Abstract Book

Table of Contents

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Oral Presentations

OP1_1

Long term metabolic outcomes and cardiovascular events after simultaneous pancreas and kidney transplant

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Background
Cardiovascular disease (CVD) is the primary cause of death in chronic kidney disease and diabetic patients. Simultaneous pancreas-kidney transplant (SPKT) improves patients' survival, but CVD remains a pivotal determinant of mortality. The aim of this study was to evaluate the metabolic control evolution and its influence on major adverse cardiovascular events (MACE) in SPKT.
Methods
We retrospectively studied 228 patients submitted to SPKT between January 1st 2000 and December 31st 2022 who had at least one year follow-up. Patients were stratified according to weight, hemoglobin A1c (A1c) and lipidic profile. MACE defined as myocardial infarction, percutaneous coronary intervention, stroke, and peripheral vascular disease requiring surgical intervention was the primary outcome.
Results
Out of the 228 patients, 120 were male, mean age at transplant was 35± 6years. At SPKT, hemoglobin A1c and total cholesterol were, 8,3±1.6% and 157±50mg/dL, respectively. At 1 year follow-up, mean hemoglobin A1c decreased approximately 3.1% to 5.3±0.6%. The remaining clinical features (recipient' s age, body mass index, time on dialysis, diabetes evolution) were similar between pre and post SPKT period.
MACE occurred in 40 (18%) patients after SPKT. These patients had a higher pre-transplant total cholesterol (161 vs 181mg/dL)(p=0.019) and higher total cholesterol (177 vs 150mg/dL)(p=0.022), LDL cholesterol (90 vs 74mg/dL)(p=0.014) and triglycerides (85 vs 117mg/dL)(0.025) at 1 year follow up. Hemoglobin A1c levels were similar between groups.
Conclusions
This study confirms that despite SPKT, patients remain at high risk of cardiovascular events. Lipidic profile correlate to MACE after transplantation. Consequently, lipid-lowering therapies should be implemented after SPKT and CVD factors should be meticulously managed.
Conflicts of interest
No conflicts declared

OP1_2

Cardiovascular Complications after Pancreas-Kidney Transplantation - A Single-Center Study

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Background
Patients (pts) with Type1 Diabetes (DM) and kidney failure have a high risk of adverse cardiovascular events (ACE). Pancreas-kidney transplantation (PKT) improves survival in these pts, but ACE may remain an important cause of morbimortality. We aimed to evaluate the prevalence of ACE after PKT and to analyze risk factors for their occurrence.
Methods
We performed a retrospective study that included 229 pts who underwent PKT between 2000 and 2022 in our transplant center. Only pts with both grafts functioning were considered. The frequency and risk factors for ACE were assessed.
Results
The study population was composed mostly by men (52.8%), with a mean age of 35.8 (20-52) years. Mean duration of DM was 24.4 (11-49)years. By the time of KPT, the prevalence of overweight/obesity, hypertension, smoking habits, peripheral artery disease (PAD), history of ischemic heart disease and cerebrovascular disease was 16.1%, 83.7%, 34.5%, 14.5%, 7.9% and 2.6%, respectively. The mean follow-up was 10.0 (1.0-23.6) years. During this time, 42 pts (18.3%) experienced an ACE, including stroke (n=9), myocardial infarction (n=11) and critical limb ischemia (CLI) requiring amputation (n=22). The average time since PKT to ACE was 6.8 years. Sensorimotor neuropathy and PAD at the time of KPT were associated with increased risk of ACE, especially CLI requiring amputation. We found that pts with history of ischemic heart disease developed ACE more frequently that pts without it (61.1% vs 17.3%, $p<0.01$). Regarding metabolic parameters, only high total cholesterol values at 3 months of PKT were associated with higher frequency of ACE. During follow-up, 33 pts died, 33.3% due to cardiovascular (CV) events.
Conclusions
ACE persist significant sources of morbimortality for PKT pts, especially for those with previous events. Pretransplant CV risk scoring and ankle brachial indices may help to identify pts who would benefit from risk factor optimization pre- and post-PKT.
Conflicts of interest
No conflicts declared

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OP1_3

A multicentric study of histomorphological characteristics of unaccepted organs for pancreas transplantation

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Background

The main limitation in the expansion of pancreas transplantation programs lies in the quality of the pancreatic graft, being macroscopic appearance the main cause of discardment (79%) based on the National Transplant Organization data. However, no objective data exists to justify this rationale. The aim of this study is to determine the histopathological characteristics of organs initially accepted for evaluation but subsequently discarded for clinical transplantation.

Methods

A prospective multicenter study was carried out including 129 non-viable organs from 4 national centers. Human pancreas allografts were recovered from DBD (76%) or cDCD (24%) and kept in formaldehyde for subsequent histopathological analysis.

Results

Out of the total, 61 organs were initially accepted for evaluation following the standard donor criteria1 but were discarded afterwards mainly due to macroscopic appearance (85%). Baseline characteristics of these donors included: 71% males; median age of 44 (35-48) years old; BMI of 24.9 (22.9-27.4) kg/m²; being cardiovascular disease (46%) the main cause of death. After histopathological analysis, parenchymal fibrosis was seen in 77% of cases, 85% presented adipose infiltration, 79% chronic inflammatory infiltration and 25% pancreatic necrosis. Diffused immunohistochemical expression of insulin, glucagon and somatostatin in pancreatic islets were present in 88.5%, 93.4% and 85.2%, respectively, translating into preserved endocrine functionality. However, significant histopathological changes associated with worse organ viability, defined as moderate (>25-50%) and/or severe (>50%) alterations, were seen in 17 (28%) specimens being adipose infiltration the most frequent finding in up to 7 cases, adipose plus chronic inflammatory infiltration in 3, adipose infiltration plus parenchymal fibrosis in 1, parenchymal fibrosis in 5 and necrosis in 1 organ. Analysis of the subgroup of potentially viable organs evidenced that 36 out of 44 pancreases (82%) were discarded for their macroscopic appearance.

Conclusions

Analysis of pancreases deemed unsuitable for clinical transplantation demonstrates that the percentage of organs discarded due to macroscopic appearance that were in fact, potentially viable according to histomorphological study, is not despicable.

Conflicts of interest

No conflicts declared

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OP1_4

Automated insulin delivery systems versus pancreas transplantation in type-1 diabetes treatment

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Background

We performed a prospective cross-sectional study to compare the effect of widely used automated insulin delivery (AID) systems with pancreas transplantation on the metabolic control parameters in type-1 diabetic patients.

Methods

31 patients after pancreas and kidney transplantation (SPK) were enrolled. They had an excellent kidney graft function (GFR 1.2 ± 0.3 ml/s/1.73 m2) (mean ± SD) and received 1 month after Tx a glucose sensor. Neither insulin nor antidiabetic drugs were administered. 377 patients were treated with AID: either the Medtronic Minimed 780G (n=200) or Tandem t-slim x2 Control IQ - CIQ (n=177). Patients after SPK were significantly older 40 ± 14.6 vs. 46 ± 9.4 years (p=0.0069), of longer diabetes duration (23 ± 11.2 vs. 30 ± 9.8 years) (p=0.00096) and had reduced GFR (1.61 ± 0.4 vs. 1.2 ± 0.3 ml/s/1.73 m2) (p=3.2x10-9) in comparison with the AID users. Mann-Whitney and Student´s t tests were used for statistical analyses.

Results

CIQ and 780G users did not differ significantly in HbA1c, mean glycemia nor SD. A notable difference was found in % of time in range (TIR) (glycemia 3.9–10 mmol/l) favorizing slightly the 780G; 72 ± 13.7 % and 75 ± 14.5 %, resp. (p=0.0051). According to close outcomes of both systems we took them as a one cohort. SPK patients had significantly lower HbA1c; 38 ± 5.8 vs. 55 ± 10 mmol/mol (p = 1.2x 10-17), mean glycemia was 6.4 ± 1 over 8.3 ± 1.4 mmol/l (p = 2x10-12), SD was 1.46 ± 0.54 vs. 2.7 ± 0.9 (p=2.4x10-13) and TIR was 90 ± 10 over 73.5 ± 14 % (p=8.2x10-11). SPK patients also spent much less time in glycemia above 10 mmol/l; 5.1 ± 7.8 over 18.8 ± 9.8 % (p = 1.3x 10-12). Time in hypoglycemia (below 3.9 mmol/l) did not differ significantly; 4 ± 5.4 vs. 2 ± 2.7 % (p=0.14). Function of the pancreatic graft remained stable (HbA1c 3 months after Tx was 39 ± 4.2 mmol/mol). 6.4% of patients treated with the AID systems had mean glycemia as good as the SPK patients, 3.2% of AID had the same TIR as SPK and 4.8% of AID users had as good HbA1c levels as the SPK patients.

Conclusions

Type-1 diabetic patients one month after pancreas and kidney transplantation had significantly better results in all important parameters of diabetes control in comparison with the automated insulin delivery systems except for hypoglycemia, where the automated systems have proven to be very safe.

Conflicts of interest

No conflicts declared

References

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OP1_6

Reducing Neutral Protease Dose During Islet Isolation Does Not Impair Islet Recovery From Pancreases Retrieved for Autologous Use

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Background

Total Pancreatectomy with Islet Auto-Transplantation (TPIAT) is an effective treatment for selected patients with severe chronic pancreatitis. Islets are isolated using a blend of collagenase and neutral protease (NP) enzymes. Due to pancreas fibrosis, most islet isolation labs use high concentrations of NP for auto islet isolation (~200 Units). However, increasing concentrations of NP can result in islet fragmentation and reduce islet viability. Here, we investigate the impact of using a lower protease dose on autologous islet isolation outcome.

Methods

Following total pancreatectomy, islets were isolated using enzymes mixtures that consisted of Vitacyte collagenase (~2,000 Units) and either 100 Units (U) or 200 U of Nordmark NP (n=5 per group). Islets were assessed for purity (%), viability (%) and number (IEQ) then infused trans-hepatically. Graft function was assessed at 6-months post-infusion. Statistical differences between the groups were determined by Mann Whitney Test. Results are presented as average ± standard deviation.

Results

All 10 pancreases were severely fibrotic with calcification. Recipient age was statistically matched between the two protease groups (100U: 35±13 years and 200U: 33±10 years). Patient and pancreas weight were also comparable. Although final islet yield was higher in the 100U group, this did not reach statistical significance (4,874±3,528 IEQ/g vs 3,268±2,406 IEQ/g). Average preparation purity was 9% in both groups, and NP dose did not significantly impact islet viability (100U: 76±4% vs 200U: 73±5%). Final tissue volume was statistically comparable across the two groups (100U: 6.9±4.2ml vs 200U: 6.4±4.6ml). NP dose did not affect post-transplant outcomes at 6-months.

Conclusions

Our preliminary experience demonstrates the feasibility of using a decreased dose of protease when isolating islets from significantly fibrotic pancreases retrieved for autologous use. Using reduced protease would result in cost saving, and our data suggests it might reduce islet fragmentation and increase islet viability. However, enzyme selection must be considered carefully for each individual organ.

Conflicts of interest

No conflicts declared

OP1_7

Islet function loss following total pancreatectomy and islet autotransplantation (TPIAT)

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Background
TPIAT is a last resort treatment to alleviate pain, improve quality of life and (partially) preserve pancreatic endocrine function for patients with chronic pancreatitis. A significant number of islets is lost during isolation and transplantation. The goal of this study was to compare the islet secretion capacity before and after TPIAT.
Methods
A multidisciplinary team assessed eligibility for TPIAT. The islet isolation took place in a Good Manufacturing Facility (GMP). The islets were infused into the portal vein after percutaneous transhepatic catheterization. A two-hour mixed meal tolerance test (MMTT) was administered at baseline, 3 months and 1 year postoperatively to assess beta cell function.
Results
Of 21 consecutive TPIAT patients, fifteen had data available at baseline and 3 months and were included in this analysis. All patients underwent TPIAT for chronic pancreatitis. The mean age was 42.1 ± 11.7 years and 13 patients were female. Mean BMI decreased from 24.5 ± 4.8 at baseline to 22.9 ± 4.5 kg/m2 after three months ($p<0.001$). Patients received a median (IQR) of 3464 (2800-4648) islet equivalents per kilogram bodyweight. Area under the curve (AUC) C-peptide during MMTT decreased from 175.6 ± 85.8 at baseline to 77.3 ± 49.2 at 3 months ($p<0.001$). AUC C-peptide/AUC glucose ratio was reduced from 0.21 ± 0.11 at baseline to 0.06 ± 0.05 at 3 months ($p<0.001$). For those patients with 1 year data, AUC C-peptide was similar at 3 months to 1 year (83.4 ± 51.3 vs 80.1 ± 62.0, $p=0.76$, $N=13$). Of 12 patients who were insulin independent at baseline, 2 remained insulin independent at 3 months after TPIAT.
Conclusions
TPIAT leads to more than 50% loss in islet secretory capacity. No further reduction in islet mass is observed up to 1 year.
Conflicts of interest
No conflicts declared

OP1_8

Impact of diabetes mellitus on quality of life in patients with or without islet autotransplantation after total pancreatectomy

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Background

Islet autotransplantation (IATx) is an optional treatment for patients undergoing total pancreatectomy (TP) for non-malignant reasons. According to reported data, the general quality of life significantly improves (based mainly on pain relief). In 30% of cases, the IATx prevents diabetes development. The rest of the patients remain on less or more intensive insulin therapy. Therefore, the presented study aimed to determine the impact of diabetes on the patient quality of life.

Methods

Patients were divided into two groups and monitored at least for three months. The first one included patients after TP treated with IATx (n=9). The second group comprised patients after TP without subsequent IATx (n=9). All of them filled out the ADQOOL questionnaire validated for quality of life in patients with diabetes. Six parameters evaluated diabetes compensation: (The mean HbA1c, The mean time in range (TIR), The mean glycemia, Variation coefficient, Time in hypoglycemia and hyperglycemia).

Results

IATx improved significantly the diabetes compensation in comparison to the control group (the mean HbA1c was 50.4 ±8 vs. 66.2 ±13 mmol/mol, p=0,036; Time in range 84 ± 15 % vs. 51.4 ± 19 % p= 0.0017). Their mean glycemia was lower (7.4 ± 1.4 mmol/l vs. 10.2 ± 2.1 mmol/l, p=0.004), and the variation coefficient was better (25 ± 5 % vs. 37 ± 5 %). Time in hypoglycemia did not differ between groups (2.4 ± 2.1 % vs. 4.6 ± 3.5 % p=0.15).

In general, patients after IATx felt their quality of life was “very good” in comparison to people after TP who consider their lives as “not good, not bad” (average weighted impact score AWI 2 ± 0.6 vs. 0.6 ± 1.0). If they did not have diabetes, they would feel their lives were the same (AWI score -0.6 ± 0.48 for the AITX group vs. -2 ± 0.63 for the TP only group).

Conclusions

IATx resulted in significantly better diabetes compensation and significantly reduced perception of diabetes negative impact on their life well-being.

Conflicts of interest

No conflicts declared

References

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OP1_9

Long-term Intention-to-Treat Analysis of Autologous Pancreatic Islet Cell Transplantation for Enhanced Glycaemic Control Following Pancreatectomy: A 15-Year Study of the Milan Protocol

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Background

Pancreatogenic diabetes, resulting from pancreatic tissue loss after pancreatectomy, is a challenge for patients undergoing pancreatic surgery. Islet autotransplantation (IAT) offers a potential solution to prevent or alleviate pancreatogenic diabetes, but until now was indicated only in subject with painful chronic pancreatitis

Methods

This study presents a 15-year clinical program experience with the Milan Protocol, which expands IAT after pancreatectomy to a broader range of patients with malignant and non-malignant pancreatic diseases. The analysis evaluates IAT's feasibility, efficacy, and safety.

Results

Between November 2008 and June 2023, IAT procedure was performed on 114 out of 147 candidates. IAT related complication occurred in 19 out of 114 patients (16.7%) and 5 were potentially serious. IAT significantly prolonged the duration of diabetes-free survival in both partial ($p<0.001$) and total pancreatectomy patients ($p<0.001$), reduces the risk of insulin dependency following surgery (HR 0.52, 95%CI 0.30-0.90, $p=0.02$), and prevented the loss of sustained C-peptide secretion (HR 0.16, 95%CI 0.088-0.28, $p<0.001$). Moreover IAT independently decreases the risk of death (HR 0.39, 95%CI 0.019-0.79, $p=0.009$) and significantly prolongs survival in patients with malignant neoplasia ($p=0.0031$), while no significant effect was observed in patients with non-malignant neoplasia ($p=0.23$). Although not statistically significant, a trend toward improved disease-free survival is observed, potentially associated with an increased likelihood of receiving adjuvant treatment

Conclusions

These findings provide insights into the benefits and of IAT as a therapeutic option for pancreatogenic diabetes after pancreatic surgery beyond painful chronic pancreatitis.

Conflicts of interest

No conflicts declared

References

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OP1_10

Examining the Long-term Metabolic Outcomes of β-Cell Replacement Therapy Following Islet Autotransplantation: An Analysis from the Milan Protocol Experience

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Background
Islet autotransplantation (IAT) is a promising approach to preserve beta-cell function in patients undergoing pancreatic surgery. This study aimed to assess the long-term metabolic outcomes of IAT according to the Milan Protocol and validate the modified Igls Criteria for evaluating beta cell graft function.
Methods
The research encompassed 114 patients who underwent IAT between November 2008 and June 2023, with only minor adjustments made to the Igls Criteria. The modified classification was validated through metabolic tests, including Arginine tests and Mixed Meal Tolerance Tests. Patients were further classified based on the extent of pancreatectomy (total vs. partial).
Results
Over the 10-year follow-up period (median 6.3 years), patients exhibited sustained C-peptide secretion, with a prevalence of optimal and good beta cell function. Metabolic tests confirm the differentiation of Igls categories. Among those who underwent partial pancreatectomy, superior metabolic outcomes were observed, including sustained C-peptide secretion and a reduced risk of developing diabetes or insulin dependence compared to those who underwent total pancreatectomy. For patients who had total pancreatectomy, the quantity of infused islets and tissue volume were identified as critical factors influencing metabolic outcomes
Conclusions
In conclusion, this study offers valuable insights into the long-term metabolic effects of IAT and validates the effectiveness of the modified Igls Criteria in evaluating beta cell function. The extent of pancreatectomy and islet yield play pivotal roles in achieving favorable metabolic results. These findings lay a solid foundation for further research and advancements in optimizing the efficacy of IAT for individuals with pancreatic diseases other than chronic pancreatitis.

Conflicts of interest
No conflicts declared

References
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OP2_1

Redefining donor eligibility criteria: Expanding pancreatic transplant horizons

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Background

The primary obstacle to expanding pancreatic transplant programs is the low number of ideal grafts. In Spain, 43% of potential pancreatic donors were discarded in 2022 from the beginning due to strict baseline trait cut-offs: age > 50 years old (45 years old in cDCD) or BMI > 30 kg/m2. However, in our country, the organ viability for transplantation in donors not complying with these criteria has not been further studied.

This study aims to evaluate pathological pancreas characteristics in donors who fall outside the current criteria but are still within an “acceptable expansion range” (group 1): BMI 30-35kg/m2 or age 50-60 years old. Viability was also studied in “non-eligible” donors: BMI >35kg/m2 and/or age > 60 years-old (group 2).

Abbreviations: donation after brain death (DBD), Controlled donation after circulatory death (cDCD).

Methods

A prospective multicenter study was conducted involving four Spanish pancreas transplant groups. A total of 129 grafts non-valid for transplant were registered, 68 being discarded from the beginning due to exclusion criteria (“marginal donors”). Organ “viability” has been defined according to four histomorphological findings: parenchymal fibrosis (PF) <25%; adipose infiltration (AI) <25%; T-lymphocytes infiltration (CI) < 25% and absence pancreatic necrosis (PN)) and pancreatic islets viability (immunohistochemistry positivity for insulin, glucagon, and somatostatin).

Results

Among marginal donors (n=50, DBD; n=18 cDCD), 17 (25%) pancreases met all the histopathological criteria for viability. In group 1 viability was 26,5% (9/34); while in group 2 viability was 23,5% (8/34). In terms of type of donor, viability was 22% in the DBD group, and 33% in the cDCD group.

Conclusions

This study highlights the potential for pancreas viability among donors falling outside standard criteria, showing a potential acceptable expansion range of 26.5% in marginal donors, and a 23.5% in organs otherwise not considered for clinical transplant. This finding suggests the need for reconsideration of donor exclusion criteria to expand pancreatic transplant opportunities.

Conflicts of interest

No conflicts declared

References

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OP2_2

Insulin independence following islet transplantation improves long-term metabolic outcomes

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Background

Pancreatic islet allotransplantation is an emerging and effective method of beta cell replacement in Type 1 Diabetes Mellitus, restoring glycaemic control and hypoglycaemic awareness. The rate of insulin independence following islet transplant varies between centres. Having surpassed 10 years of islet transplant in Scotland, we aimed to evaluate the effect of insulin independence following transplant on long-term graft survival and metabolic outcomes

Methods

A retrospective analysis was conducted on data collected prospectively between 2011 and 2022. All patients who underwent islet transplantation in Scotland and consented to follow-up were included. Primary endpoint was graft survival (90-minute C-peptide >50pmol/L). Secondary endpoints were GOLD score, HbA1c, BETA-2 score, Insulin requirement. Metabolic outcomes were compared at 1 year following transplant and long-term graft survival analysis was conducted

Results

60 patients were included. There was a 55% reduction in insulin requirement following transplant and 30% achieved insulin independence. Insulin independence was associated with significantly improved graft function at 1 year following transplant according to the igls critera. Mean graft survival was 9.0 years in the insulin-independent group compared to 4.4 years in the dependent group

Conclusions

In the largest UK single-centre study on islet transplant to date, we have demonstrated significantly improved outcomes in patients who achieve insulin independence following islet transplant

Conflicts of interest

No conflicts declared

OP2_4

Primary graft function and 5-year insulin independence after pancreas and islet transplantation for type 1 diabetes: a retrospective parallel cohort study

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Background

Allogeneic pancreas (PTx) and islet transplantation (ITx) have emerged as the best alternatives to treat the most severe forms of T1D (1). Assessing and predicting long-term graft function is an important objective for optimizing patient outcomes. In the field of ITx, long-term success has been related to the early estimate of transplanted beta-cell function, also named primary graft function (PGF) (2-3). The objective of the present study was to analyze and compare the potential association of primary graft function estimated soon after transplantation, and the 5-year rate of insulin independence in patients receiving an ITx and, for the first time, in patients receiving PTx.

Methods

This retrospective multicenter cohort study evaluated PGF in PTx and ITx recipients to assess its impact on 5-year insulin independence rates. Participants from two French cohorts were included, alongside normoglycemic, non-transplanted controls. PGF was measured early following transplantation with Beta-2 score and patients were followed up to 5 years. We then compared the predicted 5-year insulin independence rates using a validated PGF-based calculator to the observed rates in these recipients (3).

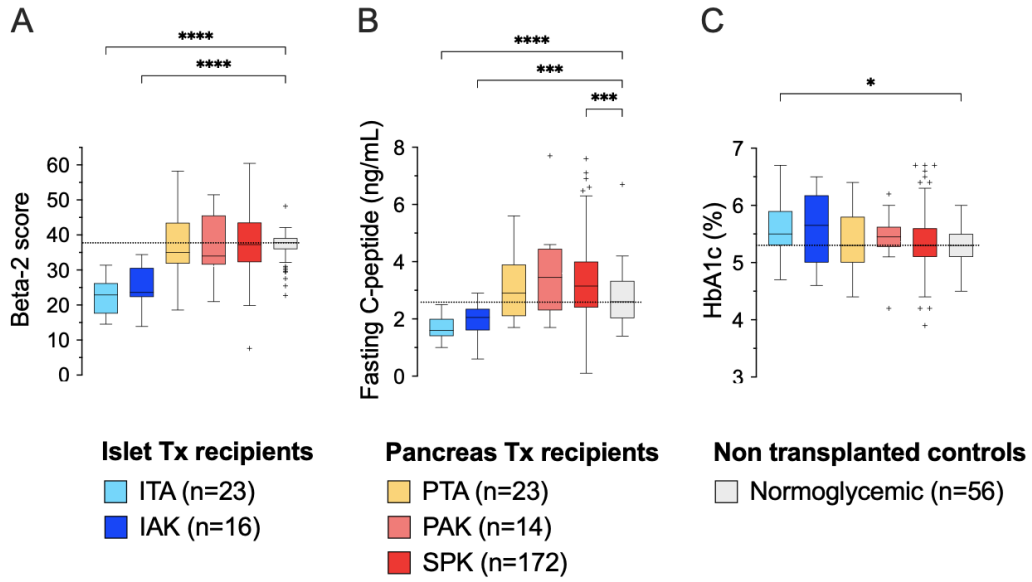
Results

PGF was comparable between ITA/IAK and PTA/PAK/SPK groups, with PTx recipients showing higher PGF than ITx recipients. ITx recipients had lower PGF compared to normoglycemic controls, while PTx recipients' PGF was similar to controls (Fig. 1). The study's PGF-based calculator precisely predicted 5-year insulin independence rates for ITx and solitary PTx but underestimated them for SPK recipients (Fig. 2).

Conclusions

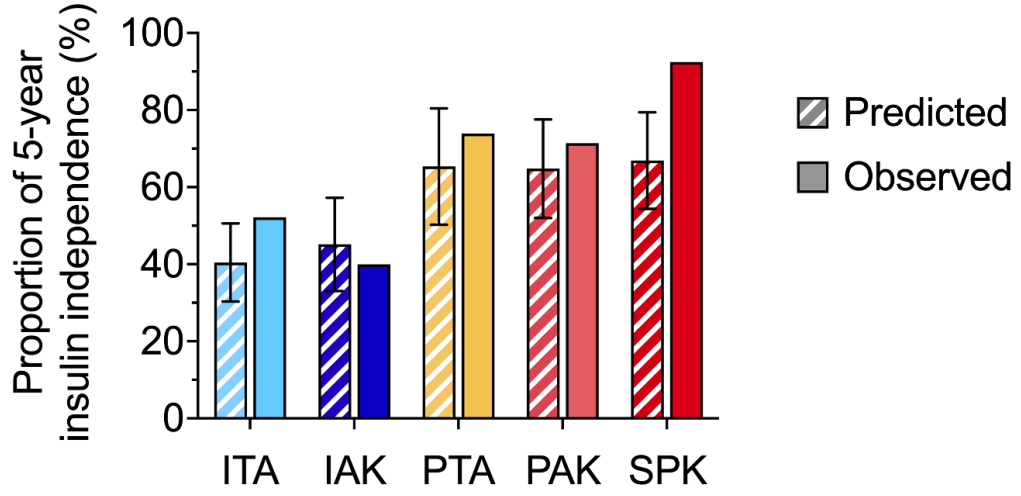
The study highlights the potential of PGF as an early indicator of long-term post-transplant success, especially in solitary pancreas recipients. Despite limitations, the findings suggest that optimal PGF is indicative of better graft function and higher chances of maintaining long-term insulin independence, underscoring its importance in managing T1D treatment.

Figure 1: Beta-2 score (A), fasting serum C-peptide (B) and HbA1c (C) values in the islet recipients, pancreas recipients and non-transplanted controls.



The distribution is represented in the form of a box plot using the Tukey method, where the line in the middle of the box is drawn at the median, the box limits represent the 25th and 75th percentiles, and the whisker limits are represented from the value of the 25th percentile minus 1.5 times the interquartile range (IQR) to the value of the 75th percentile plus 1.5 times the IQR. Outliers are represented individually. P values ≤ 0.001 are summarized with an asterisk. Groups were compared with Welch ANOVA tests. Symbol meaning: P ≤ 0.05 (*); P ≤ 0.01 (**); P ≤ 0.001 (***); P ≤ 0.0001 (****)
ITA= Islet Transplantation Alone; IAK= Islet After Kidney transplantation; PTA= Pancreas Alone; PAK= Pancreas After Kidney; SPK= Simultaneous Pancreas-Kidney

Figure 2: Predicted and observed proportion of 5-years insulin independence rates in in the islet and pancreas recipient subgroups.



Mean insulin independence predicted with 95% CI (hatched bar) and mean observed insulin independence (solid bar) are reported for the different recipients.
IA= Islet Transplantation Alone; IAK= Islet After Kidney transplantation; PA= Pancreas Alone; PAK= Pancreas After Kidney; SPK= Simultaneous Pancreas-Kidney

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Conflicts of interest
No conflicts declared

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OP2_5

Feasibility, Safety, and Function of Endoscopic Transplantation (ETx) of Endocrine Cells in the Digestive Submucosa: A Preclinical Study in Minipigs

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Background

After two decades of research, intraportal pancreatic islets transplantation is a standard treatment for unstable type I diabetes and reimbursed in France since 2021. This route is recommended despite the risk of thrombosis, hemorrhage and IBMIR's damage to nearly half of the graft. The gastric submucosa, accessible for endoscopic transplantation (ETx) has emerged as a promising alternative with encouraging results in preclinical and clinical studies.

This study aimed to assess the feasibility, safety and function of ETx into digestive submucosa using different cellular models in minipigs.

Methods

We modelized islets with inert microbeads and different cells. First we infused varying volumes (2, 1, 0.5 mL) and concentrations (100%, 50%, 33%) of inert microbeads (EMBOGOLD®, size 50-500µm) into the submucosa of pig stomach and rectum ex vivo organs to study diffusion patterns. Then ETx of autologous adipose cells to evaluate route safety and cell survival. We then evaluated autologous parathyroid cells transplantation, monitoring calcium and parathormone (PTH) levels, and using functional imaging with PET-choline. Finally, we transplanted autologous islets, with subsequent monitoring of insulin and glycemic levels after a glucose tolerance test (IVGTT).

Results

Our results showed limited diffusion of injected material, indicating the necessity for multiple infusions of small volumes. No morbidity or mortality occurred after adipose cells autotransplantation and without necrosis in histological examinations. In minipigs transplanted with parathyroid cells, there was no major hypocalcemia, PTH levels were signficantly higher at time of sacrifice following total parathyroidectomy compared to those without transplantation and PET choline imaging revealed graft survival in the submucosa (Figure 1). Finally, in a minipig transplanted with islets, insulin secretion was measured during IVGTT after local pancreatectomy (Figure 2).

Conclusions

Our study highlights the safety, reproducibility and potential benefits of ETx in the digestive submucosa in different models of endocrine cell transplantations.

Figure 1 & 2

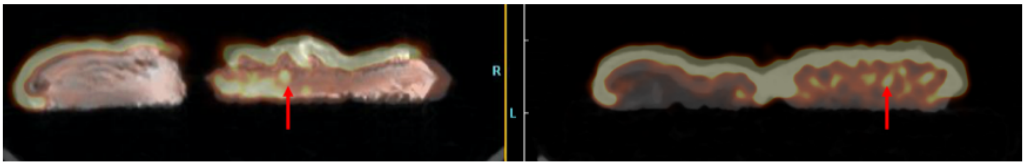

Figure 1 : Choline PET Imaging

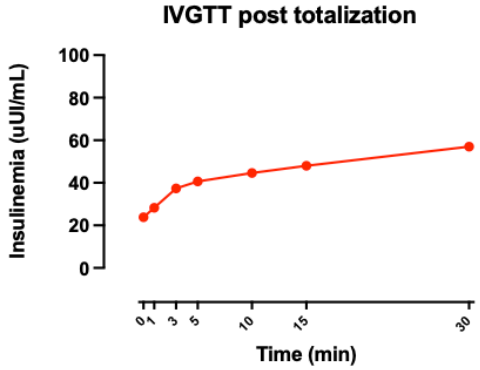

Figure 2 : IVGTT after total pancreatectomy and islet transplantation in the stomach submucosa

Conflicts of interest
No conflicts declared

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OP2_6

Prediabetic human islets : should we transplant them?

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Background
Currently, prediabetes (HbA1C ≥5.7% and < 6.5%) affects 33% of adults in the United States and 50% of adults in China. Clearly, type 2 diabetic islets show abnormal GSIS, a high a/b cell ratio, and fail to reverse diabetes in nude mice, yet little is known about prediabetic human islets. Retrospective analysis showed 42% of the 203 islet allografts transplanted in our center came from diabetic donors, 52% from non-diabetic donors, and 1% from diabetic (>6.5%) donors
Methods
In vitro and in vivo quality controls were used to compare *prediabetic* human islets with non-diabetic and diabetic islets.
Results
Prediabetic islets (n = 47 donors) showed an intermediate phenotype between non-diabetic (n = 73) and diabetic islets (n = 9) in both perifusion with elevated basal insulin secretion and altered GSIS and in oxygen consumption rates (OCR) (n = 13) with increased basal OCR and attenuated spare respiratory capacity (mitochondrial reserve). In vivo function (C-peptide/glycemia) of islets (n = 45 donors) sharply declined in our nude mouse bioassay between 5.7% and 6.5% HbA1c. Human islet grafts from 26 donors (14 non-diabetic, 10 prediabetic, and 2 diabetic) were challenged with a Hifat vs. Chow diet. Multivariate analysis showed functional and beta cell mass adaptation only in donor islets with HbA1C <5.7%; prediabetic and diabetic islets adapted poorly. A clinical outcome will be presented.
Conclusions
This is the first study to show that human islets from prediabetic donors show defects in beta cell function in vivo in the nude mouse bioassay and their adaptation in vivo to the Hifat diet, as well as altered mitochondrial function and elevated basal insulin secretion in vitro. With the exception of the "exclusion criteria ≥6.1%" of the NIH phase 3 trial, few centers disqualify pancreases based on HbA1c, yet accumulating evidence suggests progressive alterations in pre-diabetic islets. Reporting donor HbA1c levels in both clinical trials and research should become mandatory.
Conflicts of interest
No conflicts declared

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OP2_7

Culturing Isolated Human Islets in UW better protects the integrity of the Islet Basement Membrane compared to CMRL

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Background
The islet basement membrane (BM) is a specialized sheet of extracellular matrix comprising laminins, collagen IV and perlecan. It is essential for islet function and survival. During pancreas digestion, the islet BM is significantly disrupted and further BM loss occurs during the routine post-isolation culture period in CMRL media. Previous studies have shown the superiority of UW cold storage solution for maintaining islets during the culture period. However, the impact of UW storage on BM integrity during culture has not been explored. We hypothesize that the increased viability and recovery of islets following culture in UW may be due to preserved integrity of the islet BM.
Methods
Isolated human Islets (n=4) were divided into two groups, and cultured for up to 72hours in either: CMRL media at 37°C or; UW at 4°C. Islet samples were collected every 24hours from each condition, which included collection of a post-isolation sample. Samples were assessed for purity, viability, and islet yield. BM integrity was determined histologically via insulin and laminin labeling. Total protein concentration was determined by ELISA. Results from the cultured samples were normalized to the post-isolation sample. Statistical analysis was by way of ANOVA, with data presented as average ± standard deviation.
Results
Islet viability significantly declined following culture in CMRL at 48 and 72hours (reduction of 26±5%, and 25±3%, both $p<0.001$). There was no significant loss of viability for islets stored in UW, for up to 72hours. Following 72hours of culture, the remaining number of islets stored in CMRL were significantly lower than those stored in UW ($p<0.05$). At the molecular level, storing islets in CMRL resulted in a significant loss of total laminin at 24hours (49±12%, $p<0.05$), with further loss by 72hours compared to post-isolation (71±12%, $p<0.001$). Interestingly, storage of islets in UW led to minimal loss of laminin, which was maintained up until 72hours. These laminin profiles were supported by the corresponding histology data.
Conclusions
UW is significantly superior to CMRL for maintaining islets for up to 72hours. The islet BM (measured via laminin) is significantly more stable in UW storage solution, compared to CMRL. Increased islet BM survival provides important structural evidence, which may underpin the reasons for enhanced islet survival in UW culture systems.
Conflicts of interest
No conflicts declared

OP2_8

Perlecan: An islet basement membrane protein with anti-inflammatory attributes

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Background
Human islet isolation requires enzymatic digestion of islet basement membrane proteins (BMP) including Collagen-IV (C-IV), Laminin-521 (L-521) and Perlecan (PLC). Previous studies have demonstrated that culturing human islets with C-IV, L-521 or Nidogen-1 improves islet survival when exposed to hypoxia. In this study, we assessed whether PLC, used alone or combined with C-IV and L-521, also has the ability to protect human islets from hypoxia-induced damage.
Methods
Human islets (n = 7) were cultured for 4 – 5 days at 2% O2 in (A) CMRL (sham-treated controls) supplemented with (B) 40 µg/mL C-IV; (C) 10 µg/mL; L-521; (D) 10 µg/mL PLC or (E) combined BMP and characterized for yield; viability; apoptosis; reactive oxygen species (ROS) production, glucose stimulation index (SI) and TNF-a release. Data were corrected for IEQ and normalized to controls (mean ± SEM).
Results
Compared with controls (100%), islet yield was increased using C-IV (p<0.001), L-521 (p<0.001), PLC (p<0.05) or the combi (p<0.05) (Table 1). Fragmentation was also reduced compared with controls when islets were treated by C-IV (p<0.001) or L-521 (p<0.01) but not when PLC (NS) or the combi (NS) was added. Viability was increased in islets treated by C-IV (p<0.001), L-521 (p<0.05), PLC (p<0.01) or the combi (p<0.05). Compared with controls, the SI indicated intact function after using C-IV (p<0.01), L-521 (p<0.001), PLC (p<0.001) or the combi (p<0.05).
ROS production was decreased using C-IV (p<0.05), L-521 (p<0.01), PLC (p<0.001) or the combi (p<0.01) correlating with decreased TNF-a release observed after adding C-IV (p<0.05), L-521 (p<0.05), PLC (p<0.001) or the combi (p<0.01). Apoptosis was also reduced using C-IV (p<0.01), L-521 (p<0.05) or PLC (p<0.01), but not when adding the combi (NS).
Conclusions
Our findings indicate that PLC has a similar protective capacity as C-IV or L-521. Importantly, this study suggests that PLC also has distinct anti-inflammatory qualities that may be benefit post-transplant islet engraftment.

Islet BMP	Yield (%)	Fragmentation (IN/EQ)	Viability (%)	SI (20/2 mM)	ROS (%)	TNF-α (%)	Apoptosis (%)
Sham-treated (0 µg/mL)	100	100	100	0.94 ± 0.07	100	100	100
Collagen-IV (40 µg/mL)	172 ± 9.2[c]	63 ± 9.0[c]	130 ± 4.5[c]	1.47 ± 0.12[b]	55 ± 8.5[a]	54 ± 7.8[a]	68 ± 10.4[b]
Laminin-521 (10 µg/mL)	159 ± 6.1[c]	64 ± 8.1[b]	119 ± 4.4[a]	1.49 ± 0.07[c]	51 ± 6.3[b]	46 ± 3.6[a]	73 ± 5.5[a]
Perlecan (10 µg/mL)	146 ± 8.7[a]	73 ± 9.7	127 ± 6.4[b]	1.50 ± 0.07[c]	42 ± 4.7[c]	29.5 ± 2.8[c]	66 ± 6.9[b]
Combination (60 µg/mL)	142 ± 5.7[a]	85 ± 19.3	118 ± 2.9[a]	1.43 ± 0.09[a]	49 ± 6.0[b]	40 ± 3.5[b]	87 ± 7.2

[a]p<0.05, [b]p<0.01, [c]p<0.001 vs sham-treated

Table 1. Effect of different BMP on human islet characteristics after 4 – 5 days of culture in hypoxia at 2% O2 (n = 7).

Conflicts of interest
No conflicts declared

References
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OP2_9

Generation of insulin-producing cells from bile duct organoids

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Background

Islet replacement therapy offers significant benefits to individuals with type 1 diabetes; however its wide-spread use is hampered by the shortage of primary human islets. Therefore, converting other cell types into functional insulin-producing cells is an attractive approach. Bile duct epithelial cells known as cholangiocytes (COs) can easily be isolated from the bile duct and share some similarities with pancreatic duct cells in terms of gene expression and function. In this study, we investigated the possibility of using these organoids as a cell source for generating insulin-producing cells.

Methods

Human COs were isolated from bile duct by performing Endoscopic retrograde cholangiopancreatography (ERCP). Using single-cell RNA sequencing, immunofluorescent staining and western blotting, we first characterized the isolated COs. Next, COs were transduced using lentivirus vector carrying three beta cell transcription factors (TFs), NKX6.1, MAFA and NEUROG3 (TF-vector). The designed vectors were carrying GFP for detection of the transduced COs. The gene expressions of TFs and insulin gene in transduced COs were detected using quantitative PCR.

Results

Primary COs were positive for ductal markers CK19, CK7, SOX9, SOX17, GATA4, FOXA2 and PDX1 at both gene and protein levels but negative for the beta cell specific TFs, NKX6.1, MAFA and NEUROG3. Gene expression analysis of GFP-positive COs revealed positive expressions of NKX6.1, MAFA and NEUROG3, 30 days after transduction showing a successful delivery. Importantly, the over-expressions of NKX6.1, MAFA and NEUROG3 in COs were able to induce the insulin gene expression.

Conclusions

This approach lays the foundation for creating a new cell source capable of producing insulin-producing islet cells.

Conflicts of interest

No conflicts declared

OP3_4

Human Vascularized Islet Organ (hVIO) as an extracorporeal model for the study of physiopathology and drug screening in diabetes

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Background
Bio-engineered human vascularized islet organ (hVIO) can mimic the endocrine function of the islet vascular niche as predictive ex vivo tool in therapeutic development1. To assess hVIO's flexibility as an endocrine pancreas model, we studied the impact of CXCL8-CXCR1/2 inflammatory pathway on its endocrine niche. CXCL8 is a relevant released chemokine by inflamed human islets, but its pathway (CXCL8-CXCR1/2) inhibition may improve islet survival and function upon transplantation2.
Methods
hVIOs were generated using decellularized rat lung left lobe repopulated with human pancreatic islets and endothelial cells (BOEC) through trachea, pulmonary artery, and vein. The alveolus-islet niche similarity allowed us to recreate the endocrine functional unit in a complex 3D vascularized structure. Customized bioreactors enabled dynamic perfusion for 14 days, with the first 7 for device assembly (MP) and the subsequent 7 (TP) to assess endocrine and vascular function under different stimuli. MiR-375, a surrogate marker of β cell death, gauged β cell mass in MP and TP. Insulin secretion tests (IST), fluorangiography assays, and immunofluorescence (IF) were used to evaluate endocrine and vascular compartments in MP and TP.
Results
In MP, hVIO exhibited complete revascularization, with integrated pancreatic islets. MiR-375 showed islet mass preservation, fluorangiography indicated a functional vascular barrier, and IST demonstrated increased insulin production during MP, sustained in TP. In TP, hVIOs were tested as a predictor platform for inflammatory β cell damage via the IL-8-CXCR1/2 pathway. CXCR2-positive HIs, heterogeneous CXCR1/2 expression in BOECs, and preserved islet phenotype in hVIOs were observed. IL8 did not negatively affect islet function or HI β cell death in a vascularized ECM environment. In the presence of IL8, hVIOs displayed a positive trend in insulin release.
Conclusions
Validation of hVIO as a model for replicating IN using human cells open new possibilities for studying ED. Additional experiments are required to confirm the preliminary outcomes of insulin secretion during TP under inflammatory conditions, and to ensure the preservation of the vascular and endocrine compartments
Conflicts of interest
No conflicts declared

References
Citro et al. Curr Diab Rep 2015
Citro et al. Biomaterials 2019

OP3_1

3D iPSC differentiation produces functional beta cells for transplantation into a murine model

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Background
Induced pluripotent stem cells (iPSCs) can be differentiated in vitro into functional β cells. Nonetheless, several protocols are available with different efficiency. The goal of this study was to generate mature β cells capable of responding to a glucose stimulus and suitable for transplantation into a mouse model, utilizing a highly efficient differentiation protocol, modified from Barsby et al, 2022.
Methods
iPSCs are differentiated into β cells in 2D until the pancreatic progenitor stage. Subsequently, cell aggregation in micro wells (Aggrewell or Sphericalplate 5D) was implemented. In the last stage, the rotating suspension culture allowed us to obtain PDX1+/NKX6.1+/insulin+ spheroids. The expression of stage specifics markers was assessed by flow cytometry and gene expression. The ability of β cells to respond to a glucose stimulus was tested by dynamic perifusion assay (GSIS). The final differentiated β cells were transplanted under the kidney capsule, into the liver or in the femoral muscle of immunodeficient mouse (NSG). After 12-14 weeks the organs were explanted and the presence of β cell markers were evaluated by immunohistochemistry.
Results
The flow cytometry results showed that at iPSC stage cells express OCT4 (99.6%±0.5), at pancreatic precursor stage express PDX1 (59.6%±7%) and NKX6.1 (53.1%±6.1%) and at β cell stage express insulin (60.1%±6.6%) and glucagon (7.04%±1.3%). The gene expression analysis showed a significantly different expression profile of stage-specific markers between iPSC (*SOX2, NANOG, POU5F1*) and cells at different stages of pancreatic differentiation (*PDX1, NKX6.1, INS, GCG*). GSIS analysis proved that cells are able to release insulin efficiently (30 pg/mL/cluster) after a glucose stimulus. Organs explanted at 12-14 weeks after β cell transplantation showed the presence of cells positive for PDX1, Insulin and chromogranin A.
Conclusions
The study demonstrates that iPSC-derived β cells express the fundamental markers and function of mature β cells, and that these cells engraft and produce insulin upon transplantation in different mouse models.
Conflicts of interest
No conflicts declared

OP3_2

The study of cellular age during stem cell differentiation into β cells

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Background

Human induced pluripotent stem cells (iPSCs) can differentiate in vitro into insulin-producing cells. The role of cellular age in β cell differentiation and iPSC-derived β cell function is unknown. Recent studies reveal that aging leads to an increase in senescent β cells, impairing glucose sensing and insulin secretion. This study aims at investigating aging hallmarks at various differentiation stages and on β cell function.

Methods

Six clones of iPSC were differentiated into mature β cells (iβ). As aging hallmarks, the senescence-associated β-Galactosidase activity (SA-β-Gal), the phosphorylation of the histone H2AX (γH2AX) and the superoxide production (mitoSOX) were measured by flow cytometry and immunofluorescence. Genes related to the senescence-associated secretory phenotype (SASP) and cellular senescence were quantified by TaqMan custom gene arrays. The senescence state of the iβ was associated to their function, assessed by dynamic Glucose-Stimulated Insulin Secretion (GSIS). Additionally, somatic mutations were quantified during the differentiation process with ultra-high accuracy duplex sequencing.

Results

Flow cytometry showed an increase in senescence during differentiation: positive cell percentages were higher at the iβ stage compared to iPSCs for SA-β-Gal (iPSC: 9.6% ± 0.4%; iβ: 42.1% ±1.4%), γH2AX (iPSC: 3.5 ± 0.5%; iβ: 18% ± 0.9%), and mitoSOX (iPSC: 5.6% ± 0.6%; iβ: 25.4% ± 4%). Immunofluorescence confirmed γH2AX, SA-β-Gal, and superoxide presence at the iβ stage. Transcriptomic analysis showed increased gene expression related to SASP, cell cycle arrest, DNA damage, and β cell senescence in the final maturation stage. GSIS demonstrated variable insulin secretion capacities in response to glucose among iβ clones, linked to their senescence state.

Conclusions

These results suggest that the iβ senescence state may affect differentiation efficiency and function. Protection from the aging process during differentiation or depletion of senescent cells before transplantation is required for an efficient and long lasting function of stem cell-derived β cells for cell therapy.

Conflicts of interest

No conflicts declared

OP3_3

Generation of gene-modified human mesenchymal stem cells with enhanced immune-protective properties in bioartificial pancreas

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Background

The acceptance of transplanted pancreatic islets, while an important advancement in treating type 1 diabetes, remains a significant challenge. Despite the promise of restoring insulin production through islet transplantation, the immune system can identify transplanted cells as foreign substances, leading to rejection. Thus, the Vanguard consortium aim to generate a vascularized and immune-protected bioartificial pancreas by assembling insulin-producing organoids composed of islet cells, endothelial cells for system vascularization and mesenchymal stem cells to support immune protection.

Methods

Pancreatic and amniotic mesenchymal stem cells (pMSCs and aMSCs) were isolated and characterized for epithelial, mesenchymal and immunomodulatory markers, e.g. HLA-G, CD47 and PD-L1. To increase the expression of these markers, both pMSCs and aMSCs were transduced by lentiviral vectors (LV) carrying the transgenes of interest or the CRISPR-activation system to increase endogenous gene expression. FACS analysis was performed to verify the over-expression of the selected target proteins.

Results

Both pMSCs (n=2) and aMSCs (n=4) were negative for epithelial markers and expressing mesenchymal ones. In both primary cells, the basal expression of HLA-G and PD-L1 immunomodulatory proteins resulted low or absent while all cells highly express CD47. HLA-G and PD-L1 LVs transduction of both primary cells dramatically increase transgene expression while not affecting their viability or proliferation rate. Moreover, the CRISPR-activation system was validated for both selected genes with a resulting transactivation of the promoters inducing a significant increase of HLA-G and PD-L1 proteins.

Conclusions

Immunomodulatory genes were successfully over-expressed in both pMSCs and aMSCs. Further investigations are planned to assess if mesenchymal stem cells could represent a valid immune-protective cell option to be included during the assembling of pancreatic organoids.

Conflicts of interest

No conflicts declared

References

Vanguard grant No.874700

OP3_5

Transplantation of spheroids composed of primary human endothelial cells (ECs) and re-aggregated neonatal porcine islets accelerate diabetes reversal in immunodeficient mice

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Background

Engraftment of transplanted islets depends on rapid revascularization of the graft. The present study aimed to evaluate beneficial effects of endothelial cells (EC) on vascularization and maturation of re-aggregated porcine islet cells (REPI) after transplantation.

Methods

Spheroids from dispersed neonatal porcine islet-like clusters (NPICCs) and ECs isolated from human cord blood were generated by re-aggregation on 5D-spherical plates and were transplanted into diabetic NOD-SCID IL2rγ-/- (NSG) mice to investigate *in vivo* revascularization and metabolic function. Expression of proangiogenic factors and revascularization was assessed by qRT-PCR and immunohistochemistry.

Results

Spheroids expressed significantly higher levels of VEGF, TIE1 and, TM7SF2 (ANG1) as compared to re-aggregated porcine islet cells (REPI) alone ($p < 0.01$). Interestingly, in comparison to REPIs alone, spheroids exhibited increased viability. Animals transplanted with spheroids (n= 6) showed significantly faster normoglycemia development as compared to the REPI group (n= 8) (100% normoglycemia development, median 51.5 days versus 87.5% normoglycemia development, median 60.0 days) ($p < 0.05$). Additionally, spheroid grafts exhibited significantly increased vascular density ($p< 0.01$). Functional blood vessels were composed of human and mouse endothelial cells suggesting that ECs improve islet engraftment by enhancing graft revascularization.

Conclusions

Transplantation of REPI-EC spheroids induce robust angiogenesis and improves *in vivo* maturation and function of REPIs. These findings provide a novel strategy to enhance the efficacy of porcine islet transplantation.

Conflicts of interest

No conflicts declared

OP3_6

Vascularized endocrine pancreas for type 1 diabetes

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Background

This study aimed to engineer a functional, vascularized endocrine pancreas by utilizing decellularized human placental cotyledons (hPLCs) as scaffolds and seeding them with pancreatic islets and human blood outgrowth endothelial cells (BOECs). The use of hPLCs mimics the native pancreatic structure, supporting effective cell integration. BOECs hold future potential for recipient-specific sourcing, prioritizing biocompatibility and reducing immune rejection risks.

Methods

Decellularized hPLCs were initially repopulated with human BOECs and then seeded with 1500 human islet equivalents (IEQ). Recellularization was verified using histological and immunohistochemical methods. Endocrine function was assessed through glucose-stimulated insulin secretion tests. Vascularized endocrine constructs were transplanted into the subcutaneous space of streptozotocin (STZ) treated diabetic NSG mice (PLCs+Islets+BOECs). Control mice were transplanted with non-endothelialized scaffolds (PLCs+Islets) containing the same number of islets and free islets in subcutaneous space (SC) and under the kidney capsule (KC).

Results

Engineered constructs displayed a continuous network of CD31+ endothelial cells within the hPLC, embedding the islets within this vascular bed. These constructs successfully released insulin in response to glucose, mimicking normal pancreas function. Notably, 80% of mice with the PLCs+Islets+BOECs construct normalized their blood sugar levels within the first week post-transplantation, compared to 60% in the PLCs+Islets group. Mice with islets transplanted in prevascularized areas did not achieve normal blood sugar levels. Removal of the graft-bearing construct resulted in a rapid return to high blood sugar levels within 24 hours. After 90 days, the PLCs+Islets+BOECs group exhibited a larger β-cell mass and higher vessel density in the grafts compared to the PLCs+Islets group, indicating effective vascular integration.

Conclusions

The engineered vascularized endocrine pancreas provides a fully biocompatible construct that closely mimics the native islets' matrix environment and offers mechanical protection, enabling transplanted islets to engraft and function long-term.

Conflicts of interest

No conflicts declared

References

This work was supported by grants from the European Commission (Horizon 2020 Framework Program; VANGUARD grant 874700), the Juvenile Diabetes Research Foundation (JDRF; grant 3-SRA-2020-926-S-B and 3-SRA-2023-1441-S-B), the Shota Rustaveli National Science Foundation (grant FR-19-19760) and the Swiss National Science Foundation (grant 310030_173138 and grant CRSII5_209417).

OP3_7

Implantation of thin-film microwell islet delivery devices in a large preclinical minipig model

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Background

We report about the engraftment of sentinel-sized islet delivery devices in a five week long minipig study to select promising implantation sites for macro-encapsulation devices and a 3 month follow up autotransplantation proof of concept study in minipigs. The aim of this project is test the potential of an open microwell-array islet delivery device for beta cell replacement in a preclinical large model.

Methods

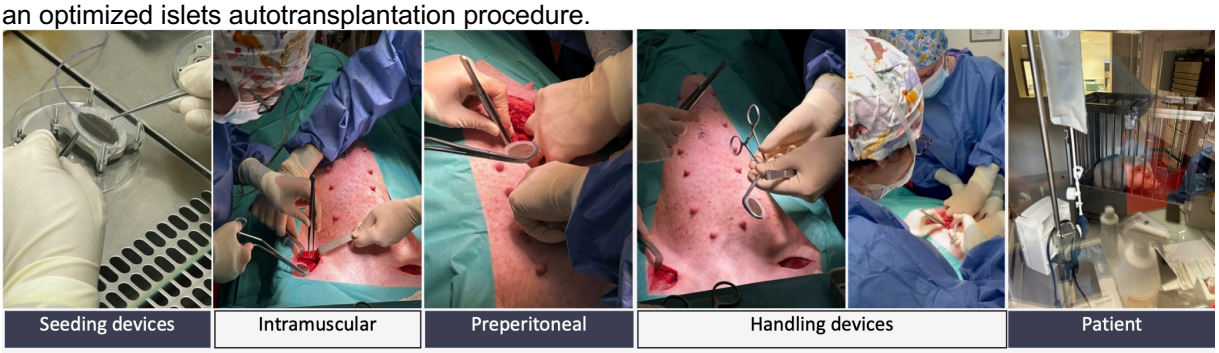
Small macroporous microwell array sentinel devices were implanted into five different locations in 4 minipigs for 5 weeks. Implantation was done subcutaneous , pre-peritoneal, omentum, kidney capsule and intramuscular. In a follow up study, we performed implantations intramuscular and preperitoneal in an autotransplantation minpig model using upscaled devices in partial pancreatectomized minipigs. Islets were isolated from part of the pancreas and seeded into devices and implanted for 3 months.

Results

The different implantation sites were histollogically evaluated for implant intactness, host tissue response, blood vessel density and fibrous capsule thickness. Out of the total 36 samples, 6 samples showed less tissue integration (3 out of 4 kidney capsules, 2 out of 8 omental and 1 out of 8 intramuscular samples). Folding of the device was only observed for a single intramuscular sample, while 32 out of 36 samples remained intact over the retrieval procedure. The pre-peritoneal space and intramuscular implanted open devices showed the thinnest fibrous layer, while both kidney capsule and intramuscular implanted open devices showed the highest vascular densities. Islets were isolated from the explanted part of the pancreas and seeded into two types of devices and implanted for 3 months in the same minipig. We developed a two-stage procedure at day 1 islets where isolated and left to recover overnight. The next day islets number was determined and accordingly multiple devices were seeded using a gravity-based method using dedicated cell seeding clamp. After cell seeding the loaded devices where immediately transported to the operating room for implantation into the same minipig. Implantation of the islet containing devices was done using standard surgical tools under general anaesthesia.

Conclusions

The intramuscular and preperitoneal sites where found to be most optimal for device implantation using an optimized islets autotransplantation procedure.



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Autotransplantation of islets isolated from partial pancreatectomized minipigs in two different sites using thin-film microwell array islet delivery devices

Conflicts of interest

No conflicts declared

References

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Juvenile Diabetes Research Foundation, grant key 3-SRA-2016-256-S-B

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OP3_8

A Hybrid implant combining a macroporous device with immunoprotective microcapsules for cell therapy applications

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Background

Cell encapsulation strategies including macro- and micro- delivery devices have been widely used in cell therapy due to their ability to provide immunoprotection to the transplanted cells. While both strategies can provide mechanical and physiochemical support for maintaining cell survival and function, they each have their limitations. In this study, we report the design and fabrication of a hybrid implant combining the advantages of both macro- and micro- cell delivery devices.

Methods

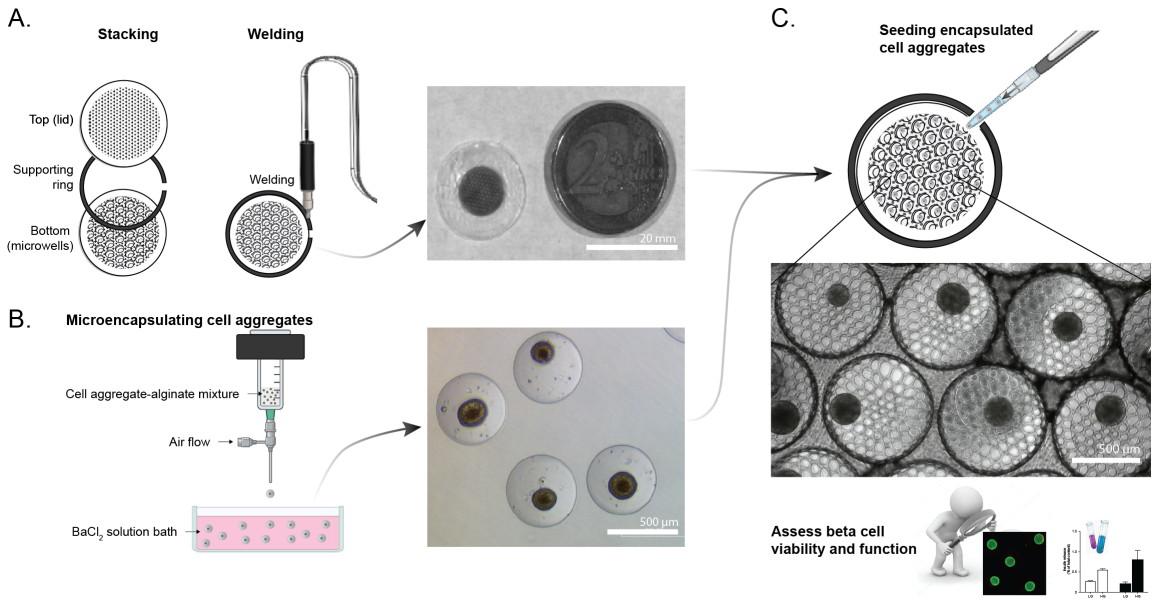
The hybrid implant comprises a microwell-array macroporous device fabricated from non-degradable clinically approved polyvinylidene fluoride (PVDF) combined with immunoprotective alginate microcapsules. Hybrid implants were seeded with microcapsules containing (pseudo)-islets, cultured, and assessed for their viability and function. The prevascularization potential of the hybrid implant was assessed by attachment and viability of human umbilical vein endothelial cells (HUVECs) cultured on macrodevice films.

Results

The microwell design provides a vessel to retain individual microcapsules, while the pores enable unhindered mass transport of nutrients and oxygen to the encapsulated cells and support vascular ingrowth. We show that both rodent pseudoislets and primary human islets maintain their viability and function inside the hybrid implant in a proof-of-concept study. Mechanically, it is strong and flexible suitable for surgical handling and for eventual retrieval for replacement. The hybrid implant also supports the growth of HUVECs across its surface allowing *in vitro* “prevascularization”, which can potentially accelerate blood vessel formation in poorly vascularized transplantation sites such as the subcutaneous space.

Conclusions

In conclusion, the hybrid cell delivery device, which confers immunoprotection and allows prevascularization, can act as a protective container during surgical handling and retrieval of microencapsulated cells opening a wide range of cell therapy applications including stem cells.



Device assembly and seeding with microencapsulated islets. A) Assembly and welding of the microporous top film, the support ring, and bottom film (including microwells) by ultrasonic welding to

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produce a hybrid implant (~240 µm thickness; 19 mm diameter; and ~ 85 microwells). B) Cell aggregates (pseudoislets or human islets) were mixed with ultrapure sodium alginate and extruded through the nozzle of the droplet generator with airflow to produce microencapsulated cells (~ 500 µm). C) Microencapsulated cells seeded into the hybrid device using a wide bore pipette tips, where each microcapsule confined into the microwells of the hybrid implant.

Conflicts of interest

Aart van Apeldoorn is founder and shareholder of Lighthouse Biomedical B.V. a start-up company aiming to commercialize beta cell delivery devices in the future.

References

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OP3_9

Insulin-independence of diabetic pigs after autotransplantation of 3D-bioprinted bionic pancreatic tissue petals

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Background

The first milestone of a new therapy for the treatment of T1D is the development of 3D bioprinted petals consisting of: pancreatic islets and biomaterials. The aim of the study was to demonstrate the functionality of pancreatic islets in in vivostudies on large animals.

Methods

Domestic pigs were the research model. The animals were divided into 4 groups:(1) healthy pigs (Control; n=3); (2) animals with T1D after pancreatectomy, treated with insulin (T1D; n=3); (3) animals after pancreatectomy and autotransplantation of pancreatic islets to the liver (LIVER; n=3); (4) animals with T1D after pancreatectomy, which were autotransplanted with bionic petals (3D-PETALS; n=3).The effectiveness of the transplantation (TX) was assessed by the concentration of glucose, insulin intake and C-peptide. The observation lasted 1 month.

Results

The results showed that in the LIVER group, insulin intake within 3 weeks decreased by 71% compared to the demand before TX. Whereas 1 month after TX, the demand was lower by 62%. The 3D-PETALS group showed that the insulin intake in 3 weeks after TX decreased by 65%, and within a month decreased by 84%. In the 4th week after TX, the insulin intake average in the T1D group was 8.17U, while in the LIVER group=2.44U and in the 3D-PETALS group=1.06U. Islet TX significantly reduced insulin intake (T1D vs LIVER; $p=0.0001$ and T1D vs 3D-PETALS; $p<0.0001$). Glucose measurement showed significant changes. After 1 month of follow-up, glucose levels were significantly lower in the LIVER vs T1D (265.8mg% vs 310.2mg%; $p<0.0001$) and 3D-PETALS & T1D (198.3mg% vs 310.2mg%; $p=0.0354$). Most importantly, glycemic levels were also significantly lower between the LIVER & 3D PETALS groups (265.8mg% vs 198.3mg%; $p=0.0021$). The concentration of C-peptide during the study was 0.14ng/ml.

Conclusions

Bioprinted petals with dECM-based bioink significantly reduces diabetic parameters. Thus, it seems to be an effective therapy for people with T1D.

Conflicts of interest

Marta Klak is the co-founders of Polbionica Sp. z o.o.Andrzej Berman is the co-founders of Polbionica Sp. z o.o.Michal Wszola is the co-founders of Polbionica Sp. z o.o.

References

The National Centre for Research and Development: STRATEGMED3/305813/2/NCBR/2017

OP4_1

Should we be transplanting the pancreas from a donor with raised amylase? National cohort study

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Background
Simultaneous pancreas-kidney (SPK) transplantation improves quality of life in people with diabetes and end-stage renal disease, and limits the progression of diabetes related complications. Many surgeons are reluctant to accept the pancreas of a donor with raised amylase, due to concern of potentially inferior outcomes. We aim to ascertain whether donor amylase and liver blood tests (a marker of visceral ischaemic injury) predict pancreas transplant outcome.
Methods
This retrospective cohort study used the NHS registry on adult SPK transplantation (2016-2021). Adjusted regressions models assessed the effect of donor amylase and liver blood tests on pancreas transplant outcome.
Results
857 SPK recipients were included (619 following brainstem death, 238 following circulatory death). Peak donor amylase ranged from 8-3300U/L (median=70). Donor peak amylase had no significant impact on pancreas graft survival when adjusting for multiple confounders (aHR=0.944, 95% CI=0.754-1.81). Median peak alanine transaminase and aspartate transaminase was 67U/L and 72U/L (range 8-5930 and 0-7910U/L). Neither of these influenced pancreas graft survival in multivariable models (aHR=0.967, 95% CI=0.848-1.102 and aHR=0.908, 95% CI=0.771-1.070, respectively). Restricted cubic splines were used to assess the relationship between donor blood tests and pancreas graft survival without assuming linear relationships; these confirmed neither amylase, nor transaminases, significantly impact on pancreas transplant outcome (Figure 1).
Conclusions
Donor amylase and transaminases do not predict transplant outcomes. Therefore, raised donor amylase or transaminases should not be considered a barrier to organ utilisation. The use of pancreas grafts from donors with hyperamylasaemia is a safe, immediate, and simple approach to expand the donor pool.

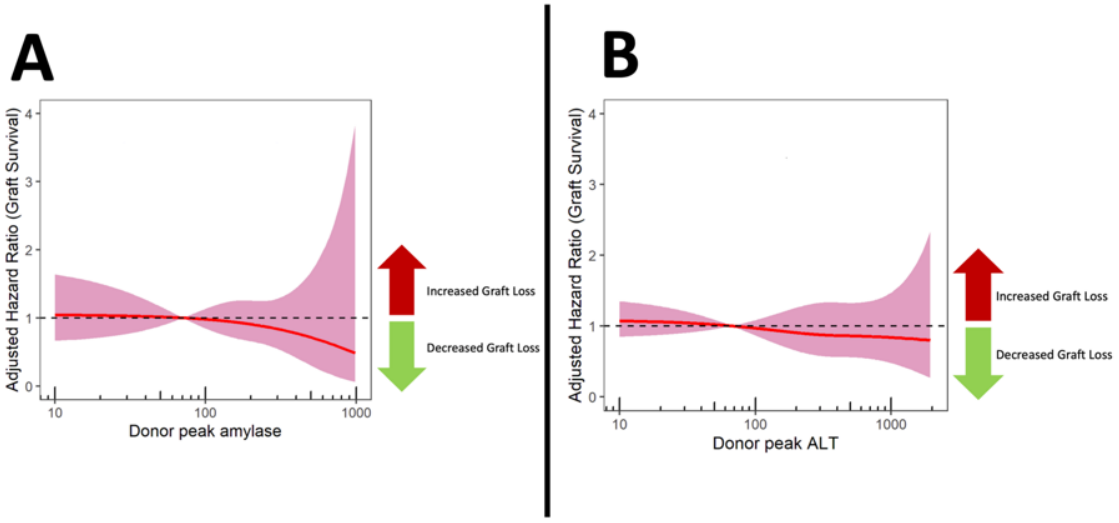

Figure 1 – restricted cubic splines demonstrating the impact of donor peak amylase (A) and peak ALT (B) on pancreas graft survival.

Conflicts of interest
No conflicts declared

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OP4_2

Cancer in Simultaneous Pancreas and Kidney Transplant Recipients – A Single-Center Study

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Background
The incidence of *de novo* malignancies is higher in organ transplant recipients due to the immunosuppression (IS) burden. Insufficient data is available on this matter regarding pancreas-kidney transplantation (PKT).
Methods
A retrospective analysis on every PKT recipient from a single center from 2000 to 2022 with >1y of follow-up, collecting data regarding the incidence, treatment, and outcomes of *de novo* malignancy.
Results
Of 231 patients, 122 (52,8%) were male with mean age at transplant of 35,8±6,4y. 24 (10,4%) developed cancer; 8 of them had >1 neoplasm, to a total of 34 neoplasia identified; the mean time for cancer diagnosis after PKT was 10,6±5,0y. The most common type of malignancy was cutaneous (17, 7,4%), followed by gynecological (6, 2,6%), hematologic (3, 1,3%), colon (2, 0,8%), lung (2, 0,8%) and a single case (0,4%) of breast, hypophysis, native kidney and native pancreas cancer; 3 had metastasis. 26 cancers were treated surgically, and 6 were treated with classic chemotherapy with/without radiotherapy or hormonal therapy; no patient was treated with tyrosine kinase inhibitors or immunologic checkpoint inhibitors. 24 (70,6%) malignancy cases had complete response. 1 (2,9%) patient had acute kidney injury related to cancer (due to lymphomatous infiltration of the kidney graft); no other direct consequence to graft function was recorded. IS was changed in 8 (23,5%), with suspension of antiproliferative agents with/without mTOR inhibitor initiation. There was no statistically significant difference for pancreas or kidney graft loss in the oncologic patients. The mean time from cancer diagnosis to death was 3.3±3,0y, having a higher mortality than the cancer free PKT population (7, 29,2% *vs* 27, 13,2%, p=0.020), with a relative risk for death of 2.2 (p=0,04).
Conclusions
The incidence of cancer in PKT is quite significant with impact on prognosis. Cancer surveillance and awareness is of upmost importance to improve this population survival.
Conflicts of interest
Nothing to declare

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OP4_3

Normothermic Regional Perfusion in Controlled Donation After Circulatory Death Pancreas Transplantation: A single center experience

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Background

In the face of organs shortage for transplantation, the transplant community is increasingly considering controlled donation after circulatory death (cDCD). There is limited information concerning the use of Normothermic Regional Perfusion (NRP) on the quality of the cDCD pancreas.The aim of this study is to report the effect of NRP in pancreas obtained from cDCD and its impact on recipients´ outcomes.

Methods

This is a retrospective, observational study describing the outcomes of pancreas transplants from cDCD donors using NRP (Nov 2019-Nov 2023).

Results

During the study period, 91 pancreas transplants were performed, including 18 pancreas transplants (17 SPK, 1 PAK) from cDCD donors.

The median donor age was 44 [IQR 25-75%, 31-46] years, with a BMI of 24.2 [IQR:22-25.1] kg/m2, 83.3% were male. The median total warm ischemia time (WIT) and the functional WIT were 18 [IQR:17-22] min. and 14.5 [IQR:7.7-18.7] min., respectively. Postmortem NRP was run for 127 [IQR:95.5-149] min. The median pancreas and kidney cold ischemia time was 6.5 [IQR:4.4-7.7] min. and 8.7 [IQR:7.8-11.6] min., respectively. Median hospital stay was 13 [IQR:9.7-24.2] days. Six patients presented with postoperative delayed kidney graft function, with one case requiring dialysis. Pancreas related surgical complications (Clavien Dindo ≥ 3) were present in 33 % of cases. After a median follow-up of 14.6 [IQR:9.7-26.2] months, 1-year pancreas graft survival was 81.4%, 1-year kidney graft survival was 93.3% and patient survival was 100%.

Conclusions

To date, this is one of the largest single center series describing the use of NRP in cDCD pancreas transplantation, displaying competitive results in terms of graft and patient survival.

SPK, Simultaneous Pancreas Kidney; PAK, Pancreas After Kidney

Conflicts of interest

No conflicts declared

OP4_4

Validating the Igls criteria 2.0 for graft outcomes in patients with islet transplantation

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Background
Reliable assessment of functional outcomes of β-cell replacement is imperative for clinical follow-up and decision making, and comparing and benchmarking different transplantation (Tx) options. Currently, multiple scoring methods are available to define graft functional outcomes after Tx. The Igls criteria 1.0 (Igls 1.0) were the first standardised approach. Recently, an update was proposed: the Igls criteria 2.0 (Igls 2.0). In this study, we validated Igls 2.0 in islet transplant (ITx) recipients.
Methods
We included data from all first ITx recipients (2007-2023) in our centre. Islet graft function was scored at 6 months post-Tx using Igls 1.0 and Igls 2.0. Both criteria categorize outcome as Optimal, Good, Marginal or Failure. Igls 1.0 is based on HbA1c, severe hypoglycaemic episodes (SHE), insulin requirements and C-peptide. Igls 2.0 distinguishes treatment outcome (Igls 2.0 T) based on HbA1c and SHE or CGM time-in-range and time-in-hypo, and graft outcome (Igls 2.0 G) based on C-peptide and insulin requirements.
Results
We analyzed 56 islet transplant alone (17.2%), islet-after-kidney (75.9%) or islet-after-lung (3.4%) transplant recipients (mean age 60.2±10.2 years; 33.9% female). According to Igls 1.0, outcome was Optimal in 9.6%, Good in 63.5%, Marginal in 19.2%, and Failure in 7.7%. According to Igls 2.0, treatment outcome T was Optimal in 50.0%, Good in 28.8%, Marginal in 21.2%, and Failure in 0.0%, and graft outcome G was 11.8%, 80.4%, 2.0%, and 5.9%, respectively. In 66.0% of cases, Igls 2.0 were different to Igls 1.0. In all (35/35) of these cases, the two Igls 2.0 domains of treatment outcome and graft outcome differed from each other (T Optimal with G Good in 60.0%, T Marginal with G Good in 25.7%), and Igls 1.0 scored similar to the lowest of these two domains, underestimating either treatment or graft function. When Igls 1.0 scored Marginal (n=9), Igls 2.0 further distinguished this to Marginal treatment outcome T with Good graft function G in 88.8%. Using CGM yielded similar treatment outcome as using HbA1c in 77.8%. When different, CGM-based treatment outcome was more similar to the corresponding graft outcome.
Conclusions
Igls 2.0 provide a finer distinction between clinical treatment outcome and graft function. The use of CGM in Igls 2.0 compared to only HbA1c seems to correspond better to graft outcome.
Conflicts of interest
No conflicts declared

OP4_5

New Technology SEPAX -SEFIA a repurposed closed system for human islet purification and processing in cGMP environment

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Background

The COBE 2991 cell processor, commonly used for pancreatic islet isolation, will no longer available in Europe affecting >17 active transplant centers, leading to a search for alternative purification procedures with equivalent efficacy or superior.

Goal of the study: Demonstrate the proof of concept that the Sepax/Sefia cGMP technology single platform, dedicated to hematopoietic stem cell processing can be repurposed for islet cell processing and purification

Methods

Islet gradient or Biocoll density gradient purifications were used to purify islets from 7 pigs and in 1 human pancreas using the Sepax 2C Pro as well as the Sefia S2000. Dithizone staining determined islet purity + trypan blue islet viability.

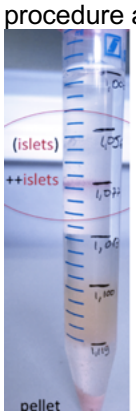
Results

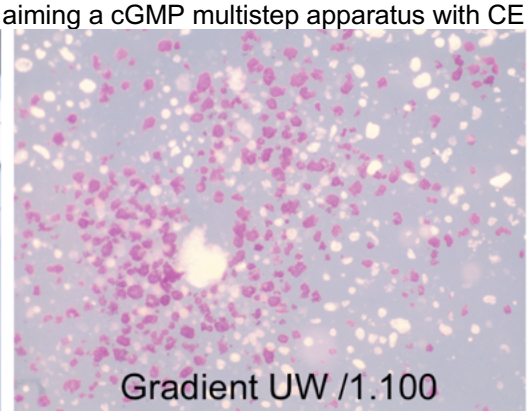
The conventional isolation procedure is used to purify islets in the COBE where multiple fractions are recovered and assembled based on purity and pellet volume. In the Sepax, only one standard density gradient is used for mononuclear cell fraction isolation with NeatCell protocol and one fraction is collected . After enzymatic digestion, we optimized recovery and volume limits through gradient tests to purify the pellet in the Sepax yielding 75%(±5% SD) pure fraction of >90% viable islets.

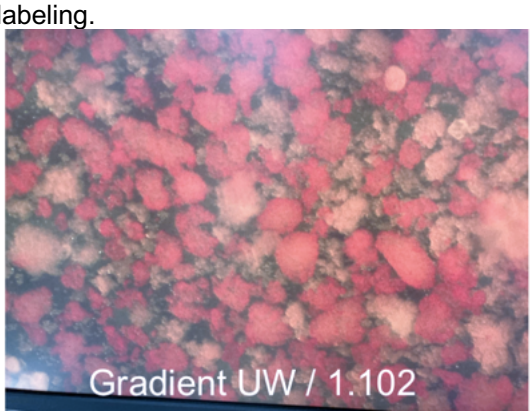
Conclusions

We have shown the proof of concept of the purification of human islet cells using Sepax and Sefia cGMP closed system technology as a strategy to replace COBE 2991. Secondly,we developed a cooling system to maintain human islets at <10°C during processing. Clinical grade human pancreases islet cell purification will start in December 2023 with evaluation in vitro and in vivo as well as setting endpoint release criteria comparing COBE 2991 purified vs SEPAX/SEFIA purified islets.

Perspectives: Ongoing development with Sepax-Sefia will enable multiple islet processing steps procedure aiming a cGMP multistep apparatus with CE labeling.







Conflicts of interest

No conflicts declared

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OP4_6

Knock-out of the activating ligand repertoire of human stem cell-derived β cells avoids chronic allograft rejection mediated by NK cells in vivo

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Background

Natural Killer (NK) represents a heterogenous population of lymphocytes that regulates several aspects of the alloimmune response in solid organ transplantation. Activation of NKs upon transplantation depends on the repertoire of ligands exposed by the target cells. In the last years, MHC-I-null stem cell-derived β cells have been proposed as alternative source for treatment of Type 1 Diabetes (T1D). However, despite MHC-I abrogration via beta-2-microglobulin (B2M) knock-out prevents T cell-mediated response, it triggers NKs via missing-self recognition leading to graft loss. As we demonstrated that the NK activating ligands CD276 and CD155 mediate NK recognition and killing of MHC-I-null stem cell-derived β cells (1), we propose their knock-out to escape NKs and ensure long-term survival of β cells after transplantation.

Methods

We generated luciferase-expressing wild type (WT) (negative control of rejection), B2M-/- (positive control of missing-self recognition) and B2M-/-/CD276-/-/CD155-/- (T-KO) human induced pluripotent stem cells (iPSCs). Gene-edited iPSCs were differentiated into β cells (iBeta) following an in vitro protocol mimicking pancreas development (2). We transplanted iBeta in hindlimb muscles of hIL-15 NOG mice humanized with donor-derived NK cells, then assessed engraftment up to 60 days through in vivo imaging system.

Results

Gene-edited iPSCs differentiated into iBeta with high efficiency (> 50% of NKX6.1+ and > 40% of INS+ cells). Strikingly, we found that T-KO iBeta successfully escaped NK-mediated allogeneic response and killing in vivo. Indeed, we observed only a slight reduction of graft area and bioluminescence signal of TKO iBeta after transplantation, comparable to the WT counterpart. Conversely, MHC-I-/- iBeta were quickly recognized and totally rejected by circulating NK cells ($p < 0.001$, $n = 9$). Furthermore, T-KO iBeta long-term persisted in vivo [60 days for T-KO ($n = 6$) vs 10±6 days for MHC-I-/- ($n = 9$); $p < 0.0001$] and properly maintained their functionality, as confirmed by c-peptide levels. Finally, histopathological analysis of explanted grafts excluded tumor formation and confirmed the maintaining of insulin-secreting phenotype.

Conclusions

We proposed genetic manipulation of the NK activating ligands as a novel strategy to make grafts invisible to human NK cells in vivo, offering new perspectives for using clinical-grade stem cell pancreatic derivatives as cell therapy for T1D treatment.

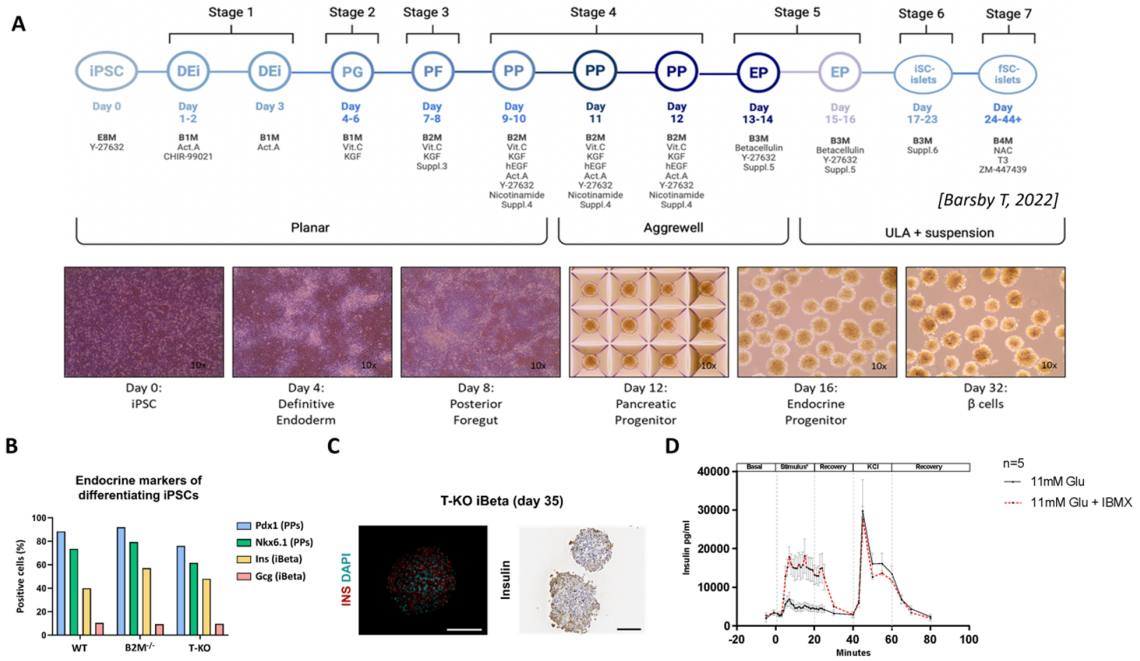

Figure 1. (A) Schedule of differentiation of stem cells into β cells with stages, steps of development, timing, stimuli, culture conditions. Below representative pictures of cells at different time points showing iPSC transformation and cluster generation in 3D culture. **(B)** Percentage of Pdx1+ and Nkx6.1+ cells at pancreatic progenitors' stage (15 days) and Insulin+ and Glucagon+ cells at the end of differentiation (30 days) by flow cytometry. **(C)** Insulin staining in IF/IHC performed on T-KO iBeta clusters after 35 days of differentiation. **(D)** Insulin release during dynamic perifusion of β cells (day 35) stimulated with 11mM glucose (with or w/o IBMX) and depolarizing KCl.

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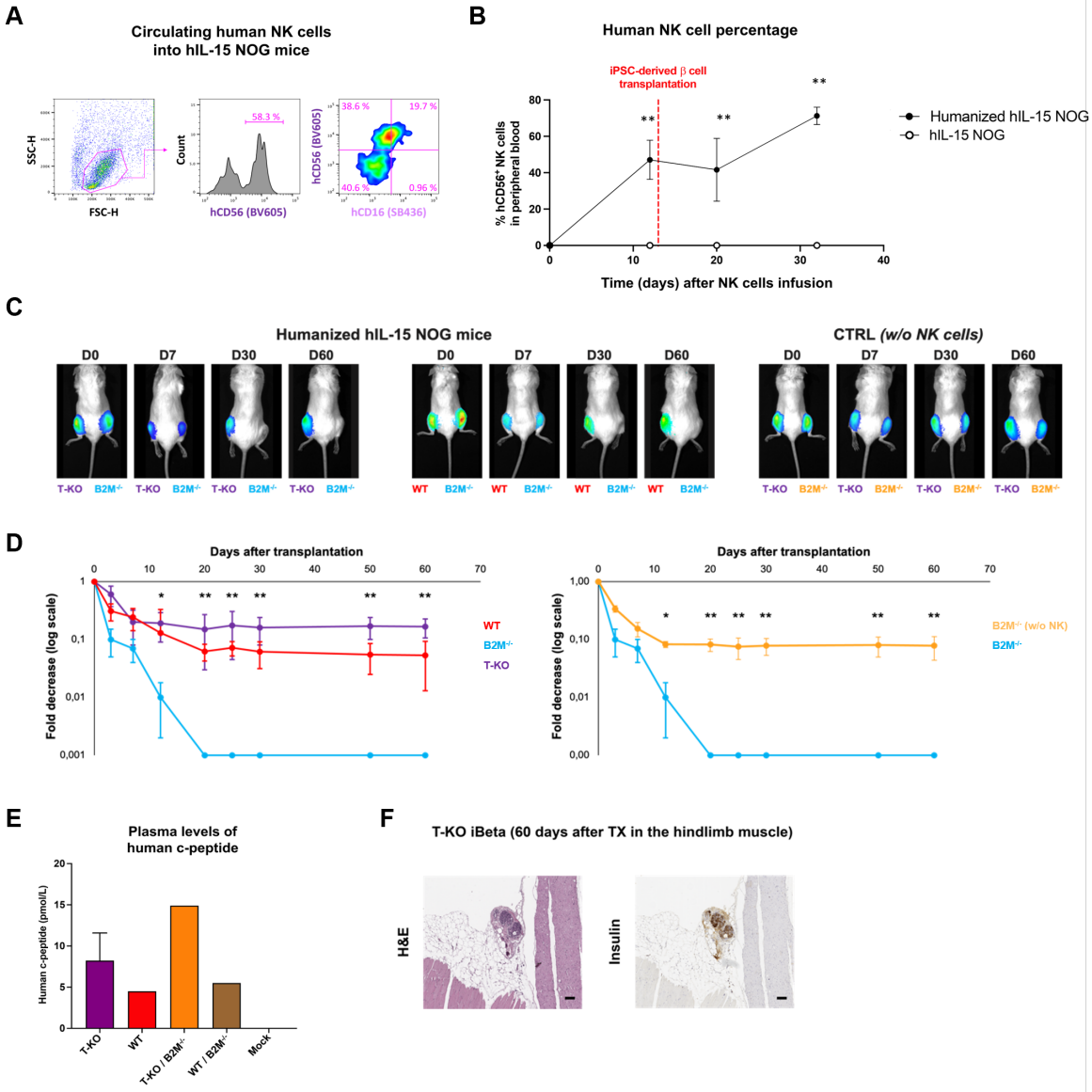

Figure 2. (A) Representative FACS plots of peripheral blood cells from hIL-15 NOG mice after 12 days from NK infusion. Cells were stained with anti-hCD56 and anti-hCD16. **(B)** Relative frequencies of hCD56+ NK cells into humanized mice (n=5) compared to mice w/o NK cells (n=3). Data are expressed as mean ± SD. ** $p < 0.001$. **(C)** Representative images of transplanted mice humanized with donor-derived NK cells and of control group mice w/o NK cells. **(D)** Fold decrease of total emission expressed as photons per second from WT, B2M-/- and T-KO iBeta transplanted in humanized mice (n=5 for T-KO / B2M-/- and n=4 for WT / B2M-/-) (left) and from B2M-/- graft in mice injected (n=9) or not (n=2) with human NK cells (right). Data are expressed as mean ± SD. * $p < 0.01$, ** $p < 0.001$. **(E)** Plasma levels of human c-peptide measured at 90 minutes after i.p. glucose administration. Data are expressed as mean ± SEM for T-KO (n=3) and mock (n=2) mice, otherwise they represent single measurements. T-KO and WT represent humanized mice that rejected B2M-/- iBeta; T-KO / B2M-/- and WT / B2M-/- are the mice w/o NK cells. Mock consists of mice w/o iBeta. **(F)** Haematoxilin and Eosin (H&E) and insulin staining show the presence of spheroid structures corresponding to T-KO iBeta close to muscular and connective tissues. Scale bars are 100 µm.

Conflicts of interest

No conflicts declared

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Clinical Cases

CC1

Continuous Glucose Monitoring in a Patient with Pancreas Graft Thrombosis

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Background

Pancreas graft artery thrombosis is a feared complication in the early posttransplant period, often diagnosed too late to preserve the graft. Using rtCGM could possibly help in earlier hyperglycaemia detection. We present rtCGM data from a patient in a perioperative rtCGM trial who suffered from graft artery thrombosis resulting in graftectomy 7 days post-transplant.

Methods

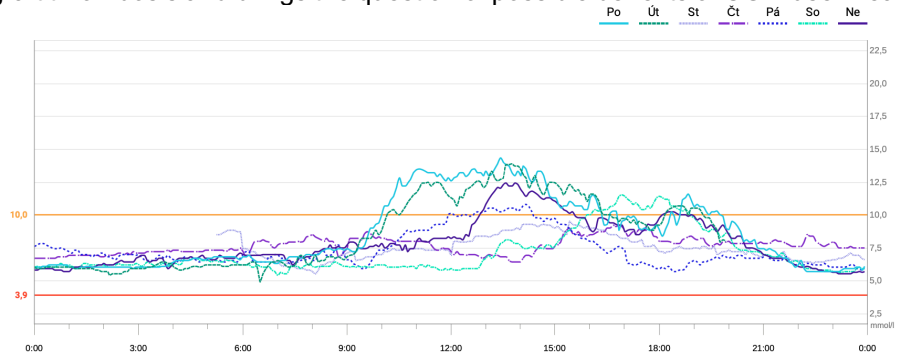
We retrospectively analysed CGM data obtained from a blinded CGM, Dexcom G6, during an ongoing prospective trial testing the accuracy and feasibility of CGM in postoperative intensive care. Sensor is inserted immediately after surgery, and kept throughout the ICU stay and, if possible, up to 10 days.

Results

50-year-old male with type 1 diabetes and advanced microvascular complications (neuropathy, proliferative retinopathy, nephropathy) was admitted for pancreas retransplantation. He had undergone simultaneous pancreas and kidney transplantation in 2011 and had lost his pancreas graft due to acute cellular rejection grade 3 refractory to anti-rejection treatment. He achieved normoglycaemia early post-transplant, with no need for insulin after switching from parenteral nutrition to oral food intake on day 3. Occasionally he had milder hyperglycaemia (11 mmol/l) after meals, which was attributed to corticosteroid therapy. He was recovering promptly, however suffered from epidydimitis on day 3, treated with antibiotics. On day 7, severe abdominal pain occurred at night, abdominal ultrasound and subsequent CT showed arterial graft thrombosis with necrotic changes, leading to immediate graftectomy. CGM data cover days 0 to 6 (when it was removed on patient request). Despite lacking the last day when the symptoms occurred, we captured postprandial hyperglycaemias especially in the afternoon starting on day 3, gradually worsening each day, daily time in hyperglycaemia rising from 9% on day 2 to 38% on day 6, average glycaemia rising from 7.3 ± 1,3 to 8.7 ± 2.7 mmol/l. These elevations were not adequately captured by frequent glucose meter measurements, his pre-prandial glycaemia levels were only mildly elevated.

Conclusions

This case study shows retrospectively captured hyperglycaemic patterns in a patient with pancreas graft thrombosis and brings the question of possible benefits of CGM use in early posttransplant period.



Conflicts of interest

No conflicts declared

CC2

Two Cases of Severe Pancreatitis Following Rejection Therapy with Methylprednisolone

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Background

We present two cases of severe graft pancreatitis following anti-rejection therapy.

Methods

Patient 1 (62-years-old woman 10 years after PTA) was referred for pancreas graft biopsy due to elevated pancreatic amylase and lipase levels and HbA1c. Patient 2 (38-year-old man 14 months after SPK) was admitted due to elevated pancreatic enzymes levels. He already had biopsy proven acute cellular pancreas graft rejection grade 1 one month post-transplant, successfully treated with methylprednisolone (2.25 g in total). However, one year later the enzymes were elevated again.

Results

In both cases percutaneous ultrasound-guided biopsy showed acute cellular rejection grade 1 and we initiated treatment with methylprednisolone. Patient 1 received 5 doses (2.5 g in total) and responded with just a mild decrease in enzymes levels. 8 days after the biopsy, abdominal pain occurred at the site of pancreatic graft, followed by diarrhoea and vomiting. Initial ultrasound did not show any changes, but the following days the graft became enlarged and surrounded by fluid collections. Inflammatory markers and pancreatic enzymes plummeted. CT scan confirmed graft pancreatitis, blood perfusion was normal. The symptoms (mostly abdominal pain, bloating and mild fever) were fluctuating. We added antibiotics to fluid and symptomatic treatment. Despite intense therapy the progression of the inflammatory changes resulted in focal graft necrosis and required graftectomy (17 days after biopsy). Histology revealed focal purulent pancreatitis with haemorrhagic and necrotic changes. In patient 2 we administered 3 pulses of methylprednisolone (1.5 g in total), nevertheless this resulted in even higher rise of enzyme levels, fever and abdominal pain 4 days after the biopsy. Ultrasound showed fluid lining around the graft, CT stated signs of graft pancreatitis, with preserved blood perfusion. Antibiotic therapy was initiated immediately, along with fluid and symptomatic treatment, and within 7 days both clinical and laboratory signs of pancreatitis resolved, and he has normal pancreas graft function since then.

Conclusions

This case reports show a rather rare and delayed adverse effect of routinely performed graft biopsy and anti-rejection treatment and raises questions of adequate monitoring and complications management.

Conflicts of interest

No conflicts declared

CC3

Early detection and treatment of hyperglycaemia with oral agents in pancreas transplant recipients without acute rejection

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Background
Treating hyperglycaemia after pancreas transplant can be challenging, especially when not in context of acute rejection. Moreover, there is little evidence on the use of oral hypoglycaemic agents after pancreas transplantation. Consequently, hyperglycaemia in pancreas transplant recipients without acute rejection often leads to early initiation of insulin or under-treated hyperglycaemia.
In Newcastle, we established a surveillance programme to identify patients with pancreas graft dysfunction with the goal of early treatment of hyperglycaemia in the presence and absence of acute rejection.
Methods
4 patients in our post pancreas transplant surveillance programme, with hyperglycaemia and no signs of acute rejection in CT scan, DSAs and C-peptide. HbA1c and intensive glucose self-monitoring were requested. After MDT discussion, oral hypoglycaemic agents were started according to glycaemic profile.
Results
Case1-2 months post-SPK, complicated by peripancreatic collections. CT scan showed enhanced pancreatic graft. Amylase, lipase normal. No DSAs. C-peptide 1,200pmol/l. Hyperglycaemia related to TPN was treated with insulin. Off TPN, insulin was stopped, but hyperglycaemia after dinner persisted. Sitagliptin was started. 6 months later glucose 4-8mmol/l, HbA1c 42mmol/l, C-peptide 1,660pmol/l.
Case2-HbA1c 50 mmol/mol and 10 kg weight gain at 2 years post-SPK. CT pancreas showed normal graft. Amylase, lipase normal. No DSAs. C-peptide 1,920 pmol/l. Insulin resistance suspected and metformin commenced. 6 months later glucose 4-9 mmol/l, HbA1c 46 mmol/mol, C-peptide 1,450 pmol/l.
Case3-19 years post-PAK, HbA1c of 52 mmol/mol. Normal amylase and lipase. No DSAs. C peptide 1,270 pmol/l. Linagliptin was commenced. 4 months later glucose levels were 3.5-8 mmol/l, HbA1c 42 mmol/mol.
Case4-8 months after treatment for acute pancreas rejection post PAK, glucose readings >12 mmol/l after meals, HbA1c 44mmol/mol. CT scan:parenchymal atrophy. No DSAs. C-peptide 520pmol/l. Commenced sitagliptin and 4 months later glucose levels 4-7 mmol/l , HbA1c 29mmol/mol, C-peptide 740 pmol/l.
Conclusions
Early experience with our pancreas transplant surveillance programme has demonstrated the potential to restore and maintain glycaemic control in patients with hyperglycaemia without signs of acute rejection. Oral hypoglycaemic agents can provide effective therapy without the burden of recommencing insulin.
Conflicts of interest
No conflicts declared

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Posters

P01

Extracellular matrix as a key component in the production of functional and physiologically stable bionic pancreatic islets using the inkjet method

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Background

There are clinical trials report using stem cell-derived β-cells as an innovative and future-proof solution for the treatment of T1D. Stem cell-derived β-cells are expected to replace non-functioning pancreatic islets. However, to make this possible, it is necessary to create their 3D conformations, which has been proven by subsequent in vitro studies. However, apart from functionality, attention should be paid to the possibility of clinical use of beta cells. The process of transplanting claster β-cells even from 3D cultures, into the portal vein carries a high risk of damage and lack of functionality, as well as the risk of an undetermined final location of the cells. The aim of this experiment was to evaluate the survival and functionality of β-cells in bionic pancreatic islets, printed with inkjet method.

Methods

Two bioinks and INS-1E cells were used as the encapsulation carrier: 2% HAMA + 20% GelMA (GROUP: H-G_INS); 2% HAMA + 20% GelMA + dECM (GROUP: ECM_INS). The control group was INS-1E in 2D culture. Cell functionality was assessed in the GSIS-test. In addition, FDA/PI vital staining was performed.

Results

During a 21-day observation period, it was shown that cells encapsulated using the inkjet method remained viable. Cells suspended in the tested hydrogel variants retained a stable structure and did not disintegrate. On the second day of the experiment, there was no difference in cell activity. The encapsulated cell groups showed significantly improved functionality starting from day 7. Both groups showed over 30% higher functionality compared to the control group. On the 14th day of the experiment, cells suspended in bioink with the addition of dECM showed a clear advantage in response to the administered glucose. Compared to the control group, the increase was over 50% ($p<0.05$), and in the case of H-G_INS it was over 30% ($p<0.05$). Day 21 of the experiment also showed a functional advantage in ECM_INS, activity higher by almost 30% compared to the control group ($p<0.05$).

Conclusions

dECM a 3D conformation of cells within a bioprinted islets is a key component for maintaining the proper functionality of insulin-secreting cells. In addition, the developed bioink composition and the method used enable the production of stable 3D structures that can be transplanted in a stable and safe manner without disintegrating in physiological temperature conditions.

Conflicts of interest

Andrzej Berman is the co-founders of Polbionica Sp. z o.o.Michal Wszola is the co-founders of Polbionica Sp. z o.o.Marta Klak is the co-founders of Polbionica Sp. z o.o.

References

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P02

Towards a living encapsulation device for beta cell replacement therapy

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Background
During the isolation process for clinical islet transplantation the extracellular matrix and microvasculature of islets are destroyed leading to poor engraftment and significant loss of transplanted cells. Despite advances in synthetic based cell delivery devices to build a islet supportive microenvironment, the issue of triggering a foreign body response remains a challenge. The creation of entirely cell based implant could help overcome this challenge and allow for improved engraftment of the transplanted beta cells. Here we report on a cell based strategy aiming to create a more natural environment for islets.
Methods
Commercially available NUNC UpCell dishes were used to culture immortalized mesenchymal stem cells into cell sheets. Cell sheets were layered on top of each other to encapsulate human donor islets and functionality of the construct was assessed. Co-culture of HUVECs and iMSCs was perform and CD31+ staining was used to assess vascular like network formation.
Results
Optimization of culture protocols for cell sheets lead to stable and transferrable iMSC cell sheets. Stacking cell sheets improves stability and handling of constructs. A multilayered construct insured good integration of human islets. Encapsulated islets stayed functional inside the construct for multiple days. Co-culture of HUVECs and iMSCs lead to a dense vascular like network within the cell sheets.
Conclusions
We showed that cell sheets can be stacked with human donor islets in between. The human islets inside the construct show a proper insulin secretion. The addition of HUVECs leads to a vascular like network in the cell sheets. More research is needed to prove suitability of the approach, including upscaling, comparison to material based methods and *in vivo* studies. The approach could be tailored to be patient specific and it might be possible to combine it with iPSC derived beta cells in the future.
Conflicts of interest
No conflicts declared

References
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P03

Generation of vascularized endocrine constructs for type 1 diabetes

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Background

The aim of this work was to investigate whether incorporation of pre-vascularized insulin-secreting organoids (PVO) into a pre-vascularized Human amniotic membrane (HAM)-derived hydrogel will provide an optimal micro-environment and support engraftment and function of the insulin-secreting endocrine constructs.

Methods

PVOs were generated with varying proportions of insulin-secreting cells (EndoC-βH1/rat islet cells), human amniotic epithelial cells (hAECs) and Blood outgrowth endothelial cells BOECs (1000 cells/organoid). HAM-derived hydrogels were produced from decellularized, lyophilized HAMs. The best performing PVOs were mixed with BOECs and loaded in hydrogel. Function and cell distribution in hydrogel was assessed *in vitro*. To evaluate *in vivo* function, a marginal mass of 1000 native islet equivalent (NI) and the PVOs containing an equivalent islet mass were mixed with BOECs (2x106BOECs/ml) and loaded in hydrogel, cultured in vasculogenic media for 2 days. The vascularized constructs and PVOs alone were then transplanted under the skin of diabetic NSG mice.

Results

In vitro, the pre-vascularized insulin-secreting organoids (PVOs) demonstrated improved functionality when embedded in HAM-derived hydrogel, both with and without additional BOECs. The PVO-hydrogel group maintained normoglycemic levels for three months, with metabolic function reflecting those of non-diabetic controls. In contrast, mice receiving transplants of equivalent-mass native islets (NI) in hydrogel did not exhibit consistent diabetes reversal. Notably, only 3 out of 7 mice achieved normoglycemia after receiving transplants of PVOs alone. Furthermore, the rapid resurgence of hyperglycemia within 24 hours post-removal of the PVOs-hydrogel constructs underscores their efficacy in glucose regulation. Analysis of retrieved grafts revealed a larger β-cell mass and significantly enhanced vascularization compared to control specimens.

Conclusions

Our findings suggest that insulin-secreting constructs composed of PVOs and BOECs-vascularized HAM-derived hydrogel could be a promising strategy of β cell replacement therapies in alternative sites.

Conflicts of interest

No conflicts declared

References

This work was supported by grants from the European Commission (Horizon 2020 Framework Program; VANGUARD grant 874700), the Juvenile Diabetes Research Foundation (JDRF; grant 3-SRA-2020-926-S-B and 3-SRA-2023-1441-S-B), the Shota Rustaveli National Science Foundation (grant FR-19-19760) and the Swiss National Science Foundation (grant 310030_173138 and grant CRSII5_209417).

P04

A novel 3D model that recapitulates human insulitis ex vivo

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Background

Insulitis is a pathophysiological process characteristic of type 1 diabetes. Non-obese diabetic mice are the gold standard experimental model to investigate insulitis. However, species differences between rodents and humans, in many cases, preempt translational application. Many human tissue collections exist to investigate this process, in a non-dynamic manner.

Methods

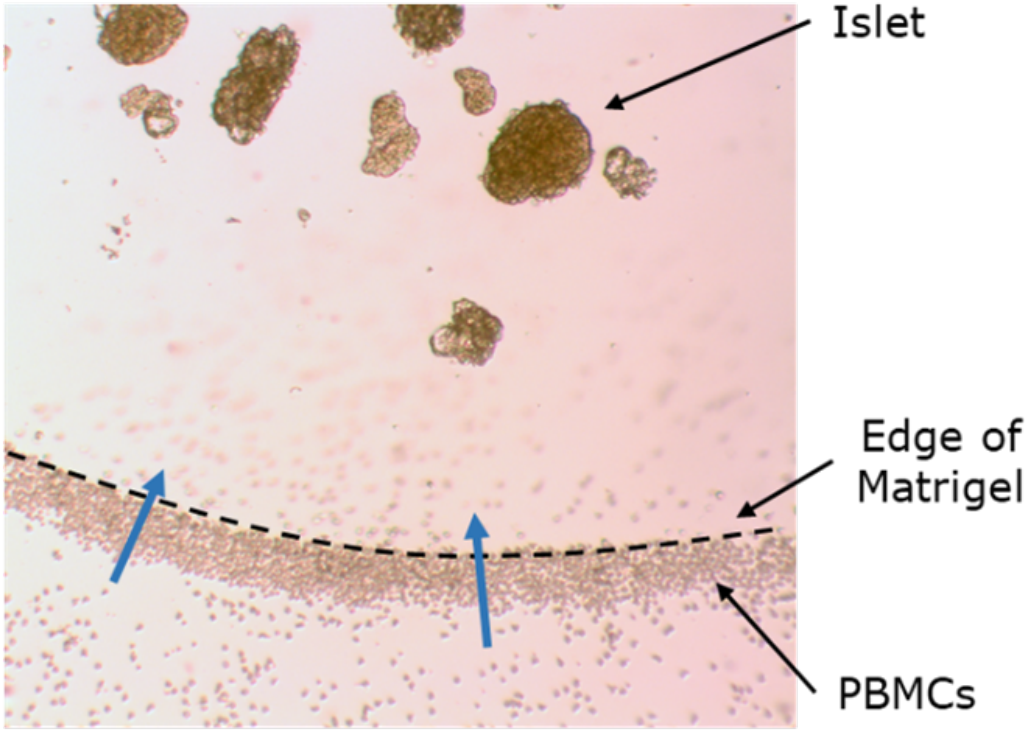
We have developed an *ex vivo* model to investigate the initiation and evolution of insulitis in humans using patient-derived human islets from cadaveric donors. Pancreas and blood samples were obtained from brain-dead organ donors upon consent from the Biotherapy Platform of the CHU of Lille, France. Highly pure (> 90 % purity) freshly isolated islets were 3D-cultured in solubilized extracellular matrix gel. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples. Islets in coculture with the same donor PBMCs were incubated with cytokines (IL-1β, TNF-α, IFN-γ). We determined the dynamic infiltration of immune cells directed to the islets over time.

Results

In our model, cytokines-induced islet-directed immune cell infiltration started as early as 18 hours and reached maximum at 48 hours. Cytokines induced the production of reactive oxygen and nitrogen species in islets and triggered cell death and apoptosis 24 hours after the insult. In this model, islets expressed higher levels of cytokines and chemokines when treated with a mix of cytokines. Islets also displayed beta cell dysfunction, measured as glucose-stimulated insulin secretion (GSIS) in a perifusion system, as early as 1 hour after the insult with cytokines, which was more accentuated 24 hours after.

Conclusions

Altogether, this model can lead to a better understanding of the landmarks of insulitis in humans to decipher novel targets of interest, and aid in the discovery or testing of novel therapeutics.



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3D human islet-PBMCs co-culture. Image of immune cells infiltrating the 3D-scaffold containing human islets from the same cadaveric donor

Conflicts of interest

No conflicts declared

References

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P05

Intraparenchymal enzyme injections improve digestion of pancreas with inadequate parenchyma distension

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Background

Incomplete enzyme distribution during pancreatic islet isolation can hinder optimal tissue digestion. Difficulty in cannulating the pancreatic duct further complicates islet isolation from diseased or damaged organs, especially for total pancreatectomy with islet autotransplantation (TPIAT). In this study, we introduced intraparenchymal injections (IPI) to enhance enzyme distribution during islet isolation and investigated whether digestion efficiency improved with the implementation of IPI.

Methods

Pancreas from organ donors and from patients undergoing total pancreatectomy were used for islet isolation which took place in a Good Manufacturing Practice (GMP) facility by experienced staff. Enzymes were perfused using retrograde or ante- and retrograde cannulation of the pancreatic duct (RC and ARC). IPI was introduced 8 years into our islet isolation program, administered concurrently into non-distended parenchyma on indication. Digestion efficiency was defined as the proportion of pancreas mass that was successfully digested.

Results

Organ donors (N=405) were on average 48.5 ± 13.2 years old, had a BMI of 26.5 ± 4.7 kg.m-2 and 43.7% were female. TPIAT patients (N=21) were 43.8 ± 13.8 years old, had a BMI of 24.5 ± 4.2 kg.m-2, 71.4% were female and 18 had chronic pancreatitis with a median duration of 5.0 years. Before IPI implementation , digestion efficiency of donor pancreases (N=162) was 84.5 ± 9.39% and after implementation (N=242), it was 86.1 ± 8.9% (p=0.07). The interquartile range changed from 80.9 - 90.4% before to 81.7 - 92.6% after implementation. After implementation, digestion was similar after RC (86.6 ± 8.9%, N=202) and RC with IPI (87.1 ± 10.1%, N=9, p=0.87). Digestion after ARC only (N=18) increased from 78.8 ± 7.75% to 88.1 ± 5.44% after ARC with IPI (N=14, p<0.001). In isolation after total pancreatectomy, digestion increased from 55.0 ± 27.4% without (N=4) to 82.0 ± 17.1% with IPI (N=17, p=0.02).

Conclusions

Our data indicate that performing IPI when distension of the parenchyma was incomplete increases digestion in what would otherwise be a poorly digested pancreas. This may be especially helpful in islet isolation of pancreas after total pancreatectomy.

Conflicts of interest

No conflicts declared

P06

Adaptation and streamlining of an advanced therapy medicinal product (ATMP) manufacturing- from laboratory scale to clinical trial

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Background
Advanced therapy medicinal product (ATMPs) are novel therapies based on genes, cells or tissues developed to treat many different diseases. However, their successful translation from laboratory research to clinical trials poses significant challenges related to manufacturing scalability, quality, and regulatory compliance.
Methods
Key considerations include cell sourcing, qualification of raw and starting materials, cell expansion and differentiation, cryopreservation, quality control, and product characterization. The importance of clear and detailed SOPs cannot be overstated as the implications of leaving anything open to interpretation can be highly deleterious and lead to increased product variability and risk of failure.
Results
At Oslo University Hospital, within the Section for Cellular Therapy, we have developed and effectively implemented a robust manufacturing process for the ATMP product, decidual stromal cells, within an academic and hospital-based Good Manufacturing Practice (GMP) laboratory. In this presentation, we will present the outline of this process.
Conclusions
By addressing these challenges, we can work toward a future where ATMPs offer safe, effective, and accessible treatments for a wide range of diseases.
Conflicts of interest
No conflicts declared

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P07

IsletNet, built-in validation tools, a multi-center validation study proposal

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Background

IsletNet, a web-based service, was developed as a digital alternative [1] to the traditional manual islet quantification approach [2]. It has been trained using islet images from interested centers [3]. We aim to propose now a multi-center validation study of IsletNet. To this end we introduce the integrated validation tools (Fig. 1A).

Methods

Simple Comparison tool is designed to compare IsletNet to a local trusted method (Fig. 1B). Pixel-to-Pixel Comparison tool facilitates a detailed comparison with annotated images. Isolated islets were counted manually; the counts were entered into an xlsx file from IsletNet. Simultaneously, images were acquired, and manual annotations were created. The manual counts, original images, and annotations were uploaded to the respective validation tools. IsletNet generated graphs (pdf) and tables (xlsx) which were downloaded for analysis.

Results

The manual islet counts (x-axis, Fig. 2A) with those automatically determined by IsletNet (y-axis, Fig. 2A). It also compares the manually estimated islet volumes (x-axis, Fig. 2B, Ricordi table selected in Fig 1B) with the automatic measurements by IsletNet (y-axis, Fig. 2B). The Pixel-to-Pixel Comparison tool depicts the islet contours and the separation lines (Fig. 2D) , compares the automated segmentation of the original images with the expert annotations (Fig. 2C,E), and it produces histograms that juxtapose the automated analysis with the manual ground truth (Fig. 2F). This tool also generates tables showing various metrics like sensitivity, specificity, accuracy, and F1 score, alongside relative errors in islet counts and volumes using different islet shape models.

Conclusions

The integrated validation tools are prepared for an independent multi-center validation study of IsletNet. A detailed proposal for this study is forthcoming, and participation from all centers is encouraged.

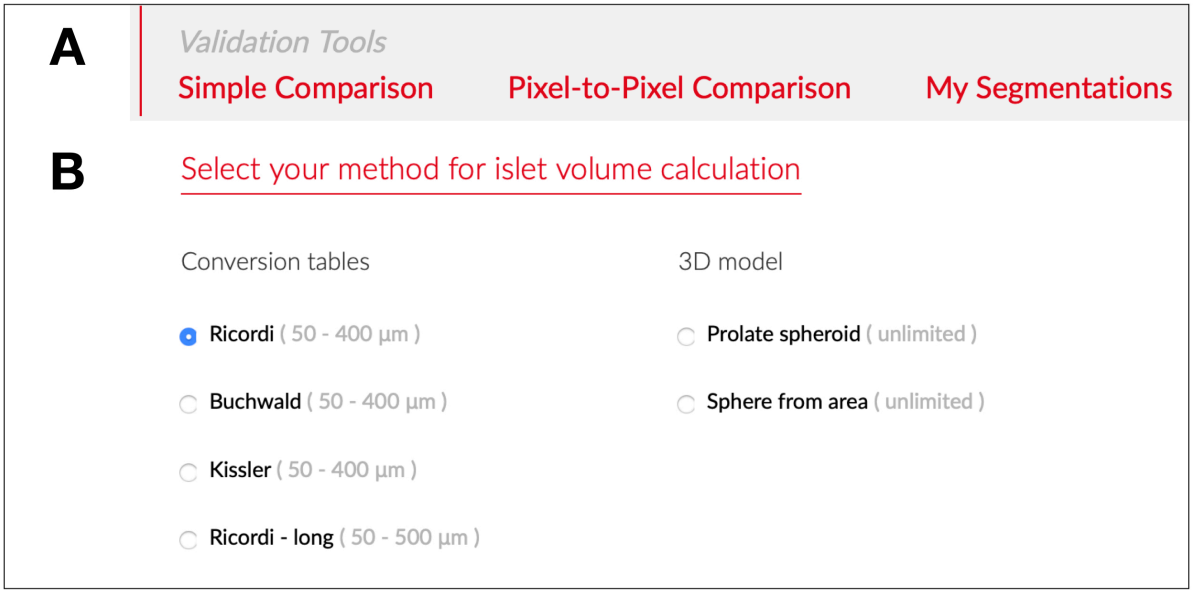

Fig. 1: The validation tools in IsletNet (screenshots).

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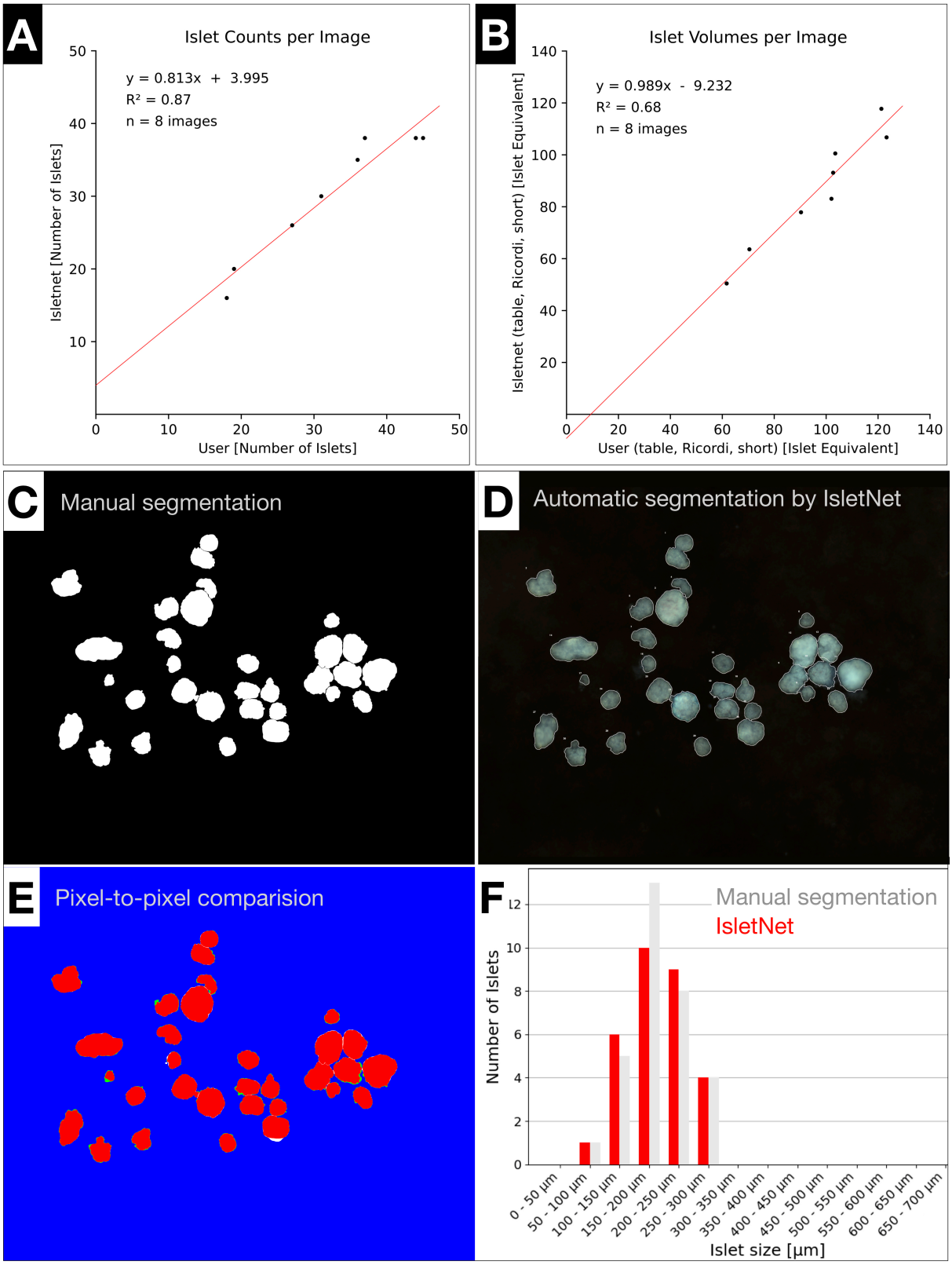

Fig. 2: The automatic output of the validation tools.

Conflicts of interest

No conflicts declared

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P08

The effects of amniotic epithelial cells and their derivatives on pancreatic islets

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Background
A considerable islet loss is observed following islet transplantation in type 1 diabetes patients due to hypoxia, inflammation, and poor vascularization. Human amniotic epithelial cells (hAECs) have been shown to have cytoprotective effects on pancreatic islets under normal and stressful conditions, due to their anti-inflammatory, immunomodulatory, and regenerative properties. This study aims to investigate whether AECs or their derivatives, such as conditioned media (CM) and extracellular vesicles (hAEC-EVs), would be able to exert similar properties and whether cell-cell contact is essential for such effects.
Methods
The conditioned media was collected from hAECs culture and the hAEC-EVs were subsequently isolated (by ultracentrifugation) and characterized (by nanoparticle tracking analysis, electron microscopy, and western blotting). Rat islets were cultured either with cells, in direct or indirect contact, CM or hAEC-EVs. The islet function was assessed by glucose-stimulated insulin secretion assay and viability by TUNEL staining.
Results
Characterization of hAEC-EVs showed the successful isolation of these vesicles. hAEC-EVs had a mean diameter of 160 nm and expressed EV markers such as CD9, CD63, and TSG101.
Rat islets cultured with AEC and exposed to the CM had an increased insulin secretion, compared to control, under normal conditions. On the other hand, less effects were observed with hAEC-EVs treated conditions. In the future, we plan to understand better the mechanisms underlying the observed effects.
Conclusions
These preliminary results suggest that hAEC-EVs might not be enough to improve islet function, whereas AECs, in direct or indirect contact, as well as their CM, are beneficial for islets in culture.
Conflicts of interest
No conflicts declared

P09

Quantification of endocrine tissue from human pancreas and effect of donor phenotype

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Background
Cellular therapy of diabetes mainly uses pancreases obtained from donors in a brain-dead state, requiring knowledge of the distribution of islets of Langerhans (IL) in the pancreatic parenchyma. Few studies have looked at the importance of quantifying endocrine tissue in a large number of human pancreases. The aim of this study was to measure the surface area of IL from a histological section taken from pancreases used in an allograft. The collected results were then compared to the parameters of the donors.
Methods
Using an anti-chromogranin A antibody, immunohistology was used to identify the IL. The sections were then digitized and subjected to analysis using image J software.
Results
In n=181 human pancreases, the surface area of IL represented on average 1.46% (median=1.14%) of the total pancreatic surface area. A 100x heterogeneity of endocrine tissue was observed, with a minimum of 0.053% and a maximum of 5.63%. A correlation study between known donor parameters (height, weight, BMI, age, HbA1c, and pancreas mass) and IL surface area was conducted to explain this inter-individual variability. Only weight (P=0.001) and BMI (P<0.0001) showed significance, despite the low coefficient of determination. Differences in donor BMI and HbA1c parameters were identified based on the status of insulin resistance (diabetic (n=12), moderate insulin resistance (n=88), low insulin resistance (n=81)) and obesity (normal (n=67), overweight (n=56), obese (n=40), and severely obese (n= 18). A higher BMI results in an increase in the pancreatic endocrine surface area. Compared to donors without diabetes (1.3%), those with type 2 diabetes (2%) appear to have a larger endocrine surface area.
Conclusions
The importance of pancreatic endocrine tissue would be influenced by the donor's weight, BMI and HbA1c, parameters that could improve the selection of pancreas donations for cell therapy.

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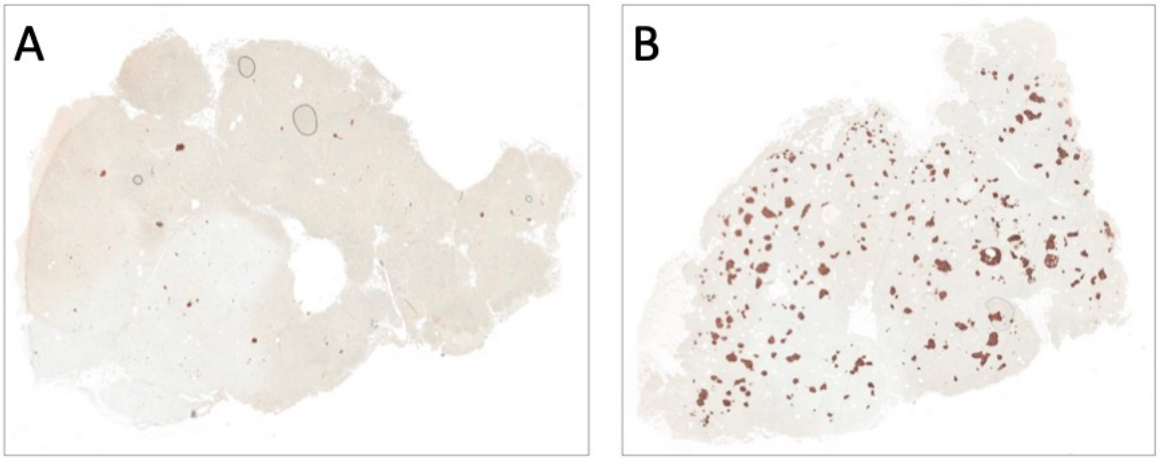

An example of the pancreatic parenchyma's islet heterogeneity. The percentage of islets in n=181 slices of human pancreatic tissue varied from 0.05% (A) to 5.6% (B).

Conflicts of interest

No conflicts declared

References

Agence de la Biomédecine, AOR Recherche et Greffe 2023

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P10

Ex vivo evaluation of the Human Bioartificial Pancreas (hBAP) for β-cell replacement purposes

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Background

In type 1 diabetes field, intrahepatic islet transplantation faces hurdles as organ scarcity, inadequate vascularization, and lack of ECM support at the transplant site. This study proposes the bioengineering of a novel human bioartificial pancreas (hBAP) for β-cell replacement using pro-vascularizing cells and ECM support. It aims to assemble endocrine cells, blood-outgrowth endothelial cells (BOEC), and human decellularized amniotic membrane (dHAM). Vascularized islet-like spheroids (SPH) from two protocols (P1 and P2) and native human islets (HI) were compared as endocrine cells for hBAP generation.

Methods

Same HI equivalents (IEQ) of matched batches were digested with P1 and P2, differing in HI digestion method. HI-derived cells were aggregated alone or with BOEC after 3 days of culture, resulting in Islet SPH and Islet SPH+BOEC respectively. P1 and P2 were compared in term of digestion efficiency, β-cells mass preservation, SPH size and harvesting efficiency. The functional and compositional evaluations of Islet SPH and Islet SPH+BOEC from P1 and P2, alongside batch-matched HI, were conducted using dynamic insulin secretion tests (DIST) and immunofluorescence analysis (IF). Furthermore, hBAP and hBAP(-BOEC) were generated using native HI and cultured ex vivo for 7 days in a customized bioreactor, followed by evaluation using DIST and IF.

Results

P1 yielded significantly more total cells after digestion than P2 ($p < 0.05$). P2 showed a significantly higher live cells percentage compared to P1 ($p < 0.05$). Both protocols demonstrated similar efficiency in recovering live cells per IEQ digested. Both Islet SPH and Islet SPH+BOEC from P1 or P2 displayed similar size distributions and insulin secretion performance but reduced insulin secretion capability compared to HI ($P \leq 0.001$). Therefore, to standardize hBAP generation, non-digested HI were employed, resulting in hBAP and hBAP(-BOEC) devices. Both bioengineered devices exhibited similar endocrine function to native islets and Ins+ cells surrounded by a vascular structure.

Conclusions

According to the results from P1 and P2, digestion protocol needs to be defined and improved. However, preliminary results on hBAP generated with HI suggested a positive impact of the vascularized dHAM microenvironment in ex vivo function suggesting promising outcomes for in vivo applications.

Conflicts of interest

No conflicts declared

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P11

Strategy for preserving pancreatic islet vascularization during transplantation: A spheroid model composed of endothelial and insulin cells

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Background
The efficacy of pancreatic islet intraportal transplantation is hampered by an early and deleterious inflammatory reaction termed IBMIR. IBMIR is associated with the secretion of pro-inflammatory cytokines, IL-1β and TNF-α , at the vicinity of the grafted islets.
We showed that IBMIR-induced cytokines induce mesenchymal transition (EndMT)of intra islets endothelial cells (IEC) in vitro and that dulaglutide, a GLP-1 analogue, counteracts EndMT.
Our aim is to study 3D interactions between IEC and β cells in a murine spheroid model and assess the effects of dulaglutide on the survival and function of both lineages.
Methods
To obtain a 2000 cells spheroid representative of a native islet, different ratios of murine IEC (MS-1) and β cells (β-TC6) were co-cultured in Aggrewell800 plates up to 96 hours. After trypsinization cell viability was measured every 24h by trypan blue and compared to values obtained in 2D cell mono-cultures. GSIS was measured in response to 3 mM and 16 mM Glucose by ELISA for 100 spheroids by condition. Cell distribution was characterized by confocal Z-stack spinning disk microscopy using an adapted optical clearing procedure. Endothelial CD31 and β cell makers (PSA-NCAM, insulin) were immunostained after 24h and 72h, at base line and in response to TGF-β2, the gold standard inducer of EndMT.
Results
Co-culture was beneficial to the survival of β cells in the spheroids (n=3). The optimal MS-1 to β-TC6 ratio was 1/20 with 80% viability as compared to the 2D β-TC6 monoculture, that was maintained up to 96h, whereas viability dropped to 60% after 72h with the ratio 1/10. β-TC6 were poorly responsive to Glucose stimulation owing to high insulin secretion baseline values (4 replicates). No overlap of CD31 and insulin labeling was observed as expected. However, full colocalization of the maturity marker PSA-NCAM and of insulin labeling was not observed suggesting different maturation states of the β cells within the spheroids.
Conclusions
We have established a spheroid model suitable for the study of EndMT and its pharmacological modulation. Further prospects are the better understanding of the role of cell junctions and ischemic gradient for optimized addressing of cytoprotective drugs to the islets and a preserved islet graft function.
Conflicts of interest
No conflicts declared

References
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P12

Pancreatic islets injury by electroporation

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Background

Microporation is a modification of electroporation technique, which can be used for introduction of macromolecules into pancreatic islet cells. Using short electric pulses, the micro pores within a cellular membrane are created. Nucleic acids can subsequently enter the cell. We tested, potential detrimental effects of microporation as a transfection method for siRNA introduction into pancreatic islet (PI) cells.

Methods

Male Brown Norway rats 10 – 12 weeks old were used as a donor of PI. PI were isolated by collagenase digestion according to a standard protocol. After overnight cultivation, microporation was used as transfection method (Neon, 2 pulses, 950 V, 30 ms). There were 3 samples: PI microporated with 200 nM siRNA, microporated without siRNA, and nontreated PI. After 24 h cultivation, islets were subjected to tests of viability and function – perifusion insulin release test, oxygen consumption rate, fluorescent test of membrane integrity (propidium iodide and acridine orange) to identify live and dead cells.

Results

All of the used methods didn´t show significant differences among treated PI versus nontreated control. The insulin release during perifusion test and oxygen consumption rate (after stimulation by glucose in high concentration) were not impaired. Using fluorescent test of membrane integrity, the percentages of dead cell in PI were: 15,9 % after microporation with siRNA, 13,7% after microporation and 10,6 % in control PI.

Conclusions

Microporation with or without siRNA did not impair PI. Microporation appears to be a safe method for transfection of PI.

Conflicts of interest

No conflicts declared

References

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P13

Cryopreservation of dissociated rat pancreatic islet cells

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Background
Long-term cultivation of isolated islets leads to dedifferentiation of endocrine cells or their death [1]. Cryopreservation of whole islets is a technically demanding process [2, 3]. To preserve islet cells for later use, we have developed a method for cryopreservation of dissociated rat pancreatic islet cells.
Methods
Isolated rat pancreatic islets were dissociated into cell suspension by Accutase (20 min, RT). Some cells were used fresh and some were frozen by controlled cooling in CMRL medium supplemented with 20% FBS and 10% DMSO. Frozen samples were stored in liquid nitrogen untill next usage. Fresh and thawed cells were tested for viability by Vi-CELL XR Cell Viability Analyzer (Beckman Coulter) and seeded into 96 well plate coated with extracellular matrix produced by bladder cancer cells (HTB-9), 50 000 cells per well. Cultivated cells were tested for glucose stimulated insulin secretion. Subsequently, beta and alpha cell were immunolabeled to analyse their occurrence.
Results
Viability of thawed cells was slightly lower than that of fresh cells, (54% versus 56%). Glucose stimulated insulin secretion was comparable in thawed and fresh cells, stimulation index was 3 and 3.5, respectively. Cultivated dissociated cells successfully adhered on the coated surface of culture plate and both beta cells and alpha cells were represented among them in similar ratio, in frozen samples there were 32 alpha cells per 100 beta cells and in fresh samples 37 alpha cells per 100 beta cells.
Conclusions
The results of this preliminary study show that cryopreserved dissociated rat islet cells have comparable functional quality to fresh cells. This approach represents a simple method for long-term preservation of islet cells.
Conflicts of interest
No conflicts declared

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P14

Concordance of the IGLS.2 classification of islet transplantation success between HbA1c-SHE and Continuous Glucose Monitoring

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Background

Results of islet transplantation (IT) may be assessed with IGLS 2.0 classification, calculated with either HbA1c and self-reported severe hypoglycaemia events (SHE), or with CGM according to TIR (time in range 70-180 mg /dL) and TBR (Time below range 54mg/dL). The 2 versions have not been compared in terms of success classification.

Methods

This retrospective single-center study used routine-care data records of a cohort of 68 T1D patients followed for a maximum of 19 years after IT and recorded before and at 3, 6, 9, 12 months and then yearly post IT corresponding to 920 venues from 475 were used for this study, the other being excluded because of missing data. The two alternative IGLS 2.0 classifications were compared using the weighed Cohen's kappa for statistical measurement of the categorical agreement between the classifications.

Results

The agreement between the CGM-based and the HbA1c-SHE based version of IGLS 2.0 classification of the clinical success of IT was 0.7, which is substantial. According to the classical approach of IGLS 2.0 classification, 49%, 24%, 17% and 10% out of a total of 475 visits were classified as optimal, good, marginal and failure, respectively, while, with the CGMS-based IGLS 2.0 classification, the percentages were 33%, 13%, 43% and 10%, respectively. These results show that the classical version classifies 73% of patients as success with optimal or good results, whereas only 46% are classified as success with the CGM version. When testing other TIR ranges as classification criteria, the highest calculated Cohen's kappa is 0.71.The majority of "optimal" and "good" datapoints presents a HbA1c level <7%, a TIR above 70% and cannot be distinguished. For TBR, optimal and good transplantation outcome have mainly a TBR <1%, but TBR >1% may still be associated with optimal function, a point which has never been reported.

Conclusions

This substantial agreement allows to use the 2 approaches of the IGLS 2.0 classification alternatively. We also show that there seems to be no threshold effect on the agreement between the two classifications when testing other ranges of TIR in the CGM based classification. The CGM version underestimates the rate of success outcome.

Conflicts of interest

No conflicts declared

P16

Effect of islet alone or islets after kidney transplantation on quality of life in type 1 diabetes : a systematic review

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Background

Islet transplantation for type 1 diabetes allows the elimination of severe hypoglycemic events (SHE) and the restoration of hypoglycemia awareness, which both all contribute to improve health-related quality of life (HRQOL). Assessing systematically the report of HRQOL outcome are therefore a major element to better understanding the benefit of islets transplantation. Therefore, we performed a systematic review of the literature to assess the impact of islets transplant alone (ITA) or islets after kidney transplantation (IAK) on HRQOL in patients with T1D.

Methods

We gathered all studies providing a quantitative estimate of the HRQOL after ITA or IAK. For inclusion, the studies were required to have the following characteristics: (i) adult recipients of islet grafts for type 1 diabetes, (ii) generic or disease-specific QOL assessment, (iii) comparison of QOL between pre and post-transplantation state or between post-transplantation state and other pretransplantation patients or general population.

Results

Seven final studies met the inclusion criteria. Overall, data on 245 patients were gathered. Questionnaires assessing HRQOL were generic ,such as SF-36 ,or disease-specific such as diabetes distress scale, diabetes quality of life questionnaires and hypoglycemia fear survey. Questionnaires encompass physical health, mental health, social health or functional health dimensions. In this systematic review, islet graft was associated with an improvement of all aspects of HRQOL compared to the pre-transplant situation.

Conclusions

Our systematic review show that islet graft allows an improvement of quality of life in T1D individuals suffering from SHE. To the best of our knowledge, this is the first systematic review assessing the impact of islet transplantation on HRQOL.

Conflicts of interest

No conflicts declared

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P17

Liver biopsies of islet transplant recipients with HLA donor specific antibodies suspected or not for rejection: about two cases

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Background

Mechanisms of rejection in islet transplantation have been suggested in animal models, yet determinants of islet graft rejection in humans remains elusive. We detail here liver biopsy findings from two islet transplant recipients who developed HLA donor-specific antibodies (DSA), with and without clinical rejection signs.

Methods

Liver biopsies were performed surgically after consent in two islet transplant recipients. Mini-invasive surgery was performed using coelioscopy and involved macrobiopsies (approximately 1cm² tissue resection) and a needle microbiopsy. Tissues were paraformaldehyde-fixed and frozen for histological analysis, including light microscopy, immunostaining for insulin, glucagon, somatostatin (metabolic markers); CD68, CD3, CD20, C4d (rejection markers) and FISH analyses for X and Y chromosomes.

Results

The first patient, receiving islets from three donors, achieved insulin independence post-third infusion. She exhibited preformed DSAs against all donors and developed new DSAs against the first donor. Two years later, while insulin-independent, she benefited from a liver biopsy during a cholecystectomy, which revealed islets in the macrobiopsy with a density of 0.4 islets/mm3, with a median diameter of 140 µm (range 108-196). The microbiopsy contained a single islet. These islets were positive for insulin, glucagon, and somatostatin, with no rejection markers detected. FISH analyses for X and Y chromosomes could differentiate male and female donors in the biopsy. The second patient also attained insulin independence after receiving islets from three donors and showed no preformed DSAs. Post-Covid19 infection, six years post-transplantation, he experienced a C-peptide decrease and resumed insulin therapy after seven years. Eight de novo DSAs emerged, targeting HLA antigens from all donors. While macrobiopsy identified islets, none were found in the microbiopsy. Surprisingly, these islets showed no signs of immune infiltration or rejection.

Conclusions

Surgical liver macrobiopsy is a reliable tool to assess islet viability in islet transplant recipients. In two patients presenting with signs of allosensitization, no infiltration or rejection could be assessed.

Conflicts of interest

No conflicts declared

P18

Slovak first islets after kidney allotransplantation in cross border cooperation and mentorship from Czech's IKEM-Prague

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Background

We report our case of Slovak first islets allotransplantation from a brain dead multiorgan donor after living donor kidney transplantation in cross border cooperation between Slovak and Czech transplant centers and mentorship from IKEM – Prague.

Methods

Surgical procedures have been performed in the University Hospital Kosice, Slovakia. Islets isolation procedure has been performed in IKEM Prague, Czech Republic. Transfer of organs and mentoring personal between centers was arranged by Air Transport due to distance between centers - 658 km. Overall time from pancreas procurement to IAKTx was 10h 40min. Recipient: 38 y. o. woman, BMI 28,7; IDDM since 1993, C-peptide negative, diabetic nephropathy G5A3, conservatively intractable hypoglycemia unawareness syndrome. The immunosuppression protocol: induction with anti-T lymphocyte globulin maintenance: tacrolimus, MMF and corticosteroids. Islets isolation procedure according to the modified Edmonton protocol. Islets after kidney allotransplantation – September 8th 2023 Open surgical procedure minilaparotomy. Islet infusion via umbilical vein to the porta of the left hemiliver Under radiological control – C-arm skiascopy Portal vein pressure monitoring (max. 25 mmHg)

Results

LDKTx was without complications, After IAKTx we observed decrease in HbA1c and measurable levels of C peptid in the bottom of normal range. CGM shows better glucose control, with less episodes of hypoglycemia and significantly less episodes of hyperglycemia. Insulin Total Daily Dose stays without decrease (yet).

Conclusions

After very short postoperative follow up, it is not possible correctly form conclusions. Personal patient valuation after transplantation is: I have new better quality of life…. Of course, longer follow up to exactly confirm the results is essentially needed. We strongly believe that this case report shows the possibility how we could offer alternative treatment option for our diabetic patients also in the future.

Conflicts of interest

No conflicts declared

References

Benefits of Islet Transplantation as an Alternative to Pancreas Transplantation: Retrospective Study of More Than 10 Ten Years of Experience in a Single Center Rev Diabet Stud (2016) 14:10-21

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P19

Becoming Transplanted - A constructivist Grounded theory Study

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Background
There are limited studies of the patient experience regarding pancreas transplant with research in pancreas transplant being weighted towards the quantitative and biomedical approach. This study took a qualitative approach in exploring the pancreas patient experience. Using a longitudinal design, this study follows the Simultaneous Pancreas and Kidney (SPK) patient from waiting list to transplant. In the understanding of this experience the hope is to inform future practice and potential interventions and identify further areas for study and development.

Methods
The study investigates patients listed for SPK transplant in Edinburgh. Data was collected using semi-structured qualitative interviews, initially face to face, then following COVID restrictions via video. Prior to each interview the researcher used meditation to enter a state of presence, empathy and congruence and facilitate a person-centred approach (Natellio, 2001).
The first interview was 6 months to transplant and the second 3 months post-transplant.

Results
The data was analysed from 19 interviews with 10 patients, 10 pre-transplant and 9 post transplantation. ***Becoming transplanted*** was developed as the core category and captures a transition through the experience of chronic disease and ***holistic illness suffering***. ***Experiential avoidance*** was shown in poor glycaemic control and fear of transplantation. ***Creative adjustment*** and ***Reframing of Self*** are found to be the positive outcome of acceptance and transplantation.

Conclusions
The individualistic experience of ***holistic illness suffering*** reiterates a need for person-centred care, as the process of alleviating suffering can only be started once identifying the cause. As health professionals we can be actively involved as facilitators to ***Creative Adjustment*** and acceptance of Self in the context of diabetes, renal failure and transplantation .

Conflicts of interest
No conflicts declared

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P21

Histopathological Insights in Discarded Pancreatic Grafts: Implications for Transplant Safety and Donor Selection

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Background

Pancreatic transplant programs are currently hindered by a lack of viable grafts; therefore, potential expanded donor criteria are being explored. However, the incidence of histopathological lesions found in these pancreases is currently unknown. A multicenter prospective study was conducted in Spain in order to describe the incidence of altered anatomopathological findings observed in discarded pancreases pertaining to these potential expanded criteria donors.

Abbreviations: donation after brain death (DBD), Controlled donation after circulatory death (cDCD), Pancreatic Intraepithelial Neoplasia (PanIN).

Methods

A total of 129 non-accepted organs for transplantation (BMI >30kg/m2 and/or age > 50 years old) were included, 76% from DBD donors and 24% cDCD donors. Incidental findings during histological analysis were registered. A detailed analysis of PanIN-related donor characteristics was conducted.

Results

Median age: 59 years [52-68], BMI: 25.1 kg/m2 [23.4-30.6], 63% males. Donor types: DBD initially accepted but discarded during extraction (18.5%); DBD discarded because of age 50-60 (7.4%), because of 50-60 years + BMI 30-35 kg/m2 (7.4%), and because of > 60 years old and/or BMI > 35 kg/m2 (37%). DCD initially accepted but rejected during procurement (7.4%), DCD rejected because of age 45-60 (14.8%), and because of > 60 years and/or BMI > 35 kg/m2 (7.4%). Diagnoses included: neuroendocrine tumor (n=2, 1.6%); low-grade PanIN (n=19, 14.7%); low-grade PanIN + chronic pancreatitis (n=2, 1.6%); low-grade PanIN + pancreatic atrophy (n=5, 3.9%); low-grade PanIN + serous cystadenoma (n=1, 0.8%); exacerbated chronic pancreatitis (n=1, 0.8%); ductal microadenoma (n=2, 1.6%); pancreatic islet hyperplasia (n=6, 4.7%); pancreatic ductular proliferation (n=1, 0.8%); septal lipomatosis (n=1, 0.8%).

Conclusions

Histological examinations can vary from incidental lesions to potential precursors of malignancies such PanIN. Understanding and interpreting these histological findings are fundamental for ensuring the safety and success of pancreatic transplants, driving advancements in donor selection protocols and refining criteria for graft acceptance.

Conflicts of interest

No conflicts declared

References

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P22

Hospital Readmission during the First Year of Simultaneous Pancreas and Kidney Transplantation: A Single-Center Analysis

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Background
Simultaneous pancreas-kidney transplantation (SPKT) is the treatment of choice for type 1 diabetes mellitus patients (pts) with end stage renal disease. Hospital readmission after SPKT is associated with increased morbi-mortality. Rates of readmission, causes and risk factors for readmission are underinvestigated.
Methods
We performed a retrospective analysis of SPKT performed at our center, from May 2020 to December 2023. The number and reasons for hospitalization during the first year (1-y) after SPKT were analyzed.
Results
260 pts (136 men) underwent SPKT. Their mean age was 35.5±6.6 years; prior dialysis and diabetes duration was 25±19 months 24.2±6 years, respectively. During the 1-y after discharge, 132 pts (50.8%) were readmitted (average of 1.8 episodes/patient). The main cause was infection (60.6%), urinary focus being the most common (56.3%), followed by surgical wound (11.3%) and peri-graft collections (10%). Some pts had more than 1 type of infection. The main etiology of systemic infections was bacterial. Of the 51 pts with identified pathogens, 68.6% had multidrug-resistant agents (MDR). Graft dysfunction (GD) was also a frequent cause of readmission: 18 pts (13.6%) had pancreatic GD, acute rejection (AR) confirmed in 7 (5 cellular, 1 humoral, 1 mixed); 4 had thrombosis, requiring pancreatectomy. Renal GD observed in 14 pts, AR in 7 (5 cellular, 1 humoral, 1 vascular). Death occurred in 3 pts during hospitalization due to infection. The recipient age, body mass index, donor age, and length of first hospital stay do not appear to correlate with increased risk of 1-y readmission.
Conclusions
Infections are the main cause of hospital readmissions, and most of the identified pathogens are MDR. Despite the pressure to reduce immunosuppression to minimize these complications, we cannot forget that 1 in every 10 transplanted pts presents with GD due to AR. The high 1-y incidence of readmissions reflects the complexity of these patients.
Conflicts of interest
No conflicts declared

P23

Short-term outcome after simultaneous pancreas-kidney transplantation with alemtuzumab vs. basiliximab induction; a single-center retrospective study

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Background

Both T-cell depletion by alemtuzumab (ALEM) as well as IL-2 receptor blocking by basiliximab (IL2R) are used as induction for simultaneous pancreas-kidney (SPK) transplantations. Multiple studies have reported higher rates of infections with ALEM versus IL2R. Due to the COVID-19 pandemic we adapted our standard induction from ALEM to IL2R for low immunological risk SPK transplantations. We compared 180-days transplantation outcomes between ALEM and IL2R induction.

Methods

Patients with low immunological risk who underwent SPK between September 2015 and June 2022 at our center were analyzed. Induction was either ALEM (30 mg, until February 2020) or IL2R (2x 20 mg, from February 2020 onwards) and triple maintenance therapy (prednisolone, tacrolimus, mycophenolate acid). All pancreas allograft transplants were performed using enteric drainage. Standard prophylaxis included antibacterial, antifungal and antiviral therapies. Valganciclovir prophylaxis for cytomegalovirus (CMV) infection was risk stratified. All patients routinely went to the intensive care unit postoperatively.

Results

Thirty-five SPK transplant recipients were included (67% males, mean age 42±10 yr). Fifty-four percent of patients were transplanted pre-emptively, 37% received organs from a DBD donor and mean donor age was 31±11 yr. Twenty-one recipients received ALEM and 14 recipients IL2R induction therapy, see Table. Two pancreas grafts were lost in the ALEM group and one kidney graft was lost in the ILR2 group. No patient deaths occurred. No differences between ALEM and IL2R groups in kidney and pancreas graft function or rejection incidence were observed. More recipients in the ALEM group suffered from bacterial (81% vs. 50%, p=0,05) and viral infections (57% vs. 38%, p=0.21) compared to the IL2R group. The duration of initial hospitalization was longer for the ALEM group compared to the IL2R group (28 [20-39] vs. 12 [10-16] days, p<0,001). The percentage of recipients with hospital readmission was equal (57%) for both groups.

Conclusions

Our experience, although limited, with IL2R induction for SPK transplants with low immunological risk has shown encouraging results with equivalent short-term graft function and decreased post-operative infection rates and hospital admission duration compared to ALEM induction.

Conflicts of interest

No conflicts declared

P24

Urinary tract infection in a randomized study of sirolimus versus mycophenolate mofetil in simultaneous pancreas and kidney transplantation

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Background
Urinary tract infections (UTI) are common in patients after simultaneous pancreas and kidney transplantation (SPK). However, the impact of specific immunosuppressive agents is not clear. Hence, we retrospectively compared the rates, severity, and risk factors of UTIs in SPK recipients under two different immunosuppressant regiments.
Methods
We retrospectively evaluated the rate of UTI in the period of 10 years after SPK in patients initially randomized in a single-center, open-label study that compared MMF vs sirolimus as a part of tacrolimus based regimens with anti-T-lymphocyte induction and early steroid withdrawal. For comparison incidence risk ratio (IRR) was assessed, with MMF as a reference group. Additionally, we analyzed the general risk factors associated with UTIs and their recurrences in SPK patients, as well as the impact of UTIs on long-term graft survival. Data from a subset of subjects were originally enrolled in the prospective study.
Results
In the intent-to-treat analysis population of 164 patients, 572 episodes of UTIs were recorded. The risk for developing UTI and consequent hospitalization was about the same in both groups (IRR 0.99 and 1.01, respectively, p >0.09), but the risk for relapse (IRR 0.75, p 0.4) or recurrence (IRR 0.55 p 0.09) was lower in the sirolimus arm. A statistically significant risk factor for developing UTI was female gender (IRR 5.25, $p < 0.05$) insertion of JJ stent after the transplantation (IRR 1.86, $p < 0.05$), and urological pathologies pre-transplant (IRR 1.75, $p < 0.05$). Non-censored 10-year kidney and pancreas graft survival was comparable between arms (77% in MMF vs 67% in the sirolimus group, p=0.16, 66% in MMF vs 64% in the sirolimus group, p 0.66 respectively).
Conclusions
Though the long-term incidence of UTIs following SPK was rather high, we found no significant difference between groups treated with MMF or sirolimus. Subjects treated with sirolimus experienced fewer episodes of UTI relapses and recurrences
Conflicts of interest
No conflicts declared

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P27

Preliminary results in the role of Hypothermic Machine Perfusion in experimental pancreas transplantation

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Background

The prevalence of diabetes mellitus as one of the fastest growing chronic diseases of the 21st century and the need for pancreatic grafts for transplantation are on the rise. However, the scarcity of pancreases with optimal donor characteristics leads to the use of pancreatic grafts from marginal donors. Hypothermic machine perfusion offers potential for application to pancreas preservation and may expand the donor pool. The objective of this study is to assess the role of hypothermic machine perfusion in the optimization of pancreatic grafts considered suboptimal for clinical transplantation.

Methods

Nine organs deemed unsuitable for transplant were placed in hypothermic machine perfusion (Waters Medical Systems®, MN). In 89% of cases, backtable vascular reconstruction was attained through an anastomosis between the splenic artery and the distal superior mesenteric artery. Organs were perfused with an IGL-1 solution via arterial flow with pulsatile ex-situ perfusion.

Results

Organ procurement was obtained from brain dead (n=6) or circulatory arrest (n=3) donors, including 6 men and 3 women with a median age of 47 (36-53) years and a BMI of 24.5 (22.4 - 28.6) kg/m². Dynamic perfusion parameters achieved were: pressure 11 (9-14) mmHg, resistance 0.26 (0.14-0.38), temperature 5.2 (4.7-6) ºC and flow 41 (31.5-64.5) ml/min. Histological examination after a median time of 11 (7.4 - 18.5) hours in ex-situ hypothermic perfusion showed a median parenchymal fibrosis of 10% (5-22), adipose infiltration of 10% (8.75-32.5), chronic inflammatory infiltration of 5% (2-7.5) and pancreatic necrosis of 30% (20-60). Focusing on cases with moderate to severe damage, only one case presented moderate fibrosis plus adipose infiltration, as well as one case of moderate adipose infiltration and one case of moderate pancreatic necrosis.

Conclusions

Histopathological analysis of pancreases placed in hypothermic machine perfusion exemplifies its potential organ viability. However, the assessment of its functionality is yet to be determined since no dynamic studies have been performed evaluating possible ischemia reperfusion injuries.

Case	Type of donor	Sex	Age	BMI	Parenchymal fibrosis	Adipose infiltration	Chronic inflammatory infiltration	Necrosis	Insulin	Glucagon	Somatostatin
1	DBD	Male	47	-	Absence	Mild	Absence	Moderate	Focal	Moderate	Moderate
2	DBD	Male	52	29.5	Mild	Moderate	Mild	Absence	Moderate	Moderate	Moderate
3	DBD	Male	34	22.2	Mild	Absence	Absence	Absence	Moderate	Moderate	Moderate
4	cDCD	Male	48	22.9	Mild	Mild	Mild	Absence	Moderate	Moderate	Moderate
5	DBD	Female	54	26	Moderate	Moderate	Mild	Absence	Moderate	Moderate	Moderate
6	cDCD	Female	39	26	Mild	Absence	Mild	Mild	Moderate	Moderate	Moderate
7	DBD	Male	43	23	Mild	Mild	Mild	Mild	Moderate	Moderate	Moderate
8	cDCD	Male	33	21.3	Mild	Mild	Mild	Absence	Moderate	Moderate	Moderate
9	DBD	Female	55	31.2	Mild	Mild	Mild	Mild	Moderate	Moderate	Moderate

Demographic and histopathological characteristics of pancreases placed in Hypothermic Machine Perfusion

Conflicts of interest

No conflicts declared

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P28

Varying efficacy of different species-specific insulins in rats

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Background

Both our previous research and already published studies suggest a varied efficacy of species-specific (rat, human, porcine) insulins in lowering blood glucose levels in rodent models. The rat insulin seems to be more compatible, eliciting a more robust response in target tissues compared to human or porcine insulins in rodents. We tested the sensitivity of the Insulin Tolerance Test (ITT) in detecting varying effectiveness among different types of insulin (rat/human) in rats.

Methods

Male Brown Norway rats (270-300g) were included in experimental groups.

ITT: To identify an appropriate insulin dosage that emphasizes differences among various insulins, we tested doses of 160, 80, 40 and 20 µg/kg.

Human recombinant insulin and rat insulin (INS1+INS2) was used in dose 20 µg/kg (0,5 IU/kg). Insulin was administered intravenously and blood glucose levels were measured from a drop of blood obtained from the tail vein using a glucometer at baseline and at 10, 20, 30, 40, 50, 60, 70, 80, 100 and 120 min after insulin injection. The decline in blood glucose levels was recalculated using the area under the curve method, applying the trapezium rule.

Results

Optimal dosage for detecting differences in glucose-lowering ability of insulin in Brown Norway rats was 20 µg/kg.

Brown Norway rats exhibited higher sensitivity to mixture of rat insulins if compared to human insulin (AUC of glucose 79,4856 vs 103,3614 (% basal). h).

Conclusions

Our results suggest that equal mixture of rat species-specific insulins (INS1 + INS2) is more effective in lowering blood glucose levels than human insulin, in Brown Norway rats.

Conflicts of interest

No conflicts declared

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