Optimizing Porcine Islet Isolation to Markedly Reduce Enzyme Consumption Without Sacrificing Islet Yield or Function

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Background. Human allogeneic islet transplantation for treatment of type 1 diabetes provides numerous clinical benefits, such as fewer episodes of hypoglycemic unawareness and tighter control of blood glucose levels. Availability of human pancreas for clinical and research use, however, is severely limited. Porcine pancreas offers an abundant source of tissue for optimization of islet isolation methodology and future clinical transplantation, thereby increasing patient access to this potentially lifesaving procedure.

Methods. Porcine islet isolations were performed using varying amounts of collagenase (7.5, 3.75, or 2.5 Wunsch units per gram tissue) and neutral protease activity (12,000, 6000, or 4000 neutral protease units per gram tissue) and perfusion volumes (1.7 or 0.85 mL/g tissue) to assess their effects on isolation outcomes. Retention of dissociative enzymes within the pancreas during perfusion and digestion was evaluated, along with distribution of the perfusion solution within the tissue. Results. Reducing enzyme usage by as much as 67% and perfusion volume by 50% led to equally successful islet isolation outcomes when compared with the control group (48 ± 7% of tissue digested and 1088 ± 299 islet equivalents per gram of pancreas vs 47 ± 11% and 1080 ± 512, respectively). Using margin-marking dye in the perfusion solution to visualize enzyme distribution demonstrated that increasing perfusion volume did not improve tissue infiltration. Conclusions. Current protocols for porcine islet isolation consume excessive amounts of dissociative enzymes, elevating cost and limiting research and development. These data demonstrate that islet isolation protocols can be optimized to significantly reduce enzyme usage while maintaining yield and function and thus accelerating progress toward clinical application.

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limited supply of suitable donor organs. The viability of islet transplantation as a treatment for type 1 diabetes, whether allogeneic human islets or xenogeneic porcine islets, is dependent upon numerous factors including safety, clinical efficacy, and the ability to obtain high quality islets. For both human and porcine islet donors, successful islet isolation is determined by several factors but is largely dependent upon the quality of the starting tissue and the digestion conditions employed to release the islets from the surrounding exocrine tissue. Although the suitability of the donor organ for islet isolation can be quickly and accurately assessed through examination of a small tissue biopsy, digestion methods vary between centers by, among other things, type and ratio of digestive enzymes, perfusion methods and volumes used for organ distension, incubation conditions, and the assessment of digestion endpoints. This variability leaves substantial room for optimization of digestion methods to improve islet isolation success.

Among laboratories in this field, the amount of enzyme used per gram of tissue differs for both the collagenase and neutral protease components. For porcine pancreas, published collagenase usage ranges from 7.5 Wunsch units (WU) per gram of tissue (our experience) to as much as 50 WU/g, with collagenase-to-neutral protease ratios similarly variable. A wide range of enzyme usage is also reported for human islet isolation. In one particularly thorough study of enzyme optimization, successful human islet isolation was attained using from 10 to 80 WU/g, with an optimal range defined from 21 to 32 WU/g. The neutral protease-to-collagenase ratio ranged from 15 to 53. In another analysis, 216 human islet isolation procedures performed at a single center averaged from 26 to 31 WU/g, depending on which of 3 different enzyme formulations were used.

A recently published study by Friberg et al has demonstrated that the majority of enzyme activity initially perfused into human pancreas fails to be absorbed by the tissue, with 65% remaining in solution after 20 minutes of recirculation. The authors note, as has been our experience with porcine pancreas, that free enzyme does not affect tissue digestion, that is, “a pancreatic biopsy taken before ductal perfusion of enzymes and subsequently placed in the digestion chamber remains macroscopically intact, whereas the pancreas containing the enzyme blends is efficiently digested.” It has also been shown in human pancreas by Cross et al that collagenase routinely infiltrates the islets of Langerhans under the standard Edmonton Protocol perfusion guidelines. This infiltrate is presumed to be damaging to islet structure, function, and overall islet isolation success, suggesting that reductions in perfusion pressure, volume, and total enzyme may be beneficial to isolation outcomes. The findings by these groups of an excess of unutilized enzyme and collagenase infiltration of the islets in a typical pancreas digestion prompted us to re-examine our process and the effect of enzyme quantity and perfusion volume on isolation outcomes and the route and extent of perfusate infiltration into the tissue.

**MATERIALS AND METHODS**

**Dye Perfusion Assays**

To determine the extent of perfusion solution infiltration into the pancreatic tissue, the splenic lobes of 10 porcine pancreases were perfused via cannulation of the main duct in the neck with margin marking dye to differentiate the perfused tissue. Black margin marking dye (American MasterTech, Lodi, CA) was prepared as a 1% solution in Cold Storage Purification Stock Solution (CSPSS) (MediTech, Inc., Manassas, VA) and manually perfused into the pancreas at 0.83 mL/g (“0.5×” volume, n = 5) or 1.7 mL/g (“1×” volume, n = 5) using a 60-mL syringe and 16 gauge cannula. Immediately on completion, 1-cm-thick transverse sections were collected from the neck (1 cm below end of cannula), midsection (midpoint of organ between point of cannulation and distal tip), and tip regions (2 cm above tip of organ furthest from cannulation site). Stained tissue sections were fixed overnight in 10% neutral buffered formalin and subsequently transferred to 70% ethanol for 24 hours. Photographs were taken of both exposed internal surfaces from each section and labeled as “proximal” (side nearest cannulation site) or “distal” (side furthest from cannulation site). Two independent scorers, blinded to the experimental conditions, manually selected the stained areas using Adobe Photoshop and the images were scored for the percentage of tissue surface area stained using Zeiss KS300 image analysis software.

A separate experiment was performed to visualize the degree to which larger perfusion volumes distend previously unaccessed areas of the pancreas versus simply further saturating already perfused tissue. To this end, blue and yellow margin marking dyes were perfused sequentially into the splenic portion of the pancreas at 0.85 mL/g for each dye (1.7 mL/g total volume). Blue-stained tissue indicates the extent of distension in the 0.5× volume condition, whereas the addition of the yellow dye mimics the 1× perfusion volume. Areas of dye overlap appear green.

**Porcine Islet Isolation**

Donor islets were prepared from retired breeder sows processed at Bob Evans Farms, Inc. in Xenia, OH. Islet isolation was performed as previously described. All isolations were performed using a single lot of Clzyme Collagenase MA and Clzyme BP Protease (VitaCyte LLC, Indianapolis, IN) reconstituted in CSPSS. Enzyme and perfusion volume calculations were based on clean pancreas weight following manual defatting. Perfusion solution was prepared in CSPSS to the indicated volume and enzyme concentrations for each donor pancreas before manual perfusion with a 60-mL syringe. The distended pancreas was subsequently placed intact into the digestion chamber, submerged in CSPSS, and maintained at 31°C for the duration of the digestion. After the dissociation and dilution phase, the undigested tissue was weighed and subtracted from the starting weight to determine the amount of tissue digested. This weight was divided by the preperfusion pancreas weight to determine the percentage digested. Islet yield, viability, and purity were determined as previously described.

The standard isolation protocol in our laboratory calls for 7.5 WU of Collagenase MA activity per gram of pancreatic tissue, 12,000 neutral protease units (NPU) of BP Protease activity per gram, and a perfusion volume of 1.7 mL/g. This control group is referred to as “1×/1×” (enzyme/volume) throughout the text. Other conditions tested were as follows: 7.5 WU/g, 12,000 NPU/g, 0.85 mL/g (1×/0.5×); 3.75 WU/g, 6000 NPU/g, 1.7 mL/g (0.5×/1×); 3.75 WU/g, 6000 NPU/g,
0.85 mL/g (0.5 × 0.5 × 0.5 ×). An entire porcine pancreas was used for each isolation.

Isolated islets were encapsulated in a 2-layer agarose macrobead at a concentration of 500 islet equivalents (IEQ) per macrobead as previously described, with the exception that all islets were encapsulated in a first-coat of 0.8% SeaKem Gold agarose (Lonza Rockland, Inc., Rockland, MD). Islet macrobeads were cultured in insulin-free RPMI 1640 containing 11.1 mM (200 mg/dL) glucose (Thermo Fisher Scientific, Grand Island, NY) supplemented with 2.5% heat-inactivated porcine serum (Biologics, Montgomeryville, IL). Culture medium was changed weekly and 24-hour post-change media samples collected for porcine insulin ELISA assay following the manufacturer’s instructions (Mercodia, Inc., Uppsala, Sweden). This assay was used to determine the daily insulin secretory capacity per macrobead in response to static glucose challenge in a persistent, supra-physiological glucose environment.

Enzyme Activity Assays

Activity assays were performed on enzyme solutions independently of islet isolation to measure the degree of enzyme retention in the pancreas during the digestion phase. For each set of samples, the splenic lobe of a single porcine pancreas was manually defatted before perfusion using the same enzyme lots as described above. Assays were performed on a total of 3 organs per group for the “1 × 1 ×,” “0.5 × 0.5 ×,” and “0.33 × 0.5 ×” conditions. Samples were collected from the perfusion solution immediately after preparation (“Perfusate” sample) and any fluid that leaked from the tissue during perfusion (“Leaked” sample). The “Leaked” volume was also measured. The perfused organ was then submerged in 250 mL CSPSS in a glass beaker and placed in a water bath at 31°C for 50 minutes. Media samples were then collected (“T50” sample), and the final volume in the beaker measured to correct for fluid loss from the tissue during the incubation period.

In addition, “nonperfused” samples were collected to assess stability of the enzyme in solution. Enzyme was prepared as for perfusion perfusion but instead placed in a 50-mL conical tube (40 mL/tube). Samples were collected from 3 independently prepared conical tubes for each of the “1 × 1 ×,” “0.5 × 0.5 ×,” and “0.33 × 0.5 ×” groups. A sample from each tube was taken immediately after preparation and designated “T(5)” (equivalent to “Perfusate” sample) and again after 5 minutes at room temperature (equivalent to “T0” sample). The tubes were then incubated in a water bath at 31°C and samples collected at 10-minute intervals from “T10” to “T50.” Samples were stored at −80°C until analyzed to ensure enzyme stability.

All samples were assayed in duplicate for collagen degrading activity (CDA) and neutral protease (NP) activity using previously validated kinetic fluorescence microplate assays. Type 1 calf skin (EMD Chemicals, Gibbstown, NJ) and bovine serum albumin (Fraction V; Elastin Products Co., Inc., Owensville, MO) were labeled with fluorescein isothiocyanate (Molecular Probes, Inc., Eugene, OR) and served as soluble substrates for the CDA and NP assays, respectively. For both the CDA and NP assays, 1 enzyme unit is defined as an increase of 1 fluorescent unit per minute at 35°C.

Statistical Analyses

Statistical analyses were performed by the Statistical Consulting Center in the College of Science and Mathematics at Wright State University. All data are presented as mean ± standard deviation. Dye perfusion quantification (Figure 1) was evaluated by 1-way analysis of variance (ANOVA) with Bonferroni correction for level of significance. For in vitro insulin production (Figure 2), total area under the curve was calculated for each group and compared by 1-way ANOVA. Collagenase and neutral protease activity assays (Figure 3) were analyzed using a paired t test, again with Bonferroni correction to control for increased potential of Type I error. Islet isolation and enzyme activity data presented in Tables 1 and 2, respectively, was compared by separate 1-way ANOVAs for each variable.

RESULTS

Dye Perfusion Assays

Representative images of a dye-perfused pancreas section and the corresponding stain-selected image used for quantification are presented in Figures 1A and B, respectively. Image analysis results, given as the percentage dye-stained surface area for each section, are presented in Figure 1C. No significant difference in surface area selected was found between scorers, indicating that the scoring method was consistently applied and minimized observer bias (intraclass correlation coefficient = 0.973). One-way ANOVA analysis further showed that no significant difference in dye distribution exists between the 0.5 × and 1 × perfusion volumes for the neck, midsection, or tip of the splenic pancreas (P = 0.59, P = 0.65, and P = 0.03, respectively).

Two-color dye perfusion was performed to further evaluate the differences in perfusate distribution between the 0.5 × and 1 × volume groups and is presented in Figures 1D–G. Surface inspection of the dye-perfused splenic pancreas indicated that the tissue was uniformly well distended along its entire length, with visible blue, green, and yellow staining (Figure 1D). Transverse sections taken from the neck of the pancreas just below the point of cannulation presented primarily blue- and green-stained tissue, with limited areas of yellow staining (Figure 1E). Staining in the midsection (Figure 1F) was very similar, with a mixture of blue, green, and yellow dye, though the exclusively yellow-stained tissue was more prominent and constituted a larger area of the section. In the tip section (Figure 1G), overall staining was reduced though still prominent. Yellow dye made up a larger proportion of the total staining in this section of the pancreas but, as in the proximal and medial sections, yellow staining co-occurred with and was centered around areas of blue and/or green staining.

Islet Isolation

Varying enzyme activity and perfusion volume resulted in no statistically significant difference between experimental groups on any standard measure of isolation outcome (Table 1). All 5 experimental groups (1 × 1 ×, 0.5 × 0.5 ×, 0.33 × 0.5 ×, 1 × 0.5 ×, 0.5 × 1 ×) were indistinguishable on the most directly comparable variables defining isolation success (% pancreas digested [P = 0.50], viability [P = 0.50], and purity [P = 0.33]). “IEQ/g starting weight,” which is perhaps
the most informative data point, also showed no significant differences between groups ($P = 0.45$), with values ranging from $892 \pm 451 \ (0.5 \times \text{total} \times 0.5 \times \text{volume})$ to $1350 \pm 285 \ (0.5 \times \text{total} \times 0.5 \times \text{volume})$. Subsequently, we completed an additional 28 islet isolation procedures using the $0.5 \times \text{total} \times 0.5 \times \text{volume}$ conditions and compared the results with the most recent 28 procedures performed under $1 \times 1 \times \text{volume}$ conditions. In this analysis, “IEQ/g starting weight” again did not differ significantly between groups ($P = 0.49$) with values of $1145 \pm 298 \ (0.5 \times 0.5 \times \text{total} \times 0.5 \times \text{volume})$ and $1056 \pm 607 \ (1 \times 1 \times \text{total} \times 0.5 \times \text{volume})$, in line with the results of the original experiments.

**Islet Function**

To evaluate functional differences between experimental groups, islet performance was assessed by measuring 24-hour insulin secretion each week for 12 weeks postisolation (Figure 2). Area under the curve analysis showed no difference in average 24-hour insulin production regardless of enzyme formulation ($P = 0.66$). Group values occurred in a tight range, from $1176 \pm 389 \ (0.33 \times 0.5 \times \text{total} \times 0.5 \times \text{volume})$ to $1473 \pm 458 \ (1 \times 1 \times \text{total} \times 0.5 \times \text{volume})$, suggesting that islets isolated under the varying conditions were functionally equivalent on this in vitro measure.
In vitro insulin production by porcine islet macrobeads. Data is presented as milliunits per macrobead per 24 hours (mU/MB per 24 h) for each experimental group (1 × 1 × 1, 0.5 ×/1 × 1, 0.5 ×/0.5 ×, 0.33 ×/0.5 ×, n = 5; 1 ×/0.5 ×, n = 3). Statistical analysis using total area under the curve showed no differences between groups.

Enzyme Activity Assays

Enzyme stability in solution was determined in nonperfused samples to confirm that data from perfused samples could be meaningfully evaluated. In the case of collagenase, enzyme function was stable over time with no apparent change in activity from the time of preparation to the end-point of the experiment after a 50-minute incubation at 31°C (Figure 3). Although statistical analysis detected a significant difference in the 1 ×/1 × group at the 40-minute time point (T40, P = 0.004) and T50 (P = 0.003) relative to the baseline at T(−5), we do not believe this variation is biologically meaningful given the minimal change in mean activity and small standard deviations between time points. Conversely, neutral protease activity was quite labile, with 30% or greater loss of activity from T(−5) to T50 in all 3 conditions tested. In addition, high levels of native protease activity from the pancreas are likely to make any evaluation of injected neutral protease function uninterpretable.12 For these reasons, further analysis of in situ function of perfused enzyme focused on collagenase activity alone.

Analysis of the enzyme solution before perfusion into the test organs confirmed the manufacturer’s specifications for collagenase and the expected differences between groups (Table 2, “Perfusate Activity”). “Leaked Activity,” which is a measure of the amount of enzyme lost during perfusion and distention, showed a volume dependent difference between groups, with the most activity lost from the “1 ×/1 ×” control, which also lost the most volume during perfusion. “Chamber Activity” was measured in a sample taken from the incubation media at T50 and reflects collagenase activity that diffused out of the pancreas during the digestion phase. The “1 ×/1 ×” group again had the greatest loss of activity due to the larger volume of fluid lost from the organ (Table 2, “Diffused Volume”). “Retained Activity” was derived from the previous values to estimate the amount of enzyme activity that was withheld inside the organ during the digestion phase. No difference was found between groups on this measure, likely due to the large within group variation between organs.

DISCUSSION

The collagenase activity and islet isolation data presented here demonstrate that our standard enzyme formulation calls for excessive use of tissue dissociative enzymes. This is noteworthy because our protocol already consumes considerably less enzyme than reported by other islet isolation laboratories for both human and porcine pancreas. Although we would expect decreased islet yield with continued reductions in perfused enzyme activity, we have not yet crossed that threshold as the lowest enzyme and perfusion volume group performed as well as any other group across all measured outcomes. Regardless, it is clear from the work presented here that current islet isolation protocols needlessly overload enzyme into the pancreas and that this excess enzyme goes to waste.

The effect of perfusion volume was initially evaluated through experiments to delineate enzyme infiltration into the tissue during perfusion. Using margin-marking dye as a surrogate, we were able to visualize the effect of differing perfusion volumes on distension of the pancreas and to quantify the tissue area accessible by the dissociative enzymes. Perfusion of black margin marking dye at 0.5 × and 1 × volumes demonstrated that the larger volume did not significantly increase the area of the pancreas reached by the perfusate. This result is remarkable given that the 1 × perfusion volume visibly and dramatically increases distension of the organ relative to a 0.5 × volume perfused pancreas.

To expand upon these results, blue and yellow dye were sequentially perfused into the splenic pancreas to mimic the 0.5 × and 1 × perfusion volumes, respectively. It is clear that the 1 × volume penetrates areas of the organ that are not accessed in the 0.5 × group as evidenced by the presence of solely yellow-stained tissue. However, it is notable that yellow staining appears almost exclusively adjacent to and in contact with blue/green areas of the tissue. These dye perfusion results may at least partially explain the maintenance of islet yield when reducing enzyme concentration and perfusion volume; the data demonstrate that the initial 0.5 × perfusion volume fully penetrates the accessible areas of the pancreas and additional volume may serve only to further distend already perfused tissue. That is, although the 1 × volume may force perfusion solution into already distended tissue, it is not opening new areas that are not at least partially infiltrated by perfusion, and thus enzyme, at 0.5 × volume. These results also provide a possible explanation for maintenance of

FIGURE 2. In vitro insulin production by porcine islet macrobeads. Data is presented as milliunits per macrobead per 24 hours (mU/MB per 24 h) for each experimental group (1 ×/1 ×, 0.5 ×/1 ×, 0.5 ×/0.5 ×, 0.33 ×/0.5 ×, n = 5; 1 ×/0.5 ×, n = 3). Statistical analysis using total area under the curve showed no differences between groups.

FIGURE 3. Collagenase and neutral protease stability in nonperfused enzyme samples. Columns represent the mean activity ± standard deviation for 3 independent samples at each sample time point (in minutes) relative to the baseline activity measured at T(−5). *P ≤ 0.005 relative to baseline.
TABLE 1.
Effect of varying enzyme activity and perfusion volume on porcine islet isolation outcomes

| Enzyme/Volume* | 1×/1× (n = 5) | 0.5×/1× (n = 5) | 0.5×/0.5× (n = 7) | 0.33×/0.5× (n = 6) | 1×/0.5× (n = 3) |
|---------------|---------------|----------------|------------------|-----------------|---------------|
| Pancreas weight, g | 269.8 ± 45.6 | 232.5 ± 30.6 | 218.3 ± 30.9 | 272.2 ± 35.4 | 256.7 ± 25.9 |
| Digestion time, min | 45.10 ± 0.65 | 51.66 ± 2.62 | 52.62 ± 1.08 | 60.25 ± 0.99 | 39.60 ± 3.38 |
| Pancreas digested, % | 47 ± 11 | 41 ± 6 | 48 ± 8 | 48 ± 7 | 52 ± 9 |
| IEQ/g starting pancreas weight | 1080 ± 512 | 892 ± 451 | 1350 ± 285 | 1088 ± 299 | 1111 ± 249 |
| Retained activity (retained volume) | 11 998 ± 5954 (79.0 ± 16.7) | 11 405 ± 6152 (49.0 ± 8.7) | 5977 ± 4135 (77.0 ± 13.5) | 13 224 ± 6230 (43.0 ± 6.2) | 8811 ± 2633 (35.7 ± 7.2) |
| Chamber activity (diffused volume) | 36 213 ± 4126 (142.7 ± 11.0) | 13 224 ± 6230 (43.0 ± 6.2) | 8811 ± 2633 (35.7 ± 7.2) | 8811 ± 2633 (35.7 ± 7.2) | 8811 ± 2633 (35.7 ± 7.2) |
| Perfusate activity (starting volume) | 54 232 ± 2309 (247.7 ± 28.9) | 25 232 ± 3,379 (94.7 ± 3.1) | 16 077 ± 2251 (122.3 ± 22.1) | 16 077 ± 2251 (122.3 ± 22.1) | 16 077 ± 2251 (122.3 ± 22.1) |

*1×/1× refers to the standard condition of 7.5 WU/g plus 12 000 NPU/g ("Enzyme") in 1.7 mL/g perfusion volume ("Volume"). All other groups are multipliers of these values as designated in column headings.

islet isolation success with reduced enzyme activity. It may be that using enzyme activity as low as 1/3 of our standard quantity (ie, 2.5 WU/g collagenase and 3000 NPU/g BP Protease), provides enough activity to saturate the perfused tissue and that delivery of additional enzyme does not provide measurable benefit.

Evaluation of collagenase retention within the pancreas during digestion also supports these interpretations of the dye perfusion and islet isolation experiments. Based on successfully using reduced enzyme and perfusion media in the islet isolation process, collagenase functional studies were restricted to the 2 experimental groups using the lowest quantities of enzyme and were compared to the control formulation. Although no significant differences in collagenase retention were found amongst the groups, it is worth noting that the average “Retained Activity” was identical between the control and 0.5×/0.5× groups despite twice as much collagenase activity being injected into the control pancreases. Our results are also in line with the findings of Friberg et al.12 in that the majority of collagenase activity remained in solution throughout the experiment with little absorption by the tissue. In our case, tissue retention ranged from 22% to 45%, consistent with the 35% retention reported for human pancreas by Friberg et al., despite the differences in perfusion and digestion protocols. Taken together, these data support the view that the areas of the pancreas that are accessible via ductal perfusion are saturated with enzyme early in the perfusion process and that additional enzyme, perfusion volume, or recirculation does little to improve isolation outcomes.

Dufrane et al17 have performed porcine islet isolation using both the Ricordi method and a static digestion (O’Neil) method similar to ours, reporting that the static and Ricordi methods provide similar outcomes in their evaluation. In addition, a recent report by Balamurugan et al18 has demonstrated that enzyme activity levels can be reduced by 50% to 60% using standard human islet techniques with “no significant main effect of Wunsch activity or CDA for any parameter measured.” Although significant differences exist between our static digestion methodology and the Ricordi method that is standard practice in human islet isolation laboratories, these findings along with the data presented here suggest that the static digestion method and/or reduced enzyme activity levels merit further evaluation for use with human pancreas.

An important corollary of these data is the potential for significant reductions in the costs of islet isolation. With current protocols calling for the use of highly purified, bovine neural tissue-free enzymes to maximize patient safety, the cost for enzyme alone can reach several thousand dollars per pancreas. The fact that many organs fail to provide suitable islets for transplantation greatly exacerbates this cost. Although islet transplantation has high short-term costs, health-economic analysis modeling suggests that islet transplantation provides superior long-term outcomes in both quality of life gains and overall expense when compared with traditional insulin therapy.19,20 The delayed financial benefit of islet transplantation is primarily due to its initial costs, which include medical procedures and follow-up, immunosuppression medications, organ procurement, and islet isolation, processing, and quality control. In addition to potentially limiting patient access, the high cost of dissociative enzymes may also delay or prevent important research progress due to laboratory budget constraints.

We have shown that, under the conditions of this study, the amount of enzyme required per porcine pancreas can be reduced by as much as 67% without detriment to islet yield, quality, or function. This change in methodology promises to substantially reduce the cost of porcine islet isolation as the field progresses toward clinical application. Laboratory

TABLE 2.
Effect of varying activity and perfusion volume on collagenase retention within the pancreas

| Enzyme/Volume* | 1×/1× (n = 3) | 0.5×/0.5× (n = 3) | 0.33×/0.5× (n = 3) |
|---------------|---------------|----------------|----------------|
| Perfusate activity (starting volume) | 54 232 ± 2309 (247.7 ± 28.9) | 25 232 ± 3,379 (94.7 ± 3.1) | 16 077 ± 2251 (122.3 ± 22.1) |
| Leaked activity (leaked volume) | 6021± 688 (26.0 ± 3.6) | 604 ± 360 (2.7 ± 1.5) | 1289 ± 990 (8.7 ± 8.0) |
| Chamber activity (diffused volume) | 36 213 ± 4126 (142.7 ± 11.0) | 13 224 ± 6230 (43.0 ± 6.2) | 8811 ± 2633 (35.7 ± 7.2) |
| Retained activity (retained volume) | 11 998 ± 5954 (79.0 ± 16.7) | 11 405 ± 6152 (49.0 ± 8.7) | 5977 ± 4135 (77.0 ± 13.5) |

*See footnotes for Table 1. Activity was calculated as “CDA U/g pancreas” for each sample. Volumes are presented in mL.
research with porcine pancreatic islets can also be expected to benefit, given that more islet isolations can be performed within the same research budget while, at the same time, additional research groups may now be able to undertake such studies. Although the current work focuses exclusively on porcine pancreas, this methodology may be adaptable for use in human pancreas and could shift the cost-benefit analysis further in favor of islet transplantation over traditional insulin treatment for select patients with type 1 diabetes.

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