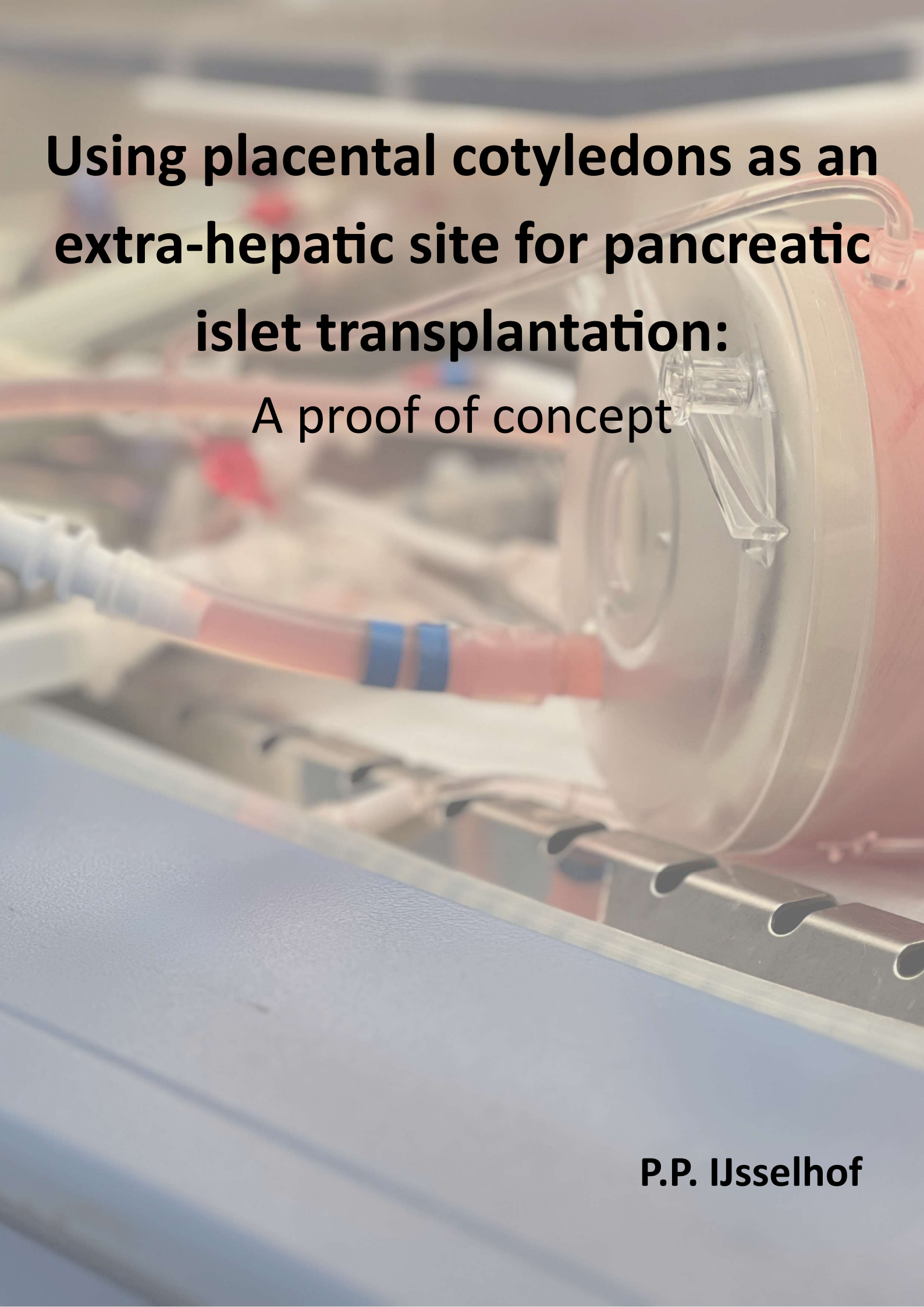

Using placental cotyledons as an extra-hepatic site for pancreatic islet transplantation:

A proof of concept

P.P. IJsselhof

Using placental cotyledons as an extra-hepatic site for pancreatic islet transplantation:

A proof of concept

Peter IJsselhof

Student number : 4728009

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Supervisors:

Dr. Marten Engelse

Dr. Jason Doppenberg

Thesis committee members:

Dr. Marten Engelse, LUMC (Chair)

Dr. Jason Doppenberg, LUMC

Dr.ir. Roos Oosting, TU Delft

Preface

This thesis marks the conclusion of my studies in Technical Medicine at Delft University of Technology, Leiden University, and Erasmus University. Over the past few years, I have explored various areas of medicine and developed a particular interest in transplantation surgery. Positive experiences during my internships inspired me to complete my graduation project at the LUMC Transplantation Center. Over the past months, I have learned a great deal, and I would like to thank the people who supported me throughout this journey.

First of all, I would like to thank my supervisors for their guidance and for giving me the opportunity to engage in meaningful and exciting scientific research. Marten, thank you for your guidance throughout the project — from brainstorming theories together in the lab to forming the overall research project. Jason, thank you for all the work you've done to enable me to embark on this graduation project. I would like to express my gratitude to Marie-Louise for the approachable and open communication we shared, and for facilitating the acquisition of placentas during cesarean sections. I would like to thank all the staff of the Perfusion Lab and the Islet Lab for their help and support throughout this project. Next, I would like to thank all the parents who made their newborn's placenta available for this research. I am truly honored to have been present during one of the most significant moments in your lives.

Finally, I would like to thank my family, friends, and Jasmijn for their unwavering support and encouragement throughout this graduation project.

P.P. IJsselhof
10-7-2025

Abstract

Introduction

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder that results in the destruction of insulin-producing pancreatic β-cells, leading to lifelong dependence on exogenous insulin. Patients without adequate glycemic control are at risk for severe complications such as retinopathy, nephropathy, and limb amputation. For these individuals, pancreatic islet transplantation has is a promising therapeutic strategy that can restore endogenous insulin production. However, current transplantation practices typically rely on infusion of islets into the hepatic portal vein—a method associated with significant drawbacks. These include immediate blood-mediated inflammatory reactions (IBMIR), limited insulin release due to the hypoxic environment, and exposure to high levels of immunosuppressants.

As a result, multiple donor pancreases are often required to achieve insulin independence, exacerbating the scarcity of donor organs. To overcome these limitations, there is growing interest in identifying alternative, extrahepatic transplantation sites that can provide a more supportive environment for islet engraftment. Human placental cotyledons, which are highly vascularized and readily available following elective cesarean deliveries, represent a novel and potentially advantageous site for islet engraftment. This study explores the feasibility of combining ex vivo placental cotyledon perfusion with islet transplantation to evaluate its potential as a new extrahepatic transplant platform.

Materials and Methods

A novel ex vivo perfusion system for human placental cotyledons was developed through iterative design, focusing on sterility, vasospasm prevention, and optimized tubing. Post-cesarean placentas were used to isolate and perfuse a single cotyledon under controlled conditions. After four hours, human pancreatic islets were injected into the intervillous space. Islet function was assessed the next day by a glucose-stimulated insulin secretion (GSIS) measurement as well as functional measurements.

Results

The perfusion setup maintained stable physiological conditions over 23 hours. Insulin secretion increased during high-glucose, showing viable and functional islets after 14 hours of perfusion. Insulin was detected in cotyledon effluent but not fetal venous return, likely due to perfusion flow and placental barriers.

Conclusion

The 23-hour perfusion demonstrated effective function without infection or tissue damage. Injected islets remained viable and glucose-responsive in the intervillous space. While the setup met several criteria for evaluating extrahepatic transplantation sites, further research is needed to address leakage from the cotyledon and to investigate changes in hormonal secretion of the cotyledon during long-term perfusion.

Nomenclature

Notation	Description
T1DM	Type 1 Diabetes Mellitus
MDI	Multiple Dose Injections
CSII	Continuous Subcutaneous Insulin Infusion
IBMIR	Instant Blood-Mediated Inflammatory Reaction
Ex Vivo	Latin for 'out of the living'
PUR	Polyurethane
OPR	Organ Preservation and Regeneration Unit
CDI 550	Inline blood gas analyzer of Terumo
LS25	Tubing diameter of Masterflex (a brand of Avantor)
RS232	Hardware standard for serial communication between devices
LUMC	Leids Universitair Medisch Centrum, a university medical center for research, education and patient care
OR	Operating Room
PRISM	PancReatic Islet Separation Method, A closed system of tissue collection, washing, buffer change, and islet purification
ELISA	Enzyme-Linked ImmunoSorbent Assay, a commonly used analytical biochemistry assay

Contents

1. Introduction

Patients with type 1 diabetes mellitus (T1DM) do not produce insulin due to a chronic autoimmune disease leading to a reduction in β-cell mass (1). In order to regulate glycemic levels and prevent damage to the microvasculature, patients inject exogenous insulin via Multiple Daily Injections (MDI) or Continuous Subcutaneous Insulin Infusion (CSII) (2,3). However, some patients with T1DM are unable to adequately control their blood glucose levels despite these treatments, putting them at risk of serious complications such as retinopathy, limb amputation, or renal failure (4,5).

To prevent the occurrence of severe hypoglycemic events, pancreatic islet transplantation may be appropriate (6). During islet transplantation, pancreatic islets are isolated from a donor pancreas and administered to the recipient. The introduction of foreign tissue necessitates the use of immunosuppressive medication to prevent rejection of the injected pancreatic islets (7).

The first step of an islet transplantation is the isolation of pancreatic islets from donor tissue. This process involves enzymatic digestion of connective tissue coupled with mechanical agitation, followed by density-gradient centrifugation to separate islets from exocrine tissue and extracellular matrix (8). The purified islets are suspended in a culture medium and stored in an incubator at 37 degrees Celsius. This period of islet maintenance in culture allows for safety, viability and functionality testing prior to releasing the islet product for transplantation (9).

During pancreatic islet transplantation, the portal vein of the recipient is cannulated, and the isolated islets are slowly infused into the portal bloodstream. An interaction between the whole blood of the recipient and the injected pancreatic islets causes the instant blood-mediated inflammatory reaction (IBMIR). Such a reaction is detrimental to islet survival (6,10). Despite this precaution, over 70% of infused islets are non-functional after transplantation (11). The surviving islets travel via the portal vein to the liver and become lodged in hepatic sinusoids. The occlusion of liver sinuses may result in portal hypertension and local liver ischemia if the infusion volume is excessive or if the islets are administered too rapidly (12).

Hepatic sinusoids are not the ideal environment for pancreatic islets to reside due to the lower partial oxygen pressure in hepatic parenchyma compared to pancreatic tissue (13). This hypoxic environment reduces glucose oxidation in β-cells, which results in reduced insulin secretion, thus making the surviving islets less effective (14). Another negative effect of the liver as a transplantation site for pancreatic islets is the pharmacokinetics of immunosuppressive drug absorption. Because of the first-pass effect, the level of ingested medication is higher in the liver than in systemic circulation, potentially exposing the transplanted islets to excessive drug concentrations (15).

This is problematic because tacrolimus, a widely used immunosuppressive drug, is known to decrease glucokinase activity and impair the revascularization of transplanted islets. (15,16). The combined negative effects of islet isolation and transplantation result in such extensive loss of functional islets that repeated transplantations are often

necessary to achieve insulin independence. The requirement for multiple donor pancreases exacerbates the existing organ shortage, limiting the number of patients who can benefit from this therapy

The negative effects of the liver as a transplantation site are known, and many possible extra-hepatic transplantation sites have been explored as alternatives. *Section 6.1 Systematic Literature Review on Extra-hepatic Transplantation Sites* presents a literature study with an overview of proposed sites and their limitations.

Based on the findings, six criteria for a suitable alternative transplantation site are established:

1) Prevent the occurrence of the immediate blood-mediated inflammatory reaction
2) Provide a microenvironment that has an adequate partial pressure of oxygen
3) Minimize the exposure of the islets to immunosuppressive medication
4) Be adequately vascularized
5) Prevent clumping of islets and central necrosis
6) Provide containment of transplanted islets and make monitoring of engraftment possible.

The literature review revealed that no current transplantation site satisfies all these conditions, highlighting the need for research into novel approaches to create suitable environments for transplanted pancreatic islets.

A novel approach to supporting isolated pancreatic islets involves using a cotyledon which is procured from an elective cesarean section and perfused ex vivo in a laboratory setting. During perfusion, isolated pancreatic islets would be injected into the intervillous space, and perfusion would be continued to allow engraftment. Once successful islet engraftment is confirmed, the cotyledon with engrafted islets could be transplanted into the recipient with T1DM. This approach could potentially reduce the number of donor pancreases required

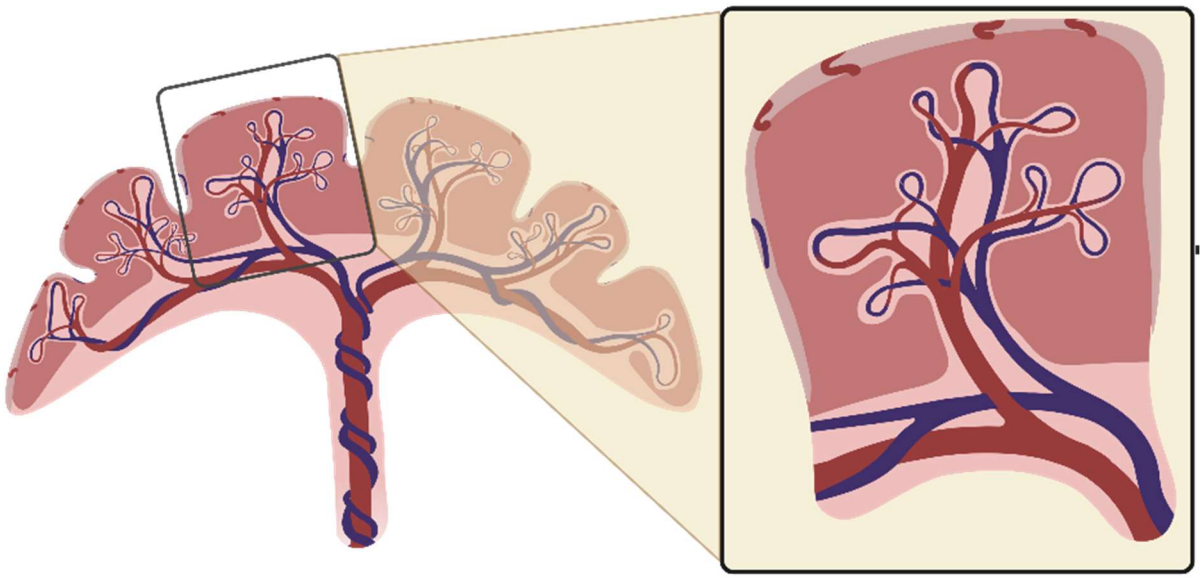

Figure 1: Cross-section of a placenta with a highlighted cotyledon showing key anatomical structures: fetal artery, fetal vein, syncytiotrophoblast membrane, intervillous space, and maternal spiral artery.

The placental cotyledon is of interest because it is a highly vascularized organ that, after birth, has no function for mother or fetus and has high availability because of the number of births per year. The placenta develops during gestation to facilitate gas and nutrient exchange between mother and fetus. It consists of approximately 20 subunits called cotyledons, each of which encompasses a fetal vasculature network, a syncytiotrophoblast membrane, and an intervillous space connected to maternal circulation. The structure of a cotyledon, shown in detail in Figure 1, enables efficient gas and nutrient exchange without mixing maternal and fetal blood, thereby preventing maternal-fetal immune reactions.

To investigate the long-term survival of pancreatic islets within the intervillous space of cotyledons, an ex vivo perfusion system must be developed to support an isolated cotyledon. This perfusion system will facilitate subsequent investigation of pancreatic islet survival following injection into the intervillous space of the cotyledon. The ex vivo perfusion of a cotyledon followed by pancreatic islet injection represents a novel approach. Although literature exists on pancreatic islet survival and on cotyledon perfusion separately, no published studies or established protocols currently describe the combined use of isolated cotyledon perfusion with subsequent pancreatic islet injection.

Cotyledon perfusion research has been established to assess the safety of new medications taken during pregnancy. Pharmacokinetic studies using placental tissue have demonstrated that placental function can be maintained during ex vivo perfusion (17). In these studies, both the maternal and fetal sides are cannulated, and oxygenated perfusate flows through both circulatory systems. Given the anatomical separation between the maternal and fetal compartments of the cotyledon, it is of interest to investigate whether islets placed on the maternal side can survive when only the fetal circulation is perfused. Since islets positioned on the maternal side are not exposed to whole blood within the fetal vasculature, they may experience reduced or absent IBMIR, which is beneficial for pancreatic islet survival.

This thesis aims to bridge this knowledge gap by first exploring the necessary materials and protocols for cotyledon perfusion and pancreatic islet injection. Subsequently, a proof-of-concept experiment will be performed to validate the functioning of the perfusion setup and protocol and to determine whether injected pancreatic islet cells survive inside the cotyledon for more than 14 hours. The findings from this research could contribute to the development of alternative transplantation strategies that improve pancreatic islet transplantation efficiency as well as potentially host stem cell derived islets.

2. Materials and methods

2.1 Preliminary experiments and protocol development

In order to create a novel perfusion setup and protocol for the perfusion of a cotyledon with injected pancreatic islets, an iterative approach is used. *Section 6.2 Preliminary experiments and protocol development* shows a description of all performed experiments. The lessons from previous experiments as well as expert opinions from academic staff are used as input for subsequent experiments

The findings of the experiments culminated in key design decisions for the final setup and protocol. For instance, it became clear that microbial growth poses a challenge during perfusion. Therefore, a regime of penicillin-streptomycin (Gibco), ciprofloxacin (Fresenius Kabi), and fungizone (Bristol-Myers Squibb) was added to the perfusate. Also, the disposable organ chamber proved hard to clean and remained a possible source of infection. For this reason, the organ chamber was switched to the 3D-printed organ chamber, which is designed for sterilization with an autoclave. Switching to sterile gloves when interacting with the placenta reduces the risk of infection by preventing microbial transfer through handling.

During the first perfusions, a point of difficulty was vasospasm of the arteries and difficulty of cannulation, which resulted in tearing of the umbilical artery and subsequent loss of perfusion capabilities. The initial reasoning for vasospasm was a reaction to cold perfusate, which gave thought to the idea that the placenta should not be cooled during procurement. In order to prevent ischemic damage to the placenta, the mobile perfusion setup was created in PLC06. Later on, it became clear that such vasospasm most probably is a reaction to the low pH of Ringer-acetate, which was used as a flush medium. With the use of the side-entry method, it was possible to cannulate the umbilical artery with a 5-French PUR cannula without tearing of the artery. This enabled cooling of the placenta in the Organ Preservation and Regeneration Unit (OPR) and maintenance of cold conditions during transportation, which was tested in PLC10 and PLC11.

The perfusion setup of PLC06 made it possible to use the same tubing and equipment during the flush as well as during perfusion. This setup increased the length of tubing and made it difficult to perform a medium change during the perfusion because of the large dead volume of the setup. With the use of the 3D-printed organ chamber during PLC10 and PLC11, it became clear that this setup has the advantage of a smaller dead volume and the ability to quickly perform a medium change.

These key insights resulted in the final setup and perfusion protocol, which made it possible to procure the placenta, transport it under static cold storage conditions, perfuse an isolated cotyledon under subnormothermic ex vivo conditions, and perform functionality testing.

2.2 Final setup and protocol

Perfusion setup

The perfusion setup consists of a Masterflex peristaltic pump (18), a TruWave pressure transducer (19), an in-house developed pressure controller, a Capiox® FX25 Oxygenator (20), a heated heated plate, an external heater/cooler, two CDI510H shunt sensors in conjunction with a CDI 550 Blood Parameter Monitoring System for inline monitoring (21), and an in-house developed 3D-printed nylon kidney organ chamber with silicone mesh. Figure 2 shows an overview of the perfusion setup. The pressure controller coupled with the pressure transducer enables closed-loop pressure control of the peristaltic pump. This makes it possible to tightly regulate the pressure provided while monitoring the arterial fluid flow.

The organ chamber is placed onto the heated plate, and the tray is filled with demineralized water to facilitate heat transfer. The outlet port of the external heater/cooler is connected to the inlet of the oxygenator. The heated dissection tray is placed in series between the outlet port of the oxygenator and the inlet port of the external heater/cooler. This ensures that the temperature inside the oxygenator, which is most critical for the temperature of the placenta, is most tightly regulated.

Tubing connecting all components together consists of puri-flex LS25 tubing (22) connected together with luer-lock connectors. Tubing section B1 comes into contact with the rollers of the peristaltic pump. This increases wear on this section of tubing. This section consists of Tygon tubing instead of silicone tubing because it is more resistant to mechanical wear. *Section 6.3: Tubing specifications* shows a table with all specifications of the tubing sections and a graph of the tubing sets with corresponding section codes.

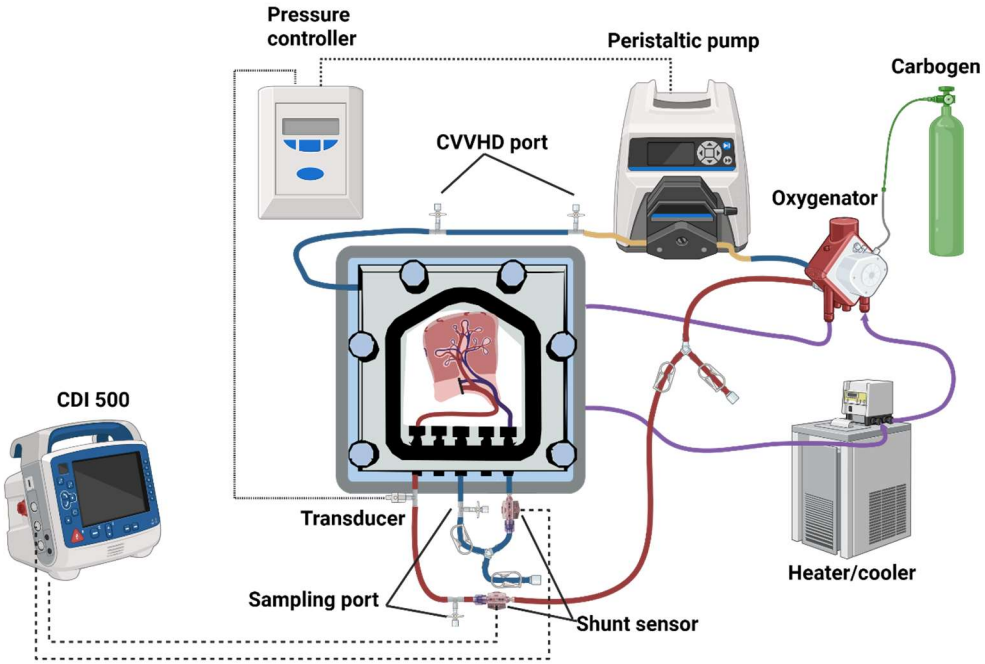

Figure 2: Overview of the final perfusion setup

Measurements

Measurement of blood parameters are done continuously with the use of the CDI550 blood gas analyzer which measures pH,PO_2, PCO_2, temperature, HCO_3 concentration, base excess, and potassium concentration. Every 6 seconds, the device outputs all calculated values via its serial port to a desktop via an RS232 to USB adapter. Using a custom Python script, all values generated by the CDI 550 are stored in an Excel sheet on the desktop for further processing. *Section 6.4 Software for reading and storing CDI550 data* provides the script for polling the CDI550 and storing all generated data points in an Excel sheet for further analysis.

To calibrate the CDI550 and measure parameters absent on the CDI550, additional measurements are performed at fixed timepoints or during interventions. These measurements are performed on the Siemens epoc Blood Analysis System (23). This point-of-care device measures pH, pCO_2, PO_2,$cHCO_3^-$, BE(ecf), BE(b), cSO_2, Na^+, K^+, Ca^{++}, Cl^-, TCO_2, Hct, glucose, lactate, BUN, urea and creatin. All measurements are stored in an Excel file for further analysis.

To quantify perfusate leaving the fetal vasculature, a percentage of venous return is measured at fixed timepoints. The peristaltic pump is switched from pressure-controlled to continuous flow mode, and flow and pressure are noted while ensuring the pressure does not exceed the previous measurement. The venous side of the tubing set is switched from closed circuit to open circuit, and effluent is collected in a 50 cc tube (Figure 3). After 3 minutes, the system returns to closed circuit mode and pressure-controlled operation. The collected perfusate weight is divided by the collection time to calculate the percentage of arterially supplied perfusate that returns venously.

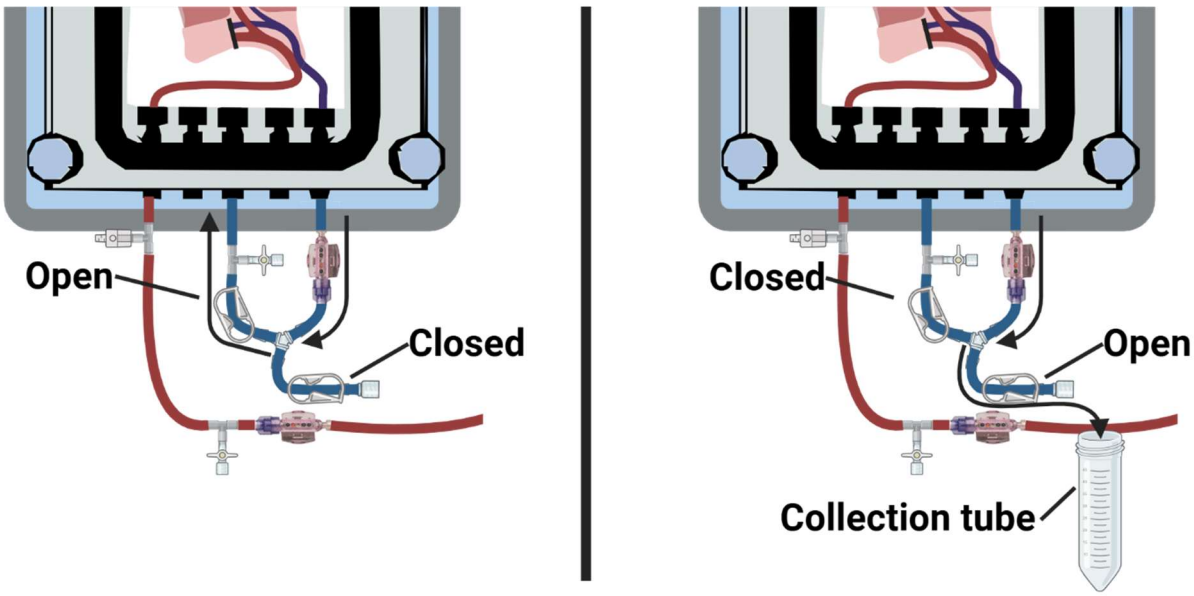

Figure 3: (Left) Venous side of tubing set in the closed circuit position; (Right) Venous side of tubing set in the open circuit position in order to measure percentage of venous return

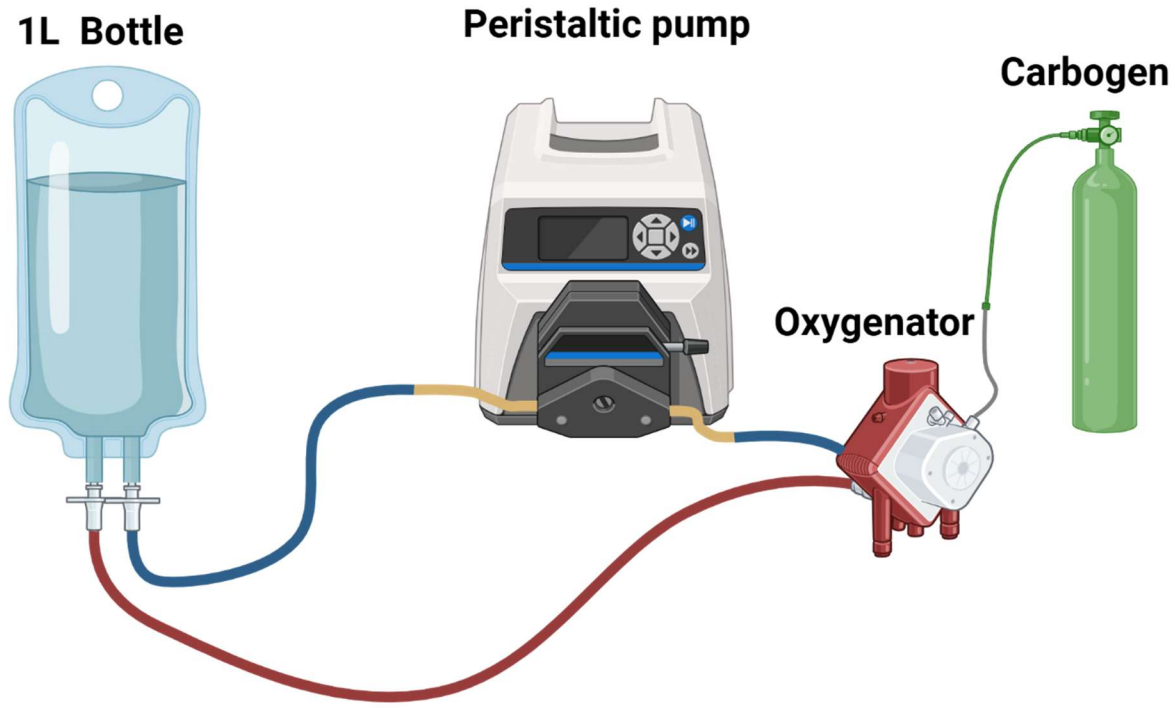

Figure 4: Overview of the setup for pre-oxygenation of GSIS fluids

Fluid preparation

The perfusate used in this experiment is a cell-free medium based on the research by Haan et al (24) and preliminary experiments. It consists of DMEM/F-12 (Gibco) with additional Human Serum Albumin (Alburex 20, CSL Behring bv), sodium bicarbonate (Gibco), penicillinstreptomycin (Gibco), ciprofloxacin (Fresenius Kabi) and fungizone (Bristol-Myers Squibb). The pH was adjusted to a range of 7.40 – 7.45 with the use of sodium bicarbonate or sodium hydroxide (Calbiochem, Merck). *Section 6.5 fluid composition* presents the description of the makeup of the perfusate and other fluids

The flush medium is prepared by closed circuit circulation and oxygenation of perfusate in the perfusion setup for 10 minutes with a carbogen flow of 1 L/min and a fluid flow of 200 ml/min. Two 50 cc syringes are each filled with 1 ml of heparin (Leo Pharma BV) and 49 ml of oxygenated perfusate. The pH is checked prior to filling the syringes and, if not in range, adjusted with sodium bicarbonate. During the procurement process, these syringes are cooled by placing them in ice to create ice-cold heparinized flush medium.

To sustain an open circuit perfusion for a minimum of 15 minutes per stage, a sufficient amount of a low glucose medium and a high glucose medium is required during the Glucose-stimulated Insulin Secretion (GSIS) measurement. Pre-bottled Ringer-acetate (Baxter) is used with two spikes placed per 1L bottle. Tubing creates a closed circuit between a peristaltic pump and oxygenator for pre-oxygenation of the bottles, with carbogen flow set at 1 L/min and fluid flow at 200 ml/min. Figure 4 shows the setup for pre-oxygenation of the bottles of GSIS fluids. Each bottle is pre-oxygenated for 10 minutes, after which the pH is checked and, if necessary, corrected with sodium bicarbonate to bring it into the range of 7.40–7.45. After pH verification, the high glucose bottle is filled with 9 ml of 40% glucose to create a 20 mmol/L glucose concentration.

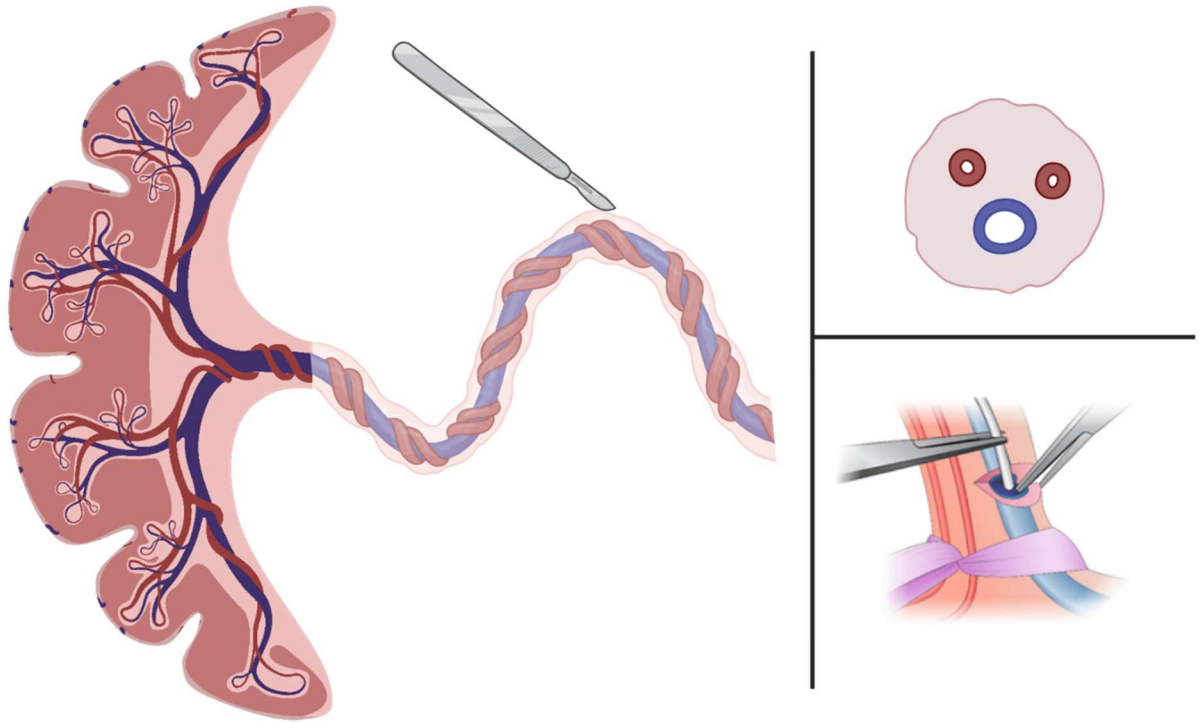

Figure 5: (Left) cross-section of a placenta and overview of an umbilical cord;(Top right) cross-section of an umbilical with two arteries and one vein; (Bottom right) Side-entry method of cannulization of the umbilical vein

Placenta procurement

The placentas used in this research originated from elective cesarean sections performed by the obstetrics department of LUMC. The placenta has no medical use or function after birth. Consent was obtained from the mother for use of the placenta for research purposes prior to the time-out procedure. After the baby is delivered, a timer of 3 minutes starts, after which the umbilical cord is clamped and cut. This waiting time allows for blood to migrate from the placenta into the neonate, thereby increasing its hemoglobin level (25). The placenta is detached from the uterus wall by the operator by pulling the umbilical cord. After delivery of the placenta, arterial and venous samples are collected by the OR staff for blood gas analyses.

After obtaining the placenta in a closed non-sterile box, it is transported to the Organ Preservation and Regeneration Unit (OPR) present inside the OR complex. The goal of the flush in the OPR is to prevent thrombosis and minimize warm ischemic time. This is achieved by flushing out blood present in the placenta, introducing heparin into the placenta, and cooling the placenta as quickly as possible. A facemask and sterile gloves are worn during the flush to prevent the introduction of pathogenic micro-organisms such as bacteria. A tray filled with ice and draped with a sterile gauze pad is prepared and placed on the operating field. The gauze pad prevents direct contact between the organ and the ice, thereby preventing freezing and damage to the placenta.

The umbilical arteries are cannulated with a 5-french PUR feeding catheter. First, an incising is made with use of the side-entry method described by Rüdinger et al (26). This method consists of fixation of the placenta and simultaneous traction and rotation of the umbilical cord. Figure 5 shows an overview of the placenta and umbilical cord, the cross-

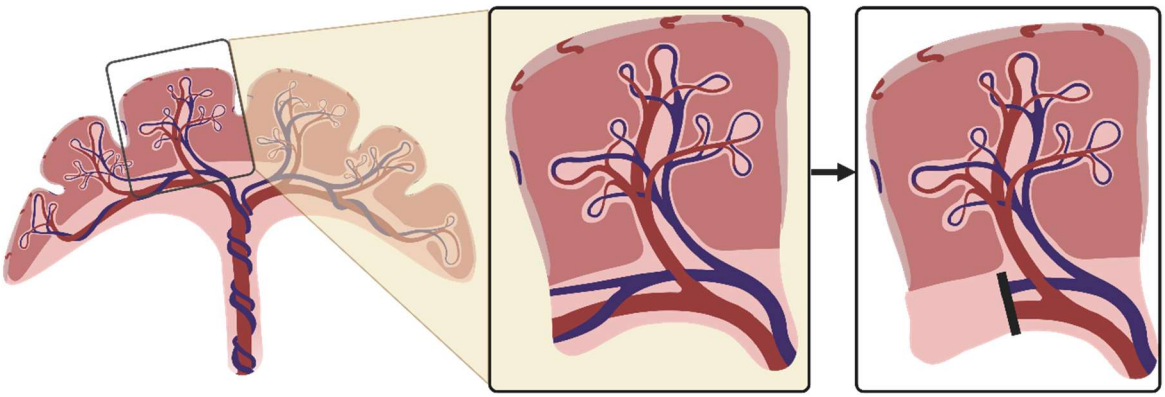

Figure 6: (Left) Cross-section of a placenta after birth; (Center) A single cotyledon; (Right) A single cotyledon with dissected vasculature sutured in order to prevent leakage

section of the umbilical cord and the side-entry method. Next the artery is brought to the surface of the umbilical cord, and a horizontal incision is made halfway transacting the umbilical artery. The exposed artery is dilated with small tweezers and cannulated with the PUR feeding catheter. In order to facilitate the outflow of the flush medium, an incision is made in the umbilical vein. Per umbilical artery a total of 50 cc of the cold heparinized oxygenated flush medium is infused with used of a syringe.

After the flush is performed, the placenta is placed in an organ transplantation bag and another organ transplantation bag is filled with 1 liter ice cold NaCl 0.9%. The organ transplantation bag with the placenta is closed and placed inside the bag with ice cold NaCl 0.9% after which this bag is also closed and is placed in a styrofoam box filled with ice and transported to the lab.

Cannulation and cotyledon isolation

In the lab, a sterile field is created with drapes inside a biological safety cabinet. The placenta is placed on an inspection tray and the maternal side is inspected for integrity of the cotyledons. A cotyledon is selected for cannulation and isolation based on visual assessment. The requirements for a suitable cotyledon are an intact structure without ruptures and the presence of a single artery and a single vein suitable for cannulation.

On the basal plate on the fetal side, an incision into the artery is made and an 8-French Bridge-to-life cannula (27) is fixated into the artery by means of a 5-0 Vicryl suture (28) which is sewn around the artery and tied down (Figure 6). When distinguishing between arteries and veins, it is important to note that, in cases where two vessels cross, the artery consistently passes over the vein. The procedure is repeated with a 12-French Bridge-to-life cannula for the vein of the cotyledon.

Ex vivo perfusion of an isolated cotyledon

After cannulation and isolation of a single cotyledon, it is placed maternal side down in the organ chamber and connected to the perfusion setup. The peristaltic pump is turned on in pressure control mode with a pressure of 50 mmHg, and the oxygenator carbogen flow is set to 0.5 L/min. Temperature is initially set to 27 degrees Celsius. The cotyledon is inspected for dissected or damaged vessels, and if present, these are sutured using a 5-0 Vicryl suture.

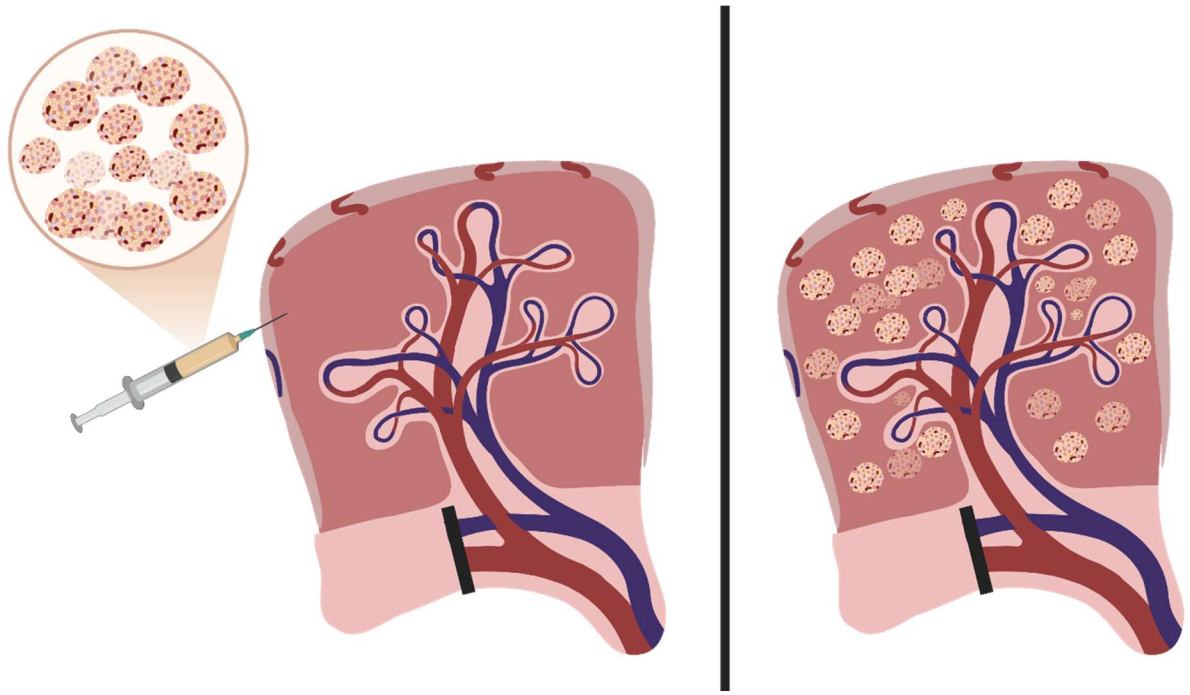

Figure 7: (Left) Injection of isolated pancreatic islets into a isolated cotyledon; (Right) Pancreatic islets residing in a single cotyledon

To prevent erythrocytes from becoming part of the closed circuit perfusate, the first 200 ml of perfusate is removed from the circulation by running the setup in non-recirculation mode until 200 ml is removed. The moment the setup is switched from open to closed circuit mode is noted as T0. The venous return percentage is measured as well as starting the CDI550 and performing a measurement with the epoc. Subsequent measurement times are noted in relationship to this first measurement. For instance, a measurement performed 3 hours after T0 is noted as T3.

Pancreatic islet injection

After four hours of closed circuit ex vivo perfusion, pancreatic islets are injected into the intervillous space of the cotyledon. The pancreatic islets are of human origin and isolated using the PRISM setup and protocol present at LUMC. The total volume of islets present in the injection fluid is noted. The total volume of injection fluid is divided into two 50 cc syringes that were connected to two 14-gauge venous cannulas. The cannulas are pushed into the intervillous space and slowly emptied (Figure 7).

After injection of the pancreatic islets, the perfusion setup is left running in the closed-circuit configuration overnight. The next day, a functionality test is performed to assess pancreatic islet survival after injection into the intervillous space of an isolated cotyledon.

Glucose-stimulated Insulin Secretion measurement

During the first stage of the GSIS measurement, the cotyledon with pancreatic islets is perfused with low glucose oxygenated perfusate in order to wash out previously made insulin and to set a baseline of insulin production regardless of glucose concentration. *Section 6.5 Fluid composition* shows the makeup of fluids used in the GSIS. One liter of pre-oxygenated low glucose perfusate is pumped under pressure-controlled conditions into the cotyledon. The bottle with perfusate is connected to the peristaltic pump, which is connected to the pressure transducer and cannula with a piece of sterile tubing. This ensures that no pancreatic islets in for instance, the oxygenator are responsible for any measured insulin production. Figure 8 shows the used perfusion setup during the GSIS.

The moment when the bottle is empty, is based the flow rate. 10 minutes before then, the first measurement is taken and is called P0. For the next 10 minutes, both cotyledon and venous measurements are taken in 5-minute intervals. Figure 10 shows a timeline of the complete GSIS protocol. Cotyledon measurements are taken by lifting the cotyledon in such a way that the effluent drips down one portion of the cotyledon and can be collected in a 5 ml Eppendorf tube. Venous measurements are performed by collecting venous return into a 5 ml Eppendorf tube. Figure 9 shows the sampling locations during the GSIS measurement.

A medium change is performed after the bottle with perfusate is nearly empty by disconnecting the pressure transducer from the rest of the tubing and switching out the bottle to a bottle with a high glucose concentration. By running the pump for a short time, any remaining low glucose perfusate is purged. Next, the tubing is reconnected to the pressure transducer and the pump is set to pressure control mode.

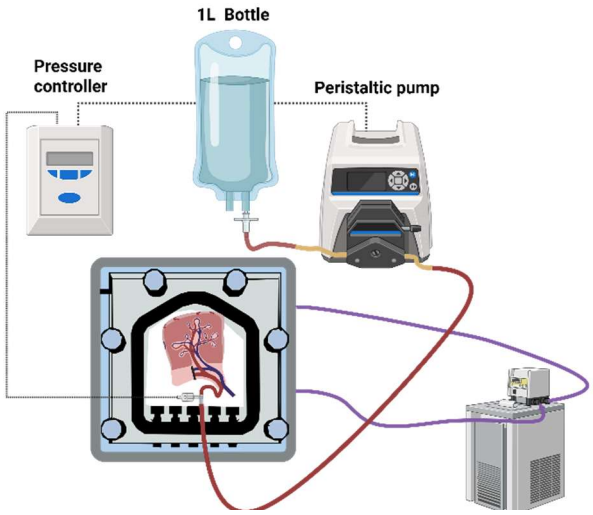

Figure 8: Overview of the perfusion setup during GSIS

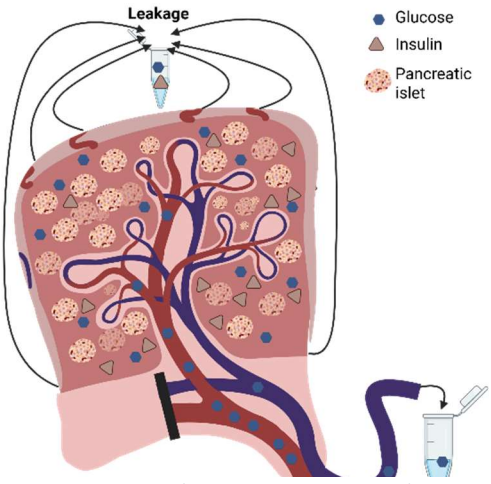

Figure 9: Cross-section of the cotyledon with injected pancreatic islets. High-glucose medium is perfused. Venous and cotyledon effluent samples are collected

Throughout the high glucose phase, measurements of both cotyledon effluent and venous return are taken at 5-minute intervals until the bottle is emptied. Once the high glucose perfusate is depleted, the medium is changed back to low glucose perfusate and measurements continue. All collected samples were stored at -22 degrees Celsius for further analyses.

To analyze the insulin and glucose concentrations in the collected samples, all samples are thawed. A point-of-care glucose tester (Abbott FreeStyle Libre 2 Reader) is used to measure the glucose concentration in the samples. A commercial human Insulin ELISA kit (10-1113-01; Mercodia, Uppsala, Sweden) is used for the measurement of insulin concentration. Absorption was measured using a microplate reader at 450 nm. A dilution series is created using a 1:2 dilution ratio to calculate results when the absorption values are too high for the absorption reader.

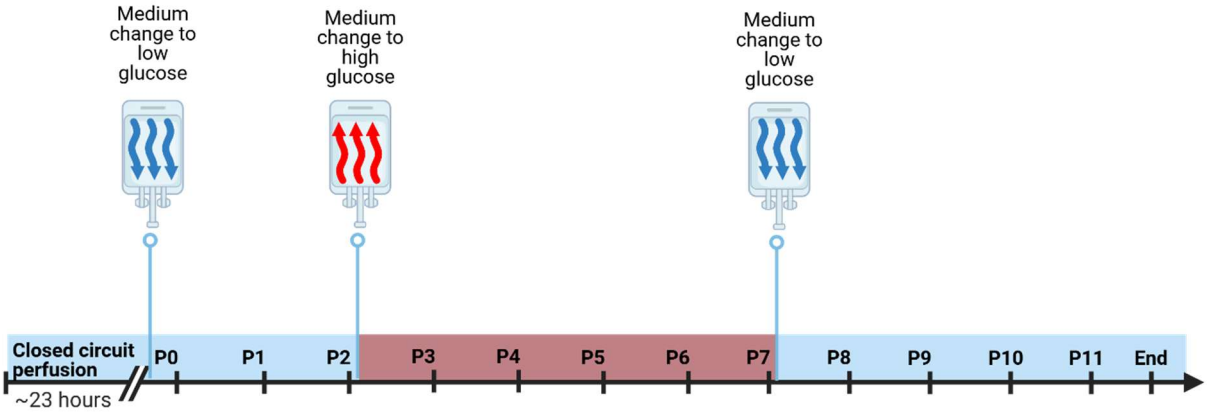

Figure 10: Overview of the GSIS time points and medium changes

3. Results

Cotyledon perfusion

The main objective of the final perfusion was to validate the functioning of the perfusion setup and protocol and to determine whether injected pancreatic islet cells can survive inside the cotyledon for more than 14 hours.

The continuous blood gas analyzer CDI550 in conjunction with a PC for recording gave insight into the state of the perfused cotyledon during the night when no measurements were taken. Both arterial and venous pH initially showed a spike due to a sodium bicarbonate correction bolus, followed by a gradual decline. Potassium levels increased steadily at a fixed rate throughout the perfusion (Figure 11). This indicates the formation of lactate due to anaerobic glucose metabolism. This was confirmed by a manual epoc measurement at T23, which showed a lactate concentration of 17 mmol/L.

The observed shift toward anaerobic metabolism may be attributed to a mismatch between oxygen supply and demand within the perfused cotyledon. One possible explanation is that the temperature of the perfusate was too high for a red blood cell-free system, particularly given that the placenta was maintained at 27 degrees Celsius. Temperature control was maintained with arterial and venous temperatures initially differing by 1.5 degrees Celsius but converging after 5 hours of perfusion (Figure 12). Temperature measurements showed no difference between arterial and venous readings beyond timepoint T10, suggesting that the perfusate had insufficient time to transfer thermal energy to the placental parenchyma which is an indicator of potential arteriovenous (A-V) shunting.

Venous return improved over time, increasing from 23% after 3 hours to 41.9% after 18 hours of perfusion (Table 1). *Section 6.6 Manual measurements of PLC12* shows an overview of all measurements performed with the epoc point-of-care device is provided in. This hypothesis is further supported by the increase in venous return percentage, which rose from 23.3% at the start of perfusion to 41.9% overnight, implying a reduced exchange across the placental microvasculature.

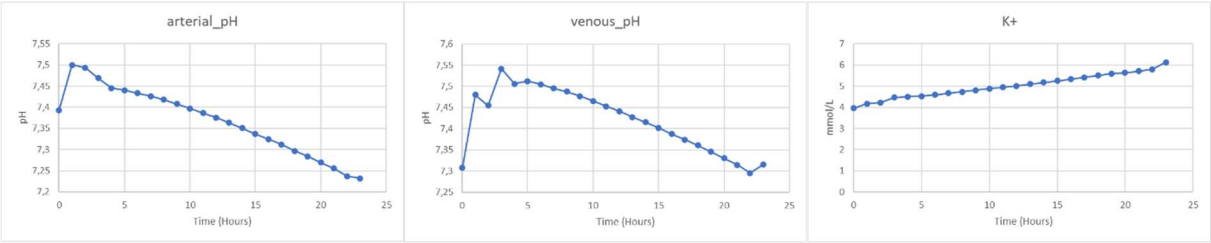

Figure 11:Continues blood gas measurements from CDI550 (Left) Arterial pH ; (Center) Arterial pH; (Right) Potassium

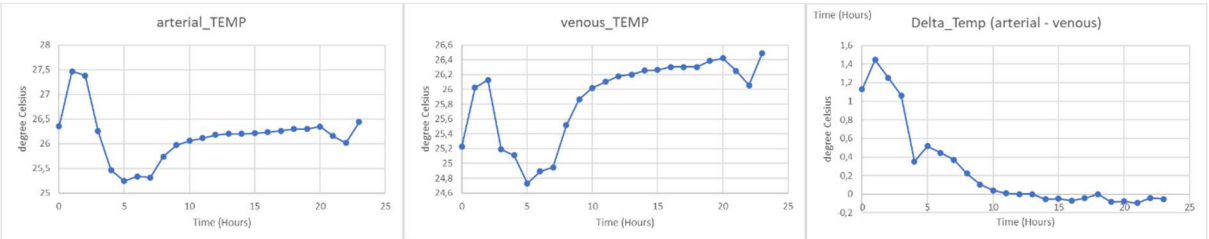

Figure 82: Continues temperature measurements from CDI550 (Left) Arterial temperature ; (Center) Venous temperature; (Right) Delta temperature between arterial and venous

Table 1: Measurements of percentage venous return

Time point	Set flow (ml/min)	Sample time (min)	Arterial volume (ml)	Collected venous volume (ml)	Percentage of venous return (%)
T3	20	5	100	23,3	23,3
T18	40	2	80	33,5	41,9

GSIS

A total of 22 samples were taken during the GSIS measurement. From these samples, a 1:2 dilution was made with demineralized water. Both the original samples and the dilutions were analyzed with the Insulin ELISA kit. Per protocol, a calibration curve was created in order to calculate insulin concentrations; this calibration curve is shown in *Section 6.7 ELISA calibration curve*. If the values of the undiluted samples were higher than the highest value of the calibration curve, then the diluted samples were used and the results doubled in order to obtain an accurate reading.

The insulin and glucose curves measured from the cotyledon effluent are shown in Figure 13. At timepoint p7 the outside of the cotyledon touched the fluid present in the organ chamber, thereby diluting this sample. This was noted and fluid was drained from the organ chamber to prevent this dilution from occurring in future measurements. Figure 14 show the insulin and glucose curves if timepoint p7 is excluded from the graph.

Insulin and glucose concentrations sampled in the venous return during the GSIS measurement are shown in Figure 14. The vertical axis of the insulin concentration was based on the vertical axis of Figure 13. In order to investigate whether there was an increase in insulin concentration over time during the high glucose phase of the GSIS, an additional figure was created. Figure 16 shows the insulin and glucose concentrations sampled in the venous return with the insulin vertical axis limited to 40 mIU/L.

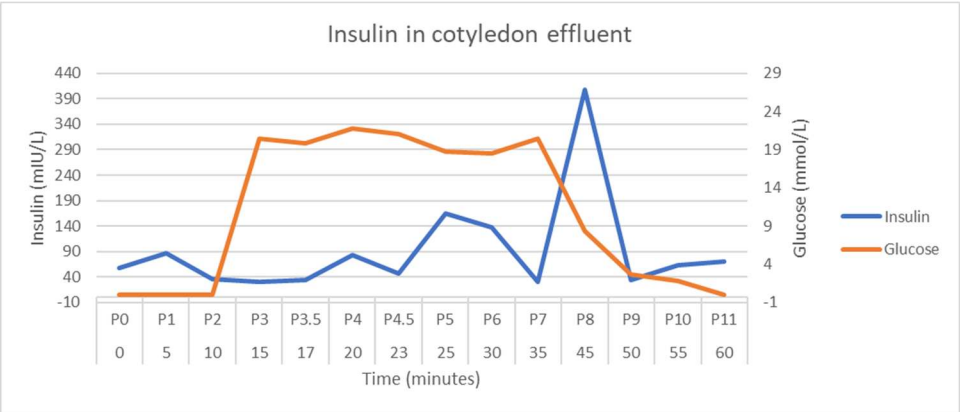

Figure 9: Insulin and glucose concentration in cotyledon effluent during GSIS

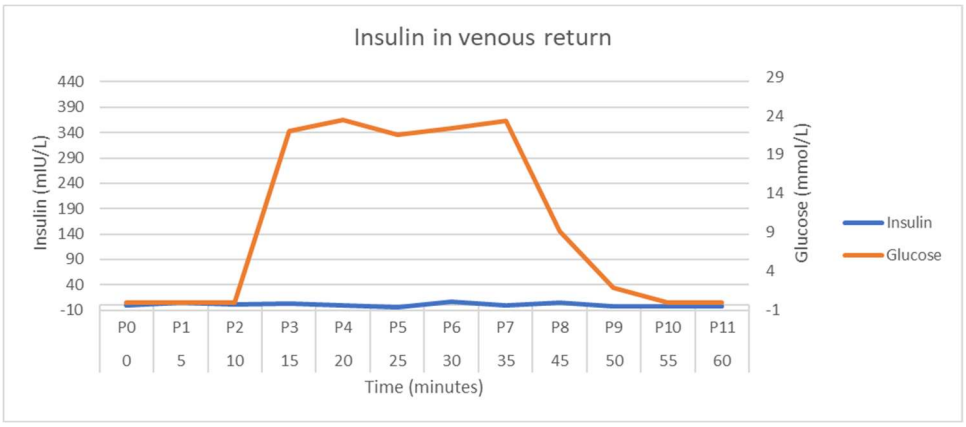

Figure 10: Insulin and glucose concentration in venous return during GSIS

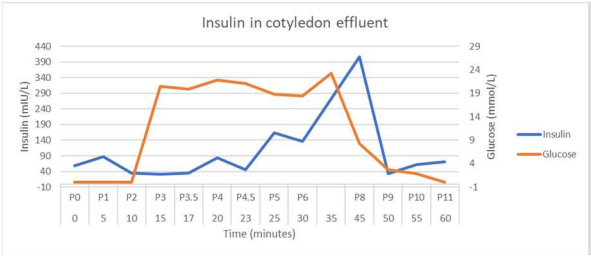

Figure 15: Insulin and glucose concentration in cotyledon effluent during GSIS with point P7 excluded

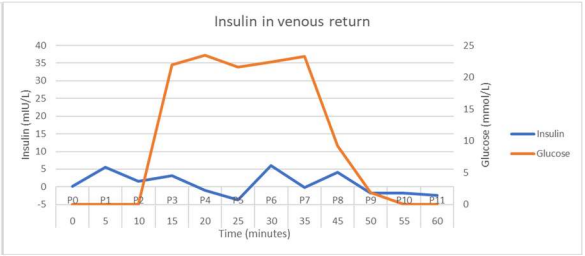

Figure 16: Insulin and glucose concentration in venous return with vertical axis limited to 40 mIU/L

4. Discussion

This thesis aimed to develop materials and protocols for the ex vivo perfusion of a single placental cotyledon and to test the feasibility of this system through a 23-hour proof-of-concept experiment, which included a 14-hour period during which pancreatic islets resided in the intervillous space. In this experiment, pancreatic islets were introduced into the intervillous space after four hours of perfusion, and their viability and function were assessed using a glucose-stimulated insulin secretion (GSIS) test at the end of the perfusion period.

Flushing the placenta in the OPR by cannulating the umbilical vessels using the side-entry method proved to be an efficient method with no signs of tearing of the umbilical artery. By cooling the placenta in the OPR and keeping the placenta cold during transportation, damage due to warm ischemia was prevented. Using pH-controlled flush medium prevented vasospasm of the umbilical arteries and placental capillaries, making it possible to infuse the cold flush medium.

The sterile techniques used during the perfusion proved highly effective as antibiotics and antifungal medication combined with sterile gloves successfully prevented any signs of infection throughout the experiment. The tubing setup demonstrated good functionality, enabling both quick medium changes during the experiment and straightforward measurement of venous return percentages through straightforward procedures.

The glucose-stimulated insulin secretion (GSIS) measurement indicated that pancreatic islets remained viable following overnight ex vivo perfusion in the maternal circulation of the cotyledon. An increase in insulin concentration in the cotyledon effluent during the high-glucose phase confirmed the presence of insulin secretion. However, the sampling frequency was insufficient to capture a detailed dose-response profile, limiting the interpretability of the insulin secretion dynamics. As a result, while insulin production was observed, the response did not exhibit the typical biphasic pattern characteristic of GSIS measurements of isolated pancreatic islets (29).

A notable finding during the GSIS measurement was the absence of an increase in insulin in samples taken from the venous return during the high glucose phase, as described in the Methods section on *Glucose-Stimulated Insulin Secretion*. One possible explanation is that the direction of perfusate flow—from the fetal circulation into the intervillous space—prevents insulin secreted into the maternal compartment from reaching the fetal venous outflow. This unidirectional flow is supported by the consistently low venous return percentage observed during perfusion. Alternatively, the lack of insulin detection may reflect a biological barrier: the syncytiotrophoblast layer, which is known to restrict insulin transfer across the placenta. Previous studies have demonstrated that insulin does not cross the placental barrier during short-term perfusion when the syncytiotrophoblast layer is intact (30,31). Further research needs to be performed on the integrity of syncytiotrophoblast layer during long-term perfusion in order to rule out the prevention of insulin transfer by the membrane.

Throughout the experiment, the percentage of venous return remained consistently low, at less than 50%. A potential cause of this is the static pressure differential between the fetal circulation and the intervillous space. In contrast, studies utilizing the placenta as a model for drug transfer often report venous return rates exceeding 90% (17). These studies typically employ a dual perfusion setup, in which the maternal side of the cotyledon is also perfused. This likely reduces the pressure gradient between the fetal and maternal compartments, thereby limiting hydrostatic fluid transfer. In the design of the current perfusion system, efforts were made to reduce leakage by minimizing resistance on the venous side of the tubing. This was done because previous research has shown that elevated venous pressures in the fetal circulation can lead to the opening of transtrophoblastic channels, which facilitates leakage from the fetal compartment into the intervillous space (32).

The placental cotyledons during gestation are endocrine organs and secrete hormones such as progesterone, hCG, and estrogens (33–35). Were an endocrine organ to be implanted in a recipient, it is to be expected that this could cause dysregulation of the hormone balance of the recipient. It is therefore of importance to investigate whether hormone production during exvivo perfusion of a placenta diminishes in order to safely transplant a single cotyledon in the future. Syncytiotrophoblasts are the producers of these hormones and originate from trophoblast stem cells. These stem cells require FGF4 and TGF-β family members for continued self-renewal (36).

During ex vivo perfusion, these are not present, which could result in the disappearance of the trophoblast stem cells in the cotyledon. As the syncytiotrophoblasts are in a post-mitotic state, this could result in the complete disappearance of syncytiotrophoblasts in the cotyledon and subsequent halting of hormone production after long-term perfusion (37). Research into the hormone production of a single cotyledon during long-term ex vivo perfusion is necessary to test this hypothesis.

The systematic literature review identified six criteria for evaluating extrahepatic transplantation sites. Injecting pancreatic islets into the intervillous space of a single cotyledon may theoretically prevent the occurrence of the immediate blood-mediated inflammatory reaction (IBMIR); however, further research is required to validate this assumption. The cotyledon demonstrated adequate partial pressure of oxygen when perfused under subnormothermic conditions with a cell-free medium. To determine whether a single cotyledon can sustain pancreatic islets during normothermic perfusion, additional studies using whole blood for fetal-side perfusion and relevant physiological measurements are necessary. Immunological aspects of the cotyledon were not evaluated in this thesis, highlighting the need for further investigation. Due to its rich vascularization, the cotyledon meets the criterion of adequate vascular supply. Because the distribution of pancreatic islets within the cotyledon was not assessed, it remains a relevant subject for future studies. Pancreatic islets injected into the intervillous space remained detectable after 14 hours of ex vivo perfusion, demonstrating the feasibility of monitoring islet viability over time.

The final experiment represents a single instance in which pancreatic islets were injected into the intervillous space of an isolated cotyledon to assess islet survival. While the presence of insulin secretion suggests that the islets remained viable, the GSIS results do

not provide a definitive conclusion about the magnitude of islet function. Further refinements to the GSIS protocol are necessary to extract more detailed information about the functional state of the transplanted islets.

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6. Supplementary information

6.1 Systematic literature study on extra-hepatic transplantation sites

Title

Alternative sites for pancreatic islet transplantation

Abstract

The liver is currently the most used site for pancreatic islet transplantation. The local microenvironment of the liver and the intraportal transplantation method results in significant losses of infused islet due to hypoxia, immunological reactions and rejection. Extensive research in animal models has been performed discussing alternative transplantation sites such as the subcutaneous space, spleen, kidney, omentum and gastric submucosa, with ongoing studies focused on improving the microenvironment of these structures to enhance transplant success. Clinical research remains limited, and in their unmodified state, these structures are not suitable as transplantation sites for islets. Therefore, further research is necessary to modify these structures to improve islet survival and transplantation success.

Introduction

Intrahepatic islet transplantation, where isolated islets are infused into the liver via the portal vein, has been a therapeutic option for a select group of type 1 diabetic patients with frequent hypoglycemic events or poor glycemic control since its first successful achievement of insulin independence in 1990 (1,2). The introduction of the “Edmonton protocol” in 2000 marked a breakthrough by introducing glucocorticoid-free immunosuppression for intrahepatic islet transplantation (3). Although the results were better than previous attempts at clinical islet transplantation, still two years after transplantation less than 50 percent of patients were insulin independent and after five years this declined to 10 percent (3). Additional research into the survival of pancreatic islets into the reveals that up to 70% of transplanted islets are destroyed immediately after transplantation (4).

The limited survival of transplanted islets necessitates repeated transfusions, limiting the widespread implementation of clinical islet transplantation (5,6). Multiple transfusions of pancreatic islets exposes the recipient to poly-human leukocyte antigen (HLA) antigens from multiple donors, complicating future solid-organ transplantations (6). The need for multiple islet donors is because of intrinsic problems of using the intraportal route as a delivery method of the islets and the microenvironment of the liver.

Intraportal islet infusion is associated with an instant blood-mediated inflammatory reaction (IBMIR), an innate inflammatory reaction elicited when islets are exposed to ABO-compatible blood that involves coagulation and complement activation (7). The infused islets are transported into the liver and get lodged in sinuses of the liver which causes portal hypertension and local liver ischemia (8). Further possible complications

of the procedure are portal embolism and bleeding at the injection site although these events are rare when using appropriate measures (9,10).

The microenvironment of the transplanted islets in the liver differs on key points from the pancreatic parenchyma, the native site of the pancreatic islet. The partial oxygen in the liver parenchyma is 10–15 mmHg, which is significantly lower than the partial oxygen pressure of the pancreas (approximately 40 mmHg) (11). This hypoxic environment impairs islet survival, causes central necrosis in larger islets and reduces the ability of surviving islets to secrete insulin due to reduced glucose oxidation (12–14).

No barrier between the immune system of the recipient and the transplanted islets is achieved during intrahepatic transplantation which necessitates the use of immunosuppressive drugs while still having the risk of allogenic rejection (1). The standard in immunosuppression is tacrolimus, which has been shown to inhibit insulin release from pancreatic islets by reducing glucokinase activity as well as inhibiting the revascularization of transplanted islets (15,16). Furthermore, it has been shown that the concentration of tacrolimus is higher in the portal vein than the systemic circulation which may result in intensifying these negative effects (16).

An alternative to the intrahepatic transplant site in which the losses of islet engraftment are minimized would ideally: 1) Prevent the occurrence of the immediate blood-mediated inflammatory reaction, 2) Provide a microenvironment that has an adequate partial pressure of oxygen, 3) Minimize the exposure of the islets to immunosuppressive medication by having the afferent blood supply not originate from the portal vein or eliminate the need for immunosuppressive medication by acting as an immune privileged transplantation site.

This paper presents a narrative review of the literature about transplantation sites other than the liver for the transplantation of pancreatic islets. The goals are to identify which anatomically structures were investigated and which advantages and disadvantages each structure has in the context of islet transplantation.

Method

This narrative review systematically gathered research papers about extra-hepatic sites for pancreatic islet transplantation. All papers available on PubMed® up to and including 8 January 2025 were included in this search. The search strategy is illustrated schematically in figure 1 with table 1 specifying search terms. The complete search strategy is given in appendix 1.

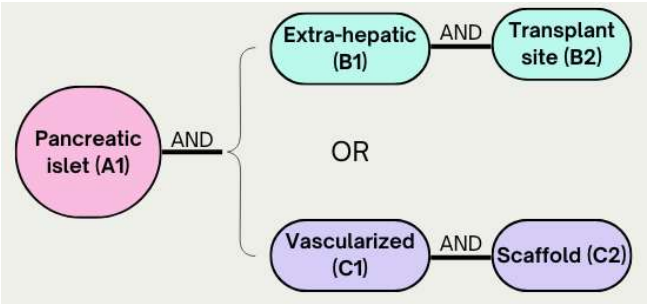

Figure 1: Schematically representation of the search strategy. The identifiers correspond with the headers of table 1.

All articles were screen based on their titles and abstracts, followed by a full-text assessment to determine their eligibility. The following inclusion and exclusion criteria are used to select the reports:

Inclusion criteria

- Report is available in full-text format
- Report is written in English
- Report describes usage of extra-hepatic transplantation sites in pancreatic islet transplantation

Exclusion criteria

- Report exclusively describes methods to produce vascularized scaffolds without transplanting pancreatic islets

After inclusion, the reports were categorized into groups based on the use of transplantation site. Reports may be included into multiple categories if they investigated multiple extra-hepatic transplantation sites.

Table 1: Overview of terms used in the search. The headers correspond with the identifiers of figure 1.

<table>
<tr><th>A1</th><th>B1</th><th>C1</th><th>B2</th></tr>
<tr><td rowspan="3">"Islet* of Langerhans"[All Fields] OR "pancreatic islet*"[All Fields] OR "beta Cell"[All Fields]</td><td rowspan="3">"Extra-hepatic"[All Fields] OR "Extra hepatic"[All Fields] OR "Non-hepatic" [All Fields] OR "ectopic"[All Fields] OR "alternative"[All Fields] OR "heterotopic"[All Fields]</td><td rowspan="3">"vasculari*"[All Fields] OR "neovasculari*"[All Fields] OR "neo-vasculari*"[All Fields] "revasculari*"[All Fields] OR "re-vasculari*"[All Fields] OR "angiogenesis"[All Fields]</td><td>"transplant* site*"[all fields]</td></tr>
<tr><td>C2</td></tr>
<tr><td>"scaffold*"[All Fields]</td></tr>
</table>

Results

A search was performed on PubMed® which returned 123 articles. Of which 113 were assessed during full-text assessment based on the exclusion- and inclusion criteria. A total of 15 papers were acquired via cross-referencing. An overview of the identification and screening process is given in figure 2.

A total of 67 papers were categorized based on used alternative transplant site. Reports were categorized as: 'Subcutaneous', 'Intramuscular', 'Peritoneum and omentum pouch', 'Bone marrow', 'Kidney capsule', 'Gastrointestinal wall', 'Spleen' or 'Other'.

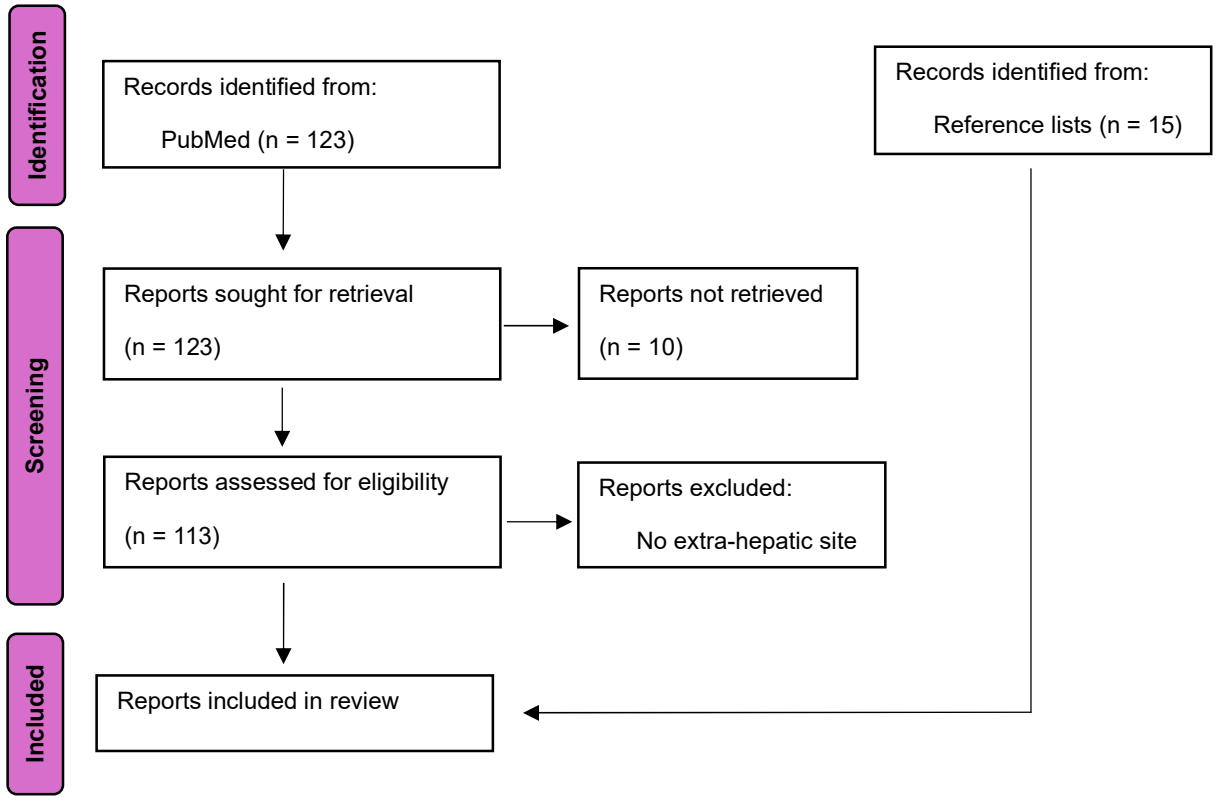

Figure 2: Overview of the screening process

Subcutaneous

The subcutaneous space has an added value over other alternative transplantation sites because of its accessibility for implantation and graft retrieval (17). A study with mice showed white adipose tissue, which is found in the hypodermis, could slow down destruction of the islets by the immune system when compared with intrahepatic transplantation (18). However, due to its low oxygen tension and poor vascularization, the unmodified subcutaneous space is a poor environment for transplanted pancreatic islets (19). To improve the density of the vasculature network of the transplantation site, pre-vascularization can be employed. By placing a nylon catheter in the transplantation site one month before transplantation, thereby inducing a foreign-body response, neovascularization can be promoted (20).

Another method uses an implantable macro-polymer device, the Cell Pouch. A study investigating the efficacy of a transplant of 500 islets to reverse diabetes when transplanted into the Cell Pouch compared to islet grafts in the kidney capsule found that the Cell Pouch functioned similarly to islets transplanted under the kidney capsule (17). A similar idea to the Cell Pouch is the use of long polytetrafluoroethylene rods surrounded by a thin, non-degradable silon monofilament mesh as a scaffold for improving vascularization. After 7 days, the rods are removed to create a space in which pancreatic islets and/or mesenchymal stem-cells can be transplanted. Mesenchymal stem-cells (MSCs) have been shown to improve blood flow when incorporated into a scaffold that is used for pancreatic islet transplantation(21).

Pancreatic islets can clump together during transplantation, creating clusters of islets. Because of the size of these clusters, oxygen diffusion is insufficient to oxygenate the pancreatic islets at the center of the cluster, causing central necrosis.
Several strategies prevent islet clumping by maintaining distance between individual islets. One such method involves using a biodegradable Vicryl mesh. The pancreatic islets are sandwiched between two layers of the Vicryl mesh which in turn are coated with a thrombin-fibrin gel. This prevents the formation of clusters of pancreatic islets and thereby prevents necrosis. The fact that the mesh is biodegradable prevents long-term foreign body reactions (22). Furthermore, another study which looked at only fibrin as a scaffold shows that again there was a positive effect on engraftment when pancreatic islets are placed in a fibrin scaffold before transplantation (23).

Intramuscular
Because of the high metabolic demand of striated muscle cells, the site is highly vascularized and capable of rapid revascularization (24). The location makes it an accessible site for transplantation and monitoring (24,25). A comparison of intramuscular islet transplantation with intrahepatic islet transplantation in rats shows that the intramuscular site has adequate partial pressure of oxygen and better revascularization. However, major problems were central necrosis and fibrosis ultimately leading to graft failure (14).

The unmodified intramuscular site performs worse in terms of islet survival when compared with intraportal transplantation in mice while using murine islets (26). A larger more recent study which used murine and human islet transplanted into multiple site (liver, kidney, muscle) found that islet transplanted into liver and muscle have similar efficiency (8). Because of ease of access and hopeful animal model results further research into clinical application is warranted although the interaction of the islet with the immune system is still of concern as the muscle is not an immune privileged site.

Recent research into the use of chemically induced pluripotent stem-cell-derived islets (iPSCs) transplanted into the abdominal anterior rectus sheath of a 25 year old patient garnered much attention (27). Following the transplant procedure, the individual no longer required insulin supplementation and glycated hemoglobin (HbA1c) levels normalized to 4.6% after 180 days and maintained a value of 4.8% at the one-year follow-up assessment (27). This pivotal paper showed the feasibility of transplanting chemically induced pluripotent stem-cell-derived islets and the use of the abdominal

anterior rectus sheath as an extrahepatic transplantation site. The paper described the preliminary findings of the ongoing TJFCH-iPS-001 trial (ChiCTR2300072200) which to date has included a total of 3 participants. Data about these additional participants is highly anticipated and further research into the use of iPSCs for transplantation is warranted.

Peritoneum and omentum pouch
The peritoneum has the advantage of a nearly unlimited volume in which pancreatic islets can be transplanted. In addition, it is able to accept unpurified islets as well as microencapsulated islet (24). Furthermore, this structure has a dense vasculature network and its efferent blood vessel drains into the portal vein(1). A disadvantage of the large space is the difficulty in retrieving the engrafted islet for monitoring or graft removal. By using a surgical approach, a pouch can be made using the parietal peritoneum and the omentum major which is called the omentum poach (28).

The omentum pouch has the added benefits that access and retrieval of the graft is relatively easy compared to the peritoneum space (29). Furthermore, there are signs that it acts as an immune privilege site (24,30). A clinical trial involving four patients undergoing a total pancreatectomy and islet autotransplantation(TPIAT) in which a partial volume of the islet was transplanted into the omentum because of persistent elevated portal pressure during intrahepatic infusion found the omentum implantation procedure to be safe and achieved similar results as patients receiving the complete islet mass via intrahepatic infusion (31). Currently there are two clinical phase two trials being performed in Miami (NCT02213003) and Milan (NCT02803905). To date there are no results published on the findings of these trials, but the omentum has the potential to be a safe and effective alternative to intrahepatic transplantation (29).

Bone marrow
An experimental study into transplanting islet into the intraossiacal space (tibia) of nondiabetic rats showed insulin and glucagon were produced by the islet and no signs of rejection were identified up to two weeks post-transplantation (32). Because no immunosuppressants were used this finding suggests that the bone marrow would be an immune privileged environment for islet transplantation. The bone marrow is an accessible transplantation site by the use of an intraosseous needle with which the islets are transplanted into a high vascularized environment without coming into contact with blood (24). Further research was performed on mice in which islets were transplanted into the femur and showed an similar response to glucose challenges as non-diabetic mice up to a year post transplantation (33).

These positive results were the basis of a clinical study with patients having contraindications for intraportal infusion (24). This phase 1 trial into the safety of the procedure resulted in positive findings such as no adverse events recorded and the presence of insulin producing cell in a biopsy taken one year after transplantation (34). A subsequent phase 2 research initiative revealed islets were not able to survive long term in the bone marrow due to the immune response (35). This resulted in loss of function of the islet four months after infusion in all but one patient independent of immunosuppression schemes. Additionally, a paper on the partial oxygen pressure in

live animals revealed that despite the rich vasculature the bone marrow has a relative low partial pressure of less than 32 mmHg (36). These results suggest that while bone marrow currently does not appear to be a suitable site for pancreatic islet transplantation, further research is needed to determine if its limitations can be overcome.

Kidney capsule
In animal models the kidney is a commonly used site for the transplantation of pancreatic islets. Benefits of the site include retrievability of the graft by nephrectomy and the relative ease of the transplantation procedure in murine models (24). When comparing the kidney capsule with the liver as a transplantation site multiple studies found lower numbers of islets were necessary to restore normoglycemia in mice (37,38). In a study with pancreatectomized primates the opposite result was found, 80% of primates receiving transplantation of islet under the kidney capsule did not achieve normoglycemia (39).

The goal of finding an extra-hepatic transplantation site is to improve the microenvironment in which the islets are placed post-transplantation. The renal capsule has a partial oxygen pressure of 15 mmHg which is relative hypoxic compared to the 40 mmhg of oxygen pressure found in the pancreas (1). Furthermore, the transplantation of islets into the kidney capsule is a more invasive procedure compared to intra-hepatic infusion (1,24). The possibility that a patient with diabetes in the future requires a kidney transplantation with subsequent loss of transplanted islets is another argument against the use of the kidney capsule as a transplant site (24). Lastly, the renal subcapsular space is inelastic and has a limited volume making the transplantation of a large amount of islets impractical (25,40).

Gastrointestinal wall
Because of its dense vascular network and drainage into the portal vein the submucosa of the stomach and duodenum are explored as possible transplantation sites. The transplantation and monitoring can be done laparoscopic or endoscopic (41). A study using mini pigs showed better glucose response compared to transplanted islets under the kidney capsule (42). This result prompted further research which showed that gastric submucosa transplantation was not able to reverse diabetes long term (mean survival time, 7.16±0.69 days) (43). Since the gastric submucosa has not demonstrated significant advantages over the liver as a transplantation site based on current evidence, it does not appear to be a promising alternative to intrahepatic transplantation.

Pancreas
The pancreas is the native home for the islets, because of this it has been investigated as an alternative site for transplantation. Animal studies involving dogs and rodents show it provides a good environment for engraftment of islet by showing minimal inflammation on the implantation site and improved glycemia (44). These findings are supported by the fact that the pancreas has a higher partial oxygen pressure compared to the liver (45). Although the pancreas has advantages over intrahepatic pancreatic islet transplantation it has no use in the clinical setting. Major disadvantages are the invasive transplantation procedure, the risk of pancreatitis and the fact that previous immune

activation because of diabetes type 1 sensitized the local immune system (24,41). Furthermore, the main function of the pancreas is to produce digestive enzymes, which poses a serious risk to the survival of transplanted pancreatic islets.

Spleen

The spleen is a richly vascularized intraperitoneal organ and has been shown in a canine study to have the ability to support pancreatic islets (41,46). The spleen drains via the portal vein, similar to the venous drainage of the pancreas, the native site of the pancreatic islets (41). This drainage into the portal vein has the physiological benefit of exposing the liver to higher concentrations of insulin compared to the systemic circulation. The liver then extracts approximately 50% of insulin that arrives through the portal vein, which helps prevent excessive insulin levels (peripheral hyperinsulinemia) in the general circulation (47).

In dogs it is found that the spleen poses a better one year graft survival compared to intrahepatic transplantation (48). Furthermore, a study examining the injection of pancreatic islets into the splenic pulp, rather than through intravascular infusion, found that only half the number of islets was needed to reverse hyperglycemia compared to intraportal transplantation in non-obese mice (49).

Reasons that prevent the clinical application of the spleen as an transplantation site are that it has no proven advantage in terms of efficacy over intra-hepatic transplantation and there are additional risks associated with transplantation into the spleen (1). Transplantation of pancreatic islets into the spleen has the risk of splenic rupture with resulting hemorrhage. In addition, the spleen is rich in lymphocytes which makes it a poor potential transplantation site.

Other

Brown adipose tissue possesses a rich vasculature network which is advantages for engraftment of islets (50). No pre-clinical studies are currently undertaken and questions about the practicality of using brown adipose tissue in clinical transplantations such as volume restrictions of the anatomical structures and difficulty of access of the structures remain.

The thymus has been identified as a possible immune privileged site which prompted investigations into the possibility of using it as an alternative transplant site. As exposure of maturing T-cells to the allogenic antigens of donor pancreatic islets would desensitize the maturing T-cells, additional interest was allocated to the thymus (1). A study using rats showed long-term survival of pancreatic islets transplanted into the thymus (51). However it was found that a large volume of islets is necessary to reverse hyperglycemia making the thymus less useful as an transplant site (1).

A novel approach to creating an extrahepatic transplant site for pancreatic islets is the formation of a venous sac in which the islets are transplanted. In a mouse model the lumbar vein was litigated after which a vasa vasorum developed in the venous sac (52). Although this paper showed a return to glucose homeostasis after transplantation and

no signs of bleeding no further research was performed on the creation of a venous sac for pancreatic islet transplantation.

Discussion

The liver is the most used site for clinical pancreatic islet transplantation despite its numerous disadvantages, and research is being performed to find an alternative transplantation site (1). The main concern is the major loss of infused pancreatic islets after transplantation into the liver, which necessitates multiple transfusions to transplant an adequate islet mass to reverse diabetes. Underlying factors that contribute to this loss of pancreatic islets are the instant blood-mediated inflammatory reaction (IBMIR), poor oxygen partial pressure in the liver capillaries, and exposure to high levels of immunosuppressive medication due to the portal vein blood supply (24,41). Alternative transplantation sites are the subject of research as these have the potential to eliminate the need for multiple transfusions for recipients. This study presents a literature review examining alternative sites for pancreatic islet transplantation.

A possible advantage of alternative transplantation sites is the prevention of the instant blood-mediated inflammatory reaction by placing the islets outside of the bloodstream. Blood transports oxygen, glucose, and insulin, which means that placing the islets outside of the bloodstream creates an additional challenge: ensuring proper oxygenation and transfer of glucose and insulin into and out of the systemic circulation. This introduces an additional requirement for adequate vascularization of the alternative transplantation site.

Some sites such as intramuscular, bone marrow, spleen, peritoneum and omentum pouch already provide a dense vascularized environment into which the islets can be transplanted. Another site with less native vascularization, such as the subcutaneous space, needs to be modified to improve vascularization in order to support the transplanted islets. This modification can be accomplished by invoking a foreign body response at the desired site prior to transplantation thus pre-vascularizing the site or by transplanting the islets into a scaffold alongside growth-factors that improve neovascularization. Still, clumping of islets can cause the formation of clusters of pancreatic islets at the transplantation site that lead to central necrosis inside these aggregated islet masses.

In order to prevent the clustering of islets it is possible either to distribute the islets during transplantation by infusing islets while moving the catheter or by placing the islets into a scaffold. This scaffold ensures there is enough space between islets for adequate diffusion of oxygen thus preventing central necrosis. The prevention of the aggregation of pancreatic islets especially in poor vascularized sites is a factor that needs to be considered during the selection of alternative transplantation sites.

During allogenic pancreatic islet transplantation, exogenous biological material is introduced into the recipient's body. This triggers a response of the immune system of the recipient that degrades the transplanted pancreatic islets. In order to prevent this interaction between the immune system and the pancreatic islets a regime of immunosuppressive medication is used during intrahepatic islet transplantation. Because of the negative effects of immunosuppressive medication ideally an alternative

transplantation site would be immune privileged thereby negating or reducing the need for immunosuppressive medication.

Some alternative sites show promising results in animal models and are for this reason explored as transplantation sites for pancreatic islets. Bone marrow for example achieved good results in rodent studies and appeared to be an immune privileged site (24). Because of its rich vascularization islets could be placed inside a rich vascularized environment via a bone marrow puncture without being exposed to the IBMIR reaction. Furthermore, the transplanted islets were contained within the bone marrow which is beneficial if in the future induced pluripotent stem cells used as a method for creating pancreatic islets (27). In this case there is a need to monitor the transplanted pancreatic islets for the formation of carcinogenic malformities.

Much insight was gained by the use of a clinical study in which the bone marrow was tested in humans as it revealed that in humans there was still allogenic rejection when islets were placed in the bone marrow (33). This disappointing result shows that bone marrow to date does not pose as a suitable alternative to intrahepatic islet transplantation and illustrates the need to use models in research that closely represent the physiological situation in humans as success in rodents is not necessarily translated into success using human participants.

Based on the findings of this narrative review a transplantation site for pancreatic islets should ideally: 1) Prevent the occurrence of the immediate blood-mediated inflammatory reaction, 2) Provide a microenvironment that has an adequate partial pressure of oxygen, 3) Minimize the exposure of the islets to immunosuppressive medication, 4) Be adequately vascularized, 5) Prevent clumping of islets and central necrosis, 6) Provide containment of transplanted islets and make monitoring possible.

Currently no alternative site for pancreatic islet transplantation satisfies the recommended requirements and proves to be a viable solution. Some alternative sites show potential but further research is needed in order to improve the microenvironment and prevent rejection of the transplanted islets. Results from phase 1 and phase 2 trials are pending and could provide valuable insight into the use of alternative sites. Still, it is advised to investigate more out of the box ideas such as using the placental cotyledon as a vascularized structure in which the pancreatic islets are to be injected ex-vivo after which the cotyledon is transplanted into the recipient.

This way islets can be injected into the intervillous space thereby circumventing the IBMIR. The cotyledon has a rich vascularization and provides both venous as well as arterial access. An optimal place inside the recipient for anastomosis of the efferent and afferent blood vessels of the cotyledon can assure adequate oxygenation of the islets and release of the produced insulin into the portal vein. Placement of the islets into the intervillous space can be done using multiple smaller injection thereby distributing the islets and preventing clumping as well as the occurrence of central necrosis. By allowing the engraftment of the islets in the structure to take place ex-vivo more control and monitoring can be applied compared to in-vivo infusion of pancreatic islets. Studies utilizing ex vivo perfusion of cotyledons are primarily limited to examining the transfer of

drugs across the placenta (53). Further research needs to be performed to get insights into the cotyledon as a possible site for pancreatic islet transplantation.

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Appendix 1

("Islet* of Langerhans"[All Fields] OR "pancreatic islet*"[All Fields] OR "beta cell*"[All Fields]) AND ((("Extra-hepatic"[All Fields] OR "Extra hepatic"[All Fields] OR "Non-hepatic"[All Fields] OR "ectopic"[All Fields] OR "alternative"[All Fields] OR "heterotopic"[All Fields])AND("transplant* site*"[all fields]))OR (("vasculari*"[All Fields] OR "neovasculari*"[All Fields] OR "neo-vasculari*"[All Fields] "revasculari*"[All Fields] OR "re-vasculari*"[All Fields] OR "angiogenesis"[All Fields]) AND ("scaffold*"[All Fields])))

6.2 Preliminary experiments and protocol development

PLC01

Goal: Gain experience in the OR obtainment of the placenta and test cannulation techniques.

The placenta was obtained in the OR and placed in an organ transport bag with 1 liter of ice-cold Ringer's acetate. In the laboratory, the placenta was placed in a rectangular non-sterile box. The umbilical cord had been detached during delivery, preventing cannulation of the umbilical vessels during this perfusion experiment.

An individual cotyledon was cannulated with a 5 French PUR cannula, but no suture was used to secure the cannula. This resulted in leakage of perfusate between the cannula and the arterial wall. An attempt to use a 14- or 16-gauge venous catheter without fixation sutures yielded similar results.

Takeaway: Future experiments should focus on developing better cannulation techniques to eliminate perfusate leakage and more secure fixation methods to prevent catheter dislodgement

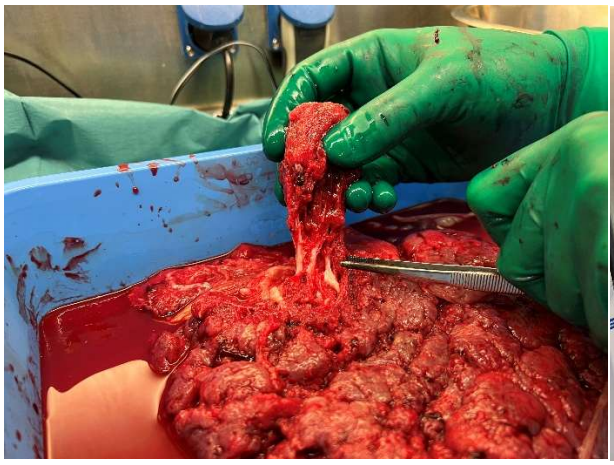

Figure 11: Picture of PLC01: A single cotyledon extended away of the basal plate

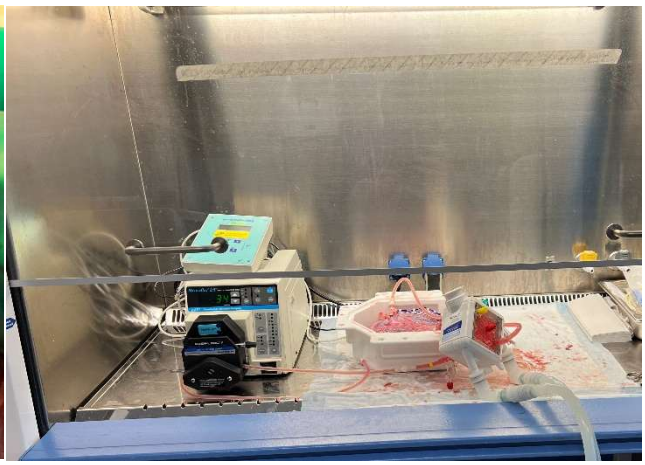

Figure 12: Picture of PLC02: Overview of the perfusion setup

PLC02

Goal: To independently procure placental tissue and evaluate suturing techniques for cannula fixation

Placental procurement in the OR followed the same protocol as PLC01. In the laboratory, the placenta was placed in a 3D-printed organ chamber and suspended on rigid plastic mesh. The perfusate was Ringer's acetate, consistent with the PLC01 protocol. An oxygenator with heat exchange capability was added to the perfusion setup to regulate perfusate temperature. No gas supply was connected to the oxygenator.

Umbilical vein and artery cannulation was performed using two 14-gauge venous catheters. Each catheter was inserted into the vessel lumen, and a suture was tied around the umbilical cord to secure the catheter. This fixation method prevented perfusate leakage when 50 mmHg pressure was applied via the peristaltic pump. The

placenta was perfused for 18 hours, after which obvious discoloration of the maternal side was observed.

Takeaway: The addition of an oxygenator and heat exchanger enables temperature control during perfusion. Cannulation of the umbilical vein and arteries can be successfully achieved using venous catheters, with sutures tied around the umbilical cord preventing perfusate leakage.

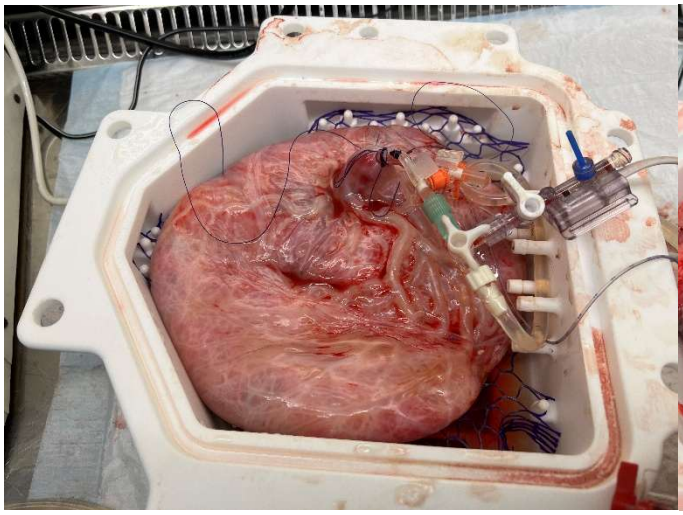

Figure 4: Picture of PLC02: Cannulation of the umbilical vein and artery with a suture around the umbilical cord to prevent leakage

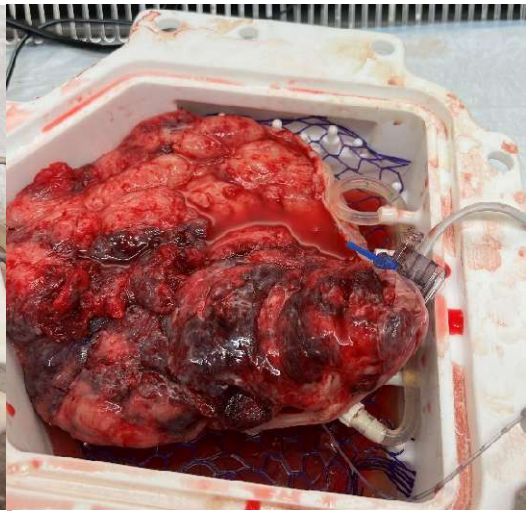

Figure 5: Picture of PLC02:Discoloration of the maternal side of the placenta after 18h perfusion

PLC03

Goal: Gain experience in canulating umbilical blood vessels

Placenta procurement followed the same protocol as PLC01. In the laboratory, cannulation of the umbilical arteries using 14-gauge venous catheters resulted in tearing of the blood vessel wall, making cannulation and perfusion impossible. A 5 French PUR cannula was investigated as an alternative, but its small diameter created high circuit resistance, making pressure-controlled perfusion difficult. Another problem was the smooth profile of both cannulation devices, which made secure fixation and leakage prevention challenging.

A Foley catheter was investigated as an alternative but proved unsuccessful. The circuit resistance was high compared to the 14-gauge venous catheter, and the inflated balloon caused damage to the vessel wall.

The placenta was placed in a heated organ chamber, and papaverine was injected into the artery to investigate whether this aided in the relaxation of smooth muscle cells in the arterial wall. The combination of heat and papaverine appeared to decrease the resistance of the capillary bed.

Takeaway: The investigated cannulation methods showed poor outcomes. Better cannulation materials and procedures are needed. Heat and papaverine seem to have a positive effect on arterial flow

Figure 13: Picture of PLC03: A single cotyledon cannulated with a foley catheter

PLC04

Goal: The study will investigate the addition of a venous reservoir and carbogen gas supply on perfusion outcomes. Additionally, cannulation and fixation methods will be evaluated.

The placenta was obtained after umbilical blood was collected for research purposes, resulting in a long warm ischemic time. After obtaining the placenta, it was handled in the OR via the PLC01 protocol. In the laboratory, the placenta was placed inside a heated organ chamber and cannulated using two 8-french bridge-to-live cannulas. An 8-french diameter ensures low resistance, and the pyramid-shaped tip aids in the fixation of the inserted cannula tip.

A venous reservoir was added to increase the perfusate volume in order to prevent the motor from running out of perfusate through the experiment. During perfusion, discoloration of the maternal side is a desired effect, as this indicates adequate perfusion of the cotyledons. A 95% carbogen supply was attached to the oxygenator with a flow of 1 L/min. After 4 hours of perfusion, the perfusate darkened, indicating an infection

Takeaway: The 8-french bridge-to-live cannula yielded positive results in preventing leakage and improving cannula fixation. Carbogen and the venous reservoir were added to the perfusion setup, and there were signs of infection after the perfusion.

Figure 15: Picture of PLC05: Discoloration of the material side of the placenta

Figure 14: PLC05: Tip of 8-french bridge-to-live cannula

PLC05

Goal: Perform a cold-flush on the OPR and test the 12- and 8-french bridge-to-live cannulas.

The placenta was obtained in the OR and transported in a non-sterile box to the OPR. In the OPR, the umbilical arteries were cannulated with an 8-French bridge-to-live cannula and a suture was placed around the individual artery. The umbilical vein has a larger diameter, which facilitates a 12-French bridge-to-live cannula. This cannula was inserted into the vein and a suture was placed around the vein.

During the flush with ice-cold heparinized Ringer-acetate, it was noted that little perfusate entered the placenta. In the laboratory, no perfusion was possible because of the high resistance of the placenta to fluid flow. The experiment was terminated because no fluid flow was achieved at a set pressure of 50 mmHg.

There are two possible mechanisms that caused the high resistance in the placenta. The first is contraction of smooth muscle cells in the arterial wall in response to the cold perfusate. Another mechanism is tearing of the artery during cannula insertion, thereby occluding the arterial lumen.

Takeaway: Investigate whether a warm flush and transport to the laboratory prevents vasospasm of the arterial smooth muscles, and search for an alternative cannulation technique to prevent tearing of the umbilical artery.

PLC06

Goal: Evaluation of the new mobile perfusion setup

A mobile perfusion setup was created with the use of a 5-liter tank of 100% oxygen, two peristaltic pumps with pressure transducers, a 2L bag of flush medium, two oxygenators, a 4-liter bag for effluent collection, and tubing. The goal is to start oxygenated perfusion on the OPR after cannulation and to transport the placenta in the mobile perfusion setup to the laboratory. In this way, a long warm ischemic time is prevented while keeping the placenta at room temperature.

The placenta was cannulated on the OPR with two 8-French cannulas using the same procedure as PLC05 for the arteries and a 12-French catheter for the vein. After cannulating the placenta, it was placed in a modified XVIVO organ chamber and perfusion was started. Pressure was set to 50 mmHg and oxygen flow was set to 1 L/min. The mobile perfusion setup was transported to the laboratory and the organ chamber was placed inside the flow hood.

After 2 liters of flush medium was perfused, the placenta was discolored, and the setup was changed to closed circuit subnormothermic perfusion. The perfusate was DMEM F12 with the addition of human serum albumin 20g/L. During the perfusion, pH was corrected with the addition of sodium bicarbonate. After 4 hours of closed circuit perfusion 20.000 ieq of pancreatic islets were injected into both arteries in a bolus volume of 15 ml perfusate. An increase in the resistance of the placenta was noted as the flow decreased with 10 ml/min.

The experiment continued overnight, but in the morning it was clear the venous reservoir was empty and air was being pushed through the placenta.

Takeway: The mobile perfusion setup made it possible to transport the placenta during ex vivo perfusion from OPR to the laboratory. The setup needs adjustments in order to prevent the occurrence of an empty venous reservoir. The introduction of pancreatic islets in the artery of the placenta causes an increase in resistance.

Figure 6:PLC06 mobile perfusion setup

Figure 16: PLC06: modified XVIVO organ chamber

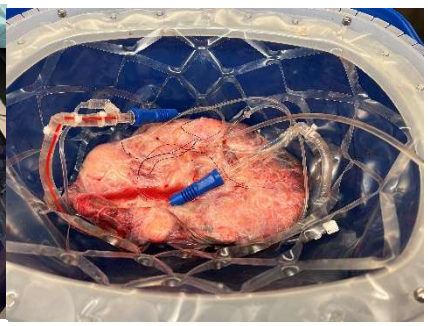

Figure 7: PLC06: cannulated placenta

PLC07

Goal: evaluate the new tubing set for the prevention of a dry venous reservoir

The placenta was obtained on the OR and canulated on the OPR. A senior medical staff member was present during cannulation and mentioned an alternative cannulation technique which reduces the risk of tearing the artery inside the umbilical cord. The placenta was perfused in the OPR and transported to the laboratory.

A medium change was performed to switch the perfusate to cell medium with human serum albumin 20 g/L. A CVVHD dialysis machine was added to the perfusion setup in order to remove waste products from the perfusate. There were a significant number of alarms generated by the CVVHD machine because of pressure differences between inlet and outlet. After 2 hours of 25 degrees Celsius perfusion with the use of the heat exchanger, the temperature was raised to 30 degrees Celsius. The placenta became too metabolically active for cell-free perfusate, which resulted in lactate buildup and a drop in pH. After 3 hours, the temperature was reduced to 27 degrees Celsius.

Takeaway: The current setup triggers alarms on the CVVHD machine due to hydrostatic pressure differences between inlet and outlet ports. There is an alternative cannulation technique which might result in better cannulation results. The maximum temperature for cell-free perfusate perfusion of a placenta is 27 Degree Celsius.

PLC08

Goal: Evaluation of the new cannulation technique and multi-day perfusion

Cannulation was performed in the OPR with the use of the side entry technique which is described in the methods section of the manuscript. No signs of tearing or trauma of the umbilical vein were noticed. After transport the placenta was put on closed circuit with a perfusate of DMEM F12 and human serum albumin 20g/L. After one night of perfusion, it became clear that there was a serious infection of the perfusate and placenta. The rest of the experiment was cancelled, and the venous reservoir and oxygenators were discarded.

Takeaway: The addition of antibiotics, antifungal medication, and sterile gloves when handling the placenta is essential for the prevention of infection. Future perfusate should include antibiotic and antifungal components.

PLC09

Goal: Evaluate the addition of anti-biotic and anti-fungal medication as well as sterile gloves for handling the placenta.

Procurement of the placenta was similar to the protocol of PLC08. In the laboratory, the placenta was successfully perfused for two days. However, during cleanup, it was noted that there remained signs of infection in the smell of the perfusate. This indicates the current setup of reused organ chamber as well as the elaborate network of tubing might be the source of persistent infection during the experiment. A senior medical staff employee noted that there is an anastomosis of the two arteries at the base of the umbilical cord. This potentially eliminates the need for cannulating both arteries.

Takeaway: A setup needs to be created which is easier to clean and ideally be sterilized.

PLC10

Goals: Evaluation of a new procurement protocol and new perfusion setup

A redesign of the perfusion setup was done in which the disposable organ chamber of XVIVO was replaced by the 3D-printed organ chamber of PLC02. This organ chamber can be sterilized using an autoclave and has a lid which seals the inside from airborne microbes. The 3D-printed organ chamber has large enough internal volume to eliminate the need for a venous reservoir which simplifies the setup and reduces the risk of infection.

The removal of the disposable organ chamber resulted in a new method for the procurement of the placenta in the OPR because the mobile perfusion setup was no longer an option. The umbilical arteries were cannulated using a 5-French PUR catheter, and 50 mL of ice-cold heparinized perfusate was infused into the placenta. The side entry method of cannulation of the umbilical artery from PLC09 resulted in no tearing of the umbilical artery. The use of perfusate instead of Ringer-acetate ensured a correct pH, which prevented vasospasm due to pH imbalances. This technique proved successful as it enabled perfusion of the placenta in the OPR and cooling of the placenta for transportation.

In the laboratory the umbilical arteries were canulated with an 8-french bridge-to-live catheter. A two-day perfusion was performed in which the flow through the placenta dropped during the first night. A possible cause of this reduction is the swelling of the Wharton's jelly in the umbilical cord which compresses the umbilical vein and arteries. A measurement of venous return percentage showed 40% venous return indicating leakage from the fetal to the maternal side of the cotyledon.

Takeaway: The new procurement protocol is less cumbersome than the protocol of PLC08, and no signs of infection are present with the new perfusion setup. The new perfusion setup is capable of perfusing the placenta, but the flow through the placenta drops during the first night, possibly due to swelling of Wharton's jelly.

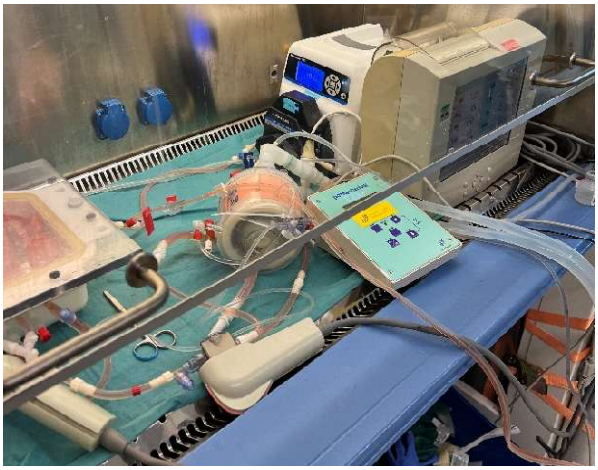

Figure 19: perfusion setup with 3D-printed organ chamber

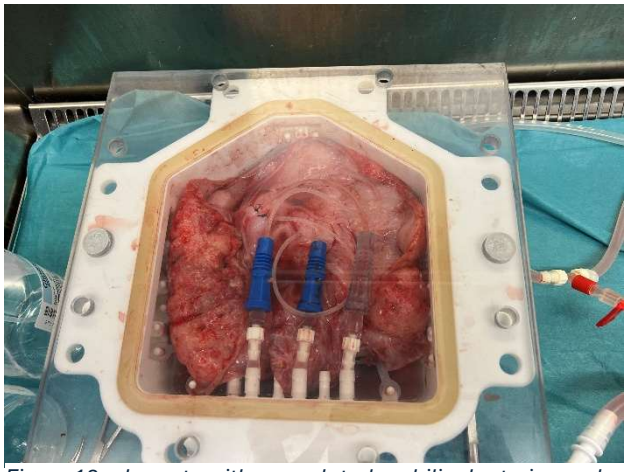

Figure 18: placenta with cannulated umbilical arteries and veins inside 3D-printed organ chamber

PLC11

Goal: Evaluate the GSIS protocol and perform a single cotyledon perfusion

The placenta was procured and transported similarly to PLC10. In the laboratory, the artery and vein of a single cotyledon are cannulated and perfused using 14-gauge venous catheter and 8-French bridge-to-live cannulas respectively. These materials were chosen because of the small vasculature of the cotyledon. Perfusion at 50 mmHg results in discoloration of a single cotyledon and makes it possible to resect all tissue not belonging to this cotyledon. Transected vessels are tied using sutures, and excess material is removed from the organ chamber and discarded.

The single cotyledon is perfused and oxygenated for 4 hours, after which 25,000 IEQ of pancreatic islets are infused into the maternal side of the cotyledon. A 14-gauge venous catheter is inserted into the cotyledon, and 50 mL of perfusate together with the islets are infused.

The next day, a GSIS was performed using Ringer-acetate which was passed through the oxygenator. This diluted the infused medium, making it difficult to interpret the results of the GSIS. The collected samples were analyzed using an ELISA for the presence of insulin, which was detected. However, no measurement of insulin production during low glucose levels were taken.

Takeaway: The injected pancreatic islets survived one night of ex vivo placental perfusion, but the setup for the GSIS needs to exclude the oxygenator in order to reduce the dead volume of the setup. It is also important to take ample measurements of the low glucose phase during the GSIS and measure glucose levels in the samples in order to create a dose-response curve.

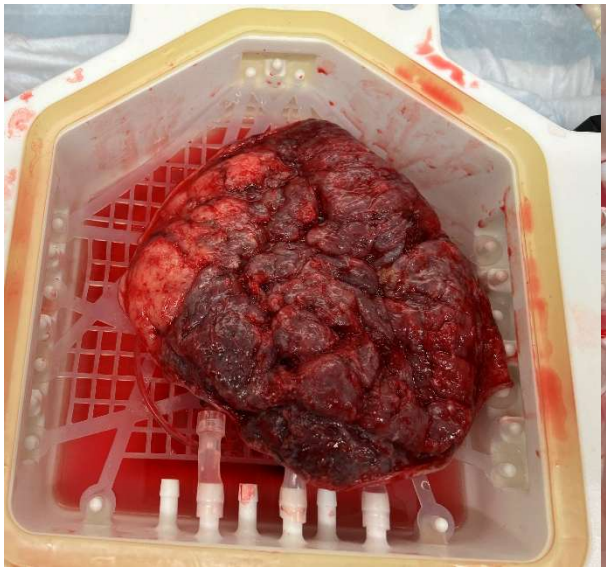

Figure 21: discolored cotyledon after perfusion of a single artery and vein

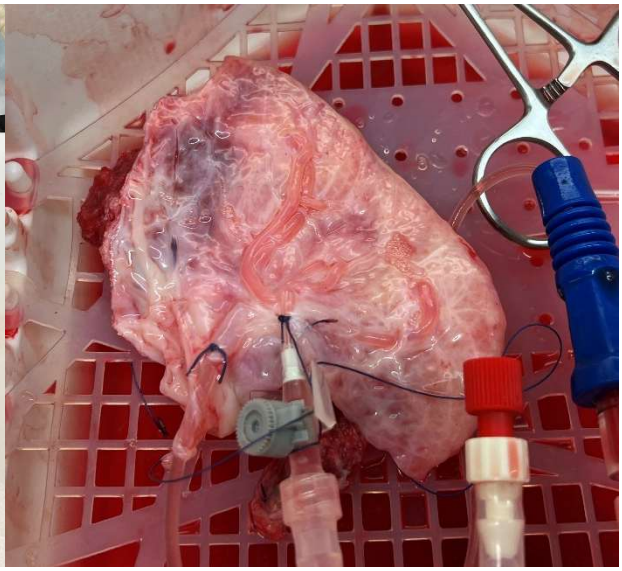

Figure 20: isolated cotyledon with sutures to prevent leakage

6.3 Tubing specifications

Name	Type	Connected (Type)	Length (cm)	Connected (Type)	Marking	Set	Note
A1	LS25	Reservoir (None)	22	A1 (Male)	1x brown	Perfusion	
A2	LS25	A1 (Male)	12	A3 (Male)	2x brown	Perfusion	
B1	LS25 Tygon	A2 (Male)	21	B2 (Female)	1x blue	Perfusion	
B2	LS25	B1 (Male)	46	Oxygenator (None)	2x blue	Perfusion	
C1	LS25	Oxygenator (None)	12	T-junction	1x blue 1x red	Perfusion	
C2 & C3	LS25	T-junction	6	None (Male) & C4 (Male)	None	Perfusion	
C4	LS25	C3 (Female)	6	D1 (Male)	1x green	Auxiliary	CDI shunt sensor placeholder
D1	LS25	C4 (Male)	6	D2 (Male)	1x red	Perfusion	
D2	LS25	D1 (Male)	3	T-junction	2x red	Perfusion	
D3	LS25	T-junction	4	Organ chamber connection 1 (None)	2x red	Perfusion	
E1	LS25	Organ chamber connection 3 (None)	3	E2 (Male)	None	Perfusion	
E2	LS25	E2 (Female)	6	E3 (Male)	1x green	Auxiliary	CDI shunt sensor placeholder
E3	LS25	E3 (Female)	9	T-junction	1x red 1x blue 1x brown	Prefusion	
E4 & E5	LS25	T-junction	9	E5 (Male) & Organ chamber connection 4 (None)	None	Prefusion	

E6	LS25	Organ chamber connection 2, 5 & 6	3	None (Male)	None	Perfusion	
F4	LS25	Organ chamber connector 6 (Female)	2	T-junction	1x green 1x brown	Auxiliary	Cleaning harness oxygenator
F5	LS25	C2 (Female)	58	None (Male)	1x red 1x green	Auxiliary	Medium change
F6	LS25	E4 (Female)	20	None (Male)	1x blue 1x green	Auxiliary	Medium change
F7	LS25	B1 (Female)	70	None (Male)	2x green 1x red	Auxiliary	Oxygenate medium
F8	LS25	C2 (Female)	27	None (Male)	2x green 1x blue	Auxiliary	Oxygenate medium

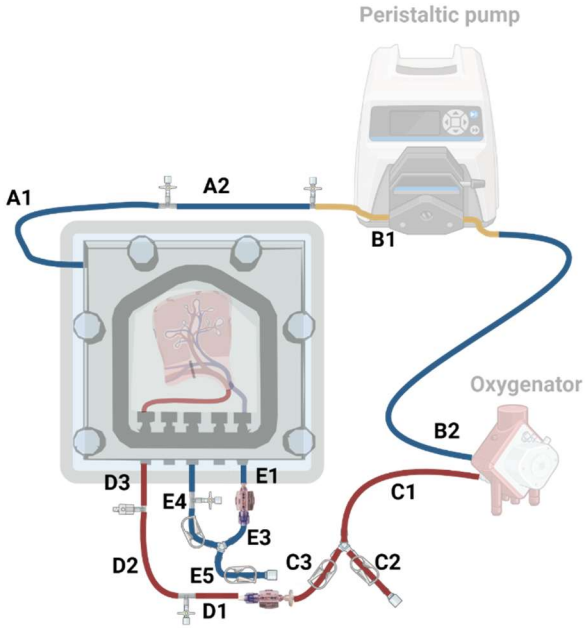

Figure 22: Perfusion setup during GSIS

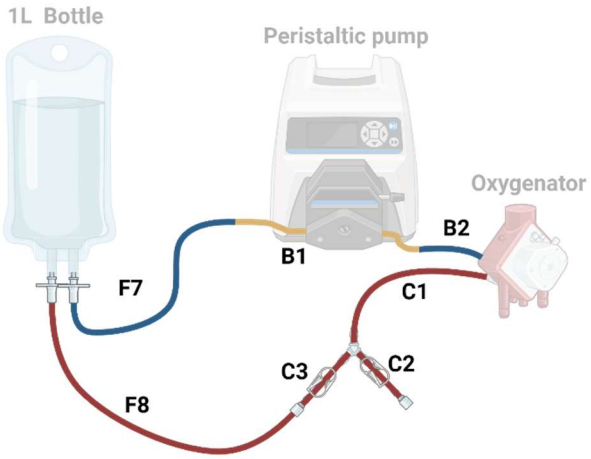

Figure 23: Perfusion setup during pre-oxygenation of fluids

6.4 Software for reading and storing CDI550 data

```
import serial
import pandas as pd
import time
import numpy as np
from datetime import datetime
import os

com__port='COM14'


def open_serial_port():
    try:
        ser = serial.Serial(
            port=com__port,
            baudrate=9600,
            bytesize=serial.EIGHTBITS,
            parity=serial.PARITY_ODD,
            stopbits=serial.STOPBITS_ONE,
            timeout=1  # Timeout for read
        )
        if ser.is_open:
            print("COM3 opened successfully.")
        return ser

    except serial.SerialException as e:
        error_message = str(e)
        if "could not open port" in error_message:
            print("Device not found! Please check the COM port and try again")
        else:
            print(f"Error: {error_message}")
        return None

def main_loop():
    ser = None

    columns_AV = ["Time", "arterial_pH",
"arterial_CO2","arterial_O2","arterial_TEMP","arterial_HCO3","arterial_BE","ar
terial_cSO2",
           "K+","VO2","Q","BSA","venous_pH","venous_CO2","venous_O2","venous_T
EMP","venous_SO2","HCT"]

    columns_A = ["Time", "arterial_pH",
"arterial_CO2","arterial_O2","arterial_TEMP","arterial_HCO3","arterial_BE","ar
terial_cSO2",
           "K+"]
    # Create dataframe for saving results
    df = pd.DataFrame(columns=columns_AV)
```

```
    # Use current datetime for creation of filename
    current_time = datetime.now().strftime("%d-%m-%Y_%H-%M-%S")
    filename = f"{current_time}.xlsx"

    # Create the OUTPUT folder if it doesn't exist
    if not os.path.exists("OUTPUT"):
        os.makedirs("OUTPUT")


    while True:
        if ser is None or not ser.is_open:
            ser = open_serial_port()
            time.sleep(3)  # Wait before retrying if not open
            continue

        try:
            if ser.in_waiting > 0:
                raw_data = ser.readline()  # Reads until '\n' or timeout
                raw_data = raw_data.decode('utf-8',errors='ignore')     #
Decode from bytes to string
                raw_data = raw_data.strip()             # Remove
leading/trailing whitespace (\r\n, spaces)
                data = raw_data.split('\t')

                if len(data) == 9:
                    new_row_columns = columns_A
                elif len(data)==18:
                    new_row_columns = columns_AV
                else:
                    continue

                new_row = pd.DataFrame([data], columns=new_row_columns)

                # Replace non-numeric values besides Time to NaN
                new_row.loc[:, new_row.columns != 'Time'] = new_row.loc[:,
new_row.columns != 'time'].apply(pd.to_numeric, errors='coerce')

                # Add time of recording computer dateTime to dataframe
                new_row.insert(0, 'Computer_dateTime', datetime.now())

                # Append the row to dataframe
                df = pd.concat([df, new_row], ignore_index=True)

                desired_order = ['Computer_dateTime'] + columns_AV
                df = df[desired_order]

                # Save dataframe to excel
                filename = os.path.join("OUTPUT", f"{current_time}.xlsx")
```

```
                df.to_excel(filename, index=False)

                # Print the last 10 rows of the dataframe
                columnsOfInterest =
['Time','arterial_pH','arterial_CO2','arterial_O2','arterial_TEMP',
                                    'venous_pH','venous_CO2','venous_O2','ven
ous_TEMP','K+','arterial_BE','arterial_HCO3']
                realTimeView = df[columnsOfInterest]
                print(realTimeView.tail(10))
                print("Press ctrl+C to terminate program")




        except serial.SerialException as e:
            print(f"Serial error: {e}")
            ser.close()
            ser = None
            time.sleep(5)  # Wait before retrying
        except UnicodeDecodeError as e:
            print(f"Decode error: {e}")
        except Exception as e:
            print(f"Unexpected error: {e}")




if __name__ == "__main__":
    try:
        main_loop()
    except KeyboardInterrupt:
        print("Program stopped manually.")
```

6.5 Fluid composition

Perfusate preparation				
	Stock [c]	**Final [c]**	**Per 1L**	**Comments**
DMEM F12, HEPES	N/A	N/A	700 mL	Cat# 11330, Gibco.
Human Serum Albumin	200 gr L^{-1}	40 gr L^{-1}	200 mL	Alburex 20, CSL Behring bv.
Sodium bicarbonate	7.5%	N/A	10 mL	Cat# 25080094, Gibco.
Penicillin-streptomycin	5000 U mL^{-1}	50 U mL^{-1}	10 mL	Cat# 15070063, Gibco.
Ciprofloxacin	2 mg mL^{-1}	6 µg mL^{-1}	3 mL	Fresenius Kabi.
Fungizone	0.25 mg mL^{-1}	0.25 µg mL^{-1}	1 mL	Bristol-Myers Squibb.
Sodium Hydroxide	1M	N/A	0-10 ml	Cat# 567530, Calbiochem, Merck. Add to perfusate until pH range is reached (7.40-7.45).

Flush preparation				
	Stock [c]	**Final [c]**	**Per 50 cc**	**Comments**
DMEM F12, HEPES	N/A	N/A	35 mL	Cat# 11330, Gibco.
Human Serum Albumin	200 gr L^{-1}	40 gr L^{-1}	10 mL	Alburex 20, CSL Behring bv.
Sodium bicarbonate	7.5%	N/A	0.5 mL	Cat# 25080094, Gibco.
Penicillin-streptomycin	5000 U mL^{-1}	50 U mL^{-1}	0.5 mL	Cat# 15070063, Gibco.
Ciprofloxacin	2 mg mL^{-1}	6 µg mL^{-1}	0.15 mL	Fresenius Kabi.
Fungizone	0.25 mg mL^{-1}	0.25 µg mL^{-1}	0.05 mL	Bristol-Myers Squibb.
Sodium Hydroxide	1M	N/A	0-0.5 ml	Cat# 567530, Calbiochem, Merck. Add to perfusate until

				pH range is reached (7.40-7.45).
Heparin	5.000 I.E./ml	100 I.E./ml	1 ml	Leo Pharma BV.

GSIS low glucose fluid				
	Stock [c]	**Final [c]**	**Per 1L**	**Comments**
Ringer-acetate	N/A	N/A	1000 mL	Baxter
Sodium bicarbonate	7.5%	N/A	10 mL	Cat# 25080094, Gibco.
Sodium Hydroxide	1M	N/A	0-0.5 ml	Cat# 567530, Calbiochem, Merck. Add to perfusate until pH range is reached (7.40-7.45).

GSIS high glucose fluid				
	Stock [c]	**Final [c]**	**Per 1L**	**Comments**
Ringer-acetate	N/A	N/A	1000 mL	Baxter
Sodium bicarbonate	7.5%	N/A	10 mL	Cat# 25080094, Gibco.
Sodium Hydroxide	1M	N/A	0-0.5 ml	Cat# 567530, Calbiochem, Merck. Add to perfusate until pH range is reached (7.40-7.45).
Glucose	40%	3,6 g/L	9 ml	

6.6 Manual measurements of PLC12

Timepoint	Timepoint (hr)	T-1	T0	T1	T2	T3	T4	T23
	Remark		11:20	12:20	13:20	14:20	15:40	10:10
Notes								
Temperature	° C		26	26	27,7	26,5	25,5	25,5
Flow	ml/min		19,5	28,1	21,1	18	21	40
Pressure	mmHg		50	50	40	40	40	40
Blood	ml/min							
Substitution	ml/hr							
Dialysate	ml/hr							
FF	%							
Glucose infusion	ml/hr							

EPOCH measurements		Arterial	Arterial	Arterial	Arterial	Arterial	Arterial	Arterial
Timepoint (hr)			*T0*	*T1*	*T2*	*T3*	*T4*	*T23*
pH	mmHg		7,259	7,506	7,508	7,455	7,386	7,141
pCO2	mmHg		55	48,4	46,9	45,7	53,1	57
pO2	mmHg		352,9	666,6	688,3	632	674,2	697,2
HCO3	mmol/L		24,7	38,2	37,2	32,1	31,9	19,5
BE(ecf)	mmol/L		-2,4	15,1	14,2		6,9	-9,5
BE(b)	mmol/L		-3,3	14,2	13,2	8	6,8	-7,6
sO2	%		99,9	100	100	100	100	100
Natrium (Na+)	mmol/L		147,0	155	156	153	159	154
Potessium (K+)	mmol/L		3,8	4,1	4,1	4,2	4,4	2,7
Calcium (Ca++)	mmol/L		0,52	0,79	0,75	0,42	0,75	0,41
Chloride (Cl-)	mmol/L		CNC	106	106	98	106	111
TCO2	mmol/L		24	38,7	37,7	38	32,5	20,2
Hct	%		CNC	<10	<10		<10	<10
Glucose	mmol/L		12,3	16,3	15,4	8,8	14,3	9,4
Lactate	mmol/L		<0,3	<0,3	<0,3	4,03	4,95	17,9
BUN	mg/dl		CNC	<3	<3		<3	4
Urea	mmol/L		CNC	<1,1	<1,1	<1,0	<1,1	1,4
Creatin	mg/dl		CNC	1,67	1,61		1,45	1,06

EPOCH measurements		Venous	Venous	Venous	Venous	Venous	Venous	Venous
Timepoint (hr)			*T0*	*T1*	*T2*	*T3*	*T4*	*T23*
pH	mmHg		7,317	7,497	7,515	7,491	7,393	7,144
pCO2	mmHg		45	48,1	46,7	43	52,7	53,8
pO2	mmHg		329,6	389,4	375,7	300	480,9	594,9
HCO3	mmol/L		23	37,3	37,7	32,9	32,1	18,5
BE(ecf)	mmol/L		-3,1	14,1	14,8		7,2	-10,5
BE(b)	mmol/L		-2,5	13,2	13,8	9	7	-8,6
sO2	%		99,9	100	100	100	100	100
Natrium (Na+)	mmol/L		143,0	157	158	154	160	156
Potessium (K+)	mmol/L		3,8	4,1	4,2	4,2	4,3	1,9
Calcium (Ca++)	mmol/L		0,6	0,71	0,65	0,45	0,69	0,39
Chloride (Cl-)	mmol/L		107	106	106	100	107	113
TCO2	mmol/L		23,4	37,8	38,2	35	32,7	19,1
Hct	%		<10	<10	<10		<10	<10
Glucose	mmol/L		13,7	12,3	11,8	8,8	11,5	7,4
Lactate	mmol/L		<0,3	0,94	2,72	4,2	6,77	>20
BUN	mg/dl		<3	<3	<3		<3	4
Urea	mmol/L		<1,1	<1,1	<1,1	<1,0	<1,1	1,4
Creatin	mg/dl		0,49	0,98	0,89		0,94	0,83

Interventions during perfusion			
Date	**Time**	**What**	**Remarks**
20-6-2025	14:00	venous return measurement	23,3%; collect 23,3ml in 5 minutes; pump set to 20ml/min; pressure 39mmHg
20-6-2025	14:15	Infusion islet	IUE 20:00 in 20 ml of medium; injected in cotyledon
21-6-2025	10:10	venous return measurement	41,9%; collect 33,5 ml in 2 minutes; pump set to 40ml/min; pressure 38mmHg

EPOCH measurements		Flush	CVVHDF Medium	Effluent T0-T24
Timepoint (hr)				
pH	mmHg	7,318		
pCO2	mmHg	45		
pO2	mmHg	677,2		
HCO3	mmol/L	23,1		
BE(ecf)	mmol/L	-3		
BE(b)	mmol/L	-2,3		
sO2	%	100		
Natrium (Na+)	mmol/L	139		
Potessium (K+)	mmol/L	2,3		
Calcium (Ca++)	mmol/L	0,57		
Chloride (Cl-)	mmol/L	106		
TCO2	mmol/L	23,5		
Hct	%	<10		
Glucose	mmol/L	18,3		
Lactate	mmol/L	<0,3		
BUN	mg/dl	<3		
Urea	mmol/L	<1,1		
Creatin	mg/dl	1		

6.7 ELISA calibration curve