

Abstract

The concept of organ preservation by perfusion dates back to the mid-19th century. Innovations since then have included temperature regulation, perfusion fluid composition and various pumping systems. Advances made in liver, heart and kidney machine preservation are now contributing to increased graft utilisation, assessment of graft viability and potentially improved graft survival. Pancreas transplantation has not benefitted to the same extent from the application of perfusion technology, although the need is just as great.

This overview, reviews current pancreas specific preservation techniques. We explore concepts, which include static cold storage, use of preservation solutions, the 'two-layer method', and machine perfusion. We also discuss ideas for future development.

Narrative review of literature from inception to December 2017 using OVID interfaces searching EMBASE, Google Scholar, and MEDLINE databases. All studies relevant to pancreas perfusion and preservation were examined for clinical relevance with no exclusion criteria. Conference papers and presentations were also reviewed and included where appropriate.

The application of recent advances in understanding in ischaemia-reperfusion as well as technical developments in machine preservation Ischaemia-reperfusion have the potential to improve organ utilisation, viability and outcome.

INTRODUCTION AND BACKGROUND

Over the last 10 years, rates of pancreas transplants have declined, the reasons for which are multiple. Insufficient number of referrals, improved non-transplant management of complex diabetes, increasing donor risk (especially obesity and age) and whether outcome concerns justify morbidity are all contributing factors. Being aware of the fine balance of risk: benefit which, combined with short waiting lists, has led to a conservative approach to donor criteria; acceptance and transplant rates. This low rate of organ utilisation has clear health-economic consequences.¹

Although the donor factors that impact upon the outcome of pancreas transplantation have been well researched, current quantification of donor risk remains insensitive.² Pancreas transplant is known to increase life expectancy and improve quality of life, but ensuring not transplanting high-risk organs clearly defines the need for better methods to assess donor pancreata and anticipate the transplant outcomes.

Techniques to preserve and perfuse organs in hypothermic and normothermic settings have been developed in kidney, liver, heart and lung models, with increasing evidence suggesting improved patient outcomes and better graft survival. Yet the prospect of perfusing the whole pancreas remains challenging, with clinical preservation techniques having changed little in the last 30 years.³ Many justify the lack of development as the procedure is regarded niche, but this is due to the lack of referrals of patients, as 1 year survival and insulin independence at 3 years are both in excess of 90%. Having more diabetics referred for transplant would substantially make better use of donor organs offered and create a significant impact into reducing the burden of disease associated with diabetes.⁴

Organ preservation is a key factor in the two major early complications of solid organ pancreas transplantation, vascular complications and reperfusion pancreatitis. The wider application of pancreas transplantation is undoubtedly limited by the resulting morbidity, and it follows that the future growth of pancreas transplantation is dependent on developing strategies that reduce the effects of ischemia-reperfusion.

SEARCH STRATEGY

An electronic search was performed and all relevant articles were identified using electronic databases Medline via PubMed (1950– Dec 2017) and Embase via Ovid SP (1950– Dec 2017). Searches were adapted to the databases and the search terms used included “pancreas perfusion”, “pancreas preservation”, “machine perfusion”, “extracorporeal perfusion”, “ex-vivo perfusion”, “normothermic” and “hypothermic”. The search was limited to English language and availability of full-text.

STATIC COLD STORAGE

Static cold storage (SCS) is the standard method all organs are currently retrieved and transported globally. The hypothermic conditions between 2-4°C are thought to reduce ischaemic injury through a reduction in cellular metabolism thereby reducing adenosine triphosphate (ATP) use and oxygen consumption. As metabolic activity has been shown to reduce up to two-fold for every 10°C drop in temperature.⁵

Early experiments comparing cold storage and machine perfusion of canine models in 1982 demonstrated graft failure rates using machine perfusion were 30% at 24hrs, and 40% at 48hrs. But there were no failures observed at 24 and 48 hours with cold storage.⁶ These results, along with the complexities associated with machine perfusion of the pancreas have made SCS the preferred and most widely used method for pancreas preservation for the last 30 years.⁷

University of Wisconsin solution (UWS) was developed by Belzer and Southard in the late 1980s, guided by the principle of using metabolically inert substances to preserve organs. The two principle ingredients are Raffinose and Lactobionate. Raffinose is a hydroxyethyl starch (HES) used to prevent oedema and it also contains free radical scavengers. The use of UWS in whole pancreas organ preservation has been shown to improve outcomes after SCS and so is the most commonly used preservation solution.⁸

An alternative to UWS is Histidine Tryptophan Ketoglutarate solution (HTK). HTK is low potassium; low viscosity solution formulated using the principle of inactivating organ function by withdrawal of extracellular sodium and calcium. This together with intensive buffering of the extracellular space by Histidine Hydrochloride underlies its mechanism of action. The electrolyte composition of HTK is similar to that of extracellular fluid and it has a similar osmolarity to plasma. In 2006, Englesbe et al reported the results of a multi-centre study comparing UWS to HTK in 77 consecutive pancreas transplants. SCS was performed either using UWS (n=41) or HTK (n=36). Outcome measures were pancreas function at 90 days, and rate of both technical graft loss and pancreatic leaks, which were equivocal between groups, with no significant differences in postoperative amylase and lipase levels.⁹

Similarly, in 2007, Becker et al reported no significant differences in patient survival or graft survival following SCS with UWS (n = 47) or HTK (n = 48). They also reported that grafts flushed with HTK appeared more oedematous; but this did not appear to impair early graft function.¹⁰ Some evidence suggests that HTK is associated with early graft rejection, graft pancreatitis and graft loss, causing UWS to be used in preference.¹¹

Celsior is an extracellular low viscosity preservation solution; where Baldan et al demonstrated that Celsior was an effective alternative to UWS for pancreas procurement in a porcine auto transplant model.¹² Uhlmann et al also in a porcine model contradicted these findings by reporting that the use of Celsior was associated with increased ischemia-reperfusion injury when compared with UWS.¹³ while Garcia-Gilet al. demonstrated that lipid peroxidation after reperfusion of pancreata preserved in Celsior and UWS was similar.¹⁴ The first prospective randomised study of SCS comparing UWS (n = 50) with Celsior (n = 50) in human models was by Boggi et al. The authors demonstrated that Celsior and UWS had similar safety profiles for pancreas preservation.¹⁵ A similar reported by Manrique et al. comparing Celsior (n = 28) with UWS (n = 44) demonstrated that 2-year recipient survival rates, 2-year graft survival rates, leak rates, and clinical graft pancreatitis rates were also similar.¹⁶

Institut Georges Lopez-1 solution (IGL-1) is another low viscosity solution, which has been shown to be equivalent to UWS and HTK in terms of feasibility and safety despite significant differences in its composition. The concentrations of Na⁺ and K⁺ in IGL-1 are inverted in comparison to UWS, which is thought to reduce oedema, and the Hydroxyethyl Starch (HES) component of UWS is replaced with Polyethylene Glycol (PEG) in IGL-1; which reduces its viscosity and stimulates nitric oxide, thought to help combat ischaemia. In a porcine pilot study, Gacia-Gil et al. found IGL-1 (n=8) to be as effective as UWS (n=8) for SCS of the pancreas.¹⁷ Which was followed by Chedid et al; reporting a series of 5 simultaneous kidney and pancreas transplants using IGL-1 during organ procurement and static cold storage. The median cold ischaemic time was 13hrs. All 5 pancreata had primary function providing insulin independence, with no graft thrombosis.¹⁸

TWO LAYER METHOD

Perfluorocarbons (PFCs); originally developed in 1962, for potential 'liquid ventilation' were used by Kylstra et al to evaluate the ability of mice to sustain gas exchange when spontaneously breathing oxygenated saline.¹⁹ Clark et al in 1966 then demonstrated that spontaneously breathing mice could survive when submerged in PFCs under normobaric conditions.²⁰

PFCs are hydrocarbons, in which the majority of hydrogen has been replaced by fluorine. The attractive and exploited characteristic of this chemical is its ability to bind reversibly large amounts of oxygen. Oxygen concentration of PFC is estimated to be at least 20 times higher than in human blood under the same conditions, whilst the molecule itself is relatively inert and nontoxic.²⁰

In 1988 the Japanese group Kuroda et al. developed a "Two-layer Method" of cold storage involving a combination of PFC's and a convention SCS perfusion fluid. This was based on the intention to combine the characteristics of SCS solution in countering the effects of cooling, with those of PFC in providing oxygen.²¹ The PFC forms a bottom layer with (lower density) UWS or Euro-Collins solution (ECS) floating on the top.

The two-layer method (TLM) supplies oxygen to the cold stored pancreas by diffusion, with the intention to allow the graft to produce adenosine triphosphate (ATP) and maintain cellular integrity. Early small animal studies suggested that this method allowed the pancreas to remain viable up to 96 hours.²² However, the efficiency and ability of oxygen penetrating deep tissue in large animal studies is unproven, with beneficial effects of PFC's being limited to the surface layer of cells in solid organs, and no convincing evidence showing superiority over traditional SCS.²³

Matsumoto et al cold stored 10 pancreas grafts using TLM and compared these with 44 cold stored in UWS alone. The mean cold ischaemic times were 16.5hrs and 18.1hrs respectively. Grafts were compared at the time of reperfusion and 3 months post-transplant. At the time of reperfusion, 0 grafts (0%) in the TLM group were oedematous compared with 10 (23.3%) in the UWS group. 7 (70%) grafts in the TLM group obtained the best overall quality score, compared with 24 (57.1%) in the UWS group. 9 (90%) recipients in the TLM group became insulin-independent during hospitalisation, compared with 31 (70.5%) in the UWS group. The time to insulin independence was no different between the two groups.²⁴

OXYGEN PERSUFFLATION

Oxygen persufflation (PSF) is the simple addition of oxygen to an organ during SCS, where oxygen is bubbled directly through vasculature into the organ. The addition of filtered and humidified oxygen, which is directly bubbled through graft vasculature, has shown to increase ATP levels and reduce metabolic stress (*see figure 1*).²⁵

PSF is not a new concept. Rudolf Magnus made the incidental finding of PSF in 1902 while examining feline heart perfusion. Compressed oxygen had accidentally been introduced into the heart perfusion circuit instead of blood, yet heart contractility continued.²⁶ In 1954, Bunzl et al compared the benefits of PSF versus liquid perfusion in a frog spinal model. This model showed that peripheral nerve reflexes and muscle contractions could be preserved for up to 6–8 hours with PSF. In addition, reduced levels of oedema and improved tissue oxygenation were noted.²⁷

A number of experimental studies suggest beneficial effects of PSF. Reddy et al showed improved preservation of a non-heart beating donor pancreas in a rat model by increasing levels of ATP using PSF.²⁸ An experimental study by Scott et al showed equivalent results where either human pancreatic or paired porcine pancreatic lobes were preserved for up to 24 hours with either PSF or TLM. PSF was performed by pumping 20 cc/min 40%-oxygen humidified gas to the superior mesenteric artery and celiac trunk averaging pressures of 10-20 mmHg. Homogeneity of PSF was assessed by magnetic resonance imaging (MRI) visualising negative contrast associated with gas in the vasculature. The ATP to inorganic phosphate ratio (ATP: P_i) was non-invasively measured and porcine islets were isolated following 6 and 24-hour preservation. The results demonstrated that pancreatic tissue was homogeneously persufflated, with elevated ATP levels and islet yields similar to that of fresh pancreas tissue.²⁹

PERFUSION TECHNIQUES

The pancreas is a low flow organ with complex vascular anatomy, and unlike other organs, ideal perfusion parameters of flow and pressure are hard to establish. Whilst high perfusion pressures can cause endothelial injury and an increased rate of thrombosis, low pressures can lead to under-perfusion and inadequate oxygenation. The first recorded attempt at perfusing an isolated organ was by Loebel in 1849, followed in 1895 by Langendorf, who created an organ-perfusion technique using apparatus consisting of a medium reservoir and a siphon tube connected to the organ. The system was non-pulsatile, and was infused by gravity without recirculation.³⁰

HYPOTHERMIC MACHINE PERFUSION (HMP)

Experience in HMP of the pancreas has been largely based upon methodology developed in the kidney HMP recirculates cold preservation solution with various methods to regulate pressure and/or flow. The purported benefits of HMP include clearance of the products of anaerobic metabolism, reduction of vascular resistance (thought to be a marker of viability) and the provision of oxygen, with commercial HMP devices for liver and kidney now available.

Perfusate used in HMP of the pancreas vary greatly with UWS used frequently or Kidney Perfusion Solution (KPS-1), which is the formulation of UWS designed, adapted for MP. The scientific basis of the perfusion solutions used in HMP is the minimisation of ischaemic injury and interstitial oedema.³¹

Several porcine and canine studies have shown the pancreas can be preserved for up to 24 hours using HMP, although oedema was noted.³² Karcz et al perfused 15 porcine pancreases at a temperature of 4-10 °C for 315 minutes, at a perfusion pressure of 15-23mmHg. Although pancreas weight gain ranged from 3.2% to 18.3%, a significant reduction in islet and acinar cell damage was observed post-perfusion.³³ It is thought that the higher degree of interstitial oedema with HMP compared to SCS may be due to disruption of the extracellular space even at low pressures. This suggests that HMP may be valuable for islet cell isolation, in which the intercellular disruption and oedema might aid enzymation digestion.³⁴

Two recent human studies have shown successful pancreas outcomes using HMP. Leemkuil et al, compared 8 human pancreases (4 DCD and 4 DBD) preserved by HMP and 8 (4 DCD and 4 DBD) preserved by SCS. HMP was performed for 6 hours with oxygenated Belzer UWS, using dual perfusion of the superior mesenteric artery and the splenic artery. Tissue biopsies and samples of the preservation fluid were collected at baseline and after 6 hours of preservation. At baseline, the ATP content in the DCD groups was, as expected, significantly lower than in both DBD groups. After preservation, the ATP levels fell in both groups preserved by SCS, but increased in both groups after HMP. Indeed, the post preservation ATP content in the DCD group treated with HMP, was similar to the pre-preservation ATP level in DBD organs. During HMP, amylase, lipase and LDH levels in the preservation fluid increased reaching a plateau after 5 hours of HMP, suggesting that there was no further accumulation of cellular injury.³⁵

Cantarovich et al has recently presented a series of 7 human DBD pancreases, which were perfused using preservation solution IGL-1 and pumped using the HMP system developed by Waters Medical Systems, the WAVE perfusion machine, which operates at a pressure of 25mmHg. Tissue biopsies at 6, 12, and 24hr of perfusion showed no evidence of oedema.³⁶

NORMOTHERMIC PERFUSION

The first normothermic pancreas perfusion was in 1926 by Babkin and Starling who perfused isolated canine pancreases. Grafts with shorter preservation periods demonstrated higher production of insulin compared with those preserved for greater than 24 hours. These results led to the hypothesis that insulin production reflected the amount of organ injury and may correlate with organ preservation.³⁷ Normothermic perfusion models are designed to reproduce physiological parameters of pressure and flow, while hypothermic models tend to operate at sub-physiological pressures (see *figure 2*). Meyer et al and Eckhauser et al perfused two canine pancreases using pressures of 75mmHg and 90-110mmHg respectively. Both showed good preservation of function, but still sustained tissue oedema.^{38, 39}

Barlow et al, has perfused five discarded human pancreases. These organs were procured for the purpose of transplantation and then declined as unsuitable (for reasons not documented). Donor ages ranged from 14 to 51 years and included both DCD and DBD organs. The median cold ischaemic time (before starting NMP) was 13 hours 19 minutes. Perfusion pressures were maintained at 50-55mmHg keeping a stable mean arterial flow of 35 ± 2.8 ml/ min/ 100g. Perfusate insulin and amylase levels and other markers were measured. Exocrine secretions were drained via a wide bore cannula in the distal duodenum, allowing measurement of lipase and amylase. Tissue biopsies were also taken to assess viability, tissue damage, and signs of ischaemia reperfusion injury. The study showed wide variation between organs, but tissue oedema in all organs. None of these organs was either transplanted or underwent islet isolation protocol, so there is indication of functional beta cell mass at the end of preservation. However, despite the small size of the series, it provides valuable insight into the possibility of assessing organ viability during machine perfusion.⁴⁰ This study also highlights one of the key technical issues: whether a normothermic perfusion device needs to be transportable

to the donor hospital. It's possible that the ill-effects of cold ischemia, even for the few hours is enough to trigger a cascade of inflammatory injury in this, the most sensitive of transplanted organs.

ISLET PRESERVATION

There is clear overlap between what is needed for solid organ transplantation and islet isolation. This topic has been covered elsewhere and for more detail we would refer the reader to this review.⁴¹

UWS has been used as a preservation solution for pancreas grafts used for islets isolation and transplant since the 1980's. As other preservation solutions became available there have been conflicting reports of their relative efficacy. Salehi et al reported that islet yields from human pancreases preserved in HTK or UWS are equivalent.⁴² Hubert et al demonstrated that the islet isolation yields from pancreases preserved with Celsior were 2.1-fold lower than those obtained with UWS, and suggested that colloid free preservation solutions might be inferior for pancreas perfusion and cold storage before islet isolation.⁴³ Solution de Conservation Des Organes et de Tissus" (SCOT solution) is a low potassium (5 mmol/L) preservation solution containing 30g/L of Polyethylene Glycol (PEG) 20kDa. Giraud et al demonstrated advantages of using SCOT solution, suggesting increase islet yield and reduced graft immunogenicity in pancreatic islet transplantation.⁴⁴

A number of early studies have reported that the Two-Layer Method was superior to simple SCS for islet preservation,⁴⁵ however two recent large-scale studies showed no benefit.⁴⁶ Despite this evidence the TLM technique has gained popularity in some islet isolation units. Diffusion is thought to be useful at oxygenating a thin layer of cells; hence its application in this setting is thought to be favourable. Goto et al demonstrated that PFC use can improve islet yield which suggests hypoxia during cell isolation can damage islets.⁴⁷ Brandhorst et al identified a simpler method, the one-layer method (OLM), solely using oxygenated perfluorocarbon as a potential alternative to the TLM. In porcine models, 3 hours of additional oxygenation using the OLM has been shown to significantly improve the ATP content of islets damaged by warm ischaemia, however no significant improvement was seen in post-transplant function.⁴⁸

Oxygen persufflation has also been used experimentally in islet preservation with an abstract from Papas et al, have shown human pancreas persufflation ameliorates hypoxia induced impairment of islet function.⁴⁹

FUTURE WORK

In perfusion models, identifying how best to measure the effect of perfusion, and which markers are the most useful in assessing tissue viability is of interest. Current models have relied on insulin, c-peptide, amylase and lipase but these may not be the most useful way to assess the organ. The potential to assess organ viability prior to transplantation, to manipulate the organ (e.g. using stem cells or gene therapy) are all opportunities waiting to be exploited. Many fundamental, issues remain to be resolved: optimal temperature, oxygen delivery, perfusate composition, functional markers, pressures, flows and others. A portable circuit that minimises cold ischemia and provides a solution to deal with the exocrine component of the pancreas would appear to be a blueprint that investigators might aspire to.

CONCLUSION

A radical change that allows optimisation of preservation and assessment of the donor pancreas is needed to reverse the slow decline in pancreas transplantation. This would enable the successful transplantation of more organs and without compromise to the outcome. Machine perfusion technology, as pioneered in the kidney lung and liver, might point the way to achieve this.

Figure Legends:

1. **Oxygen Persufflation:** Whole organ pancreas is kept in UWS cooled by ice bath and is oxygenated through direct cannulation. Either arteries or portal vein can be cannulated to offer anterograde or retrograde persufflation. Combining this method with the two-layer method would involve change of the UW medium.

2. **Normothermic Perfusion:** Shows principles of closed circuit perfusion, using a either pulsatile or continuous perfusion, measuring the pressure and flow as well as oxygenating and warming the blood. Open circuits would not recirculate, while hypothermic circuits use preservation fluid and ice, avoiding the need for additional devices in the circuit.

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