Acta Pharmacologica Sinica (2018) 39: 845–857
 1671-4083/17
www.nature.com/aps

Review Article

Organ preservation: from the past to the future

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Abstract

Organ transplantation is the most effective therapy for patients with end-stage disease. Preservation solutions and techniques are crucial for donor organ quality, which is directly related to morbidity and survival after transplantation. Currently, static cold storage (SCS) is the standard method for organ preservation. However, preservation time with SCS is limited as prolonged cold storage increases the risk of early graft dysfunction that contributes to chronic complications. Furthermore, the growing demand for the use of marginal donor organs requires methods for organ assessment and repair. Machine perfusion has resurfaced and dominates current research on organ preservation. It is credited to its dynamic nature and physiological-like environment. The development of more sophisticated machine perfusion techniques and better perfusates may lead to organ repair/reconditioning. This review describes the history of organ preservation, summarizes the progresses that has been made to date, and discusses future directions for organ preservation.

Keywords: organ transplantation; organ preservation; static cold storage; machine perfusion; organ assessment; organ repair; ischemia-reperfusion injury

Acta Pharmacologica Sinica (2018) 39: 845–857; doi: 10.1038/aps.2017.182; published online 22 Mar 2018

Introduction

Organ transplantation is the only effective therapy for patients with end-stage disease in many cases. A number of factors have contributed to the success of organ transplantation, including organ preservation, surgery, immunosuppressive medication, and post-transplantation care. A supply of high-quality donor organs is crucial to transplantation procedures; organ preservation has been described as "the supply line for organ transplantation"[1]. It allows time for preparation of the recipient, organization of staff and facilities, allocation and transportation of the organ, and laboratory tests[2,3].

Static cold storage (SCS) offers a simple and effective way to preserve and transport organs and is the most commonly used method[4]. However, a number of limitations are associated with SCS, including tissue damage induced by prolonged hypothermic preservation, difficulty in assessing donor organ function and viability, inevitability of ischemia-reperfusion injury (IRI), and limited opportunity for organ repair. Recently, the growing use of marginal organs from extended criteria donors has led to an emergence of *ex vivo* lung perfusion (EVLP) to assess donor lung function[5,6]. In addition to being an excellent graft assessment tool, EVLP has also shown potential for enabling graft repair, reconditioning, and immunomodulation[7], which inspired similar research and clinical applications in other organ systems[8–10]. The desire to extend preservation times has motivated research on optimal preservation solutions, temperatures, techniques, and therapeutic additives for organ repair and reconditioning[11–14]. By reviewing the history of organ perfusion and preservation, we noted that before the introduction of SCS in 1960s[15], machine perfusion with plasma or blood-based solutions was the clinical method for preserving isolated organs[16,17]. Reevaluating the advantages and limitations of early organ perfusion/preservation may help with the development of new techniques/solutions that enable prolonged safe preservation and the repair of extended criteria donor organs to address the organ shortage issue. Theories, preservation techniques, preservation solutions, and clinical practices are discussed.

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Received 2017-10-21 Accepted 2017-12-31

Past: a story of organ perfusion and preservation

Primitive concepts underwent a number of modifications over decades of scientific exploration to arrive at current practices in organ perfusion and preservation. It is essential to under-

stand this history to be able to evaluate the future direction of this field of research.

Organ perfusion as a primitive preservation technique

Organ preservation developed from the primitive concept of extracorporeal circulation, which first emerged in 1812 in the monography of Cesar Julien Jean Le Gallois. He speculated that "if the place of the heart could be supplied by injection—and if, for the regular continuance of this injection, there could be furnished a quantity of arterial blood, whether natural or artificially formed, supposing such a formation possible—then life might be indefinitely maintained in any portion"[18]. In 1849, German scientist Carl Eduard Loebell described in his Dissertation Inaugurals the first perfusion experiments on isolated pig kidneys. He observed that the bright red arterial blood perfused through porcine kidneys became dark and viscous upon its course through the renal veins[19]. In 1885, Max von Frey and Max Gruber constructed the first closed artificial circulation system, which shares many similarities to today's organ perfusion systems[20]. In 1895, Jacobj created a double circulation apparatus, which used an isolated lung as an oxygenator and permitted organ perfusion for several hours[19]. These early studies led to the development of extracorporeal membrane oxygenation (ECMO) and the subsequent development of perfusion systems for organ preservation[19–24].

From blood to chemically defined perfusion solution

Historically, blood was used as perfusate in early apparatuses. Primitive perfusion apparatuses required a large supply of blood to operate, whereby the volume of an animal's own blood was insufficient. People tried to substitute an animal's own blood with blood from a different animal species. The use of cross-species blood was toxic to the graft and led to its rapid decline[25]. Scientists then diluted the animal's own blood with normal saline or Ringer's solution. These methods led to the development of severe edema in organs, especially in the lung[25]. These early studies led to the realization of xenoimmunity and the development of transfusion solutions.

In 1937[26], Alexis Carrel perfused isolated cat thyroids in the Lindbergh apparatus with Tyrode's solution comprised of glucose, ions, and 40%–50% homologous serum. He found that the organs were viable for 3–21 days. However, cultivation over 6 days showed a tendency towards hyperplasia. In 1968[27], Hou *et al* cultured normal human placentas in a chemically defined culture medium. Placentas were kept viable for at least 14 days, but the stroma underwent great modification within 3 days. These studies demonstrated that organs or tissues were capable of surviving outside of the body for several days under normothermic conditions in culture medium. However, maintaining the normal histological morphology of cultured organs raised challenges, which slowed down organ culture research for several decades.

Temperature: from normothermic to hypothermic

Originally, organs were perfused at room temperature. In 1876, Bunge and Schmiedeberg added a water bath to the circuit to maintain perfusion blood at physiological temperatures[19]. Later, scientists began to speculate that the use of lower temperatures might attenuate organ damage during perfusion by abating cellular metabolism. In the 1960s, a number of experiments were performed with cooled diluted serum or heparinized blood, and kidney perfusion was extended from hours to days[28,29]. However, the use of cold blood also caused many problems, such as vascular spasm in kidney grafts[30].

From dynamic to static modality

In the 1960s, kidneys were successfully preserved for 3–5 days by continuous perfusion with cooled, oxygenated blood or plasma[28,29]. However, this method required complex and costly equipment, which limited its availability and made the transportation of organs extremely difficult. In 1969, Collins GM was able to successfully preserve canine kidneys for 12 h by immersing them in iced saline solution, and he later further prolonged cold storage time to 30 h with Collins solution[15,31]. This simple method for organ preservation was more cost-efficient and convenient for organ transportation than its predecessors. The birth of SCS replaced dynamic perfusion methods and became the standard method of organ preservation.

Present: current practice and research on organ preservation

Preservation techniques (temperature, apparatus, perfusion setting, *etc*) and perfusion solutions are the major fields of research in organ preservation.

Static cold storage and preservation solutions

Since the 1960s, SCS has gradually become the gold standard method for organ preservation. SCS involves flushing the procured organ with preservation solution at 0–4 °C, then immersing it into preservation solution at the same temperature until transplantation. The hypothermic environment is responsible for decreasing cellular metabolism, and the preservation solution reduces cellular metabolism and provides cytoprotection.

Collins solution was the first preservation solution to enter the commercial market in 1969[15]. It was used to preserve the kidney, heart, liver, and lung grafts. In 1980, Collins solution was modified via impermeant composition and improved chemical stability. The new solution was named a Euro-Collins solution, and it provided better protection during prolonged cold ischemia and was widely used[2,32]. The University of Wisconsin (UW) solution was introduced in the mid-1980s[33] and continues to be used today for abdominal organ preservation[34]. These solutions are so-called intracellular fluid (ICF)-type solutions characterized by low Na^+ and high K^+ concentrations. ICF-type solutions were intended to prevent cellular edema by maintaining intracellular ion concentrations upon cold-induced dysfunction of Na^+/K^+ pumps[35].

Adding amino acids to the preservation solution and using a histidine buffer system led to the development of histidine-tryptophan-ketoglutarate (HTK) solution, which is characterized by low K^+ and low Na^+ concentrations. It was originally

Table 1. Composition of popular cold preservation solutions.

Solutions	EC	UW	HTK (Custodiol)	Custodiol-N	Celsior	LPDG (Perfadex)	Ep4 (EP-TU)	ET-Kyoto	IGL-1
K^+	115	125	10	10	15	6	26	44	30
Na^+	10	25	15	16	100	138	141	100	125
Cl^-	15	20	32	30	71	142	103	–	–
Ca^{2+}	–	–	0.015	0.02	0.25	–	–	–	0.03
Mg^{2+}	–	5	4	8	13	0.8	4	0	5
Colloid/Impermeant	–	Pentafraction Lactobionate Raffinose	Mannitol	–	Lactobionate Mannitol	Dextran 40	Dextran 40	Pentafraction Trehalose	Lactobionate Raffinose Polyethylene glycol
Buffer	Phosphate Bicarbonate	Phosphate	Histidine	Histidine	Histidine	Phosphate THAM	Phosphate	Phosphate	Phosphate
Antioxidant	–	Glutathione Allopurinol	Mannitol Tryptophan α-ketoglutarate	Tryptophan α-Ketoglutarate	Glutathione Mannitol	–	–	–	Glutathione Allopurinol
Glucose	180	–	–	–	–	5	10	–	–
Amino acids	–	–	Histidine Tryptophan	Histidine N-acetylhistidine Glycine Alanine Tryptophan Arginine Aspartate	Histidine Glutamic acid	–	–	–	–
Others Sulfate,	–	Sulfate Adenosine		– Deferoxamine LK 614	Sucrose	–	Sulfate	Sulfate Dibutyryl cAMP Gluconate	Nitroglycerin Adenosine
Reference	[41]	[41]	[38]	[38]	[41]	[43]	[48]	[49]	[41]

Note: Values are expressed in mmol/liter unless indicated otherwise.

developed for cardiac preservation, but it also achieved comparable patient survival for abdominal organ transplants[36,37]. Custodiol-N is a modified HTK solution with additional amino acids and chemicals. It is currently undergoing clinical trials; experimental studies showed promising reductions of hypoxic and cold-induced cell injury[38,39]. Celsior solution was originally made available in the 1990s as a heart preservation solution and was later also used for both thoracic and abdominal organ preservations[40]. Like HTK solution, Celsior solution also uses a histidine buffer and a low K^+ concentration. However, its Na^+ concentration is much higher. It shows equivalent performance to UW solution at a cheaper cost[41,42].

The risk of hyperkalemia-induced pulmonary vasoconstriction led to the development of extracellular fluid (ECF)-type solutions, which have lower K^+ and higher Na^+ concentrations[43]. In the 1980s, an ECF-type solution called EP4 (or EP-TU) was introduced, which sustained a canine lung preservation model for as long as 96 h[44]. A low-potassium dextran glucose (LPDG) solution was developed and currently used as the gold standard for lung preservation[43,45,46]. ET-K solution was developed by optimizing the properties of sugar and electrolyte contents and by adding a protective component for pulmonary endothelium, which showed excellent postoperative lung graft performance[47]. ET-K and EP-TU solutions have been applied in clinical lung transplantation in Japan[48,49]. Table 1 summarizes information regarding the composition of popular cold preservation solutions.

Ex vivo machine perfusion

IR-induced injury increases the risk of early graft dysfunction and reduces long-term survival after transplantation[50] (Figure 1). Meanwhile, the shortage of donor organs has led to the use of extended criteria donor (ECD) organs. Proper donor organ functional assessment and *ex vivo* repair/reconditioning of organs prior to transplantation has become necessary. Machine perfusion is a method involving organ perfusion with a controlled flow of perfusate. It facilitates the maintenance of organ microvasculature tone, provision of oxygen and nutrients in support of tissue metabolism, and removal of toxic metabolic waste.

The cellular rate of respiration is proportional to the surrounding temperature[51]. For example, SCS at 0–4 °C reduces the metabolic rate of the organ to approximately 5% of its physiological level[52,53]. Different temperatures have been investigated for *ex vivo* machine perfusion, including normothermic machine perfusion (NMP) at 35–38 °C, subnormothermic machine perfusion (SNMP) at 20–34 °C, controlled oxygenated rewarming (COR) at 8–20 °C, and hypothermic machine perfusion (HMP) at 0–8 °C (Figure 2) (Table 2).

Hypothermic machine perfusion

HMP (0–8 °C) is based on the concept that oxidative energy production by mitochondrial electron transport is sustained at hypothermic temperatures. HMP continuously provides metabolic substrates for the generation of ATP, which enables the graft to restore tissue energy. The first clinically available HMP device was developed by Folkert Belzer in 1960s[28] and used to perform the first HMP-preserved human kidney transplant in 1968[16]. Belzer *et al* achieved perfusion of the kidney with hypothermic, diluted plasma or blood for 3 days[28]. Humphries *et al* were able to extend kidney perfusion to 5 days[29].

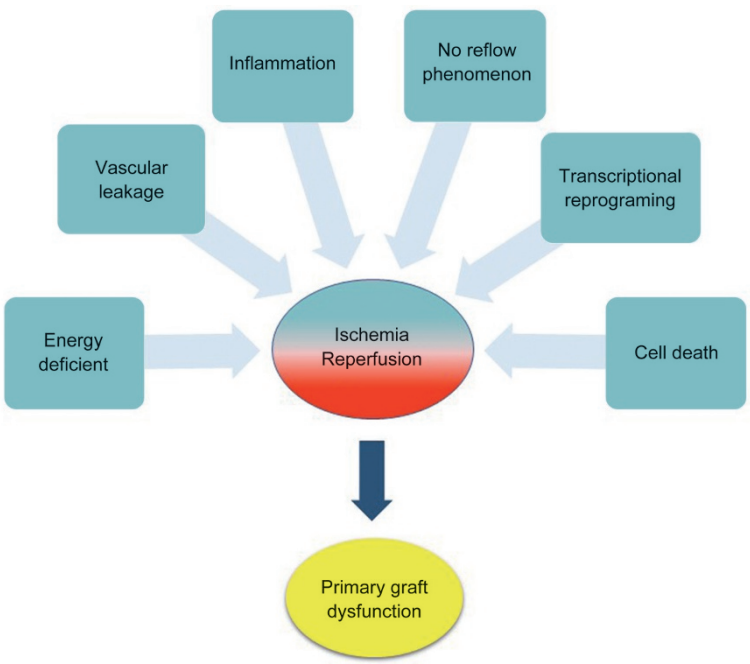

Figure 1. Biological processes induced during ischemia-reperfusion that may lead to primary graft dysfunction.

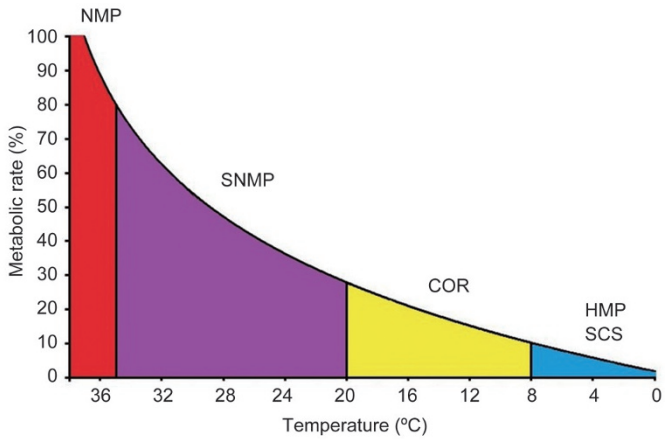

Figure 2. Metabolic rate reduces with a decrease in temperature in humans. (SCS=static cold storage, HMP=hypothermic machine perfusion, COR=controlled oxygenated rewarming, SNMP=subnormothermic machine perfusion, and NMP=normothermic machine perfusion).

The 1990s saw the resurgence in interest in HMP for kidney preservation as both the demand for organs and reliance on ECD donors grew[54]. New HMP technology showed decreased rates of delayed graft function and improved outcomes in the case of marginal donors relative to SCS. By 2015, approximately 25% to 35% of all transplanted kidneys in the United States were preserved with HMP[54].

Challenges arise in the use of HMP for liver preservation since the liver receives blood from both the portal vein and hepatic artery[2]. However, the first clinical trial of HMP-preserved liver grafts showed shorter hospital stays and reduced vascular and biliary complications as benefits[55]. Few studies on HMP in heart and lung transplants have been reported. Nakajima *et al* reported that short-term HMP (1–2 h) can improve lung tissue energy levels and ameliorate IRI by decreasing the production of reactive oxygen species in rat lungs[56,57]. Michel *et al* showed that HMP preserved the cellular structure of donor hearts better than SCS during prolonged ischemic times in pigs[58]. Additional research in the field of cardiac and lung HMP is required.

Normothermic machine perfusion

NMP (35–38 °C) is a method of perfusing organs under physiologic conditions to maintain metabolic activity and viability. NMP maintains donor organs at body temperature while providing oxygen and essential substrates. Historically, NMP was developed to assess organ function prior to transplantation[59–61] and to preserve donor organs during distant procurement[62,63]. In 2001, Steen *et al* reintroduced the EVLP technique to evaluate lungs from donation after cardiac death (DCD)[64]. In 2007, they performed the first human transplantation of a rejected donor lung after assessment with EVLP[65]. Early studies were only able to achieve perfusion times of less than 6 h in large animal models[66,67]. In 2008, Cypel *et al* in Toronto modified the EVLP technique with low tidal volume ventilation, reduced perfusion rate and acellular perfusate, and extended

Table 2. Comparison of various methods used for preservation of organs.

Methods	Temperature	Organ	Merits	Challenge	Clinical application	Reference
Cold static storage	0–8 °C	Kidney, liver, lung, heart	Low cost, simple and easy to operate	Metabolite accumulation, not for organ function assessment	Kidney, liver, lung, heart	[32–50]
Hypothermic machine perfusion	0–8 °C	Kidney, liver, lung, heart	Provides oxygen and metabolic substrates	Perfusion time is limited, not for organ assessment	Kidney, liver	[28,29,54–58]
Normothermic machine perfusion	35–38 °C	Kidney, liver, lung, heart	Provides oxygen and essential substrates; maintains metabolic activity and viability; good for organ assessment and repair	Perfusion time is limited for organ regeneration	Kidney, liver, lung, heart	[8,10,64–78]
Subnormothermic machine perfusion	20–34 °C	Kidney, liver	Newly proposed technique	To be determined	–	[85–89]
Controlled oxygenated rewarming	8–20 °C	Kidney, Liver	Slowly, gradually rise the perfusate temperature to mitigate ischemia reperfusion injury	To be determined	Liver	[90–92]

perfusion time for up to 12 h in swine lungs with stable lung function[68]. The Toronto group conducted the first clinical trial successfully and reported excellent outcomes in 2011[69]. They further reported extended clinical outcome data[70,71], and the marginal donor lungs treated with EVLP showed comparable or even better results than regular lung transplants[72].

The success of EVLP inspired many research groups worldwide to further investigate the role of NMP in other organ systems. Nicholson *et al* described that a short period (1 or 2 h) of NMP could restore function and replenish ATP after warm and cold ischemia in porcine kidneys[8,73, 74]. The first clinical study on preserving kidney grafts with NMP was reported in 2011[75]. A follow-up clinical study showed that the delayed graft function rate was significantly lower in the NMP group than in the SCS group in ECD kidney transplantation[76]. The first clinical trial on NMP in liver transplantation was reported in 2016; 16 donations after brain death livers and 4 DCD livers were transplanted after NMP. The results showed that 30-day graft survival was similar between the NMP and SCS groups, and the median peak aspartate aminotransferase level was significantly lower in the NMP group than in the SCS group[77]. Clinical studies have shown promising results with NMP in resuscitating marginal and declined donor livers[9,78]. In addition, NMP has been shown to be superior to SCS in preserving DCD hearts in dogs[79]. In pigs, DCD hearts reconditioned with NMP showed comparable function to brain death donor hearts[80]. Over a 2-year period in a clinical trial involving 159 cases of orthotopic heart transplantation, NMP showed higher recipient survival and lower incidences of primary graft dysfunction (PGD) and acute rejection than SCS[10].

Several companies have now marketed a commercial portable machine to facilitate *ex vivo* machine perfusion, such as Organ Care System™ (TransMedics, USA) for the heart, lung, or liver and Organ Assist® device (Organ Assist, The Netherlands) for the lung, liver, or kidney. These devices can be used during organ transportation, which offers a platform for normothermic organ preservation immediately after procurement, monitoring and assessing graft function continuously[11,81]. These mobile devices have demonstrated encouraging results in clinical studies, which opens new avenues for organ preservation and transportation[82–84].

Subnormothermic machine perfusion

Subnormothermic machine perfusion (SNMP, 20–34 °C) is a midway approach between HMP and NMP[85]. Although better preservation times were accomplished with NMP than with HMP, it was speculated that the cytoprotective benefits of reduced cellular metabolism under hypothermic temperatures could further improve organ preservation. Meanwhile, sufficient metabolism would be maintained for viability assessment and organ repair/reconditioning[86]. Although studies have shown that livers or kidneys perfused with SNMP are superior to grafts preserved under SCS[87,88], a recent study showed that porcine kidneys preserved under SNMP were associated with higher indices of renal and tubular injury upon reperfusion than those preserved under NMP[89]. Therefore, whether SNMP should be developed in addition to NMP should be further determined.

Controlled oxygenated rewarming

Following cold ischemic preservation, the abrupt change in temperature from hypothermia to normothermia upon reperfusion may effectuate dysfunction of the mitochondria and pro-apoptotic signal transduction, which contributes to reperfusion-induced organ injury[90]. Hypothermic preservation is meant to protect the ischemic organ by reducing metabolism. However, ischemic redox dyshomeostasis leads to impairment of the mitochondrial membrane potential through mitochondrial transition pore opening. Mitochondrial damage can be further enhanced upon reperfusion[91]. COR (8–20 °C) is an alternative organ perfusion method involving a slow, gradual rise in the perfusate temperature. The period of COR is aimed to minimize injury to the graft and improve hepatocellular function upon reperfusion, offering gentle restitution of mitochondrial function[91]. Clinical studies have shown that COR is safely transferable to clinical practice in liver transplantation[91]. By the end of 2016, COR had been effectively applied in 15 human liver transplantations[92]. Minor and colleagues demonstrated that COR following SCS had better kidney function with mitigated activity of mitochondrial permeability transition pore opening, caspase 9 activation, and apoptosis in porcine kidneys[90]. It should be noted that during EVLP, lung perfusate was gradually warmed up during the first 30 min[68]. COR could be integrated into NMP.

Organ perfusate: from chemically defined solutions to blood/blood substitute

Perfusate composition is of central importance in maintaining stable organ function *ex vivo*. Blood-based perfusates were commonly used for organ perfusion before cell culture media were developed in the 1950s[93,94]. Due to its variable nature and associated technical and ethical concerns, the use of blood or blood products was gradually replaced by chemically defined solutions. For example, Steen Solution™, a chemically defined solution, has been widely used for EVLP and machine perfusion of other organs; it contains colloid components (human serum albumin and Dextran 40) to maintain oncotic pressure, physiological ion concentrations to regulate osmolality, and buffers to retain normal pH and glucose as an energy resource. However, the supplementation of additional nutrients, including red blood cells and other blood substitutes, to Steen Solution™ is under investigation to extend perfusion time.

Blood/blood substitutes

Studies have identified that blood-based perfusate is necessary during NMP to transport oxygen and meet metabolic demands, and it provides superior functional preservation in the case of kidney, liver and heart storage[95–98]. It is still disputed whether blood or red blood cells should be involved in EVLP. Some studies have highlighted the use of blood over acellular perfusates[99], whereas others have observed spontaneous lung injury when using whole blood[100]. When look-

ing over the studies that have currently achieved the longest perfusion times, it is interesting to see that perfusates used in these studies were either whole blood or blood-based solutions[95,101–104]. One study that provided pertinent evidence in support of the essentiality of blood in perfusate is a cross-circulation study. O'Neill *et al* connected a conventional EVLP circuit to the internal jugular vein of a recipient pig so that metabolic substrates and hormones from the recipient pig were available to the perfused lungs, whereas metabolic waste produced by the perfused lungs was cleared by the recipient; they effectively perfused the lungs with recipient autologous blood for 36 h without notable changes in physiological parameters[105].

However, the use of blood-based perfusate is accompanied by a series of concerns, such as immune-mediated responses, hemolysis, thrombus formation, biochemical and humoral variations, and a risk of blood-borne infectious transmission[106]. Further development of an acellular perfusate is another major direction.

Nutrients

Currently, commonly used perfusates, such as Steen solution™ and Organ Care System (OCS) perfusate, use glucose as the only energy resource. However, during NMP, organs are perfused at body temperature. Glucose alone is not sufficient for organ metabolism. To prolong NMP for organ repair, the incorporation of more nutrients, such as amino acids, vitamins, lipids and others, should be considered. Amino acids are basic components of proteins and are essential nutrients for cell survival and proliferation. Vitamins can help cells use the provided chemical energy to process proteins, carbohydrates, and fats required for cellular metabolism[107]. Amino acids and vitamins have been used routinely in cell culture media[93,94]. Interestingly, cell culture media were used for organ culture to maintain isolated organs for days without serum or blood supplements[27]. In liver and kidney studies, amino acids and extra glucose have been added into perfusate during NMP, and this approach showed promising results in pigs[108,109].

Fetal mouse lungs cultured in a medium without growth factors showed poorly developed airways and a lack of defined acinar structures[110], which suggests that growth factors and hormones may also be required for organ rebuilding /regeneration.

To avoid the use of human blood products, interest has increased in acellular oxygen carriers, which have similar oxygen carrying capacity to human hemoglobin[111]. Initial studies on hemoglobin-based oxygen carriers have shown encouraging results, including enhanced oxygenation and improved allograft function of *ex vivo* perfused organs in normothermic/subnormothermic conditions[106,112], which opens the door for blood substitution in future.

It is reasonable to conclude that an ideal perfusate should offer oxygen carrying capacity, oncotic properties, buffers to maintain physiological pH, metabolic substrates and physiological electrolyte levels, growth factors and hormones. A blood substitute designed to replace human blood in *ex vivo* machine perfusion will be a promising direction for prolonging the preservation of isolated organs.

Future perspectives: organ repair/reprogramming with *ex vivo* machine perfusion

Prolonged *ex vivo* machine perfusion & organ repair/reprogramming

The incredible progress of organ preservation research over the past few decades has led to the booming success of clinical organ transplantation as a treatment for patients with end-stage disease. However, this demand has skyrocketed to a level that cannot be satisfied by the number of available donor organs. The use of *ex vivo* machine perfusion aspires to warranting the use of marginal donors by minimizing IRI and facilitating the repair/regeneration of suboptimal grafts in order to expand the donor pool and improve overall graft function after transplantation. For this purpose, prolonged *ex vivo* perfusion time is required (Figure 3).

Organ repair

There has been an increasing number of studies focusing on the application of *ex vivo* machine perfusion for organ repair. EVLP is among the most active areas of study. A series of therapeutic strategies have been studied using EVLP for lung repair. For example, different drugs were delivered through perfusate to mitigate IRI[113–115], therapeutic gases (NO, CO, H_2) were inhaled during EVLP to reduce inflammatory response and lung edema[116–118], mesenchymal stem cells were used to treat lung injury induced by endotoxins and infection[119], and IL-10 gene therapy was developed to prevent IRI[120,121]. When the types of injury are clear, injury-specific treatments can be used during EVLP. For example, high-dose, broad-spectrum anti-microbial agents were added to perfusate to treat human

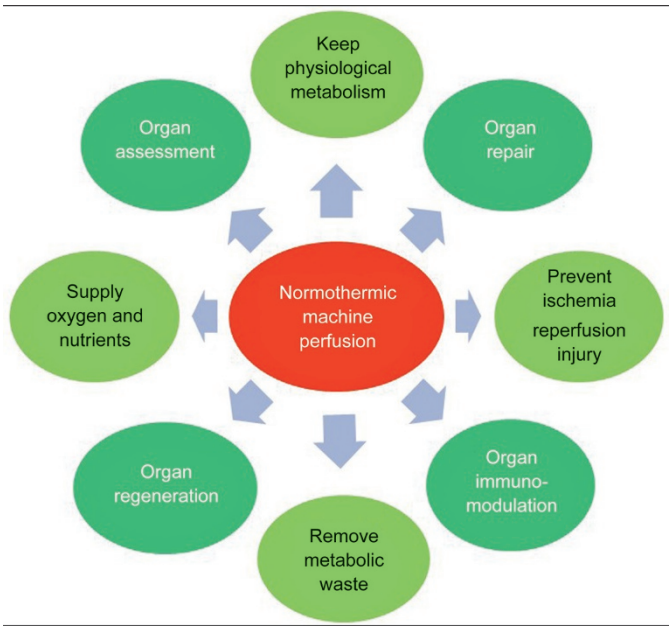

Figure 3. The advantage of the potential use of normothermic machine perfusion.

donor lung infection[122,123], lung lavage and surfactant replacement were used to treat acid aspiration-induced pig lung injury[124–126], and pulmonary thrombolysis was performed to eliminate pulmonary embolism followed by successful lung transplantation[127,128].

In kidney, Brasile *et al* delivered heme analog cobalt protoporphyrin during *ex vivo* kidney perfusion to reduce inflammatory and free radical injury by upregulating the protective gene hemoxygenase-1 in canines[129]. They also used growth factors to upregulate cellular processes to resuscitate and repair warm IRI in canines and in rejected human kidneys[130]. Hosgood *et al* delivered nitric oxide donors and carbon monoxide-releasing molecules during NMP, which enhanced renal flow and improved renal function in pigs[131]. Yang *et al* investigated the effect of adding erythropoietin to perfusate during NMP and found that erythropoietin promoted inflammatory cell apoptosis and drived inflammatory and apoptotic cells into tubular lumens, which led to inflammation clearance, renal protection, and tissue remodeling in a porcine model[132].

In liver, studies on therapeutic medications during NMP to reduce IRI showed promising results in pigs and rats[133–135]. Goldaracena *et al* delivered an antiviral drug to perfusate during normothermic *ex vivo* liver perfusion and effectively induced Hepatitis C virus resistance after pig liver transplantation[136].

Organ regeneration

In 2008, Ott *et al* reported the first whole organ engineering success. They used *ex vivo* machine perfusion as a platform, decellularized rat hearts by coronary perfusion with detergents in a Langendorff apparatus, then reseeded these constructs by perfusion with cardiac or endothelial cells; eight constructs were maintained for up to 28 days by coronary perfusion with a nutrient-rich medium in a bioreactor that simulated cardiac physiology. This study revolutionized the field of tissue engineering, kindled hope for possibility of whole organ engineering[137]. They also successfully created bioartificial rat lungs using a slightly modified approach and subsequently transplanted the regenerated left lungs orthotopically. The bioartificial lungs provided gas exchange *in vivo* for up to 6 h after extubation[138]. Using the same perfusion system, Ott's group further maintained the bioartificial rat lungs for up to 7 days with good function after implantation[139]. They later decellularized human and porcine lungs[140], which brought the matrix to clinical scale. A similar perfusion method has also been used to create kidney and liver scaffolds in animals and in clinically rejected human organs[141]. Although there are still many challenges, the use of NMP alongside stem cells for organ engineering has received increasing interest.

Organ immunomodulation

Ex vivo machine perfusion has also provided a potential platform for organ immunomodulation. Miyoshi *et al* reported that *ex vivo* perfusion of canine pancreaticoduodenal allografts using class-II-specific monoclonal antibodies delays the onset of acute rejection[142]. Brasile *et al* treated canine kidney grafts with a bioengineered interface consisting of a nano-barrier membrane during NMP for 3 h. They found that untreated control dogs experienced a mean onset of rejection on day 6, whereas the mean onset of rejection was significantly delayed until day 30 in dogs in the treatment group[143]. Martens *et al* distributed multipotent adult progenitor cells in the airway during EVLP and observed a reduction in pro-inflammatory cytokines and neutrophils in bronchoalveolar lavage fluid, which is related to innate immune system modulation and may play an important role in reducing PGD after transplantation[144].

Due to severe donor shortage from humans, xenotransplantation is gaining more attention. *Ex vivo* perfusion of porcine lungs with fresh human blood is used to study discordant pulmonary xenograft injury[145,146]. Pre-perfusion of donor organs with recipient serum[147] and the delivery of targeted drugs have been attempted to prevent hyperacute rejection[148]. *Ex vivo* machine perfusion offers an effective platform to alleviate discordant xenograft rejection by removing the recipient's xenoreactive natural antibodies[149]. Studies on the recellularization of animal organ scaffolds by human liver stem/progenitor cells with *ex vivo* machine perfusion techniques are under investigation[150,151].

Conclusion

Since the very first speculation on organ preservation made by Cesar Julien Jean Le Gallois over two centuries ago, tremendous progress has been made in this field of research. In the early days, organs were perfused with blood at physiological temperatures. The introduction of SCS in the 1960s revolutionized organ preservation. From then on, it became standard practice to statically preserve organs at hypothermic temperatures. With the recent demand to expand the organ donor pool, the currently accepted status of organ preservation is seeing a retrospective shift from SCS to theories inspired by early techniques, as these techniques provide great potential for improved graft preservation, viability assessment, and most importantly, repair/regeneration. The success of organ preservation with dynamic machine perfusion operating on the basis of blood-based perfusates at close-to-physiological temperatures has prompted further in-depth studies on organ preservation and repair/reconditioning. The need to prolong *ex vivo* machine perfusion time requires the optimization of current perfusates with the addition of essential components to meet metabolic needs. Prolonged *ex vivo* machine perfusion opens a door for organ repair and reprogramming, warranting further investigation of novel strategies to improve donor graft quality prior to transplantation.

Acknowledgements

Lei JING is a PhD candidate of the First Bethune Hospital of Jilin University and is supervised by Dr Li-ping PENG; she is supported by the First Bethune Hospital of Jilin University. This work was supported by research grants from the Canadian Institutes of Health Research (MOP-42546, MOP-119514, and PJT-148847) and an Ontario Research Fund-Research

Excellence award.

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