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Current state of hypothermic machine perfusion preservation of organs: The clinical perspective ☆

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ABSTRACT

This review focuses on the application of hypothermic perfusion technology as a topic of current interest with the potential to have a salutary impact on the mounting clinical challenges to improve the quantity and quality of donor organs and the outcome of transplantation. The *ex vivo* perfusion of donor organs on a machine prior to transplant, as opposed to static cold storage on ice, is not a new idea but is being revisited because of the prospects of making available more and better organs for transplantation. The rationale for pursuing perfusion technology will be discussed in relation to emerging data on clinical outcomes and economic benefits for kidney transplantation. Reference will also be made to on-going research using other organs with special emphasis on the pancreas for both segmental pancreas and isolated islet transplantation. Anticipated and emerging benefits of hypothermic machine perfusion of organs are: (i) maintaining the patency of the vascular bed, (ii) providing nutrients and low demand oxygen to support reduced energy demands, (iii) removal of metabolic by-products and toxins, (iv) provision of access for administration of cytoprotective agents and/or immunomodulatory drugs, (v) increase of available assays for organ viability assessment and tissue matching, (vi) facilitation of a change from emergency to elective scheduled surgery with reduced costs and improved outcomes, (vii) improved clinical outcomes as demonstrated by reduced PNF and DGF parameters, (viii) improved stabilization or rescue of ECD kidneys or organs from NHBD that increase the size of the donor pool, (ix) significant economic benefit for the transplant centers and reduced health care costs, and (x) provision of a technology for *ex vivo* use of non-transplanted human organs for pharmaceutical development research.

Introduction

The advent of clinical organ transplantation in the 1960's accelerated an interest in preserving organs outside of the body and despite some much earlier studies, notably by Nobel prize-winner Alexis Carrel and Charles Lindbergh [23], which focused principally upon perfusion at physiological temperature, increasing attention was given to hypothermic preservation. At about the same time it was established that renal function could be maintained on a pump oxygenator, and the benefits of lower temperatures on oxygen demand and ischemic tolerance in the perfused kidney were recognized [32,99]. The conceptual basis for the application of hypothermic perfusion in renal transplantation was in large part due to the early contributions of the team of investigators led by Arthur L. Humphries at the Medical College of Georgia (MCG) in the 1970's. The worldwide prominence of this group in clinical kidney transplantation was partly due to their pioneering research on *in vitro* kidney preservation [64,65,66,67]. As part of this MCG program in Augusta, Ga., Armand Karow Jr. worked to develop a means to cryopreserve kidneys, hearts, pancreatic islets and many other tissues [74,75,76,98,108,180]. Karow's vision at that time was for freezers filled with human organs for transplantation akin to a blood bank. While this vision remains to be fulfilled today, his contributions laid the foundations for on-going research as discussed in other articles in this memorial issue of the journal. Nevertheless, in parallel with Karow's research interests in organ cryopreservation, the early foundations laid down by the MCG group in hypothermic perfusion preservation (HPP) of organs [2] has been developed to the point that today clinical application is not only a reality but, in the case of kidneys, is a preferred

Abbreviations: DGF, delayed graft function; PNF, primary non-function; ECD, expanded criteria donor (ECD); NHBD, non-heart-beating donor; DCD, donation after cardiac death.

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preservation modality for so-called "expanded criteria donor organs"—a concept that is discussed more below.

It is not our intent in this article to review the history of the development of hypothermic perfusion preservation of organs for transplantation since this has recently been covered in an excellent review in this journal by Fuller and Lee, to which the reader is referred [48]. Instead we aim to provide a complementary disposition focusing on the current state of the art of HPP, often referred to as hypothermic machine preservation (HMP), as it applies clinically to the different transplanted organs.

Scientific basis for HMP

A good deal is known about the effects of cold on cells since cooling has proved to be the foundation of nearly all effective methods of protecting, and preserving cells and tissues for applications such as transplantation. Transplantation science calls for effective methods of preservation since it is unavoidable that donor cells, tissues and organs are required to withstand a period of *ischemia* and *hypoxia* as part of any transplantation procedure when the blood supply is temporarily interrupted. The basis of this hypothermic protection is that cooling can help to combat the deleterious effects of ischemia, but the consequences of cooling are not exclusively beneficial such that hypothermic storage is a compromise between the benefits and detriments of cooling. A complete understanding of the effects of hypothermia on the perfused organ calls for examination of the events at the cellular level since this is where the pathophysiology of ischemia, hypoxia and even hypothermic stress is ultimately mediated. It is not within the realm of this article to give even a brief review of the principles known to provide the basis for hypothermic protection of *ex vivo* organs, but reference is made to other recent articles that serve this purpose [19,130,158,159,160].

In essence, excision of a tissue for transplantation means that ischemia is total and inevitable even though the period may be brief. An immediate consequence of cessation of blood supply to an organ is deprivation of the supply of oxygen to the tissues, but anoxia (total) or hypoxia (partial) is only one of the many consequences of a lack of blood supply. A multifactorial cascade of events ensues following the initiation of ischemia. The pivotal event is ATP depletion, which occurs within the first few minutes of oxygen deprivation. This early event leads immediately to a shift from aerobic to anaerobic metabolism, which very quickly becomes self-limiting with the production of lactate and protons. Cell depolarization also occurs very early in the cascade leading to a breakdown of ion homeostasis, and a concatenation of other intracellular and membrane-associated events that eventually culminate in cell death by either apoptosis or necrosis. A rise in the intracellular concentration of protons and calcium is at the center of many of the mechanisms now recognized to be contributory to cell death as a result of ischemia [37,130,142,158].

The basic principle of cellular preservation for clinical application is to minimize the deleterious effects of ischemia and anoxia during the preservation interval. This can either be achieved pharmacologically by using a wide variety of cytoprotective drugs, and/or by reducing temperature. Interestingly, conventional wisdom teaches us that there is no single drug, or cocktail of drugs, that can so safely and effectively suppress metabolism and provide ischemic protection for multiple tissues and organs as the application of hypothermia can [137,138]. Here the focus will be confined to our ability as interventionalists to control the environment of cells to optimize hypothermic preservation. In this context HPP is based upon the fundamental premise that devices can be designed to facilitate the replacement of blood in the circulation of an *ex vivo* organ with specially designed fluids to maximize the protective effects of hypothermia on the ischemic tissue.

The fundamental basis of all biologic and chemical processes is molecular activity and mobility, which are governed by thermal energy, such that as temperature is lowered so molecular motion is slowed. The removal of heat from a system slows down both physical and chemical processes in proportion to the loss of heat, and therefore to the fall in temperature. Since the processes of deterioration associated with ischemia and anoxia are mediated by chemical reactions, it has proved well founded to attempt to prevent or attenuate these changes by cooling. Biochemical processes involve molecular interactions that are invariably catalyzed by enzymes in reactions that require energy input from cellular stores such as ATP or creatine phosphate. Cooling can affect all components of these reactions including the energy status of the substrate molecules, the stability of the enzyme protein, and the capacity of the cell to supply biological energy. Table 1 lists a catalogue of the most important effects of hypothermia in relation to ischemic events that forms the basis for hypothermic preservation of organs. A discussion of these effects is available in a variety of other publications [19,130,158,159,160].

Why perfuse? Continuous perfusion vs. static cold storage

Reliance upon hypothermia to counteract the effects of ischemia during organ storage has traditionally involved two modes of preservation: simple static cold storage (SCS) and continuous hypothermic perfusion. SCS has undoubtedly been the most widely used technique due principally to its simplicity, convenience and relative cost compared with machine perfusion techniques, which are inherently more complex due to the requirement for specialized perfusion devices. In fact, based on 2006 data from the Organ

Table 1
Catalogue of ischemia-related events influenced by hypothermia [158,159,160].

Ischemic events affected by hypothermia	Principal effects
General suppression of reaction rates	1. Slows metabolism and demand for oxygen 2. Reduces rate of substrate and energy depletion 3. Attenuates chemical processes that cause ischemic injury
Metabolic uncoupling	Dislocation of integrated biochemical pathways
Energy metabolism	Complex shifts from aerobic to anaerobic alter substrate dependencies and determine optimum temperature for preservation
Ion transport and cell swelling	Passive redistribution of ions and water across cell membranes demands counteractive measures
Proton activity changes	pH regulation changes demand effective buffering
Generation of oxygen-derived free radicals (ODFR)	Increased susceptibility of cells to generate ODFR and attenuates natural defense mechanisms
Structural changes	1. Membrane phase changes and loss of phospholipids 2. Thermal shock 3. Induction of stress proteins 4. Cytoskeletal changes
Mode of cell death	Apoptosis or necrosis

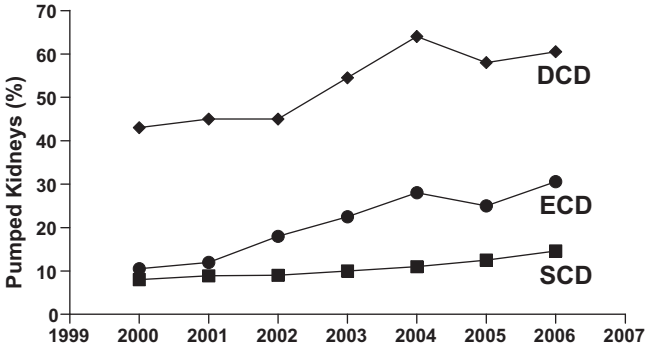

Fig. 1. Progressive annual increase in the percentage of clinical donor kidneys perfused prior to transplantation. It is clear that the highest proportion of "pumped" kidneys is found in the DCD (formerly labeled NHBD) category followed by the ECD group. In recent years, the percentage of organs in the Standard Criteria Donors (SCD) has also begun to increase. The reasons for these trends are explained in the text. (Data from the OPTN/UNOS Registry reports [150].)

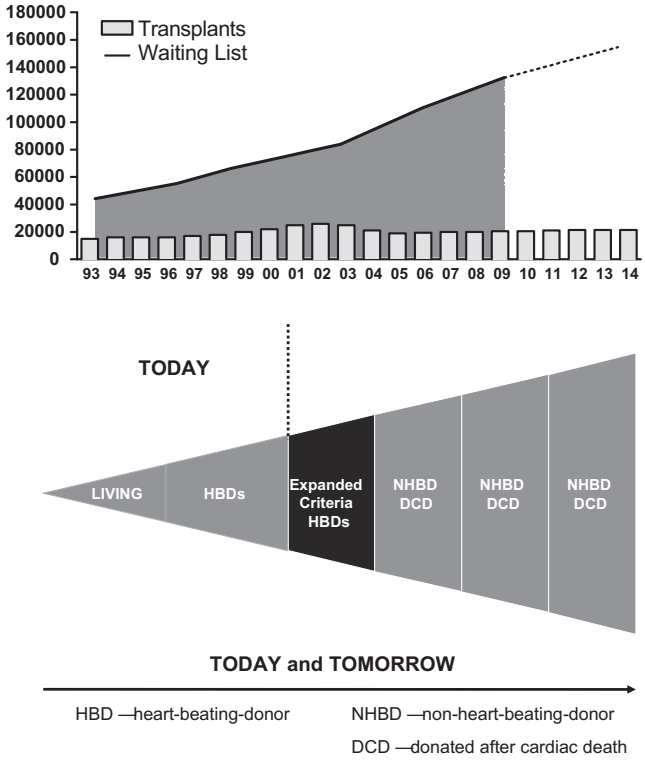

Fig. 2. Diagram illustrating the unmet demand for donor transplant organs. There is an ever widening gap between supply and demand as illustrated by the current (USA plus Europe) and forecasted numbers for kidney transplants in the top panel. The lower panel illustrates the fact that today the vast majority of transplanted kidneys are recovered from living-related donors or heart-beating cadavers [150]. The diagram also illustrates the point that there is now a recognized opportunity to increase the donor pool by considering the use of "expanded criteria organs" from heart-beating donors, or recovering organs from NHBDs as discussed in the text.

Procurement and Transplantation Network (OPTN), only about 20% of all kidneys in the USA were preserved using HMP. Nevertheless, as illustrated in Fig. 1 these proportions are changing due to a resurgence of interest in the relative merits of HMP as it applies to the potential "rescue" of so-called marginal donor organs, ECD organs, and even NHBD organs that are increasingly being adopted as a means to provide more organs for clinical transplantation. The strategic importance of this is discussed more fully in the next section.

In kidney preservation, both animal experiments and historical clinical studies have demonstrated that HMP provides better early graft function compared with SCS [106,116,140,147,181]. Associated benefits of HMP are listed in Table 2, and are discussed below in the context of the current role of HMP in clinical organ transplantation.

Clinical need and role of hypothermic perfusion preservation

Clinical kidney and liver transplantation has evolved from an experimental procedure 50 years ago to the current treatment of choice for patients with end-stage organ disease where patient and graft survival rates exceed 90% per year. However, as illustrated in Fig. 2 an ever increasing shortage of donor organs means that there is an increasing number of patients on the waiting lists for transplants such that future advances in this field are constrained by both the numbers of available organs and their quality. As a consequence, less-than-optimal donor organs are increasingly being used in an attempt to close the widening gap between supply and demand.

At the present time, with the exception of living, related donor-derived kidneys, most organs for transplantation are largely obtained from brain-dead but still heart-beating unrelated donors. The usual practice of using heart-beating donors greatly restricts the number of available organs. Many patients that die in hospital, or from trauma outside of the hospital, have potentially usable organs, particularly kidneys, which cannot be used because of uncertainty regarding their quality. In the last decade Organ Procurement Organizations (OPOs) and transplant centers began evaluating and expanding their acceptability criteria of organs from cadaver donors. The most common reluctance noted in establishing this practice was organ evaluation and cost [70]. The ability to evaluate transplantable organs from expanded criteria—defined by the advanced age of the donor or additional donor risk factors such as hypertension and diabetes—and asystolic donors by machine *in vitro* perfusion may significantly increase the number of organs available for transplantation and is one of the potential benefits currently being explored [7].

With respect to kidney alone, there are two determining factors that delineate the clinical significance of kidney recovery, transport, preservation, and assessment techniques: first, the existing

Table 2
Top ten benefits of hypothermic perfusion preservation.

- Maintains the patency of the vascular bed
- Provides nutrients, and low demand O_2 to support reduced energy demands
- Removes metabolic by-products and toxins
- Provides access for administration of cytoprotective agents and/or immunomodulatory drugs
- Increases available assays for organ viability checks
- Can facilitate change from emergency to elective surgery with reduced costs and improved outcomes
- Improves outcomes as demonstrated by reduced primary non-function and delayed graft function
- Permits use of expanded criteria kidneys, or organs from non-heart-beating donors to increase donor pool
- Economic benefit for transplant centers and reduces health care costs
- Provides technology for *ex vivo* use of non-transplanted organs for pharmaceutical research

overwhelming demand/supply imbalance, and second, the substantial cost savings and quality of life improvements provided by a transplant vs. chronic dialysis. Dialysis is currently the only other viable therapy for end-stage-renal disease (ESRD). By expanding the donor pool with "expanded criteria" and non-heart-beating donors and improving the quality of heart-beating donor kidneys, the estimated increase in the number of transplantable kidneys is 40%, the improvement in reducing DGF is 35%, and the estimated annual savings to the U.S. healthcare system could be greater than $1.5 billion.

More than 100,000 patients were registered for organ transplant on the UNOS waiting list by the end of February 2009, 78,565 of those being in need of a kidney transplant [149,150]. Three years earlier the number of kidney candidates on transplant waiting list was 66,961, 88% higher than in 1997. In 2006 there were 15,630 recovered kidneys with transplant potential, however only 76% were transplanted [149,150].

As summarized in the annual OPT/SRTR 2003 UNOS reports [26,27,38], the number of candidates on the kidney patient waiting list increased 6.3%, from 47,830 in 2001 to 50,855 in 2002. Seventy five percent of the increase occurred among patients of 50 years of age or older, these patients representing more than 50% of the total patients on the waiting list (compared to only 34% in 1993). Accordingly, the waiting time steadily increased from 235 days in 1993 to 341 in 2002. Kidney transplants from deceased donors increased from 7500 in 1993 to 8500 in 2002, and 15% of those were recovered from ECD. The use of ECD kidneys in older patients has become a common practice over the last decade, with recipients 50 years of age and older receiving 70% of these kidneys. The one-year kidney patient survival rate has been recorded at 94% and 97% for recipients of living and deceased organ donors, respectively. Five years post-transplantation the survival rate drops to 90.1% and 80.7% for the recipients of living and deceased organ donors, respectively.

The number of patients requiring kidney transplants has increased by 2400 per year since 1998 [26,27,38]. If 2400 more kidneys, from 1200 donors, either expanded criteria or NHB donors, had been procured each year, the waiting list would not have increased. Even today, the waiting list would not increase if each of the 59 organ procurement agencies in the U.S. obtained kidneys from two expanded criteria or NHB donors each month [29]. A program for procuring kidneys from NHB donors alone could result in a 40% increase in the overall supply of cadaveric kidneys. The literature indicates that there may be from two to four and a half times as many NHB donors as heart-beating donors [81].

In an attempt to improve the availability of organs from NHBDs a classification of donors within this category has been adopted, and is often referred to as the Maastrich classification (Table 3). Four categories of NHBDs have been identified [82]. There is little experience with donors of Category I due to logistics and questions about the viability of the kidneys. Category II is the main source of NHB donors. This category includes patients with myocardial infarction and cerebral bleeding, as well as trauma victims. Category III comprises patients for whom life support has been withdrawn, as in a ventilator switch-off procedure. The 10 min no touch period is particularly relevant in Categories II and III. Category IV was introduced to remind doctors of the possibility of a switch to a non-heart-beating procedure when a heart-beating procedure is complicated by an irreversible cardiac arrest.

Table 3
Categories of non-heart-beating donors: the Maastrich classification [82].

Category	Description	Location	Potential contribution
I	Dead on arrival	Outside hospital, emergency room	?
II	Unsuccessful resuscitation	Emergency room, intensive care	+++
III	Awaiting cardiac arrest	Intensive care	++
IV	Cardiac arrest while brain-dead	Intensive care	±

There is an extensive literature on transplantation of kidneys from donors without heart-beats [63,146]. Kidneys from trauma donors without heart-beats survived as well as those from donors with heart-beats [24,25,51,81,84,103,102,146,182]. Nearly half the recipients of kidneys from donors without heart-beats required dialysis during the first week after transplantation, and 4% of the kidneys in this group never functioned. Differences between the survival of kidneys from donors without heart-beats and the survival of those from donors with heart-beats were due entirely to graft failure in the first month after transplantation. This means that the disadvantage of using kidneys from donors without heart-beats could be reduced considerably, or eliminated if kidney viability measures were available to exclude poor kidneys, particularly those that will never function.

Use of kidneys from donors whose hearts have stopped beating could increase the supply of kidney transplants by a factor of 2–4.5 [81]. Currently more than 99% of cadaveric kidneys available for transplantation come from hospitalized brain-dead donors whose hearts are still beating [150]. The acceptable warm ischemia time in a NHBD has not been determined. The precardiac arrest condition of the donor, as well as preexisting disease states and age are factors in determining organ quality. Although kidneys can withstand 30–45 min of cardiac arrest, the outcome of a kidney transplant is further complicated by recipient factors, such as state of immunization and cardiovascular status.

Clinical impact of delayed graft function

The impact of delayed function on transplantation is negative, and is considered undesirable for both clinical and economic reasons. Delayed graft function is presently being addressed in several ways. The first and most common method is dialysis. The second is to adjust the patient's immunosuppression regime, e.g., by withholding cyclosporine. Schlumpf et al. [139] and Varty et al. [177] replaced cyclosporine with antithymocyte globulin and OKT3, respectively, in order to avoid cyclosporine nephrotoxicity during the first days after transplantation. The third way of decreasing delayed function is machine preservation. There is a growing body of evidence that preservation by machine perfusion is advantageous for the preservation of ischemically damaged kidneys [73], and for improving immediate post-transplant graft function [184]. Experiments have confirmed that machine perfusion preservation of kidneys damaged by warm ischemia is superior to preservation by simple cold storage. Machine perfusion has been reported to result in better survival rates and improved preservation of microcirculatory integrity [17,18].

The current resurgence of interest in the clinical use of HMP was marked by reports in the early 1990's that preservation by machine perfusion reduces delayed function rates and increases the rate of prompt function of renal grafts [10,58,83,109]. Moreover, machine perfusion with a modified ViaSpan® solution (Belzer's machine perfusate) has been found to be superior to machine perfusion with plasma-based perfusate [11]. Nevertheless, at that time the effect of machine perfusion on graft survival remained controversial [83,121]. Because machine perfusion preservation is relatively labor intensive, logistically demanding and costly, and because cold, static storage solutions are safe and effective, most transplant centers abandoned the use of preservation machines in the late 1980's in favor of the simpler static cold storage method. Nevertheless, machine perfusion was still shown to be preferable

for prolonged preservation times [107], and for preservation of ischemically damaged kidneys. However, prolonged cold ischemia times may have to be avoided in NHBD kidneys since it may lead to further delayed function [28]. Cold ischemia time may also have a detrimental effect on graft survival in combination with delayed function, HLA-DR mismatch, and acute rejection [31]. In the case of organs already damaged by warm ischemia, as in NHBD kidneys, prolonged hypothermic ischemia may have an additional negative effect on transplant outcome, even if the kidneys are preserved by machine perfusion. Therefore, hypothermic preservation times for warm ischemic kidneys are currently being kept at a minimum. Furthermore, machine perfusion may even have benefits for heart-beating donor kidneys, allowing both longer storage transport times, and better matching to more needy patients, and the promise of better short-term graft function.

Machine perfusion for preserving NHBD kidneys has been reported to result in better clinical performance when compared with simple cold storage in a study in which each machine perfusion kidney was compared with its cold-stored contra-lateral control [103]. Furthermore, earlier reports of the excellent transplant results with NHBD kidneys were, at least, partially attributable to preservation by machine perfusion [35,122]. Machine perfused kidneys from NHB donors show significantly higher early post-transplant delayed function rate when compared with matched machine perfused heart-beating donor grafts [33]. However, renal function recovers within 6 months post-transplantation, and both NHB and heart-beating donor kidneys have equal serum creatinine levels. On the other hand, NHB donor kidneys preserved by machine perfusion have less delayed function than NHB donor kidneys preserved by cold static storage [33].

More recently, at the Oxford Transplant Unit (UK) kidneys of Maastrich class III and IV of NHB donors were successfully perfused using the LifePort® Transporter (Organ Recovery Systems, Des Plaines, IL) [114]. For an average graft storage time of 13 h and perfusion flow rate of 115.5 ± 7.8 mL/min, a 72.2% immediate renal function, and 28.8% delayed graft function without any graft loss in the post-operative period were noted [114]. In Russia only kidneys of Maastrich criteria II and IV of NHB donors are allowed to be used for organ donation [132,131]. Kidneys from uncontrolled NHB donors are of dubious quality due to significant exposure to warm ischemia that in turn causes major graft ischemia–reperfusion injuries and poor long-term outcomes. To rescue those kidneys, the LifePort® Transporter was employed for their perfusion starting at the procurement site and at the time of organ recovery, in the operating room [132,131]. Systolic perfusion pressures of 30 and 40 mm Hg were used for 40–45 min WIT, and 65 min WIT kidneys, respectively [132]. Based on perfusion parameter selection, nine kidneys with extreme warm ischemia exposure of 59–65 min were successfully transplanted. Moreover, it has been observed that for immediate-function grafts the decrease in internal vascular resistance should take place within the first three hours of perfusion, whereas for delayed graft function organs, the reduction in the vascular resistance extends to the first 12 h [132,131].

Machine perfusion, when compared to static storage, has been shown to be beneficial for ECD kidneys [128,127]. The length of patient hospital stay and DGF rate in recipients of ECD donor kidneys increase by 37% each when static storage of those kidneys was used. The incidence of graft function of ECD kidneys at 6 months post-transplantation increases by 9% in the machine perfused kidneys relative to SCS kidneys. ECD kidneys with post-transplantation DGF show 29% reduction in the flow rate during pumping, and 38.5% and 40.7% increase in the vascular resistance and perfusate calcium concentration, respectively [128,127].

In Poland, the analysis of the long-term results of 415 human renal transplants preserved either by machine perfusion using the MOX-100 Waters machine (Waters Instruments, Inc., USA), or by simple cold storage revealed similar graft survival 15 months post-transplantation between the two preservation methods (87.7% and 85%, respectively) [87]. Nevertheless, in spite of the significant difference in cold ischemia time between machine perfused and static stored kidneys (33.7 ± 7.6 h vs. 27.5 ± 7.4 h, $P < 0.05$, respectively) the incidence of return to hemodialysis treatment was significantly reduced in the machine preserved grafts (7.05%) in comparison to SCS kidneys (12.2%, $P < 0.05$). Five years post-transplantation, recipients of SCS kidneys returned to dialysis twice as frequently as recipients of perfused kidneys, and the 5 year graft survival of perfused kidneys was significantly better than that of cold-stored organs (68.2% vs. 54.2%, $P < 0.05$, respectively) [87].

In summary, studies have shown that ECD kidneys preserved by SCS had an overall increased risk of reduced graft viability. However, HPP was clearly associated with a reduced incidence of delayed graft function, an overall improved graft survival, and an increased rate of clinical use [106,140,147]. Nevertheless, until recently these conclusions have remained equivocal due to the heterogeneity of existing clinical studies and especially the lack of prospective studies [174]. In recognition of the need for a controlled, prospective randomized study, Moers et al. recently published the results of such a study involving 336 consecutive deceased donors from whom one kidney was randomly assigned to pulsatile perfusion and the contra-lateral kidney to SCS. One year follow-up showed that HMP reduced the risk of DGF and graft failure and improved graft survival in the first year after transplantation [111]. Moreover, a cost-effectiveness analysis in this study suggests that the additional costs of HMP are more than compensated by savings due to the reduced costs of graft function-related complications, especially of reduced need for continued or renewed dialysis in case of DGF, PNF, or graft failure [54].

HMP of other transplant organs (heart, liver and pancreas)

Heart preservation

It is widely documented that the safe cold ischemic time for clinical heart preservation is limited to 4–6 h using principally SCS methods. However, it has been reported that continuously perfused hearts, at 8–10 °C for 5 h, have post-volume loading cardiac indices comparable to the initial preischemic values, while the myocardium of static stored hearts, at 4 °C for 5 h, is unable to reach its initial contractile properties. Hearts preserved using the two preservation techniques are biochemically and ultrastructurally similar [129]. Hypothermic perfusion facilitates early post-storage contractile capacity due to uniform myocardium cooling, constant wash-out of metabolic waste products during ischemia and continuous supplementation of the needed metabolic substances.

The resurgence of interest in the relative merits of HMP compared with SCS of other organs, notably the kidney, has led to new research studies focused on machine perfusion preservation of hearts in the past 5 years. Using a canine orthotopic transplant model, 6 months old mongrel dog hearts were transplanted after either 24 h of continuous hypothermic perfusion at 5 °C and 15 mm Hg, or 4 h of static storage at 4 °C [45]. Belzer-MP solution (KPS-1, Organ Recovery Systems, Des Plains, IL) supplemented with hydroxyethyl starch, glutathione, adenine, and fructose-1,6-bisphosphate was used for perfusion preservation. A weight gain of 27.4% was recorded in the HMP hearts [45]. No significant differences were noticed between the static stored and machine perfused hearts in terms of the required duration of cardiopulmonary bypass support, length of survival, degree of inotropic support and cardiac output. However, in comparison to static

storage, continuous hypothermic perfusion provided optimal myocardial cooling (through the native coronary circulation), and continuous substrate supplementation with limited anaerobic metabolism while washing toxic by-products [45]. Machine perfusion is effective in reducing oxidative stress damage in the myocardium, since a significant increase in oxidative damage DNA by-product (8 oxoG) and a reduction in mismatch repair enzymes (MYH, OGG1 and MSH2) were seen in the static stored hearts relative to perfused hearts [44]. Additionally, machine perfusion offers a better preservation of the myocardial metabolism through reduced intracellular lactate and increased glucose production and higher intracellular high-energy phosphate [44].

Currently, no device for machine perfusion of human allograft hearts is FDA cleared for clinical use. Key parameters for optimum preservation such as perfusate composition and temperature, perfusion pressure and flow rate still need to be determined with myocardial edema being one of the highest risks of this technique. Nevertheless, several devices and techniques are currently under investigation. For example, The LifeCradle™ HR Cardiac Perfusion System of Organ Transport Systems, Inc. (Frisco, TX) is specifically designed for hypothermic heart perfusion [30]. Supported by eight years of pre-clinical research and experience, the LifeCradle™ was originally scheduled for clinical trials to start in 2008 (www.allezoe.com/lifecradle.aspx). Another hypothermic perfusion heart transporter (Organ Recovery Systems, Inc.) has provided safe continuous hypothermic perfusion of dog hearts at set pressure for 24 h as mentioned above [45,44].

The LifeCradle™ system was also used for dog heart perfusion with Celsior at 5 °C for 10 h to study the influence of perfusate flow rate (5–30 mL/min/100 g) on myocardial flow distribution, myocardial metabolism and edema development during preservation [125]. For the majority of tested flow rates, the epicardial/endocardial perfusion ratio remained close to one. High myocardial flow resulted in low tissue lactate/alanine ratios, while hearts with low tissue flow were characterized by significant lactate accumulation or increase in lactate to alanine ratio in the myocardium. Low tissue flow organs had minimal myocardial weight gain over 10 h (11 ± 4%) whereas hearts with high tissue flow experienced significant weight gain (34 ± 4%, $P < 0.01$) and high myocardial water content [125].

An alternative approach to machine preservation of donor hearts that relies upon normothermic perfusion instead of hypothermia has been investigated in recent years [30]. The Organ Care System (OCS, TransMedics) device perfuses the hearts with a warm, oxygenated, nutrient-rich blood while the organs are maintained beating [30]. The OCS has received the CE mark for European clinical use, while a clinical feasibility study was approved by FDA in US in April 2007 to evaluate the safety and performance of OCS for human heart preservation for transplantation (www.transmedics.com). At this time we are unaware of any reports of the results of the aforementioned planned clinical trials using any of these devices or techniques.

Liver perfusion preservation

Today, liver transplantation is an effective and preferred treatment of choice for patients with end-stage liver disease. Despite some early attempts to develop HMP techniques for liver preservation, clinical practice has relied upon SCS [14,100,126,143]. Nevertheless, due in part to the resurgence of interest in hypothermic perfusion preservation of kidneys as a means to increase the donor pool by facilitating the use of marginal donors, the prospects of HMP of livers has been re-visited in recent years [69,71,72,96,175,176]. Three specific factors are identified as important for effective HMP, namely the type of perfusion solution, the characteristics of perfusion dynamics, and the need for oxygenation [175,176]. van der Plaats et al. have recently described a prototype device called the Groningen Machine Perfusion (GMP) system designed to deliver these presumed characteristics during 24 h of continuous perfusion [55,176].

Currently, there is no hypothermic perfusion machine commercially available to be used to extend the present 4–6 h limit of liver cold preservation for clinical transplantation. The technical performances of prototype machines such as the Groningen Liver Perfusion System (Netherlands) and the Organ Recovery Systems device were tested using porcine livers [112,113,175,176]. Attention was given mainly to perfusion uniformity and cellular injury generation, hypothermia and flow regime maintenance, and consistency in oxygen supply during 24 h continuous preservation. The Groningen hypothermic liver perfusion system contains an organ bath/solution reservoir, two centrifugal pumps, one pulsatile and one continuous, a hollow fiber membrane oxygenator, a battery pack and a measurement/control unit connected to a computer interface [176]. The pulsatile pump is used for hepatic artery perfusion and the continuous pump perfuses the portal vein without oxygenation, both pumps being constant pressure-controlled. Ice slush is used as the cooling source for perfusion temperature regulation between 5 and 7 °C. Following 24 h of perfusion, increased liver vascular resistance and hepatic edema was observed without endothelial cell or hepatocyte damage [176].

The Groningen Liver Perfusion system is under development by Organ Assist (Groningen, The Netherlands) [55]. Despite its compact design and portability, and excellent results so far in porcine liver transplantation, it has not yet been employed clinically. A second Dutch company, Doorzand (Amsterdam) produces Airdrive, a user-friendly, disposable, compressed air powered perfusion device, successfully tested in pre-clinical trials [55]. In the first human clinical study involving 19 cases receiving HMP livers, carried out in 2008 at Columbia University, Guarrera's team reported that patient and graft survival were very good without episodes of primary non-function or vascular complications [55].

Monbaliu's group in Belgium has also reported recently on studies using a prototype machine (Organ Recovery Systems) in which porcine livers were hypothermically perfused for 24 h with Belzer-MPS at 4–6 °C [112,113]. Pressure-controlled continuous perfusion through both the hepatic artery (<25 mm Hg) and portal vein (<7 mm Hg) was applied. The pressure-controlled perfusion provided organ protection against sudden increase in perfusion pressure, thus reducing the risk of shear stress and damage to the sinusoidal endothelial cells [112]. Throughout perfusion higher vascular resistance was registered on the hepatic artery in comparison to the portal vein; the former decreased substantially during the first 6 h of perfusion, while the latter remained almost constant. During preservation the perfusate aspartate amino transferase (AST) and LDH levels showed a slow small increase, in comparison to the liver fatty acid-binding protein (L-FABP), lactate and glucose that rose considerably in concentration by the end of perfusion relative to baseline [112]. In contrast to SCS where mean ATP levels are reduced in 24 h of liver preservation by 47% relative to baseline, after 24 h of HMP tissue ATP content increases to 166% when a low perfusion regime was applied (portal vein pressure and flow of 3–5 mm Hg and 0.5 mL/min/g, respectively; hepatic artery pressure of 20 mm Hg) [178]. Under the latter conditions maintaining an oxygen tension of 310 mm Hg throughout perfusion brought the 24 h ATP levels to 127% of baseline value. Livers perfused under low flow conditions with oxygenated solution preserved their appearance and morphology [178].

These recent studies investigating HMP of livers highlight the variability in machine preservation protocols and parameters studied, including the venous (0.14–0.5 mL/min/g) and arterial (0.1–1.2 mL/min/g) flows under both pulsatile and non-pulsatile settings [43,179]. Seventy-two hour continuous hypothermic

perfusion of pig livers through the portal vein only has also been reported. For dual perfusion low values of portal vein (3–4 mm Hg) and hepatic artery (20–30 mm Hg) pressures need to be used. Although both constant flow and constant pressure perfusion regimes can be employed, constant flow hypothermic perfusion for longer than 18 h can damage sinusoidal endothelial cells and endoplasmic reticulum due to increased vascular resistance and shear stress [43]. Hypothermic oxygenated machine perfusion is a practical potential strategy for "marginal" liver preservation, where HMP preconditions the liver cells prior to the introduction of blood cells by reactivating mitochondrial respiration and oxidizing mitochondrial electron complexes prior to reperfusion. The reported preservation temperature ranges between 1 and 18 °C, with the suggestion that lower temperatures substantially increase the perfusate viscosity that in turn can damage the sinusoidal endothelial cell lining. Moreover, there remains a lack of consensus with regard to organ viability criteria. Vascular resistance along with perfusate aspartate amino transferase (AST) concentration, tissue ATP and liver fatty acid-binding protein (L-FABP) content are some of the preferred hepatic viability markers [179].

Pancreas preservation

Earlier studies have demonstrated that pancreas hypothermic preservation by machine perfusion is feasible and can be safely extended to 24 and 48 h [5,46,97,172,173]. Dedicated renal perfusion systems have been employed mostly [46,97,172,173] after appropriate modifications required to accommodate the physiologic low flow and pressure needs of the pancreas [5]. The latter helps avoid excessive organ edema that post-segmental transplantation and reperfusion has been documented to result in sub-capsular bleeding, hemorrhagic necrosis, venous congestion, and hemorrhagic pancreaticoduodenal secretions [172]. The special case of pancreas preservation prior to islet isolation as a prelude to the treatment of Type I diabetes by islet transplantation is discussed in the "Future Applications" section below.

Future applications and current research

An increasing number of OPOs and transplant centers around the world are adopting HMP of kidneys as the preservation modality of choice in an attempt to close the ever widening gap between supply and demand by relying increasingly upon ECD kidneys or marginal donor organs. Moreover, the multifaceted advantages of HMP over conventional SCS, summarized in Table 2, have resulted in a resurgence of interest for preservation of other organs besides the kidney. The opportunity to improve the assessment of the quality of the organ during perfusion preservation in an attempt to reduce the discard rate for transplantation is a very important prospect that deserves more attention and research.

Assessment of the quality of donor organs during HMP

As discussed in the previous sections, machine perfusion improves immediate post-transplant graft function, provides better survival rates and enhanced preservation of microcirculatory integrity [17,18]. Hypothermic machine perfusion allows for extended storage and transport times and for the possibility of kidney performance assessment and metabolic support provision during perfusion [33,145]. Presently, during machine perfusion, renal flow rate and vascular resistance are the only accepted indicators of kidney *in vitro* viability [85,104,128]. A small number of biochemical parameters and ischemic injury markers have been quantified in the renal effluent [78,79,128,127], yet their use is limited, and their role in predicting kidney *in vivo* function and transplant outcome is still controversial. Thus, the need for a complete and diagnostically valuable evaluation of kidney function *ex vivo* still remains.

Machine perfusion based viability testing can be successfully used to select good quality kidneys for transplantation [86,105,115]. The Newcastle (UK) clinical viability protocol for hypothermically machine perfused kidneys from NHB donors requires perfusion flow indices of 0.4 mL/min/100 g/mm Hg or greater, and perfusate GST content of less than 100 IU/L/100 g renal mass [115]. In Japan, another perfusion apparatus, LPS-II (Nikiso, Tokyo, Japan), that consists of a non-pulsatile pump, heat exchanger, organ chamber, reservoirs and a membrane oxygenator has been used for cadaveric kidney perfusion preservation. Those kidneys, which showed perfusion flow rates of at least 0.4 mL/min/g and no increase in the perfusion pressure during machine preservation, were selected for transplantation [105]. Due to unavailability of Belzer-MPS in Japan, kidneys are normally perfused with cryoprecipitated AB positive plasma at a temperature of 8–10 °C and an average perfusion pressure of 30–50 mm Hg for 8–12 h [105]. Kidneys displaying 0.40–0.65 mL/min/g perfusion flows have a 25.7% incidence of PNF in comparison to kidneys of 0.90 mL/min/g flow rates [105]. From the LPS-II renal perfusion apparatus organs showing 1.10 mL/min/g flow rate and 55.3 mm Hg/mL/min/g vascular resistance were successfully transplanted, without any incidence of DGF post-reperfusion [86].

Identifying and developing improved methods to evaluate kidney quality (viability) during hypothermic preservation have been the objectives of many studies. A five-channel sensor used to measure temperature and ion activities (K^+, Na^+, Ca^{++}, pH) in the intersitium of human kidneys [1] detected significantly higher potassium activity in non-functioning kidneys when compared to primary functioning grafts (18 vs. 3.9 mmol/L). Changes in the glomerular dynamics (evaluated using the ureteral output), and increased renal vascular resistance at the post-glomerular level during hypothermic machine perfusion were linked to a decline in the renal perfusate flow rate [123]. The viability of machine preserved NHB donor kidneys was determined by calculating the intrarenal resistance and determining lactate dehydrogenase and α-glutathione S-transferase (αGST) concentrations [34,78]. The latter correlated well with warm ischemia damage, high levels of αGST predicting poorer post-transplant graft function or even non-function. The proximal tubule lysosomal enzyme glutathione S-transferase is the best predictor of outcome of human kidney function before transplantation because of the correlation between warm ischemia time and the release of proximal tubule cell enzyme αGST in the machine perfusate. However, while high αGST is a predictor of poor outcome, low αGST does not necessarily indicate a good perfusion result [79].

The efficacy of hypothermic pulsatile perfusion for ischemically damaged human kidneys was established by measuring perfusion parameters, perfusate electrolytes, perfusate gas values and kidney ischemic injury markers [36]. Perfusate Na^+, K^+, pH and osmolarity showed no variation between delayed function and immediate-function grafts, while flow was significantly elevated in the immediate functioning group and lactate dehydrogenase index was higher in the delayed function graft (16.48 ± 1.3 vs. 9.95 ± 2.3) [36]. Using NHB donor canine kidneys, it was shown [61] that machine perfusion with trifluoperazine added to Belzer-MPS improves kidney function by reversing the vasospastic effects of ischemia and reperfusion. However, after implantation and 4 h of reperfusion, machine perfused NHB donor kidneys showed significantly higher Na^+/K^+ ratios (6.61 vs. 2.16) and creatinine clearance levels when compared to heart-beating donor-derived kidneys [61].

The development of reliable methods for organ viability assessment is important to the reduction of delayed graft function rate in the recipients of NHB donor kidneys. Two criteria for *in vitro*

quality control of kidneys have been developed [85,104]: (1) perfusion flow rates higher than 0.4 mL/min/g with increasing flow during perfusion, and (2) no increase or reduction in renal arterial pressure over time. The addition of prostaglandin E1 (PGE1) to machine perfusate improves kidney function by ameliorating mitochondrial ischemic injury (seen in EM images) and reducing release of Ca^{++} into the perfusate [128,127]. Quantitative hydrostatic and biochemical criteria were used to evaluate kidney function and to select well perfused kidneys for transplantation: renal flow rate higher than 0.5 mL/(min g), flow resistance smaller than 0.0085 (mm Hg min)/(mL g) and perfusate Ca^{++} less than 0.002 mM/g [128]. Kidneys with a mean flow rate of 1.56 mL/(min g) and vascular resistance of 0.0027 (mm Hg min)/(mL g) showed no delayed function after transplantation. Increased ionized Ca^{++} concentration in ischemic damaged kidneys effluent reflects disruption of the structural integrity of cellular organelles with subsequent lysis and release of intracellular calcium.

In recent years we have addressed the question of *ex vivo* organ viability assessment in our own program of research to investigate kidney preservation technologies with the ultimate goal of reducing the current renal discard rate and improving the use of marginal donor kidneys. As part of this program we have begun to study the role of the perfusate in modulating kidney biochemical perturbations during lengthy machine perfusion, and to emphasize that a comprehensive assessment of kidneys *ex vivo* performance is needed for a reliable screening of viable organs for transplantation. A more complete assessment of viability has to consider renal biochemistry in addition to the traditional biophysical parameters of flow rate and vascular resistance, but apart from the few studies summarized above there have been very few studies taking this additional approach [36]. For example, we recently reported on the role of the perfusate in modulating biochemical perturbations during extended machine preservation of kidneys [9]. For this study, we compared the kidney effluent composition during 72 h HMP with either Belzer's-MPS, or the new Unisol™-UHK proprietary hypothermic blood substitute solution [157,160,166]. Unisol is designed, among other characteristics, to have a higher buffering capacity than the commonly used preservation solutions [6], and provides superior protection of the vascular endothelium [167], as well as optimum buffer capacity for acid–base regulation during prolonged HMP of kidneys [8]. Unisol™-UHK is an intracellular base solution designed for applications at profound hypothermic temperatures (<15 °C) [6,157,158,160,168]. Belzer's-MPS is the current clinical standard for machine perfusion of kidneys [128,151]. Kidney perfusion flow parameters, with similar values between the two experimental groups, satisfied the published acceptance criteria for transplantation [85,104,128,151]. Thus, from a renal flow point of view, the two perfusates, UHK and Belzer-MPS were equivalent. However, marked differences were monitored between the two experimental groups in terms of kidney biochemical and metabolic activity as determined by effluent perfusate analysis [9]. These data serve to emphasize that a comprehensive assessment of kidney *ex vivo* function during the preservation interval would facilitate a greater degree of discrimination, and allow for a more accurate and reliable selection of viable kidneys for transplantation. Nevertheless, analysis of the effluent perfusate from the perfused organ is not the only approach for improved assessment of the organ status during perfusion preservation. Recently we have advocated the use of interstitial fluid analysis using microdialysis as a potentially new approach for assessing organ function during perfusion preservation [7].

Microdialysis for organ assessment during HMP

Microdialysis is used as a bio-analytical sampling technique to monitor the chemistry of the extracellular space in living tissues without altering the fluid balance or the metabolic pathways. Information on the relative concentration of tissue interstitial fluid components can be obtained every few minutes, over long periods of time, without loss of blood or other fluids, with minimal dilution by blood and negligible metabolite breakdown. Microdialysis consists of implanting a very small dialysis probe into the examined tissue. The probe is analogous to a blood capillary vessel, with a semi-permeable bio-compatible membrane as the active part that, based on its pore size, permits only specific molecular weight components of the extracellular space to pass through. The working principle of microdialysis consists of forcing an isotonic fluid through the probe inlet port into the probe membrane, at a low, constant and controllable flow rate, in order to allow the isotonic fluid equilibration with the interstitial fluid, and collection of the dialysate at the outlet probe port. The isotonic fluid, with an identical ionic composition, physiologically resembles the targeted interstitial fluid.

The use of microdialysis has increased dramatically in recent years for a wide variety of research investigations and clinical studies [59,91,183]. For example, microdialysis has been used to measure renal interstitial adenosine concentration and its role in the regulation of renal hemodynamics during endotoxin shock and induction of acute renal failure [117]. Also, it has been proven using microdialysis that renal cortical interstitium contains detectable amounts of ATP that consistently decrease in response to reduction of the renal arterial pressure, in correlation with the autoregulation-associated alteration in renal vascular resistance [118]. Clinically, the microdialysis method applications cover a broad spectrum, that includes, among others, vascular and plastic surgery, hemodialysis, neuromonitoring in neurointensive care, neonatal investigations, diabetes, metabolism, and intra-dermal and intra-abdominal studies.

In the field of organ preservation and transplantation the microdialysis technique has been used to continuously monitor the metabolic changes in a porcine liver graft during transplantation. A microdialysis catheter of 20 kDa membrane cut-off was inserted into the liver and perfused at 0.3 μL/min with sterile isotonic solution. Microdialysates were collected at 20 min intervals during the donor operation, during 15 h of static cold preservation, and for 7 h post-transplantation. One single microdialysis probe measured simultaneously four metabolite concentrations, glucose, lactate, pyruvate and glycerol. The method proved to be a useful tool in estimating liver graft tissue injury due to cold ischemia and transplantation, through intrahepatic biochemistry [119]. Nevertheless, based on an extensive literature survey, no research or clinical studies of kidney preservation have employed the microdialysis technique to monitor renal extracellular space electrolytes and metabolites during either *ex vivo* organ perfusion or SCS. Implementation of this method is expected to bring accuracy in monitoring renal biochemical activity of hypothermically preserved kidneys. The microdialysis probe would be placed in intimate contact with the tissue, allowing the interstitial fluid components to be collected from the immediate vicinity of their production location. Consequently, the loss in their concentration, due to transport across the extracellular space into the blood vessels, and dilution in the perfusion/preservation solution would be avoided. Furthermore, the opportunity for cell products to be degraded prior to collection would be minimized.

Experimental study in porcine kidneys

To put this to the test an experimental study was performed in our laboratory to evaluate the merits of the microdialysis method in terms of its practicality for renal viability assessment, suitability and simplicity for monitoring biochemical activity of kidneys through the collection and analysis of renal interstitial fluid, during hypothermic preservation [7]. For this, porcine kidneys recovered

from 2 h-warm-ischemic-NHB donors were hypothermically machine perfused for 24 h, at 5–7 °C and 30–60 mm Hg arterial pressure, using the continuous pulsatile perfusion prototype of the LifePort® kidney preservation system. The kidneys were perfused with Belzer-MPS. Renal interstitial fluid was periodically sampled using flexible peripheral tissue-type microdialysis probes of 20 kDa molecular cut-off and 10 mm membrane length (CMA/20, CMA Microdialysis, Sweden). As illustrated in Fig. 3, a single microdialysis probe was inserted into the renal cortex (pole) of each kidney and interstitial fluid samples were collected hourly while the kidneys were perfused. The probes were perfused with isotonic fluid at a flow rate of 2.5 μL/min, and the dialysates were collected in vials placed in a 4 °C refrigerated fraction collector. Renal effluent samples were collected in parallel with the microdialysates. Both renal effluent samples and microdialysates were analyzed for pyruvate concentrations, pyruvate being one of the important metabolic activity indicators. More specifically, this pilot study included an investigation of the effect of the addition of fructose-1,6-diphosphate (FDP) as an energy substrate to promote anaerobic glycolysis in the hypoxic perfused kidneys [7]. The rationale for this approach was based upon the premise that during glycolysis the eventual pathway block by the build up of lactate, which in turn inhibits phosphofructokinase (PFK), can be circumvented by the supply of exogenous FDP [94,154,155].

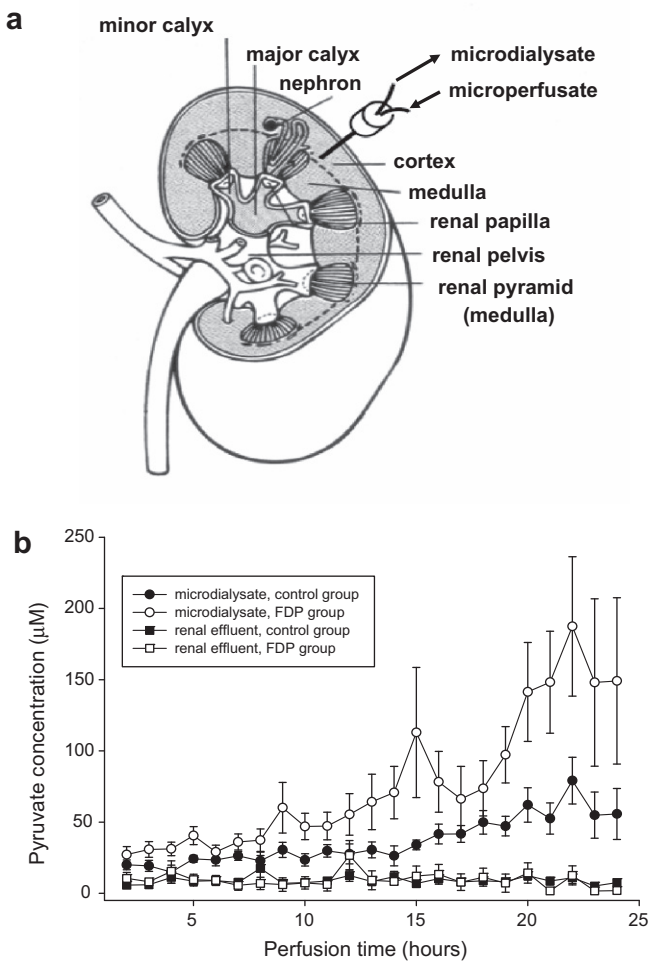

Fig. 3. (a) Schematic representation of the placement of a microdialysis probe (not to scale) in the renal cortex for sampling of the interstitial fluid. This technique is proposed as one means of improving the assessment of the quality of donor organs during the storage interval [7]. (b) Renal pyruvate concentration during hypothermic machine perfusion, as a function of time. Statistically significant higher pyruvate concentrations ($P < 0.05$) were detected in the interstitial fluid relative to renal effluent. Data were collected using the microdialysis technique depicted in (a) and are presented as the mean ± SEM. ($n = 4$ for the control group and $n = 8$ for the FDP group).

The presence of exogenous FDP in the perfusate induced no changes in the renal flow rate and vascular resistance, renal effluent biochemistry, or pyruvate concentration relative to untreated control kidneys. Significant increases in pyruvate production ($P < 0.05$), however, were observed after12 h of perfusion in the interstitial fluid of FDP-treated kidneys relative to control kidneys (Fig. 3b). After 24 h of perfusion, interstitial fluid concentrations of pyruvate were 149.1 ± 58.4 vs. 55.6 ± 17.9 μM ($P < 0.05$) in the FDP and control group, respectively. The microdialysis probe collected the interstitial fluid directly from the cellular sites of metabolic and synthetic activity, where perfusate dilution was minimal. Consequently, the biochemical changes induced by the organ metabolic activity were detected only at the interstitial level, in the microdialysates. Interstitial fluid pyruvate may be a good indicator of kidney function. The addition of FDP to the perfusion solution during ischemic kidney preservation resulted in enhanced pyruvate production in the extracellular space, indirectly reflecting an increase in anaerobic ATP production. The pyruvate will be transformed during organ reperfusion into acetyl Co-A enzyme allowing an immediate start of aerobic metabolism. This in turn can increase the amount of ATP available to the cells and may help prevent reperfusion injury upon transplantation.

This study illustrates that microdialysis is effective in monitoring biochemical changes in the organ that were not detected by analyzing the perfusate. We propose that the interstitial biochemical profiles might be a better indicator of kidney viability, enabling more effective, valid decisions to be made on suitability of NHB donor-derived kidneys for transplantation.

Pancreas HMP for islet isolation

There is now a worldwide consensus that islet transplantation may be considered a viable option for the treatment of insulin-dependent diabetes mellitus, and clinical trials are underway at many centers around the world [4,141]. As this approach for curing diabetes transitions into a routine clinical standard of care so the demand for donor islets will escalate. Moreover, the potential for xenotransplantation to relieve the demand on an inadequate supply of human pancreases will also be dependent upon the efficiency of techniques for isolating islets from the source pancreases. Procurement of donor pancreases for islet isolation and transplantation is not yet widely practiced due in part to concerns about post-mortem ischemia upon functional islet yields [88].

Islets are highly vulnerable to irreversible damage after prolonged ischemia [15,21,22,40,41,153], and cold ischemia of the cadaveric pancreas is detrimental to islet yield [16,52,60,77,88,135]. *In vitro* studies have shown a significant reduction in insulin release in response to glucose challenge even after short periods of cold storage in UW solution [52]. These observations have been seen in clinical practice as there have been no reports of successful single-donor islet transplants with prolonged cold storage beyond 10 h [60]. Ryan et al. have provided evidence of the detrimental impact of cold ischemia on post-transplant islet function [136].

Transplanted islets isolated from 24 h perfused dog pancreata have been reported to result in 60% recipient survival post-transplantation, providing similar outcome to fresh islet implantation [173]. Islets isolated from human pancreas after 13 h of SCS and 4 h of hypothermic pulsatile perfusion on a Waters RM3 system were characterized by higher viable yield and stimulation index

relative to cells isolated from organs that sustained more than 8 h of static storage alone [97]. Against this background, our studies have been designed to test the general hypothesis that, in line with our experience with clinical kidney preservation, machine perfusion will facilitate improved pancreas preservation. Moreover, it is anticipated that this will extend beyond the present clinical limit of 16 h for conventional cold storage and will improve the yield and potency of islets isolated from marginal donor pancreases that may suffer a period of warm ischemia in addition to prolonged cold storage. Since HPP technology has had a major impact on circumventing ischemic injury in kidney transplantation [33,83,109], we applied this approach to the preservation and procurement of viable islets after HMP of porcine pancreata since pigs are now considered the donor species of choice for xenogeneic islet transplantation for a number of compelling reasons [120].

Experimental study in porcine pancreas

In light of the brief history of pancreas perfusion preservation outlined above, and current resurgence of clinical interest in HMP of donor organs, we embarked upon the development of a perfusion technique for pancreas preservation prior to islet isolation [161,170]. Considerable attention to detail was necessary in the development of a successful technique for perfusion of pig pancreata on the LifePort® perfusion machine. Both the surgical dissection, including the mode of cannulation, and configuration in the organ cassette on the machine proved to be important in the development of a technique that guaranteed 24 h continuous perfusion [161,171] (Fig. 4). The LifePort® perfusion machine provided a controlled closed loop pulsatile perfusion at a set systolic pressure of 10 mm Hg (Fig. 5) [162].

These studies revealed an unexpected salutary effect of machine perfusion on islet harvesting. The progressive development of edema during extended machine perfusion of organs is a phenomenon that is generally regarded as undesirable. Steps are usually taken to minimize the problem by adjusting the mechanical perfusion parameters such as flow and pressure, as well as the composition of the perfusate, to minimize the development of interstitial edema. Having resolved a technical problem with respect to cannulation of the pancreas that affects the efficiency of perfusion as described elsewhere [161,171], we determined that 24 h of HMP resulted in moderate edema in the gland compared to the non-perfused controls that were simply flushed with and immersed in cold UW/Viaspan solution. Contrary to expectations, development of edema (up to 150%) did not prove deleterious, but was shown to be of considerable benefit by correlating with a more efficient disruption of the pancreas during enzymatic digestion to yield a significantly greater number of islets (Fig. 6). In fact, the increased yield over the fresh group of pancreases was 1.6–1.8 times greater and approximately 2–3-fold greater than for pancreases preserved by SCS in UW/Viapsan. Our hypothesis is that the edema causes sufficient disruption to the extracellular matrix and architecture of the pancreatic gland that the subsequent distension and digestion of the gland was able to proceed more effectively. This is evidenced by shorter digestion times, a more homogeneous digestion product, and better gradient purification resulting in higher yields and purity of the final islet preparation. The structure and function of the islets *per se* did not appear to be compromised by the level of tissue edema encountered in these studies [169,170,171]. Concerns that a change in the hydration of the isolated islets due to HMP might alter the buoyant density of the islets, and thereby critically alter their ability to be separated from exocrine tissue on a density gradient did not appear to be a problem. This is presumably due to the fact that any inherent edema in the islets is counteracted by the pre-gradient incubation in UW solution, which is a hypertonic medium that would dehydrate the islets during the 30 min cold incubation prior to loading on the density gradient for purification. The latter is a routine step used in islet isolation protocols [89,90].

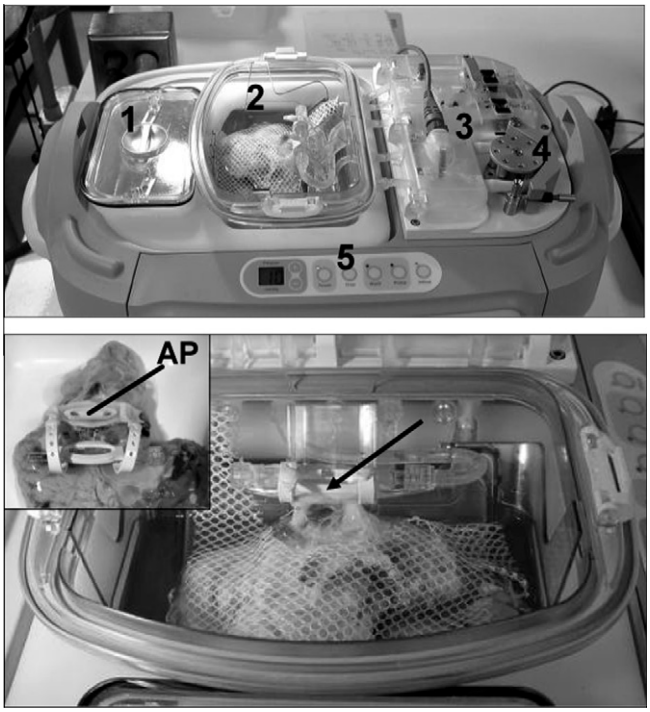

Fig. 4. Hypothermic perfusion preservation of a porcine pancreas on a LifePort® machine. The upper panel shows the features of the LifePort® including, (1) ice chamber; (2) organ cassette; (3) the tubing frame, which includes pressure and bubble sensors; (4) the pump head; and (5) the user-friendly control panel. The lower panel shows the details of a pig pancreas installed in the perfusion cassette and hooked up to the perfusion inlet line via a seal-ring cannula (arrowed). This proprietary cannula allows simultaneous perfusion of the celiac trunk (CT) and superior mesenteric artery (SMA) by way of an aortic patch clamped in the seal-ring cannula (see inset) [171]. The inset photo shows the opening of the CT and SMA in the aortic patch (AP), which was exposed for viewing by opening the seal-ring cannula.

The unanticipated mechanical benefit of HMP described above was achieved without compromising the quality of the isolated islets (Table 4). The functional ability, in terms of their insulin-secretory index, of the islets isolated from the perfused pancreases was equivalent to that of the controls including fresh pancreas. Moreover, the insulin content was significantly higher than the control group comprising pancreases stored statically in cold UW/Viaspan solution, which is currently the standard method employed clinically. It was further demonstrated that these effects and standards of preservation were achieved irrespective of the nature of the perfusate since equivalence was demonstrated using the two proprietary solutions, KPS-1 and Unisol™-UHK [171]. Further improvements and benefits to this technique are anticipated by optimizing the composition of these baseline perfusates by adding cytoprotective agents designed to minimize preservation and reperfusion injury [158].

If this unanticipated finding is validated in further studies designed to evaluate the *in vivo* function of the islets, the implication would be very significant for the field of islet transplantation. Despite many efforts to improve the technique of islet isolation the field remains constrained by the limitations and vagaries of enzymatic digestion of a gland that comprises less than 5% endocrine tissue. Consequently, isolating islets from a single donor pancreas often yields insufficient islet mass to reverse diabetes in a recipient, such that multiple donors often have to be considered for

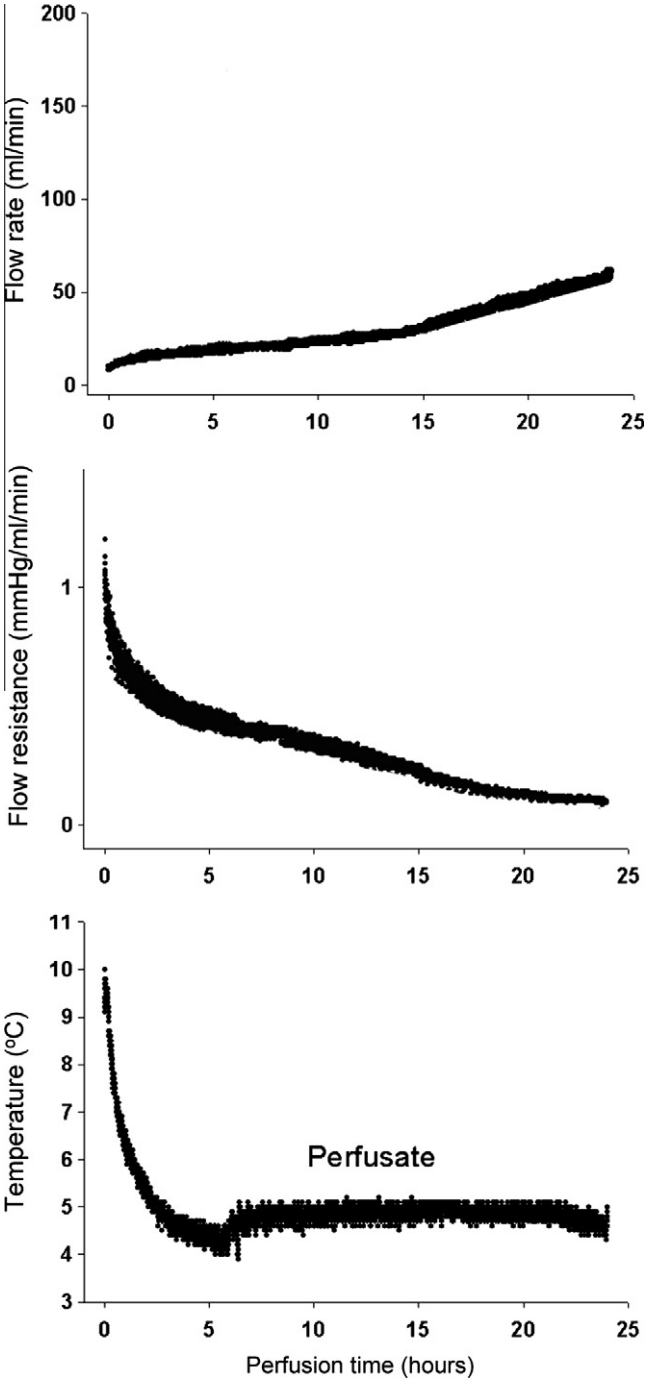

Fig. 5. Physical parameters (flow, resistance and perfusate temperature) measured on the LifePort® perfusion machine during 24 h hypothermic perfusion of a porcine pancreas. Pancreas weight = 87 g; the level of edema after 24 h = 139%, and the islet yield = 247,950 IE (islet equivalents; IE are determined using the universally accepted standard convention for quantifying islets, which inherently have a wide distribution of sizes ranging from <50 to >400 μm diameter [134].

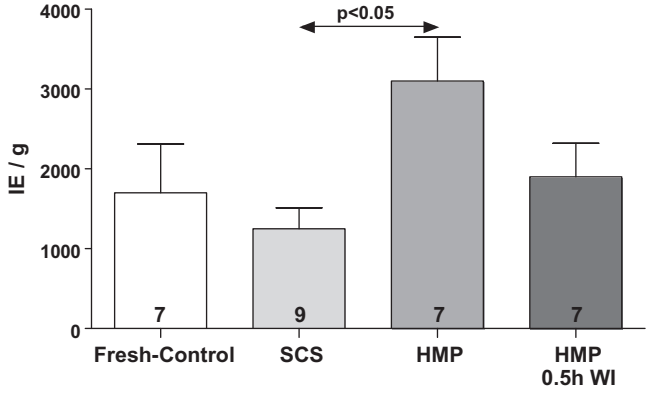

Fig. 6. Effect of HPP on islet yield expressed as islet equivalents (IE) per gram of digested pancreas (mean ± SEM).

treating a single recipient. Our initial results using HMP strongly suggest that the development of a moderate degree of interstitial edema while preserving the integrity of the islets greatly facilitates islet isolation to the extent that approximately twice as many islets may be retrieved compared with non-perfused pancreases. Moreover, the salutary effects of HMP were also manifest after prior warm ischemia [169,170,171].

The role of oxygenation during HMP

Although tissue energy requirements are minimal at deep hypothermic temperatures, there are suggestions that constant supply of oxygen along with adenosine as a precursor for the replenishment of ATP will result in superior ATP levels [42,80,92,101,126,144], and minimum oxidative and metabolic stress in preserved tissues. However, there is no clear consensus among the research community about the need of oxygen supply during hypothermia. There are concerns that low concentrations of molecular oxygen, such as that dissolved in organ preservation solutions, is sufficient to support the generation of free radicals during prolonged storage [49,124]. It is recognized therefore, that hypothermia may set the stage for a progressive development of tissue injury as a result of reactions and processes that occur during hypothermia, but that fuel changes that proceed for considerable time after normal conditions of temperature and oxygen tension are resumed. Others have shown that a moderated oxygen tension is beneficial during hypothermic preservation, which suggest that oxidative stress can lead to adaptation in tissues and increased production of antioxidants. It has been shown that rats gradually exposed to oxygen increased their production of pulmonary antioxidants [57]. Nevertheless, numerous investigations have suggested that oxygen supply is essential during hypothermic preservation of livers [93,110,175]. Recent studies on transplantation survival [96] of rat livers from NHBDs suggest that the saturation of UW solution with atmospheric air is a primary requirement for the preservation and restoration of ATP levels and mitochondrial functions [95]. Previous studies by Stubenitsky et al. have also shown that the oxygenated hypothermic preservation of warm ischemic kidney slices can restore normal tissue ATP levels [148]. In respect of oxygenation of perfused livers it has recently been determined that a partial pressure of oxygen equal to 55 kPa is needed to comply with total liver oxygen demand during HMP [152,47]. Since physiological distribution of oxygen in the liver is supplied via both the hepatic artery (65%) and portal vein (35%), a pO_2 of 35.8 and 19.2 kPa has been set by some investigators as the target supply levels in these respective lines during HMP [176].

Studies that clearly demonstrate the rapid depletion of adenine nucleotides during cold storage of organs at 0–2 °C are suggestive that mitochondrial function is severely impaired by hypothermia [20]. However it has been demonstrated, for example in the liver that the same tissues stored at 8–10 °C can reestablish ATP reserves if an adequate supply of oxygen is maintained by continuous perfusion as discussed above [50,95]. Moreover, it has also been established during hypothermic kidney preservation that the balance between glycolysis and complete oxidation of fatty

Table 4
Islet yield and function indices.

Pancreas/islet characteristics	Fresh (untreated control) ($N = 7$)	SCS (Viaspan) ($N = 9$)	HMP (KPS-1) ($N = 7$)	30WIT ($N = 6$)	30WIT/HMP (KPS-1) ($N = 7$)
Post-preservation edema (%)	–	−2.8 ± 0.7	138 ± 19	–	127 ± 21
Total islet yield (IE × 1000)	117 ± 31	75 ± 16	188 ± 37*	158 ± 27	127 ± 22
Insulin stimulation index	4.6 ± 1.3	2.5 ± 0.4	2.9 ± 0.4	6.2 ± 2.2	4.2 ± 0.4
20 mM-glucose stimulated insulin release (ng/mL/IE)	0.33 ± 0.15	0.20 ± 0.05	0.23 ± 0.08	0.22 ± 0.02	0.36 ± 0.05
Insulin content (ng/mL/IE)	8.51 ± 3.68	4.75 ± 1.00	11.80 ± 3.79*	5.41 ± 0.76	6.56 ± 1.06

* $P < 0.05$ vs. SCS.

acids at 10 °C is controlled by the oxygen tension. Pegg et al. showed that glycolysis provided the principal source of energy at 10 °C when the pO_2 = 150 mm Hg, but that oxidation of caprylic acid provided the main fuel when pO_2 was raised to 650 mm Hg [124]. Furthermore, it had previously been demonstrated by Huang et al. using well oxygenated kidney cortex slices that the preferred substrate for energy metabolism was also markedly influenced by temperature. Under normothermic conditions glucose, amino acids, ketone bodies and fatty acids were all utilized, but only short chain fatty acids and ketone bodies were consumed at 10 °C [62]. Clearly the effect of cooling on metabolism is complex, and should not be regarded as causing a simple uniform retardation of all biochemical reactions [158]. More investigation is required to arrive at a consensus on optimum oxygen tension requirement that can provide superior graft function and prevent oxidative damage.

Multiple organ perfusion *in situ* and the special case of whole-body perfusion

It will be obvious from the forgoing discussion and other recent reviews on this topic [48,56], that the technology of HPP has focused on individual transplantable organs. However, it should be recognized that organ preservation should start at the earliest possible onset of ischemia, i.e. in the donor. Optimizing organ procurement techniques will inevitably help to improve the health of the organ after preservation. To this end, some researchers have recently advocated "*in situ*" machine perfusion of multiple organs to facilitate the wash-out and cooling of all organs at the same time as providing better metabolic support to reduce the mismatch between metabolic demand and supply [168,176]. The basis for this concept is the well established techniques of extracorporeal bypass perfusion used extensively in some clinical settings, notably cardiovascular surgery [53].

Special case of whole-body hypothermic perfusion

Historically, a variety of organ preservation solutions have been developed, and while there are accepted industry standards for certain organs and applications, the concept of a "Universal" preservation solution for all tissues and organs has still to be realized in practice. In general, the solutions adopted for abdominal visceral organs (kidney, liver and pancreas) have not proved optimal for thoracic organs (heart and lungs), and vice versa. In contrast, a new approach to bloodless surgery using hypothermic blood substitution (HBS) to protect the whole body during profound hypothermic circulatory arrest (clinical suspended animation, or "*corporoplegia*"—literally meaning body paralysis [160,163] has focused on the development of a hybrid solution-design with the objective of providing "universal" tissue preservation during whole-body hypothermic vascular flushing [158,160,164,165].

We have investigated this approach based upon the "Unisol" concept [157], in which two new solutions (a *Maintenance* and a *Purge*) formulated for separate roles in the procedure have been tested [3,167]. The principal solution is a hyperkalemic, "intracellular-type" solution designed to "maintain" cellular integrity during hypothermic exposure at the nadir temperature (<10 °C). The companion solution is an "extracellular-type' purge solution designed to interface between blood and the *Maintenance* solution during both cooling and warming. This novel approach to clinical suspended animation has been established in several large animal models [160,164,165], and more recently explored for resuscitation after traumatic hemorrhagic shock in pre-clinical models relevant to both civilian and military applications [3,12,13,133,160].

In considering the efficacy of a solution for universal tissue preservation there is no better test than to expose all the tissues of the body to cold ischemia. Moreover, protection of those tissues most exquisitely sensitive to an ischemia/hypoxia insult, namely the heart and brain, provides the greatest challenge. Current interests in the development of hypothermic arrest techniques to facilitate resuscitation of hemorrhagic shock victims in trauma medicine has parenthetically provided an opportunity to examine the efficacy of new hypothermic blood substitution solutions for universal tissue preservation during multi-organ perfusion [160,164,165].

Successful application of a technique of hypothermic blood substitution to man would provide a >3-fold extension of the current limits of <1 h for "safe" arrest without a high risk of neurological complications. This novel approach to bloodless surgery would significantly broaden the window of opportunity for surgical intervention in a variety of currently inoperable cases, principally in the areas of cardiovascular surgery, neurosurgery and emergency trauma surgery. This provides further evidence for the protective properties of solutions such as the Unisol family of solutions used for global tissue preservation during whole-body perfusion in which the microvasculature of the heart and brain are especially vulnerable to ischemic injury [68,165]. Moreover, the application of solution-design for clinical suspended animation and Emergency Preservation and Resuscitation (EPR) under conditions of ultraprofound hypothermia, places HBS solutions such as Hypothermosol® (Biolife Solutions, Inc. [156] and Unisol® (Organ Recovery Systems, Inc. [157] in a unique category as universal preservation media for all tissues in the body. In contrast, all other organ preservation media, including the most widely used commercial solutions such as UW—Viaspan® (SPS-1, Organ Recovery Systems) are established for specific organs, or groups of organs, e.g. UW for abdominal organs and Celsior, Cardiosol, or Custodiol for thoracic organs [6,39]. Moreover, the demonstrated efficacy of these synthetic, acellular hypothermic blood substitute solutions justifies their consideration for multiple organ harvesting from cadaveric and heart-beating donors [168].

Future directions

Hypothermic machine perfusion preservation technologies have been explored more extensively in recent years because of the contributory role they play in narrowing the gap between supply and demand of organs for transplantation. While current HPP techniques are being successfully exploited clinically, defined areas

for improvement are recognized for future developments. Focus upon organ specific metabolic demands (oxygenation and perfusate components) and circulatory requirements (optimized flow regime, infusion flow rate and pressure) at low temperatures is necessary. These will help extend the current short preservation time limits for liver, heart and pancreas, and thus, provide the time for proper donor-recipient matching and the distribution of organ to the best matched recipient. Moreover improving evaluation methods for the condition of an organ and quality control during hypothermic perfusion by developing and establishing biomarkers and other indices of organ function and viability (currently only flow rate and vascular resistance are recognized as renal viability indicators) will help expand the donor pool (warm ischemia damaged organs), eliminate poor quality organs from transplantation, and allow for the selection of organs with low rate of post-transplant complications.

Conflict of interest

The authors have no conflict of interest with the sponsors of this publication.

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