ORIGINAL ARTICLE

Normothermic ex situ pancreas perfusion for the preservation of porcine pancreas grafts

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Abstract

Pancreas transplantation improves and extends the life of patients with insulin-dependent diabetes. Pancreata from extended criteria donors have been increasingly used due to the scarcity of available grafts. Normothermic ex situ pancreas perfusion (NESPP) can keep grafts metabolically active, potentially allowing for assessment and organ repair, and could improve outcomes of marginal grafts. A novel NESPP technique was developed and tested. Porcine pancreata were removed after a short period of warm ischemia and subjected to 6 h of NESPP. Perfusion parameters, potential graft assessment markers and graft injury were measured. Next, pancreata subjected to 3 h of NESPP were transplanted and animals were followed for up to 3 days. Graft function and injury post-transplantation were evaluated. Using this novel system of perfusion, pancreata were perfused for an extended period of time with minimal edema. Histology at the end of perfusion showed intact islet cells with only mild signs of tissue injury. NESPP transplanted grafts showed immediate function after transplantation, with glucose levels in normal range. NESPP maintains a physiologic environment and excellent graft function without causing significant graft injury. Porcine pancreas transplantation is feasible and allows for in vivo graft assessment of pancreas function and injury after NESPP.

KEYWORDS
basic (laboratory) research/science, organ perfusion and preservation, pancreas/simultaneous pancreas-kidney transplantation, tissue injury and repair

1 | INTRODUCTION

Pancreas transplantation (PTx) is a curative treatment for patients with insulin-dependent diabetes eliminating the need for insulin therapy and controlling symptoms such as hypoglycemic unawareness.[1] For decades PTx was considered a quality-of-life operation, however, recent data suggest that PTx may be 'life-saving'.[2,3] New evidence suggests that life-years are gained with PTx primarily by

reducing the risk of long-term cardiovascular disease.[4-6] However, like many fields in transplantation, due to stringent donor criteria the number of suitable donors limit PTx. Unfortunately, there have been no major advances in pancreas preservation since the development of University of Wisconsin (UW) solution in the 1980s. In addition, allo-islet transplantation also holds some promise in potentially reducing the risk from PTx, but is also limited by suitable donors and poor graft islet cell recovery and engraftment rates.[7]

Due to the severe organ shortage, grafts from extended criteria donors and donation after cardiac death (DCD) have been increasingly accepted for transplantation.[8,9] To decrease preservation injury and better assess grafts before transplantation, research studies have focused their attention on ex situ machine perfusion. Normothermic ex situ machine perfusion preserves organs in an optimal physiologic state, reduces graft injury and since grafts are metabolically active, offers a platform for graft assessment and graft repair for suboptimal organs. Normothermic ex situ machine perfusion has been successfully used for the preservation of lung,[10] liver,[11,12] and kidney[13,14] grafts. So far, ex situ machine perfusion has scarcely been explored for pancreas grafts and normothermic perfusion times have been limited due to graft edema and injury.[15,16] The aim of this study was to develop a stable system for extended normothermic ex situ pancreas perfusion (NESPP) for the preservation of pancreas grafts with the goal of eventually providing a platform for organ assessment and repair.

2 | MATERIALS AND METHODS

2.1 | Animals and study groups

The study was approved by the Animal Care Committee of the University Health Network Research Institute, Ontario, Canada. Twelve-week-old male Yorkshire pigs (~30 kg) were utilized for ex situ pancreas perfusions and 15-week-old male Yorkshire pigs were utilized for the transplant model (~40 kg). All animals received humane care and all procedures were performed in accordance with the "Principles of Laboratory Animal Care" and the "Guide for the Care of Laboratory Animals" published by the National Society for Medical Research and by the National Institutes of Health, respectively.

Pancreata were removed from 30 kg Yorkshire pigs after a short period of warm ischemia (10 min) and subjected to 6 h of NESPP ($n = 7$). Next, pancreata subjected to 3 h of NESPP were transplanted and animals were followed for up to 3 days ($n = 3$). To induce diabetes, complete pancreatectomy was performed in the recipient animal prior to transplantation. Graft function and injury were evaluated. To demonstrate that pancreatectomy in swine leads to diabetes, in two cases pancreatectomy alone was performed and animals were followed for up to 24 h. Glucose levels were closely monitored. Before sacrifice, both in the pancreatectomy alone and pancreatectomy and transplantation animals a glucose tolerance test was performed.

2.2 | Surgical protocol – Pancreas retrieval for NESPP

The anesthetic and surgical procedures were performed as previously described by our group.[17] After anesthesia induction and intubation, general anesthesia was maintained by administration of inhaled isoflurane. Next, a central venous catheter was placed into the internal jugular vein for blood collection and administration of fluids and medications.

After baseline assessment of vital parameters and blood gas analysis, a median laparotomy was performed. Inferior vena cava and distal aorta were separated from each other. Aorta branches were ligated, and renal arteries were isolated and freed from adherent tissue. Liver hilum was dissected, and liver arteries and bile duct were ligated. Portal vein was isolated from the surrounding tissue. The celiac trunk below the portal vein and the mesenteric vessels were isolated. Next, the aorta behind the diaphragm between the heart and the celiac trunk was dissected and a 2–0 tie was placed around it. Next, the pancreas with duodenum and spleen were separated from the surrounding tissue. Sodium heparin (500 IU/kg) was injected systemically. Next, 1.5 L of pig blood was collected in CPDA bags and cardiac arrest was induced by intravenous injection of 40 mEqKCl. Aorta was cannulated and previously set ties around lower aorta, renal and upper aorta were tied off. Next, flush with UW solution containing 10 000 IU/L heparin via inferior aorta was started. The inferior vena cava and portal vein were opened to vent blood and preservation solution. After the first liter of UW solution was flushed, the distal mesenteric vessels (superior mesenteric artery and superior mesenteric vein) were tied off and flush was continued with one more liter of UW. Care was taken to slowly flush the pancreas at a slower rate so that no unnecessary edema occurred. Dissection of pancreas and duodenum was completed, and the pancreas was removed en-block with duodenum, spleen, aorta and portal vein. On the back-table, all arterial branches were tied off and spleen was removed. Next, the portal vein and aorta were cannulated using 3/8" x 1/4" and 1/4" x 3/8" cannulas. The pancreatic duct and duodenum were also cannulated to allow for output measurements during perfusion. The pancreas was then placed in an organ bag and stored on ice until the perfusion was started. Shortly prior to connecting the pancreas to the ex situ system, the organ was flushed with 500ml Albumin.

2.3 | Normothermic ex situ pancreas perfusion (NESPP)

Our NESPP system includes a S3 heart-lung machine and neonatal cardiopulmonary bypass equipment consisting of a centrifugal pump, an oxygenator, a venous reservoir, an arterial bubble filter, and PVC tubing (Sorin Group Inc., Markham, Canada) (Figure 1). Additionally, a heat exchanger and a heat source were built into the system. Perfusion circuit parameters (temperature, arterial and venous pressure, and arterial flow) were continuously
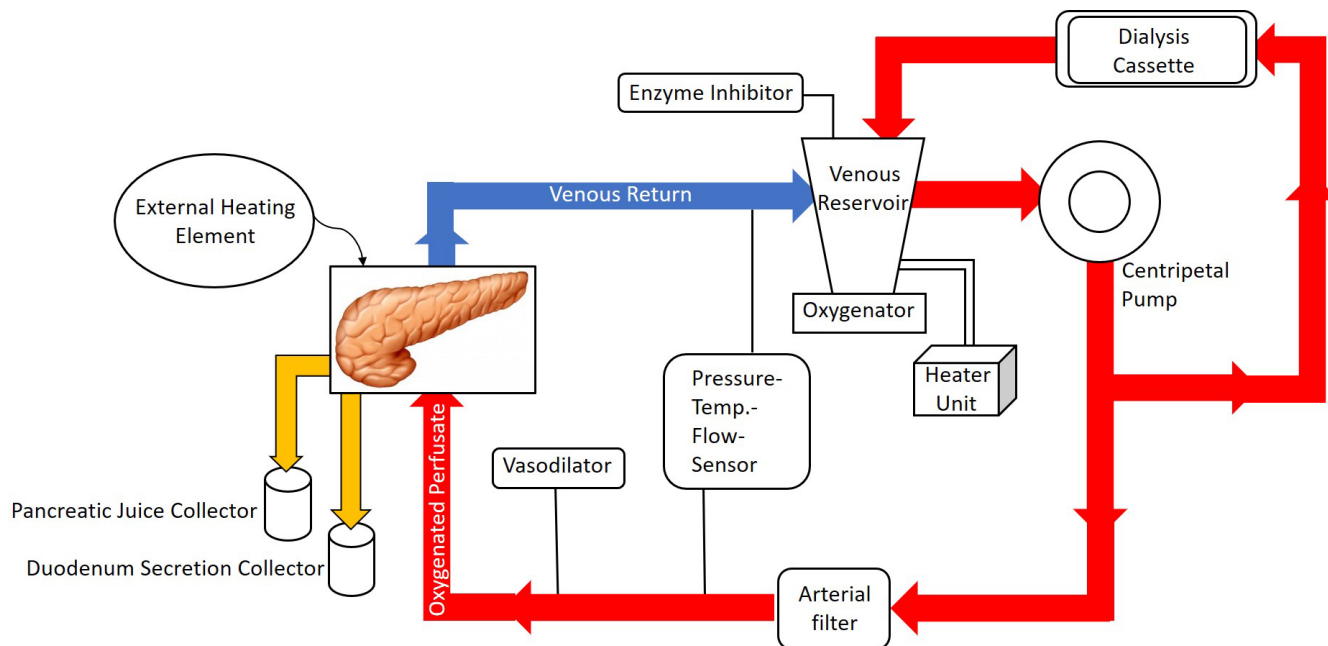
FIGURE 1 Schematic image of the perfusion system. The circuit comprises of neonatal cardiopulmonary bypass technology. A centrifugal pump propels the solution into the oxygenator, where it is enriched with oxygen and warmed to 37°C. After passing the arterial filter, the perfusate is pushed with a pressure of 25 mm Hg through the aorta into the graft located in the customized pancreas chamber. The venous outflow (−3–0 mm Hg) leads the perfusate back into the venous reservoir. The perfusion solution is collected in the venous reservoir. Syringe pumps secure the supply of additional compounds. Pancreas juice and duodenum secretion are collected throughout the perfusion. Perfusion parameters are recorded regularly

recorded. The perfusate solution is made of Ringer's lactate, STEEN Solution (XVIVO Perfusion AB, Goteborg, Sweden), washed leukocyte-filtered erythrocytes, double reverse osmosis water, sodium bicarbonate, calcium gluconate, heparin and aprotinin (Table 1). Oxygen/carbon dioxide gas (95%/5%; 2 L/min) was provided continuously during perfusion, resulting in pO_2 levels around 650 mmHg during the entire preservation time. In addition, epoprostanol (8 ml/h, 0.5 mg dissolved in 250 ml Ringer's lactate) and aprotinin (5 mg/h) were administered continuously during perfusion. Arterial pressure was initially set at 25 mmHg and maintained at 25 mmHg by adjusting the rate of the centrifugal pump. Temperature measured at the oxygenator was maintained at 36°C.

A dialysis system was built in the circuit. Coming from the main reservoir as the starting and end point, the perfusion solution is driven by a centrifugal pump through an oxygenator. After oxygenation of the solution, the perfusate runs through an arterial filter for removal of any emboli or other debris and then the circuit splits into a 60 cm long line running to a dialyzer unit and a 72 cm long line going to the aorta of the graft. The solution that runs through the dialyzer returns to the main reservoir through a 50 cm long tubing. The perfusate is drained through the portal vein back through a 37cm long tubing to the main reservoir.

Perfusate samples were collected regularly and stored at −80°C for further investigation. Additionally, pancreatic juice and duodenal output samples were collected regularly when present.

TABLE 1 Ingredients in perfusate solution and amount or rate administered

Ingredient	Amount/Rate
Stock solution	
Ringer's Lactate	200 ml
Steen Solution	150 ml
Erythrocyte cc. Leucocyte-depleted	125 ml
Double reverse osmosis filtered water (DRO)	27 ml
Sodium bicarbonate (8.4%)	8 ml
Calcium gluconate (10%)	1.8 ml
Heparin (10000 IU/10 ml)	1 ml
Solu-Medrol	250 mg
Aprotinin	30 ml (15 mg)
Continuous administration	
Flolan	8 ml/h (0.5 mg dissolved in 250 ml Ringer's)
Aprotinin	10 ml/h (5 mg/h)

2.4 | Surgical protocol – Pancreas transplantation

The anesthetic and surgical procedures for the recipient animal were performed as described above and previously by our group.[17]

Following anesthesia induction and intubation, general anesthesia was maintained by administration of inhaled isoflurane. Next, a central venous catheter was placed into the internal jugular vein for blood collection and administration of fluids and medications. Under sterile conditions, the right carotid artery was dissected and a polypropylene catheter (18 G) for invasive arterial pressure monitoring was inserted. The catheter was then secured with 2–0 silk ties.[18]

After baseline assessment of vital parameters and blood gas analysis, a median laparotomy was performed. Inferior vena cava and distal aorta were separated from each other, freed from adherent tissue and prepared for anastomosis of the pancreatic graft. Next, the native pancreas is carefully freed from all adherent tissue, making sure not to injure the bowel vasculature. Pancreas is removed completely, leaving the spleen in situ. Before removing the graft from the NESPP system, 2000 IU sodium heparin were injected systemically. The stored pancreas was then flushed with 300 ml heparinized UW and the pancreas anastomoses were sewed using 6–0 monofilament polypropylene sutures (donor portal vein end-to-side to recipient vena cava, donor distal aorta end-to-side to recipient aorta). Following reperfusion, hemostasis and tranexamic acid administration (500mg intravenous) to avoid excessive blood loss. Next, side-to-side anastomosis of the donor duodenum to the recipient small bowel. The abdomen was closed, the animals were recovered and followed for 48–72 h (two animals were followed for 48 h and 1 animal was followed for 72 h). In two animals, after pancreatectomy the abdomen was closed and animals were followed for 24 h.

NESPP perfusion in grafts that were transplanted followed the protocol presented above. Arterial pressure was maintained at 25 mmHg and venous pressure around –1 mmHg. All the other perfusion parameters were in line with the data presented above. After 3 h of perfusion grafts were transplanted and animals were followed for 48–72 h.

2.5 | Sample collection

Blood gas analyses of the perfusate were performed hourly during graft perfusion. Additionally, blood gas analyses of the donor were taken before retrieval and blood gas analyses of the recipient were taken before pancreas retrieval, before and after transplantation and every day during postoperative care. Samples were also analyzed using a point-of-care comprehensive metabolic blood chemistry analyzer (Piccolo Xpress, Union City, Canada) and part of each sample was stored at −80°C for later analysis.

For amylase, lipase and lactate dehydrogenase (LDH) measurements, samples were sent to the Toronto General Hospital Core Laboratory for analysis with the Abbott Architect Chemistry Analyzer using the manufacturer's reagents. For the grafts that only received NESPP, after 4 hours of perfusion, a glucose tolerance test was performed by adding 1ml of Dextrose 50% (Baxter Corporation, Mississauga, Canada) to the perfusate. Glucose, insulin, and c-peptide levels were measured at 1, 5, and 10 min after the glucose administration. For measurement of insulin and c-peptide

enzyme linked immunosorbent assays kit (R&D Systems, Toronto, Canada and Mercodia, Winston Salem, United States) were used according to manufacturer's instructions. For the grafts that were transplanted, no glucose test was performed during NESPP. A glucose test was performed at 48–72 h after transplantation, by administrating 50 ml of Dextrose 50% (Baxter Corporation, Mississauga, Canada) to the recipient animals. Glucose levels were monitored for two hours and samples were taken at multiple timepoints.

Graft edema during NESPP was assessed hourly on a semiquantitative scale from 0 to 3 (0-no edema, 1-mild edema, 2-moderate edema, 3-severe edema).

2.6 | Histology

For the grafts that were not transplanted, after perfusion five biopsies were taken from each graft. One biopsy was taken from the duodenum. Four biopsies were taken from the pancreas graft, which included one from each region in the pancreas; the graft was split in four regions as described by Taylor et al.[19] For the grafts that were transplanted, three biopsies were taken at sacrifice, one from the region next to the duodenum, one from the middle part and one from the tail. All samples were placed in 10% neutral buffered formalin and transferred to 70% alcohol after 36–48 h. Following paraffin-embedding and sectioning (3-μm), hematoxylin and eosin (H&E) stained sections were used to score fat and parenchyma necrosis as well as islet cell integrity on a semiquantitative scale from 0 to 3 (0-no changes, 1-mild changes, 2-moderate changes, 3-severe changes) by a pathologist blinded to the experimental groups. Additionally, duct inflammation and the integrity of islet cells were assessed. For the assessment of islet cells, additional glucagon sections were prepared for the transplanted grafts. The changes were assessed over 10 high power fields (40x) and averaged.

3 | RESULTS

3.1 | Ex situ perfusion experiments

3.1.1 | Animal and graft characteristics

Average animal weight for grafts that received perfusion alone was 32.4 ± 2.2 kg. After pancreas retrieval, all animals were euthanized in a humane manner. Average graft weight after recovery was 143.9 ± 17.6 g. After perfusion, the average graft weight was 201.4 ± 32.1 g, with an average percent graft increase of 40 ± 14.1%. Average animal weight for grafts that were transplanted was 42.5 ± 3.5 kg.

3.1.2 | Perfusion parameters

Normothermic ex situ pancreas perfusion was initiated with an arterial pressure set to 25 mm Hg and was maintained at 25 mm Hg

throughout the whole perfusion. Venous pressure was maintained at around −1 mm Hg by regulation of the height of the venous reservoir. Arterial blood flow rate was initially around 120 ± 21 ml/min. During the perfusion there was a slight decrease in the flow, and towards the end of perfusion arterial flow was around 101 ± 15 ml/min (Figure 2). Duodenum output and pancreatic juice production during NESPP are displayed in Table S1.

Blood gas analyses were performed at baseline (Table 2) and then hourly during normothermic ex situ perfusion. Measurements of acid-base homeostasis, including pH and bicarbonate concentration, remained stable during preservation time and were physiologic when compared with basal values observed in the donor pigs (Table 2, Figure S1). Hourly measurements of venous and arterial pO2 revealed the metabolic activity of the pancreas; oxygen consumption was constant over the course of perfusion, suggesting metabolic activity of the pancreas (Figure 3). Perfusate lactate levels decreased from baseline until the last hour of NESPP (9.97 ± 1.06 mmol/L vs. 2.1 ± 0.4 mmol/L) (Figure 3).

3.1.3 | Graft function and injury

Amylase and lipase were measured hourly as markers of graft injury. Pig amylase at baseline is higher than in humans, with values ranging from 1000–2300 U/L.[20] Figure 4A,B show the trend of the two enzymes over the course of perfusion. Graft edema was assessed hourly; most grafts showed only mild edema at the end of perfusion (Table 3).

Histology showed normal pancreatic parenchyma at baseline with preserved acini, islet cells and fat with intact lobular architecture. Histological features of the pancreatic graft biopsies obtained at the end of perfusion showed preserved pancreatic acini and islet cells, while pancreatic parenchyma had evidence of mild to moderate necrosis (Tables 4 and 5, Figure 5). Parenchymal necrosis was predominantly mild. C-peptide and insulin were detected at stable levels throughout the perfusion. Glucose stimulation test did not further elevate insulin and c-peptide in the perfusate (Figure S2).

Several pro-inflammatory cytokines such as IL2, IL6, IL18, TNFα, and INFγ were present in the dialysate, while anti-inflammatory

FIGURE 2 Perfusion parameters – Arterial Flow. BL, baseline; NESPP 1 – HNESPP 7, individual curves for each of the 7 cases
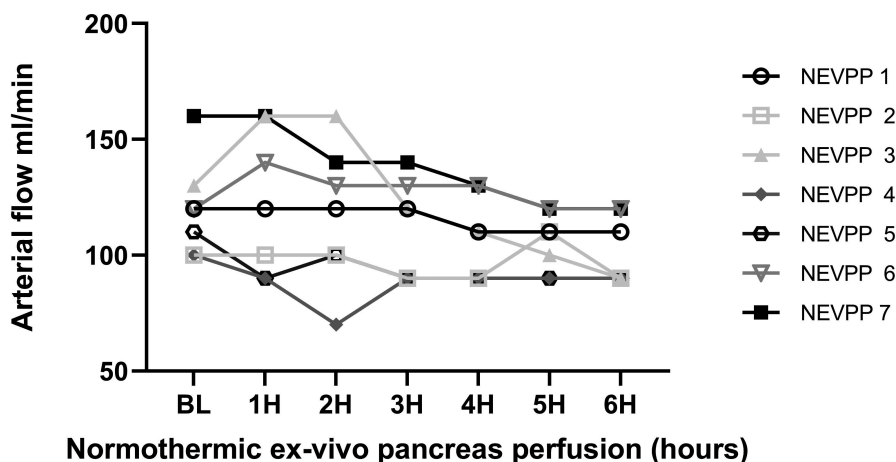
TABLE 2 Blood gas analysis and osmolarity at baseline

| Venous blood gas analysis	Physiologic values for donor pigs at baseline (n = 7)	Values for NESPP setup at baseline (n = 7)
pH	7.34 ± 0.04	7.26 ± 0.08
pCO$_2$	53.63 ± 5.74 mm Hg	44.52 ± 12.24 mm Hg
pO$_2$	52.22 ± 5.49 mm Hg	633.22 ± 31.21 mm Hg
HCO3$^-$	28.68 ± 2.98 mmol/L	19.08 ± 2.35 mmol/L
Hb	102.17 ± 6.91 g/L	67 ± 2.92 g/L
O$_2$ saturation	83.9 ± 4.9%	99.9%
Na$^+$	132.82 ± 1.3 mmol/L	137.7 ± 0.78 mmol/L
K$^+$	3.63 ± 0.28 mmol/L	3.6 ± 0.05 mmol/L
Ca^{2+}	1 ± 0.10 mmol/L	1.17 ± 0.05 mmol/L
Cl$^-$	100.33 ± 1.51 mmol/L	105.17 ± 1.6 mmol/L
Glucose	4.43 ± 0.79 mmol/L	4.08 ± 0.41 mmol/L
Lactate	1.25 ± 0.81 mmol/L	11.14 ± 0.88 mmol/L
Osmolarity	294 ± 3.8 mmol/kg	289.3 ± 1.6 mmol/kg
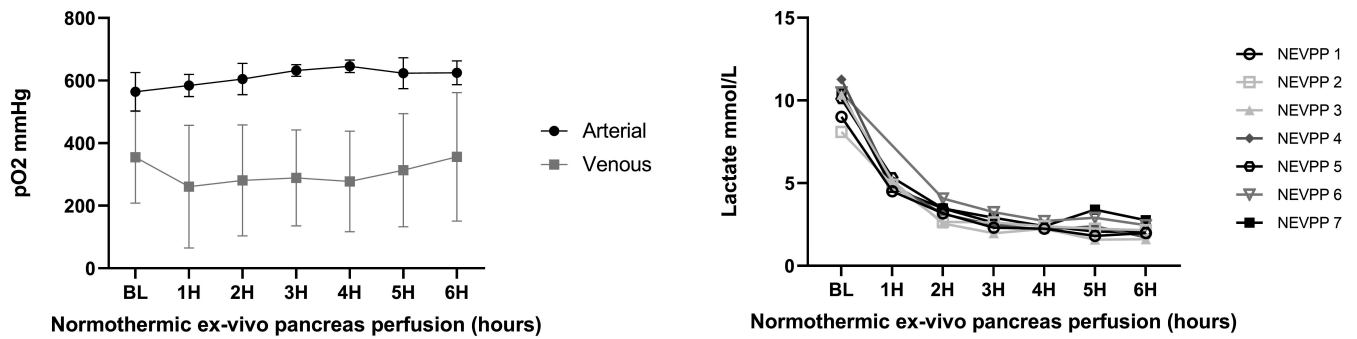
FIGURE 3 Oxygen partial pressure and lactate levels over the course of perfusion. BL, baseline; Arterial, partial pressure of oxygen in the arterial blood gas analysis; Venous, partial pressure of oxygen in the venous blood gas analysis; NESPP 1 – HNESPP 7, individual curves for each of the 7 cases. Oxygen partial pressure values are presented as mean ± SD
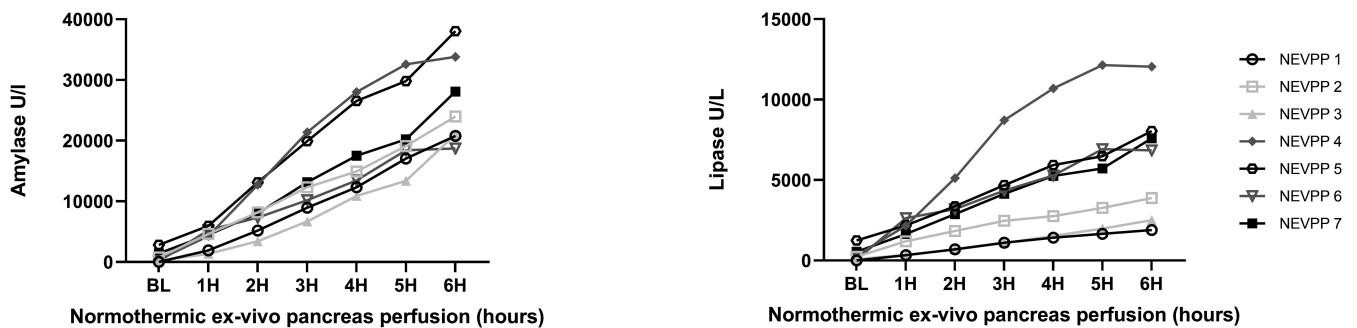

FIGURE 4 Amylase and lipase levels over the course of perfusion. BL, baseline; NESPP 1 – HNESPP 7, individual curves for each of the 7 cases

TABLE 3 Edema assessment during the course of perfusion

Case #	Baseline	1 h	2 h	3 h	4 h	5 h	6 h
NESPP 1	0	1	1	1	1	1	1
NESPP 2	0	1	1	1	1	1	1
NESPP 3	0	1	1	1	1	1	1
NESPP 4	0	1	1	1	2	2	3
NESPP 5	0	0	1	1	1	1	1
NESPP 6	0	1	1	1	1	2	2
NESPP 7	0	0	1	1	1	1	1

Note: Graft edema was assessed on a semiquantitative scale from 0 to 3 (0-no edema, 1-mild edema, 2-moderate edema, 3-severe edema).

cytokines IL10 and TGF-β accumulated in the perfusate over the course of perfusion (Figure 6).

3.2 | Pancreatectomy group

Glucose levels post-pancreatectomy were hourly measured and values are presented in Figure 7A. Due to the high glucose levels post-pancreatectomy, animals were lethargic and without appetite, therefore they were sacrificed at 24 h. Before sacrifice, a glucose tolerance test was performed (Figure 7B). C-Peptide levels post-pancreatectomy and after the glucose stimulation test were stable (Figure 7C).

3.3 | Transplantation group

During the post-operative course, all animals maintained normal glucose levels without any insulin administration (Figure 7D). All pigs were in good clinical condition during the follow-up period. Acid-base homeostasis and electrolyte levels were stable and only calcium substitution was necessary in the first 24 h post-transplantation. Amylase and lipase peaked and on first post-operative day and were almost in normal range for pigs by post-operative day 2 (Figure S3). A glucose tolerance test was performed before sacrifice; glucose normalized within the first hour after glucose administration and remained in normal range after (Figure 7E). C-peptide levels were slightly elevated on POD1 and showed a significant increase at 10 min after the glucose tolerance test was initiated. C-peptide levels normalized within the first two hours after glucose administration (Figure 7F). At sacrifice, all grafts showed pancreatic parenchyma with good preservation of pancreatic acini and islet cells and mild periacinar inflammation and edema (Figure 8).

TABLE 4 Histopathologic changes at the end of perfusion

Case #	Fat necrosis				Parenchyma necrosis			
	Pancreas region 1	Pancreas region 2	Pancreas region 3	Pancreas region 4	Pancreas region 1	Pancreas region 2	Pancreas region 3	Pancreas region 4
NESPP 1	1	1	1 to 2	2	0	0	0	1 to 2
NESPP 2	1	1	0	0	0	0	1	0
NESPP 3	1 to 2	1 to 2	1 to 2	1 to 2	1	1	1	2
NESPP 4	2	1 to 2	1	1	1	1	1	1
NESPP 5	1	2	2	2	2	1	2 to 3	2 to 3
NESPP 6	1	1 to 2	1 to 2	1	0	0	0	1
NESPP 7	2	1	1	2	1	1	1 to 2	1 to 2

Note: Fat and parenchyma necrosis were assessed on a semiquantitative scale from 0 to 3 (0-no changes, 1-mild changes, 2-moderate changes, 3-severe changes).

TABLE 5 Islet cell integrity at the end of perfusion

Case #	Islet cell integrity			
	Pancreas region 1	Pancreas region 2	Pancreas region 3	Pancreas region 4
NESPP 1	0	0	0	0
NESPP 2	0	0	0	0
NESPP 3	0	0	0	0
NESPP 4	1	0	0	0
NESPP 5	0	0	0	0
NESPP 6	0	0	0	0
NESPP 7	0	0	0	0

Note: Islet cell integrity was assessed on a semiquantitative scale from 0 to 3 (0-no changes, 1-mild changes, 2-moderate changes, 3-severe changes).

4 | DISCUSSION

The current study demonstrates that NESPP can be performed for 6 h in a porcine model. During the 6 h of perfusion, pancreata showed stable perfusion parameters, active metabolism, homeostasis and only mild graft injury. Steen solution containing albumin was chosen for this model to regulate the oncotic pressure and stimulate physiologic conditions for the pancreas. We also found that the addition of aprotinin in the perfusate reduced graft necrosis and allowed for longer perfusions. Moreover, we determined that transplantation after NESPP is feasible and safe. Grafts preserved with NESPP showed normal function post-transplantation with glucose levels in normal range without insulin administration.

Early experiments using our system in the absence of dialysis led to an edematous graft after several hours of perfusion. With the inclusion of a dialysis circuit, edema was reduced and allowed for longer perfusion times. We also noted the presence of several pro-inflammatory cytokines in the dialysate, which might have contributed to a lower inflammatory process and graft injury during ex situ perfusion.

Interestingly, a rise in cytokines (both pro-inflammatory and anti-inflammatory) was noted during the perfusion. IL6 and TNFα have been linked to the inflammation process in pancreatitis.[21] TGF-β and IL10 have been shown to have anti-inflammatory properties.[21] IL10 was reported to correlate with severity of pancreatitis, and could potentially be used as a predictive marker for pancreatitis severity.[22] Moreover, effective targeting of IL10 during ex situ lung perfusion has been shown to improve graft viability.[23] Strategies to block IL6 and other inflammatory cytokines during perfusion might further mitigate organ injury and optimize grafts before implantation.

Minimal information is known about ischemia reperfusion injury in the pancreas. In a mice model of ischemia-reperfusion injury, Lunsford et al subjected mice to different amounts of ischemia reperfusion injury and were able to demonstrate a significant inflammatory response in these pancreases as compared with controls.[24] Serum cytokine/chemokine analysis demonstrated significant up-regulation of several cytokines and chemokines including: interferonγ, TNFα, IL2, IL1β, and IL6. This was a very similar profile to the proinflammatory cytokine profile that we noted during NESPP. Interestingly, ischemic preconditioning was noted to improve the impact of ischemia reperfusion in a rat model.[25]

Previous experience with normothermic ex situ porcine pancreas perfusion is very limited. Several studies using both hypothermic and normothermic perfusion were published by the Minnesota group largely in a canine model.[26,27] Hamaoui et al. described their experience with ex situ perfusion of porcine and human pancreata a few years ago.[28] Grafts were subjected to static cold storage for circa 26.5 h, followed by either simulated reperfusion or hypothermic machine perfusion and simulated reperfusion. In this study, graphs were perfused with a pressure of 30–40 mmHg, and flow was 20–60 ml/min/100 g. In our study, despite using a lower pressure of 25 mmHg, we achieved a higher flow, which allowed for a better perfusion of the graft. Also, the authors report that due to hemolysis, amylase assessment was not possible. In our study, hemolysis was only mild, therefore amylase assessment was possible. Despite a short perfusion of only 2 h, Hamaoui et al. reported severe graft damage with moderate edema and severe necrosis of 40–90% of the samples. In our study, grafts showed mostly mild edema after 6hr of perfusion, with mild to moderate tissue necrosis.
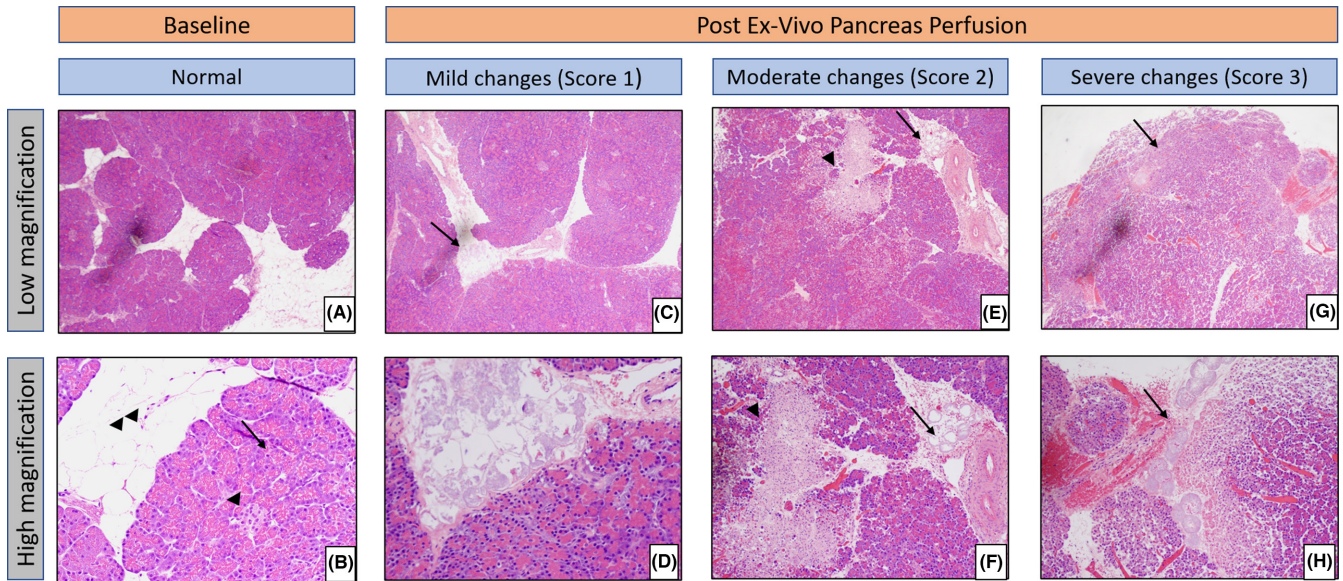
FIGURE 5 Histopathologic changes during NESPP. (A, B) Normal pancreatic parenchyma at baseline with preserved acini (arrow), islet cells (arrow head) and fat with intact lobular architecture (double arrow head). (C, D) Pancreatic parenchyma post perfusion with evidence of mild fat necrosis (arrow; higher magnification in panel D) with preservation of pancreatic acini and islet cells. (E, F) Pancreatic parenchyma post perfusion with evidence of moderate fat necrosis (arrow; higher magnification in panel F) and moderate ischemic necrosis involving patchy areas of pancreatic parenchyma, chiefly affecting the pancreatic acini (arrow head; higher magnification in panel F). (G, H) Pancreatic parenchyma with severe ischemic necrosis and fat necrosis affecting large areas of pancreatic parenchyma (arrow; higher magnification in panel H)
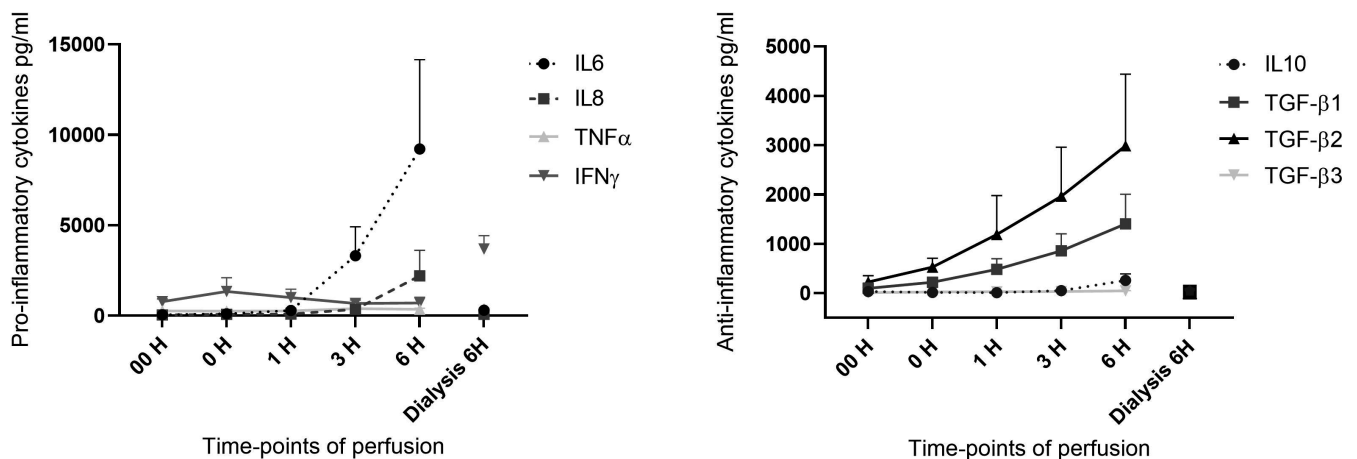

FIGURE 6 Cytokine release during NESPP in the perfusate and dialysate. Dialysis was undertaken over the whole course of perfusion, however this graft only shows cytokine release in the dialysate at 6 h of perfusion

A few years ago, Kumar and colleagues described a model of ex situ normothermic porcine pancreas perfusion.[15] Porcine pancreata were perfused at high (50 mmHg; control group) and low (20 mmHg; "low pressure" group) pressure and graft viability was compared between the two groups. Grafts in the control group achieved a mean blood flow of 140ml/min, while the ones in the low pressure group had a blood flow of only 40 ml/min. Grafts from both groups showed comparable oxygen consumption rates and pancreatic juice consumption rates. Amylase levels were lower in the low pressure group, and immunohistochemistry showed less cellular death in the low pressure group. In our study, despite using a low pressure of 25 mmHg, we

achieved higher blood flow rates (90–160 ml/min) while still maintaining pancreas tissue integrity. Unlike our current study, Kumar et al also failed to demonstrate functionality of the grafts after perfusion.

Recently, Ogbemudia et al presented their experience with pancreas ex situ perfusion. 13 porcine pancreas grafts were procured after circulatory death and subjected to 3 h of SCS. Next, grafts were either stored on ice for further 6 h or were perfused for 6 h by oxygenated hypothermic machine perfusion either in UW Machine Perfusion solution (UW-MPS) or in Institut George Lopez solution. To assess the grafts after perfusion, simulated reperfusion was performed for 1 h. During simulated reperfusion, grafts perfused in
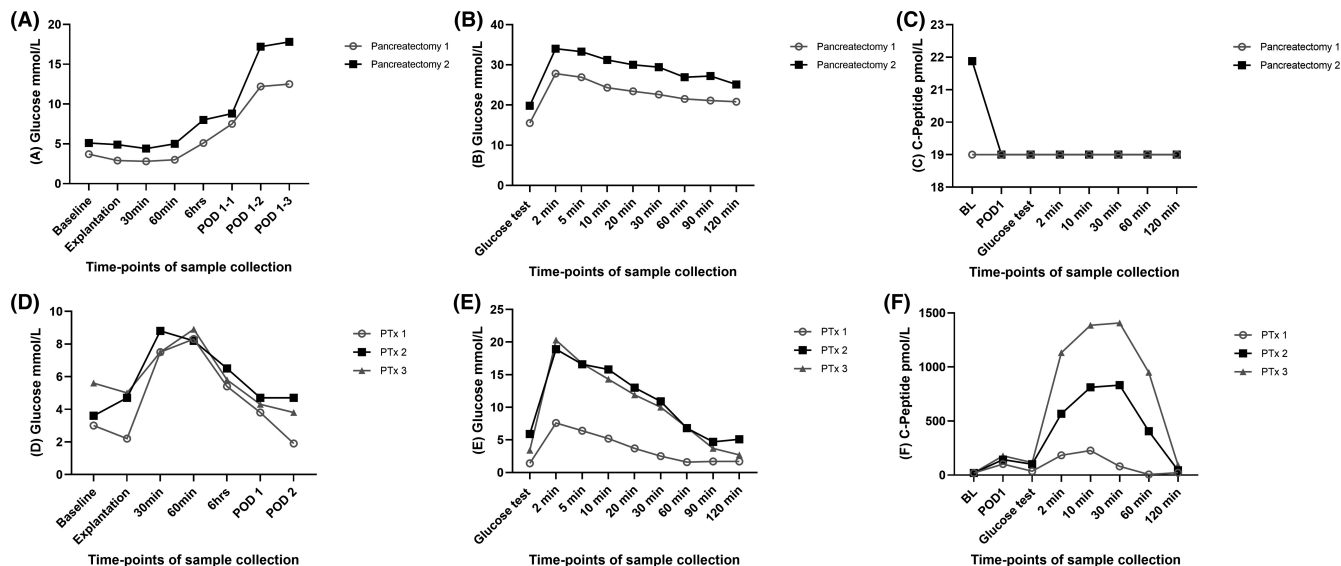
FIGURE 7 Glucose levels after pancreatectomy with/without transplantation and after glucose stimulation test. (A) Glucose levels post-pancreatectomy. (B) Glucose levels after glucose stimulation test in pancreatectomy animals. (C) C-Peptide levels post-pancreatectomy and after the glucose stimulation test. (D) Glucose levels post-transplantation. (E) Glucose levels after glucose stimulation test in transplanted animals. (F) C-Peptide levels post-transplantation and after glucose stimulation test in transplanted animals. POD, postoperative day; POD 1–1, glucose levels on POD1 in the morning; POD 1–2, glucose levels on POD1 at noon; POD 1–3, glucose levels on POD1 in the afternoon. Glucose test, glucose levels before glucose injection; 2–120 min, glucose levels at different time-points measured from the glucose administration time
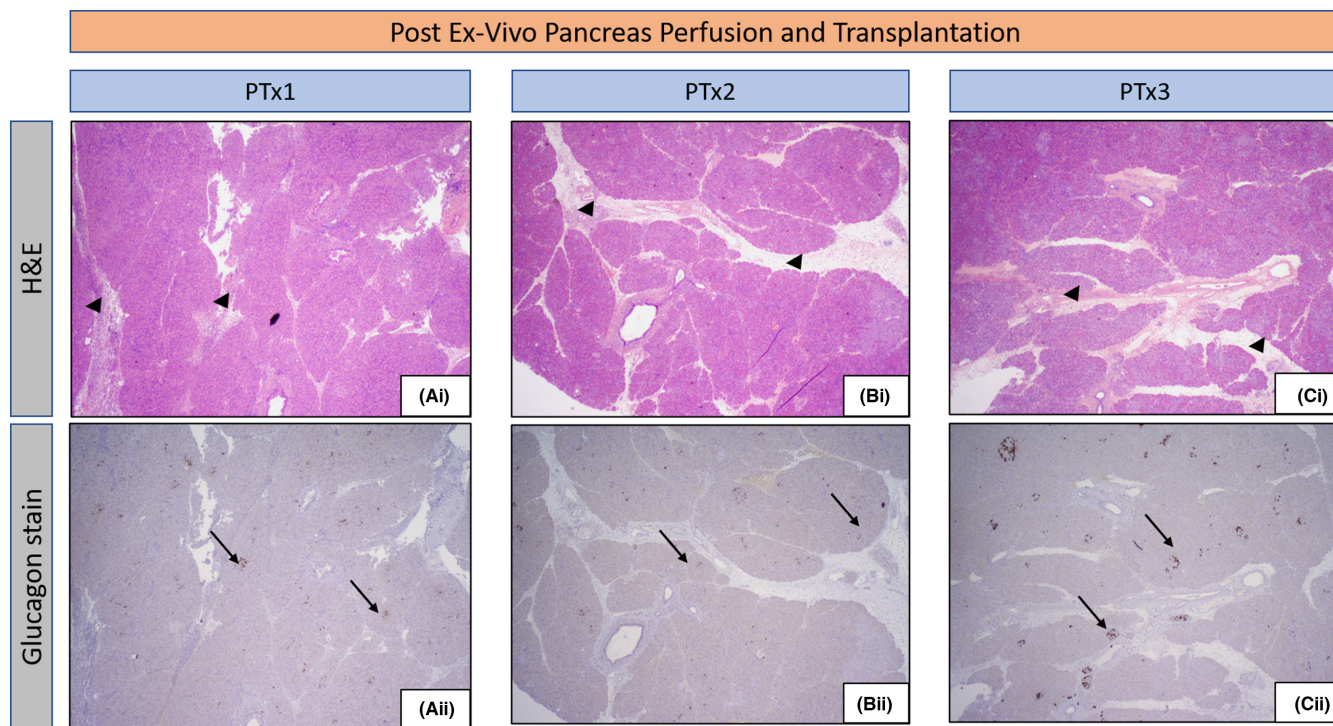
FIGURE 8 Histopathologic changes after pancreas transplantation. [A(i), B(i), C(i)] Three cases post perfusion show pancreatic parenchyma with good preservation of pancreatic acini and islet cells and mild periacinar inflammation and edema (arrow head). [A(ii), B(ii), C(ii)] Glucagon stain for the corresponding cases highlights the preserved islet cells

UW-MPS showed a higher arterial flow, normal macroscopic appearances and a decrease in tissue weight. The authors concluded that oxygenated hypothermic machine perfusion might be better than SCS. Amylase and lipase levels were significantly higher compared to our perfusion system, both during cold perfusion and simulated reperfusion, suggesting a higher injury of the grafts.

In the current experiments, during the perfusion, a continuous c-peptide release was noted from the grafts that appeared to be very constant throughout the perfusion and did not respond to glucose stimulation. Possibly this is due to the constant stimulation of c-peptide release from the basal levels of glucose present in the perfusate. Future perfusion experiments will be dedicated to developing parameters for assessing functionality of grafts while they are undergoing NESPP.

To demonstrate the reliability of our NESPP system, we transplanted grafts perfused for 3 h and observed the animals for 48–72 h. During the follow-up period we found that post-transplantation, NESPP grafts had normal function and can maintain physiologic glucose levels. Although the number of transplanted animals is limited, we demonstrated that porcine pancreas transplantation after NESPP is safe and feasible. Having a transplantation model is extremely important, since graft injury occurs not only during the ischemia period but is a complex process which continues also after reperfusion. Therefore, a transplantation survival model is best suited to fully apprehend the complexity of this process.

Our technique of normothermic ex situ pancreas perfusion has several limitations. This technique is not only challenging to implement, but also cost intensive. Moreover, the parameters we identified for graft assessment might be suitable only in our perfusion setup. Differences in modalities such as perfusion pressures, priming solution, and temperature might not allow the generalization of the assessment parameters to other perfusion models. Translation to a clinical set-up could prove challenging because perfusion characteristics and perfusate biomarkers might not have comparable levels in human and animal scenarios. Future studies will also be performed to further analyze the dialysate in an attempt to better understand its importance in the NESPP circuit and its role in minimizing graft edema during extended perfusions.

The current study demonstrates the safety and feasibility of pancreas transplantation after ex situ pancreas perfusion in a porcine model. Future studies will compare pancreas transplantation after static cold storage with pancreas transplantation after ex situ perfusion in DCD grafts. Our aim is to increase the donor pool for pancreas transplantation by extending the utility of extended criteria grafts. In particular, we aim to make DCD donation a safe alternative to donation after brain death, with a predictable good outcome after pancreas transplantation. In the future, better assessing grafts in an ex situ circulation system and actively improving the graft condition during ex situ perfusion we hope to make grafts, which have been rejected for transplantation in the past, suitable for transplantation.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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