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Persufflation Improves Pancreas Preservation When Compared With the Two-Layer Method

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Abstract

Islet transplantation is emerging as a promising treatment for patients with type 1 diabetes. It is important to maximize viable islet yield for each organ due to scarcity of suitable human donor pancreata, high cost, and the high dose of islets required for insulin independence. However, organ transport for 8 hours using the two-layer method (TLM) frequently results in lower islet yields. Since efficient oxygenation of the core of larger organs (eg, pig, human) in TLM has recently come under question, we investigated oxygen persufflation as an alternative way to supply the pancreas with oxygen during preservation. Porcine pancreata were procured from non-heart-beating donors and preserved by either TLM or persufflation for 24 hours and fixed. Biopsies were collected from several regions of the pancreas, sectioned, stained with hematoxylin and eosin, and evaluated by a histologist. Persufflated tissues exhibited distended capillaries due to gas perfusion and significantly less autolysis/cell death than regions not exposed to persufflation or tissues exposed to TLM. The histology presented here suggests that after 24 hours of preservation, persufflation dramatically improves tissue health when compared with TLM. These results indicate the potential for persufflation to improve viable islet yields and extend the duration of preservation, allowing more donor organs to be utilized.

ISLET TRANSPLANTATION is emerging as a promising treatment for patients with type I diabetes.^{1,2} The minimally invasive nature of the procedure as well as the islets' intrinsic ability to tightly control blood glucose levels on demand via insulin release offer a combination of advantages that cannot be matched by other presently available treatments, such as self-monitoring or whole-organ pancreas transplantation. Despite recent advances building upon the Edmonton Protocol³ published in 2000, most centers still require multiple transplants to achieve long-term diabetes reversal.⁴ The large dose of islets required, the high cost of the

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isolation process, and a shortage of suitable donor pancreata demand that we maximize the viable islet yield from each available organ.

A lack of proper oxygenation during preservation places hypoxic or anoxic stress on the tissue and may predispose islets to death during the isolation process.⁵⁻⁸ It has been postulated that improving pancreas preservation via enhanced oxygenation may (1) extend the effective duration of preservation, (2) expand the donor pool up to 10-fold, (3) reduce the immunogenicity of transplanted islets, and (4) reduce the number of donors needed per patient.⁹ Presently, the two-layer method (TLM) is the state of the art for pancreas preservation. TLM involves suspending the pancreas between cold preservation solution and a preoxygenated perfluorochemical with the goal to improve oxygenation. Recently, the efficacy of TLM has come under scrutiny. While it is adequate to oxygenate smaller pancreata (eg, rat, segmented dog pancreas), diffusion limitations may prevent adequate oxygenation of larger pancreata (eg, pig, human).¹⁰⁻¹¹ Several recent large retrospective studies also reported that TLM provides no significant benefit to isolation outcome, highlighting the need for continued development of pancreas preservation protocols.¹²⁻¹⁴ Persufflation (PSF; ie, vascular gas perfusion) is one such solution presented here. This method actively delivers oxygen throughout the organ for the duration of preservation, eliminating prolonged periods of cold ischemia prior to isolation.

METHODS

Procurement

Adult Landrace pigs were systemically heparinized with 100,000 U of heparin and euthanized by pentobarbital overdose. The pigs were exsanguinated and eviscerated. The aorta was opened longitudinally followed by cannulation of the celiac trunk (CT) and superior mesenteric artery (SMA) (Fig 1). The pancreas was then flushed with 5 L of heparinized cold preservation solution (CPS; ie, H-Phase II with 2% penta-starch + 1000 U heparin per liter) and the pancreatic duct cannulated and infused with 60 mL of CPS for ductal preservation. Surface cooling was maintained by applying 2 L of moist crushed lactated Ringer's solution (LRS) to the surface, and the surgical field was kept clear by irrigation with chilled LRS. The pancreas with associated vasculature was then resected en bloc from the viscera as described by Ferrer et al.¹⁵

Preservation

Upon removal from the viscera, the pancreas was divided, the connecting and duodenal lobes placed onto TLM, and the splenic lobe preserved by PSF for the duration of the experiment. All lobes were stored at 4°C throughout preservation.

Two-Layer Method

TLM was performed as previously described.¹⁶ Briefly, lobes were suspended halfway between 1 L of CPS and perfluorodecalin, which was preoxygenated for 1 hour prior to preservation by bubbling with 99% oxygen gas.

Persufflation

Following division of the pancreas, the splenic lobe was tested for vascular leaks by gentle hand perfusion followed by careful inspection for bubbles upon initiation of PSF. All arterial leaks were tied off. The lobe was then submerged in CPS, and PSF performed with 20 mL/min of humidified 40% oxygen gas supplied to both the CT and SMA at the minimum pressure from which a steady flow could be achieved (typically < 20 mm Hg) for the duration of preservation. Oxygen was supplied from a portable electrochemical oxygen concentrator (Giner Inc, Newton, Mass, USA) and chilled to 4°C before entering the lobe.

Histology

After 24 hours, the tissues were fixed in 10% buffered formalin for histological analysis. Biopsies were then collected from several regions, embedded in paraffin, sectioned at 4 μm , and stained with hematoxylin and eosin. All slides were then evaluated by an experienced histopathologist (T.D.O., who was blinded as to treatment when slides were scored for extent of autolysis/cell death).

RESULTS

Biopsies taken from organs preserved for 24 hours by PSF exhibited distended capillaries and less autolysis/necrosis as compared to tissues preserved by TLM for 24 hours (Fig 2). Of particular note are the pyknotic nuclei observed in TLM samples, which suggest that irreversible damage has occurred, with some cells in the latter stages of cell death. Occasionally, in the earlier stages of technique development, clots were observed in PSF organs, preventing gas flow to these tissues. Histologically, these regions exhibited increased autolysis correlating with the presence of red blood cells and pyknotic nuclei. When biopsies were compared from two of these organs, there was significantly greater autolysis present in nonpersufflated regions ($P = 4.82\text{E}^{-7}$; Fig 3). These findings indicate that PSF improves tissue health compared to TLM.

DISCUSSION

Improving pancreas preservation during organ transport represents a key area of interest for improving islet isolation and transplantation outcomes. Recent studies have suggested that TLM is inadequate to oxygenate large pancreata (eg, human, porcine), especially their core, and this may limit its usefulness for pancreas preservation. PSF offers an alternate way of delivering oxygen to the entire organ throughout preservation by using its native vasculature. The histological analysis presented here suggests that PSF dramatically improves tissue health when compared with static methods of organ preservation, such as TLM, for 24 hours. This agrees with recent porcine isolation outcomes in our lab, which in a paired comparison show greater numbers of islets of a higher quality following culture as determined by oxygen consumption rate to DNA ratio, a measure of viability,¹⁷ and islet morphology. In the clinical setting, PSF may expand the deceased donor pool by improving the preservation of marginal organs, in turn leading to more successful transplantations. We conclude that pancreas PSF should be investigated further to assess its potential as a novel method of pancreas preservation.

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Fig 1. Schematic illustrating how the organs were persufflated starting with the electrochemical oxygen concentrator and showing the paths taken by the gas upon entering the organ via celiac trunk and superior mesenteric artery (SMA) until exiting the organ via the portal drainage system. SMV, superior mesenteric vein.

Fig 2.
Representative histological sections from porcine pancreas preserved for 24 hours by **(a)** the two-layer method and **(b)** persufflation. The presence of pyknotic nuclei is indicated by arrows.

Fig 3. Effect of persufflation on necrosis/autolysis (%). Biopsies were examined from 11 regions with persufflation (black) and 16 regions without persufflation (white) from two porcine pancreata. Lines indicate the mean response.