Transplant International

ORIGINAL ARTICLE

Pancreas collagen digestion during islet of Langerhans isolation—a prospective study

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ABSTRACT

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. The amount of pancreas collagen (I-V) was determined using colorimetry prior to and after the digestion process in 52 human islet isolations. Collagen I-V and VI were also assessed histologically. We identified a collagen digestion threshold of ≥ 60% as an independent factor beyond which an islet preparation has a ninefold increased odds of yielding ≥ 250 000 islet equivalents (IEQ) ($P = 0.009$) and a sixfold 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. We determined that a 60% pancreas collagen digestion is the threshold beyond which an islet isolation is likely to be successful and transplanted.

Transplant International 2020; 33: 1516–1528

Key words
collagen, collagenase, extracellular matrix, islet isolation, islet of Langerhans, islet transplantation, outcomes, type 1 diabetes mellitus

Received: 31 May 2020; Revision requested: 3 July 2020; Accepted: 20 August 2020; Published online: 28 September 2020

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 islet preparation

doi:10.1111/tri.13725

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 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 are 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.

Materials 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, predigested 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 counterstained 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 postpurification IEQ to prepurification 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" [1]. 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) (Fig. 1a, Figure S1A). All were used for the colorimetric collagen content assay. The individual pancreas collagen content of the three biopsy samples is shown in Figure S1B. One-third of the postdigestion yellow appearance tissue (marked ** in Fig. 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 Fig. 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 histologic sections from an additional predigestion biopsy (Fig. 1b). Histologic quantification of the collagen on tissue sections was not

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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 (Fig. 1c, $R^2 = 0.675$, $P < 0.001$). The quantification of the collagen surface on histologic sections significantly correlated with the colorimetric measurements using the Sircol method (Fig. 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 (Fig. 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 (Fig. 2b–e). The best ratio between "successful" and "failed" isolations appeared to range between 60 and 80% collagen digestion (Fig. 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 (Fig. 2h). Sixty percent remained the optimal cut-point value when setting the successful IEQ

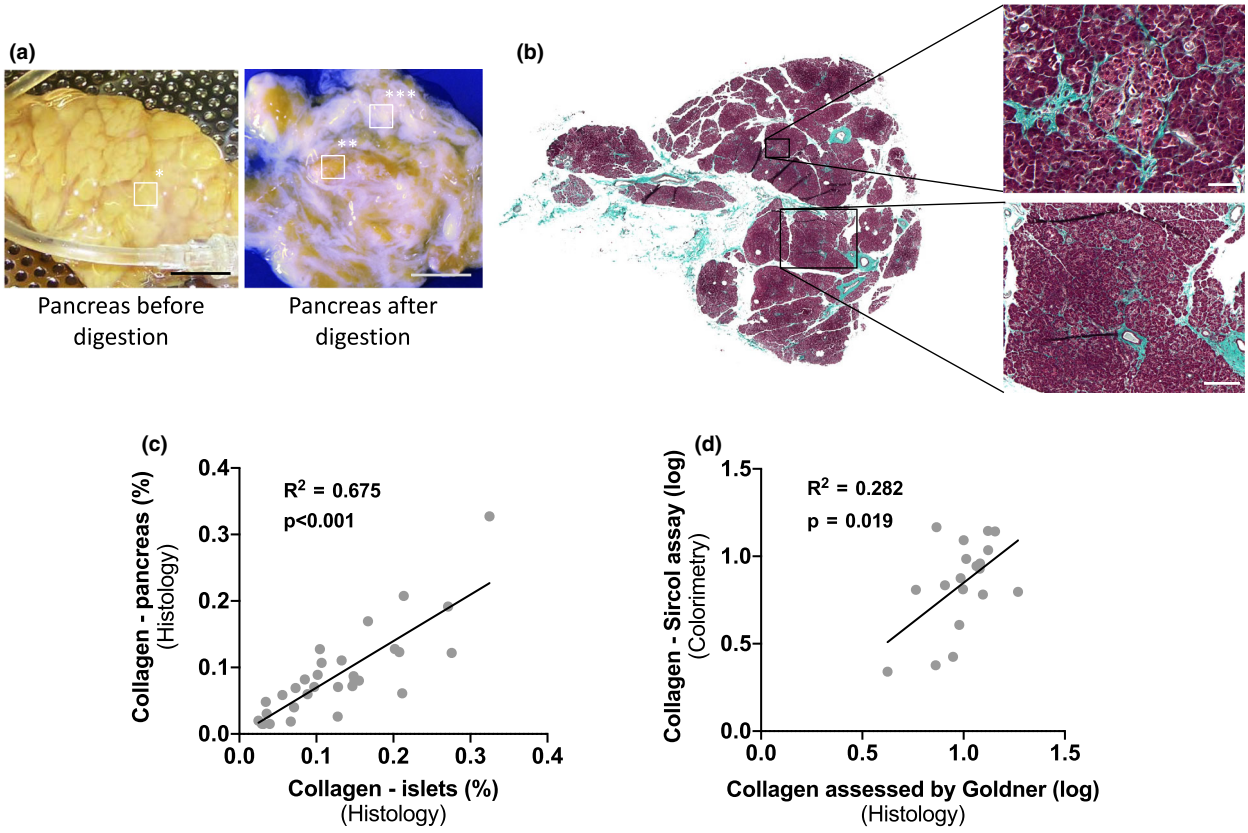

Figure 1 Histologic and colorimetric quantification of pancreas collagen. (a) Macroscopic view of the pancreas before and after digestion (scale bars: 1 cm). Areas where were taken *predigestion pancreas biopsy, **partially digested pancreas biopsy (yellow tissue appearance), and ***completely digested pancreas biopsy (white scaffold appearance) were analyzed postdigestion. (b) Representative pancreas section showing the whole biopsy (left panel) and an islet (right upper panel, scale bar: 50 μm) and exocrine pancreas (right lower panel, scale bar: 200 μm) 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

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% (Fig. 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, Figure S2). 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 (Fig. 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 (Fig. 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 (Fig. 3c). The relation between the percentage of initial collagen digested and the number of embedded/ fragmented islets is shown in Fig. 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) (Table S1). 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 Table S2. Overall, a successful digestion (i.e., ≥60% of initial collagen digested) and BMI ≥ 30 kg/m2 were independent factors associated with a nine- to 10-fold increase in odds of obtaining a successful isolation (i.e., final yield ≥ 250'000 IEQ) (Table S3). A successful digestion was the only independent factor significantly associated with the transplantation of an islet preparation (Table S4).

Assessment of collagen type VI

Since the Sircol assay only measures 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 (Fig. 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 (Fig. 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 (Fig. 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

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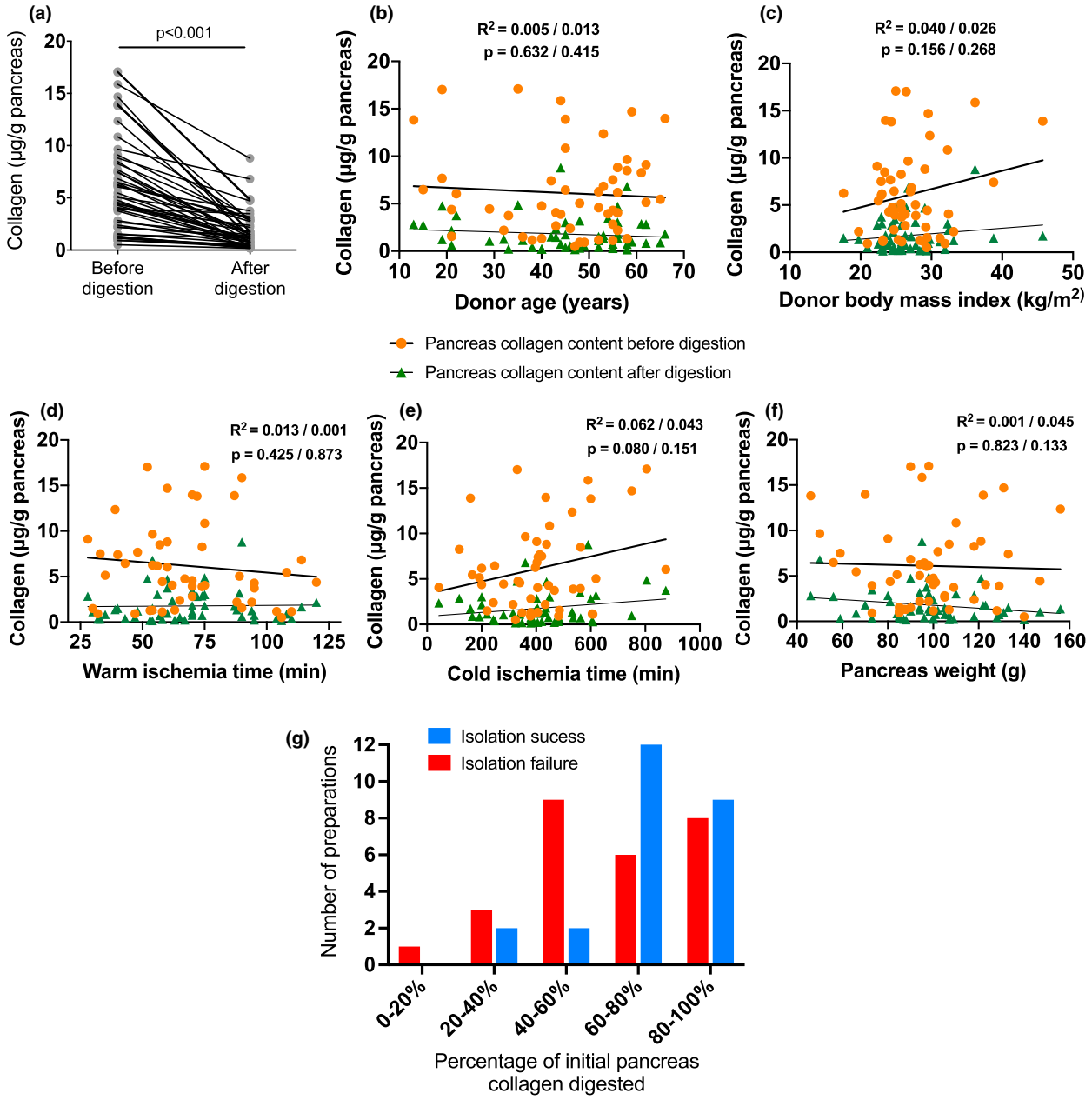

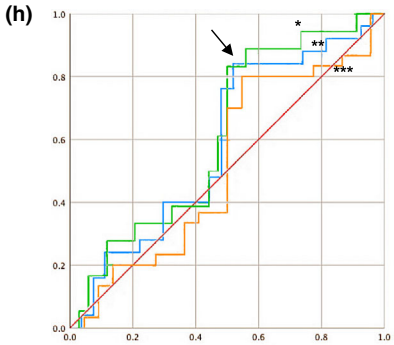

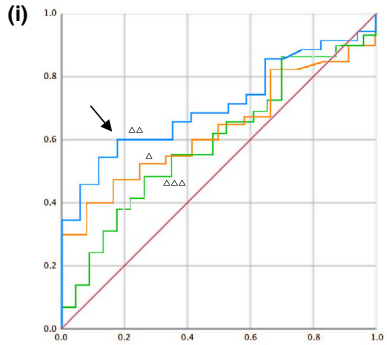

Successful isolation yield cutoff:

* **≥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 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). Group comparison was performed using paired Student's t-test. 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. R^2 and *P*-values were calculated using Pearson correlation. (g) Isolation success (i.e., a final yield of ≥ 250 000 IEQ) and failure are shown in function of percentage of collagen digested. (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

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

	Successful digestion* (n = 35)	Poorly effective digestion† (n = 17)	*P*-value‡
Age, year (mean ± SD)	47.4 ± 13.1	41.8 ± 14.6	0.168
Gender			
Male (%)	18 (51.4)	7 (41.2)	0.488
Female (%)	17 (48.6)	10 (58.8)	
BMI, kg/m^2	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 (%)	7 (20.0)	5 (29.4)	0.719
Cerebro-vascular (%)	26 (74.3)	11 (64.7)	
Suicide (%)	1 (2.9)	1 (5.9)	
Anoxia (%)	1 (2.9)	0 (0.0)	
Preservation solution			
UW (%)	5 (14.3)	0 (0.0)	0.431
IGL-1 (%)	21 (60.0)	12 (70.6)	
Celsior (%)	3 (8.6)	2 (11.8)	
Other (%)	6 (17.1)	3 (17.6)	

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

*≥60% of initial collagen content was degraded during digestion.

†<60% of initial collagen content was degraded during digestion.

‡Student's *t*-test or Mann–Whitney U-test for continuous variables and chi-square test for binary or categorical variables (global *P*-value).

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 ninefold 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 [29] and collagen IV [30] and to a lesser extent of fibronectin [31], collagen I [32], collagen III [32], collagen V [32], and collagen VI [11]. Of note, various types of integrins allow the islets to bind collagen fibers [30,33].

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

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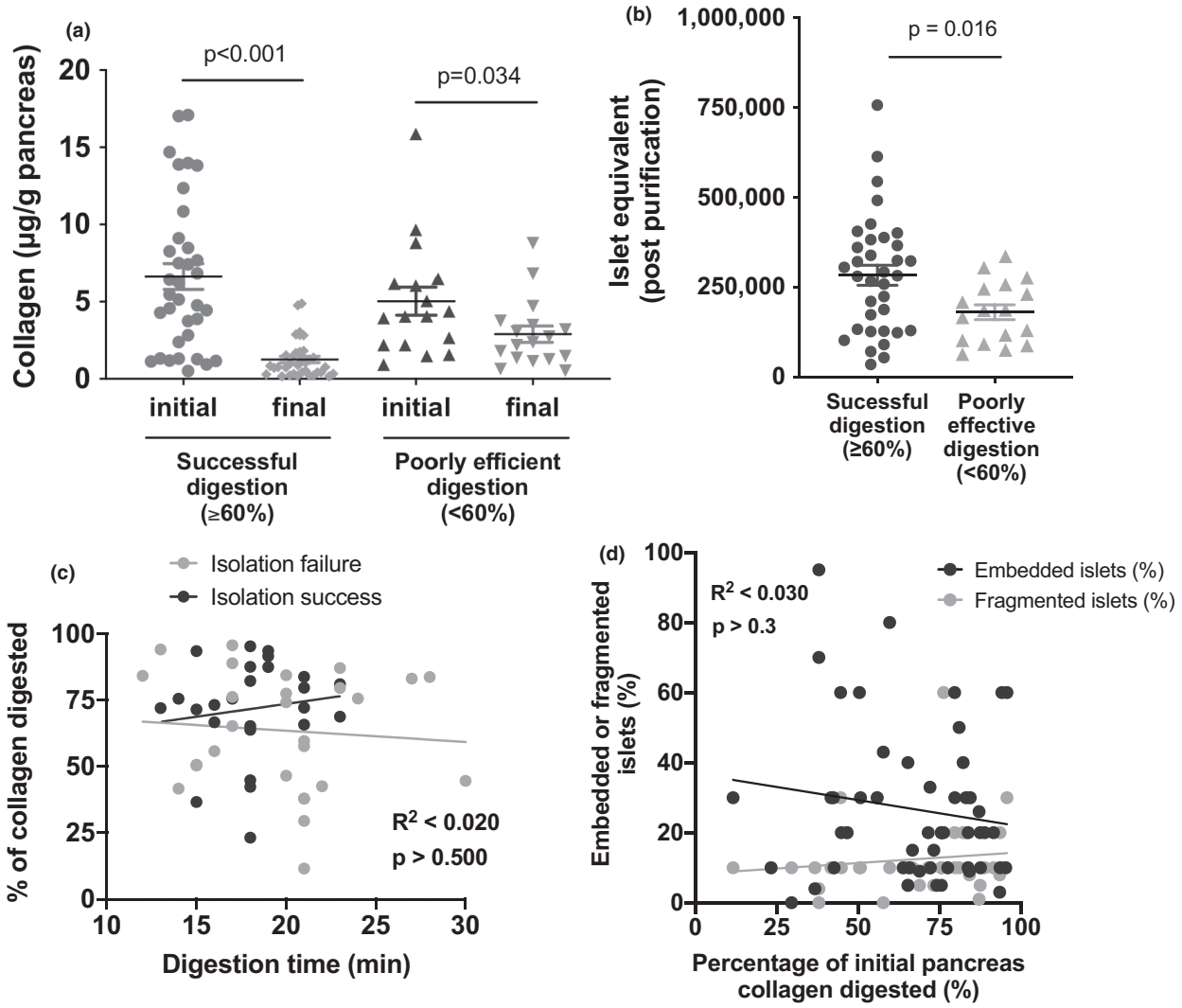

Figure 3 The percentage of pancreas collagen digestion can predict isolation outcomes. (a) Pancreas collagen content before (initial) and after digestion (final) in function of digestion efficiency. (b) postpurification final islet equivalent number in function of digestion efficiency. (c) Percentage 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

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 [34]. 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

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

	Successful digestion* (n = 35)	Poorly effective digestion† (n = 17)	P-value‡
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	0 (0.0)	1 (5.9)	0.753
Serva NB1, lot 2	6 (17.1)	3 (17.6)	
Serva NB1, lot 3	23 (65.7)	11 (64.7)	
Serva NB1, lot 4	1 (2.9)	0 (0.0)	
Serva NB1, lot 5	2 (5.7)	1 (5.9)	
Liberase, lot 1	3 (8.6)	1 (5.9)	
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, prepurification	423 964 ± 215 005	387 934 ± 152 768	0.539
IEQ, prepurification	392 159 ± 211 678	366 848 ± 218 935	0.689
IEQ per g pancreas (prepurification)	4408 ± 2257	4487 ± 2266	0.792
Mean prepurification islet size, µm	144.8 ± 53.4	138.6 ± 41.4	0.992
Embedded islets, %, prepurification	21.9 ± 16.2	36.6 ± 27.5	0.051
Fragmented islets, %, prepurification	12.9 ± 10.5	11.4 ± 8.3	0.853
Total islets postpurification	246 498 ± 123 877	150 706 ± 92 188	0.007
IEQ postpurification	283 017 ± 164 214	180 142 ± 85 397	0.016
IEQ/per g pancreas (postpurification)	3216 ± 1853	2297 ± 1108	0.066
Mean postpurification 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

IEQ: islet equivalent.

*≥60% of initial collagen content was degraded during digestion.

†<60% of initial collagen content was degraded during digestion,

‡Student's *t*-test or Mann–Whitney U-test for continuous variables and chi-square test for binary or categorical variables (global *P*-value).

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 [35]. 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 [36,37]. 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)

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

	Successful digestion* (n = 22)	Poorly effective digestion† (n = 5)	P-value‡
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

*≥60% of initial collagen content was degraded during digestion.

†<60% of initial collagen content was degraded during digestion.

‡Student's t-test or Mann–Whitney U-test for continuous variables.

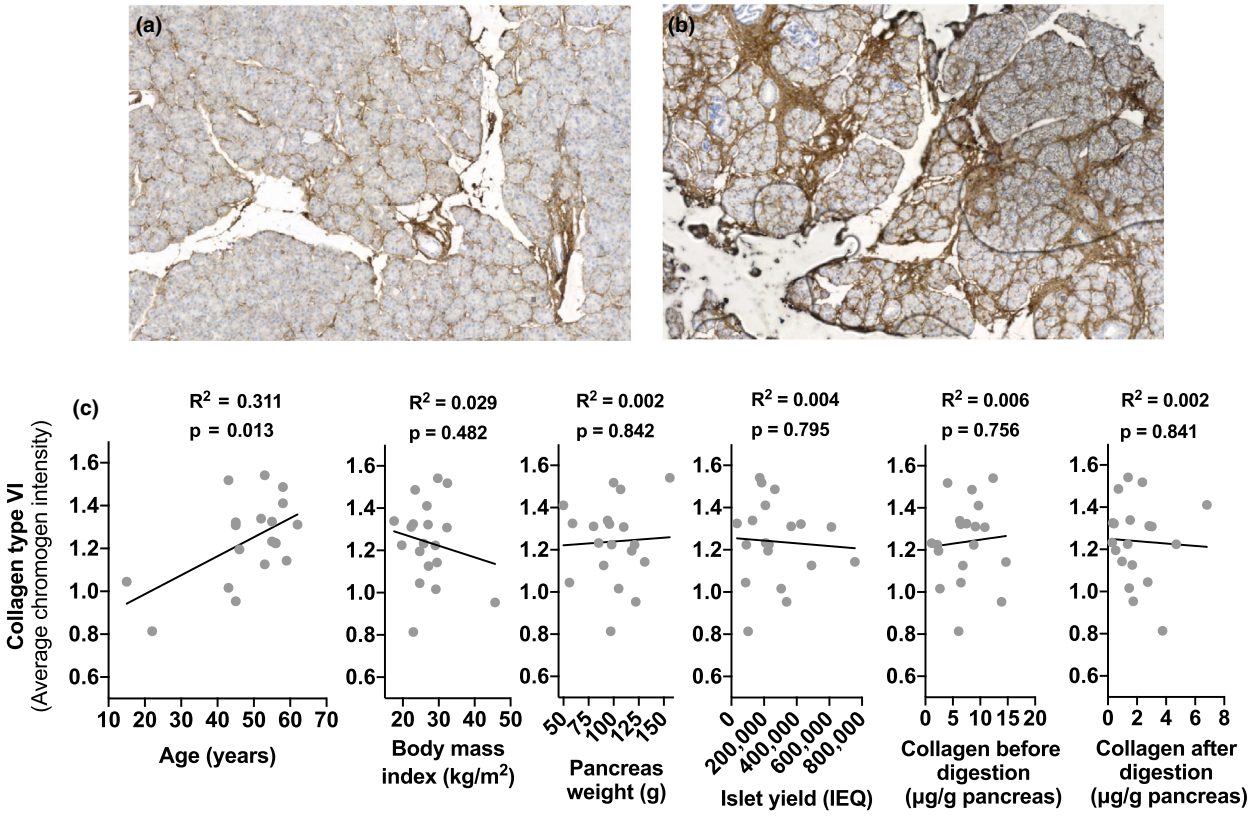

Figure 4 Collagen VI quantification in donor pancreases. Representative pancreas sections showing collagen IV stained by way of immunohistochemistry in a (a) young (15yo) and (b) older donor (52yo). (c) Pancreas collagen VI content in function of donor age, BMI, pancreas weight, final islet equivalent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

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[38], myocyte[39], hepatocyte[40], and mesenchymal stem cells [41]). 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

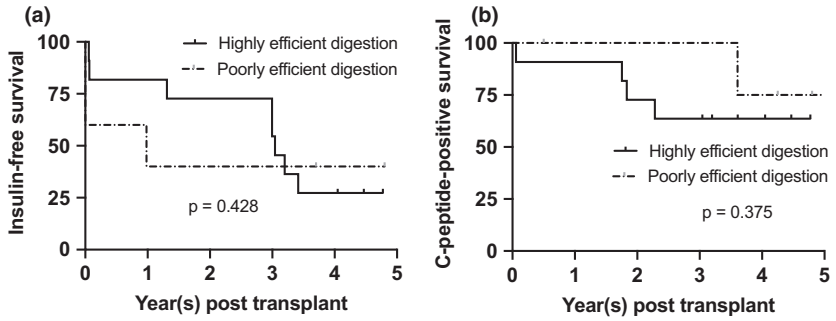

Figure 5 Survival curves for (a) insulin independence and (b) C-peptide positivity in preparation following successful versus poorly effective digestion. Gehan–Breslow–Wilcoxon test was used for survival curve comparison

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 [35].

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 [12,13,32,35]. Pancreas collagen could also be measured at other sites/steps (in the postdigestion 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.

Authorship

RM: designed the study. RM, JM, YM, and DB: performed the experiments. RM, JM, YM, BB, AA, NM, GP, SL, DB, and TB: 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: 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.

Funding

YDM was supported by the Swiss National Science Foundation (Advanced Postdoctoral Mobility Grant No. P300PB_174500).

Conflict of interest

None of the authors have any of conflict of interest to declare with regard to the content of this article.

Acknowledgments

We thank Solange Masson, David Matthey-Doret, Corinne Bosson, and Nadine Pernin for excellent technical assistance.

Data Availability Statement

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.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. (A) Macroscopic view of the pancreas biopsies taken *predigestion, and postdigestion, including **partially digested pancreas biopsy and ***completely digested pancreas biopsy (scale bars: 1 mm).

Figure S2. 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.

Table S1. Characteristics of donor pancreases stratified by successful isolation (final yield ≥ 250'000 IEQ) versus unsuccessful isolation (final yield < 250'000 IEQ).

Table S2. 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.

Table S3. Estimated odds ratios for isolation success (final yield ≥ 250'000 IEQ) using a binary logistic regression model.

Table S4. Estimated odds ratios for islet preparation to be transplanted using a binary logistic regression model.

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