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DOI:
10.1042/CS20171251

Document Version
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Link to publication record in King's Research Portal

Citation for published version (APA):
Arzouni, A. A., Vargas-Seymour, A., Rackham, C. L., Dhadda, P., Huang, G. C., Choudhary, P., Nardi, N., King, A. J. F., & Jones, P. M. (2017). Mesenchymal stromal cells improve human islet function through released products and extracellular matrix. *Clinical Science*, *131*(23), 2835-2845. Advance online publication. https://doi.org/10.1042/CS20171251

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Download date: 27. Dec. 2024

Mesenchymal stromal cells improve human islet function through released products and extracellular matrix.

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Abstract

Aims: The aims of the current study were (i) to determine whether the reported beneficial effects of mesenchymal stromal cells (MSCs) on mouse islet function extend to clinically relevant human tissues (islets and MSCs), enabling translation into improved protocols for clinical human islet transplantation; and (ii) to identify possible mechanisms through which human MSCs influence human islet function.

Materials and methods: Human islets were co-cultured with human adipose tissue-derived MSCs (hASCs) or pretreated with its products - extracellular matrix (ECM) and annexin A1 (ANXA1). Mouse islets were pretreated with mouse MSC-derived ECM. Islet insulin secretory function was assessed in vitro by radioimmunoassay. Quantitative RT-PCR was used to screen human adipMSCs for potential ligands of human islet G-protein-coupled receptors.

Results: We show that co-culture with hASCs improves human islet secretory function in vitro, as measured by glucose-stimulated insulin secretion, confirming previous reports using rodent tissues. Furthermore, we demonstrate that these beneficial effects on islet function can be partly attributed to theMSC-derived products ECM and ANXA1.

Conclusions: Our results suggest that hASCs have the potential to improve the quality of human islets isolated for transplantation therapy of Type 1 diabetes. Furthermore, it may be possible to achieve improvements in human islet quality in a cell-free culture system by using the MSC-derived products ANXA1 and ECM.

Abbreviations

ASCs, *MSCs isolated from adipose tissue*; α-SMA, *alpha-smooth muscle actin*; ANXA1, *annexin A1*; ANOVA, *analysis of variance*; ECM, *extracellular matrix*; GPCR, *G-protein-coupled receptor*; MSC, *mesenchymal stromal cell*; qPCR, *quantitative RT-PCR*; SEM, *standard error of the mean*; T1D, *Type 1 diabetes*.

1 Introduction

Pancreatic islet transplantation represents a potential cure for type 1 diabetes (T1D), with islet grafts currently offering five-year insulin-independence rates of up to 50%[1]. Despite encouraging results, the availability of islet transplantation as a therapeutic option is limited by a shortage of tissue donors, which is exacerbated by the loss of islet functional viability during the isolation procedure and the subsequent in vitro cultivation period, which expose islets to ischemia, oxidative stress and inflammatory cytokines[2-4]. The cultivation of islets prior to transplantation allows for the immunosuppressive pretreatment of the graft recipient, quality control of the islets, and for their transportation to remote transplantation centres, but this period in culture can lead to a substantial loss in the functional β-cell mass which has a negative impact on transplantation outcomes[5]. Developing methods to maintain the functional viability of isolated islets during the pre-transplantation period is therefore likely to improve the clinical outcomes of islet transplantation as a therapy for T1D.

Mesenchymal stromal cells (MSCs) are multipotent adult stromal progenitor cells[6] which can be isolated from the perivascular niche of many adult tissues and expanded in vitro[7]. MSCs are involved in physiological processes such as maintenance of cellular homeostasis and tissue repair in response to ageing or damage[8]. They are motile cells which migrate to sites of tissue damage and inflammation, where they release a plethora of bioactive molecules, including immunomodulatory and anti-inflammatory agents which act on cells of the innate and acquired immune systems[9-11]. We have previously demonstrated that mouse MSCs improve the insulin secretory responses of mouse islets both in vitro and in vivo[12-15], offering a potential method for improving islet transplantation outcomes. Our recent screening of mouse MSCs for expression of ligands acting through islet G-protein-coupled receptors (GPCRs)[16] identified the anti-inflammatory molecule annexin A1 (ANXA1) as an MSC-derived secretory product which is involved in the improved function and survival of isolated mouse islets in vitro, and in improved outcomes of islet transplantation in a mouse model of diabetes[17]. One aim of

the current study was to determine whether these observations can be extended to clinically relevant human islets and MSCs.

MSCs secrete copious amounts of extracellular matrix (ECM) to form three-dimensional scaffolds for tissue repair, and to act as a reservoir for MSC-derived biologically active molecules to localise their actions at the site of damage and repair[18]. It has long been known that ECM derived from tumour cell lines exert beneficial effects on the function and viability of isolated rodent islets[19-23], so the second aim of the current study was to determine whether MSC-derived ECM contributes to the beneficial effects of MSCs on islet function.

2 Materials and methods

2.1 Procurement of human and mouse islets

Human islets from ethically approved and next of kin-consented cadaver pancreas donors were supplied by the King's College Hospital Human Islet Unit, according to previously described protocols[24]. Islets (70%-85% purity) isolated from six donor pancreases were received within 48 hr of pancreas harvest from cadaveric donors. Mouse islets were isolated by collagenase digestion of mouse pancreas (1 mg/mL; type XI; Sigma) followed by density gradient separation (Histopaque-1077; Sigma), as previously described[12], and maintained in culture (37°C, 5% CO_2) in RPMI-1640 (Sigma) supplemented with 10% (vol./vol.) FBS (Gibco) and 1% (vol./vol.) pen-strep.

2.2 Adipose tissue-derived MSCs (AMSCs)

Human hASCs (Invitrogen) were expanded to passage 3 using the proprietary media MesenPRO RS (Gibco). Cells were seeded at a density of 5000 cells per cm^2 of tissue culture plastic and left for 24 hours to adhere. Medium was completely replaced every 3 days, and MSCs passaged every 7th day for further expansion and cryopreservation. Mouse ASCs were isolated from testicular fat pads of male C57Bl/6 mice, and maintained in culture as adherent monolayers, as previously described[14,15]. MSC

populations were assessed for their differentiation capacity along the adipogenic, osteogenic, and chondrogenic lineages, and for the expression of a range of cell surface markers, as previously reported[15]. Preliminary measurements demonstrated similar functional phenotypes of the ASCs between passages 3 and 8, so all cells used in these studies were confined to this range of passages.

2.3 Co-culture of human islets and hASCs

hASCs (passages 3-8) were seeded into 35 mm Nunclon petri dishes or 6-well plates, and cultured for 24 hr to form a confluent monolayer. For islet-MSC co-culture, 100 human islets were seeded directly onto the hASC monolayers, and the culture medium switched to RPMI-1640 (Sigma) supplemented with 10% (vol./vol.) FBS (Gibco) and 1% (vol./vol.) pen-strep. The co-cultures were incubated for 4 days at 37°C, 5% CO2. In parallel, control groups of 100 human islets alone were incubated in non-coated 35 mm petri dishes in RPMI-1640 medium.

2.4 Generating extracellular matrix (ECM) from mouse andhuman ASCs

Mouse or human ASCs (1 x 10^5 cells) were seeded into 6-well Nunclon plates and left to adhere overnight. Cells were cultured in DMEM medium supplemented with 50µM ascorbic acid for 10 days, and medium was completely replaced every 48 hours. The cell monolayer was decellularised with 0.5% (vol./vol.) Triton X-100 containing 20 mM NH_4OH in PBS for 10 sec at room temperature, and treated with DNase (100 U/ml; Thermo Scientific, MA, USA.) for 1 hr at 37°C. Adherent ECM was washed with PBS and stored at 4°C until use. For the detection of collagen strands, the adherent ECM layer was washed with PBS and incubated with van Gieson (0.05% acid fuchsin (vol./vol.) in saturated picric acid) for 5 min at room temperature. Archived formalin-fixed (4% vol./vol.) graft material from a previous in vivo study of islet and mASC co-transplantation under the renal capsule in a mouse model[12] was analysed for ECM and mASCs. Sections (5µm) were incubated (5 mins, room temperature) with van Gieson to detect collagen (as above) or immunostained for alpha-smooth muscle actin (α-SMA) as a marker for mASCs. Sections were incubated for 1h at room temperature

with a monoclonal mouse α-SMA antibody (1:1000, Dako, Agilent, USA), and visualised as described previously[12]. For immunofluorescence labelling of insulin, a polyclonal guinea pig anti-insulin antibody (1:100; Jackson ImmunoResearch Inc, USA) was used (1h, room temperature) with a Texas Red anti-guinea pig secondary antibody (1:40; Jackson; 1h, room temperature). Reagents were obtained from Sigma-Aldrich (Pool, UK), unless stated otherwise.

2.5 Pretreatment of mouse and human islets with ASC-derived ECM

Mouse or human islets (75 per well) were added to 6-well plates coated with mouse or human ASC-derived ECM, respectively. Islets maintained on ECM were incubated in RPMI-1640 supplemented with 10% (vol./vol.) FBS and 1% (vol./vol.) pen-strep for 2 days. Islets incubated in medium alone served as control groups.

2.6 Pretreatment of human islets with ANXA1

Human islets (100 per dish) were incubated in non-treated petri dishes containing RPMI-1640 supplemented with 10% (vol./vol.) FBS and 1% (vol./vol.) pen-strep, either alone or in the presence of ANXA1 (R&D Systems Abingdon, UK), for 2 days. Co-cultures of human islets and hASCs were performed in parallel as positive controls, as described above.

2.7 Assessment of islet function in vitro

Islets were harvested and assessed for glucose-stimulated insulin secretion by static incubation in buffers supplemented with 2mM or 20mM glucose, as described previously[17]. Briefly, islets were pre-incubated for 2h in RPMI containing 2mM glucose to establish a basal rate of insulin secretion. Groups of three islets were transferred into 1.5-ml microcentrifuge tubes and incubated at 37°C in a bicarbonate-buffered physiological salt solution, supplemented with 2 mM $CaCl_2$, 0.5 mg/mL BSA and either 2 or 20 mM glucose. Samples of the incubation medium were taken after 1h and stored at −20°C until assayed for insulin content using an in-house radioimmunoassay, as previously described[25]. For

the assessment of islet insulin content, islets were lysed in acidified ethanol, sonicated, and stored at -20°C before radioimmunoassay.

2.8 Identification of MSC-derived islet GPCR ligands

Total RNA was extracted from hASCs using RNeasy mini kits and RNase-free DNase sets according to manufacturer's instructions (Qiagen, Manchester, UK) and reversed transcribed into cDNA using an Applied Biosystems high capacity reverse transcription kit (Life Technologies, Paisley, UK). Pooled biological replicates of cDNAs were screened by quantitative RT-PCR (qPCR) for a total of 146 candidate ligands of known islet GPCRs[16]. Post amplification melt curve analysis was carried out and qPCR reactions showing positive melt curves were further analysed by agarose gel electrophoresis to confirm that the qPCR product was the appropriate size for each ligand. Quantitative RT-PCR of ASC biological replicate cDNAs (not pooled) was performed using QuantiTect primers (Qiagen). Relative expression of mRNAs was determined after normalisation against GAPDH as an internal reference and calculated by the $^{2-\Delta\Delta Ct}$ method. The level of expression of each ligand mRNA was classified by its Ct value relative to GAPDH-Ct18 as either high (Ct <26), medium (Ct 26-30) or low (Ct >30).

2.9 ANXA1 content and release by hSCs

hASC extracts and conditioned media were prepared as described previously for mASCs[17], and hASC-derived ECM was solubilised by pippetting in PBS. The ANXA1 content of hASC extracts, conditioned media and ECM was quantified using a commercially available ELISA kit, following the manufacturer's instructions (USCN Life Sciences Inc, Wuhan, China).

2.10 Statistical analyses

The statistical analyses were carried out using GraphPad Prism (version 5.00 for Windows, GraphPad Software, San Diego California USA). All data are expressed as mean ± standard error of the mean

(SEM). Within a given experiment, two-way analysis of variance (ANOVA) with a Bonferroni's or Dunnett's post hoc test was used. Results were considered significant at $p < 0.05$.

3 Results

3.1 Co-culture of human islets with hASCs

Human islets from six donors were independently co-cultured with hASCs, as described. The basal and glucose-stimulated rates of insulin secretion varied between the different batches of human islets. Co-culture with hASCs had no consistent effect on the basal rate of insulin secretion (2mM glucose), but enhanced glucose-stimulated (20mM) insulin secretion (Figure 1A) in all batches of human islets tested, with the hASC-induced enhancement reaching statistical significance in 4/6 batches. Co-culture with hASCs caused no statistically significant changes in insulin content in any of the batches of human islets tested (Figure 1B). These observations using human islets and hASCs are in agreement with the beneficial effects of mASCs on islet secretory function which we have previously reported using mouse tissues[14,15,17].

3.2 Pretreatment of mouse and human islets with MSC-derived ECM

Analysis of graft material from a previous study of mouse islet/mASC renal capsule co-transplantation[12] demonstrated that 28 days after engraftment there was an abundance of collagen fibres in and around islets co-transplanted with mASCs (Figure 2B). The collagen fibres were largely absent in the islet alone grafts (Figure 2C), consistent with mASCs being the predominant source of ECM within the grafts. Immunostaining for α-SMA as a marker could detect no mASCs remaining in the islet graft 28 days post-transplantation (Figure 2A), although a few α-SMA-positive cells could be detected in the non-endocrine portion of the sections (Figure 2A). The α-SMA-positive structures detected in the kidney parenchyma were not part of the subcapsular graft and are likely to be renal microvessels, confirming that the α-SMA immunostaining protocol was effective and further reinforcing the absence of MSCs in the islet graft. These observations suggest that the motile mASCs

deposit their ECM and subsequently migrate away from their site of implantation. Figure 2D and E show the generation of ECM from mouse (D) and human (E) ASCs grown as adherent monolayers on tissue culture plastic (left panels). After decellularisation and DNAase treatment, adherent fibres of ECM remained (middle panels), and van Gieson staining revealed the presence of collagen in the ECM fibres (right panels).

Pretreating mouse islets with mASC-derived ECM had no significant effect on basal (2mM glucose) insulin secretion, but caused a significant increase in insulin secretion at a stimulatory glucose concentration (20mM), as shown in Figure 3A. In six independent experiments, ECM pretreatment of islets caused a significant increase in glucose-stimulated insulin secretion when compared to islets incubated alone. Similarly, the pretreatment of three independent batches of human islets with hASC-derived ECM induced a significant increase in glucose-stimulated insulin secretion, as shown in Figure 3B. The effect of ECM to enhance glucose-stimulated insulin secretion from human islets was not as marked as that of co-culture of the islets with hASCs in parallel experiments using the same human islets (Fig. 3B). Pretreatment with hASC-derived ECM had no significant effect on the insulin content of human islets (Fig. 3C), consistent with the lack of effect of hASC co-culture.

3.3 Identification of MSC-derived islet GPCR ligands

hASC-derived ligands ("secretome") for islet GPCRs were identified using a panel of qPCR primers for 146 potential ligands to screen hASCs (passage 8). hASCs expressed mRNAs for 36 of the islet GPCR ligands (Figure 4), of which 9 were expressed at high levels (Ct <26), 14 had medium expression levels (Ct 26-30), and 13 were expressed at low levels (Ct>30). The full data set for the hASC secretome is shown in Supplementary Table 1. The pattern of mRNA expression in hASCs had many similarities to that previously reported for mASCs[17], with ANXA1, COL3A1, CXCL12 and WNT5A being among the most abundant mRNA species.

3.4 ANXA1 in hASCs

hASCs extracts contained 1.97 ± 0.6 ng ANXA1 per $2x10^5$ cells (n=5, independent preparations), and parallel analysis of hASC-conditioned media showed that approximately 50% of the ANXA1 protein produced by the hASCs was released into the medium over a 48 hr incubation period (1.00 ± 0.32 ng/well, n=3). hASC-derived ECM also contained ANXA1, comprising approximately 10% of the ANXA1 protein content produced by hASCs (0.20 ± 0.09 ng/well, n=5). These observations demonstrate that hASCs synthesize and release ANXA1, and suggest that the hASC-derived ECM acts a reservoir for secreted ANXA1.

3.5 Effects of ANXA1 on insulin secretion from human islets

Pretreatment of human islets with ANXA1 (1nM, 5nM) induced concentration dependent increases in glucose-stimulated (20mM) insulin secretion when compared to islets incubated alone, as shown in Figure 5. ANXA1 pretreatment caused a small, but not statistically significant, increase in basal (2mM glucose) insulin secretion (Fig. 5A) and had no effect on the insulin content of human islets (Fig. 5B). These effects of ANXA1 pretreatment on human islet function are consistent with those we have previously reported using mouse islets[17].

3.6 Effects of ECM and ANXA1 on insulin secretion from mouse or human islets

To determine whether the effects of ASC-derived ECM and ANXA1 were additive, mouse or human islets were pre-treated (48h) with exogenous ANXA1 (5nM), ASC-derived ECM, or both. Supplementary Figure 1 shows that in both human (Suppl. Fig. 1A) and mouse islets (Suppl. Fig. 1B), ANXA1 or ECM pretreatments individually caused a significant increase in glucose-stimulated (20mM) insulin secretion, while having no effect on basal (2mM) secretion. Pretreatment with both ECM and ANXA1 did not further potentiate glucose-stimulated insulin secretion from mouse or human islets, when compared with islets pretreated with either ANXA1 or ECM alone.

4 Discussion

Numerous studies using both small and large animal models of diabetes report the benefit of co-transplanting MSCs with islets to improve graft efficacy[12,26-34]. Many of these in vivo studies have focused on the immunoregulatory and angiogenic capacity of MSCs as the primary mechanisms for their beneficial effects on islet graft survival and function. However, we[14,15,17] and others[35-39] have demonstrated that MSCs or MSC-derived molecules can improve islet cell survival and function in vitro, suggesting that at least part of the beneficial effects of MSCs is mediated by direct effects on the β-cells, rather than via cells of the innate immune system or vascular endothelium. Since the main function of islets is to secrete hormones that are involved in the maintenance of glucose homeostasis, primarily insulin, identifying mechanisms through which MSCs enhance β-cell secretory function offers the potential to improve the outcomes of human islet transplantation as a therapy for T1D.

In the current study, we investigated the effects of hASCs and their derived products (ECM and ANXA1) on human islet function in vitro. Previous studies using mouse tissues suggest that glucose-stimulated insulin secretion is a valid in vitro surrogate measure for improved in vivo graft performance[13-15], so we measured insulin secretion from human islets co-cultured with hASCs or pretreated with their derived products. Human islets are much more variable than mouse islets in terms of insulin content and secretion, and factors such as age, body mass index and duration of organ cold ischemia time have been found to impact upon human islet isolation success and in vitro islet function[40,41]. Nonetheless, using six different human islet preparations we observed the same qualitative beneficial effects of hASC co-culture on insulin secretion, confirming and extending a previous report using human islet co-cultures with cord blood or bone marrow-derived MSCs[42]. Thus, the direct beneficial effects of MSCs on β-cell function demonstrated in experimental studies using rodent tissues are also applicable to clinically-relevant human tissues.

Cell-derived matrices consisting of fibrillar proteins, matrix macromolecules and growth factors can be employed to mimic aspects of tissue microenvironments in vitro[43], and there is a long established

role for ECM derived from cultured cells in modifying islet function both in vitro and in vivo[19-23]. Our measurements in mouse islet-MSC grafts retrieved after 28 days in vivo clearly demonstrate that the MSCs laid down an extensive ECM network at the transplantation site, which was associated with improved outcomes in terms of glycemic control[12] even though MSCs were no longer detected at the implantation site. We therefore investigated whether the beneficial effects exerted in vitro by MSCs could be attributed, at least in part, to interactions between islets and MSC-derived ECM. Our results demonstrate that both mouse and human ASCs lay down ECM in vitro, and that one of the main components of ASC-derived ECM is collagen, as previously reported[44,45]. hASCs laid down a more dense layer of ECM than mASCs, which is consistent with the much higher (> 10-fold) levels of COL3A mRNA measured in hASCs in the current study, compared to previous measurements in mASCs[17]. More importantly, ASC-derived ECM was effective in improving the in vitro insulin secretory function of both mouse and human islets, consistent with ASC-derived ECM being one mechanism through which ASCs influence islet function. This beneficial effect of ECM is in accordance with recent studies in which co-encapsulating human islets with peptides derived from ECM components enhanced their functional survival[46,47]. Our results also show that the improved insulin secretory function of human islets co-cultured with hASCs or pretreated with hASC-derived ECM is not due to differences in insulin content, in agreement with previous reports using mouse islets[15].

However, the effects of ECM did not fully mimic the beneficial effects of co-culturing islet with ASCs, suggesting the existence of additional mechanisms. We have recently identified ANXA1 as a major secreted product of mASCs which exerts beneficial effects on mouse islet function in vitro[17], and we here confirm that hASCs synthesize and release substantial amounts of ANXA1. As for mouse islets, pre-incubating human islets with human recombinant ANXA1 enhanced glucose-stimulated insulin secretion, consistent with ASC-derived ANXA1 being involved in the beneficial effects of ASCs on islet function. ANXA1 has been previously identified as a component of human bone marrow MSC-derived ECM[18], and our measurements demonstrated that a significant amount of hASC-derived ANXA1 remained associated with the ECM through the decellularisation and washing stages required to

generate the adherent ECM layers from hASC monolayers. Several studies have supported the concept of ECM acting as a reservoir for biologically active molecules to enable their targetted and maintained release at the site of tissue repair[48], so our observations are consistent with the MSC-derived ECM influencing human islet function by maintaining high local concentrations of ANXA1. Combined treatment with ECM and exogenous ANXA1 did not further enhance insulin secretion from human islets above either treatment delivered alone, which might suggest a common mechanism, but it may equally reflect a limit to the extent which any pretreatment can improve glucose-stimulated insulin secretion. In any event, ECM-associated ANXA1 is unlikely to be the sole mechanism through which hASCs influence islet function. Thus, previous studies of islet-ECM interactions have identified the importance of integrin-laminin signalling[19,22,49,50], and the current study has shown that human adipMSCs synthesise a wide variety of protein ligands for human islet GPCRs with the potential to Influence islet function (Supplementary Table 1). Nevertheless, the identification of ECM and ANXA1 as important mediators of the beneficial effects of ASCs offers a means to reproduce their beneficial effects in a cell-free environment, if only partially. The inclusion of additional MSC-derived products may lead to a more effective "cocktail" with which to treat human islets prior to transplantation.

In conclusion, we have shown that hASCs have beneficial effects on human islet secretory function similar to those reported in mouse studies, suggesting that they will have beneficial effects on the outcomes of islet transplantation. Co-transplanting hASCs with human islets via the clinically-preferred intraportal route may not be technically possible because the islets will lodge in the hepatic microcirculation whereas the much smaller hASCs will most likely end up in the lung[51]. The incorporation of beneficial MSC-derived biologically active products into the pre-transplantation protocols therefore offers a relatively simple and defined means of improving the functional survival of human islets destined for transplantation therapy of T1D.

Clinical perspectives

(i) The present study aimed to determine whether the beneficial effects of MSCs extend to clinically relevant human tissues (islets and MSCs), enabling translation into improved protocols for clinical human islet transplantation;

(ii) We show that human adipose MSCs (hASCs) improve human islet function in vitro, as measured by glucose-stimulated insulin secretion. Furthermore, we demonstrate that these beneficial effects can be partly attributed to the hASC-derived ECM and the released molecule ANXA1;

(iii) Our results suggest that hASCs and their derived products (ANXA1 and ECM) could be employed to improve the quality of human islets harvested for transplantation.

Acknowledgements

AA and PD were funded by the MRC Doctoral Training Programme at King's College London. AVS received a postdoctoral fellowship grant (BEX 8723/13-3) from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), Ministry of Education, Brazil. CR is funded by Diabetes UK (15/0005146). We are grateful to the pancreas donors and their next of kin.

Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

Author contributions

AJFK , NN and PMJ conceived the research. AAA, AVS, CLR, PD and PMJ designed experiments. AAA, AVS, CLR and PD performed experiments and analysed data. GCH and PC provided technical

expertise and reagents. AAA, AVS and PMJ wrote the manuscript. All authors edited and approved the manuscript.

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Figure Legends

Figure 1: Effects of human adipMSC co-culture on human islet secretory function.
Human islets were incubated alone (white bars) or co-cultured with a monolayer of human adipMSCs (black bars), removed from the MSCs and assessed for (A) insulin secretion in the presence of 2mM or 20mM glucose; and (B) insulin content. Data are representative of 6 (n=6) independent experiments using islet preparations from six different donors, and are presented as mean ± SEM, n=10 observations, **p<0.01 vs. islet alone at the same glucose concentration.

Figure 2: MSC-derived extracellular matrix in vivo and in vitro
In vivo: Representative sections of mouse islet-MSC co-grafts 28 days after transplantation under the kidney capsule (A), showing insulin-positive (red) endocrine aggregates, with only a few α-SMA-positive (green) cells which were confined to the non-endocrine component of the graft. The α-SMA-positive structures visible below the graft are likely to be renal microvessels and are not part of the subcapsular graft. Islet alone grafts at one month stained with van Gieson (C) only contained a small number of collagen fibres at the graft periphery. However, an abundance of collagen fibres were detected in and around the islets co-transplanted with MSCs (B). Scale bar: 100μm.
In vitro: Mouse (D) and human (E) adipMSCs maintained in vitro as adherent monolayers (left panel) deposited a layer of ECM (middle panels) which was visible in decellularised cultures, and which contained many collagen fibres, as visualised by van Gieson staining (right panel). Scale bars: 100μm

Figure 3: Effects of MSC-derived ECM on mouse and human islet function. Mouse (A) or human (B) islets were incubated alone (white bars), on adipMSC-derived ECM (grey bars), or co-cultured with adipMSCs (black bars), removed from the ECM or cell monolayer, and assessed for insulin secretion in the presence of 2mM or 20mM glucose. Human islets were also assessed for insulin content (C). Data are representative of three (B, C) or six (A) independent experiments using islet preparations from different donors, and are presented as means ± SEM, n= 10 observations, *p<0.05, **p<0.01, ***p<0.001 vs. islet alone at the same glucose concentration.

Figure 4: Expression of human adipose MSC-derived ligand mRNAs ("secretome") for human islet GPCRs. Human adipMSCs were cultured as adherent monolayers before harvesting lysates for

screening and quantification by qRT-PCR. Data are displayed as mean expression from 4 biological replicates, where individual points represent each biological replicate and horizontal line represents mean value. Full data set ranked according to Ct value is shown in Supplementary Table 1.

Figure 5: Effects of exogenous ANXA1 on human islet function. Human islets were incubated alone (white bars) or in the presence of ANXA1 (1 or 5 nM, black or grey bars, respectively), removed from the ANXA1 and assessed for (A) insulin secretion in the presence of 2mM or 20mM glucose; and (B) insulin content (B). Data are representative of 5 independent experiments using islet preparations from five different donors, and are presented as mean ± SEM, n=10 observations, **p<0.01 vs. islet alone at the same glucose concentration.

Figure 1

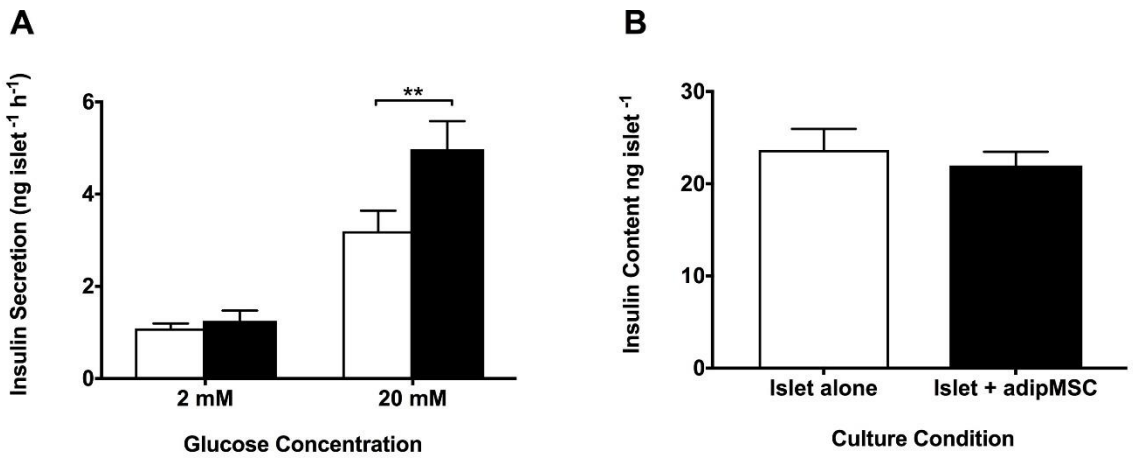


Figure 2

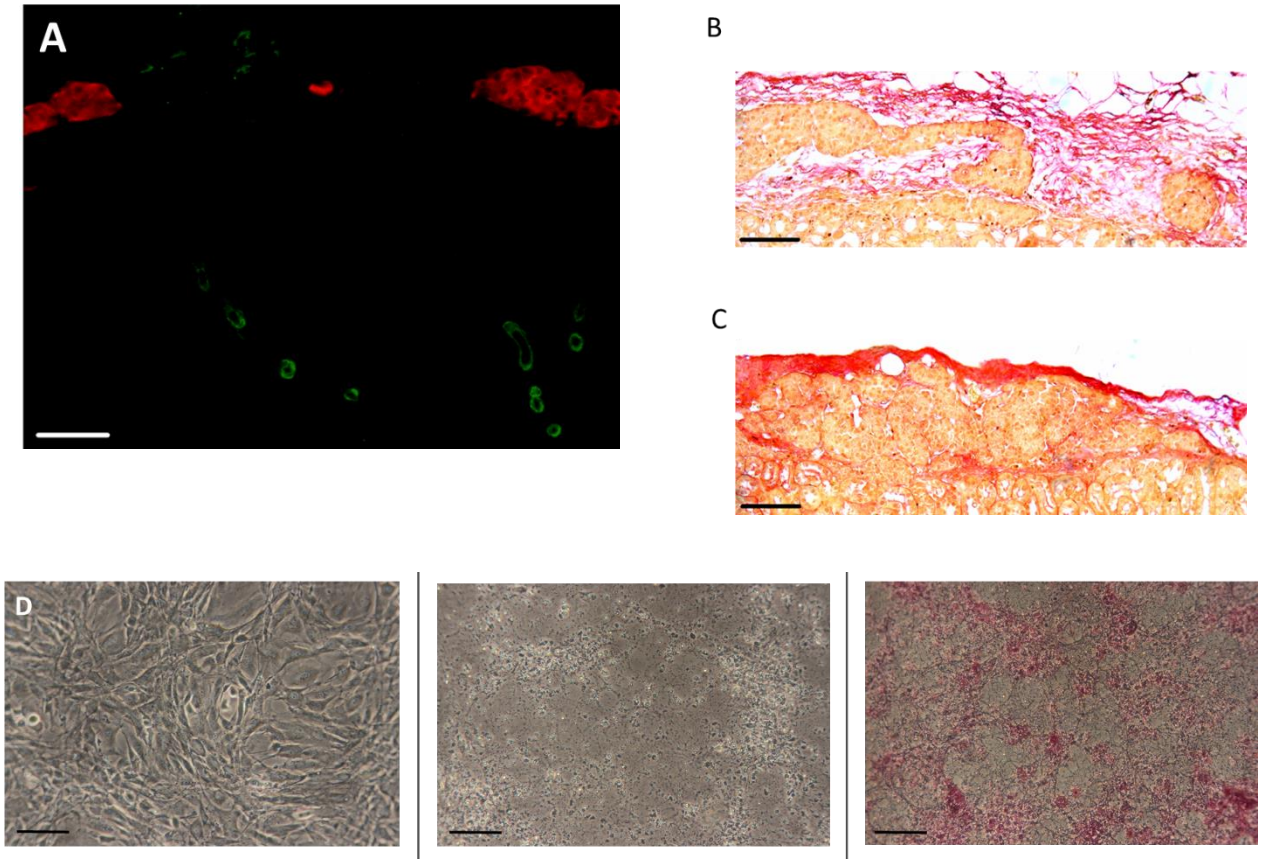


Figure 2 (cont.)

Figure 3

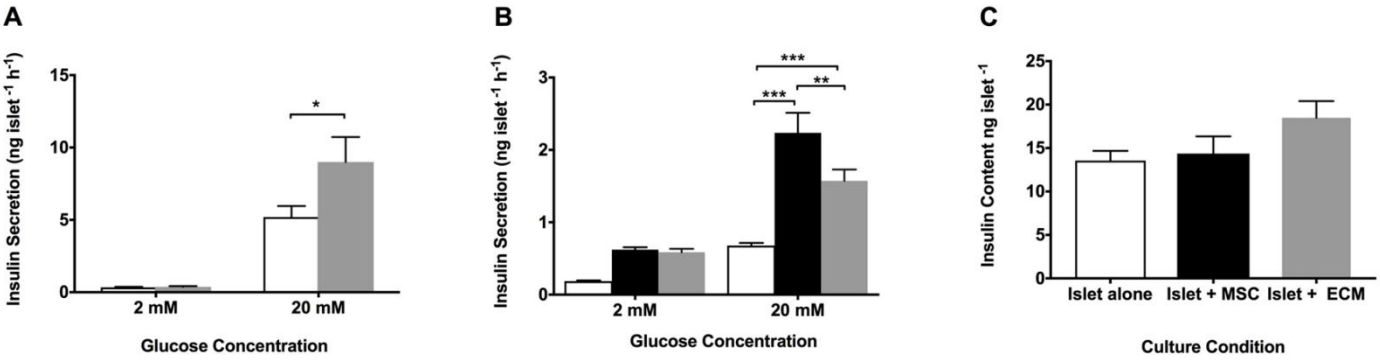


Figure 4

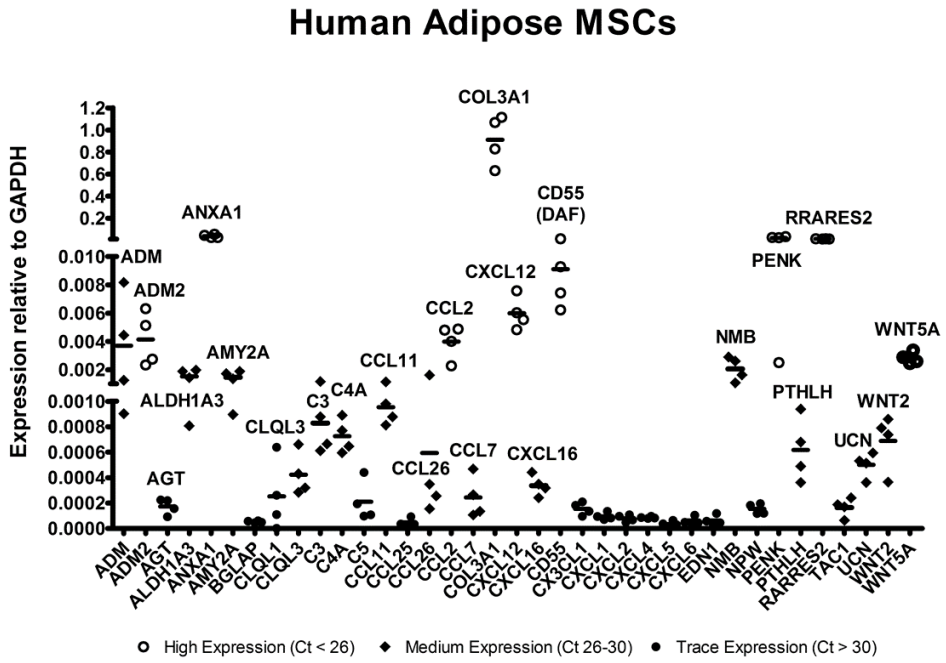
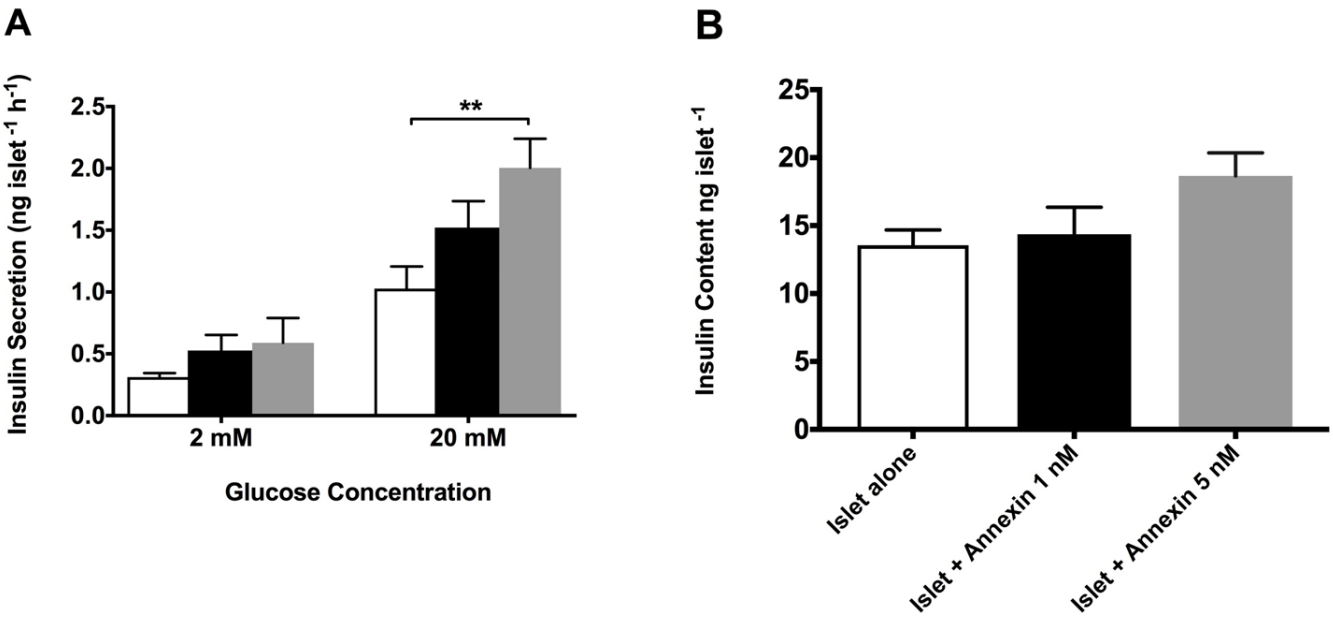


Figure 5


A
Insulin Secretion (ng islet -1 h-1)
2.5
2.0
1.5
1.0
0.5
0.0
**
2 mM
20 mM
Glucose Concentration
B
Insulin Content ng islet -1
25
20
15
10
5
0
Islet alone
Islet + Annexin 1 nM
Islet + Annexin 5 nM