Functional islets and where to find them

Doppenberg, J.B.

Chapter 7. Pancreatic Islet Separation Method (PRISM): A Novel Human Islet Isolation Technique

Jason B. Doppenberg[1,2], Marten A. Engelse[1], Eelco J.P. de Koning[1]

[1]Dept of Internal Medicine, [2]Transplantation Center, Leiden University Medical Center, Leiden, the Netherlands

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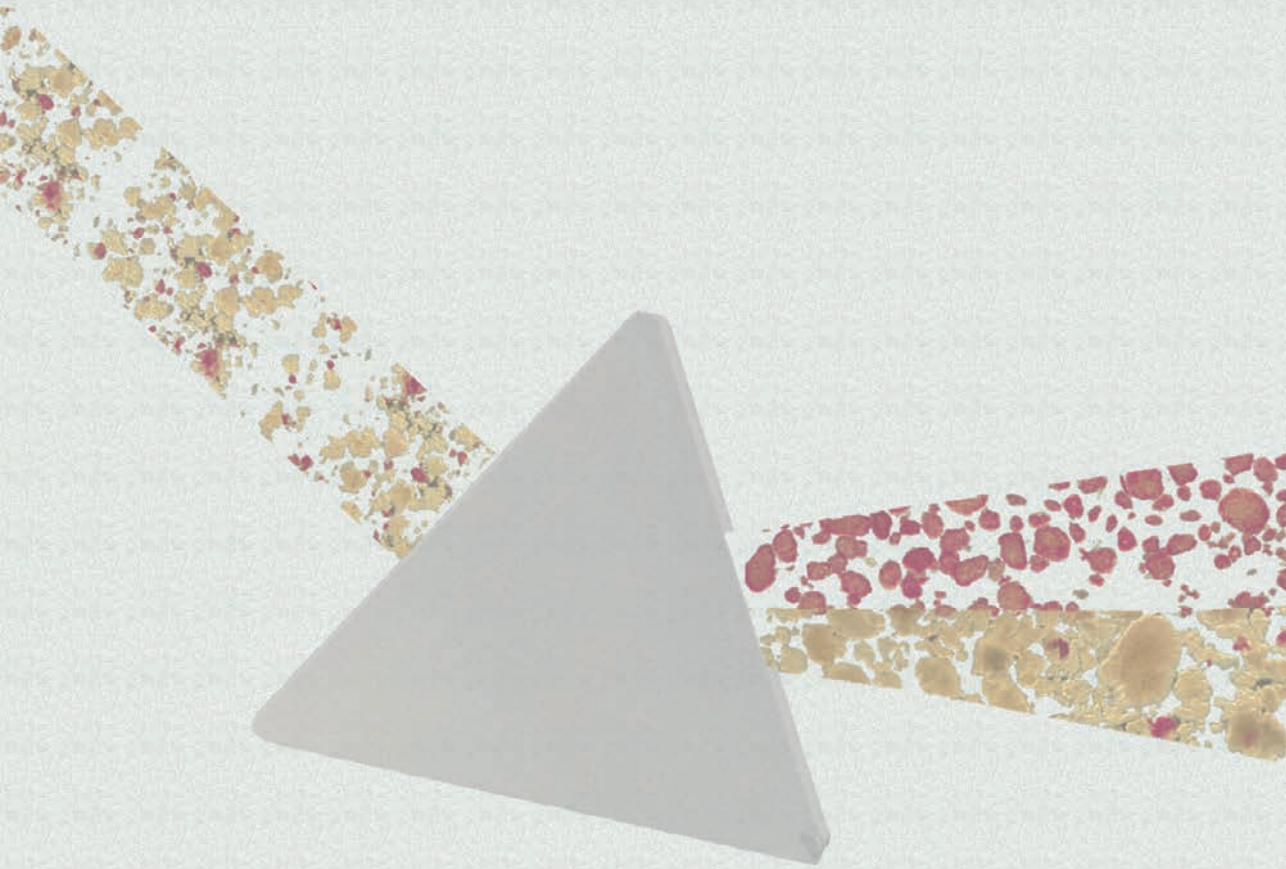


Abstract

Background: Successful pancreatic islet isolations are a key requirement for islet transplantation in selected patients with type 1 diabetes. However, islet isolation is a technically complex, time-consuming, manual process. Optimization and simplification of the islet isolation procedure could increase islet yield and quality, require fewer operators and thus reduce cost.

Methods: We developed a new, closed system of tissue collection, washing, buffer change and islet purification termed PancReatic Islet Separation Method (PRISM). In the developmental phase, pump and centrifuge speed was tested using microspheres with a similar size, shape and density as digested pancreatic tissue. After optimization, PRISM was used to isolate islets from ten human pancreases.

Results: Islet equivalents, (IEQ), viability (FDA/PI), morphology and dynamic glucose stimulated insulin secretion (dGSIS) were evaluated. PRISM could be performed by one operator in one flow cabinet. A similar islet yield was obtained using PRISM compared to the traditional islet isolation method (431,234±292,833 vs. 285,276±197,392 IEQ, p=0.105). PRISM islets had similar morphology and functionality.

Conclusion: PRISM is a novel islet isolation technique that can significantly improve islet isolation efficiency using fewer operators.

Introduction

Pancreatic islet transplantation is a treatment option for selected patients with type 1 diabetes mellitus[1]. It is generally accepted that a higher number of islets of good quality results in better functional outcome including insulin independence[2]. In practice, often two or more donor pancreases are required to achieve this goal, but this strategy is associated with an increased risk for HLA sensitization and procedure-related complications and increased costs[3,4]. Costs could be reduced by further automating the islet isolation procedure requiring fewer operators and reducing the complex logistics of islet isolations.

Since the introduction of the semi-automated method of islet isolation,[5] only minor revisions have been broadly incorporated[6]. Key in this method is the collection of enzymatically and mechanically digested pancreatic tissue followed by enzyme deactivation via dilution, cooling and/or the addition of (human) serum or serum albumin[7]. Additional washing steps, requiring several rounds of centrifugation, are necessary to dilute the enzymes to further reduce the risk of islet damage[8]. After pancreas digestion, it is necessary to purify islets from the exocrine tissue in order to reduce the tissue volume for transplantation. Infusion of more than 10 ml of tissue is associated with increased procedure-related complications[9]. Isopycnic centrifugation (density gradient separation) has been utilized since the late 1960's to purify islets, mainly using Ficoll variants[10], since density is the most pronounced physical differential characteristic of islets compared to exocrine tissue. Large scale density gradient separation became possible after the implementation of COBE 2991 cell processors which are universally used in islet isolation[11]. Still, 15-51% of islets are reportedly lost during this procdure[12].

In order to overcome these issues, we investigated whether closed whole blood processing techniques could be applied to an islet isolation setting. Autotransfusion systems contain a continuous flow centrifuge bowl which allows retention and washing of cells during centrifugation and can be used to separate blood into cell types[13]. In this study we used the principle of an autotransfusion system to include all the steps of islet isolation after enzymatic digestion in order to increase isolation speed, reproducibility and efficiency.

Methods

Mock tissue

In order to test the applicability of the continuous flow centrifuge based system, "mock tissue" consisting of 3 different categories of fluorescent polyethylene microspheres (Cospheric LLC, Santa Barbara, USA) were used. One category of microspheres had a density of 1.028 – 1.030g/mL (orange or green colored, 32 – 180 µm diameter), mimicking apoptotic tissue, one category of microspheres had a density of 1.065 – 1.071g/mL (blue colored, 150 – 180 µm diameter), mimicking islets, and the category of microspheres with a density of 1.095 – 1.100g/mL (red colored, 150 – 400 µm diameter) mimicked exocrine tissue[14]. This mock tissue was suspended in Ringer's acetate solution (B. Braun, Melsungen, Germany) supplemented with 1% human serum albumin (Sanquin Bloodbank, the Netherlands) to combat electrostatic forces.

Human Pancreases for Isolation

Human cadaveric donor pancreases were procured through a multiorgan donor program. Organs were used only if the pancreas could not be used for clinical pancreas or islet transplantation and if research consent was present, according to the local guidelines of the medical ethical committee of the LUMC and the Dutch Transplantation Foundation as the competent authority for organ donation in the Netherlands. For the islet isolation procedure using PRISM, the pancreas was transported to the Human Islet Isolation Laboratory at the Leiden University Medical Center, Leiden, the Netherlands. Islet depleted tissue (exocrine tissue) from other islet isolations was used in initial validation tests. All prior islet isolations from a nine-year period using our traditional islet isolation protocol in a similar research laboratory setting were used as controls (n=63)[15]. Islet yield is expressed as islet equivalents (IEQ) and were manually with confirmation from a second independent investigator, as previously published[16].

Pancreatic Islet Isolations using PRISM

Dissection, cannulation, enzymatic perfusion and digestion of pancreatic tissue for PRISM isolations was identical to our traditional islet isolation protocol[15]. In both methods, a blend of collagenase NB1 and neutral protease (Serva Electropheresis, Germany) was used to perfuse the organ, aiming for pancreas distension with minimal leakage.

Digestion

Digestion commenced after large pieces of tissue were placed in a digestion chamber connected to a warmed loop filled with 1 liter digestion solution, consisting of Ringer's acetate solution, supplemented with 10 mM nicotinamide (prepared by our institute's pharmacy), 0.5% penicillin-streptomycin (Lonza, Geleen, the Netherlands), 0.4% sodium bicarbonate (Lonza, Geleen, the Netherlands), 20 mM HEPES (Gibco BRL, Breda, the Netherlands), and 0.2 µl/ml Pulmozyme (Roche, Switzerland)).

The digestion was monitored for the presence of islets freed from surrounding exocrine tissue. Once there was a sufficient proportion of free islets, the digestion solution with the suspended tissue was redirected through a heat exchanger, cooling the tissue. A pump set at 8 ml/min mixed the pancreatic digest with ABO-compatible human serum (Sanquin Bloodbank, the Netherlands) into the collection tubing system until the approximately 280 ml serum bag was empty. Subsequently, the tissue entered the disposable continuous flow centrifuge bowl (Xtra Autotransfusion system, LivaNova, London, UK), spinning at 1400 RPM . Figure 1a shows a schematic overview of the initial digestion phase. After the first 1L bottle of digestion solution was drained, a preheated (37ºC) 1L bottle of dilution solution (same composition as digestion solution) was connected to the tubing system and the solution was pumped through the digestion chamber and heat exchanger into the centrifuge bowl. This was repeated for another 6 liters of dilution solution.

After all the tissue had been digested and retained in the centrifuge bowl, the tissue was resuspended manually by gentle agitation. Centrifugation was restarted at 1500 RPM (145g at the bottom of the bowl, 105g at the top of the bowl). Subsequently, 250 ml cooled UWS, supplemented with 0.8 µl/ml dornase alpha (Pulmozyme®), was pumped into the bowl, replacing all dilution solution (figure 1b). The centrifuge was paused, and the contents of the bowl were pumped back into a bag which contained the UWS.

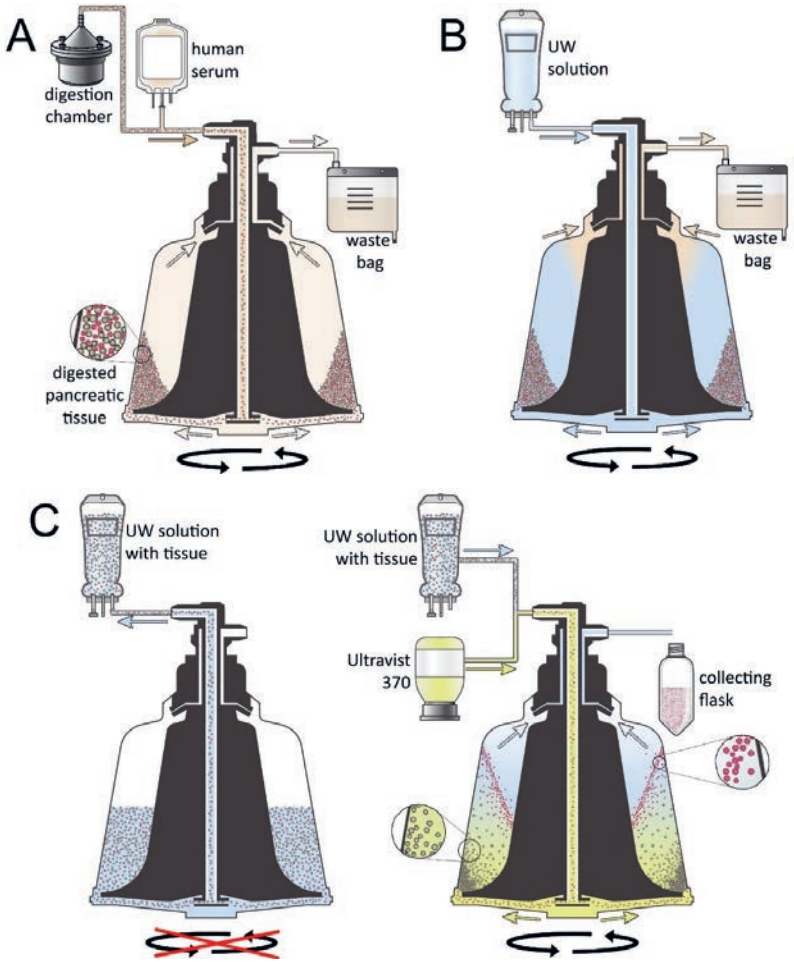


Figure 1. Schematic representation of different phases during PRISM A) Tissue collection, washing, and pooling in the centrifuge bowl. Digested pancreatic tissue (islets depicted in red, exocrine tissue depicted in green) from the digestion chamber is supplemented with human serum and pumped into a spinning centrifuge bowl through its central shaft and moved towards the perimeter of the bowl by centrifugal forces. Excess digestion solution exits trough the overflow into the waste bag. **B)** UW Solution(light blue), which is more dense than the digestion/dilution solution, is pumped into the centrifuge bowl and forces out the digestion/dilution solution (yellow). Pancreatic tissue is more dense than UWS and is therefore retained in the centrifuge bowl during this process. Excess solution exits through the overflow into the waste bag. **C)** Density gradient purification. Left panel) After the centrifuge bowl is filled with UW Solution (blue), centrifugation is stopped. The tissue suspended in UWS is pumped back into the bag which contained the UWS. Right panel) UWS is combined with an increasing proportion of Ultravist 370 (yellow) to create a density gradient. During establishment of the density gradient the less dense islets (in red) separate from the more dense exocrine tissue (in green). Fractions are obtained in collecting flasks once the centrifuge bowl is filled and the gradient solution containing the separated tissue exits the bowl.

Density Gradient Separation

A density gradient with a non-linear slope was formed starting at 1.045 g/ml and ending at 1.15 g/ml using a continuous speed of 30 ml/min (figure 2). Only UWS was pumped into the bowl for the first 25 ml, to allow apoptotic tissue to become buoyant. Subsequently the density gradient was extended to 1.060 – 1.085 g/ml for 350 ml, corresponding to a broad range of islet densities. Then, the density was increased more rapidly, correlating to densities in which embedded islets generally become buoyant (1.085 – 1.110 g/ml for 125 ml). The final stage in the density gradient (1.110 – 1.15 g/ml for 75 ml) allowed the exocrine tissue to become buoyant before exiting the continuous flow centrifuge bowl. A schematic representation of the density gradient purification phase is shown in figure 1c.

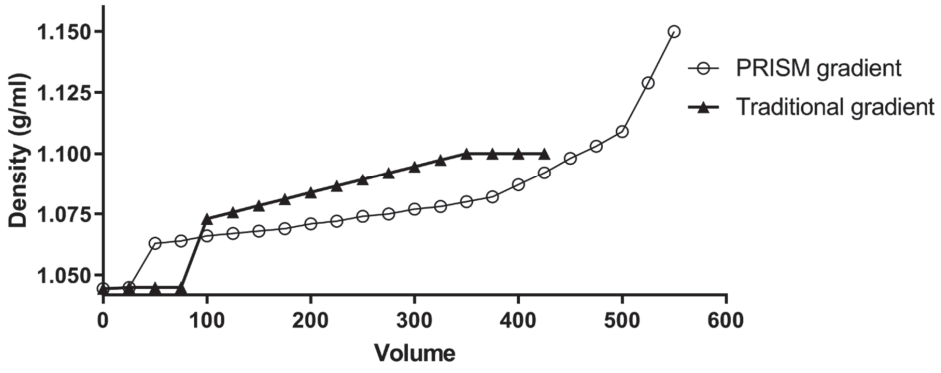


Figure 2. Density gradient for islet separation using PRISM. The density in every 25 ml of exiting density separation solution (DSS) from the centrifuge bowl during PRISM is measured (white circles). The density gradient in PRISM is made up of DSS consisting of University of Wisconsin (UW) solution and Ultravist 370. Theoretically, an unlimited volume can be used to make up the gradient. In the figure we used 550 ml. For comparison, the density was measured in every 25 ml of exiting DSS from the COBE 2991 Cell Separator (traditional gradient) (black triangles), consisting of UW and Biocoll 1.100, which is limited in volume (425 ml). In the traditional method digested tissue is added after the gradient has been set up. The gradient in PRISM is set up in a spinning centrifugation bowl during tissue infusion, allowing for simultaneous density gradient creation, loading of tissue, and collection of fractions.

Each fraction of 25 ml is collected through the overflow and washed in 225 ml of Ringer's acetate solution (supplemented with 1% human serum, 0.44% nicotinamide, 0.05% glucose, 48 units/L penicillin/streptomycin, 0.03% sodium bicarbonate, 15 mM HEPES, 46.1 mM sodium pyruvate, 1 mM calcium chloride, and 0.5% pulmozyme). Fractions of a similar islet purity and morphology were combined and cultured in CMRL 1066 (Mediatech, Herndon,

VA, USA), supplemented with 10% human serum, 10 mM HEPES, 2 mM L-glutamin, 50 µg/mL gentamycin, 0.25 µg/mL Fungizone (GIBCO BRL), 20 µg/mL ciprofloxacin (Bayer healthcare AG, Leverkusen, Germany) at 37^{0}C in 5% CO_2 for one day before evaluation.

PRISM is schematically represented in figure 3.

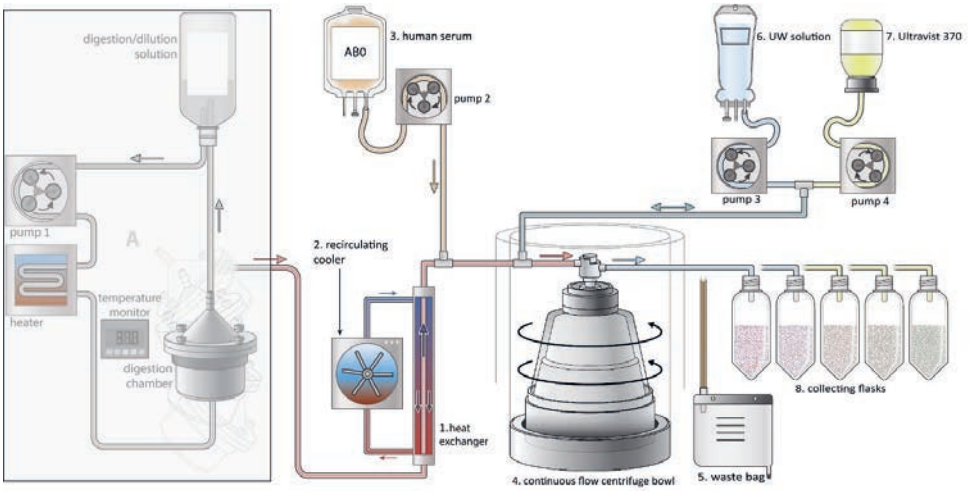


Figure 3. Depiction of PRISM. In the shaded grey area pump 1 is used to pump digestion solution through the heater and digestion chamber laden with the enzymatically perfused pancreas pieces (digestion circuit). When islets are sufficiently released from the exocrine tissue, the flow of tissue is diverted towards the heat exchanger (1) , which is connected in a separate circuit to a recirculating cooler (2) so the tissue will be cooled. The chilled tissue is supplemented with human serum (3), pumped into the main line by pump 2. Next, the tissue is collected in the spinning continuous flow centrifuge bowl (4). Digested tissue is retained in the spinning centrifuge bowl while continuously being washed with excess solution spilling over into the waste bag (5). After digestion, UWS (6) is pumped into the bowl by pump 3, replacing the digestion/dilution solution. Once the UWS has completely replaced the digestion/dilution solution, centrifugation stops, and the tissue is pumped back into the now empty bag which had contained UWS. A custom-made pump controller drives pump 3 and pump 4, creating a density gradient using Ultravist 370 (7) combined with the UWS now containing the tissue. This is pumped into the spinning centrifuge bowl and once the total volume of bowl is reached, fractions can be obtained in collecting flasks (8) via the overflow of the centrifuge bowl. The density gradient continues to be built while the remainder of the tissue is loaded.

Dynamic glucose stimulated insulin secretion test

Functionality of isolated islets was tested at day one of culture using a dynamic glucose stimulated insulin secretion (GSIS) test. Islet samples (±20 islets), were collected and were placed in filter-closed chambers (Suprafusion 1000, Brandel, Gaithersburg, USA) and perifused at 500 µl/min at 37ºC. First, islets in each channel were preconditioned by perifusion with a low-glucose (1.7 mM glucose) solution (20 mM HEPES, 11.5 mM NaCl, 0.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 2.4 mM $NaHCO_3$, supplemented with 0.2% human

serum albumin in demineralized water) for 90 minutes. Thereafter, the islets were perifused with the low-glucose solution for 15 minutes followed by a high-glucose solution (similar solution but with 20 mM glucose) for 60 minutes and finally with the low-glucose solution for 75 minutes. Fractions were collected at 7.5 minute intervals. The fractions were measured for human insulin using an immunoassay (Mercodia AB, Uppsala, Sweden). For each time-point, the insulin concentration was divided by average insulin concentration of the time-points during the second low-glucose phase, to give a stimulation index at every time-point. GSIS tests and analysis were performed as previously described[15].

Islet Viability

Islet viability was determined at day 1 of culture using a FDA/PI viability kit (Invitrogen, Carlsbad, USA) and was assessed under a fluorescent microscope (DM6000, Leica Microsystems, Wetzlar, Germany) as previously described[16].

Islet Morphology

Mechanical damage to islets was judged by the assessment of the percentage of islets in different size categories (20-400μm)[17,18] and by islet circularity ($4*Area*\pi/Perimeter^2$)[19]. Digital images of purified fraction samples were analyzed using Image Pro Premier 9.3 (Media Cybernetics, Rockville, USA). Islets were segmented from these images based on a modified logarithm calculating average diameter, area, and perimeter of each islet.[20]

Statistics

Data analysis was performed using Prism 8.1.1 for PC (GraphPad software, Inc., La Jolla, CA). Continuous measures were expressed as the mean±SD within PRISM isolations and historical controls. To calculate the area under the stimulation index curve, these curves were integrated over time and expressed as mean±SD within each group. Comparisons between groups were conducted using Mann-Whitney U-tests on non-normally distributed outcomes (IEQ and IEQ/g data) and unpaired two-tailed t-tests on normally distributed outcomes (all other data). p-Values of less than 0.05 were considered statistically significant.

Results

Validation of isolation steps

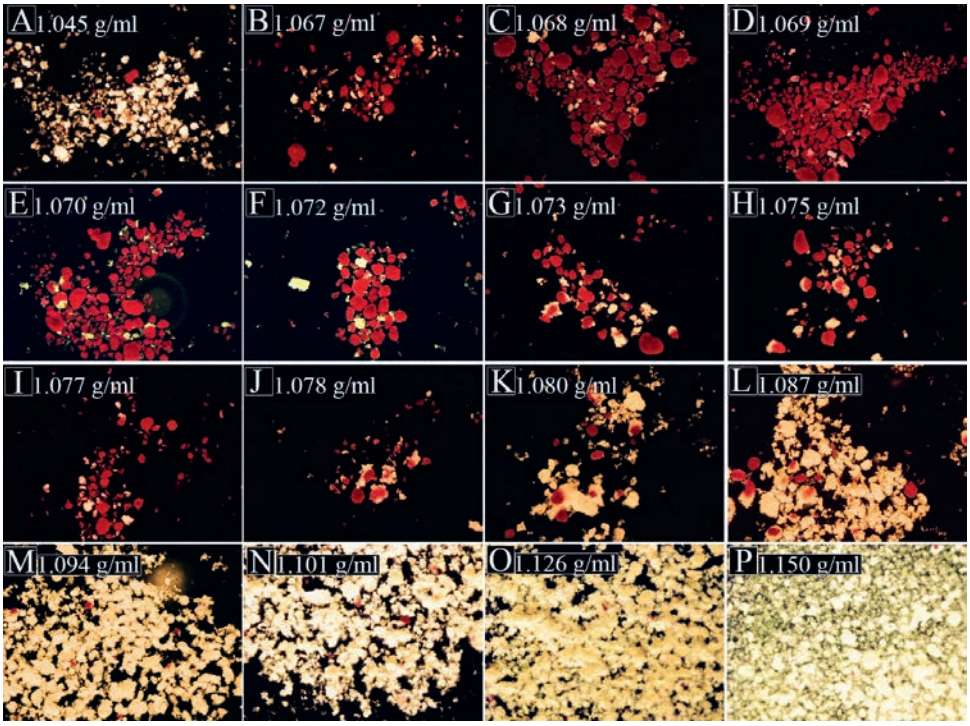
Using mock tissue, the essential steps of suspension cooling and subsequent tissue retention, collection, washing, media change and purification in the islet isolation process were simulated using a continuous flow centrifuge bowl (supplementary figures 1-3). The results of these validation tests were used to develop PRISM.

Human Pancreatic Islet Isolations

Donor characteristics of the 10 human pancreases that were used for islet isolation by PRISM and the 63 pancreas used for islet isolation using by traditional method are summarized in table 1. PRISM islet isolations were performed with one operator during the procedure and one assistant during preparation and culturing. From the start of unpacking the pancreas to the end of harvesting islet fractions, the isolations lasted approximately 3 hours. Fractions containing free islets were found mainly at a density of 1.075±0.015 g/ml (figure 4). Due to the non-linear manner in which the PRISM density gradient builds up, many fractions (of 25 mL) with a high islet purity were collected around this density (figure 4). Importantly, the tissue volume of each fraction increased only after high purity fractions were no longer obtained. A higher islet yield was obtained using PRISM (431,234±292,833 IEQ), compared to the 63 previously performed islet isolations using traditional methods (285,276±197,392 IEQ) in our center, although not statistically significant (p=0.105; figure 5a). When correcting for pancreas mass, 3,999±2,534 IEQ/g was isolated using PRISM and 2,971±1,828 IEQ/g using traditional methods (p=0.169; figure 5b).

	PRISM	Controls	*p* value
Age (years)	59.1±8.3	56.3±11.8	p=0.47
Sex (% Male)	60±49	67±47	p=0.68
BMI (kg/m^2)	27.2±6.7	26.6±5.5	p=0.76
Donor type (%DBD)	40±49	27±44	p=0.41
WIT (mins)	16.2±2.9	15.8±3.1	p=0.79
CIT (hours)	10.1±4.1	11.4±4.8	p=0.47
Pancreas mass	111.2±16.5	110.6±35.0	p=0.96

Table 1. Donor characteristics. Values are given as mean±SD. BMI: Body Mass Index, DBD: Donation after Brain Death, WIT: Warm Ischemia Time, CIT: Cold Ischemia Time. PRISM n=10. Controls n=63.



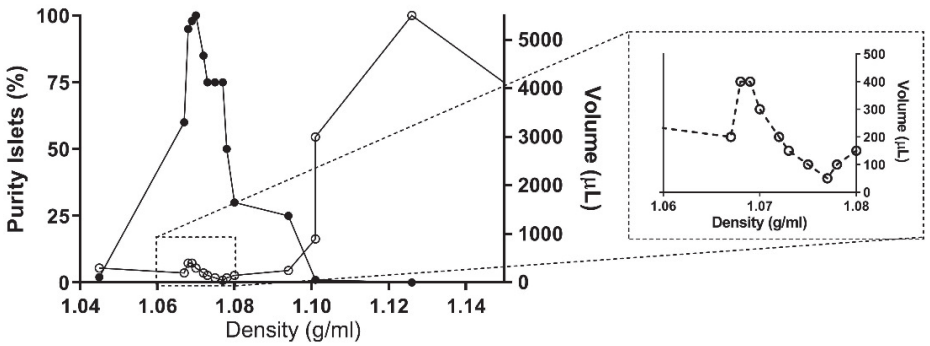


Figure 4. Upper panel: Fractions of pancreatic tissue collected after density separation during a representative PRISM isolation. Islets are stained in red using dithizone. Fraction A (1.045 g/ml) consists of exocrine and islet tissue. Often tissue in this fractions is dead or degranulated. Fractions B-F (1.067 – 1.072 g/ml) contain islets with little to no exocrine tissue. Fractions G-L (1.073 – 1.087 g/ml) contain embedded islets (islets that are attached to a substantial amount of exocrine tissue) and gradually more exocrine tissue. Fractions M-P (1.094 – 1.150 g/ml) contain almost no islets at all, merely exocrine tissue.
Lower panel: Islet purity (black circle, left y-axis) and tissue volume (hollow circles, right y-axis) for each fraction from the upper panel is shown. There is a small peak in volume when pure fractions of islets are obtained (insert).

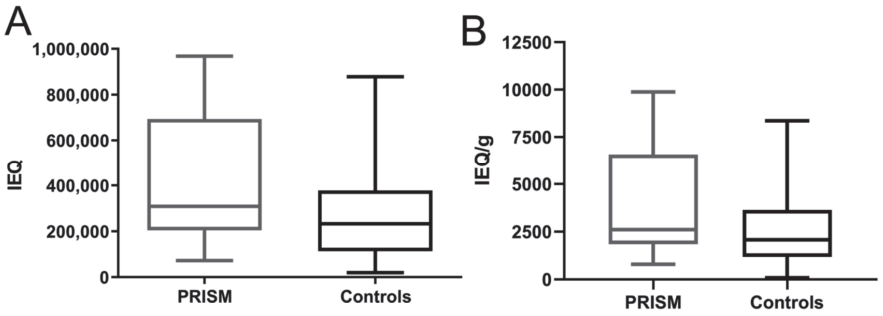


Figure 5. Islet yield after PRISM and traditional islet isolation. A) Using PRISM 431,234±292,833 IEQ were isolated (PRISM) and using our traditional islet isolation method 285,276±197,392 IEQ (Control islets, p=0.048). B) When correcting for pancreas mass, 3,999±2,534 IEQ/g was isolated using PRISM and 2,971±1,828 IEQ/g using our traditional islet isolation method (Controls, p=0.044). PRISM n=10. Controls n=63.

Dynamic glucose stimulated insulin secretion test, Viability, and Islet Morphology

After one day of culture, viability was analyzed using FDA/PI staining and dynamic glucose stimulated insulin secretion was performed. A similar biphasic insulin secretory profile was observed in PRISM islets compared to control islets (figure 6). The peak stimulation index

was not different between PRISM islets and control islets (4.6 ±3.5 and 4.5 ±2.9, respectively; p=0.916). Also the area under the insulin curve was similar (351±138 for PRISM islets and 399±546 for control islets, p=0.775).

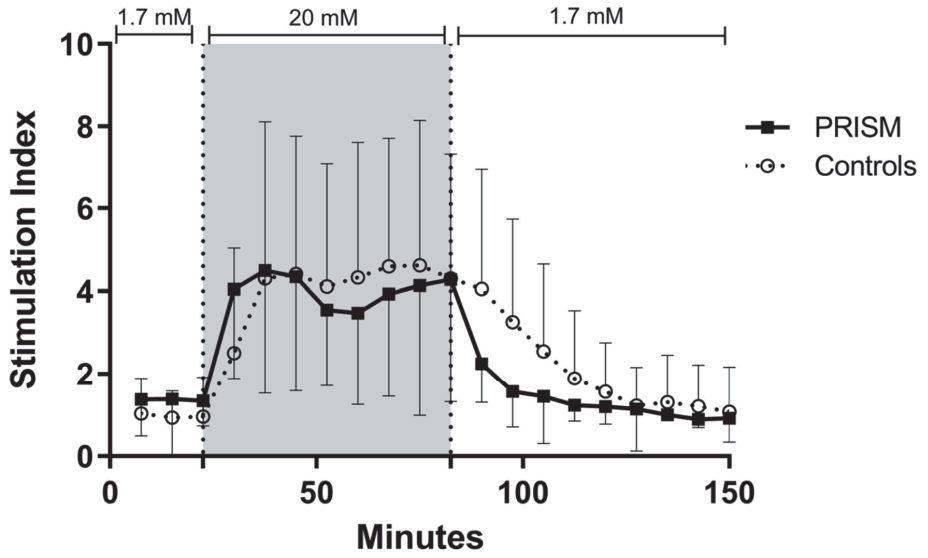


Figure 6. Functional testing of islets isolated by PRISM. Dynamic glucose stimulated insulin secretion (dGSIS) tests were performed on islets isolated by PRISM (PRISM-black squares) and by the traditional islet isolation method (Controls-white circles). PRISM islets had an earlier initial rise in insulin secretion at 30 minutes (stimulation index (SI) PRISM islets 4.0±2.2 and control islets 2.5±2.5, p<0.05)). PRISM islets had a faster decrease in insulin secretion after switching from high to low glucose medium at 90 minutes (SI PRISM islets 2.2±0.9 and Control islets 4.1±2.9, p=0.04), and at 97.5 minutes (SI PRISM islets 1.6±0.9 and Control islets 3.3±2.5, p=0.03). PRISM n=6. Controls n=40.

Islet viability was 99% (n=3), 95% (n=2) and 30% (n=1). Islet viability was not tested in 4 PRISM isolations.

No difference was found in mechanical damage parameters between PRISM and control islets. The percentage islets in each size category relative to the total number of islets did not differ between PRISM and control islets (p=0.13; figure 7a). Islets isolated using the traditional isolation method and using PRISM were similarly fragmented, as determined by circularity (0.53±0.16 for PRISM islets and 0.50±0.10 for control islets, p=0.58; figure 7b).

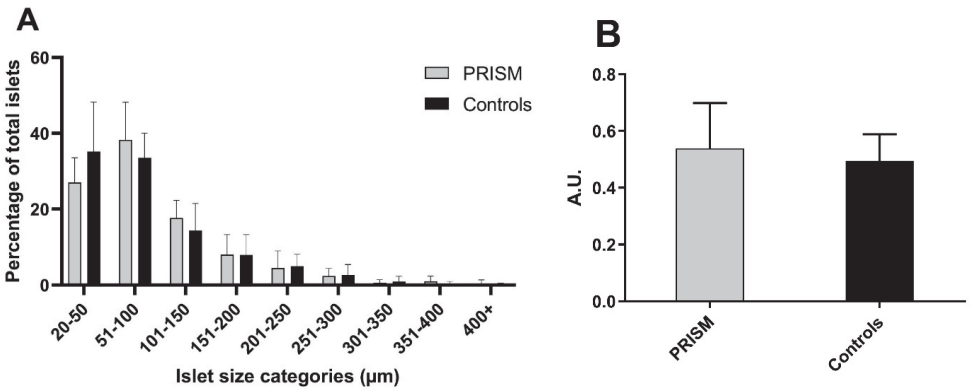


Figure 7. Islet morphology A) Proportion of islets in different islet size categories. There was no difference between islets isolated by PRISM and by the traditional method (Controls; p=0.13. B) Islet circularity in arbitrary units (A.U.) assessed by digital image analysis. PRISM islets had similar circularity as control islets (PRISM 0.53±0.16 and Controls 0.49±0.09, p=0.58). PRISM n=10. Controls n=40.

Discussion

Here we present a novel islet isolation technique based on continuous flow centrifugation. We named this technique PancReatic Islet Separation Method (PRISM). PRISM is a fast, compact, automatable system that requires less equipment and fewer operators than traditional islet isolation methods. Moreover, PRISM is a closed isolation method from the moment the pancreas, after perfusion with an enzyme solution, is placed in the digestion chamber until the collection of the purified islets, decreasing the risk of contamination with possible implications for clean room facility requirements[21].

Previous attempts have been made to isolate islets using other systems than enzymatic digestion and isopycnic centrifugation, such as selective destruction of exocrine tissue techniques[22–25], or other means of sorting digested tissue techniques[26–33], but these have not resulted in major changes in current islet isolation procedures. Many of those techniques did not simplify the process, were not reproducible, or were only applicable in (small) animal islet isolation procedures. When devising PRISM, a main condition was to incorporate well-known mechanisms of enzymatic digestion and isopycnic centrifugation for purification, as used in traditional islet isolations.

A key feature of the PRISM technique is a continuous flow centrifugation bowl, in which digested tissue is retained, washed and purified. Research in the field of interoperative blood salvage has shown that continuous flow centrifuge bowls are able to very effectively wash out fat[34], cytokines[35], and ions[36] when appropriate centrifuge and pump speeds are used. Only one cycle (volume of the bowl) is needed to achieve this result[36]. PRISM uses seven liters of liquid to wash out digestive enzymes and cell debris while collecting the digested tissue in the centrifuge bowl. Prior studies have also shown that purification of viable leukocytes can be performed using a continuous flow centrifuge bowl from whole blood or marrow[37]. These studies indicate that the maximum packed cell volume is limited to 175 ml[37]. This implies that the bowl is large enough to wash the tissue thoroughly, although comparisons are difficult due to the fact that pancreatic islets are not single cells, but larger, clusters of cells.

When developing PRISM, a major concern was the potential shear stress on the islets during the collection, washing and purification steps. Using our system of continuous centrifugation, we aimed to minimize tissue exposure to periods of acceleration/deceleration and narrow passages. The narrowest passage in the centrifuge bowl is 800µm. There is a paucity of information about fluid dynamic stress on freshly digested pancreatic tissue. One study showed that the greatest stress on islets is exerted during COBE purification, when the acceleration of flow is high in narrow tubing, causing the islets to fracture[18]. The percentage of islets in the smallest size categories is in line with results from other centers[17]. This can be quantified as changes in not only the size of the islet, but also in its fragmentation. After PRISM isolations islets did not differ in size or circularity from previous isolations using the traditional islet isolation method.

In traditional islet isolation procedures, collected pancreatic digest must be stored prior to purification to allow a density gradient to be built[38]. It was later shown that storage in UWS had the added benefit of making exocrine tissue even more dense than islets[39]. This also made UWS an attractive alternative to other solutions as the lighter component in density gradients[40]. While PRISM does not require short term storage prior to purification, we aimed to maximize the usage of UWS in order to increase density gradient separation efficiency by using a very high density solution (Ultravist 370. 1.409 g/ml[41]) as a heavy component for the density gradient and UWS as the light component. Other centers have utilized similar, earlier generation iodine-containing non-ionic radiocontrast agents such as iodixanol with success[42].

Additionally, earlier studies have shown that suspending pancreatic digest in a solution with a high osmolality (450 mOsm/Kg) may also increase the density difference between exocrine tissue and islets[43] (although this has been contested)[44]. It has also been suggested that exposure to high osmolality solutions can free embedded islets from exocrine tissue while being agitated[45]. At the highest density that we use (1.15 g/ml), the osmolality of the solution is 451 mOsm/Kg. The low viscosity of Ultravist 370 reduces the shear stress on the islets and requires less force for sedimentation during centrifugation[46]. The combination of using a high proportion of UWS to other components, proper osmolality, and low viscosity creates an ideal solution for density gradient separation in PRISM.

Purification of pancreatic digest is routinely performed using a COBE 2991 cell separator. Adding too much tissue to a density gradient set up in a COBE 2991 can result in inefficient separation, lower purity and a lower yield[40]. Existing protocol limitations range from 20ml[44,47] to 45ml[48], 50ml[40,49], or 60 ml[50]. This is partly due to the fixed size of the bag used in the COBE 2991. We realized that by using a continuous flow centrifuge, part of the gradient can be added as it enters the bowl while another part is collected. In this fashion, the volume of the density separation solution (DSS) that is required to set up an optimal DSS gradient is not limited to the size of the bowl.

Also, during optimization, we noted that more tissue could be added to the density separation solution in the bowl if the tissue is added while the gradient itself is being set up from the UWS and Ultravist. To do so, the pancreatic digest is removed from the centrifugation bowl after the buffer was changed to UWS and then returned to the centrifuge bowl resuspended in UWS. We speculated this was necessary to prevent disruption of the gradient by sample overcrowding since tissue is continuously added. Since the combined pump speed of the light (UWS) and heavy component (Ultravist 370) that forms the density separation solution in the bowl is kept at 30 ml/min and the viscosity of the density separation solution is low, the sedimentation rate of the tissue is short enough to allow for separation of the islets from the exocrine tissue while being pumped into the centrifuge bowl.

Compared to traditional isolations, PRISM requires fewer manual interactions during an isolation. The time needed to prepare the pancreas, to install tubing sets, and to collect purified islets is similar compared to the traditional islet isolation method. However, during the digestion, collection, washing and purifying stages of isolation, only one skilled and experienced operator is required to guide the processes. The only manual steps include

sample collection to monitor the digestion process and operate the pumps. With prepared solutions and a pre-installed disposable tubing set, the entire islet isolation procedure from pancreas preparation to storage of islets in the incubator can be performed by one operator in approximately 3 hours. The costs associated with disposables in order to perform PRISM isolations are similar to those needed for traditional isolations. However, dependent on the regulations of individual institutions, cost-saving measures could be implemented through personnel downsizing and reduction of cleanroom rent hours.

PRISM yielded on average more than 400,000 IEQ from pancreases disregarded for clinical use, which was more than the islet yield isolated from similar pancreases using traditional islet isolation methods. This yield had a wide range which was probably related to the quality of the pancreases. The islets were highly viable after one day of culture and were as functional as islets isolated by the traditional method. These data indicate that the islets obtained via PRISM have similar viability and functional capacity as islets obtained from traditional islet isolation methods.

Our novel pancreatic islet separation method further automates islet isolations, improves isolation efficiency, and decreases the time necessary to isolate islets from human pancreases. This can reduce the logistical burden in human islet isolations facilities.

Supplementary data

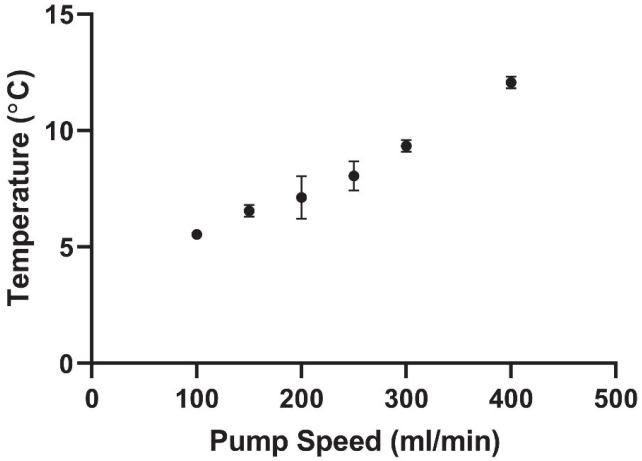


Figure S1. Relationship between pump speed and outflow temperature. In order to rapidly cool the digested pancreatic tissue and halt enzymatic activity, a medical grade heat exchanger (BIOTherm, Medtronic, Minneapolis, USA) was tested. A simulation was performed using Ringer's acetate solution (37°C) and mock tissue in suspension which was pumped through the heat exchanger. Cooling liquid (30% propylene glycol, 4°C) was pumped through the heat exchanger in the opposite direction via a separate flow system by a recirculating cooler (FP50-HE, Julabo GmbH, Seelbach, Germany) at varying speeds (n=3). The temperature of the outflowing water from the heat exchanger was assessed with a type-K thermocouple (Ebro TFN 520, Ingolstadt, Germany). At pump speeds of 200 ml/min (a common speed in traditional islet isolations), the outflowing water reached a temperature of ±7°C. At 7°C a theoretical 92% enzymatic activity decrease can be achieved. At a pump speed of 200 ml/min, this cooling is realized in approximately 41 seconds.

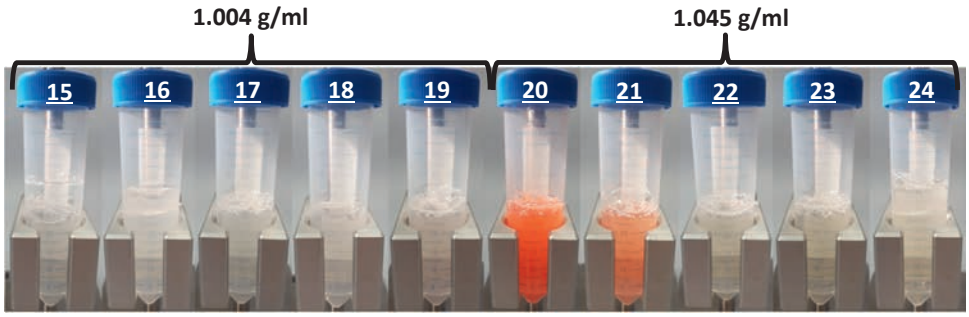


Figure S2. Centrifuge bowl outflow fractions. In order to simulate the crucial islet isolation step of changing medium from Ringer's acetate solution to University of Wisconsin solution (UWS), mock tissue was pumped into a continuous flow centrifuge bowl filled with Ringer's acetate solution (RAS). This was followed by an additional 250 ml Ringer's acetate solution (25 ml/min) and 250 ml UWS (25 ml/min). Fractions of 25 ml were collected via the overflow in 50 ml tubes. After 19 fractions of clear RAS, the 20th fraction contained mock tissue with a density of 1.030 g/ml. Subsequent fractions all contained UWS with a density of 1.045 g/ml without other densities of mock tissue. The first 14 collected fractions are not shown. The continuous flow centrifuge bowl was set at 1500 RPM (145 *g* at the bottom of the bowl and 105 *g* at the top of the bowl). An increased centrifuge speed was chosen due to the higher viscosity of UWS to RAS (3.32 vs. ≈ 1.56 mPa·s, respectively, at 4°C[41]) and a higher density (1.045 vs ≈ 1.004 g/ml, at 4 °C[53]) of UWS to RAS. Also, since the density difference between the UWS and mock tissue is much smaller than in the retention and washing phase (when the tissue is in RAS), the sedimentation rate of the mock tissue is slower, requiring more time and therefore a lower pump speed.

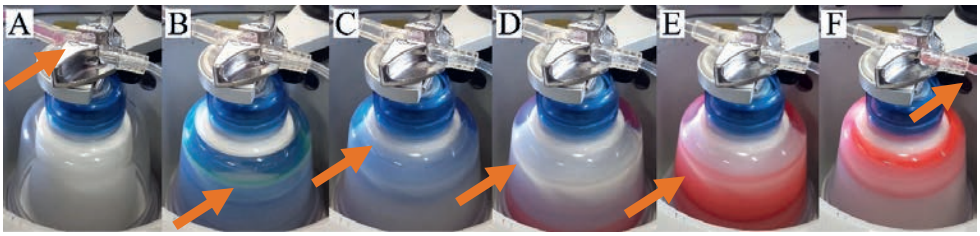


Figure S3. Simulation of density gradient purification in PRISM. A mixture of microspheres with densities of 1.040 g/ml (green), 1.071 g/ml (blue) and 1.089 g/ml (red) were suspended in UWS. Two computer-controlled Masterflex pumps (Metrohm, Barendrecht, the Netherlands) at varying speeds were used, with a constant combined pump speed of 30 ml/min. One pump controlled the flow of UWS containing the mock tissue, and the other pumped a mixture of 34% 370 mg I/ml iopromide (Ultravist 370, Bayer BV, Netherlands), with 68% UWS. A) At the start of the gradient a mixture of microspheres (arrow) enters the spinning centrifuge bowl, suspended in UWS. B) As the centrifuge bowl fills, green microspheres, which are less dense than UWS, rise to the outer rim (arrow) of the suspension. C) The green microspheres have left the bowl and blue microspheres (arrow) rise to the rim of the centrifuge bowl with increasing density of the solution. D) A clear separation of blue and red microspheres can be seen: the blue microspheres have exited the bowl, red microspheres remain in the bottom of the bowl, and a region with no microspheres is visible (arrow). E) As the density of the solution continues to increase, red microspheres (arrow) rise in the bowl. F) After the highest density solution is pumped into the centrifuge bow, red microspheres rise to the top and exit the bowl (arrow).

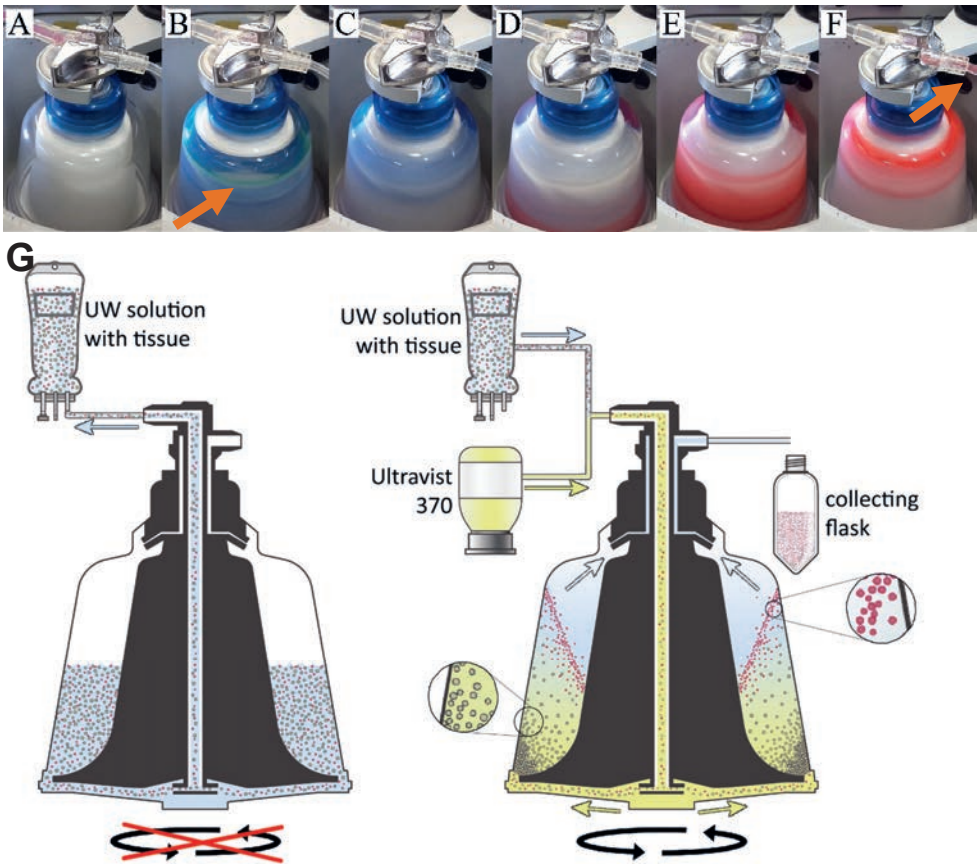


Figure S4. A-F) Simulation of density gradient purification in PRISM. A mixture of microspheres with densities of 1.040 g/ml (green), 1.071 g/ml (blue) and 1.089 g/ml (red) were suspended in UWS. Two computer-controlled Masterflex pumps (Metrohm, Barendrecht, the Netherlands) at varying speeds were used, with a constant combined pump speed of 30 ml/min. One pump controlled the flow of UWS containing the mock tissue, and the other pumped a mixture of 34% 370 mg I/ml iopromide (Ultravist 370, Bayer BV, Netherlands), with 68% UWS. A) an empty spinning bowl at the start of the gradient. B) Green microspheres on the top rim (arrow) of the suspension filling the bowl. C) Blue microspheres exiting the bowl with increasing density of the solution. D) An interphase between the blue and red mircospheres: the blue microspheres have exited the bowl and red microspheres remain in the bottom of the bowl. E) Red microspheres rising in the bowl with increasing density of the solution. F) Red microspheres exiting the bowl (arrow). **G) Schematic representation of density gradient purification during a PRISM islet isolation.** *Left panel)* After the centrifuge bowl is filled with UWS (blue liquid), centrifugation is stopped. The tissue suspended in UWS is pumped back into the bag which contained the UWS, as depicted. *Right panel)* UWS is combined with an increasing proportion of Ultravist 370 (yellow liquid) to create a density gradient. During establishment of the density gradient the less dense islets (in red) separate from the more dense exocrine tissue (in green). Fractions are obtained in collecting flasks once the centrifuge bowl is filled and the gradient solution containing the separated tissue exits the bowl.

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