Published in final edited form as:

Transplantation. 2012 April 15; 93(7): 693–702. doi:10.1097/TP.0b013e318247281b.

A new enzyme mixture to increase the yield and transplant rate of autologous and allogeneic human islet products

A.N. Balamurugan^{1,2,*}, Gopalakrishnan Loganathan^{1,2}, Mellina, D. Bellin^{1,3}, Joshua J. Wilhelm^{1,2}, James Harmon², Takayuki Anazawa^{1,2}, Sajjad M. Soltani^{1,2}, David M. Radosevich², Takeshi Yuasa^{1,2}, Mukesh Tiwari^{1,2}, Klearchos K. Papas^{1,2}, Robert McCarthy⁴, David E.R. Sutherland^{1,2}, and Bernhard J. Hering^{1,2}

¹Schulze Diabetes Institute, University of Minnesota, 420 Delaware Street SE, Minneapolis, MN, 55455, USA

²Department of Surgery, University of Minnesota, Minneapolis, MN, USA

³Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA

⁴VitaCyte LLC, 1102 Indiana Avenue, Indianapolis, IN 46202, USA

Abstract

Background—The optimal enzyme blend which maximizes human islets yield for transplantation remains to be determined. In this study, we evaluated 8 different enzyme combinations (ECs) in an attempt to improve islet yield. The ECs consisted of purified, intact, or truncated class 1 (C1) and class 2 (C2) collagenases from *Clostridium histolyticum* (Ch) as well as neutral protease (NP) from *Bacillus thermoproteolyticus rokko* (thermolysin) or Ch (ChNP).

Methods—We report the results of 249 human islet isolations, including 99 deceased donors (research $n=57$, clinical $n=42$) and 150 chronic pancreatitis pancreases. We prepared a new enzyme mixture (NEM) composed of intact C1 and C2 collagenases and ChNP instead of using thermolysin. The NEM was first tested in split pancreas ($n=5$) experiments and then used for islet autologous ($n=21$) and allogeneic transplantation ($n=10$). Islet isolation outcomes from 8 different Ecs were statistically compared using multivariate analysis.

Results—The NEM consistently achieved higher islet yields from pancreatitis ($p<0.003$) and deceased donor pancreases ($p<0.001$) than other standard ECs. Using the NEM, islet products met release criteria for transplantation from 8 of 10 consecutive pancreases, averaging 6510 ± 2150 IEQ/g pancreas and $694,681\pm147,356$ total IEQ/transplantation. In autologous isolation, the NEM yielded $>200,000$ IEQ from 19 of 21 pancreases (averaging $422,893\pm181,329$ total IEQ and 5979 ± 1469 IEQ/kg recipient body weight) regardless of the severity of fibrosis.

*Corresponding author: A. N. Balamurugan, PhD, Assistant Professor of Surgery, Schulze Diabetes Institute, Dept. of Surgery, University of Minnesota, Minneapolis, MN 55455, Tel: 651-253-0656, Fax: 612-626-5855, bala@umn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Contribution:

Authors A.N. Balamurugan, G. Loganathan, M.D. Bellin, J.J. Wilhelm, J. Harmon, T. Anazawa, S.M. Soltani, D.M. Radosevich, T. Yuasa, M. Tiwari, K.K. Papas, R. C. McCarthy, D.E.R. Sutherland, and B.J. Hering participated in design and performance of research and data analysis

Authors A.N. Balamurugan, G. Loganathan, and S.M. Soltani contributed to writing of the paper.

Potential Conflict of Interest:

Authors McCarthy has a financial interest in VitaCyte LLC. All other authors have no potential conflicts of interest.

Conclusions—A new enzyme mixture composed of *Clostridium histolyticum* neutral protease with Clzyme high intact C1 collagenase recovers higher islet yield from deceased and pancreatitis pancreases while retaining islet quality and function.

Keywords

Islet isolation; human islets; autotransplantation; pancreatitis; collagenase; neutral protease

Introduction

Clinical islet cell transplantation is a promising therapy for restoring normoglycemia and insulin independence in patients with type-1 diabetes (1–7). Although a human pancreas contains an estimated one to two-million islets, it is difficult for islet-processing laboratories to consistently isolate adequate quantities of islets from donor pancreases to achieve optimal numbers for transplantation (8, 9). Two major factors that affect human islet yields are donor organ characteristics and tissue dissociation enzymes used for islet isolation (8–10). Determining suitable enzyme blends—specifically the combination, concentration, and optimal ratio of collagenases and proteases—is critical for obtaining proper islet release from the surrounding acinar tissue (11–13).

In 1994, a purified enzyme blend, Liberase-HI (L-HI, Roche Applied Science, Indianapolis, IN), was successfully introduced for human islet isolation (14, 15). L-HI was used worldwide for clinical islet isolations, including our center (13, 16–19), until 2007, when L-HI was withdrawn from the market because of safety concerns associated with the use of bovine brain-derived materials used in the *Ch* fermentation process.

After L-HI was withdrawn, clinical islet transplant programs were forced to quickly identify a replacement enzyme blend. We evaluated many different enzyme products which are listed in Table 1A and various enzyme combinations (ECs) containing different amounts of intact or truncated C1/C2 collagenases from *Ch* used in combination with NP from *Bacillus thermoproteolyticus rokko* (thermolysin) or NP from *Ch* (ChNP). During our study, we incorporated the biochemical analysis of the collagenase enzymes as part of our evaluation criteria. Assessment of purified collagenases by collagen-degrading activity (CDA) (20) and high performance liquid chromatography (HPLC) (17) has been useful to characterize the integrity of collagenase enzymes (17). Based on the biochemical characteristics of enzymes and observations of enzyme behavior during isolations, we blended intact C1/C2 collagenase from *Ch* and ChNP into a new enzyme mixture (NEM).

In this report, we summarize the results from 99 islet isolations from deceased organ donors, utilizing different combinations of intact or truncated C1/C2 collagenase with ChNP or thermolysin. We also summarize the results of 150 isolations from pancreases obtained from patients with chronic pancreatitis.

Results

Islet isolation with different ECs

Table-1A & B and Figure-1A summarize results from 94 human islet isolations using ECs A-F and NEM. ECs were tested by HPLC and collagen degradation assays to determine enzyme quality and activity (17). Results of ECs A-F compared with NEM demonstrated statistically significant differences for digestion time ($p=0.002$), digested islet equivalent number (IEQ, $p=0.009$), digested IEQ/g pancreas ($p=0.017$), post-purification total IEQ ($p<0.001$), and post-purification IEQ/g pancreas ($p<0.001$). Mean post-purification IEQ/g pancreas achieved with NEM was statistically higher than EC-A ($p<0.001$), EC-B

($p=0.004$), EC-C ($p=0.003$), EC-D ($p=0.039$), and EC-F ($p=0.034$). Islet yields were higher with NEM than with EC-E and EC-G, but not statistically significant (Table 1C). Apart from total IEQ and IEQ/g pancreas, there were no significant differences among the donor characteristics between different groups.

Split pancreas experiment

Since the donor characteristics were same for both groups, many isolation characteristics (example, CIT, pancreas weight, digestion time, dilution time, digest count, digest volume) were not statistically significant (Table 1C). However, the NEM produced a significantly higher average total IEQ ($219,003 \pm 45,298$ vs $151,153 \pm 19,042$; $p=0.047$) and a significantly higher average percentage of islets whose diameter was >200 microns (19.7 ± 4.7 vs $8.3 \pm 2.5\%$, $p=0.021$) when compared to EC-F. Digested IEQ/g pancreas (3517 ± 969) and post-purification IEQ/g pancreas (4634 ± 1101) were also higher with NEM relative to EC-F (2840 ± 1532 IEQ/g and 3152 ± 1061 IEQ/g, respectively); however, these results were not statistically significant due to the small number of experiments performed (Table-1C). Although islet morphology score was not significant between two groups, NEM produced many solid intact islets ($>200 \mu$) because of the presence of neutral protease when compared to thermolysin in EC-F group.

Allotransplant outcome

On average, donor age was 45 ± 4.7 years with a BMI of 29.2 ± 3.6 kg/m², CIT was 6.5 ± 1.5 hours, and trimmed pancreas weight was 103 ± 29 g in the NEM group. Isolation results are contrasted with those using EC-A and EC-F (Table-2A). Eight of 10 isolations (80%) performed using the NEM achieved yields sufficient for islet allotransplantation compared to only 3 of 13 (23%) from EC-A and 7 of 19 (37%) from EC-F. Average islet yield and IEQ/g pancreas were significantly higher among successful islet isolations performed with the NEM ($694,681 \pm 147,356$ IEQ and 6510 ± 2150 IEQ/g) compared to EC-A ($516,796 \pm 101,309$ IEQ and 4635 ± 1310 IEQ/g; $p=0.003$) and EC-F ($454,789 \pm 101,742$ IEQ and 4716 ± 2070 IEQ/g; $p=0.003$). Although, there was a significant difference in terms of donor age, applying the Edmonton scoring system to all pancreases revealed no difference in organ quality amongst the various EC-groups.

Transplant outcomes are summarized in Table-2B. All recipients had a history of severe hypoglycemia in the year preceding their first islet infusion, which has not recurred post-transplant in patients who have maintained graft function. Recipients 1, 2, and 3 received islets prepared using EC-A; however, recipient 3 also received a second islet infusion from EC-F. Recipients 4–8 received islets isolated from EC-F; however, recipients 7 and 8 received islets isolated from NEM as second islet infusions. Among the 6 islet transplant recipients receiving only islets isolated with the NEM (recipients 9–14), 1 was able to completely reduce their need for exogenous insulin while 3 others were able to reduce their insulin requirement by over 70%. All patients, with the exception of recipient 14, exhibited adequate basal and stimulated C-peptide levels similar to patients in other enzyme groups. Graft failure was observed in recipient 14 on day after choosing to withdraw from immunosuppression. The average beta-score on day 75 was similar between the NEM (5.7 ± 1.5), EC-A (5.7 ± 0.5), and EC-F (5.0 ± 2.0) groups. After a second islet infusion, recipients 3, 7 and 8 increased their initial beta-score.

Islet autotransplantation outcome

Table-3 summarizes the islet isolation outcomes from autologous islet preparations using 3 different ECs. In a global statistical test, there is evidence for a statistically significant difference across the groups for total IEQ/g pancreas weight ($p=0.003$). In post hoc follow-up testing, the average total IEQ ($422,893 \pm 181,329$) and IEQ/g pancreas weight

(5327±2450) in the NEM group was statistically greater than with enzyme EC-A (230,305±111,094 IEQ and 3751±1864 IEQ/g; $p=0.006$), EC-B (193,585±128,002 IEQ and 3511±3105 IEQ/g; $p=0.008$). Notably, 19 of 21 isolations performed with the NEM yielded >200,000 IEQ/pancreas (Fig. 1B).

After transplantation, 9 patients achieved insulin independence, 4 had partial function (required lower daily doses of insulin), and 8 were insulin dependent (required a multiple daily injection regimen). Where known, HbA_{1c} levels in insulin independent recipients and those with partial function ranged from 5.1–6.2% (≥3 months post-transplant). HbA_{1c} levels were more variable in the fully insulin dependent recipients, ranging from 5.7–12%. However, all patients tested were C-peptide positive (>0.3 ng/mL), including those patients using multiple injections per day. Insulin independence rates following autologous isolations with the NEM mixture were comparable to insulin independence rates with EC-A and EC-B (Table-3). Overall, 42% of patients in the NEM group (9/21) achieved insulin independence within the first year post-transplant, compared to 36% (28/78) with EC-A and 34% (16/47) with EC-B ($p=0.78$) (Table-3).

Islet quality control

The quality control data for various ECs are summarized in Table-1B. The average viability of pure and impure islets in the NEM group was 95±0.5 and 91±0.5%, respectively. The average stimulation index calculated from GSIR assay was 3.0±1.3, and the average OCR/DNA for pure and impure islets was 118±27 and 91±16, respectively.

When NEM isolated islets were transplanted into diabetic nude mice ($n=24$), diabetes was reversed in 14 mice, diabetes failed in 7 mice, partial function was observed in 1 mouse, and 2 mice died within a few days after transplantation. Isolated islets from autologous preparations obtained using the NEM showed an average islet viability of 91.3±4.7% ($n=21$) (Table-3) and 115±19 OCR/DNA ($n=19$).

Discussion

Effective and cost-efficient islet transplant treatment requires a high percentage of successful islet isolations from a wide spectrum of donor pancreases (4, 21). To date, obtaining sufficient quantities of intact islets for transplantation is typically achieved from fewer than 45% of human donor pancreases (18, 22). The two major factors that affect human islet yields are donor and enzyme characteristics (8). Results from this study describe a new enzyme formulation that provides higher islet yields from human pancreases for clinical transplantation.

The high number of isolations performed reflects the difficulty of replacing L-HI after it became unavailable for clinical use in 2007 (23, 24). After the withdrawal of L-HI, three enzyme suppliers developed alternate enzyme products for islet isolation in an attempt to re-establish a suitable enzyme blend that consistently produces sufficient numbers of quality islets for transplantation. In 2007, NB-1 and NB enzymes (EC-A and EC-B) were the only available purified enzymes. Despite early reports describing the ability of EC-A and EC-B to produce similar islet yields to those achieved with L-HI (25, 26), many researchers found it difficult to reproduce these results. Previously, we reported that a higher proportion of intact-C1 with a molecular weight of 116 kDa (C1_{116kDa}) was found in L-HI when compared to EC-A or EC-B (17). Szot et al., however, were able to obtain high islet yield in selected donors (27).

Careful analysis of islet isolation results from ECs A-G prompted us to combine VitaCyte-CIzyme™ Collagenase-HA with ChNP in the NEM. In the presence of the same C1/C2

enzymes, islet morphology varied distinctly between islets released by ChNP and those released by thermolysin. Intact-C1 collagenase from EC-F with thermolysin produced large quantities of small islets, whereas islets isolated with EC-A or EC-B generated small quantities of larger, intact islets. We believed that, to obtain a higher quantity of large, intact islets, a NEM composed of ChNP and purified collagenase containing a high proportion of intact-C1_{116kDa}/C2 could generate islet yields superior to those obtained using predominantly truncated C1_{100kDa}, C2, and ChNP (EC-A and EC-B) or those containing C1_{116kDa}, C2, and thermolysin (EC-E, EC-F, and EC-G). The advantages of using the NEM for are remarkable; we observed a 2-fold increase in the proportion of islet isolations for clinical transplantation compared to those that used other enzymes (EC-A, EC-B, EC-F). Similarly, the results from 21 islet isolations performed for autotransplant procedures were superior to those obtained earlier using EC-A, or EC-B (Table-3). Results from 19 of 21 islet isolations achieved yields >200,000 IEQ per organ, a result considered critical for a high percentage of patients to achieve insulin independence (28).

An interesting observation is the value of using ChNP in place of thermolysin in the NEM. Direct comparison of these two proteases in split pancreas experiments showed that the NEM gave higher digested IEQ/organ, digested IEQ/g pancreas, post-purification IEQ/organ, and post-purification IEQ/g pancreas than obtained with thermolysin in EC-F. The only statistically significant differences, however, were noticed in post-purification IEQ and the percentage of islets >200 μ m. The reason for this difference in performance is not known since thermolysin and ChNP share similar selectivity for degrading defined peptides and both have the HEXXH motif in their catalytic site. Crystal structures have been reported for thermolysin in the MEROPS database (29), and the high degree of sequence homology among these enzymes suggests that both proteases contain one functional catalytic domain with a single zinc atom in the active site. These enzymes do differ in their reaction rates, however, as purified thermolysin degrades protein faster than ChNP (30).

Clinical outcomes from NEM isolated islets do not show significant differences as assessed by beta-score. Considering the multitude of recipient related factors (weight, pretransplant insulin requirements, type of immunosuppression and induction), our preliminary clinical data may indicate that insulin independence alone is not a good variable for comparison of NEM isolated islets. Our assessment of beta-score, which examines posttransplant C-peptide, HbA1c, and fasting plasma glucose levels along with insulin use, better demonstrates that transplantation outcomes achieved by the NEM are approximately equal to those of other clinical islet isolation enzymes.

One limitation of our study is the lack of a randomization scheme to assign ECs to a particular pancreas. Though a randomized study design would be a more meaningful comparison, our data suggests that the NEM has an edge over other EC's to achieve successful human islet isolations.

This is the first study comparing the effects of ChNP or thermolysin in combination with various forms of collagenases on islet release. We clearly confirmed the importance of intact-C1/C2 collagenase (8, 12, 17) and ChNP for the release of intact islets when compared to the use of truncated C1, C2, and ChNP or intact-C1, C2, and thermolysin. We conclude that human islet isolations performed with the NEM are more productive than any other EC at improving the islet transplantation rate.

Methods

This study was conducted with informed consent using protocols reviewed and approved by the University of Minnesota Institutional Review Board.

Evaluated ECs

The enzyme combinations (collagenases and proteases) evaluated in an attempt to improve islet yield are summarized in Tables-1A.

Biochemical analysis of the purified SERVA NB-1 collagenase by analytical anion exchange HPLC and/or calculation of a specific collagen degradation activity have consistently shown that these products contain primarily truncated C1 collagenase (C1_{100kDa}) and C2 collagenase (17). This molecular form has lost one collagen binding domain that is likely removed by proteolytic cleavage of the intact-C1 (C1_{116kDa}) during anaerobic fermentation of *Ch* or during the enzyme purification process. In contrast, VitaCyte-Clzyme™ Collagenase-HA enzyme and Roche-MTF collagenase contain predominantly C1/C2 collagenase. To simplify the presentation of the data, results obtained using either SERVA collagenase product will be termed truncated-C1 collagenase, whereas those results generated with the VitaCyte-Clzyme™ Collagenase-HA (HA) or Roche-MTF product will be termed intact-C1 collagenase. For purposes of clarification, this definition reflects differences in C1 not C2. Current analytical HPLC techniques do not completely resolve intact from degraded C2 in purified collagenase products. HPLC and CDA assays of various enzymes and combinations were performed as described previously (17, 20).

Human islet isolation

In compliance with federal regulations, islet isolations were performed from deceased donors (research/clinical) ($n=94$), split pancreas digestion ($n=5$), and chronic pancreatitis ($n=150$) pancreases. On arrival at the laboratory, the pancreas was trimmed, cannulated, and distended with tissue dissociation enzymes of various combinations. After ductal perfusion of the enzymes, the pancreas was digested using a modified Ricordi's semi-automated method (31). The digested tissue was then purified by continuous iodixanol (OptiPrep™, Axis-Shield, Oslo, Norway) density gradient on a COBE-2991 cell processor.

Clinical pancreases were accepted following standard organ acceptance criteria and the Edmonton pancreas donor scoring algorithm was applied to each donor (32). In clinical allograft isolations, our University of Minnesota isolation protocol (17) was used for all isolations performed with EC-A ($n=13$) and EC-F ($n=19$). For clinical isolations performed with the NEM, 2 were performed with this protocol while the remaining 8 isolations were performed with the Clinical Islet Transplantation (CIT) Consortium islet isolation protocol. In all cases of allotransplantation, the liberated islets were first cultured in CMRL-1066 supplemented medium (Mediatech, Inc, Manassas, VA) for 36–72h before being transplanted.

Autologous islet isolations were performed following total pancreatectomy as described (33–35). The isolated islets were transplanted immediately after isolation.

Split pancreas digestions ($n=5$) were performed on research pancreases to study the potency of the NEM compared to EC-F (Table-1C). Each pancreas was split into two lobes, head/body and body/tail, and each portion was digested with either intact collagenase and ChNP (NEM) or intact collagenase and thermolysin (EC-F). Digestions were performed sequentially and each lobe received alternating enzyme treatments to reduce intra-pancreatic variability.

NEM Preparation

Islet isolations were performed by preparing the NEM to contain a total enzyme activity ranging from 1567–2686 Wunsch units (WU) of collagenase and 118–186 dimethyl-casein

units (DMC-U) of NP. This was done by mixing enzymes to deliver 18–20 WU/g of collagenase and 1.25–1.5 DMC U/g of NP.

Clinical allotransplantation

Pancreases from deceased donors aged 24–48yrs and a cold ischemic time of 4–8.7h were used for isolation. Isolated islets were cultured for 36–72-h prior to transplantation (17, 36). Isolation results with the NEM were compared with results from earlier clinical isolations performed using EC-A ($n=13$) and EC-F ($n=19$) (Table-2A). Portal vein access was obtained via mini-laparotomy, and the islet preparation was infused intraportally (19).

Allotransplantation recipients

All participants had a history of type-1 diabetes complicated by severe hypoglycemic episodes and reduced awareness of hypoglycemia (Clark hypoglycemia unawareness score ≥ 4 of 7), despite intensive diabetes management. Islets isolated with the NEM were transplanted into 8 recipients ($n=6$ first infusion, $n=2$ second infusion). Recipients were enrolled in one of four transplant protocols differing in immunosuppression therapy. For immunosuppression induction, 3 patients received anti-thymocyte globulin alone, 3 received anti-thymocyte globulin with deoxyspergualin, 1 received monoclonal anti-CD3 antibody and 1 received basiliximab. Maintenance of immunosuppression consisted of low-dose tacrolimus with either sirolimus or mycophenolic acid/mycophenolate mofetil.

Patients were seen at regular intervals for the first 12 months post-transplant, with periodic metabolic assessments including HbA_{1c}, fasting glucose, and fasting and stimulated C-peptide levels. To evaluate the clinical outcome of the transplantation for the three EC groups we looked at beta-score (on day-75) as defined by C-peptide (0 points if <0.3 ng/mL, 1 point if 0.31 – 0.89 ng/mL, 2 points if >0.9 ng/mL); HbA_{1c} (0 points if $>7\%$, 1 point if 6.2 – 6.9% , 2 points if $<6.1\%$); fasting plasma glucose (0 points if >126 mg/dL, 1 point if 100 – 125 mg/dL, 2 points if <99 mg/dL); and insulin use (0 points if >0.25 u/kg/day, 1 point if 0.01 – 0.24 u/kg/day, 2 points if insulin independent) (37).

Autologous islet transplantation

The NEM was used for 21 islet isolations and the results were compared with EC-A ($n=81$) and EC-B ($n=48$). (Table-3). Graft function was monitored by HbA_{1c}, fasting/stimulated glucose, and C-peptide levels (at 3, 6, and 12 months post-transplant) (38, 39). For data analysis, short-term (within 1 year after transplant) islet graft function was stratified as: *full* for insulin independent patients, *partial* for patients requiring insulin only intermittently or long-acting insulin once daily, or *graft failure* for patients on a basal-bolus (typical diabetic) insulin regimen (40).

Islet quality assessments

Islets were assessed for viability using fluorescent diacetate/propidium iodide (FDA/PI). In allotransplant preparations, glucose-stimulated insulin release (GSIR) (41), oxygen consumption rate/ DNA (OCR/DNA) (42), and nude mouse bio-assays were also performed as reported previously (43). In selected cases OCR/DNA was performed in autologous islet preparations.

Statistical analysis

The analysis is divided into islet isolations from the following: (1) research, (2) split pancreas, (3) clinical, and (4) autologous pancreas. Summary measures were either mean $\pm$ SD or percentages for donor characteristics, islet processing, and islet transplantation outcomes. Statistical comparisons were made across A to H enzyme combinations. Because

the sample sizes were relatively small for some of the enzyme groups, Kruskal-Wallis, a nonparametric statistic, was used to test for differences across the ECs. Edmonton scoring algorithm was used for pancreases used in clinical islet isolation to assign donor points based on assessment of donor and pancreas characteristics (32). A multivariate regression model was used to test for differences in enzyme preparations in the clinical pancreas group. Insulin independence rates after autologous transplantation in the NEM mixture group was compared to insulin independence with EC-A and EC-B using a Chi-Square analysis.

Acknowledgments

The authors would like to thank Dr. Brian Flanagan, Dr. Francis Dwulet, Janet Bricher, Renae Sutcliffe, Marnee Brandenburg, Muhammad Abdulla, Tom Gilmore, Jeff Ansite, Brian Perrault, Kate Mueller, Andrew Breite, and Jennifer Kitzmann for excellent support for the completion of this manuscript. We also thank NIH-Clinical Islet Transplantation Consortium manuscript review committee members for their critical review.

Funding Source:

This project was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK-R01 DK56963), the National Center for Research Resources (NCRR) *Human Pancreatic Islet Cell Resources (ICRs)*(U42 RR16598), the National Institute of Allergy and Infectious Diseases (NIAID) (AI-065193; NIH-Clinical Islet Transplantation Consortium grant), the Juvenile Diabetes Research Foundation grants (#31-2008-336, #4-2004-372 and #4-2008-386, #6-2008-1033), and the Schulze Family Foundation. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health, the Juvenile Diabetes Research Foundation, or the Schulze Family Foundation.

Abbreviations

Ch	<i>Clostridium histolyticum</i>
C1	Class 1 <i>Clostridium histolyticum</i> collagenase
C2	Class 2 <i>Clostridium histolyticum</i> collagenase
CDA	collagen-degrading activity
ChNP	<i>Clostridium histolyticum</i> neutral protease
C1_{116kDa}	intact C1 collagenase
C1_{100kDa}	truncated C1 collagenase
DMC U	dimethyl casein unit
ECs	enzyme combinations
FDA/PI	fluorescent diacetate/propidium iodide
GSIR	glucose-stimulated insulin release
HPLC	high performance liquid chromatography
IEQ	islet equivalent number
IC	islet count
L-HI	Liberase HI
MTF	mammalian tissue free
NEM	new enzyme mixture
OCR/DNA	oxygen consumption rate per DNA
TLA	tryptic-like activity
TSE	transmissible spongiform encephalopathy

WU

Wunsch units

References

1. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*. 2000; 343(4):230. [PubMed: 10911004]
2. Bertuzzi F, Marzorati S, Secchi A. Islet cell transplantation. *Curr Mol Med*. 2006; 6(4):369. [PubMed: 16900660]
3. Froud T, Ricordi C, Baidal DA, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. *Am J Transplant*. 2005; 5(8):2037. [PubMed: 15996257]
4. Bretzel RG, Brendel M, Eckhard M, et al. Islet transplantation: present clinical situation and future aspects. *Exp Clin Endocrinol Diabetes*. 2001; 109 Suppl 2:S384. [PubMed: 11460586]
5. Berney T, Toso C. Monitoring of the islet graft. *Diabetes Metab*. 2006; 32(5 Pt 2):503. [PubMed: 17130809]
6. Gangemi A, Salehi P, Hatipoglu B, et al. Islet transplantation for brittle type 1 diabetes: the UIC protocol. *Am J Transplant*. 2008; 8(6):1250. [PubMed: 18444920]
7. Benhamou PY, Oberholzer J, Toso C, et al. Human islet transplantation network for the treatment of Type I diabetes: first data from the Swiss-French GRAGIL consortium (1999–2000). *Groupe de Recherche Rhin Rhjne Alpes Geneve pour la transplantation d'Ilots de Langerhans. Diabetologia*. 2001; 44(7):859. [PubMed: 11508270]
8. Lakey JR, Burridge PW, Shapiro AM. Technical aspects of islet preparation and transplantation. *Transpl Int*. 2003; 16(9):613. [PubMed: 12928769]
9. Johnson PR, White SA, London NJ. Collagenase and human islet isolation. *Cell Transplant*. 1996; 5(4):437. [PubMed: 8800512]
10. Niclauss N, Bosco D, Morel P, et al. Influence of donor age on islet isolation and transplantation outcome. *Transplantation*. 91(3):360. [PubMed: 21344706]
11. Kin T, Johnson PR, Shapiro AM, Lakey JR. Factors influencing the collagenase digestion phase of human islet isolation. *Transplantation*. 2007; 83(1):7. [PubMed: 17220782]
12. Bertuzzi F, Cainarca S, Marzorati S, et al. Collagenase isoforms for pancreas digestion. *Cell Transplant*. 2009; 18(2):203. [PubMed: 19499708]
13. Antonioli B, Fermo I, Cainarca S, et al. Characterization of collagenase blend enzymes for human islet transplantation. *Transplantation*. 2007; 84(12):1568. [PubMed: 18165766]
14. Linetsky E, Bottino R, Lehmann R, Alejandro R, Inverardi L, Ricordi C. Improved human islet isolation using a new enzyme blend, liberase. *Diabetes*. 1997; 46(7):1120. [PubMed: 9200645]
15. Linetsky E, Lehmann R, Li H, et al. Human islet isolation using a new enzyme blend. *Transplant Proc*. 1997; 29(4):1957. [PubMed: 9193473]
16. Cross SE, Hughes SJ, Partridge CJ, Clark A, Gray DW, Johnson PR. Collagenase penetrates human pancreatic islets following standard intraductal administration. *Transplantation*. 2008; 86(7):907. [PubMed: 18852654]
17. Balamurugan AN, Breite AG, Anazawa T, et al. Successful human islet isolation and transplantation indicating the importance of class 1 collagenase and collagen degradation activity assay. *Transplantation*. 2010; 89(8):954. [PubMed: 20300051]
18. Kin T, Zhai X, Murdoch TB, Salam A, Shapiro AM, Lakey JR. Enhancing the success of human islet isolation through optimization and characterization of pancreas dissociation enzyme. *Am J Transplant*. 2007; 7(5):1233. [PubMed: 17359501]
19. Bellin MD, Kandaswamy R, Parkey J, et al. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *Am J Transplant*. 2008; 8(11):2463. [PubMed: 18808408]

20. McCarthy RC, Spurlin B, Wright MJ, et al. Development and characterization of a collagen degradation assay to assess purified collagenase used in islet isolation. *Transplant Proc.* 2008; 40(2):339. [PubMed: 18374061]
21. Mineo D, Pileggi A, Alejandro R, Ricordi C. Point: steady progress and current challenges in clinical islet transplantation. *Diabetes Care.* 2009; 32(8):1563. [PubMed: 19638527]
22. Balamurugan AN, Bottino R, Giannoukakis N, Smetanka C. Prospective and challenges of islet transplantation for the therapy of autoimmune diabetes. *Pancreas.* 2006; 32(3):231. [PubMed: 16628077]
23. Caballero-Corbalan J, Friberg AS, Brandhorst H, et al. Vitacyte collagenase HA: a novel enzyme blend for efficient human islet isolation. *Transplantation.* 2009; 88(12):1400. [PubMed: 20029339]
24. [accessed 30 August 2009]
http://www.celltherapysociety.org/files/PDF/Resources/Risk_BSE_in_Collagenase_Enzymes.pdf.
25. Bucher P, Mathe Z, Bosco D, et al. Serva collagenase NB1: a new enzyme preparation for human islet isolation. *Transplant Proc.* 2004; 36(4):1143. [PubMed: 15194398]
26. Bucher P, Bosco D, Mathe Z, et al. Optimization of neutral protease to collagenase activity ratio for islet of Langerhans isolation. *Transplant Proc.* 2004; 36(4):1145. [PubMed: 15194399]
27. Szot GL, Lee MR, Tavakol MM, et al. Successful clinical islet isolation using a GMP-manufactured collagenase and neutral protease. *Transplantation.* 2009; 88(6):753. [PubMed: 19920770]
28. Morrison CP, Wemyss-Holden SA, Dennison AR, Maddern GJ. Islet yield remains a problem in islet autotransplantation. *Arch Surg.* 2002; 137(1):80. [PubMed: 11772222]
29. [Accessed November 30] MEROPS database. <<http://merops.sanger.ac.uk/>>
30. Breite AG, Dwulet FE, McCarthy RC. Tissue dissociation enzyme neutral protease assessment. *Transplant Proc.* 2010; 42(6):2052. [PubMed: 20692405]
31. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. *Diabetes.* 1988; 37(4):413. [PubMed: 3288530]
32. O'Gorman D, Kin T, Murdoch T, et al. The standardization of pancreatic donors for islet isolation. *Transplant Proc.* 2005; 37(2):1309. [PubMed: 15848705]
33. Anazawa T, Balamurugan AN, Bellin M, et al. Human islet isolation for autologous transplantation: comparison of yield and function using SERVA/Nordmark versus Roche enzymes. *Am J Transplant.* 2009; 9(10):2383. [PubMed: 19663895]
34. Robertson GS, Dennison AR, Johnson PR, London NJ. A review of pancreatic islet autotransplantation. *Hepatogastroenterology.* 1998; 45(19):226. [PubMed: 9496519]
35. Rutherford Y, Fomufod AK, Gopalakrishnan LJ, Beeks EC. Traumatic distal femoral periostitis of the newborn: a breech delivery birth injury. *J Natl Med Assoc.* 1983; 75(10):933. [PubMed: 6644835]
36. Hering BJ, Kandaswamy R, Ansit JD, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA.* 2005; 293(7):830. [PubMed: 15713772]
37. Ryan EA, Paty BW, Senior PA, Lakey JR, Bigam D, Shapiro AM. Beta-score: an assessment of beta-cell function after islet transplantation. *Diabetes Care.* 2005; 28(2):343. [PubMed: 15677790]
38. Blondet JJ, Carlson AM, Kobayashi T, et al. The role of total pancreatectomy and islet autotransplantation for chronic pancreatitis. *Surg Clin North Am.* 2007; 87(6):1477. [PubMed: 18053843]
39. Sutherland DE, Gruessner AC, Carlson AM, et al. Islet autotransplant outcomes after total pancreatectomy: a contrast to islet allograft outcomes. *Transplantation.* 2008; 86(12):1799. [PubMed: 19104425]
40. Bellin MD, Blondet JJ, Beilman GJ, et al. Predicting islet yield in pediatric patients undergoing pancreatectomy and autoislet transplantation for chronic pancreatitis. *Pediatr Diabetes.* 2009
41. Balamurugan AN, He J, Guo F, et al. Harmful delayed effects of exogenous isolation enzymes on isolated human islets: relevance to clinical transplantation. *Am J Transplant.* 2005; 5(11):2671. [PubMed: 16212626]

42. Papas KK, Colton CK, Nelson RA, et al. Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice. *Am J Transplant*. 2007; 7(3):707. [PubMed: 17229069]
43. Ihm SH, Matsumoto I, Sawada T, et al. Effect of donor age on function of isolated human islets. *Diabetes*. 2006; 55(5):1361. [PubMed: 16644693]

1A Islet yield with different ECs

1B Islet yield and clinical outcome with different ECs in autologous islet preparations

Fig. 1.

A: Summarized total islet yield/pancreas with different ECs. Significantly higher islet yield was obtained in NEM group when compared with other ECs: $*p < 0.001$ NEM vs EC-A, $**p = 0.004$ NEM vs EC-B, $***p = 0.003$ NEM vs EC-D, $^+p = 0.039$ NEM vs EC-F and $^{++}p = 0.034$ NEM vs EC-F. Right side graph (insert) shows the result of split pancreas experiments. When NEM was compared with EC-F, the p value was $p = 0.047$. Note that the total IEQ is expressed for half-size pancreas. Insert: Percent success and failed clinical islet preparations in three enzyme groups.

B: More frequently greater than 200,000 IEQ (Islets) were obtained when using the NEM group in autologous islet isolations (except in 2 cases). When results were compared to other ECs (EC-A, and EC-B), statistically significant improvements were obtained: $*p < 0.0001$ NEM vs EC-A, and $**p < 0.0001$ NEM vs EC-B. Insert: percent insulin independence rate one year after transplantation. The islets were isolated with 3 EC's.

Table 1

A: Evaluated Enzyme Combinations									
EC	Product Name	n							
EC-A	SERVA Collagenase NB-1 GMP grade + SERVA Neutral Protease NB GMP grade (SERVA Electrophoresis GmbH, Heidelberg, Germany)	20							
EC-B	SERVA Collagenase NB-1 Premium grade + SERVA Neutral Protease NB Premium grade (SERVA Electrophoresis GmbH, Heidelberg, Germany)	5							
EC-C	SERVA Collagenase NB-1 GMP grade (SERVA Electrophoresis GmbH, Heidelberg, Germany) + Roche Thermolysin (Roche Applied Science, Indianapolis, IN)	5							
EC-D	SERVA Collagenase NB-1 GMP grade (high TLA) (SERVA Electrophoresis GmbH, Heidelberg, Germany)	4							
EC-E	Roche MTF Collagenase + Roche Thermolysin (Roche Applied Science, Indianapolis, IN)	4							
EC-F	VitaCyte Clzyme™ Collagenase HA + VitaCyte Clzyme™ Thermolysin (VitaCyte LLC, Indianapolis, IN)	37							
EC-G	VitaCyte Clzyme™ Collagenase HA (high CDA) + VitaCyte Clzyme™ Thermolysin (VitaCyte LLC, Indianapolis, IN)	3							
NEM	VitaCyte Clzyme™ Collagenase HA (VitaCyte LLC, Indianapolis, IN) + SERVA Neutral Protease NB (SERVA Electrophoresis GmbH, Heidelberg, Germany)	12							
B: Islet isolation outcomes using different enzyme combinations									
Enzyme combination-A		B	C	D	E	F	G	H (NEM)	P value
Number of Islet Isolations	24	5	5	4	4	37	3	12	
Donor Age (yr)	40.9 ± 12.4	48.4 ± 17.3	41.2 ± 21.4	35.0 ± 15.0	43.0 ± 5.7	40.2 ± 13.5	33.0 ± 25.2	41.4 ± 9.7	0.827
Body Mass Index (kg/m ²)	30.5 ± 4.6	29 ± 3.7	29.6 ± 5.6	31.3 ± 6.7	31.7 ± 7.0	30.4 ± 7.4	28.4 ± 5.8	30.4 ± 6.3	0.998
Cold Ischemia Time (hr)	8.4 ± 3.8	10.2 ± 1.9	10.0 ± 3.9	8.0 ± 3.4	10.0 ± 4.4	7.7 ± 4.2	9.8 ± 2.8	7.3 ± 2.2	0.22
Trimmed Pancreas Weight (g)	106.8 ± 23.5	100.7 ± 31.3	91.9 ± 11.4	99.7 ± 17.3	93.9 ± 16.1	102.7 ± 25.8	122.6 ± 11.5	106.4 ± 26.7	0.651
Digestion Time (min)	23.6 ± 5.5	22.6 ± 3.4	26.0 ± 3.5	16.0 ± 4.5	20.7 ± 2.1	21.2 ± 4.2	21.5 ± 21.5	16.3 ± 5.5	0.002
Digested Pancreas Weight (%)	79.1 ± 11.8	75.5 ± 11.1	65.2 ± 28.0	68.1 ± 17.2	80.4 ± 9.6	81.2 ± 9.1	73.1 ± 37.5	81.4 ± 9.1	0.479
Digested IEQ	342,964 ± 196,956	250,965 ± 112,073	309,672 ± 235,788	427,995 ± 118,285	305,379 ± 211,200	405,235 ± 160,995	401,439 ± 286,997	685,722 ± 290,290	0.009
Digested IEQ/g pancreas	3,281 ± 1,900	2,551 ± 899	3,477 ± 2,749	4,430 ± 1,026	3,134 ± 2,040	4,087 ± 1,591	3,155 ± 2,000	6,672 ± 3,049	0.017
Digested Tissue Volume (ml)	27.8 ± 9.7	24.8 ± 20.0	20.4 ± 9.7	23.3 ± 13.5	28.0 ± 12.4	31.2 ± 12.3	24.7 ± 15.5	36.9 ± 8.0	0.077
Post Purification IEQ	231,952 ± 145,844	161,641 ± 145,844	146,605 ± 95,676	315,599 ± 77,064	214,276 ± 148,727	338,623 ± 147,061	272,766 ± 187,426	549,938 ± 246,131	<.001
Post Purification IEQ/g pancreas	2,202 ± 1,403	1,635 ± 726	1,603 ± 965	3,223 ± 855	2,217 ± 1,405	3,467 ± 1,698	2,147 ± 1,299	5,329 ± 2,519	<.001
Recovery (%)	67.0 ± 36.9	56.9 ± 29.1	58.5 ± 29.2	74.1 ± 4.8	88.8 ± 45.3	90.6 ± 47.4	68.8 ± 1.9	81.1 ± 29.3	0.091

B: Islet isolation outcomes using different enzyme combinations

	Enzyme combination-A	B	C	D	E	F	G	H (NEM)	P value
Post Purification Purity (%)	74.1 ± 19.0	71.3 ± 21.7	60.0 ± 20.9	75.0 ± 7.1	80.0 ± 5.0	76.2 ± 10.5	83.3 ± 7.6	84.7 ± 12.4	0.281
Pure Islet Viability (FDA/PI)	99 ± 0.1 (n=18)	99.5 ± 0.5 (n=5)	99.5 ± 0.5 (n=2)	99 ± 0.1 (n=3)	99.6 ± 0.1 (n=2)	97 ± 0.4 (n=33)	99 ± 0.1 (n=3)	95 ± 0.5 (n=11)	-
Impure Islet Viability (FDA/PI)	99 ± 0.1 (n=6)	99 ± 1.0 (n=2)	NA	98 (n=1)	99.4 ± 0.1 (n=2)	97 ± 0.5 (n=16)	97 ± 0.2 (n=2)	91 ± 0.5 (n=9)	-
Pure Islet (GSIR)	2.5 ± 2.0 (n=10)	1 (n=1)	NA	2.0 ± 0.5 (n=2)	3.4 ± 2.3 (n=2)	2.4 ± 1.1 (23)	3.0 ± 0.3 (n=2)	3.0 ± 1.3 (n=7)	-
Pure Islet (OCR/DNA)	134 ± 47 (n=4)	121 ± 43 (n=4)	104 ± 03 (n=2)	117 ± 21 (n=4)	132 ± 24 (n=2)	114 ± 21 (n=35)	114 ± 21 (n=2)	118 ± 27 (n=11)	-
Impure Islet (OCR/DNA)	117 ± 37 (n=2)	81 ± 3.0 (n=2)	NA	99 ± 6.0 (n=2)	122 (n=1)	91.8 ± 27 (n=17)	83 ± 42 (n=2)	91 ± 16 (n=9)	-
Nude Mice Transplantation (cured/total transplanted)	13 of 17	2 of 2	NA	2 of 6	2 of 4	32 of 53	5 of 5	14 of 22	-

C: Islet isolation outcomes from split pancreas experiments (Thermolysin vs Neutral protease)

	Intact Collagenase + Thermolysin EC-F	Intact Collagenase + NP NEM	P value
Pancreas (n = 5)			
Head-body	2 (40.0%)	3 (60.0%)	
Body-tail	3 (60.0%)	2 (40.0%)	
Donor age (yr)	49.0 ± 10.2		
BMI (kg/m ²)	30.9 ± 3.3		
Cold ischemia time (hr)	11.0 ± 4.6	11.1 ± 4.0	1.000
Trimmed pancreas weight (g)	52.2 ± 18.2	47.7 ± 5.9	0.919
Digestion time (min)	16.2 ± 1.3	17.0 ± 3.4	0.836
Dilution time (min)	22.6 ± 5.0	22.6 ± 4.5	1.000
Digested pancreas weight (%)	77.1 ± 11.1	70.3 ± 22.5	0.906
Digested IC	173,800 ± 79,860	177,400 ± 65,316	0.839
Digested IEQ	137,734 ± 70,644	166,072 ± 41,946	0.425
Digested IEQ/g pancreas	2840 ± 1532	3517 ± 969	0.546
Digested tissue volume (ml)	14.6 ± 5.9	15.1 ± 5.0	0.684
Post-purification IC	183,750 ± 41,252	172,125 ± 52,281	0.889
Post-purification IEQ	151,153 ± 19,042	219,003 ± 45,298	0.047
Post-purification IEQ/g pancreas	3152 ± 1061	4634 ± 1101	0.129

C: Islet isolation outcomes from split pancreas experiments (Thermolysin vs Neutral protease)

	Intact Collagenase + Thermolysin EC-F	Intact Collagenase + NP NEM	P value
Post-purification purity (%)	75.8 ± 9.3	65.6 ± 11.1	0.272
Percent of islets > 200 micron size	8.3 ± 2.5	19.7 ± 4.7	0.021
Morphology score (1–10)	9.1 ± 0.9	8.7 ± 1.1	0.513

EC, enzyme combination; GMP, good manufacturing practice; TLA, tryptic like activity; MTF, mammalian tissue free; CDA, collagen degradation activity; NEM, new enzyme mixture

NEM, new enzyme mixture; IEQ, islet equivalents; FDA/PI, fluorescent diacetate/propidium iodide; GSIR, glucose stimulated insulin release (Stimulation Index); OCR/DNA, oxygen consumption rate/DNA

NP, neutral protease; EC, enzyme combination; NEM, new enzyme mixture; BMI, body mass index; IC, islet count; IEQ, islet equivalents

Table 2

A: Successful and failed isolation in clinical grade pancreas using different enzyme combinations

	Truncated Collagenase + ChNP			Intact C1 Collagenase + Thermolysin		Intact C1 Collagenase + ChNP		P value
	Failure	Success	EC-A (n = 13)	Failure	Success	Failure	Success	
Islet Isolation Outcome (%)	10 (76.9%)	3 (23.1%)		12 (63.2%)	7 (36.8%)	2 (20.0%)	8 (80.0%)	
Edmonton Pancreas Donor Score	58.3 ± 12.3	66.2 ± 14.9		65.7 ± 14.1	70.3 ± 10.2	68.1 ± 11.5	63.3 ± 33.9	0.626
Donor Age (yr)	33.5 ± 10.3	26.7 ± 4.7		35.2 ± 7.9	37.0 ± 9.9	47.0 ± 0.0	43.1 ± 9.4	0.046
Body Mass Index (kg/m ²)	31.5 ± 3.3	41.3 ± 2.4		33.9 ± 3.4	33.6 ± 5.7	25.8 ± 4.6	32.6 ± 2.7	0.7489
Cold Ischemia Time (hr)	5.2 ± 1.6	7.1 ± 1.6		6.3 ± 2.0	6.1 ± 1.3	5.7 ± 1.1	7.3 ± 2.0	0.1073
Trimmed Pancreas Weight (g)	99.0 ± 17.4	113.3 ± 10.7		111.8 ± 17.5	106.7 ± 35.4	92.3 ± 28.6	113.4 ± 28.9	0.6740
Digestion Time (min)	24.2 ± 7.4	18.0 ± 3.5		20.7 ± 3.4	18.6 ± 3.6	26.5 ± 4.9	14.5 ± 2.8	0.023
Dilution Time (min)	38.0 ± 6.7	40.7 ± 11.4		35.8 ± 6.4	36.3 ± 6.0	31.0 ± 7.1	38.5 ± 8.6	0.4749
Digested Pancreas Weight (%)	82.6 ± 8.4	84.0 ± 6.1		83.0 ± 9.0	77.2 ± 10.7	79.6 ± 5.6	82.1 ± 7.6	0.7801
Digested IC	415,616 ± 135,059	489,583 ± 107,919		447,416 ± 107,919	626,922 ± 236,759	428,700 ± 298,823	796,702 ± 353,640	0.025
Digested IEQ	404,829 ± 128,720	690,408 ± 91,793		411,512 ± 95,246	506,110 ± 125,919	280,830 ± 49,257	833,441 ± 214,808	0.0004
Digested IEQ/g pancreas	4,196 ± 1,551	6,144 ± 1,116		3,800 ± 1,126	5,007 ± 1,270	2,912 ± 151	7,905 ± 2,883	0.0012
Digested Tissue Volume (ml)	32.9 ± 15.5	46.0 ± 14.0		36.1 ± 11.3	33.4 ± 12.0	40.0 ± 0.0	36.1 ± 7.1	0.9292
Post Purification IC	260,087 ± 81,340	328,500 ± 57,395		297,847 ± 133,064	420,619 ± 111,619	142,250 ± 80,963	548,000 ± 228,995	0.002
Post Purification IEQ	272,451 ± 516,796	516,796 ± 101,309		302,020 ± 75,586	454,789 ± 101,742	239,438 ± 98,091	694,681 ± 147,356	0.0007
Post Purification IEQ/g pancreas	2,700 ± 1,073	4,635 ± 1,310		2,784 ± 842	4,716 ± 2,070	2,900 ± 1963	6,510 ± 2,150	0.003
Embedded Islet (%)	10.3 ± 9.5	5.3 ± 2.7		17 ± 20.5	18 ± 16.5	25 ± 10.6	14.3 ± 9.5	0.0237
Recovery (%)	53.6 ± 35.2	74.6 ± 8.8		75.1 ± 16.7	92.2 ± 22.1	98.0 ± 62.3	85.5 ± 16.6	0.032
Post Purification Purity (%)	75.0 ± 23.9	83.3 ± 5.8		76.7 ± 8.9	80.7 ± 8.9	69.0 ± 1.4	91.3 ± 8.9	0.1356

B: Human Islet allograft outcome

Recipient #	Protocol #	Recipient body weight (kg)	Total IEQ/kg transplanted			β-Score * day 75	Day 75 basal C-peptide (ng/ml)	Day 75 stimulated C-peptide (ng/ml)	C-peptide stimulation index	% Insulin reduction day 75
			EC-A	EC-F	NEM					
Single infusion										
1	1	62.5	8239	-	-	6	1.40	3.50	2.50	80
2	1	64.0	6063	-	-	5	0.47	1.43	3.04	66
3	2	63.2	7195	-	-	6	1.10	3.60	3.27	79

B: Human islet allograft outcome

Recipient #	Protocol #	Recipient body weight (kg)	Total IEQ/kg transplanted		β-Score* on day 75		Day 75 basal C-peptide (ng/ml)	Day 75 stimulated C-peptide (ng/ml)	C-peptide stimulation index	% Insulin reduction day 75
			EC-A	EC-F	NEM					
4	1	70.4	-	6232	-	6	0.74	2.73	3.69	100
5	1	70.6	-	6282	-	3	0.15	0.47	3.13	29
6	2	51.1	-	6081	-	7	1.16	3.88	3.34	100
7	1	63.6	-	6577	-	4	1.26	2.46	1.95	66
8	2	78.3	-	5807 [#]	-	5	0.62	1.00	1.61	83
9	3	82.2	-	-	7380	5	1.55	3.03	1.95	87
10	4	67.8	-	-	6022	7	0.95	2.10	2.21	56
11	4	91.2	-	-	9040	8	1.07	2.31	2.16	100
12	3	66.0	-	-	6295	6	0.94	2.38	2.53	73
13	4	92.8	-	-	6679	6	1.27	3.14	2.47	73
14	3	71.6	-	-	10,425	2	<0.05	<0.05	Day-56 graft failed	
Two infusions										
3	2	63.2	7195	6038	-	8	2.52	7.08	2.80	100
7	1	63.6	-	6577	6503	8	1.35	3.86	2.85	100
8	2	78.3	-	5807 [#]	6084	6	1.38	2.89	2.09	100

ChNP, *Clostridium histolyticum* neutral protease; C1, class 1; EC, enzyme combination; NEM, new enzyme mixture; IC, islet count; IEQ, islet equivalents

Day 75 defined relative to final infusion

* beta score defined by C-peptide (0 points if < 0.3 ng/mL, 1 point if 0.31–0.89 ng/mL, 2 points if >0.9 ng/mL); HbA1c (0 points if >7%, 1 point if 6.2–6.9%, 2 points if <6.1%); fasting plasma glucose (0 points if >126 mg/dL, 1 point if 100–125 mg/dL, 2 points if <99 mg/dL); and insulin use (0 points if >0.25 u/kg/day, 1 point if 0.01–0.24 u/kg/day, 2 points if insulin independent)

[#] Two pancreatic islet preparations were combined for transplant (EC-F)

IEQ, islet equivalents; EC, enzyme combination

Table 3

Islet isolation outcome in autologous pancreas with different enzyme combinations

	Truncated Collagenase + ChNP EC-A	Truncated Collagenase + ChNP EC-B	Intact C1 Collagenase + ChNP NEM	<i>P</i> value
Autologous Pancreas (n)	81	48	21	
Donor Age (yr)	35.9 ± 14.3	34.5 ± 13.1	30.3 ± 10.3	0.237
Female	64 (79.0%)	38 (79.2%)	13 (65.0%)	0.233
Body Weight (kg)	68.3 ± 20.8	65.8 ± 19.3	67.8 ± 17.0	0.771
Body Mass Index	24.7 ± 5.3	24.0 ± 6.0	24.6 ± 5.5	0.781
Pancreas Weight (g)	68.2 ± 35.0	64.2 ± 27.8	81.2 ± 26.2	0.123
Digestion Time (min)	22.6 ± 5.5	23.6 ± 6.8	18.1 ± 7.0	0.003
Dilution Time (min)	31.0 ± 6.8	27.1 ± 4.9	30.8 ± 3.1	0.001
Digested Pancreas Weight (%)	80.9 ± 13.0	76.5 ± 13.5	90.3 ± 5.5	< 0.001
Digested IC	311,798 ± 159,179	278,190 ± 177,001	527,926 ± 231,756	< 0.001
Digested IEQ	253,305 ± 128,583	210,047 ± 142,617	453,072 ± 209,614	< 0.001
Digested IEQ/g pancreas	4.013 ± 1,909	3,672 ± 3,077	5,805 ± 2,678	0.003
Digested IEQ/IC	0.86 ± 0.28	0.84 ± 0.65	0.86 ± 0.26	0.949
Digested Tissue Volume (ml)	10.3 ± 8.6	7.4 ± 5.1	12.1 ± 5.7	0.025
Transplanted IC	277,276 ± 141,144	241,308 ± 145,064	451,603 ± 208,441	< 0.001
Transplanted IEQ	230,305 ± 111,094	193,585 ± 128,002	422,893 ± 181,329	< 0.001
Transplanted IEQ/g pancreas	3,751 ± 1,864	3,511 ± 3,105	5,327 ± 2,450	0.003
Recovery (%)	90.7 ± 18.8	81.3 ± 33.2	95.5 ± 12.5	0.074
Transplanted IEQ/Body Weight (kg)	3,684 ± 2,092	3,155 ± 2,207	5,979 ± 1,469	< 0.001
Embedded Islet (%)	42.4 ± 26.1	48.8 ± 25.1	42.9 ± 27.0	0.382
Islet Viability (FDA/PI %) (n=40)	93.8 ± 4.1	89.2 ± 7.0	91.3 ± 4.7	< 0.001
Insulin Independent Rate* (%)	28/78 (36%)	16/47 (34%)	9/21 (42%)	0.78

ChNP, *Clostridium histolyticum* neutral protease; C1, class 1; EC, enzyme combination; NEM, new enzyme mixture; IC, islet count; IEQ, islet equivalents; NA, not applicable; FDA/PI, fluorescent diacetate/propidium iodide

* Insulin independence calculated as number currently insulin independent/number of patients reporting (as of 10/09/2011)