Multipotent mesenchymal stromal cells derived from porcine exocrine pancreas improve insulin secretion from juvenile porcine islet cell clusters

Elisa Montanari¹ | Luca Szabó² | Alexandre Balaphas¹ | Jeremy Meyer¹ |
Nadja Perriraz-Mayer¹ | Joel Pimenta¹ | Marie-Noelle Giraud³ | Bernhard Egger⁴ |
Sandrine Gerber-Lemaire² | Leo Bühler⁴ | Carmen Gonelle-Gispert⁴

¹Surgical Research Unit, CMU-1, University Hospitals of Geneva, Geneva, Switzerland

²Group for Functionalized Biomaterials, Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, EPFL SB ISIC SCI-SB-SG, Lausanne, Switzerland

³Cardiology, Dpt EMC, Section of Medicine, University of Fribourg, Fribourg, Switzerland

⁴Surgical Research Unit, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland

Correspondence

Carmen Gonelle-Gispert, Section of Medicine, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland.

Email: Carmen.Gonelle@unifr.ch

Funding information

The study was supported by the OPO-Stiftung, the Child foundation, and the foundation La Colombe in Geneva. The authors acknowledge the financial support from the Swiss National Science Foundation (grant N° 310030E-164520) and the Foundation Insuleman

Abstract

Neonatal and juvenile porcine islet cell clusters (ICC) present an unlimited source for islet xenotransplantation to treat type 1 diabetes patients. We isolated ICC from pancreata of 14 days old juvenile piglets and characterized their maturation by immunofluorescence and insulin secretion assays. Multipotent mesenchymal stromal cells derived from exocrine tissue of same pancreata (pMSC) were characterized for their differentiation potential and ability to sustain ICC insulin secretion in vitro and in vivo. Isolation of ICC resulted in $142 \pm 50 \times 10^3$ IEQ per pancreas. Immunofluorescence staining revealed increasing presence of insulin-positive beta cells between day 9 and 21 in culture and insulin content per 500IEQ of ICC increased progressively over time from 1178.4 ± 450 µg/L to 4479.7 ± 1954.2 µg/L from day 7 to 14, $P < .001$. Highest glucose-induced insulin secretion by ICC was obtained at day 7 of culture and reached a fold increase of 2.9 ± 0.4 compared to basal. Expansion of adherent cells from the pig exocrine tissue resulted in a homogenous CD90⁺, CD34⁻, and CD45⁻ fibroblast-like cell population and differentiation into adipocytes and chondrocytes demonstrated their multipotency. Insulin release from ICC was increased in the presence of pMSC and dependent on cell-cell contact (glucose-induced fold increase: ICC alone: 1.6 ± 0.2 ; ICC + pMSC + contact: 3.2 ± 0.5 , $P = .0057$; ICC + pMSC no-contact: 1.9 ± 0.3 ; theophylline stimulation: alone: 5.4 ± 0.7 ; pMSC + contact: 8.4 ± 0.9 , $P = .013$; pMSC no-contact: 5.2 ± 0.7). After transplantation of encapsulated ICC using Ca²⁺-alginate (alg) microcapsules into streptozotocin-induced diabetic and immunocompetent mice, transient normalization of glycemia was obtained up to day 7 post-transplant, whereas ICC co-encapsulated with pMSC did not improve glycemia and showed increased pericapsular fibrosis. We conclude that pMSC derived from juvenile porcine exocrine pancreas improves insulin secretion of ICC by direct cell-cell contact. For transplantation purposes, the use of pMSC to support beta-cell function will depend on the development of new anti-fibrotic polymers and/or on genetically modified pigs with lower immunogenicity.

1 | INTRODUCTION

It is currently recognized that type 1 diabetes (T1D) patients presenting long-term complications can be treated by allotransplantation of human islets. Most transplanted patients reach improved glycemic control, but this treatment rarely leads to insulin independence.¹ An effective treatment requires multiple human pancreas donors and lifelong immunosuppression. The limited availability of human organ donors is a major hurdle for this clinical application and has driven investigation to find other sources for islets or beta cells, including xenogenic islets isolated from porcine fetal, neonatal, or adult pancreases.² The isolation of islets from adult pigs is highly inefficient due to their fragility and in contrast, protocols for the digestion of fetal, neonatal, or juvenile pancreas allow to obtain high amounts of pancreatic cell clusters or islet-like clusters.³ Cell clusters derived from the neonatal porcine are called either, neonatal porcine islets (NPI), porcine neonatal pancreatic cell clusters (NPCCs) or also neonatal porcine islet-like cluster (NPICC).⁴⁻⁷ We will use the term of islet cell cluster (ICC). ICC display no defined structure and contains only low amounts of insulin-positive beta cells (5%) and a majority of duct or acinar cells. Several protocols exist, to increase postnatal beta-cell mass in these cell clusters by beta-cell maturation in vitro.⁸⁻¹¹ ICC also mature after transplantation into immuno-deficient diabetic mice, where they respond poorly to glucose for several weeks before restoring normoglycaemia after 4-10 weeks depending on the amount of ICC transplanted.¹²⁻¹⁴ In addition, first clinical trials of transplantation of encapsulated neonatal porcine islets in humans showed a reduction in unaware hypoglycemia events suggesting a clinical benefit in patients with unstable type 1 diabetes.^{15,16}

Currently, multipotent mesenchymal stromal cells (MSC) are investigated for regenerative cell therapies and islet transplantation purposes. MSC are potentially effective in supporting function and viability of isolated islets after transplantation and also dampen inflammatory immune reactions.¹⁷ MSC are isolated and expanded from many tissues, such as bone marrow, adipose tissue, umbilical cord, and pancreas.¹⁸⁻²¹ MSC are considered as "biomolecule dispensers" since they release bioactive molecules which improve tissue regeneration by increasing vascularization,²²⁻²⁵ angiogenesis,²⁶ and reducing apoptosis.²⁷ It remains open whether MSC from different tissues have same capacities.²⁸⁻³⁰ The transplantation of rodent or human islets with syngeneic,^{23,27,31,32} autologous,³³ allogeneic,³⁴ and xenogeneic³⁵ MSC resulted in improved islet graft survival and function in rodent models. Similarly, immuno-deficient diabetic mice co-transplanted with porcine neonatal ICC and human MSC reached normoglycemia significantly earlier than mice transplanted with ICC alone.³⁶

The aim of this study was to investigate whether porcine MSC derived from the pancreatic exocrine tissue interacts with juvenile porcine ICC and is able to modulate insulin secretion. We also performed co-encapsulation and transplantation of ICC

with pMSC into immunocompetent mice to analyze their effect on graft function.

2 | MATERIALS AND METHODS

2.1 | Isolation and maturation of ICC

Fourteen (± 1) days old piglets (Appel, Switzerland) were used to isolate ICC as described previously with some modifications.³⁷ All experiments were performed with the experimentation authorization GE/151/16 delivered by the Swiss Cantonal Veterinary Office. Briefly, for each experiment 3 pancreases were harvested by surgeons and placed in the organ preservation Institute Georges Lopez 1 (IGL-1) solution (Institut Georges Lopez, SAS) on ice. Weight of each pancreas was measured and placed in HBSS (Hank's Balanced Salt Solution), modified for islets (Corning) supplemented with HEPES. Adipose tissue was removed and pancreatic tissue was manually minced with scissors into 2-3 mm² pieces. Thirty mg of Collagenase from *Clostridium histolyticum* (Sigma-Aldrich) per maximal 4.2 g of pancreas tissue was used. Pancreas tissue was digested in 50-mL tubes, containing HBSS with 2.5 mg/mL of Collagenase, in a water bath at 37°C for 15 minutes under agitation at 1g, with vigorously hand shaking of tubes for 1 minutes every 5 minutes. ICC were then washed with HBSS containing 10% FCS, 5 mmol/L HEPES 10 mmol/L glucose, and 1% streptomycin/penicillin. The digested tissue, containing cell clusters of diameters ranging between 50 and 550 μ m, were placed in beta-cell maturation media composed of HAM's-F10 culture medium containing 0.5% BSA (fraction V; Sigma-Aldrich), 50 μ mol/L IBMX (3-isobutyl-1-methylxanthine), 10 mmol/L glucose, 2 mmol/L glutamine, 10 mmol/L nicotinamide, 1.6 mmol/L sodium bicarbonate, and 1% streptomycin/penicillin, at 37°C and 5% CO₂.¹³ Tissue was placed in non-adherent flasks. From first day until day 5 of culture, 4/5 volume of media was changed every day, then media was replaced every second day. A non-negligible amount of ICC (1/3) was adherent and had to be detached from the bottom of flasks through manual pipetting every day. At day 5, flasks were changed and ICC cultured at density of ~20-25 000 IEQ/T175 flask.

For pMSC isolation and expansion, media containing exocrine tissue recovered after first 24-hour culture was used. ICC were taken after 3, 7, 14, 21, and 28 days to analyze maturation and functionality. At day 7, the final number of cell clusters was determined and expressed as IEQ.

2.2 | Immunofluorescence staining on juvenile pancreatic tissue and isolated ICC

Immunofluorescence staining was performed on sections of formalin-fixed and paraffin-embedded juvenile pancreases. ICC were collected at various time points fixed and collected using Histogel (Thermo Scientific), following the manufacturer's recommendations.

Briefly, 4- μ m sections were treated with 0.01 mol/L citrate for 15 minutes in a microwave, to unmask epitopes. To avoid nonspecific binding, slides were incubated with 0.5% BSA for 30 minutes at room temperature. For detection of insulin-positive beta cells, sections were stained with guinea pig anti-porcine insulin, diluted 1:500 (Dako), donkey anti-guinea pig coumarin AMCA (blue fluorescence) (Dako), rabbit anti-human glucagon antibody, diluted 1:200 (Dako) and Alexa Fluor 555 donkey anti-rabbit antibody (Life Technologies). For detection of mesenchymal cells and pancreatic ductal cells in the cell clusters, sections were stained, respectively, with mouse anti-human vimentin antibody (Dako) diluted 1:50 or anti-CK7 antibody (Dako, Glostrup, Denmark) diluted 1:100 and as a secondary with Alexa Fluor 488 goat anti-mouse antibody (Life Technologies). Microscope images were acquired using a fluorescence microscope (Mirax Midi, Zeiss).

2.3 | Isolation and expansion of MSC from juvenile porcine exocrine pancreas

Maturation media from the first 24-hour culture of porcine pancreatic cell cluster culture (from 3 pancreata) was centrifuged for 5 minutes at 320g to obtain a pellet of exocrine tissue. This pellet was placed into the Iscove's modified Dulbecco's medium (Cambrex) supplemented with 10% FCS, 1% streptomycin/penicillin, and 10 ng/mL platelet-derived growth factor BB (PDGF-BB, PeproTech EC Ltd) and 0.1 mmol/L Dithiothreitol (DTT; Sigma) used also for expansion of human bone marrow-derived MSC.³⁸ Cells were used after passage 3 for characterization and experiments.

2.4 | Real-time cell analysis

Doubling time was assessed by real-time cell analyzer system (RTCA). Cell index (CI) was recorded by measuring impedance. Cells at passage between 4 and 6 were seeded in duplicate at a density of 5000, 10 000, and 20 000 cells/well and their growth assessed for 120 hours using the instrument xCELLigence RTCA; (ACEA Biosciences Inc). Doubling time was calculated in the exponential growth phase with the following formula: $(t_2 - t_1) \times \text{Log}(2) / (\text{Log}(CI(t_2)) - \text{Log}(CI(t_1)))$ where t is time.

2.5 | Fluorescence-Activated Cell Sorting (FACS) analysis of porcine pancreatic MSC

100 000 porcine MSC cultured at passage 3 were trypsinized and washed with FACS buffer (PBS containing 2% FCS). Cell surface antigen staining was performed with the following labeled antibodies, anti-CD45-ECD (also known as RPhycoerythrin-TexasRed-X) (Beckman Coulter, Life Science), anti-CD34-phycoerythrin (PE) (Beckman Coulter, Life Science), and anti-CD90-allophycocyanin (APC) (Abcam). Cells were washed, and data were acquired using

FACS flow cytometer Gallios IV (Beckman Coulter) and analyzed using the Kaluza Analysis 1.3 software (Beckman Coulter).

2.6 | Differentiation into adipocytes, chondrocytes, and osteoblasts

The expanded mesenchymal cells and human bone marrow-derived MSC (positive control) were differentiated into adipocytes, chondrocytes, and osteoblasts as previously described and with minor modification.³⁹ Briefly, for adipocyte differentiation, cells were seeded at high density (20 000–25 000 cells/cm²) and cultured at 37°C in presence of 5% CO₂ for 3 weeks in adipogenic differentiation media composed of IMDM, 10% rabbit serum (Sigma), 0.5 mmol/L 3-Isobutyl-1-methylxanthine (IBMX), 1 μ mol/L hydrocortisone, 0.1 mmol/L indomethacin (all from Sigma), and P-S. Medium was changed every 3 days. To reveal triglyceride droplets in the cytoplasm of differentiated adipocytes, cells were fixed with 10% formalin for 1 hour, washed twice with water, and incubated in Oil-red-O solution (Sigma) for 2 hours at room temperature (RT). Stained cells were washed twice, mounted by coverslips, and observed under an optical microscope (Zeiss Axiophot 1, Carl Zeiss AG). For chondrogenic differentiation, expanded mesenchymal cells were trypsinized and 300 000 cells were centrifuged at 200 g for 5 minutes. Cell pellets were rinsed with PBS and cultured in Falcon tubes for 3 weeks in a chondrogenic differentiation media composed of DMEM high glucose (25 mmol/L of glucose), 0.1 μ mol/L dexamethasone, 50 μ g/mL ascorbic acid, 10 mg/L insulin, 5.5 mg/L transferrin, 5 μ g/L selenium (ITS) premix, 40 μ g/mL L-proline (all from Sigma), 10 ng/mL TGF β 3 (PeproTech EC Ltd), P-S at 37°C in presence of 5% CO₂, and medium was changed every 3 days. Cell pellets were fixed with 10% formalin for at least 24 hours. Cell pellets were dehydrated and embedded in paraffin. 5- μ m sections were stained with Masson's trichrome to reveal collagen fibrils. Slides were mounted and observed under an optical microscope (Zeiss Axiophot 1). For osteoblast differentiation, cells were cultured up to 3 weeks in control media or media for osteoblast differentiation, based on IMDM, dexamethasone 0.1 μ mol/L, beta-glycerolphosphate 10 mmol/L, ascorbic acid 200 μ mol/L (all from Sigma), and P-S. Media was changed twice a week.

For Alizarin Red S staining, cells were fixed with 10% formaldehyde for 30 minutes and washed twice with PBS. Cells were then incubated for 15 minutes at room temperature with 2% Alizarin Red S solution which had been adjusted to a pH of 4.1–4.3 with 0.5% ammonium hydroxide. Cells were then washed once with water and observed under an inverted optical microscope (Nikon, ECLIPSE Ts2-FL).

2.7 | Co-culture conditions and Insulin secretion assay

Five hundred IEQ (considered as 500 000 islet cells) were cultured either alone or with pMSC (50 000) (cell ratio 10:1). ICC and pMSC

were co-cultured in cell-cell contact or in transwell condition without cell-cell contact. Experiments were performed in triplicates using complete ICC maturation media in 24-well plates for 3 days before performing insulin secretion assay. We used an allogenic experimental setting, in which pancreas-derived pMSC were always used with ICC obtained from different pancreas donors (different piglets). Insulin secretion was measured by performing a static incubation assay. Briefly, ICC were rinsed twice with oxygenated Krebs solution containing 0.1% BSA and pre-incubated for 1 hour in Krebs solution containing low glucose (2.8 mmol/L). Then, ICC were incubated for 1 hour in each condition, low glucose (2.8 mmol/L) for basal secretion, followed by stimulation with high glucose (16.8 mmol/L) and incubated for maximal stimulation with 16.8 mmol/L glucose supplemented with 5 mmol/L of theophylline.

Supernatants were collected and total amounts of insulin extracted from ICC by ice-cold acid-ethanol extraction. Samples were frozen until insulin concentrations were determined using a porcine insulin ELISA Kit according to the manufacturer's instructions (Mercodia). All values obtained for basal and stimulated secretion were normalized to values of total insulin content. Insulin secretion was expressed as fold increase where basal insulin release was set as 1.

2.8 | Encapsulation of ICC and pMSC

For cell microencapsulation, Pronova ultrapure LVG Na-alg (Lot no: BP-1111-32) from Novamatrix (Norway) was used. A 1.5 wt% Na-alginate solution was prepared by dissolution in MOPS buffer (10 mmol/L, pH = 7.4) containing 0.4% NaCl. ICC alone or with pMSC were centrifuged at room temperature (320 g for 2 minutes), and the pellet was gently mixed with the alg solution (ICC cells/pMSC at a ratio of 10:1). Microspheres (MS) were generated under sterile conditions using the Encapsulator B-395 Pro (Büchi Labortechnik AG). The gelation bath was composed of 100 mmol/L CaCl_2 in 10 mmol/L MOPS buffer (pH = 7.4). MS formation by ionotropic interaction occurred immediately after extrusion into the gelation bath and was completed by covalent crosslinking during subsequent stirring for 2-5 minutes in the gelation bath. Ca-alg MS were collected by filtration, washed twice with HBSS containing 2 mmol/L Ca^{2+} concentration, and transplanted.

2.9 | Diabetes induction and transplantation of microencapsulated ICC in mice

MS transplantation was performed following protocols approved by the Geneva cantonal veterinary authorities (license GE/151/16). Diabetes was induced by injection of streptozotocin (SZ) (Sigma) at 220 mg/kg into the peritoneum of C57BL/6 male mice (Janvier, France). Mice were considered diabetic at blood glucose level >20 mmol/L. Three days after SZ injection, the diabetic mice were

anesthetized with isoflurane, and each mouse received 1 mL of Ca-alg MS containing 30 000 IEQ of ICC alone or with 3.10^6 pMSC (cell ratio 10:1) into the peritoneum through a small incision. Blood samples were obtained from the tail vein for glucose measurement and graft failure was stated when glucose level was >20 mmol/L for 3 consecutive measurements. Body weight of transplanted and non-transplanted diabetic mice was measured until 14 or 21 days after transplantation.

2.10 | Statistical analysis

Data are expressed as mean $\pm$ SEM of numbers (n) of independent observations. The statistical significance of differences was calculated with the Student t test or the 1-way ANOVA.

3 | RESULTS

3.1 | In situ hormone-positive cell aggregates in juvenile porcine pancreata

To evaluate the presence and distribution of endocrine cell aggregates in the pancreas of 14 days old juvenile piglets, immunostainings of alpha and beta cells were performed on random pancreas sections. Hormone-positive aggregates were varying in size, from few-cell aggregates to well-defined islets (Figure 1). In few-cell aggregates, the presence of insulin-positive beta cells was predominant whereas in small well-defined islets, the glucagon-positive alpha cells were mostly present. The well-defined small islets showed that beta and α -cells appeared to be intermingled similarly as in human islets.⁴⁰

As the scope of the study was to isolate MSCs from the exocrine pancreas, pancreas sections were stained for vimentin, a marker for mesenchymal cells. Vimentin-positive mesenchymal cells were present throughout the exocrine tissue in the juvenile pig pancreata and often in close vicinity to small hormone-positive cell aggregates.

3.2 | Juvenile porcine pancreatic islet cell cluster isolation and maturation

For each of the 11 ICC isolations, 2-3 pancreata from piglets of the same litter were digested. After digestion, the pancreatic tissue including the exocrine component was cultured in neonatal pig islet differentiation media that was changed every day. ICC were counted at day 7 in culture, when exocrine tissue had completely disappeared. Table 1 summarizes the number of islets isolated/experiment. Numbers are expressed as islet equivalents (IEQ), taking into account the size of cell aggregates. We obtained a mean number of IEQ/pancreas $132\,500 \pm 49\,600$. Further, ICC were recovered at 3, 7, 14, and 21 days of culture. As shown in Figure 2A, insulin-positive beta cells increased between day 3 and 21. In contrast,

FIGURE 1 In situ hormone-positive cell aggregates in juvenile porcine pancreas. Sections of juvenile pancreata from same litter were stained against insulin for beta cells (blue) and against glucagon for alpha cells (red). Mesenchymal cells were stained against vimentin (green), a marker for mesenchymal cells. The size of the hormone-positive aggregates in the pancreases included few-cell aggregates (A, C) and small well-defined islets (B). Size bars 200 μ m

TABLE 1 Average yield of isolated ICC per experiment (2-3 animals) and per pancreas expressed in islet equivalences (IEQ)

Isolation	Total IEQ ($\times 10^3$)	IEQ ($\times 10^3$)/pancreas
1	400	200
2	200	100
3	392	130
4	570	190
5	340	113
6	253	84
7	116	38
8	562	187
9	398	132
10	350	116
11	490	163
Mean IEQ	370	132
SD	± 142	± 49

glucagon-positive alpha cells increased already between day 3 and 7. Cell clusters remained undifferentiated containing extensive CK7-positive exocrine tissue and vimentin-positive mesenchymal cells. Insulin content was measured in samples of 500 IEQ of ICC and showed a progressive increase over time from 27.8 ± 11.47 μ g/L at day 3 to 2466 ± 637.9 μ g/L at day 14 and remained constant up to 21 days, suggesting that maximal maturation in vitro occurred until 14 days (Figure 2B). Further, to evaluate glucose responsiveness, insulin secretion assays were performed. ICC showed strongest insulin release to high glucose stimulation (5-fold increase) and maximal

stimulation with theophylline (15-fold increase) after 7 days in culture, compared to basal release (Figure 2C). Therefore, ICC after 10 days in culture were selected for further experiments.

3.3 | Expansion and characterization of MSC isolated from juvenile porcine exocrine pancreas

To investigate whether the porcine exocrine pancreas contains MSC, pancreatic exocrine tissue was recovered from media of ICC cultures after 24 hours of culture. Exocrine tissues from 3 pancreases were collected and placed in specific media for the expansion of human bone marrow-derived MSC. At passage 0 of culture, we observed morphologically heterogeneous cells, which had evolved into a homogenous fibroblast-like cell population at passage 3 (Figure 3A). Flow cytometry analysis showed that cells were CD90⁺, CD45⁻, and CD34⁻, indicating a mesenchymal phenotype (Figure 3B). To demonstrate multipotency, we differentiated these cells into adipocytes and chondrocytes. Figure 3C shows the adipocyte differentiation of expanded pig pancreas-derived MSC (pMSC) and human bone marrow-derived MSC (hMSC) obtained 3 weeks after culture in adipocyte differentiation media. Both types of MSC showed lipid droplets (stained by oil-red-O) characteristic for adipocytes. Chondrocyte differentiation was induced in pMSC pellet cultures using chondrocyte differentiation media for 3 weeks. Collagen deposition was present in chondrocytes differentiated pMSC as evidenced by Masson staining and is visible in blue (Figure 3D). For the demonstration of MSC differentiation into osteoblasts, we used Alizarin Red S to stain the calcium deposition typical for osteoblasts. hMSC showed strong staining after exposure to the osteogenic differentiation media. However, in

FIGURE 2 Juvenile pig islet cell clusters (ICC) isolation, maturation, and glucose-stimulated insulin release. A, 2000 IEQ of ICC were collected after 3, 7, 14, and 21 days of culture in maturation media. ICC were fixed, and all sections were stained against insulin (ins; blue) glucagon (glu; red) and upper row (a,b,c,d) shows vimentin staining (vim; green) and lower row shows CK7 staining (CK7; green) (e,f,g,h). Insulin-positive beta cells were increasingly present at 14 d in the periphery of the pancreatic islet cell clusters. Images are representative for 4 independent experiments. Size bars 100 μ m. B, Total insulin content was extracted from ICC (500IEQ) at days 3, 7, 14, 21, and 28 of culture and measured using a porcine insulin ELISA. Each dot represents one isolation/maturation experiment. Mean insulin content is represented by a red line. C, 500IEQ of ICC at days 7, 14, and 21 were used for insulin secretion assay. ICC were stimulated with 2.8 mmol/L glucose for basal release (basal), 18.7 mmol/L glucose for stimulated release (stim), and maximal release was induced by 18.7 mmol/L + theophylline (theophylline). The graph is representative of 5 independent experiments. The values represent one representative experiment (triplicates) and are expressed as means $\pm$ SEM

osteogenic differentiation media and in control condition, the pMSC cell monolayers constantly detached during culture and contracted into a single cell aggregate (Figure 3E, left panels). pMSC cell aggregates cultured in differentiation condition showed some Alizarin Red S staining suggesting that pMSC contain an osteoblast differentiation potential (Figure 3E, right panels). The result further suggests that the osteoblast differentiation condition we used are not optimal for highly proliferating juvenile pancreatic-derived pMSC.

Furthermore, evaluation of the doubling time by real-time cell analysis showed a shorter doubling time for pMSC (12 ± 2 hours, $n = 5$) than for hMSC (38 ± 10 hours, $n = 2$) in accordance with a study from Nishimura et al. The doubling time of neonatal porcine bone marrow-derived MSCs was also significantly shorter than that of human bone marrow-derived MSCs (17.3 ± 0.8 vs 62.0 ± 19.6 hours).⁴¹

3.4 | Co-culturing ICC in direct contact with porcine exocrine pancreas-derived MSC supports insulin secretion

Earlier studies showed that human bone marrow-derived MSC in direct cellular contact with human islets showed a beneficial effect on insulin secretion in vitro and on graft survival and function after transplantation into diabetic mice.³⁴ Therefore, we investigated the effect of human and porcine MSC on insulin secretion by ICC with or without cell-cell contact using the transwell system. ICC and pMSC at a proportion of 10 islet cells for 1 pMSC were co-cultured for 3 days as depicted in Figure 4A. As shown in Figure 4B, insulin secretion by ICC was significantly increased upon cell-cell contact with pMSC in both high glucose and theophylline stimulation. Fold increase of glucose-induced

FIGURE 3 Expansion and characterization of MSC isolated from juvenile porcine exocrine pancreas. We placed digested exocrine tissue recovered from three pancreas into human MSC expansion media. A, Heterogeneous cell population at passage 0 (P0) evolved into b) a homogenous fibroblast-like cell population after 3 passages (P3) (small rectangles are 10x magnification) B, Flow cytometry analysis of cells at P3 using antibodies against surface antigen CD90, CD45, and CD34. C, Differentiation of hMSC and pMSC (pMSC #1 and pMSC #2) into adipocytes after 3 wk of culture. *Upper images*, control media; *Lower images*, adipocyte differentiation media. Lipid droplets (in red) are stained by oil-red-o. D, Sections of pellet cell cultured in control media (control) or chondrocyte differentiation media (Chondrocytes). Collagen appears in dark blue by Masson trichrome staining demonstrating chondrocyte differentiation. Results are representative of 2 independent experiments. E, Differentiation of hMSC and pMSC into osteoblasts. *Left panels:* macroscopic images show the calcium deposition stained by Alizarin Red S (in red) of hMSC after differentiation and the cell aggregates of pMSC in differentiation and control condition. *Right panels:* Microscopy images show a strong Alizarin Red S staining in differentiated hMSC and a faint staining in the differentiation condition of pMSC cell aggregates

insulin secretion increased from 1.6 ± 0.2 for ICC without pMSC cell-cell contact to 3.2 ± 0.5 for ICC with pMSC cell-cell contact, $P = .0057$. Fold increase for maximal stimulation with high glucose and theophylline increased from 5.4 ± 2.7 for ICC without cell-cell contact to 8.4 ± 3.1 for ICC with pMSC cell-cell contact, $P = .013$. Interestingly, insulin release by ICC co-cultured with hMSC was not significantly increased with cell-cell contact. These results demonstrate that pMSC supports insulin secretion by ICC depending on cell-cell interaction.

3.5 | Transplantation of co-encapsulated ICC and pMSC is not beneficial for graft function compared to ICC alone and worsens pericapsular fibrosis

The effect of pMSC on graft function of ICC was investigated after transplantation into immunocompetent and streptozotocin-induced diabetic mice. ICC either alone or with pMSC were encapsulated in Ca-alg MS. As shown in Figure 5A, ICC and pMSC remained viable in vitro up to 3 days after encapsulation and ICC maintained their

FIGURE 4 Co-culture of ICC in direct contact with porcine- and human-derived MSC supports insulin secretion. A, Schematic representation of ICC cultured alone or with or without direct contact with MSC from porcine (pMSC) or human (hMSC) origin during 3 d prior to the insulin secretion assay. B, Insulin secretion assay was performed in the transwell system using 500 IEQ ICC and 10 000 pMSC or hMSC for each condition. Insulin release of ICC was measured after exposure to low glucose concentration 2.8 mmol/L (basal), high glucose concentration 16.7 mmol/L (stim), and maximal stimulation by 16.7 mmol/L + theophylline (Theophylline). Insulin secretion increased significantly after glucose and theophylline stimulation when pMSC were in direct contact with ICC (ICC + pMSC + contact) compared to ICC alone. Insulin secretion increased most strongly when hMSC were in direct contact to ICC and stimulated by theophylline. * ≤ 0.013 ; *** ≤ 0.000006 ; n = 3; values represent means $\pm$ SEM

FIGURE 5 Co-encapsulation of ICC with pMSC in semi-permeable Ca-alg MS. A, Upper panel: Light microscope images: ICC 3 days after Ca-alg MS encapsulation. Lower panel: ICC staining by FDA and PI shows cell viability and mortality (small rectangles). B, Insulin secretion by ICC expressed in fold increase 3 d after encapsulation. Insulin secretion was not significantly different when ICC were co-encapsulated pMSC. Insulin release from 500 IEQ encapsulated ICC was measured after stimulation with low glucose concentration at 2.8 mmol/L (basal) and high glucose concentration at 16.7 mmol/L (stim). Values represent means $\pm$ SEM, n = 3

capacity to respond to high glucose stimulation. Fold increase of insulin secretion by ICC alone or ICC with pMSC were 2.39 ± 0.25 and 3.19 ± 0.88 , $P = 0.3$ (Figure 5B). We transplanted 1 mL of cell containing MS into the peritoneum of diabetic and immunocompetent mice. The follow-up of glycemia showed a transient correction of hyperglycemia for mice receiving MS with ICC alone, whereas no effect on hyperglycemia was observed for mice receiving MS with

ICC and pMSC (Figure 6A). The follow-up of body weight showed that the total weight loss at 14 days after transplantation was not significantly different between mice transplanted with MS containing ICC alone (1.80 ± 0.17 g) compared to mice transplanted with MS containing ICC and pMSC (2.47 ± 0.93 g). In contrast, the weight loss was significantly less when transplanted mice were compared to control diabetic mice receiving no transplantation (5.9 ± 2.03 g,

FIGURE 6 Transplantation of ICC containing Ca-alg MS transiently improved diabetes in immune-competent mice. 30 000 IEQ of ICC encapsulated in Ca-alg MS either alone or with pMSC were transplanted into the peritoneum of immunocompetent and diabetic mice. Mice were treated with streptozotocin (STZ) at day 1 and transplanted (TX) at day 3 with MS containing ICC with or without pMSC. A, Follow-up of glycemia in mice transplanted with ICC (alg-ICC), ICC + pMSC (alg-ICC pMSC), or control mice (ctrl no TX) until 3 wk after STZ treatment and transplantation. B, Follow-up of body weight (g) of transplanted or non-transplanted mice. C and D, MS containing ICC alone or ICC with pMSC were recovered after 3 wk of transplantation. Upper row, light microscopy images show retrieved MS containing ICC alone or ICC + pMSC from 2 different mice (mouse #1 and #2); Lower row shows immunofluorescence images stained with FDA (green) for the detection of cell viability. Values represent means $\pm$ SEM, mice groups, $n = 3$

$P = .0007$). Microscopic observation of MS retrieved from the peritoneum at day 30 after transplantation showed increased pericapsular fibrosis around MS containing pMSC, suggesting that their presence increased the immunogenicity of the graft (Figure 6C,D).

4 | DISCUSSION

Human islets transplantation, as a treatment for type 1 diabetes patients, is limited by the shortage of human organ donors. The neonatal and juvenile porcine pancreas represent an unlimited source for the preparation of immature islets, also called ICC. These ICC contain few hormone-positive cells and mainly ductal and ductal precursor cells. Maturation media was subsequently developed to increase the proportion of endocrine cells.^{9,10} In view of future clinical applications, the

production of neonatal and juvenile porcine ICC has the advantage to be more cost-effective compared to adult porcine islets which is related to the strict hygiene conditions necessary for the breeding and housing conditions of animal donors. Further, the isolation technique of juvenile porcine islets is more efficient and cultured immature ICC showed increased viability compared to adult islets.⁴²

In this study, juvenile porcine ICC were isolated and obtained with an average yield of IEQ at $142 \pm 50 \times 10^3$ per pancreas at day 7. In the literature, the average yield of ICC for one neonatal and young porcine pancreas amounts to $30.4 \pm 1.2 \times 10^3$ and $33.3 \pm 6.4 \times 10^3$ IEQ,⁴³ respectively. In the study of Lamb and coworkers, the mean age of the young piglets was 20 days (range 4–30 days). They described an almost 2-fold increase of the average weight of pancreas from piglets aged 11–18 days (5.25 ± 1.6 g) compared to piglets aged 4–10 days (2.75 ± 1.3 g). They also described a progressive decrease

of the amount of IEQ/g of pancreas isolated from piglets aged between 11 and 18 days and those of 20 and 30 days. Therefore, the increased number of ICC obtained in this study, might be resulting from the combination of both parameters, the increased size of the pancreas of 14-day-old piglets (compared to neonatal) and the still efficient digestion of the pancreas in this range of age.

Immunofluorescence staining on pancreas sections for alpha and beta cells from same litters showed a size-disparity of the hormone-positive cell aggregates. We also observed differences in insulin contents in sampled ICC (500IEQ) at day 3 and 7 of maturation, suggesting that the size variation of hormone-positive aggregates may not reflect variations of the beta-cell mass in lobular pancreatic compartments, but may represent differences in the global beta-cell mass. Isolated ICC with initial small numbers of hormone-positive aggregates were randomly distributed throughout the ICC. After maturation, hormone-positive cells appeared more frequently at the periphery, suggesting that diffusion into ICC and action of the maturation media is limited. At all time points, the ICC contained still substantial amounts of CK7-positive ductal cells, except ICC from two isolations (data not shown).

The insulin content in ICC increased significantly between 3 and 14 days after isolation but not at later time points, indicating a limited time window for *in vitro* maturation. More importantly, even though ICC showed increased insulin content, at day 14 of culture, glucose responsiveness of ICC at day 14 was decreased compared to day 7, leading us to use ICC for experiments at day 10 of culture. This result suggests that while beta-cell maturation and/or insulin production occurs in ICC, the responsiveness of the beta cells to glucose declined. Further suggesting that the highly regulated process of glucose responsiveness, including ATP/ADP ratio changes, membrane depolarization, granule trafficking, and exocytosis are dysregulated earlier than the beta-cell insulin production under *in vitro* condition.

To analyze whether pancreatic-derived allogenic MSC (pMSC) were able to further improve function of ICC, pMSC were expanded from porcine pancreatic exocrine tissue. The resulting growing cell population showed typical mesenchymal cell morphology. Importantly, bi-lineage differentiation into chondrocytes and adipocytes, similar to human bone marrow-derived MSC occurred, suggesting that these cells were bona fide pancreatic-derived MSC. Very recently, it had been shown that human pancreatic-derived MSC are highly vimentin/nestin positive and produce a conditioned media highly enriched for proteins associated with vascular development, wound healing, and chemotaxis similar to human bone marrow-derived MSC. Further, intrapancreatic injection of these MSC reduced hyperglycemia in streptozotocin-treated mice.⁴⁴

In sections of juvenile pig pancreas, we observed strongly stained vimentin-positive cells throughout the pancreas. Further, the vimentin-positive cells remained present during the culture of the isolated ICC and whether they contribute to maturation of hormone-positive cells via secretion of paracrine factors in juvenile ICC remains to be investigated.

It is known, however, that mesenchymal cells regulate pancreatic growth at early and late development stages, probably through expansion of the pool of epithelial pancreatic precursor cells, since the mesenchymal ablation at a late development stage in mice (e13.5) leads to reduced beta- and acinar-cell mass.⁴⁵ Tracing the fate of the pancreatic mesenchyme in mice also revealed that nearly all mesenchymal cells acquire a pericyte fate at embryonic stage e13.5. Spatial distribution and abundance of pancreatic PDGFR β ⁺ pericytes further indicate that these cells represent the primary pancreatic mesenchymal cell population and are the predominant fate of the embryonic pancreatic mesenchyme.⁴⁶ The identification of the exact cell type from which the isolated and expanded pancreatic MSC originate and their relation to pericytes or other types of fibroblasts needs further investigation.^{47,48} A recent study demonstrated that *in vivo*, pericytes of multiple organs do not behave as MSC. This study challenges the current view that pericytes act as multipotent tissue-resident progenitors *in vivo* and suggest that the *in vitro* plasticity may arise from cell manipulations *ex vivo*.⁴⁹

Here we show that insulin secretion by ICC was significantly increased when co-cultured in direct cellular contact with exocrine tissue-derived pMSC for three days. High glucose and maximal stimulation with theophylline significantly increased insulin secretion compared to ICC cultured alone, suggesting that cell-cell interactions are important to support islet functionality.

This confirmed our previous studies and by other groups demonstrating that cell-cell contact between islets and MSC are necessary to improve islets function *in vitro* and after transplantation.^{32,34} The interaction of the adhesion molecule N-cadherin expressed on islets and MSC were shown to be necessary to achieve the increased insulin secretion. Further, increased islet function was also obtained in engineered cell sheets composed of human islets and supporting human bone marrow, or adipose tissue-derived MSC or fibroblasts,⁵⁰ suggesting that cell-cell contact improves the micro-environment of isolated islets. Increased functionality and survival of isolated human and murine islets was observed by Gamble et al, when islets were cultured in suspension with MSC allowing direct cellular contact.⁵¹ However, other studies showed that trophic factors alone are sufficient to induce beneficial effects. Park and coworkers showed that preconditioned murine islets through co-culture with human MSC without contact, increased survival, and function of transplanted murine islets in a syngeneic model. Increased amounts of trophic factors such as IL-6, IL-8, VEGF-A, HGF, and TGF- β measured in MSC-conditioned media have been suggested to be responsible for the activation of signaling pathways involved in angiogenesis and anti-apoptotic signaling of cultured islets.⁵² Our results do not exclude that soluble factors derived from pMSC play a role, but this effect was not sufficient to increase ICC function. Therefore, we concluded that pMSC isolated from exocrine pancreas significantly supported insulin secretion from juvenile porcine ICC. A significant beneficial effect was not observed with human MSC, suggesting that species-specific cellular interactions are implicated.

Immunoregulatory effects have been shown for allogenic MSC when transplanted in non-human primates. In cynomolgus monkeys,

intraportal co-infusion of allogenic MSC and islets increased islets engraftment and function, shown by a reduced number of islets necessary to reach normoglycemia.^{22,53}

However, in our model, after transplantation of Ca-alg MS containing ICC with pMSC into immunocompetent and streptozotocin-induced diabetic mice, a beneficial modulation of the immune and/or inflammation responses was not observed. Although encapsulated ICC alone decreased hyperglycemia up to 5–6 days, the survival and function of ICC co-transplanted with pMSC was not prolonged. The recovered MS containing ICC and pMSC showed increased pericapsular fibrosis compared to MS with ICC alone, suggesting that Ca-alg MS released increased amounts of xenoantigens. It is known that porcine xenoantigens provoke a strong adaptive immune and inflammatory response, and until today transplantation of encapsulated ICC into mice without immunosuppression has been unsuccessful. However, by using a combination therapy of anti-CD154 and anti-LFA-1 monoclonal antibodies prolonged graft survival of encapsulated neonatal pig islets in immunocompetent mice has been obtained. Under such condition, transplanted and treated mice achieved normoglycemia within 10–35 days and around 50% of treated mice remained normoglycemic for more than 100 days.⁵⁴ We concluded that in immunocompetent mice, pMSC encapsulated together with ICC in Ca-alg MS are not able to alleviate the immune response against encapsulated porcine ICC. This is also in line with a recent study indicating that encapsulated MSC do not mitigate the foreign body responses against alg MS.⁵⁵

Semi-permeable Ca-alg MS that are developed to protect allo- or xenografts present instability over time due to swelling and Ca^{2+} loss, which causes increased pore size and ultimately capsule breakage.⁵⁶ Therefore, the future use of microcapsules for long-term transplantation will depend on the ability to further develop hydrogels that are more resistant to leakage and mitigate the foreign body response, as well as the genetic engineering of pigs with lower immunogenicity.

Herein, we showed that cellular contact between pancreatic-derived MSC and ICC supports insulin secretion. Our results suggest that pMSC improves the micro-environment for isolated porcine ICC and might be beneficial for porcine ICC or islet transplantation studies. The role of pancreatic MSC in the juvenile pancreas needs further investigations. Therefore, ICC from all development stages are a potential source of islets for the treatment of type 1 diabetes in humans and also represent a complementary experimental system for studying islet development and maturation.⁵⁷

ACKNOWLEDGMENT

We thank Jean-Pierre Giliberto for his excellent work in porcine anesthesia and for surgical assistance and Marlene Sanchez for her excellent technical help.

AUTHOR CONTRIBUTIONS

EM contributed to the study design, collected data, and performed data analysis and interpretation. LS produced polymers and

contributed to the study design, collection of data, and data analysis. AB and JM performed surgery to collect porcine pancreases and contributed to research data collection. NPM, JP, and M-NG contributed to study design, research data collection, and interpretation. SG contributed to the study concept, design of polymers, critical revision of the manuscript, and securing of funding. LB and BE contributed to study concept, design, and critical revision of the manuscript and securing of funding. CGG contributed to study concept and design, wrote the manuscript, collected data, and performed data analysis and interpretation and takes responsibility of the integrity of the study. All authors approved the final version of the manuscript.

ORCID

Carmen Gonelle-Gispert <https://orcid.org/0000-0003-3789-6464>

REFERENCES

- Berman A, Wszola M, Gorski L, et al. Do we need insulin independence after islet transplantation? *Transplant Proc.* 2019;51:2775–2780.
- Kemter E, Wolf E. Recent progress in porcine islet isolation, culture and engraftment strategies for xenotransplantation. *Curr Opin Organ Transplant.* 2018;23:633–641.
- Vanderschelden R, Sathialingam M, Alexander M, Lakey JRT. Cost and scalability analysis of porcine islet isolation for islet transplantation: comparison of juvenile, neonatal and adult pigs. *Cell Transplant.* 2019;28:967–972.
- Buerck LW, Schuster M, Oduncu FS, et al. LEA29Y expression in transgenic neonatal porcine islet-like cluster promotes long-lasting xenograft survival in humanized mice without immunosuppressive therapy. *Sci Rep.* 2017;7:3572.
- Emamaullee JA, Shapiro AM, Rajotte RV, Korbitt G, Elliott JF. Neonatal porcine islets exhibit natural resistance to hypoxia-induced apoptosis. *Transplantation.* 2006;82:945–952.
- Ma X, Yang C, Zhang J, et al. Culturing with modified EGM2 medium enhances porcine neonatal islet-like cell clusters resistance to apoptosis in islet xenotransplantation. *Xenotransplantation.* 2018;25:e12358.
- Suarez-Pinzon WL, Korbitt GS, Rayat G, et al. Neonatal porcine islet xenografts in nonobese diabetic mice: effects on blood glucose and analysis of cytokines expressed in the islet grafts. *Transplant Proc.* 1998;30:654–655.
- Bakhti M, Bottcher A, Lickert H. Modelling the endocrine pancreas in health and disease. *Nat Rev Endocrinol.* 2019;15:155–171.
- Binette TM, Dufour JM, Korbitt GS. In vitro maturation of neonatal porcine islets: a novel model for the study of islet development and xenotransplantation. *Ann N Y Acad Sci.* 2001;944:47–61.
- Hassouna T, Seeberger KL, Salama B, Korbitt GS. Functional maturation and in vitro differentiation of neonatal porcine islet grafts. *Transplantation.* 2018;102:e413–e423.
- Lopez-Avalos MD, Tatarkiewicz K, Sharma A, Bonner-Weir S, Weir GC. Enhanced maturation of porcine neonatal pancreatic cell clusters with growth factors fails to improve transplantation outcome. *Transplantation.* 2001;71:1154–1162.
- Ko SH, Kwon HS, Suh SH, et al. Dexamethasone suppresses the expansion and transdifferentiation of transplanted porcine neonatal pancreas cell clusters (NPCCs) into beta-cells in normal nude mice. *Diabetes Res Clin Pract.* 2004;66(Suppl 1):S97–S101.
- Trivedi N, Hollister-Lock J, Lopez-Avalos MD, et al. Increase in beta-cell mass in transplanted porcine neonatal pancreatic cell clusters is due to proliferation of beta-cells and differentiation of duct cells. *Endocrinology.* 2001;142:2115–2122.

14. Yoon KH, Quicquel RR, Tatarkiewicz K, et al. Differentiation and expansion of beta cell mass in porcine neonatal pancreatic cell clusters transplanted into nude mice. *Cell Transplant*. 1999;8:673-689.
15. Matsumoto S, Abalovich A, Wechsler C, Wynyard S, Elliott RB. Clinical benefit of islet xenotransplantation for the treatment of type 1 diabetes. *EBioMedicine*. 2016;12:255-262.
16. Matsumoto S, Tan P, Baker J, et al. Clinical porcine islet xenotransplantation under comprehensive regulation. *Transplant Proc*. 2014;46:1992-1995.
17. Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood*. 2007;110:3499-3506.
18. Baertschiger RM, Bosco D, Morel P, et al. Mesenchymal stem cells derived from human exocrine pancreas express transcription factors implicated in beta-cell development. *Pancreas*. 2008;37:75-84.
19. da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci*. 2006;119:2204-2213.
20. Seeberger KL, Dufour JM, Shapiro AM, Lakey JR, Rajotte RV, Korbitt GS. Expansion of mesenchymal stem cells from human pancreatic ductal epithelium. *Lab Invest*. 2006;86:141-153.
21. Young HE, Mancini ML, Wright RP, et al. Mesenchymal stem cells reside within the connective tissues of many organs. *Dev Dyn*. 1995;202:137-144.
22. Berman DM, Willman MA, Han D, et al. Mesenchymal stem cells enhance allogeneic islet engraftment in nonhuman primates. *Diabetes*. 2010;59:2558-2568.
23. Figliuzzi M, Cornolti R, Perico N, et al. Bone marrow-derived mesenchymal stem cells improve islet graft function in diabetic rats. *Transplant Proc*. 2009;41:1797-1800.
24. Hirabaru M, Kuroki T, Adachi T, et al. A method for performing islet transplantation using tissue-engineered sheets of islets and mesenchymal stem cells. *Tissue Eng Part C Methods*. 2015;21:1205-1215.
25. Ito T, Itakura S, Todorov I, et al. Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function. *Transplantation*. 2010;89:1438-1445.
26. Giacca M, Zacchigna S. VEGF gene therapy: therapeutic angiogenesis in the clinic and beyond. *Gene Ther*. 2012;19:622-629.
27. Borg DJ, Weigelt M, Wilhelm C, et al. Mesenchymal stromal cells improve transplanted islet survival and islet function in a syngeneic mouse model. *Diabetologia*. 2014;57:522-531.
28. Ock SA, Baregundi Subbarao R, Lee YM, et al. Comparison of immunomodulation properties of porcine mesenchymal stromal/stem cells derived from the bone marrow, adipose tissue, and dermal skin tissue. *Stem Cells Int*. 2016;2016:9581350.
29. Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J. Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. *Stem Cells Dev*. 2012;21:2724-2752.
30. Yaochite JN, de Lima KW, Caliari-Oliveira C, et al. Multipotent mesenchymal stromal cells from patients with newly diagnosed type 1 diabetes mellitus exhibit preserved in vitro and in vivo immunomodulatory properties. *Stem Cell Res Ther*. 2016;7:14.
31. Rackham CL, Chagastelles PC, Nardi NB, Hauge-Evans AC, Jones PM, King AJ. Co-transplantation of mesenchymal stem cells maintains islet organisation and morphology in mice. *Diabetologia*. 2011;54:1127-1135.
32. Rackham CL, Dhadda PK, Chagastelles PC, et al. Pre-culturing islets with mesenchymal stromal cells using a direct contact configuration is beneficial for transplantation outcome in diabetic mice. *Cytotherapy*. 2013;15:449-459.
33. Ben Nasr M, Vergani A, Avruch J, et al. Co-transplantation of autologous MSCs delays islet allograft rejection and generates a local immunoprivileged site. *Acta Diabetol*. 2015;52:917-927.
34. Montanari E, Meier RPH, Mahou R, et al. Multipotent mesenchymal stromal cells enhance insulin secretion from human islets via N-cadherin interaction and prolong function of transplanted encapsulated islets in mice. *Stem Cell Res Ther*. 2017;8:199.
35. Perez-Basterrechea M, Obaya AJ, Meana A, Otero J, Esteban MM. Cooperation by fibroblasts and bone marrow-mesenchymal stem cells to improve pancreatic rat-to-mouse islet xenotransplantation. *PLoS One*. 2013;8:e73526.
36. Hayward JA, Ellis CE, Seeberger K, et al. Cotransplantation of mesenchymal stem cells with neonatal porcine islets improve graft function in diabetic mice. *Diabetes*. 2017;66:1312-1321.
37. Korbitt GS, Elliott JF, Ao Z, Smith DK, Warnock GL, Rajotte RV. Large scale isolation, growth, and function of porcine neonatal islet cells. *J Clin Invest*. 1996;97:2119-2129.
38. Suva D, Garavaglia G, Menetrey J, et al. Non-hematopoietic human bone marrow contains long-lasting, pluripotential mesenchymal stem cells. *J Cell Physiol*. 2004;198:110-118.
39. Baertschiger RM, Serre-Beinier V, Morel P, et al. Fibrogenic potential of human multipotent mesenchymal stromal cells in injured liver. *PLoS One*. 2009;4:e6657.
40. Brissova M, Fowler MJ, Nicholson WE, et al. Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J Histochem Cytochem*. 2005;53:1087-1097.
41. Nishimura M, Nguyen L, Watanabe N, Fujita Y, Sawamoto O, Matsumoto S. Development and characterization of novel clinical grade neonatal porcine bone marrow-derived mesenchymal stem cells. *Xenotransplantation*. 2019;26:e12501.
42. Smith KE, Purvis WG, Davis MA, et al. In vitro characterization of neonatal, juvenile, and adult porcine islet oxygen demand, beta-cell function, and transcriptomes. *Xenotransplantation*. 2018;25:e12432.
43. Lamb M, Laugenour K, Liang O, Alexander M, Foster CE, Lakey JR. In vitro maturation of viable islets from partially digested young pig pancreas. *Cell Transplant*. 2014;23:263-272.
44. Cooper TT, Sherman SE, Bell GI, et al. Characterization of a Vimentin(high)/Nestin(high) proteome and tissue regenerative secretome generated by human pancreas-derived mesenchymal stromal cells. *Stem Cells*. 2020;38:666-682.
45. Landsman L, Nijagal A, Whitchurch TJ, et al. Pancreatic mesenchyme regulates epithelial organogenesis throughout development. *PLoS Biol*. 2011;9:e1001143.
46. Harari N, Sakhneny L, Khalifa-Malka L, et al. Pancreatic pericytes originate from the embryonic pancreatic mesenchyme. *Dev Biol*. 2019;449:14-20.
47. Cano E, Gebala V, Gerhardt H. Pericytes or mesenchymal stem cells: Is that the question? *Cell Stem Cell*. 2017;20:296-297.
48. de Souza LE, Malta TM, Kashima Haddad S, Covas DT. Mesenchymal stem cells and pericytes: To what extent are they related? *Stem Cells Dev*. 2016;25:1843-1852.
49. Guimaraes-Camboa N, Cattaneo P, Sun Y, et al. Pericytes of multiple organs do not behave as mesenchymal stem cells in vivo. *Cell Stem Cell*. 2017;20(345-359):e345.
50. Imamura H, Adachi T, Kin T, et al. An engineered cell sheet composed of human islets and human fibroblast, bone marrow-derived mesenchymal stem cells, or adipose-derived mesenchymal stem cells: An in vitro comparison study. *Islets*. 2018;10:e1445948.
51. Gamble A, Pawlick R, Pepper AR, et al. Improved islet recovery and efficacy through co-culture and co-transplantation of islets with human adipose-derived mesenchymal stem cells. *PLoS One*. 2018;13:e0206449.
52. Park KS, Kim YS, Kim JH, et al. Trophic molecules derived from human mesenchymal stem cells enhance survival, function, and angiogenesis of isolated islets after transplantation. *Transplantation*. 2010;89:509-517.
53. Bartholomew A, Sturgeon C, Siatskas M, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol*. 2002;30:42-48.

54. Kobayashi T, Harb G, Rayat GR. Prolonged survival of microencapsulated neonatal porcine islets in mice treated with a combination of anti-CD154 and anti-LFA-1 monoclonal antibodies. *Transplantation*. 2005;80:821-827.
55. Fath-Bayati L, Ai J. Assessment of mesenchymal stem cell effect on foreign body response induced by intraperitoneally implanted alginate spheres. *J Biomed Mater Res A*. 2020;108:94-102.
56. Lanza RP, Jackson R, Sullivan A, et al. Xenotransplantation of cells using biodegradable microcapsules. *Transplantation*. 1999;67:1105-1111.
57. Kim S, Whitener RL, Peiris H, et al. Molecular and genetic regulation of pig pancreatic islet cell development. *Development*. 2020;147:dev186213.

How to cite this article: Montanari E, Szabó L, Balaphas A, et al. Multipotent mesenchymal stromal cells derived from porcine exocrine pancreas improve insulin secretion from juvenile porcine islet cell clusters. *Xenotransplantation*. 2021;00:e12666. <https://doi.org/10.1111/xen.12666>