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# Hypothermic Perfusion of Pancreas: Emphasis on Preservation Prior to Islet Isolation

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## Abstract

Procurement and preservation of pancreata is important for both segmental gland transplantation and islet isolation as a prelude to islet transplantation as a potentially new option for the treatment of Type I diabetes mellitus. The methods described here focus on the latter application and apply the LifePort kidney platform transporter to prolonged pancreas hypothermic machine perfusion (HMP). Twenty-four-hour HMP results in uniform fluid accumulation within the organ that in turn provides a disrupted extracellular space with beneficial effects for islet isolation without compromising islet viability and function. The method, developed and validated for juvenile porcine pancreata, can be easily applied to human and adult porcine donor pancreases, the latter being regarded as the source of choice for xenogeneic islet transplantation. The successful methods described rely strongly upon the details of pancreas surgical procurement, cannulation, and perfusion on the LifePort. Based on this method, future studies can be designed for pancreas hypothermic perfusion optimization, to develop methods of organ evaluation and quality control during perfusion in order to reliably select high-quality pancreases for clinical transplantation.

Key terms: hypothermic perfusion preservation, cold storage, pancreas preservation, porcine pancreas preservation, juvenile porcine pancreas, porcine islets

## 5.1 Introduction

The application of hypothermic perfusion technology is a topic of current clinical interest due to the potential to have a salutary impact on the mounting clinical challenges to improve the quantity and quality of donor organs and the outcome of transplantation. In the case of the pancreas, earlier studies in the late 1970s demonstrated that pancreas hypothermic preservation by machine perfusion is feasible and can be safely extended to 24 and 48 hours [1–5]. Dedicated renal perfusion systems were employed mainly [1, 2, 4, 5] after appropriate modifications required to accommodate the physiologic low flow and pressure needs of the pancreas [3]. The latter helps avoid excessive organ edema that postsegmental transplantation and reperfusion ~~has been documented to result~~ in subcapsular bleeding, hemorrhagic necrosis, venous congestion, and hemorrhagic pancreaticoduodenal secretions [4]. On the other hand, a moderate degree of perfusion-induced edema has recently been reported to assist in postperfusion processing in the special case of pancreas preservation prior to islet isolation as a prelude to the treatment of Type I diabetes by islet transplantation [6, 7]. This is a relatively new interest that provides the focus of the methods outlined here.

There is now a worldwide consensus that islet transplantation may be considered a viable option for the treatment of insulin-dependent diabetes mellitus, and clinical trials are underway at many centers around the world [8, 9]. As this approach for curing diabetes transitions into a routine clinical standard of care, the demand for donor islets will escalate. Moreover, the potential for xenotransplantation to relieve the demand on an inadequate supply of human pancreases will also be dependent upon the efficiency of techniques for isolating islets from the source pancreases. Procurement of donor pancreases for islet isolation and transplantation is not yet widely practiced due in part to concerns about postmortem ischemia upon functional islet yields. As we have reviewed recently [10], perfusion/preservation technology has had a major impact in circumventing ischemic injury in kidney transplantation [10–14]. Here we describe the application of this approach to the preservation and procurement of viable islets after hypothermic perfusion preservation of porcine pancreata since pigs are now considered the donor species of choice for xenogeneic islet transplantation for a number of compelling reasons [15].

The scientific basis for hypothermic perfusion preservation of organs is founded upon the effect of temperature on all biologic and chemical processes, which are fundamentally slowed by a reduction of temperature. Hence, the deleterious consequences of ischemia and anoxia can be attenuated by the application of hypothermia, which has provided the cornerstone of most of the effective methods of organ preservation in common use today [10, 16]. Hypothermic perfusion preservation is based upon the fundamental premise that devices can be designed to facilitate the replacement of blood in the circulation of an ex vivo organ with specially designed ~~fluids~~ to maximize the protective effects of hypothermia on the ischemic tissue.

Since the advent of clinical organ transplantation in the 1960s, a variety of perfusion machines have been developed principally for kidney preservation, but until recently these were not employed clinically due to the relatively high cost and complexity compared with simple cold storage techniques (see recent reviews [10, 17, 18]). Today, there is a growing use of machine perfusion for donor kidney preservation due to the reported

effect of improved outcome using so-called “marginal” or “expanded criteria” donor organs. This technique, therefore, has a major potential impact upon increasing the numbers of organs available for transplantation [10]. One of the commercially available machines (~~LifePort; LifeLine Scientific~~) approved for clinical use for kidneys is currently being evaluated for pancreas preservation; here we describe the methods and technological challenges associated with the application of this technique for pancreas preservation prior to islet isolation. A major technological issue to be addressed in applying the established LifePort kidney perfusion technique to the pancreas is the different perfusion parameters required by the pancreas since this is a low-flow, low-pressure organ compared with the kidney. Typically, the optimum perfusion parameters for a kidney on a LifePort machine, which by design is a pressure-controlled device, are a set perfusion pressure of 25–40 mmHg, which typically produces a flow rate of 100–150 ml/min [11]. These perfusion parameters critically impact the fluid exchange between the vascular and interstitial compartments of the organ and hence the degree of edema sustained during the perfusion interval. The method described here relates specifically to the adaptation of the LifePort for pancreas perfusion with emphasis upon low pressure (10 mmHg)-controlled perfusion of porcine pancreas as a prelude to pancreas processing for islet isolation [6, 19].

## 5.2 Experimental Methods and Materials

The success of porcine pancreas hypothermic perfusion for islets isolation strongly depends on the surgical procedure of organ procurement and pancreas cannulation for ex vivo machine preservation. The development of a porcine pancreas surgical recovery method has not been an obvious procedure. Initially, the lack of detailed pig pancreas anatomy documentation has led to improper organ vasculature preservation during pancreas procurement. Inadequate organ procurement has resulted in inconsistent and incomplete pancreas machine perfusion, thus low islet yield and viability. Nevertheless, using the pig-human pancreas physiological and topographical similarities [20, 21], we have established and implemented a porcine pancreas surgical procurement method that we have successfully validated over the last three years by using more than 100 islet isolations [6, 10, 19].

### 5.2.1 Materials

#### 5.2.1.1 Surgical Procurement of Pig Pancreas

- Animal designated research surgical facility (the OR should provide adequate environment and instrumentation to ensure proper pig anesthesia, ventilation, and vital signs monitoring during pancreas procurement);
- Domestic Yorkshire male farm pigs, 25 to 32 kg;
- Pancreas recovery cooler containing: one aortic cannula (size 18, Brad), a two spikes “Y” irrigation set (Medline), one sterile biohazard bag, 1L of cold UW solution (SPS-1, Organ Recovery Systems)—pack cooler half way with ice for organ transportation from the OR to the isolation lab;
- Lactated Ringer’s solution, 2L (B Braun Medical.).

#### 5.2.1.2 Pancreas Cannulation for Machine Perfusion

- Surgical tray and instruments (Mayo and Metzenbaum scissors, DeBakey forceps, curved and straight hemostatic forceps, micro-surgery spring scissors, needle holders);
- Gauzes (4 × 4 inches) and umbilical tape (10-inch segments);
- Sterile suture, coated Vicryl, 4-0, RB-1, 17 mm, 1/2c taper needle (Ethicon);
- Sterile ties, 0 (3.5 metric) silk, black braided (Ethicon);
- Surflo-winged infusion set, 21G × 3/4 inch, 12-inch tubing, V = 0.45 mL (Terumo);
- Sterile needles (16G, 18G) and 20 cc syringes;
- LifePort disposable 10 × 35-mm and 7 × 20-mm ~~sealring~~ cannulae (Organ Recovery Systems);
- LifePort disposable 3-, 5-, 8-mm straight cannulae and coupler (Organ Recovery Systems);
- Perfusion solution, 1L, (KPS-1, Unisol-UHK, Organ Recovery Systems, ~~Inc.~~);
- Tissue weighing scale;
- Ice machine and trays.

#### 5.2.1.3 Pancreas Machine Perfusion

- LifePort ~~k~~kidney ~~t~~transporter, pulsatile configuration (includes insulating cover, ice container, power and data acquisition cable, batteries, Organ Recovery Systems, ~~(ORS), Inc., Itasca, IL;~~
- Organ cassette (includes vented dual leads and organ cradle with cannula mount, ~~ORS~~;
- Perfusion circuit frame with built-in pressure sensor (includes filter and compliance chamber, ~~ORS~~;
- Data recording station (computer and data station software).

### 5.2.2 Methods

All animal care and handling should comply with the *Principles of Laboratory Animal Care* as formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council (National Academy Press, 1996).

#### 5.2.2.1 Surgical Procurement of Pig Pancreas

1. A team of at least two operators is recommended for pancreas procurement.
2. Follow surgical facility requirements for dress code and personal protection equipment.
3. Verify with the OR veterinary technician that the pig is intubated and under general anesthesia (i.e., ketamine 22 mg/kg, acepromazine 0.2 mg/kg, and atropine 0.025 mg/kg); confirm with the OR veterinary technician of pig anesthesia maintenance and proper ventilation.

4. Verify with the OR veterinary technician that all vital signs are monitored (ECG, heart rate, oxygen saturation level, body temperature, and so forth).
5. Verify that an electrical knife, a suction line, and canisters are available.
6. Verify that the OR back field surgical table has been properly prepared (surgical instruments, lap sponges, gauze, cold saline, umbilical tape, and so forth).
7. Verify that 2L of cold Lactated Ringer's solution have been placed on ice.
8. Verify that an i.v. pole is available near the operating table and its height is appropriate for the gravity driven in situ flushing of the organs (6 to 6.5 feet).
9. Obtain permission from the OR veterinary technician to proceed with the surgery.
10. Minimize pancreas exposure to warm ischemia to 3 minutes, unless otherwise required (e.g., experimental design to study the effects of warm ischemia).
11. When permission has been granted, perform a midline incision from the xiphoid cartilage to just above the pelvis and expose the abdominal cavity.
12. Instruct the OR veterinary technician to administer heparin to the pig (150 U/kg); allow at least 3 minutes to pass before starting in situ flushing.
13. Move and keep aside the bladder and the intestines (with the help of lap sponges) and identify the descending aorta.
14. Dissect, below the kidneys, a segment (3 cm) of the aorta apart from the surrounding tissue/vessels; place umbilical tape ties around the aortic segment; cut a small opening into the aorta between the two ties while the surgery assistant applies pressure on the aortic walls to prevent blood from squirting out.
15. Insert aortic cannula into the opening and tie it in place (make sure the umbilical tape tie is securely placed over the collar of the cannula).
17. Insert the two spikes of the irrigation set into the appropriate infusion ports of the two bags of Lactated Ringer's solution (make sure the roller clamp is closed to prevent solution loss).
18. Hang the bags of Lactated Ringer's solution on the i.v. pole and flush the irrigation set tubing to properly remove all the air; close the roller clamp.
19. Cross-clamp the inferior vena cava and the aorta above the diaphragm.
20. Connect the inlet opening of the cannula to irrigation set outflow port and open the roller clamp to initiate the gravity driven in situ flushing.
21. Cut open the inferior vena cava above the diaphragm, downstream from the clamp for blood outflow.
22. Immediately place plenty of ice inside the abdominal cavity around the pancreas and liver for organs maintenance/protection at low temperature.
23. Use the suction tubing and containers to collect the wash-out blood.
24. Make sure the solution flow from the bags, through the cannula into the aorta is not obstructed and that there is outflow from the inferior vena cava
25. When empty, remove the bag of Lactated Ringer's solution from the i.v. pole and hang the bag of SPS-1 solution (previously kept on ice); use only half of the SPS-1 solution volume to flush the organs.
26. Instruct the OR veterinary technician to euthanize the pig using a lethal dose of 5% sodium pentobarbital administered intravenously [accepted form of euthanasia according to the American Veterinary Medical Association Panel on Euthanasia (AVMA) guidelines] and complete in situ flushing.

27. Transfer the second half of the SPS-1 solution bag to the pancreas transportation biohazard bag and place the latter on ice.
28. Carefully and rapidly (less than 15 minutes) proceed to expose and dissect apart the pancreas from the surrounding tissue and organs (add ice around the visceral organs as needed); make sure pancreas capsule and integrity are maintained.
29. Keep a segment of proximal duodenum (from near pylorus and inclusive of most the duodenum second descending part) attached to the pancreas head; make sure the duodenum segment includes the opening of the pancreatic duct (Figure 5.1).
30. Ligate the splenic vein and artery prior to spleen detachment.
31. Keep a 5–7-cm-long aortic segment attached to the pancreas for future organ cannulation; the aortic segment should include the openings of both superior mesenteric artery (SMA) and celiac trunk (CT).
32. Remove pancreas from the body, and with the aortic cannula attached, quickly wash off the blood from the pancreas outer surface using cold saline; immerse the pancreas in the SPS-1 solution inside the transportation bag.
33. Place the bag with the pancreas on ice inside the pancreas cooler for transportation to the islet isolation laboratory.

*NOTE:* This surgical approach has been proven optimal for porcine pancreas machine perfusion for islet isolation. The inclusion of the duodenum segment with the pancreas head helps eliminate the leaks from the small vessels diverging from the superior pancreaticoduodenal arteries (Figure 5.1). Thus, pancreatic vasculature integrity is main-

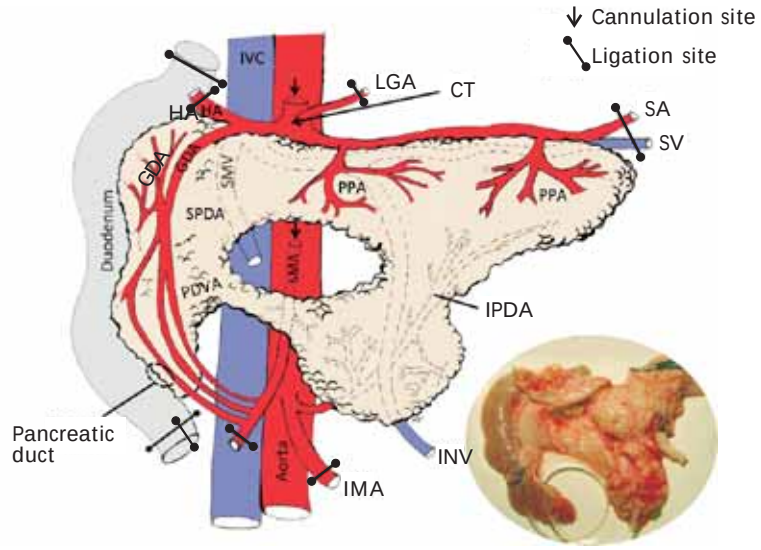


**Figure 5.1** Anatomy of the excised porcine pancreas. Schematic diagram showing the pancreas excised with a segment of the descending aorta for cannulation of the celiac trunk (CT) and superior mesenteric artery (SMA). The splenic vein (SV) and artery (SA) are ligated. All arterial branches on the margin of the gastroduodenal and hepatic side were ligated to provide uniform perfusion and effluent flow only through the portal vein. Photo insert shows an example of an excised porcine pancreas with intact segment of duodenum and catheterized pancreatic duct. *Labels*—IVC: Inferior vena cava; LGA: Left gastric artery; HA: Hepatic artery; GDA: Gastroduodenal artery; SMV: Superior mesenteric artery; SMA: Superior mesenteric artery; PPA: Posterior pancreatic artery; SPDA: Superior pancreaticoduodenal artery; PDVA: Pancreaticoduodenal vascular arcade; IPDA: Inferior pancreaticoduodenal artery; IMA: Inferior mesenteric artery; IMV: Inferior mesenteric vein.

tained and complete uniform perfusion of the pancreas head and neck is ensured. Moreover, the opening of the pancreatic duct into the duodenum is preserved. This considerably facilitates pancreatic duct cannulation by avoiding the difficulties encountered with retracted duct identification and cannulation, and preserves early ductal branches.

#### 5.2.2.2 Pancreas Cannulation for Machine Perfusion

1. A team of two operators is recommended for pancreas cleaning and cannulation.
2. Perform pancreas cannulation at the isolation laboratory in order to reduce static cold ischemia damage prior to machine perfusion.
3. Minimize pancreas exposure to static cold ischemia to less than 2 hours; static cold ischemic time is the time elapsed from the initiation of in situ flushing to the beginning of machine perfusion.
4. Transfer the pancreas from the transporting cooler to the stainless steel surgical tray; place the latter on ice and dispense 20–30 mL of SPS-1 solution from the transporting bag into the tray to help keep the pancreas moist and cold.
5. Remove the aortic cannula; clean away all miscellaneous tissue while paying attention to maintaining pancreas integrity; identify and expose the SMA and CT vessels.
6. Dissect the aortic segment at midline to expose the orifices of SMA and CT; at this point the SMA and CT orifices should be clearly seen positioned apart on the aortic cuff (1.5 cm × 4 cm).
7. Place and secure ~~in place~~ the appropriate size ~~seal-ring~~ cannula; the correct size should enclose both SMA and CT orifices without obstruction and clearly allow for their visualization through the top clear wall of the cannula.
8. Test for leaks; fill a 20-cc syringe with the solution to be used for perfusion, attach the syringe to one end of the cannula, remove the air inside the cannula, and cap the other end of the cannula; gently infuse the solution into the pancreas and identify any leaks from exposed vessels.
9. Meticulously identify and ligate all exposed leaking arterial branches on the margin of gastroduodenal and hepatic sides of the pancreas (use umbilical tape and/or silk ties appropriately).
10. Cannulate the pancreatic duct; remove the needle from the surflo-winged infusion set and use its tubing as the duct cannula; using the microsurgery scissors, cut an opening into the pancreatic duct at its originating location on the duodenum and insert the cannula; secure the latter in place by tie suturing it to the duodenum wall.
11. Measure and record pancreas weight (subtract cannula weight).

*Note:* The identification and tight ligation of all exposed vessels on the hepatic and gastroduodenal sides of the pancreas are of high importance. Usually 12–14 vessels are tied prior to perfusion to eliminate the possibility for a pathway of “least resistance” for the flow throughout the organ and to allow the effluent to emerge only from the portal vein. Leaks from open exposed vessels compromise the uniformity of the organ perfusion that in turn can lead to pressure and temperature gradients across organ surface and suboptimal pancreas preservation.

#### 5.2.2.3 Pancreas Machine Perfusion

See Figure 5.2.

1. Fill up the ice container with a mixture of ice and cold water (consult the LifePort operation manual); place the container in the transporter main enclosure.
2. Place the organ cassette inside the cassette well; install the perfusion circuit tube frame on the pump deck and close the aluminum locking arm; connect the pressure sensor to the pressure transducer.
3. Press POWER to turn on the user controls of the transporter and follow the directions of the outer display to get the transporter ready for perfusion.
4. Add 1L of cold perfusion solution to the organ cassette; set the infusion pressure to 10 mmHg on the control panel; verify that the ice container temperature, as indicated by the outer display, is below 8°C.
5. Press WASH to start the pump and circulate the perfusate throughout the circuit; make sure all the air from the circuit is removed.
6. Place the pancreas (with the duodenum attached) inside the cassette, and position the organ cannula in the cannula mount of the cradle; connect the cannula inlet port to the infusion line and open the cannula outlet port.

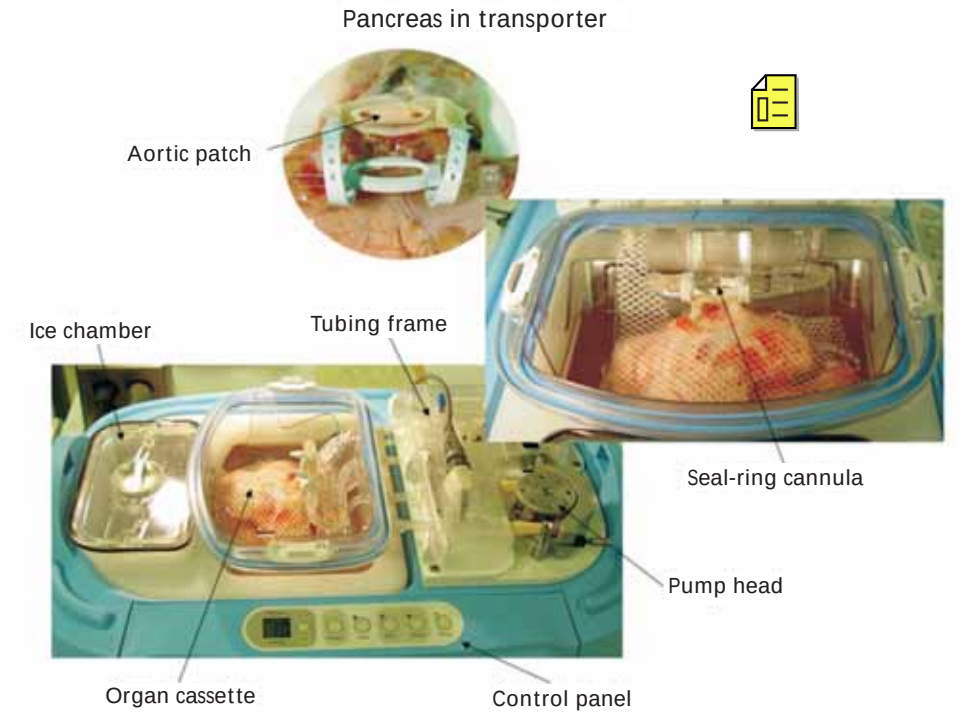


**Figure 5.2** Hypothermic perfusion preservation of a porcine pancreas on a LifePort ~~machine.~~ The lower panel shows the principal features of the LifePort. The middle panel shows the details of a pig pancreas installed in the perfusion cassette and hooked up to the perfusion inlet line via a seal-ring cannula. This proprietary cannula allows simultaneous perfusion of the celiac trunk (CT) and superior mesenteric artery (SMA) by way of an aortic patch clamped in the seal-ring cannula (see circular inset). The inset photo shows the opening of the CT and SMA in the aortic patch (AP), which was exposed for viewing by opening the seal-ring cannula.

7. Press PRIME to remove the air from the cannula and infusion line, and then cap the cannula; the latter will stop the flow and the pump based on the detected resistance.
8. Press STOP; press INFUSE to initiate the pancreas perfusion mode; watch for the pump to begin rotating and to increase its speed until the pressure set point is reached (e.g., 10 mmHg).
9. Ensure real-time visualization and recording of flow parameters on both outer display and data station; the perfusion parameters as displayed on the outer panel are: pressure set point (systolic pressure, mmHg), flow rate (mL/min), resistance (mmHg/(mL/min)), temperature (ºC, within the insulated cold section of transporter, i.e., ice container). To read the infusion temperature (ºC) and diastolic pressure (mmHg) press the scrolling arrows on the right side of the outer display to sequentially toggle through these additional parameters.
10. Allow pancreas perfusion for 24 hours; stop the pump and save the data file (includes the dynamics of all perfusion parameters).
11. Remove the pancreas from the cassette; measure postperfusion pancreas weight and record it; determine the level of fluid accumulation within the organ (edema, %).

*Note:* LifePort ~~transporter~~ allows for closed loop pancreas pulsatile perfusion at an imposed systolic pressure (10 mmHg) while the organ is immersed in cold perfusion solution inside the cassette. The described perfusion method has been developed for prolonged (at least 16-hour) hypothermic ex vivo perfusion of pancreata recovered from young porcine donors (about 2 months old).

For the specific application of islet isolation after pancreas perfusion preservation, the pancreas was removed from the LifePort ~~machine~~ by uncoupling the cannula(s) from the cassette and transferring the pancreas to a separate tray for postperfusion weighing. Thereafter, the pancreas can be processed in a conventional way for islet isolation involving ductal infusion of appropriate enzyme, trimming of the pancreas to remove extraneous tissue, and chopping into 7–9 pieces and loading into a Riccordi chamber for enzymatic digestion at physiological temperature. Finally, the washed digest is purified using a density gradient to harvest the isolated islets [19]. An alternative technique for pancreatic distension by enzymatic infusion on the LifePort without first removing the pancreas is also available [22].

## 5.3 Data Acquisition and Anticipated Results

Juvenile pig pancreata recovered, cannulated, and perfused using the aforementioned methods are successfully preserved for up to 24 hours on the LifePort ~~transporter~~. As shown in Table 5.1, prolonged hypothermic perfusion results in uniform fluid accumulation within the organ (136 ± 12%, $n$ = 19) even at low perfusion pressure (10 mmHg) [6, 19]. The edema proves to be advantageous for islet isolation. It provides a disrupted extracellular space that helps ~~free rapidly~~ more islets during subsequent enzymatic digestion and generates a more homogeneous digest, with less mantled and entrapped islets, in comparison to fresh and static stored pancreata (see Figure 5.3 and Table 5.1). The hypothermic perfusion also preserves islet function and viability (Table 5.1) [6, 19].

**Table 5.1** Islet Yield and Function Indices

| Pancreas/ Islet Characteristics | FRESH (untreated control) [N = 10] | SCS (Viaspan) [N = 9] | HMP# [N = 19] |
|---|---|---|---|
| Pancreas weight (g) | 112 ± 6 | 118 ± 5 | 101 ± 2 |
| Postpreservation edema (%) | — | –2.8 ± 0.7 | 136 ± 12 |
| Total islet yield (IEQ × 1,000) | 147 ± 31 | 75 ± 16 | 165 ± 20* |
| Insulin stimulation index | 5.8 ± 1.1* | 2.5 ± 0.4 | 3.8 ± 0.5* |
| High-glucose insulin [ng/mL/IEQ] | 0.27 ± 0.1 | 0.20 ± 0.05 | 0.25 ± 0.04 |
| Insulin content [ng/mL/IEQ] | 9.35 ± 3.1 | 4.75 ± 1.00 | 9.92 ± 1.7* |

*$p < 0.05$ versus static cold storage group (SCS).
#Pooled data for perfusion using either KPS1 (n = 9) or Unisol UHK (n = 10) perfusates for which there appears to be no significant difference in current ongoing studies [19].

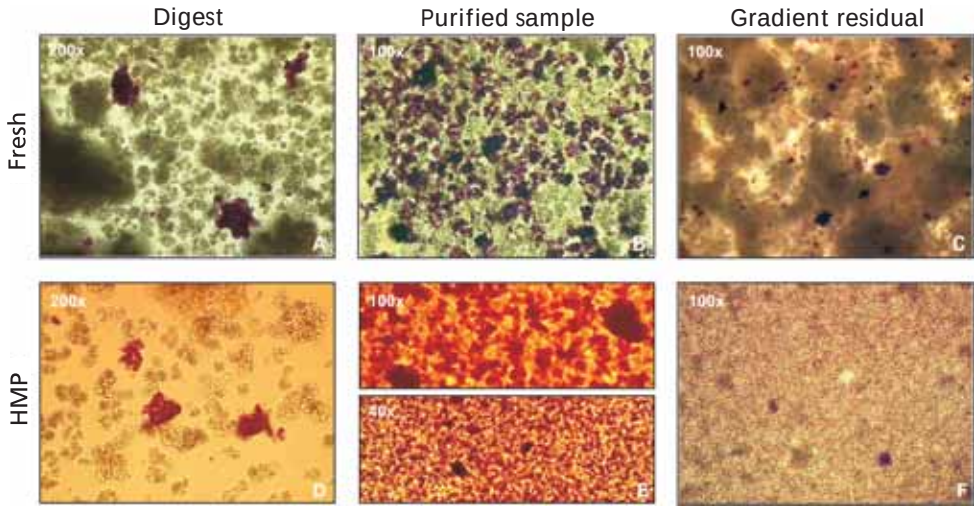


**Figure 5.3** Effect of HMP on subsequent islet isolation. Light micrographs, at various magnifications as indicated, showing the presence of isolated islets at different stages in the processing of both fresh (panels a–c) and HMP pancreases (panels d–f). Islets are identified by dithizone staining and appear purple-red in contrast to the unstained exocrine tissue, which appears gray-brown. The pancreatic digest stained during the enzymatic processing shows a typically more uniform digest and isolated cleaved islets in the perfused pancreas (d) compared with the more nonhomogeneous digest observed using freshly isolated pancreas (a). The more homogeneous digest typically derived using perfused pancreases often resulted in a cleaner separation of isolated islets on the density gradient yielding a more highly purified preparation of islets (e) compared with either fresh (b) or statically cold stored pancreas (not shown) [21]. This differential separation during gradient purification was also manifest in examination of the gradient residual, which in the case of fresh pancreas often included many trapped or embedded islets (c) compared with perfused pancreases, which showed a "clean" residual fraction with very few identifiable islets (f). This apparent differential effect on islet separation and purification was also manifest in the yield of islets obtained as an end product (see data in Table 5.1).

Pancreas perfusion on the LifePort is monitored using four parameters: perfusion pressure (mmHg, systolic, and diastolic), perfusate flow rate (ml/min), vascular resistance [mmHg/(mL/min)], and temperature (°C). The temperatures of the perfusate and the insulated cold section of the transporter (ice container) are measured. All these parameters are recorded and displayed in real time by the data recording station as illus-

trated in Figure 5.4. During perfusion, each one of these parameters dynamics can be visualized and later correlated with the perfusion and/or islet isolation outcome. Hypothermic machine perfusion of young pig pancreata is performed at an infusion temperature between 5°C and 7°C. The ice container has been specifically designed to

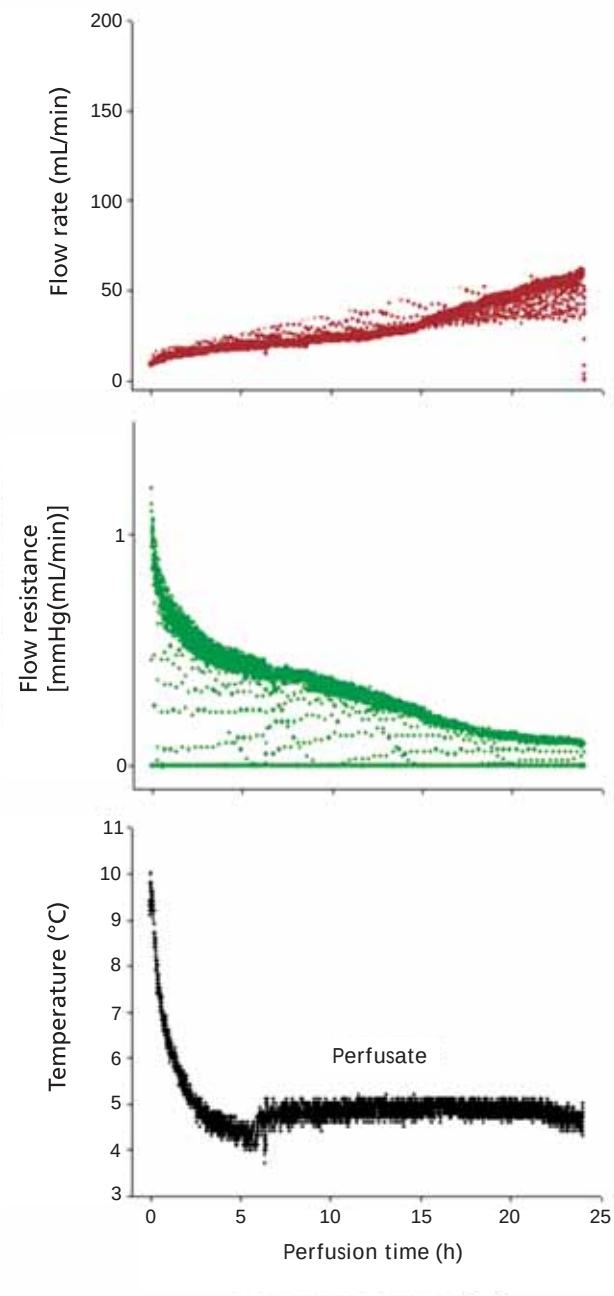


**Figure 5.4** Pancreas perfusion parameters monitored continuously during perfusion. Physical parameters (flow rate, resistance, and perfusate temperature) measured on the LifePort ~~perfusion machine~~ during 24-hour hypothermic perfusion of a porcine pancreas at a set pressure of 10 mmHg. Pancreas weight = 87g; the level of edema after 24 hours = 139%; and the islet yield = 247,950 IEQ.

accommodate this temperature range through the volume of ice/water mix and the heat exchange characteristics. Also, by design the pump is programmed to stop if the temperature of the ice container rises above 8°C, as read by the temperature sensor located outside the container in the main enclosure and in intimate contact with container wall. Under these circumstances the preservation reverts to static cold storage for the remaining duration unless there is operator intervention to restart the pump.

A properly performed organ in situ flushing and limited pancreas ischemia exposure prior to perfusion should result in low organ vascular resistance. The latter is illustrated by immediate organ perfusion initiation and/or a constant reduction in vascular resistance and increase in flow rate throughout the duration of perfusion (Figure 5.4). For open flow circuits, with leaking pancreata, tubing, and fittings, the transporter fails to maintain the imposed infusion pressure, thus resulting in erroneous perfusion and pump inactivation. This event is remedied by operator intervention to identify and correct any leaks responsible for the low vascular resistance status.

## 5.4 Discussion and Commentary

There is now a worldwide consensus that islet transplantation may be considered a viable option for the treatment of insulin-dependent diabetes mellitus, and clinical trials are underway at many centers around the world [8, 9]. As this approach for curing diabetes transitions into a routine clinical standard of care, so the demand for donor islets will escalate. Moreover, the potential for xenotransplantation to relieve the demand on an inadequate supply of human pancreases will also be dependent upon the efficiency of techniques for isolating islets from the source pancreases. Procurement of donor pancreases for islet isolation and transplantation is not yet widely practiced due in part to concerns about postmortem ischemia upon functional islet yields. As we have reviewed recently [10], perfusion/preservation technology has had a major impact in circumventing ischemic injury in kidney transplantation [10–14]. Here we applied this approach to the preservation and procurement of viable islets after hypothermic perfusion preservation of porcine pancreata since pigs are now considered the donor species of choice for xenogeneic islet transplantation for a number of compelling reasons [15].

### 5.4.1 Pancreas Perfusion on the LifePort Transporter

The LifePort pulsatile perfusion system has been successfully employed for small pig pancreas hypothermic ex vivo perfusion for up to 24 hours. The system is designed and FDA cleared for kidney hypothermic perfusion/preservation for clinical transplantation. Using the kidney system the whole pancreas of young porcine donors (25–32 kg, 2 months old) can be continuously perfused in a closed loop while being completely immersed in the perfusion solution inside the organ bath. The latter also serves as a solution reservoir, the perfusate being drawn out by the pump, forced to go through the filter, bubble trap, and the infusion port before returning to the pancreas and organ cassette. Pancreas submersion in the temperature-controlled perfusate helps eliminate temperature gradients across the organ surface and ensure high quality hypothermic preservation. Also, it ensures proper cold static storage of the organ if the pump fails and the fluid transport through the organ stops. Inside the closed transporter, a properly

filled ice container can be maintained at a temperature below 6°C for more than 24 hours, without ice replenishment. The LifePort ~~transporter~~ allows for 1 liter of perfusate recirculation at 5–7°C by a pulsatile (30 pulses/min) constant low pressure (10 mmHg) flow.

#### 5.4.1.1 Application to Adult Pig Pancreas Perfusion

Perfusion of pancreata from large adult porcine donors (more than 2 years old and over 400 lbs) can also be performed using the LifePort ~~transporter~~. However, a few adjustments need to be considered:

1. Higher perfusion pressure values need to be set to balance higher organ resistance and increased vasculature (by design, the transporter can regulate the infusion pressure between 10 and 65 mmHg);
2. The cradle needs to be removed from the organ cassette to allow for the entire pancreas immersion in the cold perfusate, for proper temperature control; or
3. The cradle needs to be removed for pancreas lobular perfusion (i.e., head, tail, and so forth can be separated and individually perfused). Straight cannulation (discussed later) of the main vessels of the selected pancreas segment is considered for lobular perfusion.

Lobular perfusion is an alternative option to whole pancreas perfusion when the organ size exceeds cassette volume. Following recovery from the body and cleaning, the pancreas lobes are identified and visually delimited from the surrounding tissue. Ferrer et al. have recently documented in great details the anatomy of the pig pancreas and the variations in its vascular and ductal configuration [23]. This paper also validates the basis for our aforementioned pancreas surgical recovery model. For lobular perfusion, the major vessel(s) of each pancreas lobe/segment are individually straight cannulated (cannula inserted into the vessel lumen). If more than one cannula is used, a coupler is employed to join all cannulae and to connect them to the infusion port as illustrated in Figure 5.5. For example, the SMA, or its branch, and the splenic artery are recommended for machine perfusion of the pancreas tail using straight cannulation.

#### 5.4.1.2 Application to Human Pancreas Perfusion

Hypothermic perfusion of human pancreata can be performed following the steps of young pig whole pancreas perfusion method, with the exception of the cannulation site and cannula type. Under normal clinical recovery protocols, human pancreata are procured without the aortic patch, but with the duodenal segment attached and with intact vasculature. In this case the SMA and splenic artery are individually straight cannulated (as discussed below) and simultaneously perfused during pancreas machine preservation (Figure 5.6). The transporter organ cassette without modification can accommodate the human pancreas. However, in comparison to the pig pancreas, the human pancreas is highly fibrotic. We believe that this fact, along with donor medical history, needs greater consideration for optimizing the perfusion pressure of the human pancreas and should be the subject of future research.

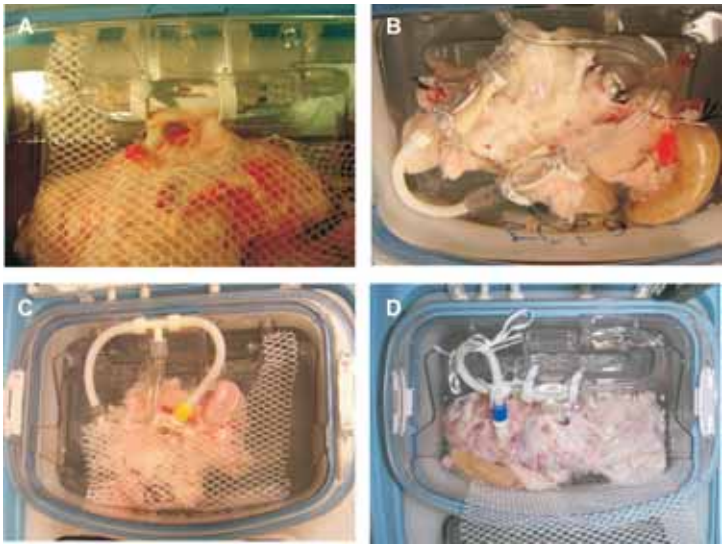


**Figure 5.5** Variations in the method of cannulation for pancreas perfusion on the LifePort ~~transporter~~. (a) Preferred and recommended method of cannulation for juvenile pig pancreas using the proprietary seal-ring cannula (~~LifeLine Scientific~~), which avoids the need to insert cannulas into the individual arteries by allowing perfusion via the openings of the CT and SMA on an aortic patch clamped inside the ~~seal-ring~~ cannula (see also Figure 5.2). (b) Dual ~~seal-ring~~ cannulas each supporting the openings of the SMA and CT on individual aortic patches and linked via a coupler. This arrangement is useful and necessary when the openings of these two main arteries are spaced too far apart to be accommodated in a single ~~seal-ring~~ cannula. (c) Straight cannulation of a large pig pancreas, or pancreatic lobe using insertion cannulas coupled together via a "T" connector for linking with the infusion port. (d) A combination of a seal-ring cannula on one artery linked to a straight insertion cannula on the other artery. This configuration can be used as variant of the arrangement in (b) for larger pig pancreases or those in which the openings are anatomically too far apart for a single ~~seal-ring~~ cannula to be used.

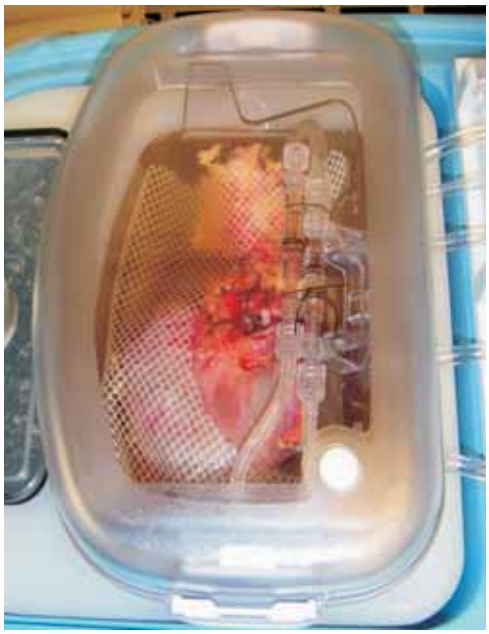

**Figure 5.6** Hypothermic perfusion of human pancreas on the LifePort ~~transporter~~. Illustration of a human pancreas hooked up on the LifePort via dual straight cannulation of the splenic and superior mesenteric arteries. The cannulas are coupled for attachment to the infusion port.

#### 5.4.1.3 Perfusates

The nature of the perfusate is also known to have a marked effect upon the quality of the perfusion preservation but this is a topic of other publications [19, 24, 25]. The methods described here were developed principally using ~~KPS1~~ (Organ Recovery Systems), which has FDA clearance for clinical machine perfusion.

### 5.4.2 Pancreas Cannulation for Perfusion

During the course of protocol development, different pancreas cannulation approaches were considered prior to establishing the final configuration. These included the aortic segment cannulation and the individual straight-cannulation of CT and SMA. Their implementation was problematic due to incompatibilities with the LifePort ~~design~~ and juvenile pig pancreas anatomy:

1. For the aortic cannulation, a 5–7 cm-long aortic segment, inclusive of both superior mesenteric and celiac trunk artery openings, is ligated at one end and straight cannulated (6.25-mm OD cannula) at the other end. The cannulated end is attached to the pump infusion port. Under this configuration, possibly due to aortic segment elasticity, the pump was unable to reach or sustain its targeted perfusion pressure. By design, under these circumstances the LifePort is configured to try to compensate by increasing its speed until the maximum allowed value is reached (240 mL/min); thereafter the pump stops. These conditions of increased pump speed inevitably result in higher fluid accumulation in the tissue as reflected in a doubling of the glandular edema. At this point, usually within 6–12 hours from perfusion onset, the pump stops and the organ preservation reverts to conventional cold static storage by fluid immersion only without circulating perfusate. This phenomenon is likely to be associated only with the research model configuration involving an aortic segment and is unlikely to have any clinical relevance or significance.
2. Using the straight-cannulation insertion method, the CT and SMA vessels are individually cannulated with 4-mm OD luer-to-barb connectors that are directly inserted inside the two vessels [Figure 5.5(c)]. The two connectors/cannulae are joined together with either a coupler (Figure 5.6), or a “T” connector [Figure 5.5(c)]. The latter is attached to the pump infusion port. In the case of young pig pancreata, this approach is dependent on organ anatomy, and in many cases has provided inconsistent perfusion and increased flow resistance that ultimately leads to flow ending, pump stopping, and incomplete organ perfusion for reasons that will now be discussed. In young pig pancreata, several small vascular branches diverge early from both the celiac trunk and superior mesenteric artery and can be blocked by the cannula tip (illustrated in Figure 5.7). Although the cannula is normally advanced only 6 mm inside the vessels (20 mm long), obstructing the flow from the cannula to the branches leads to no perfusion, or differential perfusion across the organ surface. Based on our experience, this event occurred in about one third of the attempted porcine pancreas perfusions. In marked contrast, straight cannulation of the SMA and splenic artery of human pancreata is a simple, basic procedure that is not subject to the same anatomical constraints as the porcine pancreas. As for the porcine pancreas, the two cannulae are connected with a coupler that in turn is attached to the transporter infusion port (see Figure 5.6). The human pancreas is

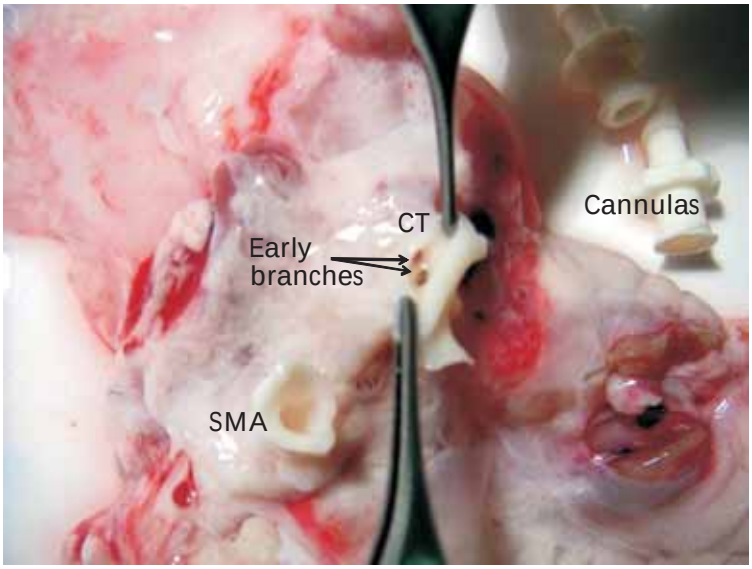


**Figure 5.7** Vascular cut-down to illustrate anatomical variants with early diverging side branches. Successful perfusion of the pancreas, especially from young pigs, via the SMA and celiac trunk requires extreme care to avoid occlusion of early side branches by inserted cannulas such as those illustrated here. These anatomical constraints are prevalent in young pig pancreata as illustrated by the vessel cut-down shown here. The risk of undesirable occlusion of these side branches is avoided by use of a ~~seal-ring~~ cannula as described in the text and illustrated in Figure 5.2.

also perfused with the duodenal segment attached. The diameters of the straight cannulae vary according to human pancreas size and anatomy; normally they cover the range of 3 to 10 mm.

For the porcine pancreas, all flow problems are eliminated by using the ~~seal ring~~ cannula for perfusion. Its geometrical design allows for direct flow to the pancreas CT and SMA vessels without interference. The cannula is placed on the aortic patch inclusive of the two vessel openings, without obstructing the vessels as illustrated in Figures 5.2 and 5.5(a). This contrasts with the use of insertion cannulas that enter the arterial lumen and potentially impede or occlude the openings to vascular side branches. The ~~seal ring~~ cannula provides a sealed flow link between the pancreas and perfusion system and ensures 24-hour continuous ~~uniform~~ perfusion without undesirable events.

### 5.4.3 Adaptation for Future Applications

Pancreas perfusion can further be applied for the preservation of organs exposed to warm ischemia prior to islet isolation and to optimize pancreas preservation solution for a better islet yield and quality. Organ preservation prior to islet isolation can allow for more time for proper donor-recipient matching and quality control of isolated cells, and offers the possibility of banking cells for increased availability to clinicians. Hypothermic machine perfusion can provide an answer to the pancreas shortage for transplantation by improving flow and reducing vascular resistance and allowing for pancreas quality evaluation prior to transplantation as we have reviewed elsewhere recently [10].

However, there is a high requirement for optimized pancreas perfusion, pancreas evaluation methods, and quality control during machine preservation. The methods of pancreas perfusion described in this chapter are based on a low (10 mmHg) constant pressure driven flow. Physiologically, the pancreas is a low flow organ. The present design of the LifePort does not accommodate infusion pressures less than 10 mmHg. Lower pressure values might be needed to clinically preserve pancreata for transplantation without inducing high levels of edema that can be irreversibly detrimental to the organ and recipient [10]. Less edema and better perfusion outcome for both islets isolation and whole pancreas transplantation may be better attained by employing a constant flow regime as opposed to constant pressure. The former requires optimization of the driven flow rate values in accordance with organ characteristics and quality (warm ischemia exposure, size, species) and is the subject of future planned research.

## 5.5 Troubleshooting

Maintaining the ~~transporter~~ clean and ready for use (according to the operation manual) should ensure its proper functioning. The ~~transporter~~'s four batteries should be properly installed within the provided compartment and the transporter should be constantly connected to a grounded AC power source to maintain the batteries fully charged. Only manufacturer approved accessories (e.g., batteries, perfusion circuits, power and data cables) should be used. For the ice container a mixture of ice and water should be employed to ensure that the organ preservation temperature requirements (< 8°C) are satisfied. To initiate the various modes of operation of the LifePort, it is important that the operator first press the STOP command to alternate between flow modes (WASH, PRIME, INFUSE) as explained in the operation manual. Following completion of each experiment, the perfusion data should be saved to an ancillary computer (Excel format).

In spite of all recommended precautions, some events can lead to device failure as outlined in the Troubleshooting Table. These are due mainly to operator's errors and/or the condition of the organ. Problems can arise during organ perfusion that compromise pancreas preservation quality and islet isolation outcome. The most frequent errors and faults are listed in the Troubleshooting Table, along with the recommended corrective actions.

## 5.6 Application Notes

The applications of hypothermic machine perfusion of isolated organs have been reviewed recently with emphasis on the clinical perspective [10]. Specific applications to the pancreas are as follows:

1. Pancreas preservation prior to segmental transplantation.
2. Pancreas preservation prior to islet isolation and subsequent infusion into Type I diabetic patients. The inherent edema that develops during prolonged HMP has been shown recently to have a salutary benefit for the specific application of pancreas preservation prior to islet isolation [6, 10, 19].

**Troubleshooting Table: LifePort Perfusion**

| Fault Message | Problem Explanation | Potential Solutions |
|---|---|---|
| Check ice | Ice container temperature above 8°C. | 1. Make sure container includes a mixture of ice and water.<br>2. Replenish ice.<br>3. Using gauze, clean the temperature sensor located behind the ice container to remove moisture and the ice film, if formed.<br>4. Press INFUSE or WASH to restart the pump. |
| Load perfusion circuit | Improper installation of tube frame. | 1. Make sure flow circuit tube frame is correctly positioned around the pump head and between bubble detectors.<br>2. Make sure the frame locking arm is in the correct position. |
| 80Check tubing | Unexpected condition in the perfusion circuit. | 1. Make sure flow circuit tube frame and locking arm are in the correct position.<br>2. Make sure all air bubbles have been removed from the infuse line.<br>3. Check for tubing occlusions.<br>4. Check valves. |
| High resistance | System is measuring resistances above 3 [mmHg/(mL/min)]. | 1. Make sure the CT and CMA are not twisted.<br>2. Make sure the cannula does not block the CMA and CT openings.<br>3. The organ might contain significant residual blood or has been damaged during procurement. |
| Can't reach pressure | Pump cannot achieve the set infusion pressure. | 1. Check for leaks in the flow circuit and tighten loose fittings or replace circuit if necessary.<br>2. Visually inspect cannula, CMA, and CT vessels for leaks; tighten cannula and close-suture the leaks in the vessels.<br>3. Visually inspect pancreas for small arterial leaks; aseptically ligate the open vessels.<br>4. The filter is clogged, replace circuit or if available, replace the filter. |
| Bubbles | Trapped air bubbles within the circuit cannot be removed without operator's intervention. | 1. Check perfusion circuit for loose fittings.<br>2. Wash the flow lines to remove air trapped in the upstream bubble detector.<br>3. Reprime the infuse line and the cannula. |
| Power up test failed | An error occurred during the power-up self test. | 1. Make sure the transporter is plugged in and/or the batteries are charged.<br>2. Make sure the power cord is properly inserted into the transporter AC plug.<br>3. Power the transporter Off then On. |

## 5.7 Summary Points

1. The LifePort ~~transporter~~, which is FDA cleared for clinical kidney preservation for transplantation, can be successfully used for juvenile pig pancreas hypothermic machine perfusion for islet isolation for transplantation. Prolonged ex vivo hypothermic perfusion of the pancreas preserves islet function and viability and facilitates, in comparison to fresh pancreata, faster release of more islets during subsequent enzymatic digestion.

2. The surgical procedure of juvenile porcine pancreas recovery is of critical importance. The pancreas needs to be recovered intact, with a segment of duodenum and an aortic segment inclusive of SMA and CT arterial openings. The latter provides the aortic cuff for unobstructed placement of a ~~seal-ring~~ cannula over the openings of the SMA and CT allowing continuous uniform perfusion of the pancreas. This technique provides the preferred method that avoids the risk of vessel occlusion when using straight insertion cannulas in young pig pancreases.
3. The back-table preparation of the pancreas for ex vivo closed-loop perfusion on the LifePort ~~transporter~~ is focused on the identification and tight ligation of all exposed marginal vessels on the hepatic and gastroduodenal sides of the organ. If left open, the leaking vessels facilitate a pathway of "least resistance" that in turn compromises the uniformity and quality of organ perfusion.
4. The present configuration of the LifePort ~~transporter~~ can easily accommodate perfusion of a human pancreas through cannulae directly inserted into the SMA and splenic artery. For the preservation of adult porcine pancreata on the LifePort ~~transporter~~, lobular perfusion needs to be considered. Due to the large size of the adult pig pancreas, individual lobes should be carefully separated surgically and the pancreatic segments (head, tail) can then be perfused individually through straight cannulae directly inserted into the corresponding lobe main artery(s).
5. The LifePort ~~transporter~~ can be further used for pancreas perfusion optimization studies (i.e., lower pressure) to establish methods of organ quality control, to improve pancreas flow, and reduce vascular resistance during machine perfusion, for both segmental and isolated islets transplantation. Pancreata with warm ischemic exposure and from expanded criteria donors can be used. A constant flow perfusion regime, as opposed to constant pressure flow (the current transporter configuration), may be advantageous for better perfusion outcome for both islet isolation and whole pancreas transplantation, but this would require reconfiguration of the standard LifePort ~~device~~.

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