an extended time period (24 hours). Histologic examination demonstrated the well-preserved parenchyma in NMP grafts, while cold-stored grafts demonstrated significant hepatocyte and biliary necrosis. In a novel analysis, the authors demonstrated increased Ki-67 staining in the biliary system of NMP grafts, consistent with biliary regeneration [11].

5.2.2. Human studies

Based on the encouraging results from several of the preclinical studies described above, normothermic ex vivo liver perfusion has entered the clinical setting. The results from a phase I trial using the transportable OrganOx Metra device (Oxford, UK) were recently reported by Ravikumar and colleagues in 2016 [12]. In this study, clinical outcomes of 20 liver transplants performed after graft preservation by NMP were compared with 40 matched controls transplanted after standard cold storage. Thirty-day graft survival was similar between groups

(100 % NMP versus 97 % cold storage), with significant improvements observed in posttransplant peak AST in the NMP group. Importantly, NMP was demonstrated to be safe, with no device-related failures in 20 consecutive cases. A second European study led by Dr. Peter Friend is currently underway comparing NMP to standard cold storage in randomized fashion. In North America, a prospective, non-randomized phase I clinical trial has been recently initiated at the University of Toronto and the University of Alberta, also using the OrganOx Metra device (**Figure 3**).

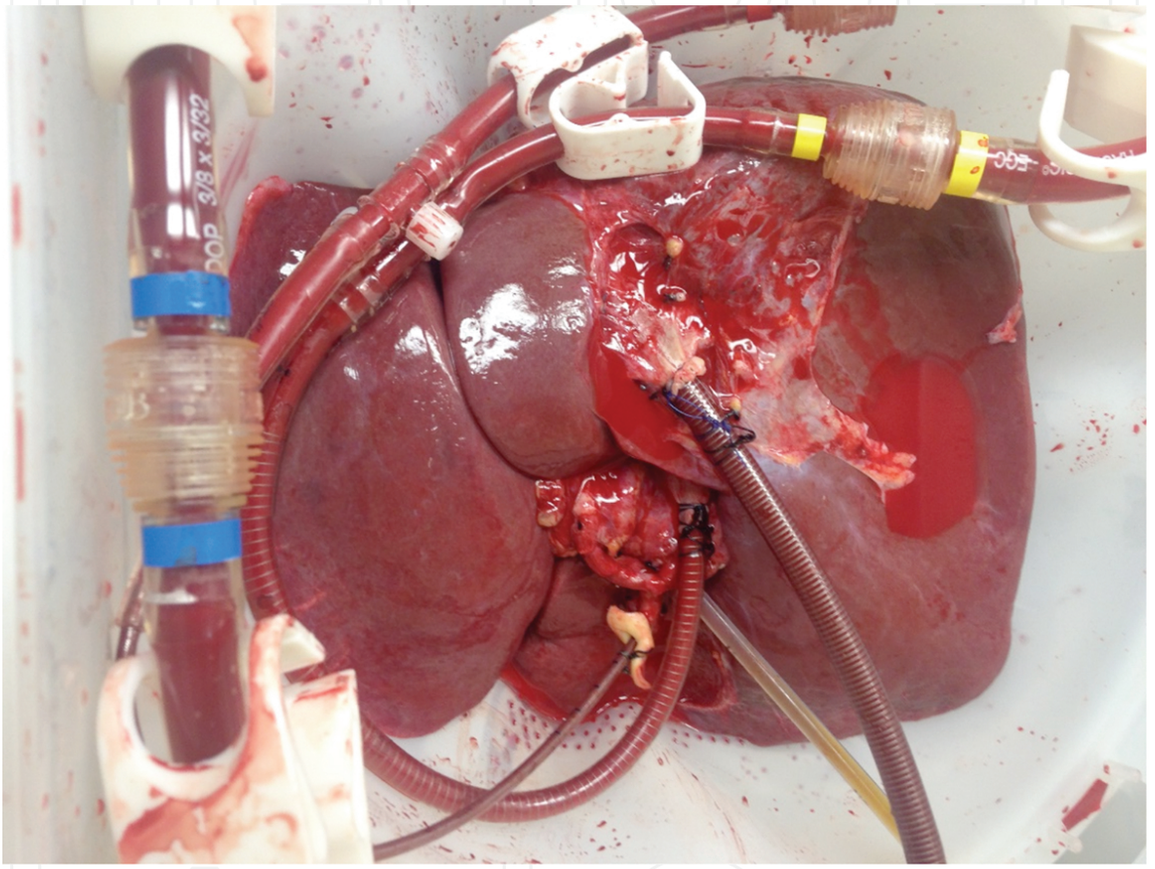

Figure 3. Normothermic perfusion of a DCD organ at the University of Toronto using the Metra device.

While clinical trials are necessary to establish the safety and efficacy of NMP, recent case studies describing the use of NMP to rescue extremely marginal grafts highlight what may become possible in the future. In 2016, Perera and colleagues in the UK reported the use of NMP to resuscitate a DCD graft with extended warm and cold ischemia time far outside of traditionally accepted parameters (109 minutes of in situ warm ischemia followed by 422 minutes of cold storage) [13]. After initiating NMP and observing evidence of good graft function including normalized lactate levels in the perfusate and robust bile production, the authors proceeded with transplantation. The recipient had an unremarkable posttransplant recovery and no evidence of ischemic-type biliary strictures at 15 months post transplant. In 2016, Watson and colleagues reported a similarly impressive clinical outcome using NMP to resuscitate a DCD graft from a 57-year-old donor with 160 minutes of warm ischemia time

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(WIT) followed by 350 minutes of cold storage [14]. After establishing NMP, the assessment phase demonstrated decreasing lactate levels in the perfusate and bile production, and the graft was successfully transplanted. The recipient had an uncomplicated postoperative course and no evidence of ischemic-type biliary strictures at 6-month follow-up.

6. Conclusion

In the last decade, warm ex vivo liver perfusion has made tremendous progress and transitioned from animal studies to clinical use. It has demonstrated great potential to improve organ preservation, particularly for extended criteria grafts. As the portability and expense associated with perfusion technology improve, wider clinical application will become feasible and may facilitate expansion of the donor pool. The potential future benefit of warm machine perfusion may extend beyond rescuing marginal grafts. Due to the restoration of cellular metabolism facilitated by warm ex vivo perfusion, this technology provides an ideal platform for a variety of graft interventions including alteration of the graft response to hepatitis C infection, prevention of hepatocellular carcinoma recurrence, decreasing the graft immune response, and the application of stem cell and gene therapy. Further research in these exciting avenues has great potential to improve liver transplantation outcomes in the future.

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