UPPSALA UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1633

Challenges in Islet Transplantation and Strategies to Improve Beta-Cell Function

HANNA LILJEBÄCK

ACTA UNIVERSITATIS UPSALIENSIS UPPSALA 2020

ISSN 1651-6206
ISBN 978-91-513-0858-6
urn:nbn:se:uu:diva-402501

Dissertation presented at Uppsala University to be publicly examined in Room B22, BMC, Husargatan 3, Uppsala, Friday, 6 March 2020 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Professor of Regenerative Medicine for Diabetes James Shaw (Newcastle University).

Abstract

Liljebäck, H. 2020. Challenges in Islet Transplantation and Strategies to Improve Beta-Cell Function. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1633. 52 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0858-6.

The incidence of type 1 diabetes is increasing worldwide and therapies of islet transplantation and potential cell-based therapies are rapidly evolving. Choosing the optimal site for such therapies is crucial for safety and for obtaining the best possible outcome. The liver is currently the site of choice, but is unfortunately associated with disadvantages for graft survival.

In paper I, intraportally transplanted human islets were evaluated for hypoxia, apoptosis, and beta-cell survival. This revealed a substantial graft loss of approximately 50 % of transplanted islet mass at one month posttransplantation. At the same time, revascularization was increased, yet still lower than that of native islets. The highest rate of apoptosis was associated with prolonged time in culture prior transplantation.

Due to progressive loss of graft function, repeated islet transplantation is often performed. A mouse model, used in paper II, demonstrated an increased survival rate of islets transplanted one week after a first transplant. This finding may reflect an improved engraftment environment "primed" by the first islet injection. No difference in islet vascular density could be ascribed to it.

As stem cell-based therapies improve, graft monitoring possibilities and retrieval are of importance for safely introducing these techniques into the clinic. Islet grafts to omentum and muscle cured diabetic mice in paper III. Gene expression was unaltered or increased for genes important for beta-cell function.

Decidual stromal cells (DSCs) have immunomodulatory properties that could prove useful for treatments of autoimmune or inflammatory conditions. In paper IV, DSCs were found to be easily isolated from human placenta. The cells were characterized by surface markers, differentiation capacity and gene expression during culture. Co-culture with human pancreatic islets was also conducted. DSCs were observed to be very similar to other types of mesenchymal stromal cells. Greatest change in gene expression was seen between passage 2 and 5. The effect on human islet function may depend on islet viability prior to co-culture.

Keywords: Islet transplantation, Type 1 diabetes, Mesenchymal stem cells

Hanna Liljebäck, Department of Medical Cell Biology, Box 571, Uppsala University, SE-75123 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-513-0858-6
urn:nbn:se:uu:diva-402501 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-402501)

All things are so very uncertain,
and that's exactly what makes me feel reassured

- *Tove Jansson*, Moominland Midwinter

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Liljebäck H, Grapensparr L, Olerud J, Carlsson PO. (2016) Extensive loss of islet mass beyond the first day after intraportal human islet transplantation in a mouse model. *Cell Transplantation,* 25(3):481-9

II Liljebäck H, Quach M, Carlsson PO, Lau J. (2019) Fewer islets survive from a first transplant than a second transplant: evaluation of intraportal islet transplantation in mice. *Cell Transplantation*, 28(11):1455-1460

III Espes D, Liljebäck H, Quach M, Lau J, Franzén P, Carlsson PO. Function and gene expression of islets experimentally transplanted to muscle or omentum. *Manuscript submitted.*

IV Liljebäck H, Iredahl Z, Singh K, Carlsson PO. Alterations of decidual stromal cells in culture and their effect on human beta-cells in vitro. *Manuscript.*

Contents

Abbreviations

ANOVA	Analysis of variance
ATMP	Advenced therapy medicinal products
ATP	Adenosine triphosphate
bmMSC	Bone marrow derived mesenchymal stromal cells
BS-1	Bandeiraea simplicifolia
CD	Cluster of differentiation
CMRL	Connaught Medical Research Laboratories
DAB	3,3´-Diaminobenzidine
DAPI	4´,6-diamidino-2-phenylindole
DMEM	Dulbecco's modified Eagle medium
DPBS	Dulbecco's phosphate-buffered saline
DSC	Decidual stromal cell
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
GABA	Gamma aminobutyric acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCK	Glycokinase
GFP	Green fluorescent protein
GLUT2	Glucose transporter 2
GPD2	Glycerol-phosphate dehydrogenase 2
GVHD	Graft versus host disease
HBSS	Hank's balanced salt solution
HIF-1	Hypoxia-inducible factor 1
IAPP	Islet amyloid polypeptide
IBMIR	Instant blood mediated inflammatory reaction

IEQ	Islet equivalent
IL	Interleukin
ISCT	International Society of Cellular Therapy
IT	Islet transplantation
ITT	Insulin tolerance test
IVGTT	Intravenous glucose tolerance test
KRBH	Krebs-Ringer bicarbonate Hepes buffer
LSR	Laser selective reflow
MHC	Major histocompatibility complex
MSC	Mesenchymal stem cell
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDX1	Pancreatic and duodenal homeobox 1
RPMI	Rosewell Park Memorial Institute
SEM	Standard error of the mean
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TNF-α	Tumor necrosis factor-α
VEGF	Vascular endothelial growth factor

Introduction

Islets of Langerhans

The pancreas is an unpaired gland that has both exocrine and endocrine function. Clusters of endocrine cells distributed throughout the pancreas form the islets of Langerhans, named after the German physician Paul Langerhans, who was the first to histologically describe them in 1869. Today, we know that these clusters consist of different hormone-producing cells which make up the endocrine function of the pancreas and play an indispensable role in the homeostasis and metabolism of glucose.

The major hormone-secreting cell types are alpha-cells, beta-cells, delta-cells, PP-cells, and epsilon-cells which, produce glucagon, insulin, somatostatin, pancreatic polypeptide, and ghrelin, respectively. In healthy human adults, the pancreas contains 3.2-14.8 million islets[1-3]. A loss of beta-cell function or mass leads to insulin deficiency, causing elevated blood glucose levels and resulting in the condition known as diabetes mellitus.

Diabetes

Diabetes mellitus is a heterogeneous disease, which can arise due to a patient's genetic load and/or environmental risk factors associated with inflammation, autoimmunity and metabolic decompensation. Type 1 diabetes (T1D) usually presents in childhood or adolescence and is characterized by an almost complete autoimmune destruction of the pancreatic islets[4]. Type 2 diabetes (T2D) is characterized by hyperglycemia and insulin resistance often associated with obesity. There are also other variants of diabetes displaying mixed phenotypes with different degrees of autoimmunity and insulin resistance, i.e. late autoimmune diabetes in adults (LADA), and maturity onset diabetes of the young (MODY)[5]. According to the International Diabetes Federation, diabetes is one of the fastest growing global heath emergencies of the 21st century with more than 463 million people suffering from the disease. T1D, one of the most common chronic diseases affecting children, accounts for 5-10 %, whereas T2D represents around 90 % of diabetes worldwide.

The incidence of T1D is increasing, but there are considerable regional variations[6,7]. To date, Sweden has the second highest incidence rate of T1D in the world (after Finland) in children aged 0-14 years (43.2 per 100,000 per year)[8].

Treatment of Type 1 Diabetes

The standard treatment of T1D includes life-long insulin administration, after groundbreaking experiments by Fredrick Banting and Charles Best in 1921. New insulin formulas and technical devices, such as continuous glucose monitoring and closed loop systems, have substantially improved treatment options and contributed to better health[9,10]. For the majority of patients with T1D, standard insulin therapy is effective for maintaining euglycemia and minimizing diabetes-associated complications (cardiovascular disease, nephropathy, retinopathy, and neuropathy)[11-13]. It is difficult to mimic the extremely precis glucose control of a healthy pancreas. Patients with T1D have an increased mortality and a reduced life expectancy compared to the general population, in certain studies by more than 10 years[14-16].

Additionally, a small number of patients, despite intensive treatment, show extreme glycemic variability and suffer repeated and unpredictable hypoglycemic episodes. This leads to an unawareness of hypoglycemia and is often associated with defective counter-regulatory mechanisms and autonomous neuropathy[17]. For this group of patients, it is possible to achieve a restoration of pancreatic endocrine function through transplantation of either a whole pancreas or islets of Langerhans only. However, the clinical utility is limited because of the necessity of life-long immunosuppressive treatment, which is afflicted with adverse side effects such as opportunistic infections, malignancies, and drug toxicity[18].

Pancreas and Islet Transplantation

Currently, the outcome after whole pancreas transplantation is an average graft survival rate of 50 % after 10 years. Pancreas transplantation can either be performed alone or as a simultaneous pancreas-kidney transplantation in patients with T1D and chronic renal failure[19]. The procedure is associated with risks of technical failure, thrombosis, infections, pancreatitis and acute or chronic graft rejection[20]. However, the success rate is improving and patient survival rate is 90 % at 4 years[20,21].

For patients where insulin therapy is unsuccessful and it is not suitable to receive a whole pancreas, islet transplantation (IT) has become an optional treatment. The islet isolation technique using collagenase, was developed in the 1960s by Lacy et al.[22], and the first experimental trials performed in the 1970s[23]. It is, as opposed to whole pancreas transplantation, a minimally invasive procedure in which a radiologically guided percutaneous transhepatic injection allows islets to reach the liver via the portal vein. The procedure is associated with low morbidity and low risks of adverse effects, such as bleeding (7 %) and portal thrombosis (3.7 %)[24]. Ever since Shapiro et al. introduced the Edmonton protocol[25] with a greater transplanted islet mass and steroid-

free immunosuppression, IT got a revival and outcomes have since become increasingly better, owing to ongoing improvements in isolation techniques and refined immunosuppressive regimes[26]. The number of patients who achieve insulin independence after IT has dramatically improved during the last two decades (44 % at 3 years after transplantation) and is currently almost on par with the results of whole pancreas transplantation[26,27].

Islet Isolation and Engraftment

Studies have revealed that, although islets only constitute 1-2 % of the pancreatic mass[28], they receive 10-15 % of the total pancreatic blood flow. The vessels inside and surrounding the islet cells are fenestrated, further implying a close connection between the vasculature and the endocrine function of the islets[29]. These integrated vessels account for 8-10 % of total islet volume[30] and are believed to play a role in paracrine signaling[31], beyond supplying islets with oxygen and nutrients.

As islets are isolated, they are inevitably deprived of their native vascular network. Consequently, the isolated islets must solely rely on diffusion of oxygen and nutrients during culture and during the initial phase after transplantation. Beyond the fact that islets are already subjected to hypoxic conditions during culture, additional factors (islet size, reduced viability, and thrombus formation) can further aggravate the hypoxia, resulting in an anoxic islet core[32,33]. Hence, reestablishment of vasculature is a vital part of the engraftment process, i.e. the adaptation of islets to the surrounding tissue at the implantation site. Engraftment also encompasses reinnervation and reorganization of adjacent stroma, and the conditions for successful engraftment differ between implantation sites[34].

Islet Transplantation to the Liver

Recent advances in IT outcome have been accomplished through enhanced procurement of islets and refined immunosuppressive therapies. However, IT often needs to be repeated and even in autologous or in experimental, syngeneic transplantations, a great amount of islet mass is quickly lost[35]. Factors affecting islet quality, and thus transplant success, are: donor condition, ischemic time, isolation procedure, degree of exocrine contamination, and culture time[36-39]. Later on, allogeneic islets grafts are further exposed to autoimmunity, alloimmune rejection and toxicity of immunosuppressive treatment.

Both before and after transplantation, islets are exposed to hypoxic and mechanical stress, as well as activation of inflammatory cytokines (IL-1β, TNF-α and HIF-1), leading to impaired function and beta-cell death[40,41]. Inflammation is induced by both innate immune cells, islet resident macrophages, and

specialized macrophages in the liver (Kupffer cells)[41-46]. Studies have shown that anti-inflammatory treatment and strategies to increase levels of antioxidants inside the islets have improved islet function[47]. Treatment of islets with mesenchymal stem/stromal cells are suggested to mediate their effect in a similar way[48].

The use of liver as implantation site is associated with further disadvantages linked to islet infusion and engraftment. First, when islets are injected through the portal vein, an immediate blood mediated inflammatory reaction (IBMIR) occurs, which has been shown to damage the endocrine cells and cause a loss of islet mass by up to 50 % in the earliest phases after transplantation[49,50]. Second, once the islets have settled in the liver, gluco- and lipotoxicity[51], amyloid formation[52], and immunological attack[53] may all influence graft survival to various extents. Hyperglycemia per se has also been shown to hamper the islets, if euglycemia is not fully reached after transplantation[54,55].

Furthermore, the liver does not provide optimal conditions for revascularization and islet oxygenation. Even under normal physiological conditions, the oxygen tension of the liver is only 5-10 mmHg, as compared to 40 mmHg in native islets[56,57]. In the liver, islets have been shown to get revascularized from the hepatic artery[58,59] A new vascular network is gradually established from the first week after transplantation. However, even after revascularization is complete, the oxygenation of intraportally transplanted islets remains low[60,61].

Islet grafts implanted in liver in mice have, in previous studies, exhibited an altered gene expression compared to control islets. These genes, vital for beta-cell function, include PDX-1, a marker of differentiation. This marker was decreased in islets transplanted to liver as well as to pancreas[62]. Similar findings have recently been demonstrated in humans, where all beta-cells in allogeneic islet grafts were lacking the differentiation marker urocortin-3[63]. In the same report, cells were found positive for both glucagon and insulin which indicates that islet cells exhibit some plasticity. It is unclear exactly what causes these changes. Of note, treatment with GABA has been shown to induce alpha-to-beta-cell conversion *in vivo*[64].

Alternative Implantation Sites

The renal capsule has historically been readily used for islet transplantations in experimental models, but translating studies into humans have failed[65]. In humans, the renal capsule is not suitable due to volume restriction. Despite the numerous sites that have been evaluated, in both experimental and clinical setting (muscle, omentum, bone marrow, pancreas, gastric submucosa, kidney and spleen[26,62,65-72]), none have in humans reported improved outcomes when compared to the intraportal site. Table 1 lists comparative studies of islet transplant sites in mice.

Each site is associated with specific technical complications, volume capacity and engraftment conditions. Furthermore, studies show that the liver appears superior to bone marrow and renal capsule from an immunological point of view[73,74]. Also, the liver is the target organ for insulin, and choosing sites with a portal drainage is presumably preferable to systemic insulin release in order to mimic a physiological pancreatic insulin secretion[75].

Table 1. Comparative studies of islet transplant sites in C75BL6J, modified from review in Cell Medicine[76].

Authors	Implantation sites	Islet type	Islet volume tx	Major outcome
Korsgren O et al. 1993 [77]	Liver, spleen and renal capsule	Murine	300 islets	Better nerve in growth in renal capsule compared to liver and spleen
Lau J et al. 2007 [62]	Pancreas vs liver	Murine	200 islets	Glucose stimulated insulin release and oxidation rates were markedly decreased in liver
Kim HI et al. 2010 [34]	Renal capsule, liver, muscle and omentum	Murine	marginal mass	Renal capsule, liver, muscle and omentum required 100, 600, 600, 200 islets to cure 50 % of engrafted diabetic mice, respectively. Kidney had shortest time to reach euglycemia (3 days), muscle the longest (27 days)
Christoffersson G et al. 2010 [78]	Muscle vs liver	Human and murine	300 islets	Improved revascularisation and response to glucose challenge in intramuscular transplantation, on par with intrahepatic transplantation
Espes D et al. 2016 [79]	Omentum vs liver	Human and murine	200-300 islets	Normalized vasculature and better response to IVGTT 1-month post-transplant in the omentum
Stokes RA et al. 2017 [80]	Renal capsule, muscle, liver, spleen capsule, liver capsule	Human and murine	220-250 islets / 2000 IEQ human islets	Renal capsule best site for murine and human IT (Renal capsule used as control). Muscle and intraportal site had similar cure rate for human islets, however both inferior to renal capsule
Cantarelli E et al. 2017 [73]	Bone marrow vs liver	Murine (two different strain	450 IEQ	Treating the animals with anti-CD3, islet rejection is prolonged when transplanted to liver compared to bone marrow

Stem Cell Therapy in Type 1 Diabetes

The development of stem cell research has enabled cellular therapies in various disorders, including diabetes. The term “stem cells” is wide and includes

cells found in the embryo as well as adult stem cell populations present in most tissue, on standby, ready to participate in tissue regeneration. Of current interest, protocols for induced pluripotent stem cells have come into practice, opening for personalized stem cell treatments.

Stem cells are capable of self-renewal and possess either totipotency, pluripotency (embryonic stem cells) or multipotency of differentiation into various tissues from one or more germ layers. Ideally, these cells could provide a source for beta-cell replacement, and thus resolve the issues of both immunosuppression and lack of donors[81,82]. Nevertheless, the underlying autoimmunity of diabetes would not be avoided.

The biology of stems cells is complex, and there is no common standardization and few guidelines. Before safely introducing stem cells into clinical trials, investigations need to be conducted regarding mechanism of action, and protocols developed for isolation, culture, and administration, all of which affect the phenotype of stem cells.

Mesenchymal Stromal Cells

A commonly used type of stem cell is the mesenchymal stromal cell (MSC), first described in the 1960-1970s[83,84]. MSCs are classified as multipotent stem cells due to their capacity to differentiate into various lineages that develop from mesoderm[85,86]. MSCs have been used in a great number of animal models for human disease as well as in veterinary medicine[87]. Furthermore, they have been evaluated for clinical cell-based therapies in a wide range of ailments, such as osteogenesis imperfecta, GVHD, myocardial infarctions, autoimmune diseases, and diabetes[88-91].

These cells were first identified in bone marrow[84], but today we know that cells fulfilling MSC criteria can be isolated from most tissues. The International Society of Cellular Therapy (ISCT) proposed the following criteria in 2006[92]:

1. Plastic adherence
2. >95 % of the cells must express surface molecules such as CD105, CD73, or CD90
3. <2 % expression of CD45, CD34, CD14 and MHC II
4. Multipotent differentiation potential

The initial hypothesis for the regenerative properties of MSCs was based on their multipotent capacity. Early on, MSCs were shown capable of differentiating into non-mesodermal cells[93,94], and studies have even demonstrated that MSCs can develop into insulin producing cells[95]. Currently, MSCs are believed to mediate their main effect through soluble trophic and immunomodulatory factors, regulating the surrounding environment[96] (Figure 1).

Contributing reasons for the frequent use of these cells are the property of self-renewal (although only for a limited time), permitting *in vitro* expansion, and that they are "immunologically privileged", allowing allogeneic MSC transplantation without the need for immunosupression[97].

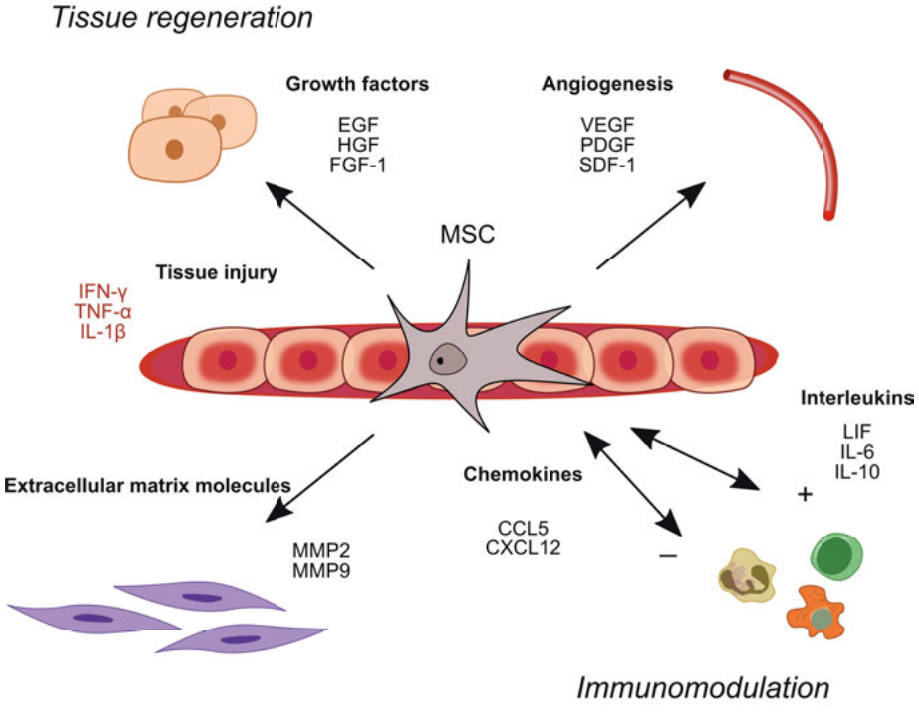

Figure 1. Simplified model of potential mechanism of actions facilitated by MSCs. When tissue injury occurs (due to damage or disease), MSCs can either home, if systemically injected, or be locally administered to the site of event. Inflammatory cytokines may then stimulate/activate MSCs, which will respond by releasing growth factors, producing immunoregulatory chemokines and interleukins, and remodeling the extracellular matrix, all of which will affect the inflammatory process.

MSC Safety and Legislation

Registered data from clinical trials show few and minor adverse events of treatment with MSCs[91]. Their genomic stability and the fact that MSCs do not seem to engraft inside the recipient may explain why few cases of ectopic tissue formation or malignancy are reported[98,99]. Human stem cells can be extensively expanded in culture, and several studies show maintained karyotype for >30 passages[100,101]. After too many passages, however, the biological function has been reported to be lost[98,102]. Notably, only 1 % of systemically administered MSCs persist longer than a week following injection[103]. Still, an

immunomodulating effect may be lasting, a phenomenon known as "hit-and-run". Even if intravenously administered MSCs have been shown to end up in the pulmonary bed, they can still mediate a systemic action[104]. The route of administration is not unimportant, considering that some MSC sources express tissue factor, and thereby are much more prone to thrombus building and to induce IBMIR, resembling the response seen in islet lodging. However, bmMSCs express relatively low levels of tissue factor, and when used for coating of islets, and subsequent exposure to blood (in an *in vitro* tubing system), no increased reaction was found compared to uncoated control islets[105].

MSCs are considered to be an advanced therapy medicinal product (ATMP), and thus guidelines from European Medicines Agency (EMA) require potency assays that can predict clinical effect. This demands an understanding of the mechanism of action.

MSC and Islet Transplantation

Additional positive effects beyond immunomodulation are seen in several studies using MSCs in IT research[106,105]. In rodents, MSCs have contributed to enhanced engraftment of islets implanted to muscle, renal capsule, and omentum, with reduced formation of fibrosis and increased vascularization[107-109]. The mechanism for this effect is MSC production of angiogenic factors, including VEGF, IL-6 and IL-8, which supports islet graft revascularization[108,110-113]. Also, MSCs express markers important for homing[114], which has enabled intravenous administration where the cells migrate to implanted islets (renal capsule) and contribute to neovascularization and islet cell proliferation[110]. Islets and MSCs have also successfully been co-transplanted to the liver[111,115]. However, donor and recipient species (for both islets and MSCs) vary, as well as administration routine, culture condition, number of passages, and site of implantation, which makes comparisons difficult. Furthermore, MSC and islet co-culture can be performed allowing physical cell-cell interactions or in an indirect fashion where islets only come in contact with MSC-secreted factors, alternatively in conditioned medium[116-118]. A review on the topic presented that a higher viability was obtained when choosing an indirect contact system as opposed to culture with direct cell-cell contact[119]. However, results are not conclusive and specific MSC source is likely to affect the results[120,121].

Placenta as a Source of MSCs

During pregnancy, the decidual layer is formed from the uterine lining (the endometrium). The process of decidualization creates a specialized tissue composed by glands, immune cells, blood and lymph vessels, and decidual

stromal cells (DSCs)[122]. It forms part of the placenta, the functions of which are to provide exchange of nutrients, oxygen and excretion, whilst maintaining the fetomaternal tolerance.

DSCs are a type of MSCs that, just like bmMSCs, are easily expanded and exhibit potent immunosuppressive effects[123,124]. Studies comparing bmMSCs and DSCs have found many similarities with regards to appearance in light microscope, size, and surface marker phenotype[125]. The ability of mesodermal differentiation has been described as equally good or somewhat reduced compared to bmMSCs[125,126]. DSCs are currently used to improve acute inflammatory disorders such as steroid-refractory GVHD and radiculomyelopathy, where no other effective therapy exists[127,128]. The placenta, otherwise discarded after a birth, may provide eligible MSCs in large quantities for evaluation of their possible cell-therapeutic utility and mechanism of action in islet research.

Aims

The overall aim of the work presented in this thesis was to investigate central factors in islet transplantation and finding strategies to improve beta-cell function and transplant outcome. The specific aims for each study were:

Paper I

To investigate human islet engraftment and graft survival within the first month after intraportal transplantation

Paper II

To investigate the possibility of enhancing islet engraftment to the liver through repeated islet transplantation

Paper III

To compare islet graft function and gene expression following transplantation to greater omentum or abdominal muscle

Paper IV

To isolate and characterize decidual stromal cells from human placenta, their alterations in culture, and investigate their effect on human islets

Materials and Methods

Experimental Animals

Papers I-III

The animal housing and all experimental procedures were approved by the local Animal Ethics Committee at Uppsala University, Sweden. All animals were housed under standardized conditions with free access to food and water. Adult male immunodeficient C57BL/6 (nu/nu) mice, obtained from Taconic M&B, Bomholtgaard, Denmark, were used as recipients for human islets in paper I. In paper II, a transgenic mouse model that expresses green fluorescent protein (GFP), under control of the mouse insulin I gene promoter (MIP) on C57BL/6 background (Taconic M&B), was used as donors for islet isolation for the first transplantation. C57BL/6 male mice, wild type and transgenic, purchased from Taconic M&B, Ejby, Denmark, were used for transplantation and islet isolation in papers II and III.

Isolation and Culture of Human Islets

Papers I & IV

The regional ethical board in Uppsala approved the use of human pancreatic tissue. Pancreata from donors after brain death were generously provided by the Nordic Network for Clinical Islet Transplantation and isolated at the human islet isolation facility, Rudbeck Laboratory, Uppsala University Hospital. The human islets were cultured according to the standard protocol at the Rudbeck laboratory. For the first 24 hours, islets were incubated in 95 % O_2 and 5 % CO_2 at 37°C, thereafter at 25°C until the islets were transferred to our laboratory and to CMRL 1066 medium (Gibco, Grand Island, NY, USA) containing 10 % vol/vol fetal calf serum, 50 mmol/L L-glutamine (Sigma-Aldrich, St Louis, MO, USA) and 5 U/mL Penicillin Streptomycin (Roche, Sigma-Aldrich). The mean culture time before transplantation was 6.2 ± 0.5 days.

Isolation and Culture of Murine Islets

Papers II & III

Pancreatic islets from non-diabetic C57BL/6 mice were isolated using collagenase digestion and density gradient purification as previously described[61] (Figure 2). Briefly, mice were anesthetized with sodium pentobarbital (200 mg/kg, Apoteket AB, Stockholm, Sweden). Collagenase A from Clostridium histolyticum (2.5 mg/mL Roche Diagnostics, Mannheim, Germany) suspended in cold HBSS (National Bacteriological Laboratory, Stockholm, Sweden) was injected via the common bile duct. The pancreas was inflated, surgically removed and placed in 37°C for 18 minutes. Density gradient centrifugation by adding Histopaque-1077 and serum-free RPMI 1640, both purchased from Sigma-Aldrich, was performed for separation of islets from exocrine tissue. Islets were handpicked and cultured free-floating at least overnight in 5 mL of culture medium consisting of RPMI 1640 (Sigma-Aldrich) supplemented with L-glutamine (2 mmol/L; Sigma Aldrich), 10 % vol/vol fetal calf serum (Sigma-Aldrich) and Penicillin Streptomycin (100 U/mL and 0.1 mg/mL, respectively; Roche Diagnostics) in paper II. In paper III, the antibiotic used was Benzylpenicillin (100 U/mL; Roche Diagnostics), otherwise the procedure was the same as detailed above.

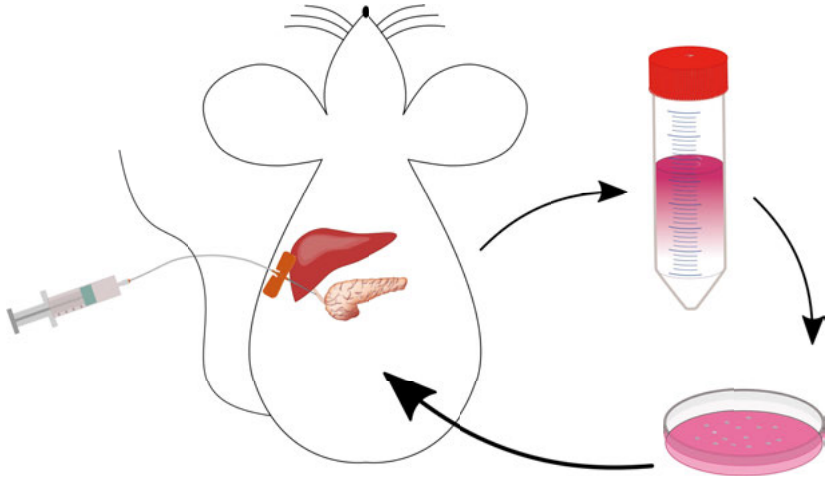

Figure 2. Islet isolation through density gradient purification after retrograde injection of collagenase I digestion solution through the common bile duct. In papers I and II, islets were transplanted after having recovered for 1-2 days in culture before transplanting them to the liver of recipients through the portal or appendicular vein. In paper III, islets were instead implanted to either the greater omentum or abdominal muscle.

Induction of Diabetes

Paper III
Diabetes was induced in C57BL/6 mice by a single intravenous injection of alloxan (75 mg/kg, Sigma-Aldrich). Mice were defined as diabetic when repeated blood glucose concentrations exceeded 15 mmol/L on two consecutive days, using blood glucose reagent strips (Freestyle Lite, Abbot, Alameda, CA, USA). Blood glucose concentrations were monitored daily for the first week posttransplantation and then every fifth day.

Islet Transplantations

In papers I, II, and III, all animals were anesthetized by an intraperitoneal injection of 0.02 mL/g body weight Avertin (Kemila, Stockholm, Sweden). In paper II, IT through the appendicular vein to the liver was made under spontaneous inhalation of isoflurane (first transplantation). All transplantations to the liver were made after a midline abdominal incision and islets were infused into the portal vein system through a butterfly needle. In paper I, islets were transplanted selectively into the right liver lobe by temporarily clamping other portal vein tributaries during the islet infusion[61]. This procedure enabled us to evaluate a greater proportion of islets transplanted, and is not believed to affect the engraftment negatively.

Paper I
Human islets (300 islets per transplantation) were injected in the livers of C57BL/6 (nu/nu) mice, as detailed above. Two hours before animals were killed by cervical dislocation, the hypoxia marker pimonidazole (60 mg/kg; Hypoxyprobe, Burlington, MA, USA) was injected intravenously through the tail vein of awake animals.

Paper II
Two groups of animals received IT twice, one week apart, while mice in a control group received a sham transplantation with saline infusion only, followed by one IT a week later (Figure 3). The first islet transplant consisted of 125 syngeneic GFP-expressing islets, while the second transplant consisted of 125 syngeneic non-GFP-expressing islets of similar size (50-100 μm). Islets were infused into the appendicular vein or portal vein with a butterfly needle (25G). The total volume of infusion at each time was ≤ 100 μL. The animals were killed 1 day or 1 month after the second transplantation. Animals in the control group were killed 1 month after IT.

Paper III
200 mouse islets were transplanted under aseptic conditions into the abdominal muscle or omentum of recipient non-diabetic or alloxan-diabetic C57BL/6 mice. Islets were transplanted to the abdominal muscle after a midline incision in the skin, as previously described[129]. In order to transplant islets to the greater omentum, a midline incision in the skin and the abdominal muscle was made and the stomach was exposed. The greater omentum was localized and carefully extended by holding the omental fat and a ligature was placed, but not fixed, around the omentum. An opening between the sheets of the omentum was made with a cannula, a braking pipette filled with islets was inserted, the islets were infused into the pouch, and the ligature was thereafter closed. The abdominal muscle was carefully sutured, followed by suturing of the skin.

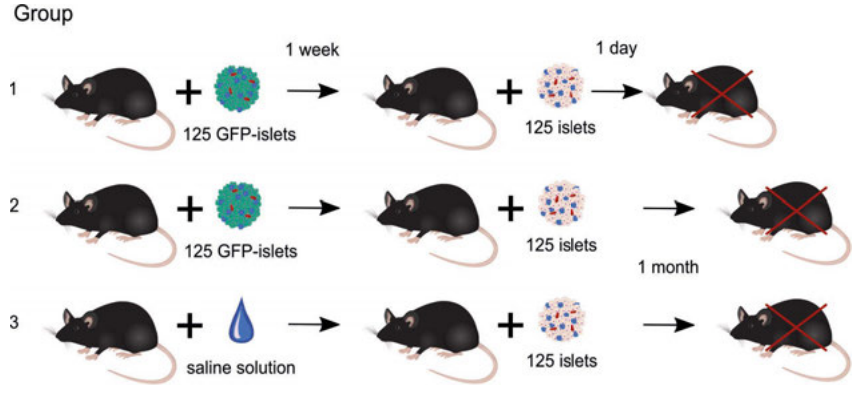

Figure 3. Experimental design paper II. A first transplantation was performed with either GFP-positive islets or a shame saline infusion to a control group. After one week, a second transplantation to the liver with non-GFP C75BL/6 murine islets was performed in all groups.

Function of Transplanted Islet Grafts

Paper III
Thirty days posttransplantation, an intravenous glucose tolerance test (IVGTT, 2 g/kg) was performed in cured animals (defined as a non-fasting blood glucose concentration <12 mmol/L for two consecutive measurements on separate days). Blood glucose concentrations were monitored for two hours following glucose injection. On the following day, an intravenous insulin tolerance test (ITT; 2 U/kg NovoRapid, Novo Nordisk, Bagsvaerd, Denmark) was performed, during which blood glucose concentrations were monitored for two hours. Non-diabetic C57BL/6 mice were used as controls for both the IVGTT and the ITT. When the animals subsequently were killed, the pancreas

was surgically removed, placed in acid ethanol (0.18 mol/L HCl in 95 % vol/vol ethanol), sonicated in order to disrupt the cells and subsequently frozen. The remaining insulin content in the pancreas was measured with an insulin enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden) in order to exclude endogenous beta-cell regeneration. An insulin content > 10 % of that of the pancreas in control non-diabetic animals was used as an exclusion criterion for the study.

Immunohistochemistry

Sampled tissue was either fixated in 4 % vol/vol paraformaldehyde before paraffin embedding or snap-frozen for cryosectioning. Frozen tissue was imbedded in TissueTek OCT (Sakura Finetek, Torrance, CA, USA) and sectioned using Leica CM 1950 cryostat (Leica Biosystems, Germany).

Paper I

The paraffin embedded liver lobes were sectioned to a thickness of 7 µm and every fifth slide was stained for insulin. Adjacent slides were stained, respectively, for blood vessels, amyloid, hypoxia and apoptosis according to Table 2 and counter-stained with Mayor's hematoxylin (HistoLab Products AB, Gothenburg, Sweden). Insulin and caspase-3 were dye developed using 3,3´-Diaminobenzidine (DAB) (Dako, Glostrup, Denmark).

Sections from human pancreata were simultaneously stained with the lectin Bandeiraea simplicifolia (BS-1) and evaluated for vascular density, as previously described[130], for comparison between native and transplanted islets.

Paper II

Graft bearing livers were initially fixated in 4 % vol/vol paraformaldehyde overnight at 4°C, transferred to 15 % wt/vol sucrose in PBS (2 h) and finally to 30 % wt/vol sucrose in PBS overnight at 4°C, to preserve GFP-expression. The livers were sectioned (10 µm) to a depth of 4.0 mm. Islet grafts were identified in sections stained with hematoxylin, while consecutive sections were saved for later performed immunostainings (Table 2). Only islets with an area exceeding 50 μm^2 were evaluated in order to avoid misestimations based on single cell residues.

Paper III

Tissue sections were collected from the same tissue block for insulin staining of the islet graft. Cryosections and paraffin sections were prepared for immunohistology using antibodies outlined in Table 2. ProLong Gold Antifade reagent with 4´,6-diamidino-2-phenylindole (DAPI) (Life Technologies, Rockville, MD, USA) was used for mounting and nuclei staining for frozen

sections. Hoechst 1:10,000 (Life Technologies) was used for nuclei staining of paraffin embedded pancreas and grafts from muscle and omentum.

Table 2. Stainings and antibodies used for histological evaluations

Primary antibody	**Dilution**	**Manufacturer**	
Guinea pig anti-insulin	1:400	Fitzgerald Industries International Concord, MA, USA	Paper I
Rabbit anti-caspase-3	1:100	Cell Signaling Technology, Danvers, MA, USA	Paper I
Lectin *Bandeiraea simplicifolia*	1:100	Sigma-Aldrich	Paper I
Mouse anti-pimonidazole	1:100	Bioscience Research Reagents, Temecula, CA, USA	Paper I
Alkaline Congo Red		VWR International, Radnor, PA, USA	Paper I
Rat anti-mouse CD31	1:100	BD Biosciences, San Jose, CA, USA	Paper II
Guinea pig anti-insulin	1:400	Ken-EN-Tec Nordic, Tåstrup, Denmark	Paper II
Rabbit anti-Ki67	1:250	Abcam, Cambridge, UK	Paper II
Guinea pig polyclona	1:400	Fitzgerald, Acton, MA, USA	Paper III
Mouse monoclonal anti-glucagon	1:800	Abcam, Cambridge, UK	Paper III
Anti-mouse FABP4	10 µg/mL	R&D systems	Paper IV
Anti-human Aggrecan	10 µg/mL	R&D systems	Paper IV
Anti-human Osteocalcin	10 µg/mL	R&D systems	Paper IV
Secondary antibody	**Dilution**	**Manufacturer**	
Swine anti-rabbit antibody	1:40	Dako, Glostrup, Denmark	Paper I
Biotinylated goat anti-rabbit antibody	1:300	Southern Biotech, Birmingham, AL, USA	Paper I
TrekAvidin-AP Lable and Vulcan Fast Red		Biocare Medical	Paper I
Biotinylated goat anti-mouse		Southern Biotech	Paper I
Alexa Flour 647 donkey anti-rat	1:300	Jackson ImmunoResearch Laboratories, West Grove, PA, USA	Paper II
Alexa Flour 488 donkey anti-guinea pig	1:300	Jackson ImmunoResearch Laboratories	Paper II
Alexa Flour 488 donkey anti-rabbit	1:300	Jackson ImmunoResearch Laboratories	Paper II
Alexa Fluor 488 goat anti-guinea pig	1:1000	Invitrogen, Carlsbad, CA	Paper III
Alexa Fluor 594 donkey anti-mouse	1:250	Invitrogen	Paper III
Alexa Fluor 594 donkey anti-goat	1:300	Jackson ImmunoResearch Laboratories	Paper IV
Alexa Fluor 594 donkey anti-mouse	1:300	Jackson ImmunoResearch Laboratories	Paper IV

Paper IV

To evaluate differentiation capacity of DSCs in paper IV, cells were stained with Oil Red O (Santa Cruz Biotechnology, CA, USA), Alizarin Red (Sigma-Aldrich), and Alcian Blue (Sigma-Aldrich) following fixation with 4 % or 10 % vol/vol paraformaldehyde. Parallel fixated cells or, for chondrogenic differentiation performed in 3D-culture, sections of the fixated pellet, were stained with mFABP4, osteocalcin, and anti-aggrecan antibody (R&D Systems, McKinley Place, MN, USA).

Cell death assessment was performed by staining with Live-or-Dye NucFix Red Staining Kit (Biotium, Fremont, CA, USA) and bisbenzimide 20 μg/mL (Hoechst 33324, Sigma-Aldrich).

Image Analysis

Paper I

Images were captured with Leica Leitz DMRBE microscope (Leica Microsystems, Wetzlar, Germany). Assessment of islet vascular density and evaluation for percentages of caspase-positive cells were made using ImageJ (National Institutes of Health, Bethesda, MD, USA). In order to determine the intra-islet capillary density, only vessels surrounded by islet tissue were counted and compared to native human pancreas, assessed with the same criteria.

The percentage of pimonidazole-positive islets was assessed. Islet amyloid was evaluated by fluorescence microscopy and polarized microscopy, in the latter amyloid displays an apple-green birefringence[131].

Paper II

Images were captured using the laser scanning confocal microscope Zeiss LSM 780 (Carl Zeiss AG, Oberkochen, Germany) with Plan-Apochromat 10x/0.45 M27 and 20x/0.8 M27 objectives. For image analysis of islet area and vascular density, a macro was built in Fiji software with the assistance of staff at the BioVis facility platform of Uppsala University.

Paper III

Light microscopy images were acquired with a Leica LMD6000 laser microdissection microscope (Leica Microsystems). Fluorescent immunohistochemistry images were acquired with Zeiss LSM780 confocal (Zeiss).

The respective areas of insulin and glucagon were calculated using the image software Imaris (Bitplane AG, Zurich, Switzerland) with a fixed intensity cut-off value for each staining. The percentage in each image was combined into an average for each animal and considered as one experiment.

Paper IV
A macro using Fiji software performed threshold-based calculations of the ratio of dead cells, taken as a relative measure of islet resistance to cytokine stress.

Glycogen Assay

Paper III
Glycogen concentration in muscle and liver was measured from 10 mg of homogenized tissue from cured and control animals by a Glycogen Assay Kit II (Abcam, Cambridge, UK). Reading of the plate was performed using a Spark Microplate Reader (Tecan, Männedorf, Switzerland).

Decidual Stromal Cell Isolation

Paper IV
Human placentas were obtained from mothers at Uppsala University Hospital. The following inclusion criteria were used: healthy women planned for routine caesarian section in week 37-42 of gestation. Exclusion criteria: positive test for HIV, hepatitis or Treponema pallidum, smokers, patients suffering from diabetes, preeclampsia, placenta accreta or known tumor disease. After collection of the placentas, the isolation process started within four hours. For decidual stromal cell isolation, the protocol from Pelekanos et al was used[132]. The placenta was washed inside a biosafety cabinet and the decidua was dissected into pieces of 5 g. The tissue was washed in HBSS, minced, divided into portions of 10 g and incubated in 25 mL of the following digestion solution: Dispase 2.4 U/mL (Thermo Scientific, Waltham, MA, USA), DNase I 15μL, ≥ 2500 U/mL (Thermo Scientific) and Collagenase I 100 U/mL (Thermo Scientific). Incubation was sustained until the solution assumed a cloudy appearance, approximately 1.5 hours at 37°C during vigorous shaking. The tissue was then washed, pulse centrifuged and passed through a 100 μm filter. The single cells were seeded in T75-bottles (Corning, New York, NY, USA) in DMEM culture medium with low glucose from Thermo Scientific, supplemented with FBS (Sigma-Aldrich) and Penicillin Streptomycin (100 U/mL and 100 μg/mL respectively, Thermo Scientific). Medium was changed every three days. When 80-90 % confluency was reached, cells were passaged by trypsinization with TrypLE (Thermo Scientific). Dissociated cells were pelleted by centrifugation 200 rcf for 5 minutes, the supernatant discarded and cells resuspended in culture medium.

DSC Characterization

STR Analysis

Short tandem repeats (STR) were analyzed to confirm the origin of expanded DSC culture. DNA was recovered from blood samples and from cell cultures by Qiagen DNA Mini Kit (Qiagen, Hilden, Germany) and STR analysis was performed by Uppsala Genome Center.

Differentiation Capacity

Multilineage differentiation capability was evaluated by a differentiation kit (R&D Systems). For differentiation experiments, cells from passage 2 were seeded in 24-well dishes (Sarstedt, Nümbrecht, Germany) and differentiation medium was changed every third day. As controls, cells seeded or pelleted at the same cell density and cultured in standard culture medium were used.

Flow Cytometry

Cells from passages 1-10, isolated from different donors (n=15), were detached from flask using TrypLE, washed in DPBS (Thermo Scientific) and stained with a multifluorescent panel for MSC markers CD29-APC, CD90-FITC, CD73-BV605, CD105-BV421 (Biolegend, San Diego, CA, USA); hematopoietic markers CD45-PE, CD34-PE; endothelial marker CD31-PE, immunological markers HLA-ABC-PE-Cy7, HLA-DR-BV786; and the co-stimulatory molecule CD80-PE. All antibodies except CD105 were purchased from BD Biosciences (San Jose, CA, USA). The stained cells were assessed on the LSRFortessa (BD Biosciences) at the core facility BioVis at Uppsala University. Results were analyzed in FlowLogic software (Inivai Technologies, Australia).

Islet and DSC Co-Culture

Human islets were co-cultured with DSCs in either direct contact conditions upon a DSC monolayer (group 1) or in an indirect setting (group 2), where islets were placed inside a cell insert with pore diameter 0.4 μm, which allowed medium exchange but not cell-cell contact with a DSC monolayer. DSCs (group 3) and human islets (group 4) were also cultured separately.

Analysis of Inflammatory Biomarkers

Inflammatory biomarkers were measured in medium and lysates from DSCs and human islets after 48 hours of co-culture. Analysis was performed with an inflammatory biomarker panel provided by Olink (Uppsala, Sweden).

Cytoprotective Properties of DSCs on Human Islets

Islets were cultured in the presence of cytokines IL-1β 50 U/mL, IFN-γ 1,000 U/mL, and TNF-α 1,000 U/mL (PeproTech, London, UK) for 24 hours in the different co-culture conditions detailed above.

Islet Function

Duplicates of 50 islets, after DSC co-culture or not, were evaluated for insulin release in response to glucose and forskolin perifusion. The islets were initially perifused with KRBH-buffer, then primed to low glucose solution (2.0 mM) before exposure to high glucose (20.0 mM). The same procedure was repeated with addition of forskolin 1 μM. Samples were assessed for their insulin content by a human insulin ELISA (Mercodia, Uppsala, Sweden).

Gene Expression Analysis

Papers III and IV

For complete descriptions of preparation of tissue for microdissection, RNA isolation, PCR, and microarray analysis, see respective paper. Genes analyzed in paper III are specified in Table 3.

Table 3. Specifications of genes analyzed in paper III.

Gene	Name	Function
GCK	Glucokinase	Glucose metabolism
GLUT2	Glucose transporter type 2	Glucose transporter
GPD2	Glycerol-phosphate dehydrogenase 2, mitochondrial	Mitochondrial enzyme, glycolysis
INS1	Insulin 1	Peptide hormone
INS2	Insulin 2	Peptide hormone
LDHA	Lactate dehydrogenase A	Anaerobic glycolysis
PCX	Pyruvate carboxylase	Mitochondrial enzyme
PDX1	Pancreatic and duodenal homeobox 1	Transcription factor, beta cell differentiation marker
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	Glycolytic enzyme, *reference gene*
HPRT	Hypoxanthine guanine phosphoribosyl transferase	Metabolic enzyme, *reference gene*

Statistical Analysis

Statistical analysis was performed using GraphPad Prism. All values are expressed as means ± SEM. Comparisons between two groups were performed by unpaired or paired two-tailed Student's t-test. For all comparisons, p-values $< 0,05$ were considered statistically significant.

Paper I
Pearson's product moment correlation was used for linear regression analysis. Multiple comparisons between data were performed using analysis of variance (ANOVA) and Bonferroni post-hoc test. Kruskal Wallis test was used for non-parametric data (the data was considered non-parametric based on Shapiro-Wilk normality test).

Paper II
Comparisons between three groups were performed using ANOVA and Tukey's multiple comparisons test. For the multiple comparisons regarding the angiogenic markers a two-way ANOVA was performed with Sidak's multiple comparisons test.

Paper III
For comparison of relative gene expression data, a non-parametric one-way ANOVA was applied with Dunn's post-hoc test using native pancreatic islets as control. For comparison of blood glucose measurements between the three groups, a one-way ANOVA with Tukey's post-hoc test was applied. Comparison of time to normoglycemia was performed as a survival curve using Log-rank test (Mantel-Cox).

Paper IV
For comparisons between more than two groups, one-way ANOVA was performed using Dunnett's correction for multiple comparisons. One-way ANOVA was calculated on AUC for islet function. Inflammatory biomarkers were analyzed with multiple t tests using False Discovery Rate (FDR) approach, where FDR was set to 1% and using the two-stage step-up method of Benjamini, Krieger and Yekutieli.

Results and Discussion

In this thesis, factors affecting islet transplantation and methods for improving its outcome were investigated. Human islet engraftment and surviving beta-cell mass after intraportal transplantation, the site of golden standard, was evaluated in paper I. Islets expressing GFP were used in paper II for repeated IT. Abdominal muscle and greater omentum were assessed as alternative sites in paper III. Finally, DSCs, a type of MSC isolated from human placenta, were isolated and characterized for possible use in IT.

Beta-Cell Loss after Intraportal Transplantation

The question if the liver is the optimal site for islet transplantation has yet to be answered. Understanding which factors affect engraftment are of importance in order to optimize intraportal transplantation or to find a site more suitable for islet transplantation. In paper I, we investigated the engraftment of human islets intraportally transplanted to nude mice. We have previously investigated the oxygenation of intraportally transplanted islets in mice[60], and comparing those results to human islets were of interest since the architecture of human and murine islets differ[133,134]. When performing an islet transplantation to the liver, an instant loss of graft tissue occurs due to IBMIR. We compared beta-cell mass 1 day and 30 days after transplantation and investigated the degree of hypoxia.

We found that human islets undergo substantial graft loss beyond the immediate transplantation phase. The graft loss in our study was found to depend on both necrotic damage of islets and a high rate of apoptosis at 1 day, which remained at 30 days posttransplantation (21 % and 34 % respectively). Necrotic areas were common at 1 day posttransplantation and were also found to affect the surrounding liver parenchyma. The high rate of caspase-3, an apoptosis marker, at 30 days correlated with the time the islets had spent in culture, in line with what has previously been reported[39]. Consequently, a negative correlation was observed between a high rate of apoptosis at 30 days post-transplantation and a low retained islet mass. The rate of apoptosis was even higher at 30 days than 1 day posttransplantation, which indicates that more regulated cell death persists in intraportally transplanted human islets than what was observed in murine islets, using the same apoptosis marker[60].

The total graft area was reduced by 52 % after 30 days compared to that found at 1 day posttransplantation.

The vascular density doubled within the first month, but still remained only one third of that in native human islets, again demonstrating a hypoxic propensity in the liver[135]. In line with a low rate of revascularization, 44 % of islets were found positive for the hypoxia marker pimonidazole at 1 day posttransplantation, which indicates a tissue oxygen tension < 10 mmHg[136,137]. Since necrotic and apoptotic cells do not accumulate pimonidazole[138], the degree of hypoxic cells at 1 day may even have been underestimated.

Amyloid Formation in Transplanted Islets

IAPP, predominantly produced in beta-cells, can form amyloid fibrils, known to be toxic for beta-cells[139]. To evaluate amyloid content, consecutive slides of islet-bearing sections were stained with Cong Red. No amyloid or very low amounts were found in islets at 1 day posttransplantation, similar to levels observed in control islets, whereas 27 % of islets contained amyloid deposits 1 month posttransplantation. Islets that already did contain amyloid at 1 day might reflect a known tendency for amyloid formation during culture[52] or even preexistent amyloid in the islet donor. The amyloid observed at 30 days supports previous findings of a predisposition for progressive amyloid formation when human islets are transplanted to renal capsule as well as to the liver[140]. A case report with autopsy material from a patient that received allotransplanted islets showed that 43 % of the islets contained amyloid[141]. One possible reason for this may be inadequate drainage, since substantial formation of amyloid has previously been reported in avascular setting studies of both microencapsulated human islets and recombinant human IAPP murine islets[142,143]. Although mostly associated with type 2 diabetes, extensive amyloid formation in transplanted islets are likely to contribute to graft failure[144].

Beneficial Effect of Repeated Islet Transplantation

The need of repeated islet transplantation in the clinic is common when islet graft function starts to decline. To evaluate the differences in engraftment between a first and second transplant, we performed intraportal islet transplantation to the same animal twice. We found a consistently better-preserved islet mass (in terms of both remaining number of individual islets and total islet area) from the second islet transplant. A control group, which was injected with a saline infusion instead of a first transplantation, showed an islet mass on par with the first islet injection in the double transplanted group. A tentative explanation that we investigated was whether the first transplantation induced an angiogenic niche through tissue expression of hypoxia, inflammatory

processes and liver microinfarctions. The latter was actually observed in paper I. However, there was no increase in angiogenic factors in serum immediately preceding the second transplantation and also no increase in islet vascular density between the control group and double transplanted group at 30 days post-transplantation.

The reason for the obtained findings is therefore obscure, but it is likely that the lodging of the second transplant in the liver differs from the first transplant due to some of the portal vein tributaries already being obstructed. Moreover, it is possible that gene expression in the liver parenchyma is changed by a first transplantation, inducing hypoxia and survival genes not necessarily reflected in circulating concentrations of angiogenic factors.

It is interesting to note that disruption of islets and compensatory growth (after unilateral nephrectomy or partial hepatectomy) in recipient tissue have previously been reported to have a positive impact on islet implantation[61,145]. Autologous IT following pancreatectomy has demonstrated increased vascular density[146].

Islet Transplantation to Muscle or Greater Omentum

Numerous sites have been tested in attempts to improve islet transplantation. Along with new cell therapies and beta-cell replacement by stem cells, the requirements for a site with regards to monitoring and retrieval are even greater.

Islets implanted into muscle and into omentum are known to rapidly revascularize with a restoration of vascular network superior to the liver[78,79]. Syngeneic transplantation of 200 islets to striated muscle or omentum was sufficient to cure the majority of diabetic animals in paper III.

Of the two investigated sites, islets transplanted to omentum had a slightly more favorable response to both intravenously administered glucose and insulin. This dysfunction in the muscular site might be due to exposure to prevailing hypoxia in the first week after transplantation, as previously reported by our group[129]. Another explanation may be a marginal surviving islet mass in the intramuscular site, resulting in graft exhaustion. Substantial fibrosis formation has previously been reported for IT to striated muscle[147,148]. In a study comparing different sites in the same mouse model, a higher number of islets were required, and it took longer time to reach euglycemia, for the mice receiving an IT to the intramuscular site as compared to the omentum[34]. Of note, the surgical technique used in that study may differ from ours.

Blood glucose levels two hours after insulin injection were increased in animals with intramuscular islet grafts when compared to controls, indicating an exaggerated counter-regulatory response to hypoglycemia. Previous studies indicate that the glucagon response to hypoglycemia may be site-dependent, and a dysregulation of alpha-cells has been reported for the intraportal

site[149,150]. A more recent study reported that glucagon secretion does increase in response to insulin-induced hypoglycemia, although not to the same extent as in healthy controls[151].

A difference between the sites investigated in this study is their venous drainage. Since the hepatic cells are a major target for insulin, the importance of portal drainage (as in omentum) has been one of the main arguments for using the liver as an islet implantation site. It has also been shown that a systemic venous drainage of islet grafts induces hyperinsulinemia through a decreased insulin clearance rate, causing insulin resistance[75]. Since neither insulin nor C-peptide levels were measured in the current study, it cannot be excluded that the reduced clearance of glucose during IVGTT in animals with intramuscular islet grafts in part reflects an insulin resistant state. The initial glucose lowering response during ITT was identical to controls in both groups of transplanted animals, which at least is indicative of a normal response to insulin in peripheral tissue. Glycogen content in liver and muscle did not differ, indicating a functional glucose uptake regardless of transplantation site.

In contrast to our previous observations of a decreased percentage of glucagon-positive cells in islets experimentally transplanted to the liver[59], in this study we found that islet composition was unaltered in both islets transplanted to muscle and to omentum.

Gene Expression of Islet Transplants

The studied genes are involved in glucose metabolism within the beta-cells, which in turn secrete insulin, a prerequisite for the glucose uptake of almost every other cell type. Glucose is the major catabolic and anabolic substrate for our cells, and in itself, glucose regulates gene transcription, enzyme activity, and hormone secretion[152]. This explains the huge range of consequences and complications, short- and long-term, that occur when normal glucose control is lost.

Gene analysis demonstrated an upregulation of GLUT2 (glucose transporter), PCX (metabolic enzyme), PDX1 (beta-cell differentiation marker), and INS1 and INS2 in islets transplanted both to omentum and muscle, compared to native islets (as opposed to humans, mice have a two-gene system for insulin transcription[153,154]). GCK (enzyme), regulating the initial step of glucose-induced ATP synthesis, was unaltered. LDHA (enzyme) was downregulated, indicative of a low production of lactate. When LDHA was overexpressed in a beta-cell line, lactate stimulated insulin secretion even in the absence of glucose[155]. Moreover, in contrast to our findings, previous experimental studies have observed altered gene expression in islets transplanted to liver[62,156], renal capsule[157], and pancreas[62].

In this study, as well as in papers I-II, normoglycemic recipients were used to avoid differences in blood glucose levels, which may affect islet grafts. We

have previously not observed any differences in engraftment and function of islet transplants to normoglycemic or successfully cured diabetic mice[156,158,159].

Using Placenta as a Source of MSCs

More than 1,000 studies involving MSCs were registered with ClinicalTrials.gov as of January 20th, 2020. Human MSCs with the desired characteristics (rich secretome, immune-privileged, accessible, demonstrated genomic stability) and that fulfill the ISCT criteria can be obtained from numerous sources[160]. Bone marrow is a commonly used source where MSCs only represent 0.01–0.001 % of the total amount of cells, and the proportion declines with age[86,161]. Several studies demonstrate loss of function when using cells from later passages, e.g. better survival when using passages 1-2 instead of passage 3-4 bmMSCs, as a therapy for GVHD[98]. MSCs from placenta would be a suitable source to avoid unwanted expansion in culture. Prolonged culture is also associated with a risk of malignant transformation, even though this risk appears low in human MSCs[162]. The almost non-existent proportion of cell engraftment *in vivo* may also contribute to the low occurrence of ectopic tissue formation reported[98,99]. The genomic stability of adult stromal cells is another major advantage of MSCs as compared to embryonic stem cells and induced pluripotent stem cells.

Few ethical considerations are connected to harvest of cells when using the placenta, a tissue that would otherwise be discarded. Adequate testing of blood-borne disease prior to isolation can easily be performed during routine visits at the maternity ward for safe handling. Using elective caesarian section, instead of placenta after vaginal delivery, minimizes stress on the cells, resulting in a great number of viable cells without the need of too many passages.

Alterations of DSCs in Culture

Surface marker evaluation of DSCs was undertaken and demonstrated a typical display of MSC markers, with a decreasing donor variability the longer the cells stayed in culture. The uneven proportion of DSCs (PE-negative population) seen in early passages (1-2) likely reflects additional cell types that came with the isolation procedure but regressed later on. From passage 5-10, the cell populations were quite homogeneous from this aspect. The HLA-DR marker was only expressed in the first 1-2 passages. The generally low alloreactivity mobilized by MSCs is believed to be due to the lack of MHC II molecules, which we also confirmed for DSCs. Of note, this expression may however change *in vivo*.

Donor variability has been cited as a contributing reason why clinical trials with MSCs have not delivered the hoped-for results[163,164]. Our characterization of surface marker expression and subsequent gene analysis from different passages is promising for a "steadiness" of DSCs without too large inter-donor variation. The passage number had a greater impact than the donor in regards to the number of differentially expressed genes, and the changes seen were relatively small. The relative limitation of age and gender regarding the donors might also work to our advantage in the quest for an unswerving DSC protocol[163]. Additionally, cells were shown to be of maternal origin and exhibited a limited capacity of multilineage differentiation, in line with what has been previous described[126]. The differentiation capacity and the other ISCT criteria do not necessarily predict biological outcome, which is most likely dependent on the DSC secretome[165].

Impact of DSCs on Human Islets *in Vitro*

Previously, MSCs have been used to increase both rodent and human islet viability and to improve transplantation[115,166]. These effects are mainly mediated by anti-inflammatory effects, secreted angiogenic factors, and induction of tolerance for allogeneic grafts[106,111,167]. The type of co-culture (direct vs indirect cell contact) is of importance, but exact mechanisms remain unclear[119]. To investigate if similar effects are induced by DSCs, a co-culture experiment using both direct and indirect contact culture, the latter with a transwell system, was set up.

Assessment of medium after 48 hours from all co-culture groups (including control DSCs and islets) revealed consistently elevated levels of inflammatory markers in DSC-containing medium. Co-culture of islets and DSCs resulted in altered levels (compared to control DSCs) of several markers, including VEGF-A, CXCL11 and PD-L1, involved in chemotaxis and T-cell activation. The same markers were analyzed in cell lysates where no differences were observed between islets from the co-culture settings. Meanwhile, DSCs from indirect culture displayed altered levels of 31 out of 65 detectable inflammatory biomarkers.

DSCs had no cytoprotective effect on islets during 24 hours of exposure to a cytokine mixture. Yeung et al. demonstrated a protective effect of MSC-islet co-culture; however, co-culture was initiated 24 hours before cytokine exposure[48]. Different culture times, dosage of cells, culture set up, as well as cell and islet source may affect the contrary results obtained in our experiment. The absolute levels of inflammatory markers measured cannot be determined by the arbitrary NPX-unit, used for relative quantification in this study. Nevertheless, incubation in a pro-inflammatory milieu for too long will likely hamper islets, especially if their prior condition adds to a "pro-inflammatory" state of DSCs[88,168,169].

Islet function after co-culture exhibited three different responses to high glucose and forskolin perifusion, likely reflecting the variability of human islet condition and its importance for the interactions that take place between islets and DSCs. However, our results suggest that the choice of co-culture system does not alter the effect of DSCs on islet function. Forward, assessing islet viability beforehand would be useful in order to understand which sets of islets would or would not benefit from DSC treatment.

Gene expression analysis demonstrated no differentially expressed genes in DSCs from co-culture groups, whereas islets from the direct contact culture group demonstrated a greater number of differentially expressed genes compared to control and indirect cultured islets. Further evaluation of these changes is needed to elucidate what categories of genes constitute these changes and how they may alter the islets. Co-culture setup that allows cell-cell contact has demonstrated alterations in MSCs in close connections to the islets, which started expressing insulin and PDX1, a key protein for insulin production[118]. Recent findings demonstrate mitochondrial transfer as a mechanism of MSC effect on beta-cells in co-culture[170]. This exchange is also reported to depend on cellular stressors of human islets, which increased the mitochondrial transfer compared to undamaged murine islets.

Conclusions

Paper I

- Substantial loss of approximately 50 % of human islet tissue occurs between 1 and 30 days after intraportal transplantation
- The vascular density increases after 30 days but is only one third of that in native islets
- One third of human islets contain amyloid after 30 days and those islets express a higher rate of apoptosis

Paper II

- Islets from a second transplantation show better survival if performed 1 week after a primary islet transplantation
- This was not due to improved islet vascular density

Paper III

- The majority of diabetic animals were cured by 200 islets transplanted to omentum or muscle
- The islet grafts maintained or increased the expression of genes important to beta-cell function
- The glycogen levels in muscle and liver were not deranged depending on systemic or portal insulin release

Paper IV

- DSCs can easily be isolated from human placenta
- DSCs display similar characteristics and low inter-donor variability
- Gene expression of DSCs is altered in culture, especially between passage 2 and 5
- Changes in medium content as well as gene expression occur in human islets after co-culture with DSCs, and the functional outcome may be dependent on islet viability

Founding

Financial support for the work presented in this thesis was generously provided from:

- the Swedish Research Council
- the Swedish Diabetes Foundation
- the Swedish Child Diabetes Fund
- the Swedish Juvenile Diabetes Foundation
- Diabetes Wellness Sverige
- the Novo Nordisk Foundation
- EXODIAB
- Familjen Erling-Perssons Stiftelse

Sammanfattning på svenska

Diabetes typ 1 är en sjukdom som beror på brist på insulin, ett hormon som gör att kroppens alla celler kan ta upp socker från blodbanan. När betacellerna som bildar hormonet förstörs måste personer tillföra insulin via dagliga injektioner för att överleva. Forskningen har lett till väldiga framsteg i diabetesbehandling och det går rentav att bota sjukdomen hos de som trots insulininjektioner har svårt att kontrollera sin diabetes. Det kan göras genom transplantation av så kallade Langerhanska öar, cellgrupper innehållande bland annat just betaceller, som finns spridda i bukspottskörteln och kan isoleras från organdonatorer. I de flesta fall brukar man transplantera öar till levern.

Att inte alla kan erbjudas transplantation beror på flera faktorer, dels bristen på donatorer, dels att effekten av en transplantation avtar med tiden och att ingreppet därför ofta behöver upprepas, och dels behovet av immunhämmande behandling för att inte transplantatet ska stötas bort.

Delarbeten

Olika faktorer, både före och efter transplantation, inverkar på hur många av öarna som överlever och därmed avhjälper diabetes. I det första arbetet undersöktes transplanterade öar i mikroskop för att utvärdera deras tillstånd. Öar som isolerats från människa transplanterades till levern på en musstam som tillåter xenogen (mellan arter) transplantation. Mängden betaceller minskade med hälften under första månaden. Kärlförsörjning till öarna återetablerades till viss del men efter en månad var den fortfarande endast en tredjedel av den hos normala öar. I många öar bildades också amyloid, ett plack av det felveckade proteinet IAPP som produceras av ö-cellerna. Detta fenomen ses annars hos personer med diabetes typ 2 där sjukdomsutvecklingen istället för immunangrepp är förknippad med insulinresistens och överproduktion av insulin och IAPP, som på sikt leder till utmattning och celldöd.

I delarbete II fick samma mus två transplantationer för att se om öarnas infästning i levern förändras. För att kunna skilja på första och andra transplantationen var de första öarna infärgade med en grön markör. I denna studie överlevde genomgående fler öar från andra transplantationen. Vi hittade ingen skillnad i t.ex. kärlinväxt så varför det blev så går inte att säga säkert. Möjligtvis påverkar den första transplantationen var nästkommande öar hamnar, alternativt påverkas levern på ett sätt som inverkar positivt på andra omgångens öar.

Många studier på djur har undersökt andra möjliga ställen dit öar kan transplanteras, som på vissa sätt kan innebära bättre förutsättningar för deras överlevnad. I delarbete III undersökte vi möjligheten att bota diabetiska möss med transplantation av öar till bukmuskel eller tarmkäx. Mössen blev botade men efter sockerbelastning hade vissa möss högre blodsockernivåer vilket kan tyda på att funktionen inte är optimal. När öarna studerades med mikroskop och deras genuttryck undersöktes liknade de normala öar. De uppvisade i vissa aspekter bättre resultat än öar transplanterade till lever eller mjälte, undersökta i tidigare studier. En annan fördel med muskel och tarmkäx jämfört med levern är att man lättare kan övervaka (med röntgenteknik) men också ta ut transplantat vilket inte går om öarna är utspridda i levern. Med nya metoder där man använder stamceller istället för öar är dessa förutsättningar mycket viktiga ur ett säkerhetsperspektiv och för att kunna utvärdera resultaten.

I sista arbetet isolerades en typ av stamceller från moderkakor efter kejsarsnitt. Ett stort antal celler kunde då utvinnas utan några risker för donatorn. Dessa celler har liknande egenskaper som andra stamceller från t.ex. benmärg, men för att isolera stamceller från benmärg krävs ett kirurgiskt ingrepp och cellerna behöver växa till i odling. Varianter av stamceller från människa och mus har tidigare visat sig kunna bidra till bättre resultat vid transplantation av öar men mekanismerna för detta är inte klarlagda. För att ta reda på om stamceller från moderkaka kan användas undersöktes celler från flera donatorer för att se hur de skiljer sig och vad som sker under odling. Cellerna har samodlats med öar varefter genuttryck samt faktorer som frisätts från cellerna har analyserats för att bättre förstå deras funktion.

Acknowledgments

Getting to work with pancreatic islets, through isolations, transplantations and by microscopy, you become amazed by their resilience and complexity, and likewise impressed by how laboratory techniques been developed and refined by extensive islet research. Not least by many researchers at *the Department of Medical Cell Biology* where the majority of the work presented in this thesis was carried out. Many people have contributed and supported me during my time as a PhD student and I would like to express my sincere gratitude to:

My supervisor Professor *Per-Ola Carlsson,* for your enthusiasm, brilliance and unmatched effectiveness! Thank you for accepting me as a PhD-student, always answering emails the same day/night and making everything seem possible!

My co-supervisor Professor *Leif Jansson,* for your infinite knowledge of vessels, everything else, and most importantly, birds!

My co-supervisor Associate professor *Joey Lau,* for the laboratory protocols and support in the lab.

Liza Grapensparr and *Sara Ullsten,* former PhD students in the group, for your endless help, support and lasting friendship.

Lisbeth Sagulin, for your vast knowledge of histochemistry, fun chemicals and for being world class in islet picking.

My Quach, for your surgical expertise, great concern for the experimental animals and our nice collaborations!

Monica Sandberg, for all the laboratory skills that I lacked (lack?) and you generously shared.

Zhanchun Li, for all the great advice and help with the antibodies.

Kailash Singh, for the indispensable introduction to FACS (the hardest thing I had to do) and continuous support, even when you were away. You are the master of flow and a wonderful friend.

Eva Törnelius, Johan Olerud and *Anders Ahlander,* for the lovely chats during hours of stainings and encouragement during my first time at the lab. *Eva*, you are the queen of pimonidazole.

Zacharias Iredahl, for awesome cell culture and multi-staining capacity!
Daniel Norman, for expert advice on ELISA.

All members of the POC (extended) lab: *Andris Elksnis (for reading this), Anja Brboric*, *Carl Johan Drott, Daisy Hjelmqvist, Daniel Espes, Julia Torn, Louise Magnusson, Svitlana Vasylovska, Victoria Strömberg, Karin Kjellström, Rebecka Hilmius, and Maria Krüger.* Thank you!

Petra Franzén, for assisting me with RNA isolation and for kind pep talks.

Professor *Gunilla Westermark*, for always taking time to answer all possible questions and for sharing your knowledge of islet amyloid.

Present and former Heads of the Department, Professor *Nils Welsh* and Professor *Erik Gylfe.*

Björn Åkerblom, for the endless times you listened to my problems and for coming up with neat solutions along this entire way, thank you!

Marianne Ljungkvist, Rikke Wulff K. and *Sara Bohman,* for your kindness and good times in the lunch room.

Martin Blixt, for your enthusiasm for teaching and extraordinary philosophical reasoning.

Göran Ståhl, for fixing everything, and I am sorry for all things I destroyed.

Anders Tengholm, for being the one telling me about Sofosko during a seminar about calcium in the spring of 2011. Everything is your fault.

And to all present and former PhD students, professors, scientists, colleagues and friends at this wonderful department where I truly felt at home – thank you!

The BioVis staff, *Jeremy Adler, Matyias Molnar* and *Dirk Pacholsky*, I wouldn't have survived without you and the imaging courses.

SYLF Uppsala, for giving me a purpose.

Campus 1477, for keeping me moving.

Och sist men inte minst,

Mina fantastiska vänner, framför allt *Tina, Emma* och *Sara*. Jag är så tacksam för att jag har er!

Min bonusfamilj, *Eva, Per* och *Lotta Hessman, Emilia* och *Fredrik Poulsen.* För all stöttning, uppmuntran och för telefonsupport under svåra Endnotekriser.

Min älskade familj, mina föräldrar *Roland Larsson* och *Gunnel Liljebäck*, min syster *Ebba* och *Dan,* för att ni alltid finns där och stöttar mig i allt jag tar mig för.

Min älskade *Jon,* för att du givit mig mer kärlek, glädje och vänskap än jag någonsin trodde var möjligt. Och för alla fåglar vi ännu inte letat rätt på.

References

1. Hellman B. Actual distribution of the number and volume of the islets of Langerhans in different size classes in non-diabetic humans of varying ages. Nature 1959;184(Suppl 19):1498-9.
2. Saito K, Iwama N, Takahashi T. Morphometrical analysis on topographical difference in size distribution, number and volume of islets in the human pancreas. Tohoku J Exp Med 1978;124(2):177-86.
3. Ionescu-Tirgoviste C, Gagniuc PA, Gubceac E, Mardare L, Popescu I, Dima S, Militaru M. A 3D map of the islet routes throughout the healthy human pancreas. Sci Rep 2015;5:14634.
4. Keenan HA, Sun JK, Levine J, Doria A, Aiello LP, Eisenbarth G, Bonner-Weir S, King GL. Residual insulin production and pancreatic ss-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes 2010;59(11):2846-53.
5. Molbak AG, Christau B, Marner B, Borch-Johnsen K, Nerup J. Incidence of insulin-dependent diabetes mellitus in age groups over 30 years in Denmark. Diabet Med 1994;11(7):650-5.
6. National Diabetes Statistics Report. 2017.
7. Patterson C, Guariguata L, Dahlquist G, Soltesz G, Ogle G, Silink M. Diabetes in the young - a global view and worldwide estimates of numbers of children with type 1 diabetes. Diabetes Res Clin Pract 2014;103(2):161-75.
8. International Diabetes Federation, IDF Diabetes Atlas, 9th edn. International Diabetes Federation, 2019. 2019.
9. Bolinder J, Antuna R, Geelhoed-Duijvestijn P, Kroger J, Weitgasser R. Novel glucose-sensing technology and hypoglycaemia in type 1 diabetes: a multicentre, non-masked, randomised controlled trial. Lancet 2016;388(10057):2254-2263.
10. Beck RW, Riddlesworth T, Ruedy K, Ahmann A, Bergenstal R, Haller S, Kollman C, Kruger D, McGill JB, Polonsky W and others. Effect of Continuous Glucose Monitoring on Glycemic Control in Adults With Type 1 Diabetes Using Insulin Injections: The DIAMOND Randomized Clinical Trial. Jama 2017;317(4):371-378.
11. Intensive Diabetes Treatment and Cardiovascular Outcomes in Type 1 Diabetes: The DCCT/EDIC Study 30-Year Follow-up. Diabetes Care 2016;39(5):686-93.
12. Hammes HP, Kerner W, Hofer S, Kordonouri O, Raile K, Holl RW. Diabetic retinopathy in type 1 diabetes-a contemporary analysis of 8,784 patients. Diabetologia 2011;54(8):1977-84.
13. Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, Sosenko JM, Ziegler D. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. Diabetes Care 2017;40(1):136-154.
14. Livingstone SJ, Levin D, Looker HC, Lindsay RS, Wild SH, Joss N, Leese G, Leslie P, McCrimmon RJ, Metcalfe W and others. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008-2010. Jama 2015;313(1):37-44.

15. Matuleviciene-Anangen V, Rosengren A, Svensson AM, Pivodic A, Gudbjornsdottir S, Wedel H, Kosiborod M, Haraldsson B, Lind M. Glycaemic control and excess risk of major coronary events in persons with type 1 diabetes. Heart 2017;103(21):1687-1695.
16. Lind M, Svensson AM, Kosiborod M, Gudbjornsdottir S, Pivodic A, Wedel H, Dahlqvist S, Clements M, Rosengren A. Glycemic control and excess mortality in type 1 diabetes. N Engl J Med 2014;371(21):1972-82.
17. Federlin K, Pozza G. Indications for clinical islet transplantation today and in the forseeable future--the diabetologist's point of view. J Mol Med (Berl) 1999;77(1):148-52.
18. Lechler RI, Sykes M, Thomson AW, Turka LA. Organ transplantation--how much of the promise has been realized? Nat Med 2005;11(6):605-13.
19. Maffi P, Secchi A. Islet Transplantation Alone Versus Solitary Pancreas Transplantation: an Outcome-Driven Choice? Curr Diab Rep 2019;19(5):26.
20. Humar A, Ramcharan T, Kandaswamy R, Gruessner RW, Gruessner AC, Sutherland DE. Technical failures after pancreas transplants: why grafts fail and the risk factors--a multivariate analysis. Transplantation 2004;78(8):1188-92.
21. Gruessner RW, Sutherland DE, Gruessner AC. Mortality assessment for pancreas transplants. Am J Transplant 2004;4(12):2018-26.
22. Lacy PE, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 1967;16(1):35-9.
23. Largiader F, Kolb E, Binswanger U. A long-term functioning human pancreatic islet allotransplant. Transplantation 1980;29(1):76-7.
24. Kawahara T, Kin T, Shapiro AM. A comparison of islet autotransplantation with allotransplantation and factors elevating acute portal pressure in clinical islet transplantation. J Hepatobiliary Pancreat Sci 2012;19(3):281-8.
25. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000;343(4):230-8.
26. Barton FB, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B, Oberholzer J, Odorico JS, Garfinkel MR, Levy M and others. Improvement in outcomes of clinical islet transplantation: 1999-2010. Diabetes Care 2012;35(7):1436-45.
27. Bellin MD, Kandaswamy R, Parkey J, Zhang HJ, Liu B, Ihm SH, Ansite JD, Witson J, Bansal-Pakala P, Balamurugan AN and others. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. Am J Transplant 2008;8(11):2463-70.
28. Jansson L, Hellerstrom C. Stimulation by glucose of the blood flow to the pancreatic islets of the rat. Diabetologia 1983;25(1):45-50.
29. Henderson JR, Moss MC. A morphometric study of the endocrine and exocrine capillaries of the pancreas. Q J Exp Physiol 1985;70(3):347-56.
30. Jansson L, Barbu A, Bodin B, Drott CJ, Espes D, Gao X, Grapensparr L, Kallskog O, Lau J, Liljeback H and others. Pancreatic islet blood flow and its measurement. Ups J Med Sci 2016;121(2):81-95.
31. Narayanan S, Loganathan G, Dhanasekaran M, Tucker W, Patel A, Subhashree V, Mokshagundam S, Hughes MG, Williams SK, Balamurugan AN. Intra-islet endothelial cell and beta-cell crosstalk: Implication for islet cell transplantation. World J Transplant 2017;7(2):117-128.

32. Suszynski TM, Avgoustiniatos ES, Papas KK. Oxygenation of the Intraportally Transplanted Pancreatic Islet. J Diabetes Res 2016;2016:7625947.
33. Giuliani M, Moritz W, Bodmer E, Dindo D, Kugelmeier P, Lehmann R, Gassmann M, Groscurth P, Weber M. Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia. Cell Transplant 2005;14(1):67-76.
34. Kim HI, Yu JE, Park CG, Kim SJ. Comparison of four pancreatic islet implantation sites. J Korean Med Sci 2010;25(2):203-10.
35. Naziruddin B, Iwahashi S, Kanak MA, Takita M, Itoh T, Levy MF. Evidence for instant blood-mediated inflammatory reaction in clinical autologous islet transplantation. Am J Transplant 2014;14(2):428-37.
36. Nijboer WN, Schuurs TA, van der Hoeven JA, Fekken S, Wiersema-Buist J, Leuvenink HG, Hofker S, Homan van der Heide JJ, van Son WJ, Ploeg RJ. Effect of brain death on gene expression and tissue activation in human donor kidneys. Transplantation 2004;78(7):978-86.
37. Kuhtreiber WM, Ho LT, Kamireddy A, Yacoub JA, Scharp DW. Islet isolation from human pancreas with extended cold ischemia time. Transplant Proc 2010;42(6):2027-31.
38. Lakey JR, Helms LM, Kin T, Korbutt GS, Rajotte RV, Shapiro AM, Warnock GL. Serine-protease inhibition during islet isolation increases islet yield from human pancreases with prolonged ischemia. Transplantation 2001;72(4):565-70.
39. Noguchi H, Naziruddin B, Jackson A, Shimoda M, Ikemoto T, Fujita Y, Chujo D, Takita M, Peng H, Sugimoto K and others. Fresh islets are more effective for islet transplantation than cultured islets. Cell Transplant 2012;21(2-3):517-23.
40. Barshes NR, Wyllie S, Goss JA. Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: implications for intrahepatic grafts. J Leukoc Biol 2005;77(5):587-97.
41. Moritz W, Meier F, Stroka DM, Giuliani M, Kugelmeier P, Nett PC, Lehmann R, Candinas D, Gassmann M, Weber M. Apoptosis in hypoxic human pancreatic islets correlates with HIF-1alpha expression. Faseb j 2002;16(7):745-7.
42. Arnush M, Scarim AL, Heitmeier MR, Kelly CB, Corbett JA. Potential role of resident islet macrophage activation in the initiation of autoimmune diabetes. J Immunol 1998;160(6):2684-91.
43. Arnush M, Heitmeier MR, Scarim AL, Marino MH, Manning PT, Corbett JA. IL-1 produced and released endogenously within human islets inhibits beta cell function. J Clin Invest 1998;102(3):516-26.
44. Berney T, Molano RD, Cattan P, Pileggi A, Vizzardelli C, Oliver R, Ricordi C, Inverardi L. Endotoxin-mediated delayed islet graft function is associated with increased intra-islet cytokine production and islet cell apoptosis. Transplantation 2001;71(1):125-32.
45. Kaufman DB, Gores PF, Field MJ, Farney AC, Gruber SA, Stephanian E, Sutherland DE. Effect of 15-deoxyspergualin on immediate function and long-term survival of transplanted islets in murine recipients of a marginal islet mass. Diabetes 1994;43(6):778-83.
46. Matsuda T, Omori K, Vuong T, Pascual M, Valiente L, Ferreri K, Todorov I, Kuroda Y, Smith CV, Kandeel F and others. Inhibition of p38 pathway suppresses human islet production of pro-inflammatory cytokines and improves islet graft function. Am J Transplant 2005;5(3):484-93.

47. Chhabra P, Brayman KL. Current status of immunomodulatory and cellular therapies in preclinical and clinical islet transplantation. J Transplant 2011;2011:637692.
48. Yeung TY, Seeberger KL, Kin T, Adesida A, Jomha N, Shapiro AM, Korbutt GS. Human mesenchymal stem cells protect human islets from pro-inflammatory cytokines. PLoS One 2012;7(5):e38189.
49. Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Kallen R, Ostraat O, Salmela K, Tibell A, Tufveson G and others. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. Lancet 2002;360(9350):2039-45.
50. Eich T, Eriksson O, Lundgren T. Visualization of early engraftment in clinical islet transplantation by positron-emission tomography. N Engl J Med 2007;356(26):2754-5.
51. Lee Y, Ravazzola M, Park BH, Bashmakov YK, Orci L, Unger RH. Metabolic mechanisms of failure of intraportally transplanted pancreatic beta-cells in rats: role of lipotoxicity and prevention by leptin. Diabetes 2007;56(9):2295-301.
52. Westermark P, Eizirik DL, Pipeleers DG, Hellerstrom C, Andersson A. Rapid deposition of amyloid in human islets transplanted into nude mice. Diabetologia 1995;38(5):543-9.
53. Abreu JR, Roep BO. Immune monitoring of islet and pancreas transplant recipients. Curr Diab Rep 2013;13(5):704-12.
54. Biarnes M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E. Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. Diabetes 2002;51(1):66-72.
55. Vasir B, Jonas JC, Steil GM, Hollister-Lock J, Hasenkamp W, Sharma A, Bonner-Weir S, Weir GC. Gene expression of VEGF and its receptors Flk-1/KDR and Flt-1 in cultured and transplanted rat islets. Transplantation 2001;71(7):924-35.
56. Carlsson PO, Liss P, Andersson A, Jansson L. Measurements of oxygen tension in native and transplanted rat pancreatic islets. Diabetes 1998;47(7):1027-32.
57. Carlsson PO, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. Diabetes 2001;50(3):489-95.
58. Andersson A, Korsgren O, Jansson L. Intraportally transplanted pancreatic islets revascularized from hepatic arterial system. Diabetes 1989;38 Suppl 1:192-5.
59. Lau J, Jansson L, Carlsson PO. Islets transplanted intraportally into the liver are stimulated to insulin and glucagon release exclusively through the hepatic artery. Am J Transplant 2006;6(5 Pt 1):967-75.
60. Olsson R, Olerud J, Pettersson U, Carlsson PO. Increased numbers of low-oxygenated pancreatic islets after intraportal islet transplantation. Diabetes 2011;60(9):2350-3.
61. Henriksnas J, Lau J, Zang G, Berggren PO, Kohler M, Carlsson PO. Markedly decreased blood perfusion of pancreatic islets transplanted intraportally into the liver: disruption of islet integrity necessary for islet revascularization. Diabetes 2012;61(3):665-73.
62. Lau J, Mattsson G, Carlsson C, Nyqvist D, Kohler M, Berggren PO, Jansson L, Carlsson PO. Implantation site-dependent dysfunction of transplanted pancreatic islets. Diabetes 2007;56(6):1544-50.

63. Anderson SJ, White MG, Armour SL, Maheshwari R, Tiniakos D, Muller YD, Berishvili E, Berney T, Shaw JAM. Loss of end-differentiated beta-cell phenotype following pancreatic islet transplantation. Am J Transplant 2018;18(3):750-755.
64. Ben-Othman N, Vieira A, Courtney M, Record F, Gjernes E, Avolio F, Hadzic B, Druelle N, Napolitano T, Navarro-Sanz S and others. Long-Term GABA Administration Induces Alpha Cell-Mediated Beta-like Cell Neogenesis. Cell 2017;168(1-2):73-85.e11.
65. Jindal RM, Sidner RA, McDaniel HB, Johnson MS, Fineberg SE. Intraportal vs kidney subcapsular site for human pancreatic islet transplantation. Transplant Proc 1998;30(2):398-9.
66. Bertuzzi F, Colussi G, Lauterio A, De Carlis L. Intramuscular islet allotransplantation in type 1 diabetes mellitus. Eur Rev Med Pharmacol Sci 2018;22(6):1731-1736.
67. Baidal DA, Ricordi C, Berman DM, Alvarez A, Padilla N, Ciancio G, Linetsky E, Pileggi A, Alejandro R. Bioengineering of an Intraabdominal Endocrine Pancreas. N Engl J Med 2017;376(19):1887-1889.
68. Maffi P, Nano R, Monti P, Melzi R, Sordi V, Mercalli A, Pellegrini S, Ponzoni M, Peccatori J, Messina C and others. Islet Allotransplantation in the Bone Marrow of Patients With Type 1 Diabetes: A Pilot Randomized Trial. Transplantation 2018.
69. Maffi P, Balzano G, Ponzoni M, Nano R, Sordi V, Melzi R, Mercalli A, Scavini M, Esposito A, Peccatori J and others. Autologous pancreatic islet transplantation in human bone marrow. Diabetes 2013;62(10):3523-31.
70. Wszola M, Berman A, Ostaszewska A, Gorski L, Serwanska-Swietek M, Gozdowska J, Bednarska K, Krajewska M, Lipinska A, Chmura A and others. Islets Allotransplantation Into Gastric Submucosa in a Patient with Portal Hypertension: 4-year Follow-up. Transplant Proc 2018;50(6):1910-1913.
71. Wszola M, Berman A, Gorski L, Ostaszewska A, Serwanska-Swietek M, Krajewska M, Lipinska A, Chmura A, Kwiatkowski A. Endoscopic Islet Autotransplantation Into Gastric Submucosa-1000-Day Follow-up of Patients. Transplant Proc 2018;50(7):2119-2123.
72. White SA, London NJ, Johnson PR, Davies JE, Pollard C, Contractor HH, Hughes DP, Robertson GS, Musto PP, Dennison AR. The risks of total pancreatectomy and splenic islet autotransplantation. Cell Transplant 2000;9(1):19-24.
73. Cantarelli E, Citro A, Pellegrini S, Mercalli A, Melzi R, Dugnani E, Jofra T, Fousteri G, Mondino A, Piemonti L. Transplant Site Influences the Immune Response After Islet Transplantation: Bone Marrow Versus Liver. Transplantation 2017;101(5):1046-1055.
74. Kamei T, Yasunami Y. Demonstration of donor specific unresponsiveness in rat islet allografts: importance of transplant site for induction by cyclosporin A and maintenance. Diabetologia 1989;32(11):779-85.
75. Guan J, Zucker PF, Behme MT, Zhong R, Atkinson P, Dupre J. Insulin resistance prevented by portal delivery of insulin in rats with renal subcapsular islet grafts. Diabetes 1997;46(3):372-8.
76. Liljebäck H, Espes D, Carlsson P-O. Unsurpassed Intrahepatic Islet Engraftment - the Quest for New Sites for Beta Cell Replacement. Cell Medicine 2019.
77. Korsgren O, Jansson L, Andersson A, Sundler F. Reinnervation of transplanted pancreatic islets. A comparison among islets implanted into the kidney, spleen, and liver. Transplantation 1993;56(1):138-43.

78. Christoffersson G, Henriksnas J, Johansson L, Rolny C, Ahlstrom H, Caballero-Corbalan J, Segersvard R, Permert J, Korsgren O, Carlsson PO and others. Clinical and experimental pancreatic islet transplantation to striated muscle: establishment of a vascular system similar to that in native islets. Diabetes 2010;59(10):2569-78.
79. Espes D, Lau J, Quach M, Ullsten S, Christoffersson G, Carlsson PO. Rapid Restoration of Vascularity and Oxygenation in Mouse and Human Islets Transplanted to Omentum May Contribute to Their Superior Function Compared to Intraportally Transplanted Islets. Am J Transplant 2016;16(11):3246-3254.
80. Stokes RA, Cheng K, Lalwani A, Swarbrick MM, Thomas HE, Loudovaris T, Kay TW, Hawthorne WJ, O'Connell PJ, Gunton JE. Transplantation sites for human and murine islets. Diabetologia 2017;60(10):1961-1971.
81. Jiang W, Shi Y, Zhao D, Chen S, Yong J, Zhang J, Qing T, Sun X, Zhang P, Ding M and others. In vitro derivation of functional insulin-producing cells from human embryonic stem cells. Cell Res 2007;17(4):333-44.
82. Zhang D, Jiang W, Liu M, Sui X, Yin X, Chen S, Shi Y, Deng H. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. Cell Res 2009;19(4):429-38.
83. Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 1976;4(5):267-74.
84. Friedenstein AJ, Piatetzky S, II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol 1966;16(3):381-90.
85. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9(5):641-50.
86. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284(5411):143-7.
87. Cyranoski D. Stem cells boom in vet clinics. Nature. Volume 496. England2013. p 148-9.
88. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell Prolif 2019:e12712.
89. Xu JY, Lee YK, Ran X, Liao SY, Yang J, Au KW, Lai WH, Esteban MA, Tse HF. Generation of Induced Cardiospheres via Reprogramming of Skin Fibroblasts for Myocardial Regeneration. Stem Cells 2016;34(11):2693-2706.
90. Peng Y, Chen X, Liu Q, Zhang X, Huang K, Liu L, Li H, Zhou M, Huang F, Fan Z and others. Mesenchymal stromal cells infusions improve refractory chronic graft versus host disease through an increase of CD5+ regulatory B cells producing interleukin 10. Leukemia 2015;29(3):636-46.
91. Carlsson PO, Schwarcz E, Korsgren O, Le Blanc K. Preserved beta-cell function in type 1 diabetes by mesenchymal stromal cells. Diabetes 2015;64(2):587-92.
92. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(4):315-7.
93. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A 1999;96(19):10711-6.
94. Woodbury D, Reynolds K, Black IB. Adult bone marrow stromal stem cells express germline, ectodermal, endodermal, and mesodermal genes prior to neurogenesis. J Neurosci Res 2002;69(6):908-17.

95. Chen LB, Jiang XB, Yang L. Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells. World J Gastroenterol 2004;10(20):3016-20.
96. Ayala-Cuellar AP, Kang JH, Jeung EB, Choi KC. Roles of Mesenchymal Stem Cells in Tissue Regeneration and Immunomodulation. Biomol Ther (Seoul) 2019;27(1):25-33.
97. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. Blood 2003;101(8):2999-3001.
98. von Bahr L, Sundberg B, Lonnies L, Sander B, Karbach H, Hagglund H, Ljungman P, Gustafsson B, Karlsson H, Le Blanc K and others. Long-term complications, immunologic effects, and role of passage for outcome in mesenchymal stromal cell therapy. Biol Blood Marrow Transplant 2012;18(4):557-64.
99. von Bahr L, Batsis I, Moll G, Hagg M, Szakos A, Sundberg B, Uzunel M, Ringden O, Le Blanc K. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. Stem Cells 2012;30(7):1575-8.
100. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science 1998;282(5391):1145-7.
101. Mitalipova M, Calhoun J, Shin S, Wininger D, Schulz T, Noggle S, Venable A, Lyons I, Robins A, Stice S. Human embryonic stem cell lines derived from discarded embryos. Stem Cells 2003;21(5):521-6.
102. Crisostomo PR, Wang M, Wairiuko GM, Morrell ED, Terrell AM, Seshadri P, Nam UH, Meldrum DR. High passage number of stem cells adversely affects stem cell activation and myocardial protection. Shock 2006;26(6):575-80.
103. Zangi L, Margalit R, Reich-Zeliger S, Bachar-Lustig E, Beilhack A, Negrin R, Reisner Y. Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells. Stem Cells 2009;27(11):2865-74.
104. Kota DJ, Wiggins LL, Yoon N, Lee RH. TSG-6 produced by hMSCs delays the onset of autoimmune diabetes by suppressing Th1 development and enhancing tolerogenicity. Diabetes 2013;62(6):2048-58.
105. Duprez IR, Johansson U, Nilsson B, Korsgren O, Magnusson PU. Preparatory studies of composite mesenchymal stem cell islets for application in intraportal islet transplantation. Ups J Med Sci 2011;116(1):8-17.
106. Ding Y, Xu D, Feng G, Bushell A, Muschel RJ, Wood KJ. Mesenchymal stem cells prevent the rejection of fully allogenic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9. Diabetes 2009;58(8):1797-806.
107. Fransson M, Brannstrom J, Duprez I, Essand M, Le Blanc K, Korsgren O, Magnusson PU. Mesenchymal stromal cells support endothelial cell interactions in an intramuscular islet transplantation model. Regen Med Res 2015;3:1.
108. 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(5):1127-35.
109. Solari MG, Srinivasan S, Boumaza I, Unadkat J, Harb G, Garcia-Ocana A, Feili-Hariri M. Marginal mass islet transplantation with autologous mesenchymal stem cells promotes long-term islet allograft survival and sustained normoglycemia. J Autoimmun 2009;32(2):116-24.

110. Cao XK, Li R, Sun W, Ge Y, Liu BL. Co-combination of islets with bone marrow mesenchymal stem cells promotes angiogenesis. Biomed Pharmacother 2016;78:156-164.
111. Ito T, Itakura S, Todorov I, Rawson J, Asari S, Shintaku J, Nair I, Ferreri K, Kandeel F, Mullen Y. Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function. Transplantation 2010;89(12):1438-45.
112. Kim KS, Lee HJ, An J, Kim YB, Ra JC, Lim I, Kim SU. Transplantation of human adipose tissue-derived stem cells delays clinical onset and prolongs life span in ALS mouse model. Cell Transplant 2014;23(12):1585-97.
113. Figliuzzi M, Cornolti R, Perico N, Rota C, Morigi M, Remuzzi G, Remuzzi A, Benigni A. Bone marrow-derived mesenchymal stem cells improve islet graft function in diabetic rats. Transplant Proc 2009;41(5):1797-800.
114. Son BR, Marquez-Curtis LA, Kucia M, Wysoczynski M, Turner AR, Ratajczak J, Ratajczak MZ, Janowska-Wieczorek A. Migration of bone marrow and cord blood mesenchymal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases. Stem Cells 2006;24(5):1254-64.
115. Rackham CL, Dhadda PK, Le Lay AM, King AJ, Jones PM. Preculturing Islets With Adipose-Derived Mesenchymal Stromal Cells Is an Effective Strategy for Improving Transplantation Efficiency at the Clinically Preferred Intraportal Site. Cell Med 2014;7(1):37-47.
116. Park KS, Kim YS, Kim JH, Choi B, Kim SH, Tan AH, Lee MS, Lee MK, Kwon CH, Joh JW and others. Trophic molecules derived from human mesenchymal stem cells enhance survival, function, and angiogenesis of isolated islets after transplantation. Transplantation 2010;89(5):509-17.
117. Jung EJ, Kim SC, Wee YM, Kim YH, Choi MY, Jeong SH, Lee J, Lim DG, Han DJ. Bone marrow-derived mesenchymal stromal cells support rat pancreatic islet survival and insulin secretory function in vitro. Cytotherapy 2011;13(1):19-29.
118. Scuteri A, Donzelli E, Rodriguez-Menendez V, Ravasi M, Monfrini M, Bonandrini B, Figliuzzi M, Remuzzi A, Tredici G. A double mechanism for the mesenchymal stem cells' positive effect on pancreatic islets. PLoS One 2014;9(1):e84309.
119. de Souza BM, Boucas AP, Oliveira FD, Reis KP, Ziegelmann P, Bauer AC, Crispim D. Effect of co-culture of mesenchymal stem/stromal cells with pancreatic islets on viability and function outcomes: a systematic review and meta-analysis. Islets 2017;9(2):30-42.
120. Reinisch A, Etchart N, Thomas D, Hofmann NA, Fruehwirth M, Sinha S, Chan CK, Senarath-Yapa K, Seo EY, Wearda T and others. Epigenetic and in vivo comparison of diverse MSC sources reveals an endochondral signature for human hematopoietic niche formation. Blood 2015;125(2):249-60.
121. Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W and others. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 2005;33(11):1402-16.
122. Vinketova K, Mourdjeva M, Oreshkova T. Human Decidual Stromal Cells as a Component of the Implantation Niche and a Modulator of Maternal Immunity. J Pregnancy 2016;2016:8689436.
123. Karlsson H, Erkers T, Nava S, Ruhm S, Westgren M, Ringden O. Stromal cells from term fetal membrane are highly suppressive in allogeneic settings in vitro. Clin Exp Immunol 2012;167(3):543-55.

124. Ringden O, Erkers T, Nava S, Uzunel M, Iwarsson E, Conrad R, Westgren M, Mattsson J, Kaipe H. Fetal membrane cells for treatment of steroid-refractory acute graft-versus-host disease. Stem Cells 2013;31(3):592-601.
125. Barlow S, Brooke G, Chatterjee K, Price G, Pelekanos R, Rossetti T, Doody M, Venter D, Pain S, Gilshenan K and others. Comparison of human placenta- and bone marrow-derived multipotent mesenchymal stem cells. Stem Cells Dev 2008;17(6):1095-107.
126. Erkers T, Nava S, Yosef J, Ringden O, Kaipe H. Decidual stromal cells promote regulatory T cells and suppress alloreactivity in a cell contact-dependent manner. Stem Cells Dev 2013;22(19):2596-605.
127. Sadeghi B, Remberger M, Gustafsson B, Winiarski J, Moretti G, Khoein B, Klingspor L, Westgren M, Mattsson J, Ringden O. Long-Term Follow-Up of a Pilot Study Using Placenta-Derived Decidua Stromal Cells for Severe Acute Graft-versus-Host Disease. Biol Blood Marrow Transplant 2019;25(10):1965-1969.
128. Sadeghi B, Ersmark B, Moretti G, Mattsson J, Ringden O. Treatment of radiculomyelopathy in two patients with placenta-derived decidua stromal cells. Int J Hematol 2019.
129. Espes D, Lau J, Quach M, Banerjee U, Palmer AF, Carlsson PO. Cotransplantation of Polymerized Hemoglobin Reduces beta-Cell Hypoxia and Improves beta-Cell Function in Intramuscular Islet Grafts. Transplantation 2015;99(10):2077-82.
130. Carlsson PO, Palm F, Mattsson G. Low revascularization of experimentally transplanted human pancreatic islets. J Clin Endocrinol Metab 2002;87(12):5418-23.
131. Westermark GT, Johnson KH, Westermark P. Staining methods for identification of amyloid in tissue. Methods Enzymol 1999;309:3-25.
132. Pelekanos RA, Sardesai VS, Futrega K, Lott WB, Kuhn M, Doran MR. Isolation and Expansion of Mesenchymal Stem/Stromal Cells Derived from Human Placenta Tissue. J Vis Exp 2016(112).
133. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci U S A 2006;103(7):2334-9.
134. Kim A, Miller K, Jo J, Kilimnik G, Wojcik P, Hara M. Islet architecture: A comparative study. Islets 2009;1(2):129-36.
135. Lau J, Carlsson PO. Low revascularization of human islets when experimentally transplanted into the liver. Transplantation 2009;87(3):322-5.
136. Raleigh JA, Chou SC, Arteel GE, Horsman MR. Comparisons among pimonidazole binding, oxygen electrode measurements, and radiation response in C3H mouse tumors. Radiat Res 1999;151(5):580-9.
137. Arteel GE, Thurman RG, Yates JM, Raleigh JA. Evidence that hypoxia markers detect oxygen gradients in liver: pimonidazole and retrograde perfusion of rat liver. Br J Cancer 1995;72(4):889-95.
138. Olsson R, Carlsson PO. A low-oxygenated subpopulation of pancreatic islets constitutes a functional reserve of endocrine cells. Diabetes 2011;60(8):2068-75.
139. Gurlo T, Ryazantsev S, Huang CJ, Yeh MW, Reber HA, Hines OJ, O'Brien TD, Glabe CG, Butler PC. Evidence for proteotoxicity in beta cells in type 2 diabetes: toxic islet amyloid polypeptide oligomers form intracellularly in the secretory pathway. Am J Pathol 2010;176(2):861-9.

140. Westermark GT, Westermark P, Nordin A, Tornelius E, Andersson A. Formation of amyloid in human pancreatic islets transplanted to the liver and spleen of nude mice. Ups J Med Sci 2003;108(3):193-203.
141. Westermark GT, Westermark P, Berne C, Korsgren O. Widespread amyloid deposition in transplanted human pancreatic islets. N Engl J Med 2008;359(9):977-9.
142. Bohman S, Westermark GT. Extensive amyloid formation in transplanted microencapsulated mouse and human islets. Amyloid 2012;19(2):87-93.
143. Carlsson PO, Espes D, Sedigh A, Rotem A, Zimerman B, Grinberg H, Goldman T, Barkai U, Avni Y, Westermark GT and others. Transplantation of macroencapsulated human islets within the bioartificial pancreas betaAir to patients with type 1 diabetes mellitus. Am J Transplant 2018;18(7):1735-1744.
144. Udayasankar J, Kodama K, Hull RL, Zraika S, Aston-Mourney K, Subramanian SL, Tong J, Faulenbach MV, Vidal J, Kahn SE. Amyloid formation results in recurrence of hyperglycaemia following transplantation of human IAPP transgenic mouse islets. Diabetologia 2009;52(1):145-53.
145. Dunger A, Korsgren O, Andersson A. DNA replication in mouse pancreatic islets transplanted subcapsularly into the kidney or intraportally into the liver. Influence of unilateral nephrectomy or partial hepatectomy. Transplantation 1990;49(4):686-9.
146. Johansson M, Jansson L, Carlsson PO. Improved vascular engraftment and function of autotransplanted pancreatic islets as a result of partial pancreatectomy in the mouse and rat. Diabetologia 2007;50(6):1257-66.
147. Svensson J, Lau J, Sandberg M, Carlsson PO. High vascular density and oxygenation of pancreatic islets transplanted in clusters into striated muscle. Cell Transplant 2011;20(5):783-8.
148. Lund T, Korsgren O, Aursnes IA, Scholz H, Foss A. Sustained reversal of diabetes following islet transplantation to striated musculature in the rat. J Surg Res 2010;160(1):145-54.
149. Gupta V, Wahoff DC, Rooney DP, Poitout V, Sutherland DE, Kendall DM, Robertson RP. The defective glucagon response from transplanted intrahepatic pancreatic islets during hypoglycemia is transplantation site-determined. Diabetes 1997;46(1):28-33.
150. Kendall DM, Teuscher AU, Robertson RP. Defective glucagon secretion during sustained hypoglycemia following successful islet allo- and autotransplantation in humans. Diabetes 1997;46(1):23-7.
151. Rickels MR, Fuller C, Dalton-Bakes C, Markmann E, Palanjian M, Cullison K, Tiao J, Kapoor S, Liu C, Naji A and others. Restoration of Glucose Counterregulation by Islet Transplantation in Long-standing Type 1 Diabetes. Diabetes 2015;64(5):1713-8.
152. Thorens B, Mueckler M. Glucose transporters in the 21st Century. Am J Physiol Endocrinol Metab 2010;298(2):E141-5.
153. Weiss MA. Proinsulin and the genetics of diabetes mellitus. J Biol Chem 2009;284(29):19159-63.
154. Shiao MS, Liao BY, Long M, Yu HT. Adaptive evolution of the insulin two-gene system in mouse. Genetics 2008;178(3):1683-91.
155. Ishihara H, Wang H, Drewes LR, Wollheim CB. Overexpression of monocarboxylate transporter and lactate dehydrogenase alters insulin secretory responses to pyruvate and lactate in beta cells. J Clin Invest 1999;104(11):1621-9.

156. Mattsson G, Jansson L, Nordin A, Andersson A, Carlsson PO. Evidence of functional impairment of syngeneically transplanted mouse pancreatic islets retrieved from the liver. Diabetes 2004;53(4):948-54.
157. Ahn YB, Xu G, Marselli L, Toschi E, Sharma A, Bonner-Weir S, Sgroi DC, Weir GC. Changes in gene expression in beta cells after islet isolation and transplantation using laser-capture microdissection. Diabetologia 2007;50(2):334-42.
158. Korsgren O, Jansson L, Andersson A. Effects of hyperglycemia on function of isolated mouse pancreatic islets transplanted under kidney capsule. Diabetes 1989;38(4):510-5.
159. Mattsson G, Jansson L, Nordin A, Carlsson PO. Impaired revascularization of transplanted mouse pancreatic islets is chronic and glucose-independent. Transplantation 2003;75(5):736-9.
160. Hass R, Kasper C, Bohm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal 2011;9:12.
161. Caplan AI. The mesengenic process. Clin Plast Surg 1994;21(3):429-35.
162. Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG and others. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. Cancer Res 2007;67(19):9142-9.
163. Siegel G, Kluba T, Hermanutz-Klein U, Bieback K, Northoff H, Schafer R. Phenotype, donor age and gender affect function of human bone marrow-derived mesenchymal stromal cells. BMC Med 2013;11:146.
164. Murgia A, Veronesi E, Candini O, Caselli A, D'Souza N, Rasini V, Giorgini A, Catani F, Iughetti L, Dominici M and others. Potency Biomarker Signature Genes from Multiparametric Osteogenesis Assays: Will cGMP Human Bone Marrow Mesenchymal Stromal Cells Make Bone? PLoS One 2016;11(10):e0163629.
165. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008;2(2):141-50.
166. Gamble A, Pawlick R, Pepper AR, Bruni A, Adesida A, Senior PA, Korbutt GS, Shapiro AMJ. Improved islet recovery and efficacy through co-culture and co-transplantation of islets with human adipose-derived mesenchymal stem cells. PLoS One 2018;13(11):e0206449.
167. Xu DM, Yu XF, Zhang D, Zhang MX, Zhou JF, Tan PH, Ding YC. Mesenchymal stem cells differentially mediate regulatory T cells and conventional effector T cells to protect fully allogeneic islet grafts in mice. Diabetologia 2012;55(4):1091-102.
168. Romieu-Mourez R, Francois M, Boivin MN, Bouchentouf M, Spaner DE, Galipeau J. Cytokine modulation of TLR expression and activation in mesenchymal stromal cells leads to a proinflammatory phenotype. J Immunol 2009;182(12):7963-73.
169. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM. A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. PLoS One 2010;5(4):e10088.
170. Rackham CL, Hubber EL, Czajka A, Malik AN, King AJF, Jones PM. Optimizing beta cell function through mesenchymal stromal cell mediated mitochondria transfer. Stem Cells 2020.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1633

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-402501

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020