Extracorporeal Normothermic Pancreas Perfusion

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Discipline/School: Discipline of Surgery, School of Medicine
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Abstract

Pancreas and islet transplantation are important treatment options for insulin dependent diabetes. However, one of the main challenges in pancreas and islet transplantation lies in organ (pancreas) preservation. Ischaemic injury post-retrieval causes significant damage the organ function and reduction in islet yield. It was hypothesised that extracorporeal normothermic perfusion of the pancreas would improve graft function post transplantation and pancreatic islet isolation when compared to traditional methods of organ preservation.

The aims and objectives of the project included:

- Conducting a systematic review of the literature in extracorporeal machine perfusion of the pancreas
- Establishing an extracorporeal normothermic perfusion model of the porcine pancreas
- Comparing the addition of kidney to the circuit of extracorporeal pancreas perfusion model

A thorough systematic review of the available literature concluded the potential benefits of machine perfusion in pancreas preservation. Important insight into the experimental setup, perfusion parameters and vital outcome measures was also attained.

A model of normothermic hemo-perfusion of the porcine pancreas with and without addition of the kidney as a dialysis organ was subsequently established. The organs were perfused for 120 minutes with stable perfusion parameters but sub-optimal acid-base environment. Addition of the kidney did not result in significant improvement of the acid-base environment.
I would like to acknowledge the immense support and guidance provided by my supervisors Professor Guy Maddern and Mr Markus Trochsler during my candidature. In addition, I would also like to thank Dr Santosh Olakkengil (Transplant Surgeon, Royal Adelaide Hospital) for his help in the organ retrieval operations, Mr Peter Frantzis (Head of Perfusion, Royal Adelaide Hospital) for his technical input and Dr John Finnie (Senior Veterinary Pathologist, SA Pathology) for histopathological analysis of specimens.
Pancreas or islet cell transplant is the only curative treatment for insulin dependent diabetes mellitus. Traditionally associated with major morbidity and mortality, recent improvements in immunomodulation therapy, graft preservation and surgical techniques have made transplant increasingly popular. However, organ preservation remain a major hurdle to expanding the donor pool and improve graft function.

Machine perfusion as a preservation method has been attempted decades ago with little success and rapidly fell out of favour due to static cold storage, which is simple and costs less. However, with increasing organ demand and improving technology in pump and monitoring equipment, machine perfusion is gaining attention once again.

Machine perfusion is well established in other organs (heart, lung and kidney) but remain relatively novel in the pancreas. In order to establish a model of extracorporeal normothermic perfusion of the pancreas, a thorough literature review was crucial to obtain insight into previous attempts, perfusion parameters, outcome measures and encountered complications.

The systematic review titled “Extracorporeal Machine Perfusion of the Pancreas: Technical Aspects and Its Clinical Implications – A Systematic Review of Experimental Models” forms the first part of this thesis. It was published in Transplantation Reviews on 19th June 2015.

Subsequently, work began on establishing the model of extracorporeal normothermic perfusion of the pancreas and investigating the role of addition of the kidney as a dialysis unit. The process of establishing the model is detailed in the manuscript “A Study of Normothermic Hemo-Perfusion of the Porcine Pancreas and Kidney” which forms the other part of the thesis. The study was accepted for publication in Artificial Organs on 21st March 2016. Attached in the thesis is the acceptance letter and author proof of the accepted article, expected to appear in the July 2017 edition of the journal.

Based on the examiners’ and supervisor’s reports received on 6 June 2016, grammatical and syntax errors were corrected. An additional segment was also added to the thesis, titled “Operative description and illustrations” to further expand the operative details of organ retrieval as suggested.
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Yours sincerely,

Dr Kean Guan Kuan

Student ID:  1660895
Program:  Masters of Philosophy (Surgery)
**Statement of Authorship**

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<th>Extracorporeal machine perfusion of the pancreas: technical aspects and its clinical implications - a systematic review of experimental models</th>
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**Principal Author**

| Name of Principal Author (Candidate) | Koen Guan Kuan |
| Contribution to the Paper | Conceptualising, literature search, data analysis, writing and submission of manuscript |
| Overall percentage (%) | 60 |

Certification: This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

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**Co-Author Contributions**

By signing the Statement of Authorship, each author certifies that:

I. the candidate's stated contribution to the publication is accurate (as detailed above);
II. permission is granted for the candidate to include the publication in the thesis; and
III. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| Name of Co-Author | Mau Nam Woea |
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Extracorporeal machine perfusion of the pancreas: technical aspects and its clinical implications – a systematic review of experimental models

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Abstract

Pancreas or pancreatic islet transplantation is an important treatment option for insulin-dependent diabetes and its complications. However, as the pancreas is particularly susceptible to ischaemic-reperfusion injury, the criteria for pancreas and islet donation are especially strict. With a chronic shortage of donors, one critical challenge is to maximise organ availability and expand the donor pool. To achieve that, continuous improvement in organ preservation is required, with the aims of reducing ischaemia-reperfusion injury, prolong preservation time and improve graft function. Static cold storage, the only method used in clinical pancreas and islet cell transplant currently, has likely reached its plateau. Machine perfusion, hypothermic or normothermic, could hold the key to improving donor pancreas quality as well as quantity available for transplant. This article reviews the literature on experimental models of pancreas machine perfusion, examines the benefits of machine perfusion, the technical aspects and their clinical implications.

1. Background

Whole pancreas and pancreatic islet transplantation are considered as important treatment options for insulin dependent diabetes [1]. Traditionally indicated for type 1 diabetes, pancreas transplantation for type 2 diabetes patients have increased significantly over recent years [2]. While exogenous insulin treatment including insulin pumps has improved over the years, it is still riddled with problems of wide glucose level fluctuation and risk of severe hypoglycaemia, resulting in annual mortality rates of up to 3-6% [3].

Traditionally, solid organ pancreas transplant is performed as a simultaneous-pancreas-kidney (SPK) transplant or occasionally, pancreas-after-kidney (PAK) transplant for patients with renal failure as a complication of diabetes. Long-term data for solid organ transplants is encouraging. Overall patient survival rate is at 95% at 1 year and 85% at 5 years. Graft survival rates are 85% and 60% at 1 and 5 years respectively [3]. Islet cells transplant has been a less durable but low risk alternative. Recently, with the introduction of the Edmonton protocol, the outcome of islet cell transplant has improved significantly and is comparable with pancreas-transplantation-alone (PTA) in its 1 and 5 year graft survival data [4,5].

As short and long term results of both solid organ and islet cell transplant continue to improve, the demand for organs will consequently escalate. One critical challenge in maximising availability of organs for transplant is to expand the donor pool to include extended criteria donors (ECD) and reducing organ discard due to dubious quality. Thus, one of the key issues to address is organ preservation – more advanced methods to prevent any further damage during the preservation period and possibly improve its function.

1.1. Static cold storage

The mainstay of pancreas preservation for both solid organ and islet cells transplant is static cold storage [6]. It is the only pancreas preservation method used clinically. Hypothermic conditions (2-4 °C) prevent ischaemic injury by reducing cell metabolism, oxygen consumption and ATP use [7]. Preservation solutions are designed to counteract the detrimental effects of cold preservation, however their effects are somewhat limited. University of Wisconsin (UW) solution is the most widely used cold preservation solution worldwide, followed by histidine-tryptophan-ketoglutarate (HTK) solution and Celsior solution [1,6]. Very little difference in the preservation solutions have been reported, and UW remains the gold standard for static cold storage [1,7]. Several other methods and variations of cold preservation has been trialled, including two-layer method and persuf...
Static cold preservation is cost-effective and simple to carry out. However, it carries several limitations. The basis of using cold temperature is based on the equation that metabolic activity is reduced by 1.5–2 fold for every 10 °C reduction in temperature. However, metabolic activity does not completely cease. In fact, anaerobic metabolism carries on at about 10% and continues to accumulate intracellular toxins and products of metabolism [9]. ATP continues to be depleted and adenosine diphosphate and lactic acid accrue, contributing to ischaemia-reperfusion injury [8]. As the preservation is static, metabolic toxins accumulated during warm-ischaemia period, organ retrieval and organ preservation are not cleared from the tissue. Therefore, the preservation time is limited [9,10]. Direct cold damage can also occur. As the temperature plunges below 4 °C towards 0 °C, cells can freeze and lyse upon reperfusion [11]. Furthermore, there is no method to assess the organ’s viability prior to transplant, which makes it less ideal for usage in marginal or extended criteria donors (ECD).

1.2. Machine perfusion

Machine perfusion (MP) was first attempted more than 150 years ago [12] and gained popularity in the 1960s. It was soon replaced by static cold storage due to the latter’s cost-effectiveness, technical ease and portability [11,13]. While probably sufficient for solid organ transplant of carefully selected grafts, static cold storage has likely to have reached its plateau in pancreas preservation, and further drastic improvements to accommodate expanded criteria donors (ECD) or marginal grafts are unlikely. The potential benefits of MP are particularly attractive when considering organs of suboptimal quality, and had led to a decreased rate of ECD organ discard [14]. Therefore, there is a recent increase in the interest of machine perfusion as a method of organ preservation [15]. Machine perfusion have been studied in hypothermic, subnormothermic and normothermic temperatures in many organs; heart, lungs, liver, kidney and pancreas [16–22]. The promising results have led to increasing use of MP in clinical kidney transplants. It is reported that 20% of all renal transplant in the US now utilise hypothermic MP [23]. Three systematic reviews published recently on hypothermic MP versus SCS in kidney transplantation agreed that the former is associated with reduced incidence of DGF in DCD kidneys [24–26]. Hypothermic MP of the liver has also recently been trialled in a human study, demonstrating early promising results of reduced delayed graft function, no vascular complications and shorter lengths of stay [11]. An experimental study on hypothermic MP of the pancreas suggests a potential to reverse effects of warm ischaemia, achieving improvement in inflammation and cell damage [27].

1.3. Potential benefits of MP

1.3.1. Circulation of oxygen and nutrients

The fundamental difference between MP and SCS is the circulation of the perfusate of choice to tissues of the entire organ. As pointed out earlier, metabolism continues at a rate of 10% even at clinical hypothermic temperatures of 2–4 °C [7]. Therefore, a continuous delivery of oxygen and nutrients is necessary to support metabolism of the cells to prevent further ischaemia.

1.3.2. Elimination of metabolic waste and toxins

Circulation of the perfusate also allows metabolic wastes, toxins and free radicals to be ‘washed-out’. Prevention of accumulation of waste toxins is essential in prevention of ischaemia-reperfusion injury when the organ is revascularised [11]. This is especially relevant in ECD or marginal grafts, where DCD organs with longer WI time have already caused accumulation of metabolic by-products and pro-inflammatory mediators. Elimination of these toxins with MP can potentially improve organ function during preservation and contribute to improve graft function and reduced complications [23,28–30].

1.3.3. Maintenance of vasculature and endothelial protection

MP usually consists of a pulsatile pump or centrifugal, non-pulsatile pump. While the type of flow (pulsatile vs non-pulsatile), flow rate and pressure are still topics for debate [31–34], it is accepted that maintenance of the vascular bed via flow generated through the microvasculature during the preservation period is beneficial to endothelial gene expression and function [12,35,36]. However, this needs to be delicately balanced against injury to the endothelium by high pressure and flow rate.

1.3.4. Viability assessment

SCS of organs does not offer any means of viability assessment until the organ is transplanted. In contrast, MP holds this significant benefit, which is especially vital when utilising sub-optimal grafts. Parameters to measure viability vary for each organ. Various methods have been suggested that reliably predict DGF and PGF [37–39], but they still need to be validated in large scale human trials. Vascular resistance during perfusion has been found to be a key viability marker [40,41]. The sensitivity of viability testing can be further improved with development of a multifactorial index based on multiple risk factors, such as age, warm and cold ischemia time, perfusion parameters and enzymatic markers [42].

1.3.5. Therapeutic manipulation and gene therapy

Another benefit of MP is the ability to administer therapeutic drugs or even gene therapy to improve the preserved organ. Various additives have been experimented with variable success in animal and clinical trials in attempts to reduce or repair ischemia-reperfusion injury (IRI). These include p52 inhibitor pifithrin-alpha (anti-apoptosis agent), calcium channel blockers, lazaroids (iron-mediated lipid peroxidation inhibitor), superoxide dismutase (free radical enzyme scavenger), allopurinol and HO-1 (heat shock protein). An attractive recent advancement is gene therapy with great potential to reduce IRI and modify the host-immune response. Gene transfer generally requires a longer period of organ perfusion to allow effective transfection and is more effective at normal physiologic metabolism, thus particularly attractive for normothermic MP [8,9,43].

1.3.6. Economic benefits

SCS first gained popularity over MP when it was introduced due to its simplicity as well as low-cost. However, recent resurgence of interest in MP has led to a re-examination of costs associated with MP. A meta-analysis published by Wight et al. suggested that MP would be cheaper and more effective than SCS in the long-term for both DCD and DBD organs. This is primarily due to a significant reduction in DGF and its associated healthcare costs [28]. This finding was supported by an analysis of the United Network for Organ Sharing in the US [29].

1.3.7. Long distance sharing and elective operation

Use of MP may also confer the benefit of long-distance organ sharing. Finger and associates reported a significantly higher risk of mortality and complications from using SPK grafts imported from interstate in the US, due to the longer preservation period [44]. However, with the ability of MP to prolong preservation time, long-distance sharing of organs is a possibility. This potentially allows scheduling of the operation as elective rather than emergency, which has obvious benefits in overall survival, morbidity and costs [45]. It is also widely recognised that transplant recipients are best served with immune-matched donors. The longer preservation duration may enable better tissue matching to improve outcomes [46].

This literature review is carried out to examine available articles on machine perfusion of the pancreas, specifically analysing the technical aspects of perfusion - comparing effects of warm ischaemia, perfusion duration, pulsatility of flow, pressure and flow rate, choice of perfusate and oxygenation - and their clinical implications.
2. Methods

2.1. Search methods

An electronic search was performed and all relevant articles were identified using electronic databases Medline via PubMed (1950–2014) and Embase via OvidSP (1980–2014). 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.

2.2. Types of studies

Randomised, non-randomised, prospective and retrospective studies were included in screening. Exclusion criteria were studies that did not specify the technical aspects of machine perfusion or those not reporting results of machine perfusion. Review articles were screened for their references for further relevant studies. References in the identified articles were used to identify more relevant studies.

2.3. Selection of studies and data collection

One author (KK) screened all the titles and abstracts of articles included in the initial search for relevance and suitability. Obviously irrelevant articles were excluded at this stage and full texts of remaining articles were obtained and assessed for eligibility for inclusion by two independent reviewers (MW, WC). Disagreements were resolved via discussion. Data from included studies was extracted by the primary author (KK) (Fig. 1).

3. Results

3.1. Technical aspects of pancreas MP

A total of 8 articles on normothermic MP and 13 articles on hypothermic MP were included in this review. All the studies of normothermic MP (Table 1) were of experimental nature to establish normothermic perfusion models (dogs: n = 6, rats: n = 1). In the hypothermic MP (Table 2) group, 6 studies included transplantation after perfusion/preservation (dogs: n = 6) and 3 studies compared islet isolation and yield after perfusion/preservation (humans: n = 1, pigs: n = 2). Detailed data extracted from the reviewed studies are included in Tables 1 and 2.

3.2. Donation after Brain Death (DBD) vs Donation after Cardiac Death (DCD)

All studies were conducted with heart-beating donors (comparable with DBD) except 4 studies conducted with non-heart-beating donors (simulating DCD) [27,47–49]. WI time was generally reported to be less than 3 minutes or 'minimal' in DBD-simulating models. In DCD models, WI time were reported to be 25 minutes and 26 minutes in the two studies that specified.

Taylor et al. compared the effects of 30 minutes WI time with minimal (<3 minutes) WI time prior to 24 hours of hypothermic MP in porcine pancreas. They reported a significant difference in islet yield (1396 ± 243 vs 2242 ± 449 IEQ/g) and insulin secretion (3.28 ± 0.53 vs 5.9 ± 1.89 ng/IE), in favour of shorter WI time [50]. Westbroek and associates also compared the effects of WI time (30 min vs none) in a model of allogenic transplant after 24 hours of hypothermic perfusion in a dog model. Post-transplant survival was found to be significantly shorter in the group with WI time (11.0 vs 15.1 days).

Fig. 1. PRISMA figure of literature search strategy.

Table 1

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<th>Author, Date, Journal</th>
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<th>Experimental setting</th>
<th>Perfusion parameters</th>
<th>Temperature</th>
<th>Flushing solution &amp; perfusate</th>
<th>Time perfused</th>
<th>Investigations &amp; Results</th>
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<tr>
<td>Eckhauser et al., 1980, Journal of Surgical Research [21]</td>
<td>Dogs, Mongrel (20-25 kg) n = unspecified</td>
<td>Heart-beating donors. Total pancreatectomy in donors preserving pancreas with aorta and portal supply. Graft underwent normothermic perfusion for 4-5 hours</td>
<td>Pulsatile pump. Flow: 20 ml/min Pressure: 90-110 mmHg initially, normalised to 30-50 mmHg Oxygenation: 95% O₂, 5% CO₂ Dialysis unit (ultrafiltration machine) used.</td>
<td>37 °C</td>
<td>Flushing solution: unspecified Perfusate: Balanced electrolyte solution + autologous red blood cells + bovine serum albumin (+ 4 g/dL) + heparin (1000u/ml) + glucose (150 mg/dL)</td>
<td>4-5 h</td>
<td>Exocrine function: 3 units/min secretin infusion. Baseline secretion 0.035 ml/min. Increased to 0.28 ml/min post stimulation. Normalise when ceased. Histology: No observable acinar, nuclei or cytoplasmic variation in perfused specimen vs control (normal dog pancreas)</td>
</tr>
<tr>
<td>Eloy et al., 1974, European Surgical Research [68]</td>
<td>Dogs, Mongrel (15-25 kg) n = 15</td>
<td>Heart-beating donors. Total pancreatectomy in donors preserving pancreas with aorta and portal supply. Graft underwent normothermic perfusion for 100 min</td>
<td>Pulsatile pump. Flow: 38-46 ml/min/100 g Pressure: Unspecified Oxygenation: 24% O₂, 3% CO₂, 73% N₂</td>
<td>37 °C</td>
<td>Flushing solution: unspecified Perfusate: Synthetic culture medium + homologous RBC + bovine albumin (40 g/L) + dextran (50 g/L)</td>
<td>100 min</td>
<td>Endocrine function: 420 mg, 900 mg, 1700 mg glucose per 100 ml solution added in 15 minute intervals. Insulin output measured per minute. Insulin secretion constant (17000 μUIRI/min) until arterial glucose concentration reached 140 mg/100 ml, after which continued to rise in response to arterial glucose concentration (max 65000 μUIRI/min). Exocrine function: Secretin infusion 0.5U/min over 90 min. CCX (0.165 U/min) added after 30 min and continued for 60 min. Baseline secretion 0.4-0.5 ml/hour. Increased to 2.4 ml/10 min post stimulation. Normalised after cessation.</td>
</tr>
<tr>
<td>Meyer et al., 1973, European Surgical Research [64]</td>
<td>Dogs, Mongrel (14-16 kg) n = unspecified</td>
<td>Heart-beating donors. Total pancreatectomy in donors preserving pancreas with aorta and portal supply. Graft underwent normothermic perfusion for 3 hours.</td>
<td>Pulsatile pump. Pressure: 75/40 mmHg Flow: 80 ml/min Oxygenation: 24% O₂, 3% CO₂, 73% N₂</td>
<td>37 °C</td>
<td>Flushing solution: unspecified Perfusate: 50% whole allogenic blood, 50% Ringer’s lactate</td>
<td>3 h</td>
<td>Endocrine function: 1.5 g glucose stimulation at 60 min. Insulin secretion increased to 600µU/100 ml plasma post stimulation. Exocrine function: 20 units secretin stimulation. Baseline secretion &lt;1 mU/hour. Increased to 4 mU/5 min, over 60 minutes. Histology: Oedema after 3 h of perfusion. Gross appearance: 1. Normal</td>
</tr>
<tr>
<td>Study</td>
<td>Species, Weight</td>
<td>Heart-beating donors</td>
<td>Total pancreatectomy in donors preserving pancreas with aorta and portal supply.</td>
<td>Pressure</td>
<td>Perfusion:</td>
<td></td>
<td></td>
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<td>-----------------------------------------</td>
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<tr>
<td>Prieto et al., 1988, Journal of Heart Transplant [66]</td>
<td>Dogs, Mongrel (20-25 kg) n = 5</td>
<td>Heart-beating donors. Multi-organ simultaneous preservation (heart, lung, kidney, liver, pancreas).</td>
<td>Blood-perfused control (n = 6) 1. Blood-perfused control (n = 6) 2. Blood-perfused injury (induced pancreatitis) (n = 6) 3. Fluosol-perfused control (n = 6) 4. Fluosol perfused injury (n = 6)</td>
<td>Blood-perfused: 116–125 mmHg initially, plateauing to 60–95 mmHg  Fluosol-perfused: 61–65 mmHg initially, plateauing to 66–76 mmHg</td>
<td>37 °C</td>
<td>Whole blood</td>
<td></td>
</tr>
<tr>
<td>Wahlberg et al., 1989, Transplant International [43]</td>
<td>Dogs, Mongrel (15-25 kg) n = 26</td>
<td>Heart-beating donors.</td>
<td>Heart as pump.</td>
<td>37 °C</td>
<td>Flushing solution: Cold UW solution (0-4 °C) Perfusion: UW solution 350 ml + dextran 5 g</td>
<td></td>
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</tr>
<tr>
<td>Loubatieres-Mariani et al., 1980, Diabetologia [89]</td>
<td>Rat, Wistar (350 g) n = unspecified</td>
<td>Heart-beating donors.</td>
<td>Total pancreatectomy in donors preserving pancreas with aorta and portal supply.</td>
<td>Pulsatility unspecified Flow: 2.4 ml/min Pressure: 35 cmH2O</td>
<td>37.5 °C vs 28 °C</td>
<td>Flushing solution: Unspecified Perfusion: Krebs-Ringer bicarbonate buffer + bovine albumin (2 g/L) + glucose (0 to 3 g/L) 93% O2, 7% CO2</td>
<td></td>
</tr>
</tbody>
</table>

**Pressure:**
- Blood-perfused: 116–125 mmHg initially, plateauing to 60–95 mmHg
- Fluosol-perfused: 61–65 mmHg initially, plateauing to 66–76 mmHg

**Oxygenation:**
- 95% O2, 5% CO2

**Flushing solution:**
- Cold UW solution (0-4 °C)
- UW solution 350 ml + dextran 5 g

**Perfusion:**
- Whole blood

**Effects of hypothermia on insulin secretion (ng/min):**
- Low glucose, 28 °C: 8.2
- Low glucose, 37.5 °C: 41.4
- High glucose, 28 °C: 25
- High glucose, 37.5 °C: 240

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Please cite this article as: Kuan KG, et al, Extracorporeal machine perfusion of the pancreas: technical aspects and its clinical implications—a systematic review of experimental models, Transplant Rev (2015), http://dx.doi.org/10.1016/j.trre.2015.06.002
Table 2
Hypothermic MP.
Table summarising experimental data from thirteen studies reviewed for hypothermic MP of the pancreas. Data includes animal model, experimental setting, perfusion parameters (pulsatility, flow, pressure, oxygenation), temperature, flushing solution & perfusate, total time perfused, investigations and results of the study.

<table>
<thead>
<tr>
<th>Author, Date, Journal</th>
<th>Animal model</th>
<th>Experimental setting</th>
<th>Perfusion parameters</th>
<th>Temperature</th>
<th>Flushing solution &amp; perfusate</th>
<th>Time perfused</th>
<th>Investigations &amp; Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brynger et al 1975 EurSurg Res [67]</td>
<td>Dogs, Mongrel (11-22.5kg) n = 20 Groups: i. n=9 ii. n=7 iii. n=4</td>
<td>Heart beating donors. Total pancreatectomy in donors. Transplanted (allogenic to recipients post perfusion/preservation.</td>
<td>Pulsatile pump. Flow: 95ml/min Pressure: 50/40 mmHg Oxygenation: 99% O2, 1% CO2</td>
<td>6-8°C</td>
<td>Flushing solution (same for all groups): 20ml of 1% lidocainhyd rochloride + 200ml dextran + 10% invert sugar + 1.4% sodium bicarbonate at 4°C</td>
<td>24 hrs</td>
<td>Post-transplant survival: 1. Death from post-op haemorrhage n=3 2. Death from hypoglycaemia n=2 3. Graft rejection n=1</td>
</tr>
<tr>
<td>Histology: Graft examined after animal sacrificed. All appeared normal except one rejected grant in group iii</td>
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<tr>
<td>Blood-glucose post-transplant:</td>
<td>All recipients hyperglycemic prior to transplant (post-pancreatectomy) All recipients normalised within 24 hours Lower glucose levels in group ii and iii on D2 to D3 post-op Episodes of hypoglycaemia – 1 in group ii (died), 3 in group iii (all asymptomatic)</td>
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<tr>
<td>Serum insulin:</td>
<td>All recipients subnormal before transplant All recipients above normal on D1 post-transplant Normalised by D5 post-transplant</td>
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<tr>
<td>Glucose tolerance test: Similar results to pre-pancreatectomy. No difference between 3 groups.</td>
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<tr>
<td>Serum amylase: Marked amylasemia. No significant differences between 3 groups.</td>
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<tr>
<td>Curry et al., 1968, Endocrinology [53]</td>
<td>Rats (250-350g) n = unspecified</td>
<td>Heart-beating donors. Pancreas harvested with cannulated celiac axis and portal vein. Graft underwent hypothermic perfusion for 2 hours.</td>
<td>Pulsatility unspecified Flow: 10-15 ml/min Pressure: unspecified Oxygenation: 95% O2, 5% CO2</td>
<td>7°C</td>
<td>Flushing solution: Unspecified Perfuse: Balanced electrolyte solution + 4% albumin</td>
<td>2 hrs</td>
<td>Effects of addition of calcium on islet cells and oxygen consumption: Concentration of perfusate with no calcium added - 0.68 mEq/L. Concentration of perfusate with calcium added – 13.3 mEq/L.</td>
</tr>
<tr>
<td>No difference in islet cells on histology. No difference in oxygen consumption</td>
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<tr>
<td>Effects of perfusate flow on oxygen consumption: Oxygen consumption increases proportionate to flow rate up to 10-12ml/min, after which becomes independent.</td>
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<tr>
<td>Florack et al., 1983, Journal of Surgical Research [56]</td>
<td>Dogs, Mongrel (14-30kg) Groups</td>
<td>Heart-beating donors. Pancreatectomy with left lobe of pancreas with splenic vessels auto-transplanted to iliac fossa</td>
<td>Pulsatility unspecified Flow: unspecified Pressure: 30mmHg (fixed)</td>
<td>7°C</td>
<td>Flushing solution: 1. Cold Ringer’s solution 2. 24 to 48 hrs</td>
<td>Preservation failure rates: Includes graft failure, pancreatitis, haemorrhage 2a. 20% 2b. 50% 2c. 0%</td>
<td></td>
</tr>
</tbody>
</table>
after perfusion/preservation.

Groups:
1. Control, auto-transplant unpreserved pancreatic grafts (n=20)

2. Static cold storage:
   2a: Collins solution 24hrs (n=12)
   2b: Collins solution 48hrs (n=10)
   2c: SGF-I solution 24 hrs (n=12)
   2d: SGF-I solution 48 hours (n=12)
   2e: SGF-I solution 72hrs (n=10)

3. Pulsatile machine perfusion:
   3a: SGF-I solution 24 hrs (n=12)
   3b: SGF-I solution 48 hrs (n=8)
   3c: SGF-II solution 24 hrs (n=12)
   3d: SGF-II solution 48hrs (n=10)

Flow: 4.5ml/min initially to 6.3-6.5ml/min at end of perfusion

n = heparin

2d: 0%
2e: 57%
3a: 40%
3b: 83%
3c: 30%
3d: 37%

Significant failure rates of machine preservation with SGF-I compared to cold storage with SGF-I.

Long term function success rate:
Includes both technical (arterial thrombosis, pneumonia, peritonitis) and preservation failures and 4 week function post-transplant

1. 80%
   2a. 67%
   2b. 40%
   2c. 75%
   2d. 75%
   2e. 30%
   3a. 50%
   3b. 12%
   3c. 58%
   3d. 50%

No significant difference between preservation method in endocrine function of grafts surviving long-term.

Amylase levels (mean ± SE):
- No significant correlation between amylase levels and long term function
  - Cold storage 24 hours – 1993 ± 121
  - Cold storage 48 hours – 3080 ± 308
  - Perfusion 24 hours – 2259 ± 264
  - Perfusion 48 hours – 4014 ± 407
- Post-transplant, serum amylase significantly lower in machine perfusion groups.
  - Control – 3953 ± 365
  - Static cold preservation – 4226 ± 327
  - Machine perfusion 2988 ± 228

Flow rate (mean ± SE):
Correlation with long term outcome
- Grafts with successful long term function - 5.0 ± 0.6 ml/min
- Grafts with preservation failures – 8.3 ± 1.0 ml/min

Histology and oedema:
Oedema observed post-preservation.
Histology obtained post-preservation and at 4 and 12 weeks post-transplant.
Significant oedema in cold static storage beyond 24 hours and all machine perfused grafts.
No difference between groups in long-term functioning grafts.

<table>
<thead>
<tr>
<th>Study</th>
<th>Pigs (60-90kg)</th>
<th>Non-heart beating donors. Mean warm-ischaemia time 25 mins.</th>
<th>Pulsatile pump.</th>
<th>Flushing solution: UW solution</th>
<th>Perfuse: UW solution</th>
<th>Weight gain (%)</th>
<th>Histology:</th>
<th>Acinar cell damage</th>
<th>Islet cell damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karcz et al, 2010, Experimental and Clinical Transplantation</td>
<td>n = 15</td>
<td>Pancreas aorta en-bloc segment. Graft underwent hypothermic perfusion for 315 minutes</td>
<td>147 ml/min</td>
<td>15-23 mmHg Un-oxygenated</td>
<td>315 mins</td>
<td>Recorded before and after perfusion</td>
<td>Biopsies taken before and after perfusion. Graded as:</td>
<td>Grade 0: no damage</td>
<td>Grade 0: no damage</td>
</tr>
<tr>
<td>Study</td>
<td>Species</td>
<td>Donors</td>
<td>Preparation</td>
<td>Pulsatility</td>
<td>Flow</td>
<td>Pressure</td>
<td>Oxygenation</td>
<td>Flushing Solution</td>
<td>Perfusion</td>
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<tr>
<td>Kowalewski et al., 1978</td>
<td>Dogs, Mongrel</td>
<td>Heart-beating donors</td>
<td>Pancreas aorta en bloc segment including stomach. Graft underwent hypothermic perfusion for 5-6 hours.</td>
<td>unspecified</td>
<td>110-140 ml/min</td>
<td>unspecified</td>
<td>97% O₂, 3% CO₂</td>
<td>Flurocarb on emulsion</td>
<td>Flurocarb on emulsion + glucose</td>
</tr>
<tr>
<td>Kojima et al., 1984</td>
<td>Rats</td>
<td>Non-heart beating donors</td>
<td>Warm-ischaemia time not specified. Pancreas with duodenum, stomach and spleen, with preservation of celiac axis and portal vein.</td>
<td>unspecified</td>
<td>10 ml/min</td>
<td>unspecified</td>
<td>Unspecified</td>
<td>Flurocarb on 4°C</td>
<td>Flurocarb on 4°C</td>
</tr>
<tr>
<td>Leeseer et al., 2004</td>
<td>Human</td>
<td>Non-heart beating donors</td>
<td>Warm ischaemia</td>
<td>unspecified</td>
<td>unspecified</td>
<td>unspecified</td>
<td>Unspecified</td>
<td>Flurocarb on 4°C</td>
<td>Flurocarb on 4°C</td>
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<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Methodology</th>
<th>Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuan et al. (Transplant Rev)</td>
<td>-</td>
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<tr>
<td>Taylor et al. (Cell Transplant Rev)</td>
<td>-</td>
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<tr>
<td>Tersigni et al. (Ann Surg)</td>
<td>-</td>
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<tr>
<td>Toledo-Pereyra et al, 1979, Surgery, Gynecology &amp; Obstetrics [55]</td>
<td>Dogs, Mongrel (17-24 kg) Groups: n = 4 n = 6 n = 6</td>
<td>Heart beating donors. No warm ischaemia time. Total pancreatectomy performed in donors. Grafts are preserved or perfused for 24 hours, before allologenic transplantation to recipients with prior total pancreatectomy. Pancreas and duodenum en-bloc with celiac trunk and portal vein Groups: 1. Apancreatic control. 2. Control, grafts transplanted (allologenic) immediately after pancreatectomy. 3. Grafts perfused for 24 hours prior to allologenic transplant. 4. Grafts preserved with cold static preservation for 24 hours prior to allologenic transplant.</td>
<td>Pulsatile pump</td>
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<tr>
<td>Dogs, Mongrel (14-27 kg) Groups: 1. n = 10</td>
<td>Heart beating donors. No warm ischaemia time. Total pancreatectomy performed in donors. Grafts are preserved or perfused for 24 or 48 hours</td>
<td>Pulsatile pump</td>
<td>5-7 °C</td>
</tr>
</tbody>
</table>
2. n = 10  
3. n = 10  
4. n = 10

hours before islet cell isolation via minimal collagenase digestion and autotransplanted into the spleen.

Pancreas and duodenum en-bloc with celiac trunk and portal vein

Groups:
1. Apancreatic control. Pancreactectomy performed with no auto-islet transplant.
2. Control. Islet isolation and autotransplant immediately after pancreactomy.
3. Perfusion for 24 hours prior to islet isolation and autotransplant.
4. Perfusion for 48 hours prior to islet isolation and autotransplant.

<table>
<thead>
<tr>
<th>Gr</th>
<th>Days post transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 3 7 14 30 60</td>
</tr>
<tr>
<td>1</td>
<td>78 17 29 37 40 -</td>
</tr>
<tr>
<td>2</td>
<td>75 12 14 13 10 92 89</td>
</tr>
<tr>
<td>3</td>
<td>74 13 16 14 10 10 97</td>
</tr>
<tr>
<td>4</td>
<td>81 14 17 16 12 11</td>
</tr>
</tbody>
</table>

No significant difference between 24 hour perfusion group and fresh transplant. Glucose levels higher in 48 hour perfusion group.

<table>
<thead>
<tr>
<th>Gr</th>
<th>Days post transplant</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1 3 7 14 30 60</td>
</tr>
<tr>
<td>1</td>
<td>9.5 12 0.5 -</td>
</tr>
<tr>
<td>2</td>
<td>11.5 13 12 11 10.2</td>
</tr>
<tr>
<td>3</td>
<td>7.1 12 14 13 14.2</td>
</tr>
<tr>
<td>4</td>
<td>12.0 15 18 19 20.1</td>
</tr>
</tbody>
</table>

Insulin levels not significantly higher in 48 hour perfusion group compared to fresh and 24 hour perfusion groups.

**IV glucose challenge test at 14 days post-transplantation:**

<table>
<thead>
<tr>
<th>Glucose levels (mg/dL)</th>
<th>at time (min) after IVGCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10 30 60 90 120</td>
</tr>
<tr>
<td>1</td>
<td>85 220 195 165 150 120</td>
</tr>
<tr>
<td>3</td>
<td>87 272 200 178 142 125</td>
</tr>
</tbody>
</table>

No significant difference between control and 24 hour perfusion groups.

**Pigs, Landrace n = 3**  
Non-heart beating donors. Warm ischaemia unspecified.

Total pancreatectomy performed in donor pigs (n = 3). The pancreas is divided - splenic lobe used for hypothermic perfusion and duodenal and connecting lobe used as control with 3 hours cold ischaemia time.

Islets are then isolated from control and perfused pancreas and transplanted into kidney capsule of 10 diabetes-induced nude mice.

**Weegman et al, 2012, Cell Med**

**Dogs, Beagle (8-13kg) n = 3**  
Total pancreatectomy performed in donors. Pancreas aorta en-bloc segment. Exocrine duct ligated.

**Westbroek et al, 1974, Transplantation**

**Pulsatile pump.**  
Pressure: 100mmHg

**Flushing solution:**  
Ringer's lactate solution

**Perfusate:**  
KPS1 solution - kidney perfusion solution

**Exocrine function:**
Pancreaticoduodenal secretions (ml) collected

24 hours: 42 ± 8.5 ml  
48 hours: 35 ± 6.7 ml

**Serum amylose:**
No difference between in all groups

**Blood glucose levels (mg/dL):**

**Serum insulin levels (μU/ml):**

**Percentage of diabetes recovery on day-7 post transplantation (%):**

**Post-transplant survival (days, mean):**

Interestingly, their control group (immediate transplant with no preservation or perfusion) survived only 6.1 days [51]. Likewise in a study by Tersigini and associates, grafts with hypothermic MP recorded a better post-transplant survival of 18.3 days compared to controls (immediate transplant) of 14.6 days [52].

3.3. Anatomy

Most studies utilised a model of total pancreatectomy preserving the segment of aorta supplying the pancreas and portal outflow, with or without a duodenal segment. 4 studies (dogs: n = 3, rat: n = 1) only preserved the celiac axis as arterial supply [52–55] and another [56] only preserved the splenic lobe of the (dog) pancreas via the splenic artery. The anatomy of the dog pancreas allows most of its blood supply from the celiac axis and its tributaries [21]. In contrast, the porcine pancreas which is divided into 3 distinct lobes – duodenal, connecting and splenic receives its blood supply from both the celiac trunk and superior mesenteric artery [57] Fig. 2.

3.4. Perfusion duration

All articles reviewed specified the perfusion duration. Studies on normothermic MP of the pancreas report perfusion duration of 90 minutes to 6 hours, usually with whole blood or modified plasma with red blood cells. In contrast, hypothermic MP experiments are usually perfused for significantly longer, with majority of them for 24 hours. Florack et al. reported hypothermic perfusion of dog pancreases comparing perfusion durations of 24 and 48 hours. They reported perfusion failure rates of 40% (24 h) vs 83% (48 h) with SGF-I solution and 30% (24 h) vs 37% (48 h) with SGF-II solution. Long term function (4 weeks post-transplant) success rates was similarly better in 24 hour perfusion groups – 50% (24 h) vs 12% (48 h) with SGF-I and 58% (24 h) vs 50% (48 h) with SGF-II [56]. Similarly, in a study by Toledo-Pereya et al., 60% of dogs received pancreas undergoing hypothermic MP for 24 hours survived for more than 60 days. However, when the perfusion duration was extended to 48 hours, only 40% of recipients survived. There were no significant difference in serum glucose levels and IV glucose challenge test at 14 days post-transplantation between the 24 hour perfusion group and control (immediate transplant) but glucose levels were higher in the 48 hour perfusion group [54].

At normothermic temperatures, the pancreas is in its full functional metabolic state and therefore requires full oxygen and nutrient support. Moreover, the balance of pressure and flow needs to be even more delicate to ensure minimal damage to the vascular endothelium to avoid platelet activation and vascular thrombosis [15]. The upper limit of normothermic MP of the pancreas that has been studied is around 5 to 6 hours. While technology and experience in normothermic MP of the pancreas are not as matured as other organs, positive results in other organs should provide impetus for studies with longer perfusion duration at normothermic temperatures [16,19,37,58].

3.5. Pulsatile flow

Two types of pumps are used in MP – pulsatile (roller pump, oscillating pump) or non-pulsatile (centrifugal). Pulsatility of flow in MP remains a topic for debate. 9 studies utilised pulsatile pumps (including one study utilising the heart as pump) while 3 studies used non-pulsatile pumps. The remaining was unspecified. There is however no study comparing pulsatile vs non-pulsatile flow in pancreas MP.

Pulsatile flow has been suggested to induce vasoprotective endothelial genes. One of the most crucial, Kruppel-like factor 2 (KLF-2) is thought to inhibit pro-inflammatory responses, thereby protecting the vascular endothelium. It is expressed in the endothelium and is rapidly metabolised once pulsatile flow ceases. KLF-2 has also been attributed to play a role in the production of nitric oxide, a potent vasodilator, and thrombomodulin, an anti-thrombotic gene [12,59–61]. Two other studies suggests pulsatile flow improves microcirculation as well as energy charge in the kidney and liver [62,63] while Uchiyama et al. found no difference between pulsatile or non-pulsatile flow in liver MP [34].

As the pancreas is considered to be a physiologically low flow organ and particularly susceptible to barotrauma [10], studies comparing pulsatility in other organs may not automatically apply and warrants further investigation.

3.6. Pressure and flow rates

Optimal flow and pressure is crucial in MP. Among the reviewed articles, 16 and 15 studies specified the flow rates and pressure respectively. Normothermic MP studies tend to utilise perfusion pressures similar to physiologic levels while hypothermic MP pressure are much lower, usually around 10–30 mmHg [21,27,47–50,52–56,64–66]. While high perfusion pressure may cause endothelial injury and increased rate of vascular thrombosis in the delicate pancreas [15], at normothermic temperatures, the organ is at its full metabolic demand. Low perfusion pressure can lead to underperfusion of the microvasculature and inadequate oxygen and nutrients supply. Several studies discussed in this
Normothermic MP requires a medium to carry and deliver oxygen to the tissue perfused. Autologous red blood cells are usually the oxygen carrier of choice. They exist in the form of perfusates as whole blood [64–66] or combination of balanced albumin and electrolyte solutions with packed red blood cells [21,68]. Various other components are added in attempts to improve the perfusate, including heparin (prevention of thrombosis), glucose, insulin (nutrients), albumin, dextran (reduce oedema), methylprednisolone (dampen inflammation), antibiotics and various electrolytes (MgSO4, KCl, NaHCO3). O’Malley et al. compared autologous blood and fluosol, an oxygen-carrying emulsion, and reported worse inflammation in the blood perfused group (serum amylase levels: 4253 vs 1285 IU/dL) but little difference on histological examination [65].

Perfusates used in hypothermic MP of the pancreas varies greatly as well. Several studies utilised existing SCS solutions like UW solution, Belzer Kidney Perfusion Solution, UHK solution (Unisol) or KPS1 kidney perfusion solution (Organ Recovery Systems) [27,48–50]. Many others developed solutions usually consisting of an oncotic agent (albumin, dextran), cryoprecipitated plasma, mannitol and other critical nutrients, anti-inflammatory agents, antibiotics and electrolytes as described above.

Oncotic pressure of the perfusate needs to be matched with the perfusion pressure to reduce interstitial oedema during MP [42]. Tersigni and associates demonstrated early-on that the addition of a colloid (albumin) to the perfusate greatly improves oedema as well as post-transplant survival (18.3 vs 4.5 days) and perfusion related complications (vascular thrombosis, hemorrhagic necrosis and ischaemic necrosis) [52].

Florack et al. compared addition of insulin and mannitol (SGF-I solution) to a perfusate containing plasma, albumin, prednisolone, dextrose and electrolytes (SGF-I solution) and found a significant improvement in preservation failure rates and long-term function success rates [56]. Hypothermic MP using KPS1 solution was reported to be slightly superior to UHK solution in both islet yield and insulin secretion. There was also less weight gain and oedema with KPS1 solution [50]. Karcz et al. used UW as perfusate and reported an improvement in acinar cell and islet cell damage as well as inflammation and oedema after 6 hours of hypothermic perfusion, following 25 minutes of warm ischaemia time [27]. Kowalewski et al. also demonstrated successful preservation of dog pancreas for 5 to 6 hours using fluorocarbon emulsion, a compound designed as ‘blood substitute’ with high oxygen carrying capacity [69].

As the mechanism of MP and SCS is inherently different, further studies need to be conducted to compare the different perfusates, use of colloids and various additives to identify the ideal composition of a MP perfusate.

3.8. Perfusate oxygenation

The concept of normothermic MP is based on creating a physiological milieu achieved by circulating oxygen and nutrients to the tissue. This is usually done via oxygenated erythrocytes or synthetic oxygen-carrying compounds. The perfusate in normothermic MP is usually oxygenated via a membrane oxygenator prior to circulation into the organ. Most studies used 95% O2 and 5% CO2 while several others oxygenates the perfusate with room air (24% O2, 3% CO2, 73% N2). While oxygenation of the perfusate is essential in normothermic MP, its role in hypothermic MP is unclear. Several studies have suggested that a controlled oxygen tension is beneficial in hypothermic preservation. Metabolic demand is drastically reduced at hypothermic temperatures, oxygenation can lead to a replenishment of ATP supply while minimising metabolic stress in tissues [23]. A study led by Kojima investigated the role of oxygenation and hyperbaric oxygenation in hypothermic MP. The number of degenerated islets at the end of a 6 hour perfusion period favours oxygenation (unoxgenated 40% vs oxygenated 30%). They also reported hyperbaric oxygenation to be harmful.

**Fig. 2.** Porcine pancreas graft with aorta and portal vein cannulation (Department of Surgery, University of Adelaide).
(50% vs 30% degenerated islets). In addition, glucose challenge test results were favourable in groups with oxygenation [47].

4. Discussion

4.1. Expanded criteria donors (ECD) and marginal grafts

In the United States, the number of pancreas transplants has fallen by about 20% in the past decade since a peak in 2004. However, numbers in Europe, Australia and South America continued to rise [3]. In Australia and New Zealand, candidates placed on the waiting list has increased by 63% in the 10 years from 2003 to 2012 [70–79]. A report based on the United States Organ Procurement and Transplantation Network (OPTN) registry reported that only approximately 20% of potential donor pancreases are retrieved, and among them, 27.7% are disposed of [80]. In Australia and New Zealand from year 2003 to 2012, only 39.9% of pancreas grafts were harvested from available and consented donors. Furthermore, 52.7% of the pancreata that were harvested were finally discarded [70–79]. The pancreas thus hold the unwanted honour of having the highest rate of retrieved organs which are then discarded due to unsuitability for transplantation [5].

The pancreas is particularly susceptible to ischaemic damage and probably less forgiving than other abdominal organs for transplant. The exact mechanism of ischaemia–reperfusion injury is complex and intricate, and is a subject of extensive ongoing investigations. Organs undergo a series of insults – warm ischaemia (WI), cold ischaemia (CI) during preservation and reperfusion after anastomosis of vasculature [11]. These insults result in delayed graft function, graft failure, rejection, vascular thrombosis, pancreatitis and anastomotic leakage [81]. It is estimated that islet yields are slashed by 40% after 8 to 10 hours of cold ischemia, and up to 60% when the CI time is extended to 16 hours [10].

Due to the inherent high risks of the operation and complications associated with graft failure, including significant risk of mortality, criteria for pancreas donation is especially strict. The ‘ideal’ pancreas donor is identified as [10,81,82]:

- 15–45 years old
- Male
- BMI 20–25 kg/m²
- Donation after brain death (DBD)

A recent long-term report from the International Pancreas Transplant Registry has confirmed that strict donor selection resulted in superior short and long-term graft function [82]. Prolonged warm-ischaemia time is obviously undesirable, therefore donation after cardiac death (DCD) has traditionally been avoided in pancreas transplants (only 3% of pancreas transplants are from DCD) [3]. Moreover, prolonged periods of hypotension and extensive use of inotropes are also best avoided.

Significant improvement in preservation techniques may allow us to utilise expanded criteria donors and marginal grafts, those that would otherwise be discarded due to dubious quality, potentially making solid-organ pancreas and islet cell transplantation more widely available and safer. This balance between quantity and quality obviously needs to be carefully balanced to achieve the desired short and long term results.

The Pre-Procurement-Pancreas-Suitability-Score (P-PASS) was introduced by the Eurotransplant Pancreas Advisory Committee to support clinicians in screening for potential donors and accepting grafts. The scoring system ranges from 9 to 28 points, including parameters such as age, BMI, ICU stay, duration of cardiac arrest, use of inotropes and biochemical markers, with a score of <17 the likely cut-off for accepting a donor [83]. This score will naturally evolve with time as preservation techniques are improved.

DCD for pancreas has generally been avoided until recently. Warm-ischaemia (WI) represents a major insult to the organ, resulting in severe ischaemic-reperfusion injury (IRI), primary graft non-function (PNF) or delayed graft function (DGF). While several large studies comparing the results of DCD and DBD showed no significant difference in patient survival and graft function, they are considerably skewed in their patient selection. Selection of DCD donors included in the study was notably more stringent with younger age, lower BMI and shorter ischaemic time [84–86]. While generally avoided for solid-organ transplants, studies have found that pancreas donors aged above 50 or BMI >30 kg/m² may in fact be preferentially suitable for islet cell transplant, due to fatty infiltration of the pancreas facilitating islet isolation [10].

MP has the unique benefits of potentially expanding the donor pool to include many donors who do not fit into the traditional pancreas donation criteria, due to concerns of suboptimal quality grafts. The potential to include these donors has got widespread implications and could make islet cell transplant, a procedure with much lower morbidity but requires multiple infusions, much more available and feasible. In addition, we can also expect to see a much lower organ discard rate as a result of superior preservation.

4.2. MP vs SCS in pancreas preservation

The debate for utilisation of MP in clinical pancreas and islet cell transplant centres around the potential of MP to extend preservation times and improve graft function, especially in marginal grafts. Despite being much more technically challenging, MP offers several key advantages as discussed above.

MP of the pancreas is mainly focused on graft preservation for islet cell transplantation. Early studies has favoured SCS over hypothermic MP in preservation failure and post-transplant survival rates [55,56,67]. However, in more recent studies, perhaps due to improvements in technology and better understanding of optimal parameters, results have been superior in MP.

Leeher et al. perfused the pancreas with the Water RM3 kidney perfusion pump with Belzer Kidney Perfusion Solution for 4 hours. Gross islet yield was reported to be lower than SCS with UW solution for <8 hours (3435 vs 5134 IEQ/g), but islet viability (86% vs 74%), insulin secretion index (6.4 vs 1.9) and percentage of islets suitable for transplant (according to Uni. of Maryland Criteria) were all superior in the MP group [48]. Similarly, Weegem et al. reported lower gross islet yield in the MP group compared to SCS, but higher purity and percentage of diabetes recovery on day 7 post xenotransplantation of islet cells (MP 69.33% vs SCS 48%) [49]. In another study comparing pancreas SCS and MP, both islet yield (1002 vs 2242 IEQ/g) and insulin secretion (2.37 vs 5.9 ng/IE) has been demonstrated to be superior in the MP group [50].

Pancreas grafts undergoing MP inevitably demonstrate a greater degree of oedema even at low pressures. While interstitial oedema may be an undesirable effect for solid organ transplant, it is favoured in grafts used for islet cell transplant. Histological examination of pancreas grafts perfused with MP show more consistent and uniform digestion of islet cells, as oedema aids in the enzymatic digestion process, leading to better islet yield and purity, with no loss of function [22].

4.3. Normothermic vs hypothermic MP

As more clinicians embrace MP as the preservation method of choice, we might have also come a full circle in recognising that the organ is best preserved in a state closest to its physiological milieu. This concept was first explored at the turn of the 20th century and has been studied sporadically since [87]. In recognising the potential of normothermic MP as the next breakthrough in organ preservation, we are making a u-turn on the concept of cold organ preservation that has been firmly held for the past 5 decades.

Normothermic MP involves perfusion of the organ at a temperature close to body temperature (37°C). The basic set-up includes a perfusion pump (pulsatile or centrifugal), oxygenator, blood reservoir and heat exchanger (e.g. circulating water bath). Tubes circulate the perfusate carrying oxygen and nutrients to the organ and metabolic toxins are...
carried away from the tissue. A dialysis unit, such as the kidney, when added to the circuit may further improve the function of the preserved organ [20].

As discussed earlier, the preserved organ is at its full metabolic demand at normothermia and therefore requires continuous oxygen and nutrient support. The aim of normothermic MP is to support the organ in its physiological environment and maintenance of metabolic support should, at least theoretically, allow preservation to continue indefinitely.

Normothermic MP carries several potential advantages over hypothermic MP and SCS. While metabolic demand is minimal at hypothermic temperatures, it is not completely eliminated and will continue to inflict ischaemic-reperfusion injury. Preservation of organs at cold temperatures also carries several inherent risks, particularly direct cold damage to the cytoskeleton and plasma membrane lipids [88]. The hypothermia state also disables the ion-exchange pumps in the cell membrane, which may cause swelling of the organ and cell lysis and can affect organ function like insulin secretion [9,89].

Several experimental studies examined the potential advantages of organ preservation at normothermia. In a study by Schon et al., pig livers which have undergone warm ischemia of one hour were successfully transplanted survived beyond 7 days after 4 hours of normothermic MP. On the contrary, the group preserved via SCS all developed primary non-function within 24 hours [90]. Magliocca et al. reported a 33% increase in potential donor pool for abdominal organs by implementing extracorporeal perfusion support for DCD donors [91]. Although still in experimental stages, normothermic MP could develop into the preferential preservation method for DCD and has the potential to optimise such grafts, offering the prospective of increasing utilisation of ECD.

4.4. Potential drawbacks of MP and limitations of this review

Despite its potential benefits, there are several drawbacks of MP that has limited its progress to become more readily utilised. Inherent difficulties in the anatomy of the pancreas’ blood supply makes it prone to small vessel venous thrombosis and haemorrhage. Outflow thrombosis accounts for up to 70% of all technical failures in pancreas preservation [27]. In addition, the physiological “low-flow” of the pancreas vessels compounds the intricacy in avoiding excessive oedema while providing adequate perfusion pressure to supply the organ’s oxygen and metabolic demands. Retrieval of an organ for MP needs to take into consideration the anatomical and technical complexities in connecting the organ to the perfusion circuit. Furthermore, maintaining an organ at its full metabolic state requires impeccable monitoring and maintenance of its physiological milieu, an obvious hurdle where the minimal deficiencies could have widespread devastating results. MP also requires much more advanced equipments, increased cost, has reduced mobility and demands a highly specialised team of professionals (surgeons, nurses, perfusionists and technicians) to be carried out successfully. Vigilant monitoring of various perfusion parameters is essential during the entire preservation period.

The use of machine perfusion in the pancreas is currently still in the pre-clinical experimental phase therefore all studies except one reviewed in this article are experimental animal studies. Studies in this topic is considerably limited and dated, with all but one study included transplantation post-perfusion as an end-point. While most clinical data on the benefits of MP are derived from other organs where the use of MP is more matured, early experimental models have shown promise that MP may offer similar advantages. This review article is intended to serve as a basis and impetus for future experimental work in this topic.

5. Conclusion

Moving forward, more experimental studies are required to establish the optimal parameters for pancreas perfusion. Key factors including pressure, flow, hypothermic vs normothermic, duration of perfusion, perfusate composition etc. needs to be robustly examined and clearly defined. The balance of optimal pressure and flow in the pancreatic microvasculature is crucial to graft function as discussed. Gross measurements of pressure and flow of the circuit may not accurately reflect the conditions in the microvasculature. A potential method of obtaining this information is utilising a Doppler guidewire and pressure transducer as described by Escaned et al. and Ng et al. in coronary artery microvasculature studies [92,93]. Non-invasive methods of assessing microvasculature has also been described utilising Sidestream Dark-Field imaging, which may be explored in the pancreas [94,95]. Furthermore, a detailed computed-tomography (CT) angiography may be considered to better define the vascular anatomy and assist in planning this challenging harvest procedure, even in the experimental animal model. A closed system with controlled ambient temperature and humidity similar to those utilised in heart transplants may likewise be adapted for the pancreas. Future studies should also include function and survival of the pancreata and islet cells post-transplantation to examine its true effectiveness.

In conclusion, machine perfusion could hold the key to revolutionising organ preservation, especially with increasing use of marginal grafts and emerging therapeutic options to optimise the organ during preservation. However, its use in the pancreas requires further optimisation and we look forward to further studies in this promising field.

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References


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| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidate and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |

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A Study of Normothermic Hemo-Perfusion of the Porcine Pancreas and Kidney

Abstract: Background: Normothermic machine perfusion has enormous potential in improving organ preservation and expand the organ donor pool. It is well established in other organs but not the pancreas, which has especially strict organ acceptance criteria. We established a model of normothermic hemo-perfusion of the porcine pancreas with and without addition of the kidney as a dialysis organ. Methods: Four pancreases were harvested and perfused for 120 min with autologous whole blood at body temperature, two with parallel perfusion of the kidney and two without. The organs and perfusion circuit were evaluated for gross appearance, pH, histology and perfusion parameters. Results: The organs maintained steadily increasing flow rate and perfusion pressure. Gross appearance of organ was stable but appears grossly ischaemic towards end of perfusion period. Histology demonstrated necrosis centred in acinar tissue but islet cells were preserved. pH was significantly alkalotic toward the end of the perfusion, likely due to pancreatic tissue damage. Conclusion: Normothermic perfusion of the pancreas is still in the experimental stages but holds great potential. Further studies to optimise perfusion parameters will significantly improve results. Parallel perfusion of the kidney may facilitate improvement in the acid-base environment. Key Words: Normothermic perfusion—Extracorporeal perfusion—Pancreas—Kidney—Transplant.

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BACKGROUN

Solid organ pancreas or islet cell transplant remain the only definitive treatment for insulin dependent diabetes mellitus. Advancements in immunomodulation therapy and islet isolation techniques have significantly improved overall patient and graft survival (1,2). However, shortages in both quantity and quality of donated organs continue to hamper the development of this therapy.

Pancreas donation requires fulfilment of very strict criteria and has the highest rate of discard after retrieval due to unsuitability for transplantation when compared with other organs (3). Only about 20% of potential donor pancreases are retrieved and close to 28% is then discarded due to unsuitability for transplant (4).

Interest in machine perfusion (MP) as a method of organ preservation, first attempted more than a century ago, has only been reignited in the last decade after falling out of favor to static cold storage (SCS) (5,6). The potential benefits of machine perfusion include improved circulation of oxygen and elimination of metabolic waste; maintenance of vascular bed; opportunity for viability assessment; therapeutic manipulation and gene therapy; possibility of long distance organ sharing (7). These advantages hold the key to the critical step of expanding the organ donor pool and improving organ utilisation.

Normothermic MP in particular is increasingly gaining attention for its prospective benefits over hypothermic MP. Studies on normothermic MP of the pancreas are still in the experimental stage. It is important to note most studies were attempted between the 1970s and 1980s, all of which were animal models (7), until Barlow et al. recently reported the first use of normothermic MP to assess viability in discarded human pancreases (8).

In their study, five discarded human pancreases were perfused with normothermic ABO-compatible packed red blood cells. A perfusion period of 1 to 2 h was achieved with stable perfusion parameters.

Perfusion of the organ close to normal body temperature with an oxygen and metabolite...
carrier, most commonly autologous whole blood, should theoretically allow preservation to be carried on for prolonged periods and allow superior viability assessment close to the organ’s physiological state. In this scenario, a dialysis unit, for example, the kidney or a hemodialysis machine may be added to the circuit for the purpose of eliminating metabolic toxins and maintaining physiological milieu (9). Certainly, the concurrent preservation of pancreas and kidney is a logical one, as solid organ pancreas transplantation is frequently performed as simultaneous-pancreas-kidney (SPK) transplants.

We have set out to establish a model of extracorporeal normothermic MP using porcine pancreas, with and without the addition of the porcine kidney as a dialysis unit, as a feasibility study.

METHODS

Four experiments were conducted—two with the porcine pancreas alone and two with addition of the porcine kidney. The experiments were conducted between July 2014 and December 2014, with full animal use ethical approval from the relevant animal ethics committees.

Pancreas retrieval

Domestic white pigs of 40–50 kg were used for the experiments. In brief, the pigs were intubated and put under general anaesthesia for the organ retrieval process to mimic a donation after brain death (DBD) model. 5000 IU of heparin was given peri-operatively for prevention of thromboembolism. The organ retrieval process was performed by a team of experienced transplant and hepatobiliary surgeons. Following a midline laparotomy, a splenectomy was carried out close to its hilum. The duodeno-jejunal flexure was divided and duodenal Kocherised to expose the pancreas. The pancreas was mobilised of its attachments, carefully preserving all supplying vessels and capsule. The superior mesenteric vessels were ligated distal to its pancreatic supply. The abdominal aorta below the diaphragm to just above the renal arteries was mobilised. Tributaries of the abdominal aorta excluding the coeliac trunk and superior mesenteric artery were carefully ligated and divided. The aorta and portal vein was then cannulated with 12-french catheters and secured in place. It was similarly flushed with cold (0–4°C) Hartmann’s solution until all residual blood and clots were removed.

Kidney retrieval

In addition to the pancreas retrieval process as described above, the left kidney was mobilised from its retroperitoneal position. The left renal artery, vein and ureter were cannulated with 12-french catheters and secured in place. It was similarly flushed with cold (0–4°C) Hartmann’s solution until all residual blood and clots were removed.

Perfusion set-up

The perfusion set-up consists of a SARNS 8000 extracorporeal roller pump (3M, MN, US), Baby RX venous reservoir and membrane oxygenator (Terumo, MI, US), metal organ chamber, PVC tubings (Cellplex, VIC, AU) and water bath temperature regulator.

Perfusate

1.8 to 2 L of autologous whole blood collected via exsanguination was used as the perfusate. Additions to the perfusate included 1 g cephazolin (antimicrobial), 500 micrograms epoprostenol sodium (vasodilatation) and 500 IU heparin (microclots prevention).

Perfusion parameters

The temperature of the water bath was set at 37°C and oxygenation at 2 L flow per min. The perfusion was commenced with the organs connected to the circuit. Initial flow rate was set at 0.2 L per min, adjusted to maintain at mean arterial pressure (MAP) to the pancreas at 70–80 mmHg and kidney at 90-100 mm Hg. Hartmann’s solution was added periodically to the perfusate to replace duodenal drainage and urine production.

Outcome measures

Ischaemia time (warm and cold) was recorded. Parameters of the perfusion circuit were recorded for flow rate and perfusion pressure; and the organs for gross appearance (oedema, congestion, haemorrhage), urine output and duodenal drainage at 30 min intervals. Blood samples were also taken for complete blood examinations, biochemical

Artif Organs, Vol. 00, No. 00, 2016
analyses and blood gasses analyses. Tissue samples were also taken in 1 cm³ blocks from the duodenal, connecting and splenic lobes of the perfused pancreas at one hourly intervals and at completion of preservation period. The samples were embedded in paraffin, sliced and prepared with hematoxylin and eosin.

RESULTS

Ischaemia time
The warm ischaemia time (commencement of exsanguination until cold flush complete) averaged 8.25 ± 6.55 min. The average cold-ischaemia time (cold flush complete to commencement of normothermic perfusion) was 34 ± 7.78 min.

Perfusion duration
The first pancreas-only organ was perfused for 4 h. After 150 min of perfusion, the pancreas had already appeared grossly ischaemic and haemorrhagic macroscopically. It was decided to continue perfusion up to 4 h to study its progression. The remaining 3 experiments (1 pancreas-only, 2 pancreas plus kidney) were run for 2 h.

Gross appearance
The gross appearance of the pancreas was graded on oedema, congestion and haemorrhage on a scale of 0 (none), 1 (mild), 2 (moderate), and 3 (severe). All pancreases displayed mild oedema after cold flush was completed, prior to commencement of perfusion. All perfused pancreases had appeared moderately to severely oedematous, congested and haemorrhagic after 90 min of perfusion (Fig. 1). Similarly, the perfused kidneys appeared oedematous and congested after 60–90 min of perfusion. The average volume of urine collected from the cannulated ureter is 0.35 mL/min. The urine collected becomes macroscopically blood stained from around 30 min into the experiment.

Perfusion parameters
Perfusion pressure was individually measured for the pancreas and kidney with a pressure probe immediately proximal to the arterial cannulation. The rate of perfusate flow to each organ was adjusted individually to maintain MAPs of 70–80 mm Hg for the pancreas and 90–100 mm Hg for the kidney. The total flow rate of the circuit increased from an average of 0.23 L/min (30 min) to 0.37 L/min (120 min) for pancreas-only perfusion and 0.29 L/min (30 min) to 0.46 L/min (120 min) for pancreas and kidney perfusion.

Blood investigations
Blood analysis demonstrated significant drop in haemoglobin (Hb) levels as the perfusion duration prolonged. Average Hb levels were 134.75 ± 9.77 g/L at commencement of experiment and gradually reduced to 84.25 ± 14.34 g/L at the end of 2 h. The reason for drop in Hb levels is likely to be due to haemolysis. Arterial pH of the circuit
was progressively alkalotic, with average pH of 7.38 at commencement to 7.89 (pancreas-only) and 7.81 (pancreas + kidney) at the end of perfusion. Biochemical analysis of the blood samples were unable to be performed due to the significant hemolysis.

Histopathological results
The tissue samples were examined by an experienced veterinary histopathologist (Fig. 2). All pancreatic tissue samples displayed worsening degrees of acinar damage, inflammation, and thrombosis as the perfusion period progressed. Inflammation was mostly found in the peripancreatic tissue, predominantly neutrophilic infiltration, and some degree of inflammation in areas of significant acinar necrosis. The islet cells examined histopathologically demonstrated a small degree of cytoplasmic microvascularization (progressive with perfusion period) but no gross evidence of necrosis or inflammation when compared with the exocrine pancreatic tissue.

DISCUSSION
This study was conducted as a feasibility study in establishing an extracorporeal normothermic perfusion model of the pancreas, with and without addition of the kidney simultaneously as a dialysis unit, primarily to investigate the major technical aspects of MP—porcine pancreas anatomy, choice of pump, perfusion parameters and challenges of a two-organ perfusion.

Perfusion duration
Similar to the study recently published by Barlow et al., we achieved a perfusion period of around 2 hours with the perfused organ appearing grossly ischaemic toward the end of the experiment. Levels of oedema, congestion, and haemorrhage on gross appearance of the perfused organ all steadily increased during the perfusion period. Histopathological analysis also consistently revealed inflammation in the peripancreatic tissue and acinar necrosis. This is still well short of the “ideal” 12-h cold-ischaemia-time accepted for pancreas preserved via SCS (10). Normothermic MP, when perfected, has the theoretical ability to allow for prolonged preservation periods. This however will require extensive optimisation of the circuit and perfusion parameters. Nevertheless, in its current state, normothermic MP still offers valuable information in viability assessment of preserved pancreatic grafts.
Anatomy

The porcine model was chosen for its similarity in size to humans. However, the anatomy of the porcine pancreas made this model a challenging one. The porcine pancreas differs from the human pancreas in the number and distribution of pancreatic lobes and their blood supply. It consists of 3 major lobes—duodenal (DL), connecting (CL) and splenic (SL) (11). The DL and SL are supplied by the coeliac trunk (CT)—DL via the gastroduodenal artery and superior pancreaticoduodenal artery; SL via the splenic artery. The major blood supply of the CL is by the superior mesenteric artery (SMA). There is an anastomosis between CT and SMA by the pancreaticoduodenal vascular arcade (PDVA).

In contrast, the human pancreas draws the vast majority of its blood supply from the CT via the splenic artery. The head is supplied by the superior and inferior pancreaticoduodenal arteries, tributaries of the CT and SMA, respectively, with rich anastomosis between the vessels (12). When adapting the perfusion to the human pancreas, it is crucial to take into consideration these variations in arterial supply.

Pulsatile versus nonpulsatile pump

The SARNS 8000 roller pump is a positive displacement pump most commonly utilised in cardio-pulmonary bypass operations, which generates a pulsatile flow. The use of pulsatile versus nonpulsatile flow (centrifugal pumps) in organ preservation remain debatable (7). Several studies have suggested pulsatile flow mimicking physiological environment inhibits proinflammatory responses and improve microcirculation in the preserved kidney and liver (13–15). Our study observed significant haemolysis of the whole blood perfusate during the course of the perfusion. This may be attributed to the use of a roller pump, where too much or too little compression of the tubing, the working mechanism of the roller pump, induces hemolysis (16).

Centrifugal pumps in contrast greatly reduce the risk of haemolysis but generate a nonpulsatile flow. Comparison of these modalities will require further investigation.

Perfusion parameters

Perfusion pressure and flow rates are widely suggested to predict graft viability. Normothermic MP relies on pressure and flow to the organ which mimics its physiological environment at full metabolic demand. Inadequate perfusion pressure results in collapse of the microvascular bed while high pressure may cause endothelial injuries and thrombosis (7). It has been reported that pancreatic grafts with better survival had higher and stable flow rates at defined pressures—maintaining a stable resistance index (17). Previous groups have utilised perfusion pressures between 75 and 110 mm Hg for normothermic MP with good endocrine functions but associated with graft oedema (18,19). As the pancreas is a physiologically “low-flow” organ, we adopted the lower range of perfusion pressure of 70–80 mm Hg. Our study was consistent with that of Karcz et al. which suggested that flow volume gradually increased in the first 60 min, becoming stable thereafter (20). However, it is important to note that the optimal perfusion parameters for pancreas MP has yet to be established and require further studies for better definition.

Simultaneous pancreas-kidney perfusion

An issue faced in normothermic perfusion of organs is the accumulation of metabolic toxins and breakdown products as the organ is in full metabolic function. A circuit containing the pancreas in particular is in danger of accumulating lipase and proteases which may result in autolysis of the organs. The high pH of the circuit observed in our study is an interesting result. Potential explanation include possibility of bicarbonate secreted by the exocrine pancreas or released as a result of pancreas necrosis accumulating to toxic levels. A well perfused and functioning kidney or dialysis unit may aid in correction of the acid–base environment. Chung et al. reported the development of a liver-kidney normothermic perfusion model which demonstrated significant improvements in glucose levels, acid–base balance and electrolyte values with addition of the kidney (9,21). pH levels were observed to be significantly lower when compared to a liver-only preservation, which tended to be alkalotic. However, this result was not observed in our small series.

To optimally perfuse both organs in one circuit is a substantial challenge, albeit a worthy one, given its potential benefits. A simultaneous pancreas-kidney perfusion circuit has the ability to allow for advanced viability assessment in the frequently used transplant model of SPK, which accounts for 78.9% of all pancreas transplants (22).

Summary and limitations

We achieved simultaneous normothermic perfusion of the pancreas and kidney for 2 h, with a slow but steady increase of perfusate flow rate.

Artif Organs, Vol. 00, No. 00, 2016
Gross appearance and histology correlated well with gross necrosis mainly around acinar tissue. Interestingly, islet cells remain relatively spared. Addition of the kidney did not demonstrate significant improvement in the acid–base environment, which was profoundly alkalotic towards the end of perfusion, likely due to release of bicarbonate by damaged pancreatic tissue.

Intended as a feasibility study, it is limited by the small number of organs experimented on. Future work will focus on perfusion of the pancreas to optimise perfusion parameters, minimise tissue damage and improve the perfusion milieu. Addition of the kidney will be revisited at a later stage to investigate its role as a dialysis unit and improving the acid–base environment of the perfusate. Further investigations will also look into the role of MP of pancreas in islet isolation and transplant.

CONCLUSION

MP as a preservation method is well established in several other organs (heart, lung, and kidney) and have demonstrated reasonable advantages. Its use in the pancreas however is still in infancy and significant amounts of work is required to further define the optimal perfusion parameters before noteworthy progress can be expected. At its current state, normothermic MP have limited benefits over SCS in pancreas transplants. Further optimisation will provide a good opportunity for explant assessment and potentially introduce therapeutic improvements during organ preservation. Our work and those of others recently has demonstrated this model is potentially practicable and more efforts in this field can be expected in the near future.

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Authors’ contributions: KGK participated in writing of paper, conducting of experiments and data analysis; MNW participated in conducting of experiments and data analysis; WYC participated in editing and data analysis of paper; RK participated in editing and data analysis of paper; STM participated in editing and data analysis of paper; AD participated in paper design and editing of paper; GM participated in paper design and editing of paper; MT participated in conducting of experiments, writing and editing of paper.

Conflict of Interest: • • •

REFERENCES


Operative description with illustrations

All four experiments of this study was conducted at an approved animal facility at the Queen Elizabeth Hospital in a temperature controlled operating theatre. The operative and support team included the main author, Mr Markus Trochsler (consultant upper gastrointestinal/hepatobiliary surgeon, the Queen Elizabeth Hospital), Mr Santosh Olakkengil (consultant transplant surgeon, Royal Adelaide Hospital), Dr Mau Wee (surgical registrar) and Mr Matthew Smith (animal facility manager).

Domestic pigs of around 50kg were used for the experiments to best represent the size of an adult patient. Ketamine was administered as a single dose (10mg/kg) prior to induction followed by intubation with an endotracheal tube and continuous general anaesthesia via 2% isoflurane gas in 2 litres/minute oxygen. This model mimics the organ retrieval process of a donation after brain death (DBD) scenario. A 16G intravenous catheter was inserted into the external jugular vein via open cut-down technique. 5000 IU of heparin was administered peri-operatively for prevention of thromboembolism to the microvasculature of the pancreas. The animal was monitored closely throughout the operation for its pulse rate, blood pressure, temperature and oxygen saturation, with any deviations from normal investigated and rectified. 1 to 1.5 litre of 0.9% sodium chloride solution was administered during the duration of the operation to replace intra-operative third space fluid loss.

Laparotomy and pancreas retrieval

A midline incision was performed from the xiphisternum to the pubic symphysis. The abdominal organs are inspected for any congenital or acquired injuries/ abnormalities. Splenectomy is then carried out by ligating (with 0 vicryl ties) and dividing the splenic artery and vein pedicle close to its hilum to avoid compromising blood supply to the tail of the pancreas [Figure 1].
The duodenal-jejunal (DJ) flexure was identified by localising the ligament of Trietz. The duodenum was Kocherised and pancreas exposed. The duodenum was then divided distal to the pylorus and proximal to the DJ flexure with Covidien GIA 80mm blue staplers [Figure 2]. The pancreas was then carefully dissected off its attachments to the retroperitoneum and surrounding organs, preserving the duodenum, taking extreme care not to injure the pancreatic tissue [Figure 3]. All supplying blood vessels to the pancreas was carefully identified and preserved, including the left gastric artery (LGA), splenic artery, gastroduodenal artery (GDA), superior pancreaticoduodenal artery (SPDA), superior mesenteric artery (SMA) and pancreaticoduodenal vascular arcade (PDVA) [Figure 4]. Its draining veins – splenic vein and superior mesenteric vein (SMV) – were also carefully preserved.
Figure 2 Pancreas exposed with duodenum divided distal to pylorus and proximal to DJ flexure. i - Vessel loop around supra-coeliac aorta; ii - duodenum; iii - pancreas; iv - vessel loop around infra-renal aorta; v - left kidney
Figure 3 Pancreas dissected and mobilised off retroperitoneum tissue and attachments. i - retroperitoneal attachments; ii - jejunal loops reflected inferiorly
The abdominal aorta segment from the median arcuate ligament to just proximal to its renal tributaries was then mobilised of its attachments. All tributaries of the mobilised abdominal aorta excluding the coeliac trunk and SMA was ligated with vicryl ties and divided [Figure 5]. The SMA was also ligated and divided distal to its pancreatic supply.
The abdominal aorta, proximal to coeliac trunk and distal to the SMA, as well as the portal vein, after the confluence of splenic vein and SMV, were cannulated with 24-french catheters and secured with 2-0 vicryl sutures [Figure 6]. The distal end of the divided duodenum was also cannulated to collect intestinal and pancreatic secretions. A separate 24-french cannula was inserted proximal to the cannulated segment of aorta for purpose of blood collection.
The animal was then euthanised by exsanguination and the blood collected via the inserted cannula. 1.8 to 2 litres of the animal’s whole blood was collected for use as perfusion medium in the perfusion model. As soon as exsanguination is commenced, the abdominal cavity was filled with crushed ice to minimise warm ischaemic time of the pancreas. When exsanguination is complete, the cannulated aortic segment supplying the pancreas was flushed with 1.5 litres of cold Hartmann’s solution at 0-4ºC. The cold flush was continued until portal vein output is clear. The aorta segment supplying the pancreas was then removed en-bloc and transferred to the back bench in preparation for perfusion. The “organ retrieval time” averaged 90-100 minutes.

**Kidney retrieval**

In the two experiments where the kidney was added to the perfusion circuit, the above described pancreatic retrieval process was followed by retrieval of the kidney [Figure 7]. The left kidney was selected for its longer renal vein. Prior to exsanguination, the left kidney was identified and Gerota’s fascia incised to expose the kidney and its hilum. The renal artery, vein and ureter were carefully cannulated with 12-french catheters and secured with 2-0 silk ties. Similarly, the kidney was flushed with cold Hartmann’s solution once exsanguination is complete. It is then retrieved and transported to the back bench for the perfusion process.
Figure 7 Left kidney in-situ. i - Pancreas reflected superiorly; ii - Left renal vein; iii – Infra-renal aorta.