

Review

Research progress on hepatic machine perfusion

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Abstract

Nowadays, liver transplantation is the most effective treatment for end-stage liver disease. However, the increasing imbalance between growing demand for liver transplantation and the shortage of donor pool restricts the development of liver transplantation. How to expand the donor pool is a significant problem to be solved clinically. Many doctors have devoted themselves to marginal grafting, which introduces livers with barely passable quality but a high risk of transplant failure into the donor pool. However, existing common methods of preserving marginal grafts lead to both high risk of postoperative complications and high mortality. The application of machine perfusion allows surgeons to make marginal livers meet the standard criteria for transplant, which shows promising prospect in preserving and repairing donor livers and improving ischemia reperfusion injury. This review summarizes the progress of recent researches on hepatic machine perfusion.

Key words: hepatic machine perfusion; hypothermic machine perfusion; subnormothermic machine perfusion; normothermic machine perfusion; liver transplantation; extended criteria donors

Introduction

At present, the shortage of donor livers remains very serious worldwide. Donor livers from dead Chinese citizens accounted for 83.55% of the total number of livers for transplantation by 2015. But only about 0.2% people were willing to donate organs after death in China. (<https://www.codac.org.cn>) The percentage of European patients waiting for donor livers for less than 3 months has fallen from 90% in the 1980s to slightly over 50% since 2000 [1]. In 2018, only 46.5% of American patients could get liver transplantation within one year. Each year, thousands of patients died of disease aggravation due to lack of qualified liver transplantation [2]. In this regard, the discrepancy between donor organ demand and supply is increasing and patients are suffering from longer waiting list. Expanding the donor pool and using all available organs is an imperative need. Several methods, such as living donation, split-liver and marginal grafts, have been used to expand the donor pool. The most promising one is marginal grafts, which come from extended criteria donors (ECD) and donation after circulatory death (DCD) rather than standard criteria donors (SCD). However,

the use of ECD or DCD often leads to adverse outcomes and increased risk of infection (Table 1) because they are more sensitive to ischemia-reperfusion injury (IRI) [3]. Therefore, improving the quality of marginal grafts will improve the prognosis of patients to some extent.

Table 1. Common complications of ECD and DCD

	Definition	Common complication after transplantation
ECD	No precise definition; Frequently cited characteristics are [11]: Advanced age; macrovesicular steatosis; DCD; organ dysfunction. Cause of death: anoxia, cerebrovascular accident. Infectious disease: Hepatitis B, Hepatitis C, HIV. Extrahepatic malignancy. Cold ischemia time (CIT) greater than 12 hours.	Early allograft dysfunction (EAD); Biliary complication Recurrence of Hepatitis Poor prognosis (higher mortality) [12,13]
DCD	Organs donated after cardiac death of the donors	EAD; Primary nonfunction (PNF); Biliary complication; Transplant failure [14-17]

Nowadays, static cold storage (SCS) is widely used to preserve donor livers. SCS slows down

metabolism and reduces oxygen consumption by lowering the temperature of the donor liver, which is able to avoid rapid functional damage in a longer time of ischemia. However, preserving ECD or DCD by SCS is unsatisfactory [4]. As an alternative method, machine perfusion may have more advantages. Machine perfusion can not only provide oxygen and nutrients for the liver, but also remove metabolic waste and reduce the damage to hepatic metabolism caused by warm ischemia or hypothermia. In addition, machine perfusion can detect the function of grafts *in vitro* before transplantation [5,6] and administer drugs for therapeutic intervention, which cannot be achieved by SCS. Lots of livers that could have been transplanted were abandoned due to the lack of objective and effective prediction and evaluation of the marginal function [7]. And transplantation can also be avoided for the livers with irreversibly loss of function [8]. Machine perfusion may change this predicament. A clinical randomized controlled trial by Nasralla et al. [9] showed that machine perfusion does contribute to improving utilization and prolong the lifespan of grafts. Under this background, machine perfusion is considered to be one of the great advances in the field of transplantation in decades [10]. This review summarizes the progress of recent researches on hepatic machine perfusion.

Mechanism of Machine Perfusion

The purpose of machine perfusion is to maintain organ vitality, repair and pretreat the organs. During machine perfusion, the donor organ is usually connected to a pressure-controlled perfusion apparatus that continuously pumps the perfusate through the blood vessels of the organ (Figure 1).

There are three main methods for liver preservation by machine perfusion, from hypothermic (4-10 °C), subnormothermic (20-25 °C) to normothermic (35-37 °C) [18]. No precise division of the temperature is defined. Another classification includes hypothermic (0-12 °C), subnormothermic (25-34 °C) and normothermic (35-38 °C) [19]. Recently, a new concept of controlled oxygenated rewarming (COR) was put forward. The process of COR was gradually increase the graft temperature (up to 20 °C) with sufficient oxygenation of the liver tissue [20].

Hypothermic Machine Perfusion (HMP)

Apart from achieving cooling effect same as SCS, HMP can also transport metabolic waste out of the donor liver and administer drugs. The easy transformation into SCS in condition of machine breakdown renders HMP a safe technique as well. A latest meta-analysis [21] showed that the incidence of

post-transplantation one-year survival was increased with an OR of 2.19 (95% CI 1.14-4.20, $p=0.02$) in HMP preservation compared to SCS.

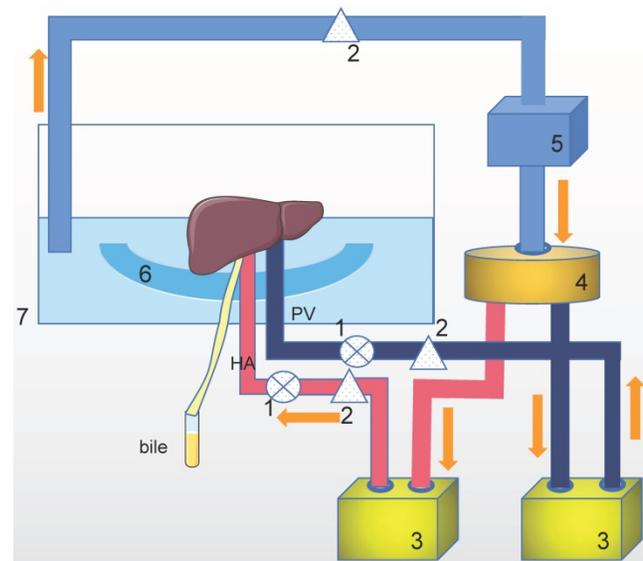


Figure 1. The mechanism of machine perfusion. This is only one example of a perfusion setup with a Y-configuration. There are some other setups with single (via the hepatic artery or portal vein) perfusion or two completely parallel circuits. Technical of oxygenation, pump and temperature control vary in different setups. HA: hepatic artery. PV: portal vein. The perfusion system includes: 1. Pressure sensor, 2. Flow meter, 3. Pump, 4. Oxygenator/heat exchange, 5. Centrifugal pump, 6. Organ holder, 7. Organ chamber.

HMP can be performed through either portal vein (PV) or portal vein and hepatic artery (HA), also known as dual perfusion [22]. In adult liver transplantation, the perfusion pressure of PV and HA are usually set to 3-5 mmHg and 20-30 mmHg, respectively [23]. Werner et al. [24] reported the first case of pediatric DCD liver transplantation via HMP. Werner pointed out that the perfusion pressure of PV and HA in children should decrease proportionally in accordance with adults' mean arterial pressure. HMP has also been proved to have a good effect on preservation of split liver [25]. Gillooly et al. [26] reported that adding short interfering RNA (siRNA) to the perfusate could inhibit chemically mediated liver failure in mice, suggesting that taking advantage of siRNA may bring a better outcome of liver transplantation.

Hypothermic oxygenated perfusion (HOPE), developed along with HMP, additionally provides oxygen to the donor liver at an approximate pressure of 60-80 kPa during perfusion [27]. Studies have shown that HOPE performed shortly after graft extraction can reduce metabolic waste production, promote mitochondrial function recovery [28,29] and improve liver ATP level, thus ameliorating hepatic function [23] and reducing the incidence of post-transplantation intrahepatic biliary complications [30,31]. Ravaioli [32] believes that HOPE should last at

least an hour. The safety and efficacy of HOPE have also been confirmed. Rayar et al. [33] reported two cases of successful liver transplantation using HOPE to preserve ECD livers in their center. In one of the cases, the donor was over 80 years old, with steatosis greater more than 20% and cold ischemia time (CIT) greater than 10 hours. Inspiringly, the hepatic function recovered quickly after surgery, indicating that HOPE can improve the IRI and restore the function of the donor liver favorably. Even if choosing donor liver with advanced age means higher risk of post-transplant complication [34], the result of this research may still present elder donor liver as an option for surgeons. Schlegel et al. [35] compared the differences of the 5-year survival rate of DCD liver transplantation between HOPE and SCS. The results showed that the 5-year survival rate of the former was 94%, while that of the latter was only 78%. Despite higher risk of failure, the 5-year survival rate after DCD liver transplantation is similar to that of donation after brain death. HOPE can also extend the lifespan of donor livers [36,37], providing convenience for liver distribution.

Subnormothermic Machine Perfusion (SNMP)

Subnormothermic machine perfusion attempts to maximize hepatic metabolism while minimizing reperfusion injury. With the temperature within subnormothermic range, the cell's demand for energy significantly declines while sufficient metabolism is maintained to monitor and restore liver function [38]. The effect of SNMP on preserving DCD liver has been verified in pig liver transplantation [39]. SNMP can reduce portal venous resistance and increase bile production compared with HMP, therefore better utilizing the DCD liver [40]. Morito, N et al. [41] pointed out that SNMP consumes higher oxygen than HMP, which restores the function of the donor liver and reduces the incidence of endothelial cell injury, therefore making better use of DCD liver and expanding the donor pool. However, Kanazawa [42] pointed out that a noticeable delay exists between the beginning of machine perfusion and the full perfusion of the peripheral liver tissue. Short as it is, the delay exposes the insufficiently perfused areas directly to room temperature. In another word, SNMP is not as effective as HMP in protecting liver tissues with inadequate perfusion.

Ciria, R et al. [43] applied SNMP to extreme ECD, including long periods of cold and warm ischemia, and the restoration effect was inspiring. Although the post-transplantation prognosis is uncertain, lactic acid descending trend and bile output are prominent. At present, there is no agreed conclusion on whether it is beneficial to use oxygen

carriers in SNMP. Shonaka, T [44,45] added hemoglobin-based oxygen vesicles (HbV) as oxygen carriers to UW solution in SNMP for porcine DCD liver, and proved that the reperfusion injury of the donor liver could be ameliorated. Karimian et al. [46] have shown that there are ATP accumulation, energy charge ratios and glutathione consumption in steatosis liver after SNMP, which may weaken the antioxidant capacity of grafts [47]. Although there are no clinical reports of steatosis liver transplantation via SNMP, it is necessary to beware of the possibility of severe ischemia-reperfusion injury and a high incidence of EAD.

Huang, V et al. [48] reported that a split-liver perfusion model could be established by SNMP *in vitro*, which allows simultaneous perfusion of left and right lobes and one lobe to serve as control for the other. Similar to whole-liver perfusions, the arterial resistance and lactic acid levels of each lobe in the split-liver model decreased gradually during the whole perfusion, and there was no significant difference between the left and right lobes. This model avoids the problems caused by the heterogenous nature of discarded human liver that troubled previous researches, and is conducive to the development of preclinical studies. Obara, H et al. [49,50] suggested that installation of leukocyte filter or replacement of purified perfusate in SNMP could reduce hepatic artery pressure and release the levels of AST, LDH and ALP in porcine liver donated after cardiac death. This method is expected to further improve the ischemia-reperfusion injury in ECD. Tabka et al. [51] reported that the addition of angiotensin IV to the perfusate could improve the function of hepatic endothelial cells and reduce oxidative stress and cell injury. Future research may focus on optimizing the composition of perfusate to further improve the quality of graft and the prognosis of transplantation.

Normothermic Machine Perfusion (NMP)

NMP provides oxygen and nutrients to the liver at 37 °C, keeping the liver in a complete functional state *in vitro* [52]. The perfect mimicking of physiological condition enhances the metabolic activity of the donor liver and reduces the ischemic graft injury [53], allowing for more active repair and providing the opportunity for therapeutic intervention to a functioning organ before it is transplanted (Table 2). Therefore, NMP may be more helpful for the repair of ECD. One of the main advantages of NMP is that the functional status of grafts can be evaluated by measuring liver metabolic indicators such as bile production and liver enzymes before transplantation [54]. The first case of ECD

transplantation by NMP in Asia was reported recently [55]. Multicenter randomized controlled trials have shown that NMP can reduce graft injury, prolong organ preservation time and improve organ utilization [9,56]. Stephenson et al. [57] proposed the possibility of split-liver in NMP, and proved that the two parts of the split-liver could maintain function, meaning two cases of liver transplantation could be completed successfully.

Table 2. Major categories of therapeutic intervention of *ex vivo* NMP in liver transplantation

Category	Agents	Advantages	Limitations/Further studies
Gene silencing with RNAi	SiRNA [26,61]	Opened up new opportunities for specific therapeutic targeting of genes; More efficient delivery, lower doses and cost-saving; None/Fewer side effects to other organs; Organ-specific; Administration method clinically more applicable.	Risks of delivering the medication systemically; Chemical structural challenges related to uptake; Off-target effects; Application requires machine preservation expertise.
Cell therapy	Regulatory T-cell (Treg) [62]	Alleviate or prevent graft-versus-host disease	Difficult to deliver therapeutic cells to target organ; Lack of standardisation of manufacturing processes; High cost
Extra-cellular vesicles	HLSC-EV [63]	Reduce liver injury during hypoxic NMP; Mimic most of the cell effects (including apoptosis inhibition and mitogenic activity) by transferring proteins, mRNAs, and micro-RNAs Represent an innovative approach to recondition organs before transplant	Mechanism of HLSC-EV on reducing hypoxic injury unclear
Vasodilator agent	Prostaglandin E1 [64,65]	Improve extracellular stress associated with microcirculatory failure and hypoxia; Suppress the production of cytokines from macrophages and proinflammatory cytokine	Need more research on actual transplantation experiments using large animal models Frequency of biliary complications unclear.
Antibiotics/Antiviral agents	miravirsin [66]	Make a liver resistant to reinfection; Lower risk for long-term complications.	Lack of suitable animal models; Need more longer-term follow-up studies.
Defatting cocktail	Combination of multiple defatting agents [67-69]	Enhance lipid metabolism and mitochondrial functioning of hepatocyte; Improve organ functional recovery; Decrease vascular resistances; Anti-inflammation and reduce markers of hepatocellular injury Improve biliary function.	The effectiveness of the drugs after ischemic injury remain dispute; Lack of clinical markers for some defatting agents; Unknown livers after defatting cocktail suitable for transplantation.
Opioid agonist	Enkephalin [70]	Protective against oxidative; Decrease the metabolic demand; May serve as therapeutic target for improved liver protection;	Lack of strong clinical relevance; Need more research on test DADLE using alternative <i>in vitro</i> models of oxidative stress;

Category	Agents	Advantages	Limitations/Further studies
		Preserved the mitochondrial function of oxidatively stressed hepatocytes; Lengthen the preservation time of donor organs during normothermic perfusion.	Mechanism of DADLE on decreasing hepatocellular metabolism unclear.

siRNA: small interfering ribonucleic acid; HLSC-EV: Human liver stem cells extracellular vesicles; DADLE: delta opioid agonist [D-Ala2, D-Leu5] enkephalin.

Perfusate of NMP requires sufficient oxygen carriers to transport oxygen and maintain physiological osmotic pressure. At present, most perfusate use red blood cells as oxygen carriers. However, it has several disadvantages, including immune-mediated responses and hemolysis [58]. Even so, the safety and efficacy of fresh frozen plasma in NMP were confirmed in controlled trials [59].

NMP is more technically challenging and expensive than SCS, especially during the preservation period, therefore more human resources are required. Selzner, M [60] believes that the maintenance process of the liver in NMP is extended 2 hours due to the processes of preparation, intubation, equipment connection, etc. Any failure in these techniques may have a disastrous impact, rendering the organ nonviable.

Controlled Oxygenated Rewarming (COR)

The temperature of COR is similar to that of SNMP. Metabolism of the graft was decreased in SNMP, while increased in the process of COR. The first clinical trial of COR was carried out by Hoyer et al. [71] in 2016 with a short-term follow-up of 6 months. Six donor livers were stored by SCS and then performed COR with no prolonged cold ischemic time before transplantation in this study. Afterwards, they extended the clinical trial to 18 liver transplantation and assessed the long-term outcome of recipients [72]. No significant decrease in postoperative outcome was observed. These studies demonstrated the feasibility and safety of COR in the clinic.

Conclusions and Future Perspectives

There are many clinical trials on hepatic machine perfusion, but we are unable to completely replace SCS with machine perfusion due to economic factors and lack of strong evidence. At present, most experiments of machine perfusion are carried out on isolated liver models or transplantation studies in animal models, which cannot fully simulate the physiological environment and metabolism of the human body. More data from liver transplantation models are needed to verify some of the experimental results. Only in the case of ECD and DCD can the

benefits of machine perfusion be predicted. However, what needs to be solved at present is that the indications for machine perfusion are not clear, which means there is no consensus on when should we consider machine perfusion [73]. Moreover, there is no agreed idea on which machine perfusion is the best under different circumstances [74]. Since organ transport takes a long time in most cases, the duration to achieve the most effective resuscitation of the donor liver has not been determined [42]. There are also continuous studies on how to better evaluate graft function [75]. With the development of machine perfusion research in transplant centers all over the world, each center has its own experience in choosing perfusion equipment, composition of perfusate, and perfusion time and evaluating graft function [76]. Researches will focus on forming a consensus in order to facilitate its application.

In addition, combination of multiple perfusion methods may be promising in making better use of the advantages of different perfusion methods. For example [77-79], combining HOPE, controlled oxygenated rewarming (COR) and NMP can reduce oxidation-induced tissue damage and increase the energy storaction of the liver. De Vries et al. [80] showed that human livers can be stored with the combination of supercooling below zero (-4 °C) and SNMP, effectively prolonging the duration of human liver preservation to 27 hours. Eshmunov [81] and his team designed a modified perfusion machine, which can simulate the transport of nutrients and bile salt from the intestine to the liver through the portal vein. In order to keep the liver moving continuously, a device to mimic diaphragm oscillations was also integrated. At the same time, it can also control the hemodynamics of the liver and regulate the level of blood sugar through feedback. Six out of ten injured human livers that had been abandoned by all European centers were successfully preserved by this new system at 34 °C for a week and might meet the criteria for transplantation. With the ongoing development of machine perfusion, the definition of ECD will continue to be extended, but how to balance the pros and cons of the shortage of donor pool and marginal donors remains to be studied in the future.

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Competing Interests

The authors have declared that no competing interest exists.

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