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DR. RAPHAËL P H MEIER (Orcid ID : 0000-0001-9050-0436)

PROF. THIERRY BERNEY (Orcid ID : 0000-0002-4230-9378)

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Pancreas collagen digestion during islet of Langerhans isolation

Raphael P.H. Meier[1,2], Jeremy Meyer[1], Yannick D. Muller[1, 2], Gregory L. Szot[2], Benoît Bédat[1], Axel Andres[1, 3], Nathalie Massé[3], Sandrine Lablanche[4], Giacomo Puppa[5], Domenico Bosco[1], Thierry Berney[1,3]

Affiliations:

[1] Cell Isolation and Transplantation Center, Department of Surgery, University of Geneva Hospitals and School of Medicine, Geneva, Switzerland

[2] Department of Surgery, University of Maryland School of Medicine, Baltimore, MD, United States

[3] Division of Transplantation, Department of Surgery, University of Geneva Hospitals and School of Medicine, Geneva, Switzerland

[4] Grenoble Alpes University, Grenoble, France; Inserm U1055, Laboratory of Fundamental and Applied Bioenergetics Grenoble, Grenoble, France

[5] Diagnostic Department, Geneva University Hospital, and Department of Pathology and Immunology, University of Geneva Medical School, Geneva, Switzerland

Address for correspondence:
Raphael P.H Meier, MD, PhD

Transplant Surgery, University of California San Francisco, 505 Parnassus Ave, San Francisco, 94143 CA, United States
Phone: 415 514 0332 / Fax: 415 353 8709
E-Mail: Raphael.Meier@unige.ch

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Abbreviations:

Islet equivalents (IEQ), standard deviation (SD), body mass index (BMI), Good Manufacturing Practice (GMP)

Authorship statement:

RM designed the study. RM, JM, YM and DB and performed the experiments. RM, JM, YM, BB, AA, NM, GP, SL, DB, and TB and collected the data. RM, JM, YM, GP, DB, and TB analyzed the data. RM performed statistical analysis. RM, JM, YM, GZ, AA, NM, GP, SL, DB, and TB interpreted the data and wrote the manuscript. RM, JM, YM, DB, and TB and had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure

The authors have no conflict of interest to disclose.

Abstract

Background:

The success of pancreas islet isolation largely depends on donor characteristics, including extracellular matrix composition of which collagen is the main element. We hypothesized that isolation yields are proportional to collagen digestion percentage, and aimed to determine a threshold that predicts isolation success.

Methods:

The amount of pancreas collagen (I-V) was determined prior to and after the digestion process in 52 human islet isolations. Collagen I-V and VI were assessed histologically.

Results:

We identified a collagen digestion threshold of ≥60% as an independent factor beyond which an islet preparation has a 9-fold increased odds of yielding ≥250,000 islet equivalents (IEQ) (p=0.009) and a 6-fold increased odds of being transplanted (p=0.015). Preparations with ≥60% collagen digestion (n=35) yielded 283,017±164,214 IEQ versus 180,142±85,397 in the <60%-collagen-digestion group (n=17) (p=0.016); respectively 62.9% versus 29.4% of those were transplanted (p=0.024). Common donor characteristics, initial collagen content, enzyme blend, and digestion times were not associated with collagen digestion percentage variations. Donor age positively correlated with the amount of collagen VI (p=0.013). There was no difference in islet graft survival between high and low digestion groups.

Conclusion:

We determined that a 60%-pancreas-collagen-digestion is the threshold beyond which an islet isolation is likely to be successful and transplanted.

Introduction

Since the introduction of the Edmonton Protocol, pancreas islet transplantation has become an effective treatment modality for patients with type 1 diabetes mellitus [1-4]. The purity and the number of islets, isolated after pancreatic digestion and gradient-based separation, are essential in determining whether an islets preparation is eligible for clinical infusion. A successful clinical islet isolation depends on numerous donor variables including age [5, 6], body mass index (BMI) [7], warm and cold ischemia time and as well as donor glucose homeostasis [8]. Another important factor is represented by the ability of a Good Manufacturing Practice (GMP)-collagenase and neutral protease blends to properly digest the pancreas without injuring islets [9]. While a lot of effort has been made in testing different GMP collagenase products, however, much less is known about the structural factors of the extracellular matrix and the surrounding islet basal membranes effect on a successful islet isolation [10, 11]. The pancreas extracellular matrix is exquisitely complex and constituted of numerous (≥120) different proteins [12] (including collagens, laminins, and fibronectin), collagen being by far the most abundant [13]. Notwithstanding this complexity, we hypothesized that collagen digestion may be an appropriate estimate of the overall digestion process during islet isolation. Another important point is that collagen I, III, IV, V [14] and VI [11] are key components of the islet-exocrine interface of the adult pancreas. Furthermore, Collagen IV (along with pan-laminin, perlecan and Laminin α5) is degraded during the islet isolation process and their over-digestion seems to negatively affect islet survival [10]. Enzymatic activity and timing of the digestion process is therefore key to obtaining sufficient and functional islets. We further hypothesized that the success of an islet isolation preparation can be, in part, estimated by the quantity and quality of pancreas collagen content degradation. A detailed understanding of collagen degradation process may potentially offer the opportunity to isolate and recover islets from younger, more difficult, pancreas donors [5, 6] and from patients with chronic pancreatitis (i.e. major scarring and fibrosis) [15].

It seems likely that isolation yields are proportional to collagen digestion percentages, however, to the best of our knowledge, a quantitative assessment of pancreas collagen content during pancreatic islet isolation has never been reported, nor has a threshold been established. The objective of this study was to quantify pancreas collagen before

and after islet isolation and to correlate these results against successful or unsuccessful islet isolation and clinical outcomes.

Material and methods

Donors

In this study, we included 52 consecutive human islet isolations performed at our institution. The study protocol was approved by the local research ethics committee (protocol no. 2017-01230). Pancreases were retrieved from brain dead donors procured by Swiss and French centers participating to the GRAGIL collaborative project [16-18]. Pancreases were shipped to the islet isolation facility in cold preservation solution with an average cold ischemic time of 6.8 ± 2.8 hours.

Islet isolation

Isolations were performed as previously described, according to a local adaptation of Ricordi's semi-automated technique [19, 20]. Either collagenase NB1 (Serva Electrophoresis, Heidelberg, Germany) or Liberase HI (Roche, Indianapolis, IN) were used for pancreas digestion. Pancreases were weighed at the start of the isolation process, and pancreatic tissue remnants were weighed at the end of the digestion phase. Digestion percentage was defined as 100x (pancreas weight - remnant weight)/pancreas weight (%). Islets were purified on a continuous Biocoll gradient (Biochrom, Berlin, Germany) using a refrigerated COBE cell processor (COBE 2991; Cobe, Lakewood, CO).

Collagen quantification (Sircol assay)

A ≤1 mm^2 biopsy was taken from the neck/body junction of the pancreas, at the site the pancreas was incised prior to the insertion of the digestion cannula. This location was chosen in order to minimize the impact of the biopsy on the islet isolation process. Two other ≤1 mm^2 biopsies were taken from the digested (white scaffold) and undigested

tissue (undigested (yellow) pancreatic tissue) following the digestion in the Ricordi chamber. The biopsy was digested overnight at 4°C with pepsin (0.1 mg/ml) in 0.5M acetic acid. Collagen I to V content was then assessed using Sircol soluble collagen assay per manufacturer instructions (Biocolor, Carrickfergus, UK). Results of the quantification were expressed as µg of collagen per g of pancreatic tissue.

Histology

Formalin-fixed pancreas tissue was fixed in 10% Formalin for 24 hours and then embedded in paraffin. Serial sections of 5 µm thickness were prepared and collagen fibers were stained using Goldner's trichrome and Sirius histochemistry. Briefly, pre-digested pancreas sections obtained immediately before digestion were incubated for 1 h at 60°C in Bouin's solution. Slides were then washed and stained with ponceau acid fuchsin, phosphomolybdic acid-orange g solution and light green solution. Finally, slides were rinsed in 2% acetic acid and rapidly dehydrated in ethanol/xylol and coverslipped. Collagen VI was assessed by immunohistochemistry. Briefly, paraffin sections were incubated with rabbit recombinant monoclonal Collagen VI antibody (Abcam, Cambridge, UK) at 1/250 dilution, followed by horseradish peroxidase polymer for rabbit/mouse IgG, and counter stained with Hematoxylin. Images from tissue sections were acquired with Mirax system (3DHISTECH, Hungary). The collagen surface was determined on two to five low magnification pancreas field using morphometric quantification (MetaMorph software, Universal Imaging, West Chester, PA and Definiens Software, Germany) of the green area (collagen I to VI) or brown area (collagen VI), normalized to unstained area (endocrine and exocrine tissue). 19 donor pancreas biopsies were available for the collagen VI analysis.

Islet quantity and quality assessment

Islet counting and purity assessment were performed before and after purification as previously described [21]. The number of islet equivalents (IEQ) was calculated using computer-assisted digital image analysis [22] by normalizing the islets to a diameter of

150 µm [23]. Islet size (µm) was calculated using the following formula: 150 x IEQ/islet number [23]. The recovery rate (%) was estimated by dividing post-purification IEQ to pre-purification IEQ numbers. Transplant tissue volume, viability, purity, endotoxin levels, and insulin secretion capabilities were assessed prior to transplantations (n=27). Islet viability was assessed by fluorescein diacetate and propidium iodide staining as previously described [24]. Endotoxin levels were measured using the Endosafe-Portable Test System (Charles River Laboratories, Wilmington, MA). A static glucose-stimulated insulin secretion assay was used to assess islet preparation's function and calculated as an insulin concentration ratio between high glucose (16.7 mM) and low glucose (2.8 mM) conditions.

Recipients

Among the 52 islet preparations, 27 were transplanted. Sixteen patients had follow-up-clinical data available. Allogeneic transplants were performed in the GRAGIL network [21] (simultaneous islet kidney (n=2), islet after kidney (n=6), islet transplant alone (n=6) or islet after lung(n=2)) within different previously reported protocols [2, 25-28]. All recipients received the islet preparation intraportally through a percutaneous transhepatic approach. Immunosuppression consisted in steroid-free regimens modified from the original "Edmonton protocol"[29]. Islet graft survival and function were assessed at 1, 6, 12, 24, 36 and 48 months after the first islet injection. Patients were excluded at these time points if they received another islet preparation with a different digestion pattern.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD). Categorical variables are presented as frequency (%). Statistical analysis was performed using the IBM SPSS 26 software (IBM SPSS, Chicago, IL). Unpaired Student's *t*-test (normal distribution) or Mann-Whitney U-test (non-normal distribution) was used for group comparison. Chi-square test was used to compare categorical data. Pearson correlation

was used to compare different collagen quantification methods and collagen VI with age. An exact two-sided p value of less than 0.05 was considered statistically significant.

Results

Collagen quantification in the pancreas

One ≤1 mm^2 pancreas biopsy was taken before digestion and two ≤1 mm^2 biopsies were taken after pancreas digestion including digested issue (white scaffold appearance) and partially digested tissue (yellow appearance) (Figure 1A, Supplementary Figure 1A). All were used for the colorimetric collagen content assay. The individual pancreas collagen content of the three biopsy samples is shown in Supplementary Figure 1B. One third of the post-digestion yellow appearance tissue (marked ** in Figure 1A) had minimal/no collagen content reduction (less that 5% decrease) in the initial collagen content and therefore likely represent an intermediate/partial stage of digestion from minor remnant parts of the pancreas not well perfused with the collagenase initially. This part was therefore deemed unsuitable to assess digestion efficiency. The completely digested tissue biopsy sample (white appearance, marked *** in Figure 1A) was therefore determined as the ideal candidate to determine the accurate percentage of collagen digestion. As a quality-control method, we analyzed the initial collagen content using a Goldner staining on pancreas histological sections from an additional pre-digestion biopsy (Figure 1B). Histological quantification of the collagen on tissue sections was not possible after digestion because of tissue disruption preventing adequate formalin fixation and staining. The intra- and peri-islet collagen surface correlated with the overall total pancreas collagen surface measured by histologic Sirius staining (Figure 1C, R^2=0.675, p<0.001). The quantification of the collagen surface on histological sections significantly correlated with the colorimetric measurements using the sircol method (Figure 1D, R^2=0.282, p=0.019). We therefore used the sircol colorimetric assay to assess collagen contents before and after digestion.

Pancreas collagen contents before and after enzymatic digestion and classification according to digestion efficiency

As expected, the pancreas biopsy pairs taken before and after digestion, and assessed with the colorimetric method, showed a consistent and significant reduction of collagen content (Figure 2A). The efficacy of collagen digestion was variable between donors but did not significantly correlate with age, sex, BMI, warm or cold ischemia time, and pancreas weight (Figure 2B-E). The best ratio between "successful" and "failed" isolations appeared to range between 60 to 80% collagen digestion (Figure 2G). We used receiver-operating characteristic (ROC) curves to determine the cutoff of initial content of collagen that was efficiently digested (expressed as a percentage of initial collagen content) and found that reduced collagen content can be predictive of the success of an islet isolation; arbitrarily determined by a final islet yield of ≥250,000 IEQ. Digestion of sixty percent or more of the initial collagen contents, was identified as the optimal cut-point value for a successful islet isolation (Figure 2H). Sixty percent remained the optimal cut-point value when setting the successful IEQ cutoff at 200,000 or 300,000 IEQ. The digestion success/failure (as defined by the "≥60%" threshold) determined a final islet yield optimal cut-point value of ≥255,000 IEQ with a sensitivity of 60% and a specificity of 84% (Figure 2I). Sensitivity/specificity were 66%/65% for a final IEQ yield of ≥200,000 IEQ and 46%/94% for a final IEQ yield of ≥300,000 IEQ. Digestion efficiency thresholds of ≥50% or ≥70% had inferior area under the ROC curve (AUC); 0.632 and 0.595, respectively. The AUC for taking the criteria 60-80% versus all other digestion percentages was also inferior to the "<60% versus ≥60%" ROC curve (AUC of 0.684 versus 0.707, Supplementary Figure 2). Accordingly, islet isolation procedures were classified in two groups: a "successful" efficient digestion group (where ≥60% of the initial collagen was digested), n=35, and a "poorly" efficient digestion group (where <60% of the initial collagen was digested), n=17.

The percentage of pancreas collagen digestion can predict isolation outcomes

Characteristics of pancreas donors with successful versus poorly effective digestion are compared in Table 1. Pancreas donors in the two groups had similar age, sex, gender,

BMI, number of ICU days, warm and cold ischemia times, cause of death and preservation solution (all p-values>0.05). The initial collagen content was not statistically different between both groups. The remaining collagen content after digestion was lower in the successful digestion group with 79.9 ± 9.5% of the collagen degraded as compared to 42.0 ± 12.3% in the poorly effective digestion group. The initial amount of collagen decreased significantly in both groups following the digestion process (Figure 3A). Of note, pancreas weight and undigested tissue weight were similar in both groups. There was no difference in terms of enzyme type/blend and digestion time between the two groups. The successful digestion group had a significantly higher total IEQ (Figure 3B), recovery rate, total islet numbers, and higher successful transplant rates compared to the poorly effective digestion group (Table 2). There was no significant correlation between digestion time and amount of initial collagen efficiently digested regardless of the isolation outcome (Figure 3C). The relation between the percentage of initial collagen digested and the number of embedded / fragmented islets is shown in figure 3D. The quality control data showed no significant differences in terms of transplant volume, viability, purity, endotoxin content and stimulation index between the two groups (Table 3). We further searched for additional variables potentially associated with a successful isolation (i.e. final yield ≥250'000 IEQ) (Supplementary Table 1). Higher BMI was associated with an increased likelihood of isolation success. The comparison of isolation outcomes between islet isolation procedures in which either a successful isolation (final yield ≥250'000 IEQ) or unsuccessful isolation (final yield <250'000 IEQ) was achieved is presented in Supplementary Table 2. Overall, a successful digestion (i.e. ≥60% of initial collagen digested) and BMI ≥30 kg/m2 were independent factors associated with a 9-10-fold increase in odds of obtaining a successful isolation (i.e. final yield ≥250'000 IEQ) (Supplementary Table 3). A successful digestion was the only independent factor significantly associated with the transplantation of an islet preparation (Supplementary Table 4).

Assessment of collagen type VI

Since the Sircol assay only measure collagen type I to V, we conducted a specific analysis of collagen type VI on pancreas sections available for immunostaining before digestion. Higher collagen VI content was found in older donors compared to younger donors (Figure 4A-C). The average brown chromogen intensity on histologic sections was 1.1±0.3 in the first age quartile (n=5, range 15 to 45 years) versus 1.3±0.1 in the three remaining quartiles (n=14, range 45 to 62 years), p=0.017. We found no correlation between collagen VI content and donor BMI, sex, pancreas weight, final IEQ numbers and initial or final pancreas collagen I-V contents (Figure 4D).

Transplantation outcomes of pancreas donors as a function of digestion efficiency

Sixteen patients had clinical follow-up data available. Eleven patients received an islet preparation from the successful digestion group and 5 patients received an islet preparation from the poorly effective digestion group. Kaplan-Meier survival curves did not reveal significant differences in insulin-free and C-peptide-positive survival rates between the two groups (Figure 5).

Discussion

In this study, we have quantified pancreas collagen contents before and after digestion by collagenase and investigated the possible associations with pancreas donor characteristics and islet isolation outcomes. Even though it seemed likely that an optimal collagen digestion percentage would translate into better isolation yield, we conducted this analysis for the first time and were able to establish the threshold of ≥60% beyond which a given preparation has a nine-fold likelihood to reach a yield of ≥250’000 IEQ and therefore being transplanted.

During the isolation process of pancreatic islets, the required enzymatic digestion step disrupts extracellular tissue surrounding the islets [10]. The exact implications of this process are not well understood, and it is likely that variations in pancreas extracellular matrix and digestion characteristics significantly affect isolation, islet survival, islet

function, and transplant outcomes [14]. The peripheral extracellular matrix of adult human islets has been reported to be composed of laminin [30] and collagen IV [31] and to a lesser extent of fibronectin [32], collagen I [33], collagen III [33], collagen V [33], and collagen VI [11]. Of note, various types of integrins allow the islets to bind collagen fibers [31, 34].

In our investigations, we focused on collagen as a major compound of the islet extracellular matrix [14]. We used a colorimetric quantitative assay to measure the pancreas collagen content before and after pancreas digestion, therefore virtually eliminating observer bias. We confirmed our observations using a histologic quantification of collagen on corresponding pancreas tissue sections. Interestingly, the collagen surface present within and around the islets correlated with the overall pancreas collagen surface. When 60% or more of the initial collagen was digested, we could predict a successful isolation with a specificity of 82%.

Currently, the only way to assess the efficiency of pancreas digestion in real-time is to take serial samples during the process and visually assess the number of embedded and fragmented islets, and assess the progress of the digestion by looking at the exocrine tissue aspect [35]. The ability to separate endocrine tissue from exocrine part during purification phase retrospectively indicates if the digestion was adequate. The digestion timing required to extract pure, undamaged, islet is difficult and is variable between donors. An error in digestion judgment can lead to either an insufficient digestion or an over-digestion that both preclude the success of the isolation. We therefore believe that a better understanding of the fate of pancreas extracellular matrix components during islet isolation could improve isolation outcomes and strategies.

A previous study by Cross et al. focused on a histologic assessment of collagen IV, laminin and perlecan of human islet basement membrane during islet isolation [10]. They showed that collagen IV, pan-laminin, laminin-alpha5 and perlecan were partly or entirely lost during the digestion process, with no restoration following culture, suggesting the potential harmful role of this process to islet survival. In this investigation, we hypothesized the initial quantitative collagen content might influence the isolation process. We were not able to identify significant associations between donor variables

such age, sex, BMI, ischemia times and pancreas weight. We did identify a positive trend with cold ischemia time and may reflect the fact that longer preservation time is associated with pancreas edema and an insufficient digestion[7]. We could however demonstrate an association between older donor age and increased pancreas collagen VI content. This association was previously evoked by Hughes et al. [11]. This observation confirms that the extracellular composition between young and older donor pancreases is different as previously suggested in other species [36]. The collagen VI content itself had no immediate correlation with the isolation yield, indicating that the relative composition, rather than a single component may have an impact on isolation outcomes.

Another key interest of studying pancreas extracellular matrix is that this could allow the use of essential components identified as “promoter” of islet *in vitro* and *in vivo* function and survival. The identification of molecules interacting with islet cadherins could help understanding the positive effect that some stromal support cells have on islets [37, 38]. In the emerging era of cellular therapies, islet transplantation is the first and currently the only clinically accepted procedure where one isolate cells from a solid organ (i.e. the pancreas) to transplant them in another solid organ (i.e. the liver). Other solid organ tissue-derived cell transplantation remains largely experimental (e.g. neural cell[39], myocyte[40], hepatocyte[41], and mesenchymal stem cells [42]). Therefore, understanding the islet dependence upon surrounding stromal tissue could benefit other fields of cell transplantation.

Our study has some limitations. Although the colorimetric quantification of collagen is much quicker compared to a histologic quantification, this technique is not able to provide a real-time assessment of collagen content. Further development of this technique will be required in order to implement this test during an islet isolation procedure. However, we believe that our investigations bring key information to further develop a preemptive assay that directs and optimizes the digestion process in real-time. We also acknowledge that we failed to identify modifiable factors influencing digestion efficiency. For example, we were not able to link the donor age, digestion time, or enzyme blend with modifications in collagen degradation efficiency. It is therefore likely that the digestion time and enzyme blend were accurately chosen during each individual procedure,

allowing a successful outcome even in challenging isolation (e.g. juvenile pancreases) as well [6]. Moreover, age and initial collagen quantity do not influence islet yields in pigs as well [36].

We also acknowledge the fact that some preparations with ≥60% collagen digestion were finally found to be isolation failures (n=14/52). We attribute this lack of sensitivity (i.e. 60%) to the fact that other factors (such as donor or retrieval factors) or the digestion of the numerous other extracellular matrix proteins in the pancreas as well as the collagen subtypes studied here are at least equally important for determining isolation success. Since collagen is the major component of extracellular matrix, we believe that our measures offer an acceptable estimate of the overall digestion process. We however acknowledge the complexity of the extracellular matrix [12, 13] and recognize the fact that future studies will be important to determine the role of individual component in determining isolation success and islet survival. Another important point is that, in our clinical setting, it was not possible to take multiple biopsies of the pancreas head, neck, body and tail to ensure homogeneity. Of note, previous reports identified only minor variations between different pancreas areas and species [12, 13, 33, 36]. Pancreas collagen could also be measured at other sites/steps (in the post-digestion pellet, in impure layers, throughout the pancreas and at all steps of the isolation process), however this would require an important number of pancreases attributed to research use only.

Overall, we believe that our findings will pave the way for future research efforts to improve pancreas collagenase-based digestion. Finally, we did not identify a significant impact of digestion efficiency on clinical outcomes. The number of patients available for comparison was low and it is possible that when a sufficient number of IEQ is met, the overall efficiency of digestion does not play a significant role after transplantation. On the other hand, we must not forget that the integrity of the islet basement membrane seems to be an important factor for islet engraftment [10].

In conclusion, a successful collagen digestion during a pancreatic islet isolation procedure is associated with favorable outcomes in terms of transplantability. A better understanding of the pancreas extracellular matrix components has the potential to further improve beta-cell isolation, culture, and transplantation.

Legend to figures

Figure 1: Histologic and colorimetric quantification of pancreas collagen. (A) Macroscopic view of the pancreas before and after digestion (scale bars: 1cm). Areas where were taken *pre-digestion pancreas biopsy, **partially digested pancreas biopsy (yellow tissue appearance) and ***completely digested pancreas biopsy (white scaffold appearance) were analyzed post digestion. (B) Representative pancreas section showing the whole biopsy (left panel) and an islet (right upper panel, scale bar: 50µm) and exocrine pancreas (right lower panel, scale bar: 200µm) at higher magnification, stained with Goldner coloration highlighting collagen I-V fibers in green. (C) Graph showing collagen content within islets plotted against collagen content on a section including mainly exocrine tissue (n=31). (D) Graph showing collagen content assessment by histology against collagen content assessment by colorimetric quantification (n=19). R^2 and p-values calculated using Pearson correlation.

Figure 2: Pancreas collagen content before and after enzymatic digestion and classification according to digestion efficiency. (A) Pancreas collagen content before and after digestion (each pair represents measurements done during one pancreatic islet isolation). The pancreas collagen content is shown in function of (B) donor age, (C) donor body mass index, (D and E) warm and cold ischemia time, and (F) pancreas weight. (G) Isolation success (i.e. a final yield of ≥250,000 IEQ) and failure are shown in function of percentage of collagen digested. Group comparison was performed using paired Student's t-test. R^2 and p-values were calculated using Pearson correlation. (H) Receiver-operating-characteristic (ROC) curves showing the collagen digestion percentage optimal cut-point value (60%, arrow) for three different isolation success cutoffs (200,000, 250,000 and 300,000 islet equivalent (IEQ)). (I) ROC curves showing the final islet yield optimal cut-point value (255,000 IEQ, arrow) for three different digestion cutoffs (50%, 60% and 70%). AUC: area under the curve. IEQ: islet equivalent.

Figure 3: The percentage of pancreas collagen digestion can predict isolation outcomes. (A) Pancreas collagen content before (initial) and after digestion (final) in function of digestion efficiency. (B) Post purification final islet equivalent number in

function of digestion efficiency. (C) Percentage of pancreas collagen digested in function of digestion time by collagenase and classified by isolation success and failure (i.e. a final yield of ≥250,000 IEQ). (D) Percentage of embedded and fragmented islets in function of the percentage of pancreas collagen digested. R^2 and p-values calculated using Pearson correlation. Group comparisons were performed using Student's t-test.

Figure 4: Collagen VI quantification in donor pancreases. Representative pancreas sections showing collagen IV stained by way of immunohistochemistry in a (A) young (15yo) and (B) older donor (52yo). (C) Pancreas collagen VI content in function of donor age, BMI, pancreas weight, final islet equivalent number (IEQ), and pancreas collagen before and after digestion. R^2 and p-values calculated using Pearson correlation.

Figure 5: Survival curves for (A) insulin-independence and (B) C-peptide positivity in preparation following successful versus poorly effective digestion. Gehan-Breslow-Wilcoxon test was used for survival curves comparison.

Supplementary Figure 1: (A) Macroscopic view of the pancreas biopsies taken *pre-digestion, and post-digestion, including **partially digested pancreas biopsy and ***completely digested pancreas biopsy (scale bars: 1mm). (B) Comparison between pre-digestion pancreas collagen content, partially digested pancreas collagen content, and completely digested pancreas collagen content.

Supplementary Figure 2: Receiver-operating-characteristic (ROC) curve showing the final islet yield optimal cut-point value for the digestion cutoff “60%to 80%”. AUC: area under the curve.

Legends to tables

Table 1: Characteristics of donor pancreases stratified by either successful or poorly effective digestion.

Table 2: Outcomes comparison between islet isolation procedures in which either a successful or poorly effective digestion was achieved.

Table 3: Quality control data of transplanted islet preparations stratified by either a successful or poorly effective digestion.

Supplementary Table 1: Characteristics of donor pancreases stratified by successful isolation (final yield ≥250'000 IEQ) versus unsuccessful isolation (final yield <250'000 IEQ).

Supplementary Table 2: Outcomes comparison between islet isolation procedures in which either successful isolation (final yield ≥250'000 IEQ) or unsuccessful isolation (final yield <250'000 IEQ) was achieved.

Supplementary Table 3: Estimated odds ratios for isolation success (final yield ≥250'000 IEQ) using a binary logistic regression model.

Supplementary Table 4: Estimated odds ratios for islet preparation to be transplanted using a binary logistic regression model.

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Availability of data

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table 1: Characteristics of donor pancreases stratified by either highly effective digestion or poorly effective digestion.

	Successful digestion [a] (n = 35)	Poorly effective digestion [b] (n = 17)	p-value [c]
Age, yr (mean ± SD)	47.4 ± 13.1	41.8 ± 14.6	0.168
Gender Male (%) Female (%)	 18 (51.4) 17 (48.6)	 7 (41.2) 10 (58.8)	0.488
BMI, kg/m2	26.8 ± 4.9	27.2 ± 4.5	0.572
ICU stay, days	2.2 ± 1.9	2.5 ± 1.7	0.220
Warm Ischemia Time, min (±SD)	66.8 ± 23.8	72.3 ± 22.9	0.451
Cold Ischemia Time, min	410.1 ± 154.2	408.3 ± 205.9	0.972
Cause of Death - Cerebral trauma (%) - Cerebro-vascular (%) - Suicide (%) - Anoxia (%)	 7 (20.0) 26 (74.3) 1 (2.9) 1 (2.9)	 5 (29.4) 11 (64.7) 1 (5.9) 0 (0.0)	0.719
Preservation Solution - UW (%) - IGL-1 (%) - Celsior (%) - Other (%)	 5 (14.3) 21 (60.0) 3 (8.6) 6 (17.1)	 0 (0.0) 12 (70.6) 2 (11.8) 3 (17.6)	0.431

[a] ≥60% of initial collagen content was degraded during digestion

[b] <60% of initial collagen content was degraded during digestion

[c] Student *t*-test or Mann-Whitney for continuous variables and χ^2 test for binary or categorical variables (global p value)

BMI: body mass index, ICU: intensive care unit, SD: standard deviation, UW: University of Wisconsin solution, IGL-1: Institut Georges Lopez-1 solution

Table 2: Outcomes comparison between islet isolation procedures in which either highly or poorly effective digestion was achieved.

	Successful digestion [a] (n = 35)	Poorly effective digestion [b] (n = 17)	p-value [c]
Initial collagen content (mg/g of pancreas)	6.6 ± 4.9	5.0 ± 3.7	0.344
Collagen content after digestion (mg/g of pancreas)	1.3 ± 1.2	2.9 ± 2.2	0.001
Percentage of initial collagen degraded after digestion, %	79.8 ± 9.5	42.0 ± 12.3	<0.001
Pancreas weight, g	101.9 ± 24.7	90.9 ± 19.0	0.111
Undigested tissue weight, g	11.7 ± 7.1	11.1 ± 10.9	0.358
Digested tissue weight, g	90.3 ± 24.0	79.8 ± 14.7	0.107
Digestion rate %	88.2 ± 8.1	88.7 ± 8.7	0.824
Enzyme used (%) Serva NB1, lot 1 Serva NB1, lot 2 Serva NB1, lot 3 Serva NB1, lot 4 Serva NB1, lot 5 Liberase, lot 1	 0 (0.0) 6 (17.1) 23 (65.7) 1 (2.9) 2 (5.7) 3 (8.6)	 1 (5.9) 3 (17.6) 11 (64.7) 0 (0.0) 1 (5.9) 1 (5.9)	 0.753
Digestion time, min	18.9 ± 3.7	19.2 ± 3.9	0.755
Tissue volume, ml	47.1 ± 16.5	41.8 ± 12.5	0.245

Total number of islets, pre-purification	423,964 ± 215,005	387,934 ± 152,768	0.539
IEQ, pre-purification	392,159 ± 211,678	366,848 ± 218,935	0.689
IEQ per g pancreas (pre-purification)	4,408 ± 2,257	4,487 ± 2,266	0.792
Mean pre-purification islet size, µm	144.8 ± 53.4	138.6 ± 41.4	0.992
Embedded islets, %, pre-purification	21.9 ± 16.2	36.6 ± 27.5	0.051
Fragmented islets, %, pre-purification	12.9 ± 10.5	11.4 ± 8.3	0.853
Total islets post-purification	246,498 ± 123,877	150,706 ± 92,188	0.007
IEQ post-purification	283,017 ± 164,214	180,142 ± 85,397	0.016
IEQ/per g pancreas (post-purification)	3216 ± 1853	2297 ± 1108	0.066
Mean post-purification islet size, µm	173.0 ± 60.0	194.8 ± 81.2	0.598
Recovery rate, %	82.7 ± 47.8	60.0 ± 39.2	0.049
Isolation success (i.e. final yield ≥ 250'000 IEQ) (%)	21 (60.0)	4 (23.5)	0.014
Transplanted preparation (%)	22 (62.9)	5 (29.4)	0.024

[a] ≥60% of initial collagen content was degraded during digestion

[b] <60% of initial collagen content was degraded during digestion

[c] Student *t*-test or Mann-Whitney for continuous variables and χ^2 test for binary or categorical variables (global p value)

IEQ: islet equivalent

Table 3: Quality control data of transplanted islet preparations stratified by either highly effective digestion or poorly effective digestion.

	Successful digestion [a] (n = 22)	Poorly effective digestion [b] (n = 5)	p-value [c]
Packed transplant volume (ml)	2.0 ± 0.9	1.9 ± 0.8	0.710
Viability (%)	89.1 ± 4.0	92.0 ± 2.7	0.135
Purity (%)	64.7 ± 19.2	66.2 ± 20.5	0.883
Endotoxin contents (EU/ml)	0.56 ± 0.16	0.50 ± 0.08	0.906
Stimulation index	1.4 ± 0.6	1.4 ± 0.5	0.950

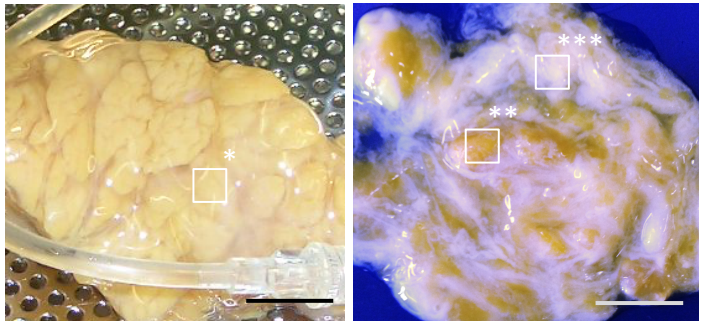
[a] ≥60% of initial collagen content was degraded during digestion

[b] <60% of initial collagen content was degraded during digestion

[c] Student *t*-test or Mann-Whitney for continuous variables

Figure 1

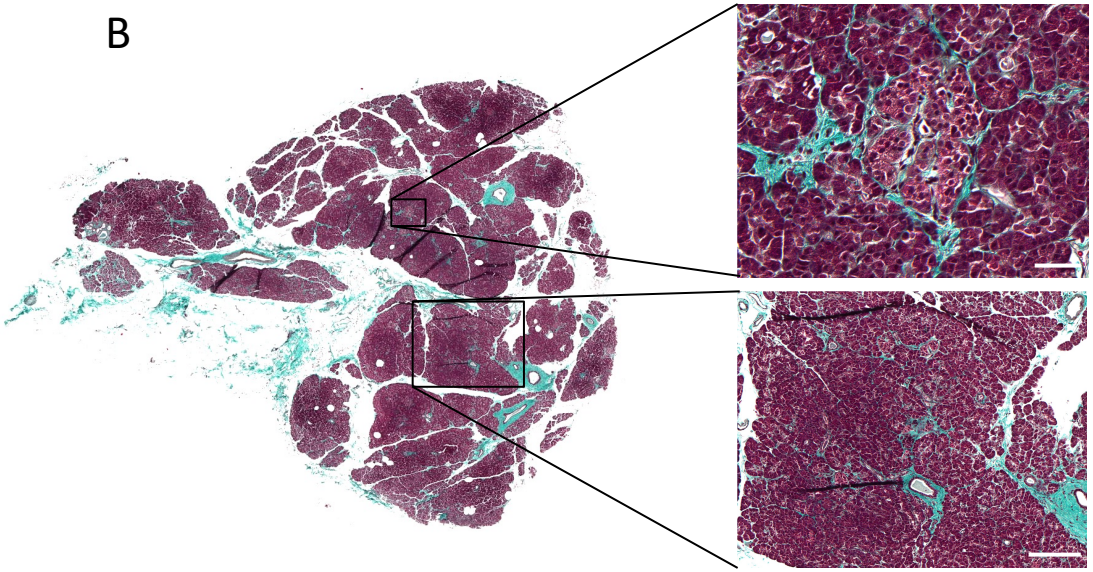
A



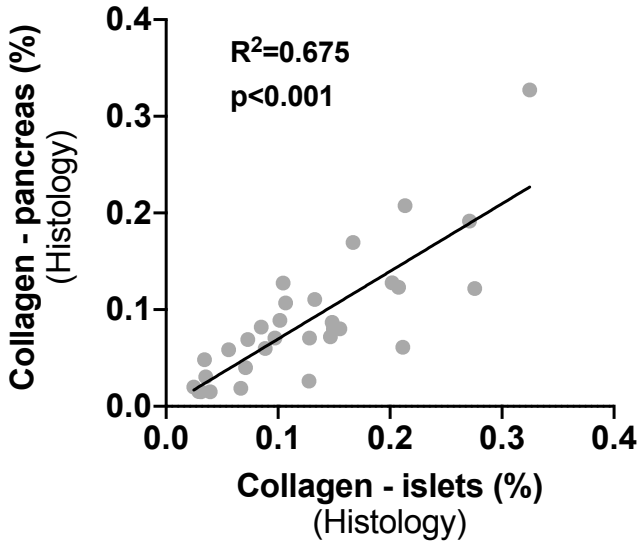
Pancreas before digestion

Pancreas after digestion

B



C



D

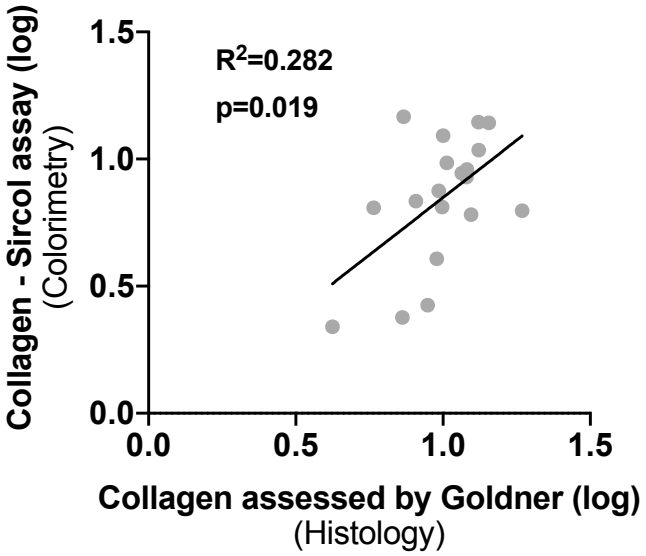


Figure 2

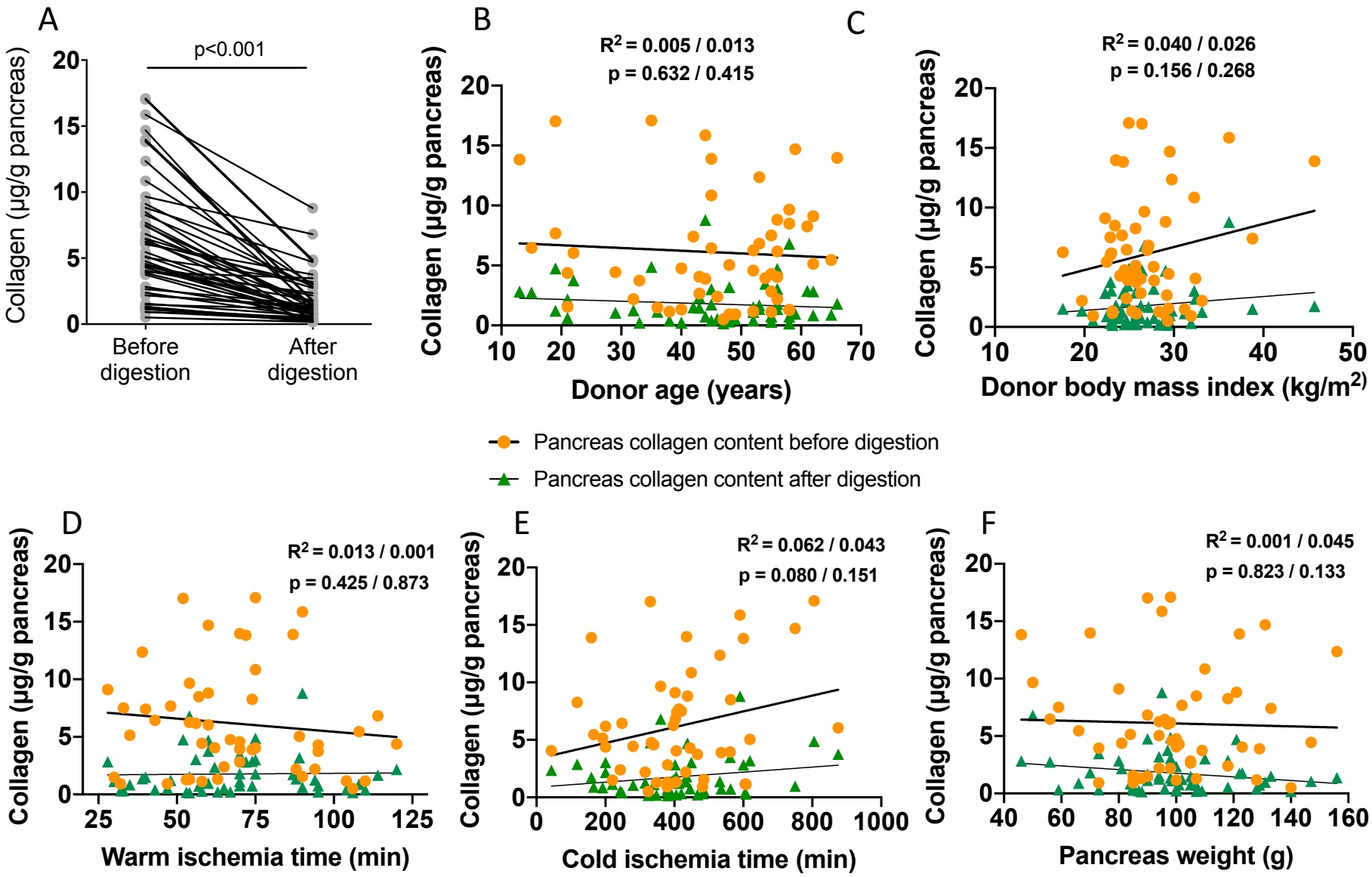
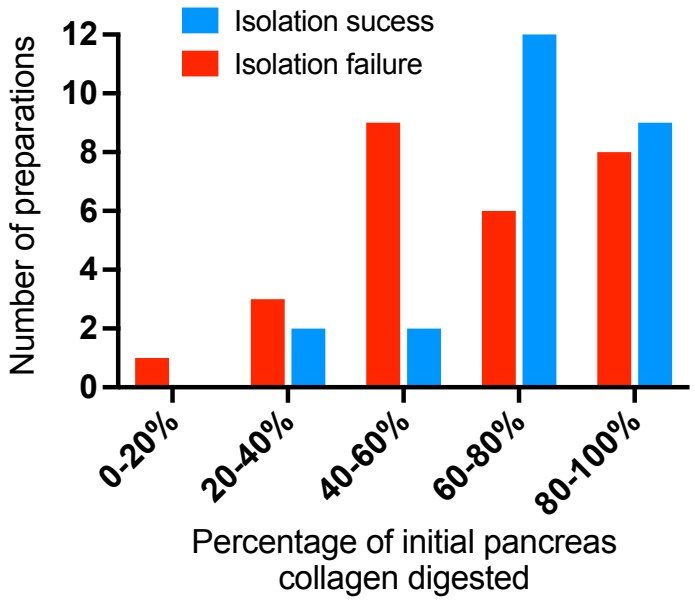
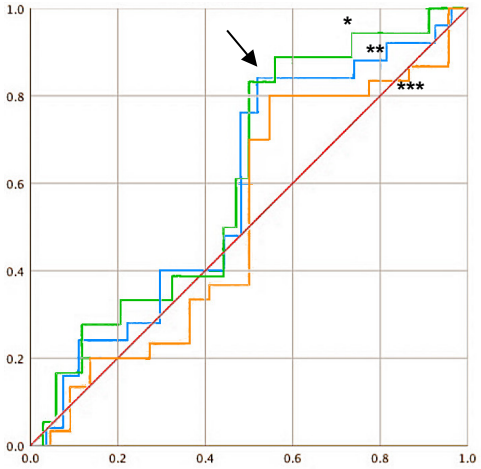

A
p<0.001
Collagen (µg/g pancreas)
Before digestion
After digestion
B
R² = 0.005 / 0.013
p = 0.632 / 0.415
Collagen (µg/g pancreas)
Donor age (years)
C
R² = 0.040 / 0.026
p = 0.156 / 0.268
Collagen (µg/g pancreas)
Donor body mass index (kg/m²)
Pancreas collagen content before digestion
Pancreas collagen content after digestion
D
R² = 0.013 / 0.001
p = 0.425 / 0.873
Collagen (µg/g pancreas)
Warm ischemia time (min)
E
R² = 0.062 / 0.043
p = 0.080 / 0.151
Collagen (µg/g pancreas)
Cold ischemia time (min)
F
R² = 0.001 / 0.045
p = 0.823 / 0.133
Collagen (µg/g pancreas)
Pancreas weight (g)

Figure 2 (continued)

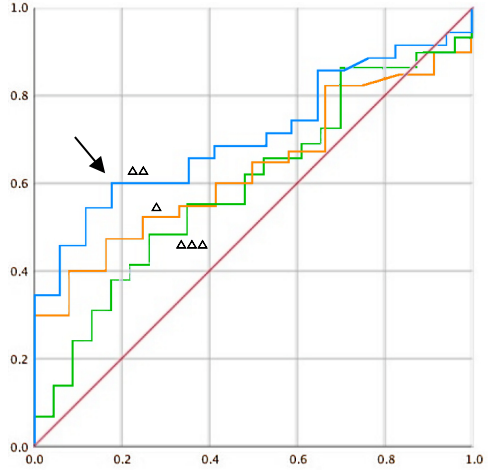
G



H



I



Successful isolation yield cutoff:

* **≥200,000 IEQ** : AUC±95%CI: 0.518 (0.352 – 0.685), p=0.824

** **≥250,000 IEQ :** AUC±95%CI: 0.587 (0.429 – 0.745), p=0.284

*** **≥300,000 IEQ :** AUC±95%CI: 0.614 (0.458 – 0.771), p=0.178

Successful digestion cutoff:

△ **≥50%** collagen digest.: AUC±95%CI: 0.632 (0.478 – 0.786), p=0.168

△△ **≥60%** collagen digest.: AUC±95%CI: 0.707 (0.568 – 0.845), p=0.016

△△△ **≥70%** collagen digest.: AUC±95%CI: 0.595 (0.440 – 0.750), p=0.079

Figure 3

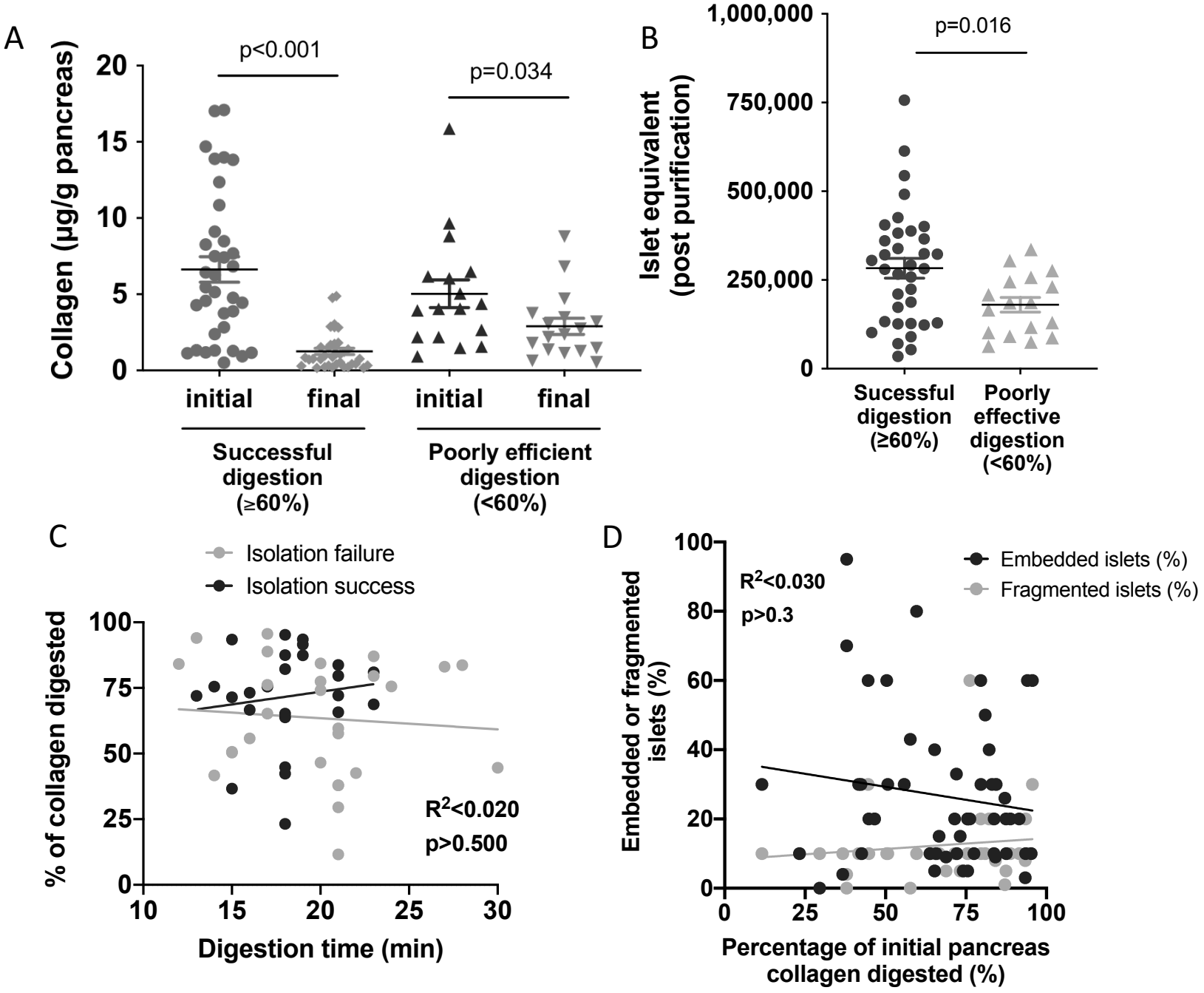
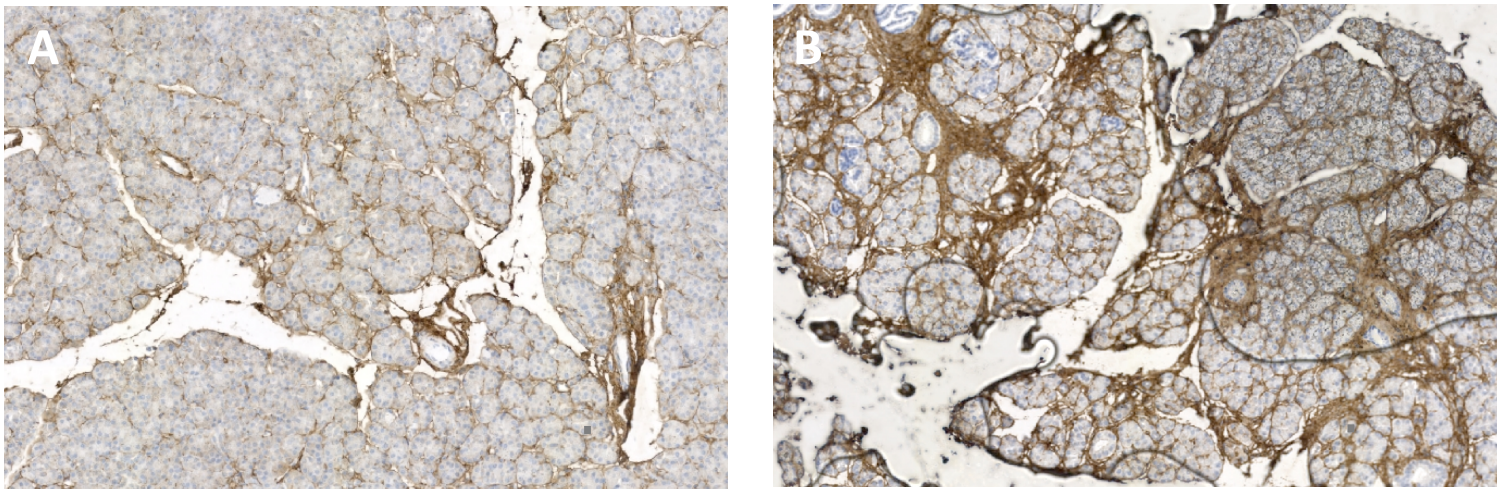

A
Collagen (µg/g pancreas)
20
15
10
5
0
p<0.001
p=0.034
initial
final
initial
final
Successful digestion (≥60%)
Poorly efficient digestion (<60%)
B
Islet equivalent (post purification)
1,000,000
750,000
500,000
250,000
0
p=0.016
Sucessful digestion (≥60%)
Poorly effective digestion (<60%)
C
Isolation failure
Isolation success
% of collagen digested
100
75
50
25
0
10
15
20
25
30
Digestion time (min)
R2<0.020
p>0.500
D
Embedded islets (%)
Fragmented islets (%)
Embedded or fragmented islets (%)
100
80
60
40
20
0
0
25
50
75
100
Percentage of initial pancreas collagen digested (%)
R2<0.030
p>0.3

Figure 4


A
B

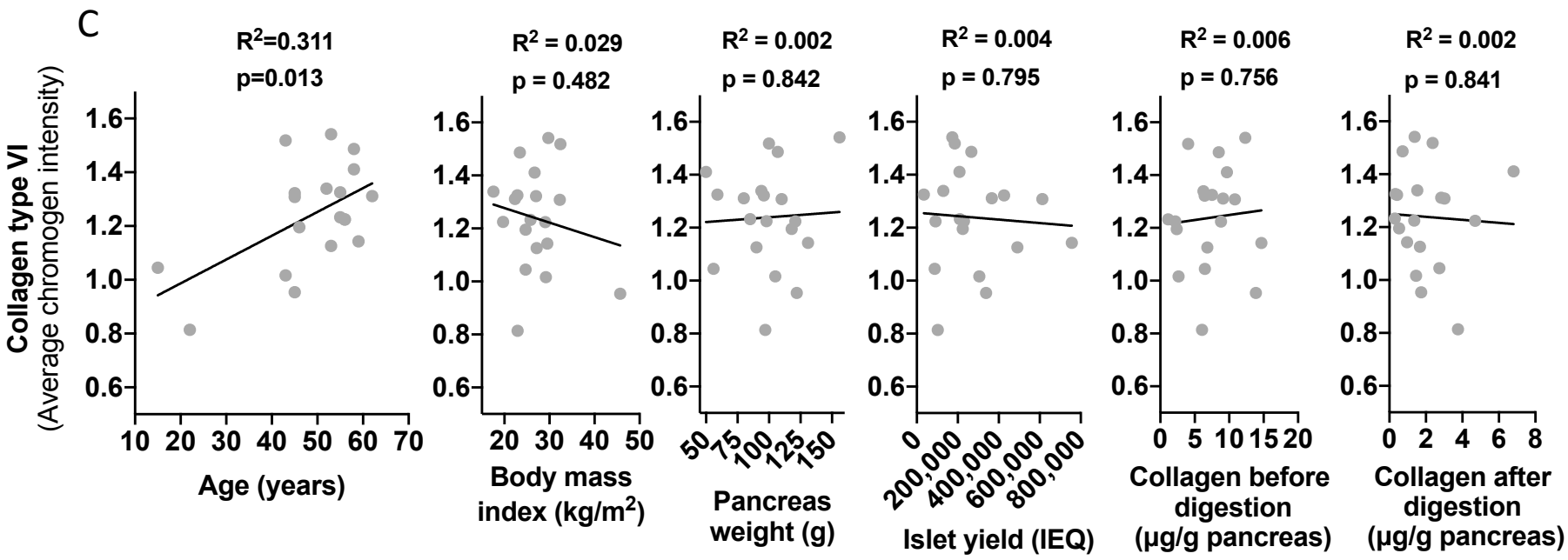
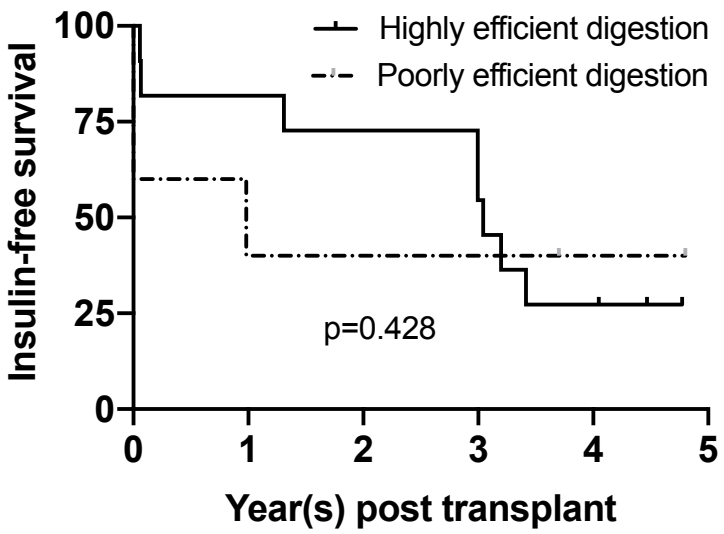
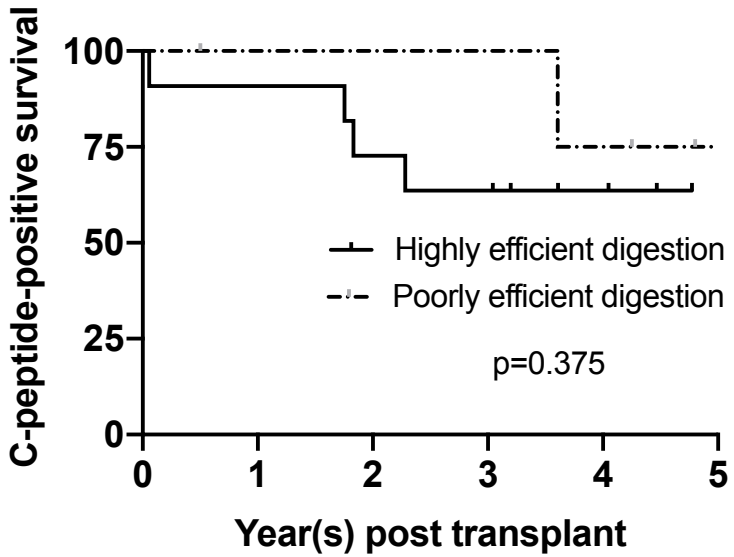

C
Collagen type VI
(Average chromogen intensity)
R2=0.311
p=0.013
Age (years)
R2 = 0.029
p = 0.482
Body mass index (kg/m2)
R2 = 0.002
p = 0.842
Pancreas weight (g)
R2 = 0.004
p = 0.795
Islet yield (IEQ)
R2 = 0.006
p = 0.756
Collagen before digestion (µg/g pancreas)
R2 = 0.002
p = 0.841
Collagen after digestion (µg/g pancreas)

Figure 5

A

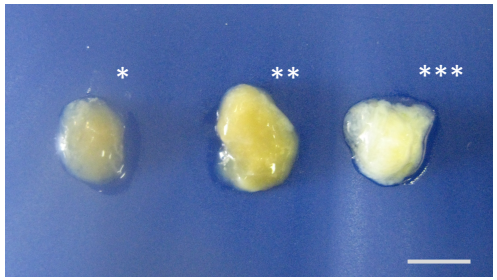

Insulin-free survival
100
75
50
25
0
0
1
2
3
4
5
Year(s) post transplant
Highly efficient digestion
Poorly efficient digestion
p=0.428

B

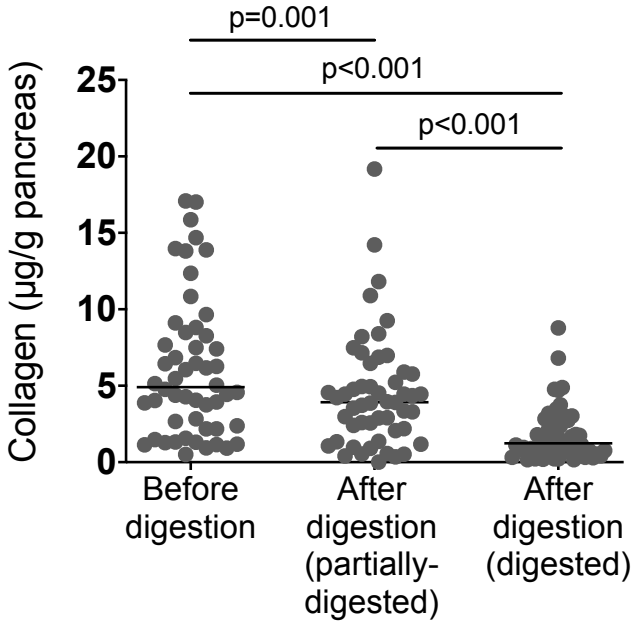

C-peptide-positive survival
100
75
50
25
0
0
1
2
3
4
5
Year(s) post transplant
Highly efficient digestion
Poorly efficient digestion
p=0.375

Supplementary Figure 1

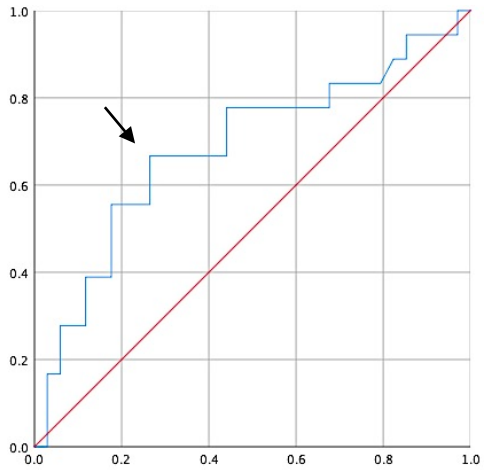
A



B



Supplementary Figure 2



Successful digestion cutoff:
60-80% of the initial collagen was digested
AUC±95%CI: 0.684 (0.522 – 0.846)
p=0.030