Rescue Purification Maximizes the Use of Human Islet Preparations for Transplantation

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The relative inefficiency of the islet purification process may hamper obtaining enough islets for transplantation even with adequate pre-purification counts. In this study, we determined the effect of an additional purification step on total islet yields and pancreas utilization at our center.

Twenty-five pancreata were processed using the automated method followed by continuous gradient purification (CGP), and the less pure islet fractions were subjected to additional rescue gradient purification (RGP). CGP and RGP islets were combined and transplanted into patients with type 1 diabetes.

CGP and RGP islets showed no significant differences in cell viability, insulin secretion \textit{in vitro} and function when transplanted into chemically diabetic mice. Mean RGP contribution to the final preparation was 27.9 ± 19.9%. In 12 of 25 preparations, CGP yielded <5000 IEQ/kg of recipient body weight, and inclusion of RGP islets to the final preparation allowed to obtain the minimal islet number required for transplantation. Transplanted islets resulted in sustained C-peptide production, HbA1C normalization and insulin-independence or reduced insulin requirements.

Taken together, our data suggest that RGP islets are comparable in terms of viability and potency to CGP islets. RGP may be of assistance in maximizing the number of islet preparations successfully used in transplant protocols.

Key words: Islet transplantation, density gradient purification, islets of Langerhans, potency test, graft function, rescue purification

Introduction

Recent improvements in islet isolation and immunosuppression have allowed transplantation of human islets of Langerhans to become a viable treatment for patients with long-standing type 1 diabetes mellitus (T1DM)\textsuperscript{(1–3)}. Ongoing worldwide clinical trials have shown that insulin-independence can be consistently achieved when a sufficient number of islets is implanted, generally with an islet graft mass >10 000 IEQ/kg of recipient body weight (r.b.w.)\textsuperscript{(1–3)}. Notably, this desirable goal is often obtained by the means of sequential or single infusions of islet preparations obtained from more than one donor pancreas (generally 2–4), and less frequently from a single preparation (1–7).

Despite the significant progress in the methods of human islet isolation and purification, it is still difficult to recover sufficient number of islets from a single cadaveric donor pancreas. An average islet isolation generally yields about 50% of the estimated over 1 million islets present in an adult human pancreas (8). Several factors may contribute to this phenomenon, including donor characteristics (age, body mass index, brain death, cause of death, time in intensive care unit), and other factors such as organ procurement, storage and ischemia time (9–12).

The isolation procedure consists of a mechanically enhanced enzymatic digestion of the pancreas, which results in dissociation of the islets from the surrounding acinar tissue. During this process islets are exposed to a number of insults that may result in cellular damage and functional impairment, which ultimately lead to a reduction of the viable islet mass recovered. The purification step allows for the separation of the islets, which represent only 1–2% of the total pancreatic tissue, and multiple fractions of variable degrees of islet purity are usually recovered (13–14). This procedure reduces the volume of tissue that will be implanted in the patient, therefore minimizing the risks associated with intra-portal islet infusion (i.e. increased portal pressure and thrombosis)\textsuperscript{(1–7,15)}. Additionally, it still remains controversial whether purification of the islet preparation may also lower the immunogenicity of the graft (16–18). On the other hand, the compromise between islet purity
and yields penalizes the latter, as purification might result in decreased number of islets recovered and also be traumatic for the islets.

It is not uncommon that human islet preparations with adequate quality and potency cannot be used for clinical transplantation because the final (post-purification) islet yield falls below the required minimum number of 5000 IEQ/kg of r.b.w. This is frequently observed even when pre-purification counts are adequate. The islet purification procedure may result in the recovery of a relatively small proportion of islets in the higher purity fractions, while a substantial proportion of the islets may be found in the less pure fractions after centrifugation.

The need for more than one donor pancreas per recipient represents a major limitation for islet transplantation to be the treatment of choice for β-cell replacement in T1DM. Implementation of methods that maximize the recovery of islets from cadaveric donor pancrea and improve the efficiency of the costly isolation procedure are needed in order to reduce the donor-to-recipient ratios and increase the success rate of islet transplantation (19–22).

The aim of the present study was to evaluate the effectiveness of performing an additional purification step (rescue gradient purification, RGP) in case of insufficient islet yields when pre-purification counts are adequate. The islet purification procedure may result in the recovery of a relatively small proportion of islets in the higher purity fractions, while a substantial proportion of the islets may be found in the less pure fractions after centrifugation.

The aim of the present study was to evaluate the effectiveness of performing an additional purification step (rescue gradient purification, RGP) in case of insufficient islet yields obtained using continuous gradient purification (CGP), in order to recover islets that would otherwise be lost in the less pure fractions.

Methods

Human islet isolation

In order to assess the effects of an additional purification step (RGP) on the recovery of human islets from cadaveric donor pancrea, we have analyzed a series of isolation procedures performed between July 2001 and February 2004 at the Human Islet Cell Processing Facility of the Cell Transplant Center (at the University of Miami School of Medicine) that resulted in transplantation into patients with T1DM. From a total of 113 pancreata processed during the study period for the purpose of transplantation, 48 preparations were eventually transplanted (43%). We have identified a total of 25 transplanted islet preparations for which the fractions with lower islet purity and pellets obtained after CGP were subjected to an additional RGP step.

Human pancreata were obtained from multi-organ cadaveric donors (mean age 49.6 ± 12.8 years, body mass index 36.3 ± 7.6, 14 males, 11 females). The glands were cold-preserved with either pre-oxygenated (30 min) two-layer perfluorocarbon/University of Wisconsin solution (FFC/UW) or with UW alone (23). The average cold ischemia time was 9.0 ± 3.0 h. Following cold preservation, islets were isolated using a modification of the automated method (24) using seven different lots of the enzyme Liberase HI (Roche, Indianapolis, IN), and a standard CGP step, as described previously (7,15,24).

Islet purification procedures

Islets were purified using a computerized semi-automated cell processor (Cobe 2991; COBE Laboratories, Inc. Lakewood, CO) (25–27) in a refrigerated (4 °C) cell processing room. The standard procedure for the purification of human islet preparation consisted of a CGP performed by loading 1.100 g/mL (osmolality 320–373 mOsm/kg H2O) and 1.077 g/mL (285–327 mOsm/kg H2O) Ficoll-based density gradients in the doughnut-shaped bag using a gradient maker device, followed by top-loading of the pancreatic digest (≤20 mL of tissue/run) in UW solution (27). After 5 min of centrifugation at 2400 rpm, approximately 15 fractions were collected, and examined for purity.

RGP was performed when pre-purification counts were adequate for transplantation and standard purification procedure (CGP) resulted in relative low islet yields, or when a considerable number of islets was still observed in the impure layers generally not considered for transplantation. This additional purification step was performed in order to recover and further purify the islets entrapped in the low purity fractions. Briefly, the low purity fractions and pellet were pooled and washed in modified RPMI media by centrifugation (420 g, 1 min, at 4 °C). The tissue (<30 mL of tissue/run) was then resuspended in stock Ficoll (density 1.132 g/mL; osmolality 420–440 mOsm/kg H2O) and loaded in the bottom of the COBE bag (26). The discontinuous gradient was obtained by applying subsequently Eurofocoll solutions (Mediatech, Herndon, VA) with density 1.108, 1.096 and 1.037 g/mL (75 mL each). After a 3-min centrifugation at 2400 rpm, four fractions were collected: a 100-mL waste layer, a 1.037/1.096 interface layer usually yielding the purest islets, a 1.096/1.108 interface layer with less pure islets and a third 1.108/1.132 interface with the least pure islets (27). Islet preparations were considered for transplantation if the final purity (pooled CGP and RGP islets) was ≥50% and the final volume to be implanted was ≥10 mL (2–7).

Islet culture prior to transplantation

Following isolation, islets were cultured at 37 °C in humidified mixed 95% air/5% CO2 in non-tissue-treated 175 cm2 flasks (Corning, Acton, MA) at a density of ~20 000 IEQ in 30 mL of Miami-defined media 1 (MM1) containing 0.5% human serum albumin. After 24 h of culture, 15 mL of culture media was removed and replaced with fresh MM1 and islets were transferred at 22 °C (5% CO2) for the remaining 24–48 h preceding intra-portal infusion.

Fluorescein diacetate-propidium iodide viability staining

Islet preparations were assessed for islet viability using cell membrane exclusion dyes. Briefly, a small aliquot of islets was obtained at the end of each isolation procedure, and transferred in phosphate-buffered saline (PBS) to a 10 × 35 mm counting Petri dish. Fluorescein diacetate (FDA) and propidium iodide (PI) stock solutions were added to the sample at a final concentration of 0.67 μM and 75 μM, respectively. Using a fluorescent microscope, 50 islets were then assessed for cell viability by estimating the percentage of viable cells (green) versus the percentage of non-viable cells (red) within each islet (28). The mean and standard deviation of viable cells were then calculated for each preparation. Product release criteria for transplantation included viability of islet cells ≥70% (2–7).

Fluorescence labeling with TMRE, JC-1 and 7-AAD

Human islets were dissociated into single cell suspensions using Accutase (Innovative Cell Technologies, Inc, San Diego, CA). Aliquots of 1000 IEQ were resuspended in 1-mL Accutase in a 15-mL tube, incubated at 37 °C for 10–15 min, and then dispersed by gentle pipetting. For the assessment of apoptosis, single islet cell suspensions were incubated with 100-ng/mL tetramethylrhodamine ethyl ester (TMRE; Molecular Probes, Eugene, OR) or JC-1 (Molecular Probes) for 30 min at 37 °C in PBS without Ca2+ and Mg2+ (29,30). These two dyes selectively bind to mitochondrial membranes, allowing for the assessment of cells with functional mitochondria, and therefore can be used as markers for cell viability: loss of staining is considered an early indicator of apoptosis (31,32). Cells were then stained with 7-Aminoactinomycin D (7-AAD; Molecular Probes) that, similar to PI, binds to DNA when cell membrane permeability is altered after cell death.
Analysis was performed using the CellQuest software on a FACScan cytometer (Becton & Dickinson Co., Mountain View, CA).

**Glucose-stimulated insulin release**

**Static incubation:** To determine the in vitro potency of isolated islets, static glucose challenge was performed (27,33). After overnight culture, islets (50–100 IEQ) were incubated with either 2.8-mM or 20-mM glucose in culture medium for 2 h at 37°C. The supernatant was collected and stored at −80°C for insulin assessment by ELISA (Alpco, Salem, NH). Glucose-stimulated insulin release was expressed as stimulation index, calculated as the ratio of insulin released during exposure to high glucose (20 mM) over the insulin released during low glucose incubation (2.8 mM).

**Perfusion:** Selected islet preparations were analyzed for their response to a dynamic stimulation assay in vitro (34). Islets were pre-perfused in a chromatography column (Bio-gel Fine 45–90 mm; Bio-Rad) with a buffer containing 125-mM NaCl, 5.9-mM KCl, 1.28-mM CaCl₂, 1.2-mM MgCl₂, 25-mM HEPES, 0.1% bovine serum albumin and 3-mM glucose for 20 min, at 37°C. Islets were perfused in the same buffer for 10 min and then sequentially exposed to 11-mM and 3-mM glucose, and 25-mM KCl. Fractions of the perfusate were collected every 2 min during perfusate with 3-mM glucose, and every minute during stimulation. The collected fractions were then assayed for human insulin concentrations by ELISA.

**In vivo assessment of islet potency:** Animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee and by the Institutional Review Board. Male athymic nu/nu (nude) mice (Harlan Laboratories, Indianapolis, IN) were housed at the Division of Veterinary Resources of the University of Miami Virus Antibody-Free rooms in microisolated cages, having free access to autoclaved chow and water. Animal procedures were performed at the Translational Research Laboratory of the Cell Transplant Center (Diabetes Research Institute of the University of Miami School of Medicine).

Animals were rendered diabetic via single intravenous administration of 200 mg/kg of Streptozotocin (Sigma, St Louis, MO). Non-fasting blood glucose was assessed by the use of a portable glucometer (Elite, Bayer; Tarrytown, NY). Mice with sustained hyperglycemia (>300 mg/dL) were used as islet graft recipients.

Human islets (n = 3 preparations) were transplanted under the kidney capsule of diabetic immunodeficient mice (33,35). For each islet preparation, up to 3 mice were transplanted with 2000 IEQ each obtained from either the first purification (CGP) or the rescue purification (on discontinuous gradients, RGP). Briefly, under general anesthesia (Metofane, Shering-Plough Animal Health, Atlanta, GA) a breach was made in the kidney capsule and islets were gently deposited in the subcapsular space through a polyethylene catheter. The breach was then closed and the surgical wound sutured (33,35). After transplantation, non-fasting blood glucose values were assessed three times a week. Reversal of diabetes was defined as stable non-fasting blood glucose <200 mg/dL. Nephrectomy of the graft-bearing kidney was performed to confirm prompt return to hyperglycemia and exclude residual function of the native pancreas in animals achieving normoglycemia after transplantation.

**Intra-peritoneal glucose tolerance test:** At least 1 month after transplantation, mice were fasted overnight and then given a glucose bolus of 2 g/kg b.w. intra-peritoneally (35). Blood glucose was monitored at selected time points after injection by collecting blood from the tail vein using a portable glucometer. Glucose clearance was calculated after intra-peritoneal bolus by calculating the area under the curve (AVC) of the glycemic profile, as previously described (36).

### Statistical analysis

Statistical analysis was performed using Microsoft Excel software and paired Student’s t-test. Data were considered statistically significant for p values <0.05.

### Results

**Human islet preparations for clinical transplantation**

The current release criteria for human islet preparations for clinical transplantation require that yields >5000 IEQ/kg of r.b.w. are utilized. Islet preparations that do not meet such criteria are, unfortunately, not suitable for release and therefore are not transplanted. The drawbacks of such unfortunate situation include the economical impact on the islet transplant center (utilization of resources and costs for the whole procedure), and the less than optimal utilization of the organs available for islet transplantation.

In an attempt to maximize the utilization of our resources, we have analyzed the data obtained from 25 human islet isolations performed at our center, in which an additional RGP step was performed and that resulted in transplantation. The number of islets obtained from CGP, RGP or total CGP + RGP was calculated with regard to the body weight of the recipient, and expressed as IEQ/kg of r.b.w.

In 12 of 25 islet preparations (48%), the yield from CGP was less than the minimum required 5000 IEQ/kg. Performing an additional RGP step for these preparations was critical to overcome the cutoff number, rendering the final IEQ/kg adequate for clinical islet transplantation. Should the RGP not have been performed, none of these preparations could have been transplanted (Figure 1A). The final preparations were utilized for transplantation in patients with T1DM in the ongoing clinical trials. The total IEQ obtained from each islet preparation before purification and after each of the purification steps (CGP and RGP) is summarized in Figure 1B.

Six patients received the preparation as first infusion, and 6 received it as second infusion. Two of 12 preparations were shipped to our partner transplant center (Baylor College of Medicine, Houston, TX) after isolation, and were transplanted there. The final volume infused in the recipients was always ≤10 mL with purity >50%, and all islet infusions resulted in a measurable function after implant, as evidenced by a substantial reduction of insulin requirements, increased C-peptide levels and improved HbA1c (Table 1). One of the preparations transplanted as first infusion at Baylor College of Medicine resulted in insulin-independence. The other patients achieved insulin-independence after receiving an islet preparation released thanks to the RGP contribution as second infusion.

The mean yield after CGP was 331 963 ± 140 958 IEQ (3176 IEQ/g of pancreatic tissue), while the mean
recovery of islets with purity >50% after the second RGP on discontinuous layers was 129,469 ± 112,770 IEQ (1238 IEQ/g of pancreatic tissue). The total islet recovery following the two purification steps, and obtained by combining both CGP and RGP fractions was 461,422 ± 141,002 IEQ (4416 IEQ/g of pancreatic tissue). Therefore, the RGP contributed 27.9 ± 19.9% to the total IEQ recovered per pancreas. The pancreata that yielded sufficient islet numbers after the first CGP run had similar characteristics to those that required the additional RGP step to achieve for transplantation after RGP.

**Assessment of islet cell viability**

Islet cell viability evaluated by FDA/PI staining showed no significant differences between islets obtained by CGP (CGP islets; 90.1 ± 5.8%) and those obtained by CGP.
Maximizing Human Islet Preparation Utilization for Transplantation

Table 1: Performance of human islet preparations in which RPG allowed meeting the required minimal islet mass for transplantation

<table>
<thead>
<tr>
<th>Recipient</th>
<th>IEQ/Kg</th>
<th>%Rescue</th>
<th>Exogenous insulin use (U/kg/day)</th>
<th>C-peptide (ng/mL)</th>
<th>Glycosylated hemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-Tx</td>
<td>Post-Tx</td>
<td>Pre-Tx</td>
</tr>
<tr>
<td>1st Tx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>8207</td>
<td>77.6</td>
<td>0.56</td>
<td>0.17</td>
<td>&lt;0.30</td>
</tr>
<tr>
<td>B</td>
<td>7480</td>
<td>44.2</td>
<td>0.53</td>
<td>0.29</td>
<td>&lt;0.30</td>
</tr>
<tr>
<td>C</td>
<td>5262</td>
<td>51.0</td>
<td>0.60</td>
<td>0.23</td>
<td>&lt;0.30</td>
</tr>
<tr>
<td>D</td>
<td>6345</td>
<td>32.8</td>
<td>0.32</td>
<td>0.20</td>
<td>&lt;0.30</td>
</tr>
<tr>
<td>E</td>
<td>5352</td>
<td>15.2</td>
<td>0.55</td>
<td>0.25</td>
<td>&lt;0.30</td>
</tr>
<tr>
<td>F</td>
<td>7233</td>
<td>56.1</td>
<td>0.56</td>
<td>None</td>
<td>&lt;0.30</td>
</tr>
<tr>
<td>2nd Tx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>35.5</td>
<td>0.23</td>
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<tr>
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<td>28.6</td>
<td>0.14</td>
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<tr>
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<tr>
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<td>43.4</td>
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</tr>
<tr>
<td>L</td>
<td>5523</td>
<td>26.2</td>
<td>0.11</td>
<td>None</td>
<td>1.60</td>
</tr>
</tbody>
</table>

Tx = Transplantation.
% Rescue = (IEQ from RGP/Total IEQ) × 100.

Figure 2: In vitro viability assessments of CGP or RGP islets. Panel A. CGP or RGP islets were assessed for islet viability using PI and FDA, showing unremarkable differences between the two groups. Data are presented as mean ± SD of five independent islet preparations. Panel B. Viability assessed on dissociated islet cells by FACS showed similar results for the two groups. 7-AAD (dead cell marker), JC-1 or TMRE (MMP indicator). Data are representative of 5 individual islet preparations showing the same pattern.

followed by RGP (RGP islets 91.4 ± 4.3%)(Figure 2A). It is noteworthy that the sensitivity of this method is extremely low (Ichii H et al., unpublished data). We therefore evaluated islet cell viability using two types of fluorescence dyes: 7-AAD is a DNA-binding dye similar to PI, which is used for the detection of dead cells; TMRE and JC-1 allow for the evaluation of mitochondrial membrane potential (MMP) and are more sensitive methods to discriminate viable versus apoptotic cells. The combination of these dyes allows for the detection and quantification of dead, apoptotic and viable cells in fresh, unfixed single cell suspensions using FACS analysis. The percentages of dead cells (7-AAD positive) in CGP islets and RGP islets were comparable (7.2 ± 5.4 vs. 7.5 ± 4.3%), and the results were similar to those obtained with FDA/PI staining. Assessment of islet cell viability by JC-1 and TMRE within the 7-AAD negative population also showed comparable results between CGP islets and RGP islets (65.2 ± 5.6 vs. 67.4 ± 7.6% in JC-1, 64.7 ± 6.7 vs. 63.2 ± 6.6% in TMRE) suggesting that the viability and mitochondrial integrity of
islets are not altered by the additional purification step (Figure 2B).

**In vitro assessment of islet function**

Islet potency was assessed by static glucose challenge *in vitro*. There were no differences in the amount of insulin secretion between CGP islets and RGP islets, and stimulation indices were comparable (Figure 3A). The dynamics of insulin release under stimulation were also examined by perifusion. Again, no differences were observed when comparing the dynamics of insulin release after stimulation with 11-mM glucose and 25-mM KCl between the two experimental groups (Figure 3B).

**In vivo performance of islets transplanted into diabetic immunodeficient mice**

Three human islet preparations were transplanted into chemically diabetic nude mice in order to assess their ability to reverse diabetes *in vivo* (Table 2). Two islet preparations resulted in reversal of diabetes in all transplanted mice, regardless of the purification procedure performed (CGP alone or after RGP)(Figure 4A). One islet preparation failed to correct diabetes in the mice transplanted with CGP islets (0/2), while 1 of 3 mice transplanted with RGP islets corrected hyperglycemia (Table 2). These data suggest that human islets obtained from RGP are able to restore and maintain metabolic control in an *in vivo* model of diabetes.

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**Figure 3: In vitro potency assessments of CGP or RGP islets.** Islets potency was assessed by static glucose challenge (Panel A). (3G = glucose 3m; 11 = glucose 11m) No differences were observed in the stimulation indices between the experimental groups (mean ± SD of five independent islet preparations). The dynamics of insulin release under stimulation were examined by perifusion (Panel B). The data presented are representative of 5 individual islet preparations showing the same pattern.

**Table 2: Reversal of diabetes in transplanted nude mice**

<table>
<thead>
<tr>
<th>Prep</th>
<th>Purity (%)</th>
<th>Reversal proportion</th>
<th>Days to reversal (mg x min)/dL</th>
<th>CGP- islets</th>
<th>RGP- islets</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90</td>
<td>0/2</td>
<td>N/A</td>
<td>11 116 ± 2,431</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>2/2</td>
<td>4, 4</td>
<td>2221 ± 380</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>3/3</td>
<td>1, 2, 2</td>
<td>3209 ± 879</td>
<td>80</td>
</tr>
</tbody>
</table>

*Animals achieving euglycemia post-transplant.
†Mean AUC of the two animals that did not achieve euglycemia post-transplant.
in a manner comparable to that of islets obtained from CGP.

Furthermore, when assessing graft function during a glucose metabolic challenge in transplanted animals (IPGTT), glucose clearance profiles were comparable in both experimental groups (Figure 4A), and the calculated AUC showed no statistical differences between groups (Table 2). These data also confirm that similar performance is obtained when comparing CGP islets and RGP islets.

**Discussion**

Successful human islet transplantation for the treatment of T1DM is dependent on the quality and quantity of islets infused. Clinical trials ongoing worldwide have clearly demonstrated that insulin-independence can be reproducibly obtained in patients with T1DM after allogeneic islet transplantation when a sufficient number is transplanted (1–7). The current release criteria for transplantation of human islet preparations is the achievement of $\geq$5000 IEQ/kg of r.b.w. In spite of recent improvements introduced in the procurement and preservation of cadaveric pancreatic glands and in isolation technology, it is still difficult to produce consistent islet yields that meet these minimal requirements for transplantation. The ideal graft mass of $\geq$10 000 IEQ/kg r.b.w. needed to achieve insulin-independence is generally attained by sequential infusion of more than one islet preparation per recipient (1–7). This represents a major drawback due to the shortage of cadaveric donor pancreata available for islet transplantation, and has a significant impact on the costs (resources and reagents) for the islet transplantation center.

The efficiency of the islet isolation and purification are crucial to obtain adequate islet numbers for transplantation. The progress in this field has been steady in the recent years thanks to the introduction of the automated method in the 1980s (24), the availability of improved dissociation enzyme blends with lower endotoxin contamination (19) and the use of the semi-automated computerized purification method in the early 1990s (25–26) that have been of invaluable assistance in improving the efficiency of human islet isolation. In addition, the introduction of improved preservation techniques for cadaveric donor pancreata, such as the utilization of moieties able to provide oxygen to the organ during preservation by the two-layer method has contributed to a better utilization of the organs, including marginal organs that were considered unsuitable for transplantation otherwise (21,37–39).

Density gradient centrifugation using the semi-automated computerized COBE-2991 cell processor is the most effective method for the purification of human islets as it allows to run large volume of pancreatic digest in reasonably short times (13,14,25,26). CGP with top-loading of the pancreatic digest is considered the gold-standard method at the present time (1–7). An important advantage of CGP is that it allows collecting serial fractions with different degrees of purity that can be assessed separately in order to select those with the highest purity for transplantation (1–7,13). In addition, top-loading has the potential advantages of allowing for keeping the digested tissue in a physiological medium for the maximum possible time and minimizing centrifugal forces (13,27). On the other hand, the efficiency of CGP is not ideal, and potential limitations include lower effective cell load, possible accumulation of cells at interfaces that may impede migration of tissue, and lead to aggregation (13).

It is not uncommon when performing human islet isolation to observe high discrepancies between pre-purification and post-CGP islet counts. Even when islet quality and numbers are adequate in the pre-purification counts, low yields after CGP may result in failure to meet the minimal requirements for transplantation. In particular, we have found that it is difficult to successfully recover mantled, embedded or aggregated islets using CGP.

In an attempt to improve the efficiency of human islet isolation procedures and maximize the utilization of islet preparations (and therefore of processed cadaveric donor pancreata), we have assessed the effects of an additional purification step in a series of procedures performed for clinical transplantation at our center. The additional purification step on discontinuous gradients (RGP) was performed on the fractions with lower purity and the pellet obtained after CGP. During the RGP, islets are resuspended in high-density ficoll solution (bottom layer) and overlaid with solutions of lower density (13,27). It is conceivable that exposure to high-density polysucrose solutions may result toxic for islet cells. On the other hand, exposure to high osmolality media may have the advantage of decreasing acinar tissue swelling and edema, which may result in enhanced density differences between acinar tissue and islets, therefore favoring separation (13,14). Based on these premises, it is our working hypothesis that the islets not recovered by CGP may be ‘rescued’ by discontinuous gradient purification.

Assessment of islet cell viability by the means of membrane exclusion dyes and of mitochondrial membrane potential showed no differences between CGP and RGP islets within the same islet preparation, suggesting that the additional purification step had no impact on this parameter. Furthermore, endocrine function was evaluated by measuring the potency of isolated islets during in vitro stimulation in both static incubation and dynamic perfusion. These tests indicated that there was no significant difference between CGP and RGP islets.

We also compared the in vivo potency of CGP and RGP islets by transplanting selected islet preparations into chemically diabetic immunodeficient mice. The proportion of animals reversing diabetes, and the time leg to restore
normoglycemia after transplantation were comparable in both groups, suggesting that islets obtained by RGP had quality and potency comparable to those obtained by standard CGP. In addition, the glucose clearance during glucose tolerance test was similar when comparing the RGP and CGP islets within the same islet preparation. Interestingly, the islet preparation that failed to correct hyperglycemia in the recipients of RGP islets was paralleled by failure to reverse diabetes also in the recipients of CGP islets obtained from the same pancreas. Overall, these data indicate that the additional RGP step has minimal or no effect on islet potency, since similar results are observed within the same preparation when assessing CGP islets.

In our study, performing RGP resulted in improved recovery of islets from the less pure fractions of the standard CGP step. Importantly, the islets recovered by RGP contributed a substantial proportion of the final islet yields (including both CGP and RGP islets) that were instrumental to meet the minimal requirements for transplantation in 12 of 25 islet preparations. Graft function after transplantation of islet preparations including RGP was comparable to that of patients receiving islets obtained with CGP alone. It is worth noting that should RGP not been performed, the 12 islet preparations that yielded sufficient islets for transplantation would have been otherwise discarded.

In three additional islet preparations with adequate pre-purification counts, however, sufficient islets for transplantation were not obtained despite RGP (not shown). The relative small number of failures of RGP to recover sufficient islet numbers for transplantation precludes at present time an analysis of the relative contribution of each variable to the outcome of the procedure. Future studies addressing this important issue are granted, as they may allow for the definition of criteria guiding the decision on when to perform rescue purification.

It is conceivable that performing a rescue purification procedure using continuous gradients and top-loading may also allow for improved islet recovery from the less pure layers collected after the first purification. It will be of
interest to perform side-by-side comparisons of the two techniques in the future in order to determine the most efficient method to improve islet yields. Based on our experience, the use of bottom-loading and discontinuous gradient purification can be considered both time- and cost-effective. Only three fractions are collected and assessed after centrifugation on discontinuous gradients, making the procedure less lengthy than CGP; furthermore, the reagents are also relatively less expensive. In addition, after the first CGP, low purity fractions and pellet are already exposed to high density/osmolality gradients, and therefore the advantage of top-loading as a means to maintain the preparation in the physiological solution for the longest time possible might no longer be an issue.

Taken together, our data suggest that performing an additional purification step for the isolation of human islets from cadaveric donor pancreata allows for improved efficiency, measured as increased islet yields with viability and potency comparable to that obtained by CGP. RGP may be of assistance in maximizing the number of islet preparations that can be successfully used for transplantation. Although there is an additional cost when performing the additional discontinuous rescue step, the benefits of obtaining enough islets for transplantation from one pancreas far outweigh the total cost of an untransplantable preparation due to insufficient number of islets. To this should be also considered the additional costs of subsequent pancreas procurement and islet isolation to again attempt to obtain islet yields suitable for transplantation.

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