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Transplantation of stem cell-derived islets as a treatment for type 1 diabetes

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Abstract

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Type 1 diabetes (T1D) is an autoimmune disease that leads to an immune attack on insulin-producing beta cells, necessitating lifelong insulin therapy. For individuals with brittle diabetes and poor metabolic control, the option of pancreatic human islet transplantation exists. However, the shortage of organ donors and the need for life-long immune suppressive agents pose significant challenges. Stem cell-derived islets (SC-islets) present a promising alternative for diabetes treatment.

This thesis explores the differentiation and transplantation of SC-islets as a treatment for diabetes. **In paper I**, three months post-transplantation, the ingrowth of recipient blood vessels and the neural density was higher in SC-islet grafts compared to human islet grafts. Furthermore, there was a higher tendency of blood flow, whereas the oxygenation was twice as high in SC-islet grafts. Both transplanted SC-islets and human islets had formation of amyloid depositions, which can affect the long-term survival and function of transplanted cells. **In paper II**, a humanized mouse model transplanted with SC-islets or human islets was validated. Transplanted SC-islets or human islets were not completely rejected 11 days after injection with human peripheral blood mononuclear cells (PBMCs). *In vivo* imaging and flow cytometry confirmed the presence of injected human immune cells, demonstrating an effective model for studying the human immune responses of allogeneically transplanted islets or SC-islets. **In paper III**, positron emission tomography (PET) imaging, using the DGCR2 affibody, for monitoring transplanted beta cells revealed successful binding to SC-islets and human islets *in vitro*. PET imaging *in vivo* demonstrated successful detection of the affibody in transplanted SC-islets. Although, the affibody could be optimized since the signal vanished 30 min after administration. However, DGCR2 remains a promising marker for SC-islet imaging. **In paper IV,** nanofiltration with a virus clearance filter paper during SC-islet differentiation was evaluated. Filter SC-islets expressed essential markers for beta cells during differentiation in comparable amounts as the control. The filtered SC-islets demonstrated physiological insulin-releasing function similar to that of the control. Nanofiltration did not seem to affect the differentiation of SC-islets.

In conclusion, transplantation of SC-islets is a promising future treatment for diabetes, however, long-term effects need to be evaluated.

Keywords: Type 1 diabetes, Islet of Langerhans, Stem cell-derived islets, Islet transplantation, Humanized mouse model, Positron emission tomography, Nanofiltration

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”Ju mer man tänker, ju mer inser man att det inte finns något enkelt svar”
– Nalle Puh

Till min familj och mina vänner,
som stöttat mig längs vägen

List of Papers

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

I. **Thorngren, J**., Brboric, A., Vasylovska S., Hjelmqvist D., Westermark, G.T., Saarimäki-Vire, J., Kvist, J., Balboa, D., Otonkoski, T., Carlsson, P-O., Lau, J. (2024) Efficient vascular and neural engraftment of stem cell-derived islets. *Submitted*

II. **Thorngren J**., Luo Z., Brboric A., Vasylovska, S., Singh, K., Lindsay, R., Christoffersson, G., Lau, J. (2024) An *in vivo* experimental model to study human immune responses towards transplanted human pluripotent stem cell-derived islets or primary human islets. *Manuscript*

III. Cheung P.*, **Thorngren, J**.*, Zhang, B., Vasylovska, S., Lechi, F., Persson, J., Ståhl, S., Löfblom, J., Korsgren, O., Eriksson, J., Lau, J.*, Eriksson, O.* (2023) Preclinical evaluation of Affibody molecule for PET imaging of human pancreatic islets derived from stem cells. *EJNMMI Research*, 13(1):107

IV. **Thorngren, J**., Vasylovska, S., Blanc, J., Wu, L., Manukyan, L., Mihranyan, A., Lau, J. (2024) Differentiation of human pluripotent stem cells into insulin-producing islet-like clusters using nanofiltered cell culture medium. *Front. Membr. Sci. Technol,* 3:1338366

***** Equal contribution

Contents

Abbreviations

5-HTP	5-Hydroxytryptophan
APC	Antigen-presenting cells
BCM	Beta cell mass
BLT	Bone marrow/liver/thymus model
BSA	Bovine serum albumin
CTLA-4	Cytotoxic T lymphocyte Associated Protein 4
CXCR4	The C-X-C chemokine receptor type 4
DAB	3.3'-Diaminobenzidine
DE	Definitive endoderm
DGCR2	Di-George syndrome critical region gene 2
DTBZ	Dihydrotetrabenazine
FSC	Forward Scatter
GAD	Glutamic acid decarboxylase
GLP-1	Glucagon-Like Peptide 1
GRP44	G-protein-coupled receptor 44
GSIS	Glucose stimulated insulin secretion
HBSS	Hanks's Balanced Salt Solution
hESCs	Human embryonic stem cells
HILOs	Human islet-like organoids
HIP	Hypoimmune pluripotent
HLA	Human Leukocyte antigen
HSC	Human hematopoietic stem cells
IA-2	Islet antigen 2
IBMIR	Instant blood-mediated inflammatory reaction
IDF	International Diabetes Federation
IFN	Interferon
$IL2rg^{null}$	Mutation in the IL2-receptor gamma chain
iPSCs	Induced pluripotent stem cells
IVGTT	Intravenous glucose tolerance test
KCl	Potassium chloride
KRBH	Krebs Ringer-Bicarbonate HEPES Buffer
LEA	*Lycopersicon esculentum lectin*
NF-L	Neurofilament light chain
NKX6.1	NK6 homeobox 1
NOG	$NOD.Cg\text{-}Prkdc^{scid}I12rg^{tm1Sug}$

NSG	NOD.Cg-Prkdc^{scid}I12rg^{tm1Wjl}
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PD-L1	Programmed Death Ligand 1
PDX1	Pancreatic and duodenum home box 1
PET	Positron emission tomography
pFTAA	Pentameric formyl thiophene acetic acid
PP cells	Pancreatic polypeptide cells
PSCs	Pluripotent stem cells
SC-islets	Stem cell-derived islets
SSC	Side scatter
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TGF-beta	Transforming growth factor beta
TH	Tyrosine hydroxylase
VAChT	Vesicular acetylcholine transporter
WHO	World Health Organisation
ZnT8	Zinc transporter 8

Introduction

Diabetes mellitus and global prevalence

According to the World Health Organization (WHO), 422 million people are living with Diabetes Mellitus disease. Diabetes is a metabolic disease where the blood glucose levels increase due to impaired insulin release from the pancreatic insulin-producing beta cells. Chronic hyperglycemia is associated with long-term damage and failure of organs such as kidneys, heart, and eyes (1). There are different classifications of diabetes, and the generally dominating forms are type 1 diabetes (T1D) and type 2 diabetes (T2D). According to the International Diabetes Federation (IDF) diabetes atlas 2021, the estimation is that by the year 2045, the number of people diagnosed with diabetes will rise to 783 million patients (2). T2D has the highest prevalence globally. However, T1D is extensively increasing around the world. The Scandinavian countries, specifically Finland and Sweden, are two of the top countries with the highest prevalence of T1D (3, 4).

T1D is a complicated autoimmune disease in which the body's immune system attacks the endogenous pancreatic beta cells that produce insulin. It is a multifactorial disease, and the exact mechanism of immune cell activation is not yet fully understood. The immune system's destruction of the beta cells leads to a deficiency in insulin secretion. T1D often manifests in childhood or early adulthood (1, 5). It is a complex condition requiring regular self-monitoring of the blood glucose and repeated insulin injections several times daily. Inadequate metabolic control increases the risk for complications and shortens life due to events of hyperglycemia. Furthermore, the risk for hypoglycemic events can induce diabetic coma which can lead to death (1). The onset of diabetes is typically acute, nevertheless, the symptoms persist as a chronic condition during the lifetime. However, in the pre-diabetes phase before the onset of the disease, circulating autoantibodies such as glutamic acid decarboxylase (GAD), islet antigen 2 (IA-2), and zinc transporter 8 (ZnT8) can be detected (6-11). Due to the destruction of beta cells, the beta cell mass (BCM) decreases during the disease progression and with time (12).

T2D is characterized by deficient compensatory insulin secretory response in combination with insulin resistance (1). T2D is often related to obesity and commonly occurs in adults, but the prevalence in children is increasing (13).

Usually, in the early onset of T2D and the first years of living with the disease, insulin is not crucial for survival, as in T1D. However, hyperglycemia develops gradually, resulting in late notice of symptoms, and many patients go undiagnosed for longer. While achieving efficient metabolic control is possible through of proper nutrition and physical activity, the insulin levels will still be abnormal, and there is an increased risk, as in T1D, for chronic complications in the heart, eyes, and kidneys with the progression of the disease.

The pancreas and islets of Langerhans

The pancreas is located in the abdomen, behind the stomach. It is an organ consisting of two functional compartments: one exocrine compartment consisting of acinar and ductal cells participating in digestion, and one endocrine compartment secreting hormones (14). The endocrine pancreas constitutes of a cluster of endocrine cells called islets of Langerhans, that compose of five different cell types each releasing a specific hormone: beta cells (insulin), alpha cells (glucagon), delta cells (somatostatin), pancreatic polypeptide cells (PP cells) (pancreatic polypeptide), and epsilon cells (ghrelin). Endothelial cells are integrated into the islets to facilitate proper blood supply, providing oxygen and nutrition to cells. The composition of these cells varies between species, where rodents have a core center of beta cells and alpha cells distributed in the periphery. In humans, the composition of cells is irregular throughout the islet. The number of endocrine cells also varies between species. Human pancreatic islets comprise ~ 60 % of beta cells, ~ 30 % of alpha cells, and the remaining 10 % constitute delta cells, PP cells, and epsilon cells (15-17). These species variations are important to consider when drawing conclusions from animal studies to humans. Understanding the complexities and physiological function of the islets of Langerhans is paramount, especially in the context of diabetes. Insights into the physiological function are important for improving new diagnostics and discovering new medical and pharmaceutical possibilities.

Type 1 diabetes and immune infiltration

Under normal physiological conditions in healthy individuals, there are circulating immune cells in the islets of Langerhans removing dead or nonfunctional cells or potentially harmful pathogens. However, at T1D, the host immune cells are activated or triggered and the endogenous beta cells are destroyed by the leukocyte infiltration of the islets (insulitis) (18). The mechanism and cause behind T1D and beta cell destruction are complex and not fully understood. Multifactorial causes such as genetic, environmental, and

immunological are suggested to be related to increased risk for the development of T1D. Environmental causes in the form of viral infections have been related to autoimmunity and the progression of T1D; Coxsackie enterovirus is one example of such a virus related to T1D (19-21). Other environmental factors, such as nutrition and special bacteria in the microbiota, may influence the risk of developing T1D (20).

Genetic factors play a significant role in the risk for diabetes, with over 50 genetic loci associated with the predisposition of T1D (22, 23). This underscores the importance of genetic research in understanding and potentially preventing T1D. The human leukocyte antigen (HLA) is related to 30-40 % of familial disposition of T1D, where HLA DQ and DR are associated with increased risk for T1D (24). However, in some individuals there is no manifestation of the condition even though they have expressions of high-risk genes or relatives with T1D. The complex nature of T1D and its varied etiology pose challenges in fully comprehending why some individuals develop the disease while others remain unaffected.

The stress or death of beta cells triggers the release of autoantigens and upregulation of HLA. Additionally, the release of pro-inflammatory cytokines like interferon (IFN) gamma and tumour necrosis factor (TNF) can induce overexpression of HLA class I and II (25). This cascade of events ultimately leads to antigen-presenting cells (APC) presenting antigen to T cells. Activated CD4 T cells can then activate CD8 T cells, B cells, and macrophages (18, 26). The expansion of autoreactive CD4 and CD8 T cells is presumed to have a central role in beta cell destruction and autoimmunity within T1D. The autoimmunity of T1D is a complex interplay of various immune components. Despite contemporary advancements in understanding autoimmunity and insulitis, numerous unresolved aspects persist, necessitating further investigation.

Islet transplantation as treatment for T1D

Currently, only a subgroup of patients with brittle diabetes undergo human pancreatic islet transplantation. The introduction of the Edmonton protocol in 2000, with a modified immunosuppressive treatment, marked a significant improvement in the efficacy of clinical islet transplantations for T1D patients (27). Islet transplantation is performed by infusion through the portal vein into the liver. While it is less invasive than whole pancreas transplantation, the need for lifelong immunosuppressive treatment poses significant challenges. These regimens are causing adverse side effects such as infections and an increased risk of malignancies (28). Further limitations with islet transplantation include the loss of islet grafts due to instant blood–mediated inflammatory reaction (IBMIR) (29, 30). The risk of beta cell death is high due to acute

hypoxia because of inferior oxygen delivery of the grafts even after revascularization compared to native islets in the pancreas (31-33). Moreover, the high demand for isolated human islet sources and the shortage of islet donors further complicate the situation. To address these issues, new strategies for beta cell replacements are emerging, and insulin-producing cells or islets derived from stem cells could serve as an alternative. Improved beta cell replacement therapy strategies have been made over the past decades, particularly since the generation of stem cell-derived islets (SC-islets) started emerging as a promising treatment for T1D. Today, it is possible to produce SC-islets from human pluripotent stem cells (34, 35).

Differentiation of pluripotent stem cells towards islets

Differentiating human pluripotent stem cells (hPSCs) into islets is a significant scientific endeavor. The multistep protocol is designed to mimic human embryogenesis. Stem cells have the unique ability to differentiate into any cell type in the body (i.e., totipotent or pluripotent cells). Human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) have the potential to be directed toward islets, resembling native human pancreatic islets. The isolation of hESCs cell lines from human blastocyst in 1998 by Thomson and colleagues and Reubinoff and colleagues in 2000 marked a significant milestone in the field (36, 37). Despite the ethical and political controversies, surrounding hESCs their indefinite self-renewal potential has made them the golden standard for cell differentiation. Furthermore, the generation of iPSCs, which have fewer ethical dilemmas, is also a promising alternative. The differentiation of hESCs or iPSCs towards islets could potentially revolutionise the field of stem cell research, opening up new avenues for treatment and understanding of various diseases.

Differentiation of hPSCs towards islets *in vitro*, is a process involving sequential stages, each finely regulated by specific molecular signals, growth factors, and transcriptional regulators to mimic the development *in vivo*. Several protocols have been developed over the past years (35, 38-41), but the general approach is similar. First, hPSCs are induced toward definitive endoderm (DE). The signal molecule Activin A is important for mimicking the *in vivo* Nodal signalling to drive the stem cells towards DE (42, 43). The C-X-C chemokine receptor type 4 (CXCR4), an endocrine marker, or the transcript factor SOX17, is used to measure the efficiency of the endoderm induction (43, 44). Cells are then guided towards the primitive gut tube and posterior foregut. To further induce pancreatic progenitors, addition of retinoic acid, epidermal growth factor, and nicotinamide, combined with inhibition of Sonic hedgehog and bone morphogenic protein signalling, are added to direct cells towards pancreatic progenitors (35, 40, 45-49). To confirm the efficiency of

cells forming pancreatic progenitor cells, expression of pancreas and duodenum homeobox 1 (PDX1) and the transcription factor NK6 homeobox 1 (NKX6.1), which are required to establish beta cells, are evaluated (46, 50, 51). All endocrine hormone-producing cells can be derived from pancreatic progenitor cells. During the last stages, to guide progenitor cells towards immature and mature SC-islets, there is a transient expression of the transcription factor neurogenin 3, and thereafter, the notch signalling is inhibited by gamma-secretase inhibitors and transforming growth factor beta (TGF-beta) inhibitors. Inhibition of TGF-beta signalling is enabling beta cell maturation and insulin expression (52). Betacellulin is involved in differentiation protocols in the final steps to increase the expression of PDX1 and insulin. Fully differentiated SC-islets should express insulin and C-peptide in combination with transcription factors NKX6.1 and PDX1, then the differentiation of the SC-islets are considered successful (46, 48, 53, 54). The generation of SC-islets is a highly regulated process. While there is potential for further optimization of protocols for enhanced functionality, the generation of SC-islets represents a noteworthy achievement in advancing stem cell research.

Stem cell-derived islets as treatment for diabetes

Over the past decade, in response to the high demand for islet transplantations and the scarcity of donated human islets, extensive research has been conducted on alternative sources of beta cells. The research has yielded promising results, with several researchers successfully generating insulin-producing cells or SC-islets derived from hESCs (38) or iPSCs (35, 41). These cells have demonstrated the ability to release insulin when stimulated by glucose *in vitro*. Animal experiments further validated their potential, showing that transplanted human SC-islets can normalize insulin levels *in vivo* in diabetic mice (45). While these SC-islets are not fully mature after *in vitro* differentiation, they continue to mature after transplantation *in vivo* (38, 45, 55). Although they are not yet equivalent to adult human pancreatic beta cells, they exhibit a similar magnitude of insulin secretion response (35, 40), offering a potential future treatment for T1D.

Furthermore, a phase I/II clinical trial is currently evaluating the efficacy, tolerability, and safety of VX-880, a stem cell-derived therapy that holds great promise as a treatment for T1D (56). The patients underwent allogeneic transplantation of fully differentiated SC-islets via a hepatic portal vein infusion, in conjunction with a standard regimen of immunosuppressive agents. The initial evaluation of the first transplanted patient, conducted 90 days post-transplantation, revealed restoration of basal insulin production, leading to a decrease in HbA1c levels. Importantly, the daily insulin dose has been gradually reducing over time, with no significant severe adverse events related to VX-

880 reported (56-58). Additional patients have been transplanted since then, and the long-term data from the first-year follow-up of the two first transplanted patients had become insulin independent, without severe adverse effects related to the VX-880 (59). In total, 14 patients have been transplanted and the clinical trial is currently paused for review of collected data, due to two patient's death, that are not related to the treatment with VX-880 (60). Despite this, these progresses instil hope for the future of T1D treatment. One concern regarding the transplantation of SC-islets is the risk of tumour or teratoma formation in grafts, which needs to be evaluated in long-term perspective in a clinical approach. Previously, it has been shown that grafts of pancreatic endoderm cells developed teratoma when transplanted to mice (61). Transplantation of more mature cells is hopefully less prone to develop into teratomas or tumours (35, 40). Furthermore, if transplantation occurs at a site that enables graft retrieval, there is an opportunity to halt the developmental progression upon the initial detection of teratoma signs. The generation of SC-islets is a significant breakthrough and a step closer to a therapy strategy for T1D. However, there is still a need for treatment without the need for lifelong treatment of immunosuppressive drugs, increasing the possibility that a larger population of patients (i.e., also including children and patients with T2D), not only patients with brittle T1D, could be treated.

Humanized mouse model

A humanized mouse model enables the study of different autoimmune diseases, where human tissue, cells, or immune cells can be studied *in vivo* in the mouse engrafted with human cells or tissues (62). There are different humanized mouse models, depending on the disease of interest to study. The development of the immunodeficient mice with a mutation in the IL2-receptor gamma chain ($IL2rg^{null}$) was a significant development in humanized mouse models. The gamma receptor domain is essential for the high affinity and binding of several cytokines, such as IL-2, IL-4, IL-7, IL-9, IL-15, and IL-12 (63). The $NOD.Cg\text{-}Prkdc^{scid}I12rg^{tm1Sug}$ (abbreviated to NOG) mouse has a mutation in the interleukin-2 (IL-2) receptor γ locus (I12rg, also known as γ_c). This mutation results in the NOG mouse lacking T- and B cells as well as natural killer (NK) cells because the mutation greatly impacts the development and function of these cell types (64). Other strains of the $IL2rg^{null}$ mutation, such as $NOD.Cg\text{-}Prkdsc^{scid}I12rg^{tm1Wjl}$ (NSG) and $C;129S4\text{-}Rag2^{tm1Flv}I12rg^{tm1Flv}$ (referred to as $BALB/c\text{-}Rag2^{null}$ $IL2rg^{null}$ mice or BRG), entirely lack the gamma chain.

There are different strategies to establish a humanized mouse model with a human immune system, and several types of humanized mouse models exist.

One model is the Hu-PBL-SCID, where peripheral blood leukocytes are injected into the mouse. This model offers rapid engraftment of human immune cells, making it useful for studying immune responses, particularly T cell function. However, it has limitations, such as the lack of development of a complete immune system and the potential for graft versus host rejection, which restricts the experimental window (65). Another model, the Hu-SRC-SCID, is engrafted with $CD34^+$ human hematopoietic stem cells (HSC) by intravenous or interfemoral injection to reconstruct a human immune system (66, 67). HSC can be isolated from bone marrow, umbilical cord blood, or fetal liver. This model has a more complete immune system compared to the previous model. However, granulocytes, red blood cells, and platelets are observed at deficient levels and the model lacks some specific factors to fully mimic human T cell development. The so-called BLT model (bone marrow/liver/thymus) is a third method for establishing a human immune system *in vivo.* In this model, the human fetal liver and thymus are transplanted under the kidney capsule in the mouse. HSCs are isolated from the same human fetal liver and injected into the transplanted mouse to develop a human immune system (63, 68, 69). Hence, the BLT model has the capacity to develop a complete human immune system. However, like the Hu-PBL-SCID model, the BLT model is prone to develop graft versus host rejection, limiting the experimental window (63). Regardless of which model is used, humanized mouse models are effective for studying human immune responses *in vivo*.

Monitoring beta cells *in vivo* using PET imaging

Monitoring beta cells and BCM could facilitate the diagnostic of diabetes and track the disease progression. A significant reduction in the BCM often occurs before the onset of clinical symptoms. Currently, the focus is on techniques that assess the beta cell function, such as measuring the insulin levels, C-peptide or HbA1c. These markers effectively evaluate endogenous beta cells function in patients or monitor the function of transplanted islets or SC-islets. However, it does not provide insights into BCM or cell localization.

Positron Emission Tomography (PET) imaging is suggested as a noninvasive imaging technique to image and monitor beta cells and BCM. It utilizes the detection of two photons of opposite directions and the annihilation event of an electron with a positron emitted from a radioactive probe. PET has a high resolution and high sensitivity, enabling detection of lower signals and potentially reducing pharmacological side effects from the tracer. However, there are challenges using PET for beta cell visualization, such as the small islet size and the non-uniform microarchitecture. To optimize beta cell imaging, the radioactive marker probe and the imaging target should be specific and highly sensitive. The signal should ideally be significant higher in endocrine tissue

to create a distinct signal well separated from exocrine tissue and surrounding background tissue.

Dihydrotetrabenazine (DTBZ) is an analog approved by the FDA. The radioligand ^{18}F-DTBZ has been used for the characterization of BCM and *in vivo* imaging of insulin in clinical studies in patients with T1D (70). The G-protein-coupled receptor GPR44 is a potential candidate and a radiolabelled antagonist coupled to carbon-11 is a promising candidate for the visualization and quantification of BCM (71, 72). Another marker that has been tested in T1D patients is the ^{11}C-5-HTP (serotonin's direct precursor 5-Hydroxytryptophan), which was used to measure pancreas volume variations during the progression of T1D. However, the observation of pancreas volume and beta cell function was not optimal (73). The Di-George syndrome critical region gene 2 (DGCR2) protein is a promising protein for the visualization of human islets and SC-islets. It has been identified in human pancreatic beta cells and in pancreatic progenitor cells derived from hESCs (74, 75). This protein could potentially serve as a marker for BCM in the future.

Challenges to image BCM and localization of transplanted cells persist. However, PET holds potential for diabetes management and disease progression tracking.

The future of stem cell-derived islets

Transplantation of SC-islets is a potential future treatment for diabetes mellitus. Nevertheless, SC-islet transplantation carries a potential risk of immune-mediated rejection, primarily through antibody-mediated killing of transplanted allogeneic cells and graft rejection (76). This necessitates the use of immunosuppressive drugs. Transplantation of pancreatic human islets or SC-islets without immune suppression would enable treating a larger group of patients, including children.

To overcome this, different encapsulation methods and gene editing of cells are suggested to protect transplanted cells from immune attack. Encapsulation of islets or SC-islets emerges as a viable approach to offer a barrier to protect islets from the immune system while allowing nutrient and oxygen exchange. However, such studies showed poor efficacy and survival of cells due to hypoxia and poor vascularisation (77). A capsule engineered to facilitate vascularization could enhance cell survival, but immune suppressive treatment is needed (78). Human islets encapsulated in an oxygen device and transplanted to patients with T1D showed survival and protection of islets several months after transplantation. However, the function was limited, and C-peptide levels decreased with time (79). A new phase I/II will study the survival and function

of encapsulated SC-islets for transplantation in T1D patients without immune suppressive treatment, that might be promising (60).

To further overcome the need for immune suppressive treatment, gene editing of islets and SC-islets is emerging as a new approach to evade the immune system. The primary purpose is to edit specific cell genes or molecules related to immune attacks or protective molecules. Programmed Death-Ligand 1 (PD-L1) is a protein that has a crucial role in regulating and suppressing the activation of the T cells. Human islet-like organoids (HILOs) with expression of PD-L1 were able to maintain glucose homeostasis over 50 days when transplanted to diabetic C57BL/6J mice compared to control (80). Suppression or reduced expression of HLA class I and II is another approach where reduction in these has effectively inhibited activation of NK cells and protection from T cell-mediated attack *in vivo* (81).

Currently, hypo-immune pluripotent (HIP) stem cells are evaluated in nonhuman primates *in vivo*. Generating HIP, with a combination of inactivating major histocompatibility complex (MHC) class I and II and overexpression of CD47, can evade the immune system and lose its immunogenicity (82). Human HIP cells differentiated to pancreatic islets from iPSCs, improved diabetes during four weeks when transplanted to immunocompetent diabetic mice. HIP cells generated from rhesus macaque were transplanted to allogenic rhesus macaque and survived for 16 weeks in immunocompetent allogeneic recipients (83). Recently published data from the research group of Schrepfer demonstrate that allogeneically gene-edited hypo-immune primary pancreatic islets, isolated from rhesus macaque, evaded the immune system when transplanted to diabetic cynomolgus monkey (84). Six months post-transplantation, the transplanted diabetic cynomolgus monkeys were insulin independent and the gene-edited islets evaded the immune system despite no immunosuppressive treatment.

Transplantation of SC-islets is a promising future treatment for diabetes. SC-islets have reached clinical studies in humans and are showing promising results. There is research in immune protective strategies ongoing and has a potential future for the clinic. However, questions regarding the safety and long-term effects need to be evaluated before it can be used as a standard treatment for T1D.

Aims

The overall aim of the thesis was to elucidate the potential of stem cell-derived islets as a future treatment for transplantation purposes for patients with diabetes. The aims for each specific paper are:

I. To study the engraftment of transplanted human stem cell-derived islets compared to transplanted human islets.
II. To validate a humanized mouse model to study human immune responses towards transplanted stem cell-derived islets or human islets.
III. To evaluate the potential of DGCR2-affibody as a PET marker for monitoring transplanted stem cell-derived islets *in vivo*.
IV. To investigate the impact of filtration of cell culture media with a nanocellulose-based virus clearance filter on the differentiation and functionality of stem cell-derived islets.

Materials and methods

Generation of stem cell-derived islets

Paper I-IV.

Human embryonic stem cells (H1 cell line, Wicell®) were differentiated into SC-islets, through a seven-stage protocol, previously published (38, 85). Details about the differentiations are found in the method sections of papers II, III, and IV. Details of specific molecular components added at each stage are found in paper IV, Table 1. In short, human recombinant Laminin 521 coated plates were used as a matrix during the propagation and differentiation of cells. Cells were propagated in mTeSR-Plus medium (STEMCELL Technologies, Vancouver, Canada) and seeded in a density of two million cells /3.5cm dish or 16 million cells /10cm dish and differentiation started 24 hours later. During stages 1 to 6, MCDB131 Medium (Gibco, Life Technologies Limited, Paisley, UK) was used and during the final stage 7 the medium was changed to CMRL 1066 medium (Corning, Mediatech Inc. Manassas, VA, United States). Media was changed every day during stages 1-5 and during stages 6-7 media was changed every second day. Cells were differentiated on monolayer until stage 4 day 3, where cells were dissociated to single cell suspension using TrypLE (Gibco, Life Technologies Corp., Grand Island, NY United States), and seeded in AggreWell^{TM}400 (STEMCELL Technologies) for uniform cluster formation. From stage 6 until the end of stage 7, cells were cultured in suspension on rotation in ultra-low attachment 6-well plates (Corning).

Functional evaluation of stem cell-derived islets

Paper I-IV

During the stages of differentiation the cells were monitored with flow cytometry of important pancreatic markers, CXCR4 (endoderm marker), PDX1 and NKX6.1 (pancreatic definitive marker), and C-peptide.

At the end of stage 7, between day 25 to 42, the functionality of cells was evaluated with a static glucose-stimulated insulin secretion test (GSIS) and dynamic perifusion test. GSIS was performed on 10-15 SC-islets in 200 µl

Krebs Ringer-Bicarbonate HEPES Buffer (KRBH) containing 2 mg/ml bovine serum albumin (BSA) with different glucose concentrations. SC-islets were pre-incubated in 3.3 mM glucose solution for 90 min followed by incubation in 3.3 mM glucose for 30 min, 16.8 mM for 30 min, and lastly 3.3 mM with the addition of 30 mM potassium chloride (KCl) for 30 min. SC-islets were incubated in physiological conditions during GSIS and supernatant was saved after each incubation.

Dynamic perifusion was performed, where 50 human islets or SC-islets were loaded into filtered-covered perifusion chambers (Suprafusion 1000, 6 channel system, Brandel, Gaithersburg, MD, United States). Cells were perifused with a flow of 200 µl/min in KRBH, containing 2 mg/ml BSA and stepwise change of glucose solutions. Samples were collected every fourth minute during the perifusion. Human islets or SC-islets were perifused in 2.8 mM glucose for 30 minutes, followed by 16.8 mM glucose for 32 min. Thereafter, cells were perifused in 16.8 mM glucose with the addition of 10 nM exendin-4 for 20 min, followed by perfusion with 2.8 mM glucose for 16 min. Lastly, the cells were perifused in 2.8 mM glucose with the addition of 30 mM KCl for 24 min.

The concentration of insulin was measured using a human Insulin ELISA kit (Mercodia, Uppsala, Sweden), according to manufacturer's instructions.

Transplantation of cells

Paper I-III.

NOD.Cg-Prkdc^{scid}I12rg^{tm1Sug} (NOG) (papers I and II) or NMRI nu/nu (paper III) mice were transplanted with either donated human islets from the Nordic Network of Islet Transplantation (Rudbeck Laboratory, Uppsala, Sweden) or SC-islets differentiated from human pluripotent stem cells. In paper I, 700-800 IEQ (islet equivalent) human islets or SC-islets were transplanted beneath the kidney capsule in NOG mice. The kidney was exposed and a small opening to the kidney capsule was made. Human islets or SC-islets were packed in a PE-50 tubing, and injected with a Hamilton syringe. In paper II, 300 human islets or SC-islets were transplanted into the abdominal muscle of NOG mice. In paper III, 200 or 800 SC-islets were transplanted to the muscle in the neck or abdominal muscle of NMRI nu/nu mice. Briefly, mice were sedated with a constant flow of isoflurane throughout the transplantation. For transplantation to the muscle (abdominal and neck, papers II and III) the muscle was exposed with a small cut in the skin in the abdomen/neck. Human islets or SC-islets were packed in a butterfly needle with Hank's Balanced Salt Solution (HBSS; Statens Veterinärmedicinska anstalt, Uppsala, Sweden) and injected into the

intramuscular layer. Mice were anesthetized with isoflurane during all transplantations, and subcutaneous injection of analgesia carprofen 5 mg/kg (Norocarp or Rimadyl Bovis vet) was used as analgesia. An additional dose of analgesia was given the day after transplantation.

Blood flow and oxygen tension measurements

Paper I

Blood flow and oxygen tension measurements were performed as described in (86, 87) and are described in detail in paper I. Briefly, animals were anesthetized with 0.02 mL/g body weight Avertin (2.5 % v/v solution of 10 g 97 % v/v 2,2,2-tribromo-ethanol (Sigma-Aldrich) in 10 mL 2-methyl-2-butanol (BDH Merck Ltd., Poole, U.K.) by intraperitoneal administration. Blood flow in the graft and kidney was measured using laser Doppler flowmetry (Transonic BLF21 series, probe diameter 1.2 mm; Transonic, Ithaca, NY). Blood flow values were recorded in arbitrary tissue perfusion units (TPUs). Oxygen tension measurements in graft and kidney were performed using custom-made Clark microelectrodes (tip diameter 2-5 µm, Unisense, Aarhus, Denmark). All measurements (≥ 5 per location) were considered as one experiment and a mean was calculated from all measurements for each mouse.

PBMC isolation and engraftment of a humanized mouse model

Paper II

To establish a humanized mouse, peripheral blood mononuclear cells (PBMC) were isolated from collected intravenous blood of healthy donors. Isolation was done by gradient isolation (88) with the use of SepMate tubes (STEMCELL Technologies, Vancouver, Canada) according to the manufacturer´s instructions. Two weeks after transplantation 20 x10^6 PBMCs/200 µl saline were injected into each mouse. To control mice (transplanted with either SC-islets or human islets), 200 µl saline was injected. Four or 11-12 days after PBMC injection, *in vivo* imaging recording was performed followed by harvesting of grafts.

Live *in vivo* imaging

Paper II

Graft infiltration and movement of immune cells was analyzed by *in vivo* imaging. Five minutes prior to imaging, a cocktail of antibodies targeting $CD4^+$ T cells (anti-human CD4 directly conjugated with FITC, clone RPA-T4, eBioscience, Carlsbad, CA, USA), $CD8^+$ T cells (anti-human CD8 directly conjugated with APC, clone RPA-T8, eBioscience) and blood vessels (anti-mouse CD31 directly conjugated with Alexa Fluor 594, clone 390, BioLegend, San Diego, CA, USA) was injected intravenously into the mice. The skin was opened, and muscle containing transplanted cells was exposed and held in place during microscopy with the help of a custom designed vacuum device (89). Z-stacks and time-lapse movies were obtained using a Leica SP8 scanning confocal microscope.

Functional evaluation *in vivo*

Paper I

The functional ability of insulin release of transplanted SC-islets and human islets was evaluated with an intravenous glucose tolerance test (IVGTT). 2 g/kg glucose was injected through the tail vein. Blood glucose measurements were done at the following time points: 0 (before injection of glucose), 5, 10, 20, 30, 60, 90 and 120 minutes after injection. At 10 minutes post glucose injection, blood was collected from the saphenous vein for human C-peptide measurements.

Human C-peptide measurements

Paper I-III

In paper I, one and three months after transplantation, 10 min post-injection of intravenous glucose 2 g/kg, stimulated human C-peptide was measured from saphenous vein blood. Before sacrifice, mice were sedated and blood was collected from the heart by puncture with a needle and syringe. Blood was centrifuged at 2000 x g, at 4 °C, for 5 min. Plasma was saved, and human C-peptide was measured using an ultra-sensitive C-peptide kit (Mercodia, Uppsala, Sweden).

PET-imaging

Paper III

For evaluation of the DGCR2 protein, PET imaging was performed on transplanted NMRI nu/nu mice. Injection of 400 kBq/g of the radioactive tracer [^{18}F]Z_{DGCR2} was injected into the lateral tail vein. A dynamic PET scan was recorded for 60 min followed by an additional MRI or CT scan for acquisition and alignment of PET scan. After the PET scan, mice were euthanized and the graft was removed for measurement of ionized radiation in a NaI counter and thereafter saved for immunohistochemistry.

Frozen section of autoradiography

Paper III

Frozen sections of HEK293 cells, SC-islets or human islets were incubated with Phosphate-buffered saline solution (PBS) and the DGCR2 affibody [^{18}F]$Z_{DGCR2:AM106}$ at room temperature for 60 min. Sections were washed in PBS twice followed by one wash with deionized water. Samples were then air-dried and left for exposure for 120 min. Thereafter, the resulting digital image readout was obtained using an Amersham Typhon storage phosphor imager (GE healthcare).

Nanofiltration of cell culture media

Paper IV

Cell culture media were filtered with a 22 nm cut-off virus clearance cellulose-based filter during differentiation of H1 cells for biosafety purposes. A schematic illustration of the filtration of media is found in paper IV, Figure 1. Basal cell culture medium was prepared prior to each stage of the differentiation and filtered with a 0.22 µm PEP-vacuum filtration system (Corning or WWR). Before each media change during the differentiation, the addition of molecular components important for each respective stage was added. After the addition of molecular components, the cell culture medium was filtered additionally with a 22 nm cut-off cellulose-based filter before medium change in the experimental group. In the control group, the bulk media were only filtered without additional filtration with a virus clearance filter.

Processing of tissues for cell suspension

Paper II

Tissues related to immune cell responses, such as bone marrows and spleens were harvested from mice after live *in vivo* imaging. Tissues were processed to single cell suspensions, previously described in (90-92). Briefly, bone marrows were flushed from the femur with PBS and spleens were squeezed in HBSS. Thereafter, red blood cells were lysed from spleen- and bone marrow cells in 0.2 M ammonium chloride buffer (NH_4Cl) washed and resuspended in HBSS.

Panel design and flow cytometry staining

Paper II

To determine the presence of different immune cells participating in the immune response towards transplanted grafts, flow cytometry staining was performed. In paper II, a three-multicolour panel was used to target human immune cells and mouse immune cells, as presented in Table 1 in paper II. The method of flow cytometry staining is described in detail in paper II. In short, cell suspensions were stained for fluorescent surface markers, including live/dead Fixable Dye Viability e780 (eBioscience) marker for 40 min on ice, followed by fixation and permeabilization of cells overnight at 4˚C, using a Foxp3 staining and permeabilization kit, according to manufacturer's instructions (eBioscience). Subsequently, cells were stained for intracellular fluorescent antigens for 40 min at 4˚C. Samples were run on a FACS Aria II (B.D. Bioscience, San Jose, CA, USA) or Cytek Northern Lights (Cytek). Recording limits were set to either one million recorded cells, or a maximum count recorded within 3 minutes.

Gating of flow cytometry data

Paper II

Flow cytometry results were analyzed and gated with Flow Logic (Invai Technologies, Victoria, Australia). The population of leukocytes were gated from total cells counted based on forward scatter (FSC) and side scatter (SSC). From the leukocyte population, single cells were gated first by height and width of FSC followed by height and width of SSC. Subsequently, live cells were gated based on the intensity of the live marker Fixable Dye Viability e780. Since this live/dead marker bind strongly to dead cells, the top population with highest intensity was considered as dead cells, and the population

below with lower intensity was gated as live cells. Then, each immune cell type of interest and subpopulations within that cell type was gated. In the human panel, cells with strong intensity for human CD45 was gated, and within this population, immune cells such as T cells, B cells and APC were gated (details of panels are found in paper II, Table 1). The T cell panel was gated in a similar way as the human panel, with the exception that T cells were not gated within the human CD45 population. This was due to the limitation of the number of markers that could be used in the same samples on the flow cytometer. Data were exported as a percentage of live cells as well as a percentage among parents (i.e., gated cells within another cell population).

Immunohistochemistry staining

Paper I-III

To preserve the integrity of the graft as well as blood vessels and nerves, the retrieved muscle grafts or kidney grafts were fixed in 4 % paraformaldehyde (PFA) diluted in PBS overnight at 4˚C followed by incubation in 15 % sucrose-PBS for 2-3 hours and then 30 % sucrose-PBS overnight at 4˚C. Subsequently, the grafts were embedded in cryopreserve liquid (HistoLab Products AB, Gothenburg, Sweden) frozen and stored at -80˚C. Human islets and SC-islets were fixed in 4 % PFA in PBS for 20 minutes at room temperature, washed in PBS and then frozen. Cryosections were prepared for muscle grafts with a thickness of 10 µm and for human islets, SC-islets and kidney grafts of 8 µm.

The immunostainings of the grafts are presented in detail in each paper. In short, in paper I, tissue sections were incubated with primary antibodies overnight, followed by secondary antibodies for 1 hour at room temperature. Nuclei were stained with Hoechst (1:10,000). Detailed information on antibodies used in paper I, is found in supplementary Table 4 in paper I.

In paper II, muscle grafts were incubated with a primary antibody targeting insulin (polyclonal, guinea pig, dilution 1:4000, Fitzgerald, Acton, MA, USA) overnight at 4˚C. Secondary peroxidase-conjugated antibody (1:1000, donkey anti-guinea pig, Jackson ImmunoResearch, PA, United States) was incubated for 30 minutes at room temperature, followed by development with the substrate 3.3’-Diaminobenzidine (DAB, Dako, Glostrup, Denmark) and counterstained with Mayer's Hematoxylin (HistoLab Products AB).

In paper III, SC-islets and muscle grafts were stained with primary antibodies targeting human insulin (polyclonal, guinea pig, dilution 1:400, Fitzgerald)

and DGCR2 (polyclonal, rabbit, dilution 1:400, Thermo Fisher Scientific, Regensburg, Germany), incubated in 4˚C overnight. The grafts were thereafter incubated with secondary antibodies Alexa Fluor 488-conjugated donkey anti-rabbit and Alexa Fluor 594-conjugated donkey anti-guinea pig (dilution 1:300, Jackson Immunoresearch Laboratories, West Grove, PA, United States), for 1 hour in room temperature. Nuclei were stained with DAPI.

In paper IV, cells or SC-islets were stained with primary markers expressed during differentiation. Primary and secondary antibodies used are found in Table 2 in paper IV. In short, at stage 2, day 1, antibodies targeting endoderm marker SOX17 were used. Pancreatic progenitor markers PDX1 and NKX6.1 were stained at stage 4 day 3. At late stage 7, SC-islets were stained with primary antibodies targeting human insulin, somatostatin, and glucagon. Primary antibodies were incubated at 4˚C overnight, and secondary antibodies were incubated the day after, at room temperature for 1 hour. Nuclei were stained with DAPI or Hoechst.

Image analysis

Paper I-II

In paper I, composition analyses were done in the ZEN program. The number of cells positive for each marker was divided by the total amount of cells stained for DAPI (nuclei).

Vascular density, neuron density and the amount of amyloid formation in grafts post transplantation were determined using the software Imaris (Bitplane). Chromogranin A was used to delineate the endocrine cells. Immunolabeling of mouse CD31 or human CD31 was used to determine blood vessels and the marker Neurofilament light chain (NF-L) was used to determine nerves. For amyloid quantification, the oligotiophene probe pentameric formylthiophene acetic acid with cyano (pFTAA-CN) was used. To calculate the percentage of each marker, the surfaces positive for each marker (mCD31, hCD31, NF-L or pFTAA) were transformed into volume surfaces area and then calculated to percentage out of total volume surface area positive for Chromogranin A. Sections were double stained with pFTAA and mouse CD31 to elucidate the distance between the nearest blood vessel and amyloid.

Beta cell apoptosis (stained for cleaved caspase-3 and ingrowth of sympathetic (stained for tyrosine hydroxylase; TH) and parasympathetic nerves (stained for vesicular acetylcholine transporter; VAChT) was calculated using the software Fiji (Image J 1.53t, National Institute of Health) (93). Using a macro, a region of interest was created of the area positive for insulin. Surfaces outside

insulin were cleared, and the surfaces positive for caspase-3, TH or VAChT inside of the insulin-positive area were analyzed, creating a surface of region of interest. The area of caspase-3, TH or VAChT was divided by the area of insulin to calculate the percentage of sympathetic TH or parasympathetic VAChT within the insulin area.

To confirm the analysis of CD31 blood vessels, additional staining was done with *Lycopersicon esculentum lectin* (LEA) together with mouse CD31 and Chromogranin A. Blood vessels was analyzed with Fiji (Image J 1.53t) software using a macro. Surface positive for Chromogranin A was analyzed by creating an area of region of interest. Surfaces outside the ones positive for Chromogranin A were cleared and thereafter an area was created of surfaces positive for mouse CD31. Surfaces outside of mouse CD31 positive surfaces were cleared and surfaces positive for LEA inside of mouse CD31 positive areas were analyzed, creating an area within the region of interest.

In paper II, the blood vessel area and the number of immune cells were estimated from recordings of live *in vivo* imaging using Imaris software (Bitplane). The area of the CD31 positive blood vessel was transformed into a volume surface area. The graft area was estimated of the total volume of the image presented as μm^3. The results were presented as a ratio of blood vessel volume and the total volume of the image (i.e., area of graft site). The number of immune cells was calculated using the spot function for CD8 positive and CD4 positive cells in the graft site. The amount of cells was estimated from the total volume of the image presented as the number of cells/10^6 μm^3.

Statistical analysis

In paper I, one biological replicate corresponded to one mouse transplanted with human islets from one human donor or SC-islets from one differentiation. Exceptions were made for blood flow and oxygen tension measurements where two mice were transplanted with human islets from the same human donor and four mice transplanted with SC-islets from two different differentiations. For analyses of cell composition and vascular density, one-way ANOVA and Šídák's multiple comparisons test were performed. The Mann-Whitney test was used for the analysis of beta cell apoptosis. Student's unpaired two-tailed t-test was used for analysis of amyloid, neural density, blood flow and oxygen tension measurements. In paper II, immune cell response in human islets and SC-islets, day 4 and day 11 after PBMC transfer was analyzed with one-way ANOVA with Holm-Šídák's test for multiple comparison. In paper III, the analysis of the binding of human recombinant DGCR2 protein was compared with control using an unpaired t-test, whereas when comparing binding of the [^{18}F] DGCR2 affibody to SC-islets, human islets and HEK293

cells was analyzed with one-way ANOVA with Tukey's multiple comparison test. In paper IV, the functional evaluation of filter and control SC-islets were compared using an unpaired two-tailed student´s t-test.

In all papers, $P<0.05$ was considered statistically significant. All values are presented as mean ± SEM. The statistical analyses were performed using GraphPad Prism 9.0 or 8.4.3 (GraphPad Software, San Diego, CA).

Results and discussion

Paper I

Transplantation of SC-islets holds the potential of reversing diabetes as a future treatment. However, hypoxia and cell death occur quickly after transplantation due to IBMIR and insufficient engraftment of blood vessels. Ingrowth of blood vessels, as well as nerves, into the graft, is crucial for the engraftment of cells, proper insulin-releasing function, and the survival of the cells. Therefore, in paper I, we evaluated the engraftment of human islets and SC-islets when transplanted to immune deficient NOG mice. Three months after transplantation, there was a change in the composition of cells in SC-islet grafts. Post transplantation, there was a decrease in insulin-producing cells together with an increase of glucagon-positive cells (40 % respectively 55 % cells), in comparison to before transplantation (50 % of insulin-producing cells and 40 % of glucagon-producing cells) (Figure 1, paper I). Human islet grafts remained similar in composition of cells three months after transplantation compared to before transplantation. After the seven stages of differentiation, the cells are considered fully differentiated and similar to human islets. However, when transplanted *in vivo,* the SC-islets continue to mature, and the insulin-releasing capacity can improve further (38, 39). However, changes in the composition may occur due to cell maturation. On the other hand, the change in cell composition might be due to transdifferentiation of cells (i.e., beta cells differentiation towards alpha cells). However, it needs to be further investigated if this transdifferentiation happened after transplantation of the SC-islets.

Immunostainings for maturity markers such as MAFA, NeuroD1, PDX1, NKX6.1, NKX2.2, and PCNA showed similar expression levels in human islet grafts and SC-islet grafts before and after transplantation (Figure 2). The immunostainings indicated that transplanted SC-islets were fully differentiated and did not continue to proliferate after transplantation. The insulin-releasing function remained when transplanted to mice. The stimulated human C-peptide values 10 min post-injection were 440.6 ± 53.22 pmol/L (n=6) for human islet grafts and 592.7 ± 157.6 pmol/L (n=6) for SC-islet grafts one month after transplantation. Three months after transplantation, the stimulated human C-peptide levels were 564.9 ± 35.8 pmol/L (n=6) for human islet grafts and 1054.0 ± 402.0 pmol/L (n=5) for SC-islets (Figure

S1). The human C-peptide levels demonstrated that SC-islets could release insulin and that the insulin-releasing function improved during the three months transplantation *in vivo*.

The total vascular density was similar in human islet grafts, and SC-islet grafts three months post-transplantation (Figure 4A). However, a mixture of ingrowing recipient mouse vessels and remnant human endothelial cells in human islet grafts contributed to vascular density. In contrast, predominantly ingrowth of remnant mouse blood vessels in SC-islets contributed to the vascular supply. The vascular density was confirmed with additional staining for the vascular marker *Lycopersicon esculentum agglutinin* (LEA). Similar to mCD31, the LEA-staining showed that the ingrowth of blood vessels was two to three times higher in transplanted SC-islets compared to human islet grafts. The functionality of the blood vessels was investigated, especially since some of the vascular structures of human origin may be remnant and not perfused. The blood perfusion was slightly higher in transplanted SC-islet grafts ($p=0.067$), while the oxygen tension was doubled in SC-islet grafts compared with human islet grafts three months after transplantation. This indicates that SC-islets had higher vascularization than transplanted human islets. This observation may be because human islets have unperfused vascular structures from donor endothelial structures remnant, resulting in lower perfusion and less functional blood vessels in human islet transplants.

The ingrowth of blood vessels and neurons is essential for the survival and function of transplanted cells. Double staining of the neural marker NF-L and mouse CD31 showed that nerves were growing in close vicinity of the blood vessels in both human islets and SC-islets. The neural density was 4-5 times higher in SC-islet grafts than in human islet grafts (Figure 5). Additional staining was done for sympathetic nerves (TH) and parasympathetic nerves (VAChT) to elucidate the origin of ingrowing nerves further. The parasympathetic nerves were similar in SC-islet grafts and human islet grafts. Interestingly, SC-islet grafts had more ingrowth of sympathetic nerves compared to human islet grafts (Figure S5). Previously, it has been reported that in human islets the vascular signals are essential for the innervation of both sympathetic and parasympathetic nerves (94).

The apoptotic rate (cells positively stained for caspase-3) in cells was 4-5 times higher in human islet transplants compared to SC-islet transplants (Figure 6A-E). Therefore, SC-islets potentially have a higher chance of survival from a long-term perspective at transplantation. However, amyloid depositions (stained positively for pFTAA) were formed in human islet grafts and SC-islet grafts three months post-transplantation (Figure 3). Amyloid depositions were detected in pancreatic biopsies sampled from patients with recent

onset of T1D (95). The formation of amyloid deposition in transplanted human islets in a patient with T1D was observed several years after transplantation (96). Amyloid depositions have also been observed in human islets transplanted in mice, potentially affecting the function and survival of transplanted cells (97, 98). The formation of amyloid depositions has been associated with hyperglycemia in mice and is related to cell stress. Insufficient vascularisation of transplanted cells may also be related to amyloid deposition due to cell stress. The formation of amyloid may affect cell survival and be related to graft loss in the long term (99).

Paper II

Today, the generation and transplantation of SC-islets is a reality. However, immune processes occurring against transplanted SC-islets are not fully understood. There is a need to study immune processes without risking the patient´s safety. A humanized mouse model, engrafted with human tissue, is an alternative to studying human tissue, cells or immune responses. In paper II, we wanted to validate a humanized mouse model to study immune responses toward allogeneically transplanted SC-islets and human islets. Immune deficient NOG mice were transplanted with human islets or SC-islets into abdominal muscle followed by intravenous injection of human PBMCs. The humanized mouse enabled the study of immune responses of transplanted cells with different techniques such as live *in vivo* imaging, flow cytometry, and immunohistochemistry staining. Immunohistochemistry staining for insulin demonstrated the presence of remaining beta cells in muscle grafts of mice transplanted with human islets or SC-islets in both control mice and mice injected with human PBMCs (Figure 1A-D). Insulin-positive cells were expected to remain in control mice since no human immune cells were injected. However, the findings in PBMC-injected mice indicated that transplanted human islets and SC-islets were not abolished in the humanized mouse during the time frame in our study.

At further evaluation of the function of remaining beta cells, human C-peptide from plasma was still detectable 11 days after PBMC injection in both mice transplanted with human islets (19.16 ± 1.6 pmol/L, n=3) and SC–islets (27.42 ± 12.5 pmol/L, n=6) (Figure 1E). During the studied time frame, transplanted insulin-positive cells were not abolished by transferred human immune cells and still had insulin-releasing functions when transplanted. The presence of transferred immune cells was investigated with *in vivo* imaging and flow cytometry. *In vivo* imaging showed the presence of $CD4^+$ and $CD8^+$ T cells in mice transplanted with human islets and SC-islets 11 days post PBMC injection (Figure 2 A-B). *In vivo* imaging is a valuable technique to monitor the movement and localization of immune cells at the actual graft site (89). *In vivo*

imaging, combined with immunostainings of the graft, creates a view of what is going on at the site of transplanted cells. Furthermore, *in vivo* imaging possesses an image of processes occurring in the native tissue environment at the actual time point.

We evaluated the alteration of immune cells in the spleens and bone marrow of transplanted mice with flow cytometry. The general T cell marker CD3 was investigated, and the presence of human $CD3^+$ of single live cells was observed in spleens and bone marrows of mice transplanted with human islets and SC-islets. There was a tendency ($p=0.0542$) of higher percentages of $CD3^+$ of single live $CD45^+$ cells in spleens of mice transplanted with SC-islets at day 11 post-PBMC transfer compared to day four after PBMC transfer (Figure 3C). The percentages were similar between the two groups in the bone marrows of transplanted mice. When further elucidating the presence of CD4 T helper cells and CD8 cytotoxic T cells, there was the presence of $CD4^+$ of single cells in spleens and bone marrows of mice transplanted with human islets but no difference was seen at day 11 compared to day four post-PBMC injection. In mice transplanted with SC-islets, $CD4^+$ of single cells was increased on day 11 post-PBMC injection in spleens and bone marrows, compared to day four post-PBMC injection (Figure 3E-F). The percentages of $CD8^+$ of single cells in spleens and bone marrows of mice transplanted with human islets and SC-islets were similar at day 11 compared to day four post-PBMC transfer (Figure 3G-H). T cells, specifically CD8 and CD4 cells are one of the primary critical cells in the destruction of beta cells at T1D (100, 101). These flow cytometry data demonstrated that injected human PBMCs could expand in the model and generate a graft-targeted T cell immune response.

Lastly, we investigated the activation of $CD4^+$ and $CD8^+$ immune cells by evaluating the percentages of CD44 and CTLA-4. CD44 is associated with the activation and migration of inflammatory immune cells. At the same time, CTLA-4 is an activation marker that acts as an immune checkpoint molecule on CD4 and CD8 cells. In spleens and bone marrows of mice transplanted with human islets, there was a higher percentage of $CD44^+$ of single live $CD4^+$ T cells at day 11 post-PBMC transfer compared to day four post-PBMC transfer (Figure 4A-B). The percentages of $CD44^+$ of single live $CD4^+$ cells were similar at day 11 post-PBMC injection compared to day four post-PBMC injection in spleens and bone marrows of mice transplanted with SC-islets (Figure 4A-B). However, in the spleens of mice transplanted with SC-islets, the percentages of $CD44^+$ of single live $CD8^+$ cells were higher at day 11 compared to day four post-PBMC transfer (Figure 4C) while there was no difference in mice transplanted with human islets at the studied time points. On the contrary, the percentages were higher in bone marrows of mice transplanted with human islets at 11 days post-PBMC injection (Figure 4D). However, in

bone marrows of mice transplanted with SC-islets, the percentages of $CD44^+$ of single live $CD8^+$ cells were similar 11 days compared to four days post-PBMC transfer. Percentages of $CTLA4^+$ of single live $CD4^+$ cells did not differ day 11 compared to day four post-PBMC-injection in spleens of either mice transplanted with human islets or SC-islets. Furthermore, the percentages of CTLA-4^+ of single live $CD8^+$ cells were similar in spleens of mice transplanted with human islets or SC-islets at day 11 post-PBMC injection compared to day four post-PBMC injection. However, percentages of CTLA-4^+ of single live $CD8^+$ cells were higher in bone marrows of mice transplanted with SC-islets on day 11 compared to day four post-PBMC injection. These results indicated that injected human immune cells could expand and accumulate in the immune-deficient mouse model. King et al. (2008) have established a similar model to study immune responses when transplanting human islets beneath the kidney capsule to immune-deficient mice (65, 102). However, one limitation of the presented humanized mouse model is that it does not develop a complete immune system compared to other humanized mouse models, where innate immune cells can also be studied (103). However, the validated model is robust and easily set up, and it is suitable for studying the immune responses of transplanted human islets and SC-islets.

Paper III

Transplantation of SC-islets is a possible alternative source for beta cell replacement. However, there are challenges in monitoring localization and survival of transplanted beta cells. PET imaging is a potential tool to monitor transplanted beta cells. However, there are no optimal PET tracers for monitoring transplanted beta cells. In paper III, we evaluated the potential of DGCR2 as a marker for PET imaging purposes.

The DGCR2 affibody was synthesized in three steps, and details of the synthesis are found in paper III. This included binding the radioactive isotope fluorine 18 to an activated ester to facilitate radioactive labelling of the interested affibody binding to DGCR2. In short, the radioactive probe [^{18}F]MeTz was synthesized in three steps. First, the radioactive isotope [^{18}F] was reacted and connected to the precursor N,N,N-trimethyl-5-((2,3,5,6-tetrafluorophenoxy) carbonyl) pyridine-2-aminium trifluoromethanesulfonate to form an activated ester, [^{18}F]F-Py-TFP. The activated ester was then linked with a tetrazine amine to form [^{18}F]MeTz, and the precursor was removed. To perform the radiolabelling of the ready-to-use [^{18}F]$Z_{DGCR2:AM106}$ affibody, the affibody molecule TCO-$Z_{DGCR2:AM106}$ was connected with the tetrazine amine, and after a short incubation in room temperature the [^{18}F]$Z_{DGCR2:AM106}$ affibody was radiolabelled and ready-to-use.

The DGCR2 protein has been identified as a biomarker for human islets (74). Immunostaining of DGCR2 has also been shown to have a strong binding to insulin-positive hESC-derived pancreatic progenitor cells (75). The [^{18}F]$Z_{DGCR2:AM106}$ binding was higher in frozen sections of SC-islets compared to frozen sections of 90 % purity of human pancreatic tissue (Figure 5). The uptake in SC-islets and human pancreatic islets of 90 % purity was higher than in HEK293 cells. Therefore, the [^{18}F]$Z_{DGCR2:AM106}$ binding was successful to the SC-islets as well as human islets. Immunohistochemistry staining of SC-islets before transplantation demonstrated the presence of the DGCR2 (Figure 8A). The DGCR2 co-localized with insulin-positive cells. Therefore, the DGCR2 protein might be a potential target for PET imaging of beta cells.

SC-islets were transplanted to the abdominal muscle or neck muscle in immune-deficient NMRI nu/nu mice for *in vivo* evaluation of the [^{18}F]$Z_{DGCR2:AM106}$ affibody binding. The SC-islets grafted in the neck had an identifiable signal in the early PET frames during 1-10 minutes after administration. However, the signal faded quickly and was not visible during the later PET-frames (30 minutes after administration, the signal vanished) (Figure 6A). The signal of administered [^{18}F]$Z_{DGCR2:AM106}$ was not detectable in the grafted SC-islets in the abdominal muscle due to a large spill-over signal from the kidney. The high binding of the radioactive tracer to the grafted SC-islets implies that the DGCR2 protein could be a suitable biomarker for tracking SC-islets. However, this pilot study demonstrates that the DGCR2 radioactive tracer has a rapid delivery. However, the binding of the radioactive tracer might be reversible to the DGCR2 protein with a modest dissociation rate from the protein. Therefore, it disappeared shortly after the early PET frames during the scan. Additional staining for evaluation of the DGCR2 expression was done after PET imaging on the muscle grafts. Four weeks post-transplantation, the expression of DGCR2 was still detectable and in co-localization with insulin-positive cells (Figure 8B-C).

Biodistribution of the [^{18}F]$Z_{DGCR2:AM106}$ was evaluated in rats and pigs. In rats, the [^{18}F]$Z_{DGCR2:AM106}$ uptake was low in the liver and there was low tissue background (Figure 9). PET/MRI demonstrated mainly a renal clearance that peaked at 60 min post-administration. No accumulation was seen in the native pancreas of the [^{18}F]$Z_{DGCR2:AM106}$. An early uptake was observed in the liver in pigs during PET/MRI, which was gradually cleared during imaging. There was a strong renal clearance of the radioactive tracer 30 minutes after tracer administration, which lasted throughout the scan (Figure 10). The tracer had a marginal uptake in other abdominal organs. The brain had minimal uptake, demonstrating that the tracer does not diffuse over the blood-brain barrier.

The exact function of the DGCR2 protein is unknown. The DGCR2 protein has been identified in beta cells, and expression has been reported on extracellular vesicles released from beta cells (104). With some modifications of the tracer and enhancement to an optimal dissociation rate, the binding of $[^{18}F]Z_{DGCR2:AM106}$ might be enhanced and a promising future affibody in the perspective of tracking SC-islet grafts in long-term perspectives.

Paper IV

Differentiation of SC-islets is a time-consuming protocol involving numerous media changes. There is a risk of contamination by different pathogens, and the highest risk of contamination comes from animal- and human-derived components (105). To minimize the risk of contamination and enhance sterility, media and components are usually sterile filtered with a 0.2-0.22 µm filter system to remove bacteria efficiently. However, many viruses have a size range below that, and standardized sterile filter systems commonly used in cell-based therapy culturing are inefficient in removing viruses. Therefore, in paper IV, we wanted to evaluate the effect of nanofiltration using a nanocellulose-based virus clearance filter paper and its potential impact on the differentiation of hESCs into SC-islets.

Mihranyan´s research group has previous tested and demonstrated that the clearance filter paper has efficiently removed virus from filtered media (106, 107). However, the effect on differentiation of hESCs into SC-islets have not been tested. Cells were monitored with flow cytometry and immunostaining during the differentiation. The transcription factor SOX17 is an essential biomarker for definitive endoderm formation in hESCs (108). It is crucial for several species' endoderm formation, hepatocyte differentiation, and liver development (109). Both filter SC-islets and control SC-islets expressed SOX17 and in a comparable expression level (Figure 4). Moreover, the definitive endoderm marker CXCR4 percentage was similar in the filter SC-islets and control SC-islets (Figure 3). The expression of essential markers remained similar between filter SC-islets and control SC-islets throughout the differentiation.

At the final stage seven, the expression of important markers PDX1 and NKX6.1 on cells was comparable between the control and filter groups (Figure 7A-B). PDX1 and NKX6.1 are essential for the functional insulin-releasing ability of beta cells and are expressed early during pancreatic beta cell development (51, 110). Moreover, the expression of C-peptide and NKX6.1 on cells was similar in filter SC-islets and control SC-islets and implemented that the hESCs differentiated towards beta cells (Figure 7C-D). Immunostaining of the SC-islets demonstrated that both filter and control SC-islets expressed insulin, glucagon, and somatostatin in comparable amounts (Figure

6A-B) at the final stage. The hESCs had fully differentiated into SC-islets, with endocrine cells important for physiological SC-islets. A virus clearance filter may be a robust method for virus clearance in media. However, the filters may be prone to clogging of proteins, and thereby depleting molecular components necessary for the differentiation of cells, and therefore negatively affect the differentiation. However, in this study, no differences were observed in the cell composition of the SC-islets from the media filtration. The present findings implicate that the filtration with the investigated virus clearance filter paper does not affect the differentiation of the cells.

The functional evaluation at the end of stage seven showed that the filter SC-islets had a similar stimulation index as the control SC-islets (Figure 8B). At the dynamic perifusion, filter and control SC-islets showed a low insulin release at high glucose (Figure 8A). The addition of exendin-4, a peptide agonist of the glucagon-like peptide (GLP-1) receptor was needed to increase the release of insulin during high glucose exposure. The filter SC-islets had a delayed insulin release compared to the control SC-islets with a little higher insulin release. Both filter SC-islets and control SC-islets returned to basal insulin secretion when exposed to low glucose. The dynamic perifusion showed that insulin secretion had a regular physiological releasing pattern. The delay in the filter SC-islets might be due to cell maturation. Balboa *et al.* have previously shown that at the end of differentiation, SC-islets are functional and have an insulin-releasing ability (38). However, the SC-islets are still not fully mature after the final stages of differentiation. Although the insulin secretion of the filtered SC-islets was delayed during dynamic perifusion, the filter SC-islets could release insulin when stimulated by glucose, and the differentiated hESCs were not affected by the nanofiltration of cell culture media. To minimize the risk of contamination and enhance sterility, media, and components are usually sterile filtered with a 0.2-0.22 µm filter system to remove bacteria efficiently. However, many viruses have a size range between 18 and 200 nm. Filtration of cell culture media with nanocellulose-based virus clearance filter is a step closer to biosafety when generating SC-islets and aiming toward the clinic.

Conclusions

Paper I

SC-islets had a superior vascular and neural engraftment capacity compared to human islets. Ingrowth of recipient blood vessels was 2-3 times higher in SC-islet grafts compared to human islets. The functionality studies demonstrated twice as high oxygenation and a higher tendency of blood flow in SC-islets compared to human islets three months post-transplantation. Ingrowth of nerves was 4-5 times higher in transplanted SC-islets than in human islets. Amyloid depositions were detected three months post-transplantation in both SC-islet grafts and human islet grafts. Amyloid depositions might affect the survival and function of the transplanted cells in long-term perspectives.

Paper II

Immune processes towards allogeneically transplanted human islets and SC-islets were investigated in a humanized mouse model. Transplanted SC-islets and human islets were present 11 days after injection of human PBMCs, demonstrated by human C-peptide values and immunohistochemistry staining. *In vivo* imaging and flow cytometry demonstrated that injected human immune cells, specifically T cells such as CD4 and CD8 cells were present 11 days post-PBMC injection. The validated humanized mouse model can be used to study immune processes towards transplanted SC-islets or human islets.

Paper III

The fluorine-18 labelled affibody molecule targeting DGCR2, $[^{18}F]Z_{DGCR2}$, had an effective binding to both SC-islets and human islets *in vitro*. Furthermore, the $[^{18}F]Z_{DGCR2}$ affibody was successfully detected and visible during the PET imaging of SC-islet grafts *in vivo*. However, the signal faded and was gone 30 min after injection due to the dissociation rate. The sensitivity of the tracer could be optimized in order to prolong the signal. Although, the DGCR2 is a promising marker for molecular imaging of SC-islets.

Paper IV

The effects of nanofiltration using nanocellulose-based virus clearance filter paper were evaluated on the differentiation of hESCs towards SC-islets. The filtered SC-islets expressed essential differentiation molecular markers through the differentiation similar to the control SC-islets. At the end of the differentiation, the filtered SC-islets expressed markers related to mature islets (i.e., insulin, glucagon, and somatostatin) comparable to the control. Filtered SC-islets had physiological insulin-releasing ability comparable to the control SC-islets in the end of the differentiation. Filtration of cell culture media with the virus clearance filter paper did not seem to affect the differentiation of the SC-islets.

Populärvetenskaplig sammanfattning

Bukspottkörteln, eller pankreas, är ett körtelorgan som utsöndrar enzymer viktiga för matsmältningen samt frisättning av viktiga hormoner. I bukspottkörteln finns de Langerhanska öarna som är ett kluster av celler som frisätter viktiga hormoner. Det finns fem olika celltyper i de Langerhanska öarna. Dessa är; betaceller som frisätter insulin vid högt blodsocker, alfaceller som frisätter glukagon vid lågt blodsocker, deltaceller som frisätter somatostatin, pp-celler som frisätter en pankreatisk polypeptid och epsilon celler som frisätter hormonet ghrelin.

Insulinet fungerar likt en nyckel som öppnar dörren in till våra celler så att glukos kan ta sig in i cellen och ge den energi. Däremot vid sockersjuka (diabetes) saknas eller fungerar inte insulinet eller nyckeln lika bra som hos en person utan diabetes. Diabetes kan delas in i olika typer, där denna avhandling har fokuserat på typ 1 diabetes (T1D). T1D börjar vanligtvis i ung ålder. Det är inte helt utrett idag varför vissa får sjukdomen och vad det är som startar den. Det som är känt för T1D är att kroppens eget immunförsvar aktiveras och attackerar samt förstör de insulinproducerande betacellerna. Därmed kan inte kroppen längre producera tillräckligt med insulin, vilket leder till ett kraftigt förhöjt blodsocker och kroppen får inte tillräckligt med energi.

Den vanligaste behandlingen vid T1D har länge varit, och är ännu idag, injektioner av insulin. Trots noggrant kontrollerande av blodsocker och kost, kan sjukdomen vara svårbehandlad och en person med diabetes kan uppleva att blodsockret svänger under dagen. Vissa patienter har en väldigt svårkontrollerad diabetes och kan uppleva upprepade toppar av blodsockernivåer, där insulin inte är tillräckligt. Det är också en stor risk att blodsockret blir farligt lågt, där patienten kan falla i koma, eller i värsta fall dö. Hos dessa patienter är transplantation av antingen bukspottkörtel eller humana Langerhanska öar en alternativ behandling. Däremot finns det problematiska aspekter med detta; för det första är det nödvändigt med immunhämmande läkemedel efter transplantation, för att undvika att de transplanterade cellerna attackeras av immunförsvaret, som i sig har allvarliga biverkningar. För det andra, upprepade transplantationer är ofta nödvändigt då de transplanterade cellerna oftast dör en tid efter transplantation. Ett tredje problem är att det idag råder donatorbrist och

brist på organ till transplantation. Därför försöker man hitta alternativ till organtransplantation.

De senaste årtiondena har flera forskare arbetat med att tillverka kluster av celler som liknar kroppens egna Langerhanska öar som är framtagna av stamceller. Dessa cellkluster namnges som stamcells öar eller ”SC-öar” i detta arbete. Dessa tillverkas genom användning av stamceller som har möjlighet att bilda alla celler i vår kropp. Genom att tillsätta olika molekyler i flera steg kan man styra dessa celler till att bli SC-öar. Dessa framställda celler kan frisätta hormoner som insulin, glukagon och somatostatin som humana Langerhanska öar. Forskningen har kommit långt, och de första patienterna har redan transplanterats med SC-öar i en studie i USA. Ännu kvarstår många frågor om dessa SC-öar, framförallt transplantation av dessa i ett långtidsperspektiv.
Denna avhandling har syftat till att utforska SC-öar och deras potential som en behandling för T1D.

I delarbete I, transplanterade vi humana SC-öar och humana Langerhanska öar i njuren på möss med nedsatt immunförsvar. I mössen undersökte vi efter 3 månader om de transplanterade cellerna överlevde och om kärl och nerver kunde växa in i cellerna under denna tidsperiod. Det vi fann var att en större andel blodkärl växte in i de transplanterade SC-öarna. Vi såg också och att SC-öar som transplanterats hade en större inväxt av nerver (4-5 gånger större) i jämförelse med transplanterade humana öar. Vi undersökte även överlevnad av transplanterade celler och såg något fler döda celler i transplanterade humana öar jämfört med transplanterade SC-öar. Till sist fann vi att båda typer av celler hade uppkomst av ett protein vid namn amyloid. Amyloid uttrycks när betacellerna är stressade och detta protein kan påverka överlevnaden och frisättningen av insulin i ett långtidsperspektiv. Sammanfattningsvis var det större inväxt av kärl och nerver i de transplanterade SC-öarna, men proteinet amyloid som fanns i både SC-öar och humana Langerhanska öar kan påverka de transplanterade cellerna i ett längre perspektiv.

I delarbete II, var syftet att utvärdera en mus modell (med nedsatt immunsvar) som hade fått injektion av mänskliga immunceller (en så kallad humaniserad musmodell på forskarspråk) för att studera hur de mänskliga immuncellerna beter sig i närheten av transplanterade SC-öar och humana Langerhanska öar. Vi studerade immuncellerna vid fyra dagar och elva dagar efter att mänskliga immunceller hade injicerats. Vi förväntade oss att de mänskliga immuncellerna skulle attackera de transplanterade cellerna då de var från olika personer. Resultaten visade att insulinproducerande betaceller inte var totalt förstörda av de injicerade mänskliga immuncellerna. Vi såg även att de transplanterade cellerna fortfarande kunde frisätta insulin, vilket ytterligare tydde på att de transplanterade SC-öarna och Langerhanska öarna inte var förstörda av immuncellerna inom den valda tidsperioden på 11 dagar. Vi kunde se att

det fanns mänskliga immunceller så som vita blodkroppar närvarande i olika organ i musen vilket innebär att de injicerade mänskliga immun cellerna hade överlevt och kunde öka i antal i musen under den valda tidpunkten. Slutsatsen var att vi lyckades injicera mänskliga immunceller i den transplanterade musen, och denna mus skulle kunna användas för att studera immunceller och deras försvar vid transplanterade celler.

I delarbete III, transplanterade vi SC-öar till möss med nedsatt immunförsvar. Med hjälp av en teknik som heter positronemissionstomografi (PET) kan man märka in olika organ med en radioaktiv molekyl och undersöka i en PET kamera. Syftet med denna studie var att undersöka om en ny radioaktiva markör (PET markör) som heter DGCR2 kan användas för att ”filma” betaceller och transplanterade celler. Vi observerade att DGCR2 PET markören kunde binda bra till SC-öar och humana Langerhanska öar. När vi sedan undersökte i de levande mössen, såg vi att PET markören hade bundit till de transplanterade SC-öarna. Dock var markören synlig i kameran endast upp till 10 minuter, därefter försvann den. Därmed behövs troligen DGCR2 PET markören modifieras i strukturen för att kunna förlänga tiden. Sammanfattningsvis, är DGCR2 PET markören en lovande markör för att kunna undersöka och följa transplanterade betaceller med hjälp av PET kamera. Däremot behöver molekylen ändras för att kunna studera transplanterade betaceller under en längre tid.

I delarbete IV, var syftet att studera ett filter som var speciellt tillverkat för att eliminera virus. Vid framtagning av SC-öar från stamceller behöver man tillsätta en lösning med olika molekyler till cellerna (cellmedium), vid flera tillfällen till dess att cellerna har blivit SC-öar. Då detta sker i många steg är det en stor risk att cellerna kontamineras av bakterier, jästsvampar eller virus (patogener). Detta leder till att cellerna får näringsbrist på grund av konkurrens med kontaminerande bakterier, jäst eller virus, och i värsta fall dör cellerna. För att minimera risken av sådan kontaminering brukar lösningarna filtreras genom speciella filter, men dessa är inte tillräckliga för att ta bort virus. Därför var syftet att studera ett filter som var speciellt framtaget för att ta bort virus (virusfilter) och om det kan påverka tillverkningen och funktionen av cellerna. Cellmedlet filtrerades genom virusfiltret innan det tillsattes till cellerna medan kontrollgruppen fick medium som inte var filtrerat genom virusfiltret. Vi såg inga skillnader mellan virusfilter gruppen och kontrollgruppen under tillverkningen av SC-öar. Båda grupperna hade uttryck av viktiga markörer för att styra cellerna åt rätt håll. I slutet, när stamcellerna hade blivit till SC-öar, hade gruppen med virusfilter förväntad funktion och kunde frisätta insulin, precis som kontrollgruppen. Dock tydde resultaten på att gruppen med virusfilter var något eftersläpande i sin frisättning av insulin jämfört med kontrollgruppen. Sammanfattningsvis, verkar inte filtrering med virusfiltret påverka framställningen av SC-öarna.

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References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37 Suppl 1:S81-90.
2. International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium. Available at: https://www.diabetesatlas.org2021.
3. Patterson CC, Karuranga S, Salpea P, Saeedi P, Dahlquist G, Soltesz G, et al. Worldwide estimates of incidence, prevalence and mortality of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107842.
4. Ogle GD, James S, Dabelea D, Pihoker C, Svennson J, Maniam J, et al. Global estimates of incidence of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Atlas, 10th edition. Diabetes Res Clin Pract. 2022;183:109083.
5. Mitchell AM, Michels AW. Self-Antigens Targeted by Regulatory T Cells in Type 1 Diabetes. Int J Mol Sci. 2022;23(6).
6. Arvan P, Pietropaolo M, Ostrov D, Rhodes CJ. Islet autoantigens: structure, function, localization, and regulation. Cold Spring Harb Perspect Med. 2012;2(8).
7. Ferrannini E, Mari A, Nofrate V, Sosenko JM, Skyler JS, Group DPTS. Progression to diabetes in relatives of type 1 diabetic patients: mechanisms and mode of onset. Diabetes. 2010;59(3):679-85.
8. Morran MP, Casu A, Arena VC, Pietropaolo S, Zhang Y-J, Satin LS, et al. Humoral Autoimmunity against the Extracellular Domain of the Neuroendocrine Autoantigen IA-2 Heightens the Risk of Type 1 Diabetes. Endocrinology. 2010;151(6):2528-37.
9. Wenzlau JM, Kirstine J, Yu L, Moua O, Sarkar SA, Gottlieb P, et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A. 2007;104(43):17040–5.
10. Murgia C, Devirgiliis C, Mancini E, Donadel G, Zalewski P, Perozzi G. Diabetes-linked zinc transporter ZnT8 is a homodimeric protein expressed by distinct rodent endocrine cell types in the pancreas and other glands. Nutr Metab Cardiovasc Dis. 2009;19(6):431-9.
11. Baekkeskov S, Høiriis Nielsen J, Marner M, Bilde T, Ludvigsson J, Lernmark A. Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. Nature. 1982;298:167-9.
12. Christoffersson G, Rodriguez-Calvo T, von Herrath M. Recent advances in understanding Type 1 Diabetes. F1000Res. 2016;5.
13. DeForest N, Majithia AR. Genetics of Type 2 Diabetes: Implications from Large-Scale Studies. Curr Diab Rep. 2022.
14. Zhou Q, Melton DA. Pancreas regeneration. Nature. 2018;557(7705):351-8.
15. Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, et al. Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. J Histochem Cytochem. 2005;53(9):1087-97.

16. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren P-O, 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.
17. Ionescu-Tirgoviste C, Gagniuc PA, Gubceac E, Mardare L, Popescu I, Dima S, et al. A 3D map of the islet routes throughout the healthy human pancreas. Sci Rep. 2015;5:14634.
18. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Analysis of islet inflammation in human type 1 diabetes. Clin Exp Immunol. 2009;155(2):173-81.
19. Dotta F, Censini S, van Halteren AGS, Marselli L, Masini M, Dionisi, S., et al. Coxsackie B4 virus infection of β cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci U S A. 2007;104(12):5115-20.
20. Vehik K, Lynch KF, Wong MC, Tian X, Ross MC, Gibbs RA, et al. Prospective virome analyses in young children at increased genetic risk for type 1 diabetes. Nat Med. 2019;25(12):1865-72.
21. Laitinen OH, Honkanen H, Pakkanen P, Oikarinen S, Hankaniemi MM, Huhtala H, et al. Coxsackievirus B1 Is Associated With Induction of β-Cell Autoimmunity That Portends Type 1 Diabetes. Diabetes. 2014;63(2):446-55.
22. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet. 2009;41(6):703-7.
23. Robertson CC, Inshaw JRJ, Onengut-Gumuscu S, Chen WM, Santa Cruz DF, Yang H, et al. Fine-mapping, trans-ancestral and genomic analyses identify causal variants, cells, genes and drug targets for type 1 diabetes. Nat Genet. 2021;53(7):962-71.
24. Noble JA, Valdes AM. Genetics of the HLA region in the prediction of type 1 diabetes. Curr Diab Rep. 2011;11(6):533-42.
25. Richardson SJ, Rodriguez-Calvo T, Gerling IC, Mathews CE, Kaddis JS, Russell MA, et al. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. Diabetologia. 2016;59(11):2448-58.
26. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, et al. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med. 2012;209(1):51-60.
27. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000;343(4).
28. Espes D, Lau J, Carlsson PO. MECHANISMS IN ENDOCRINOLOGY: Towards the clinical translation of stem cell therapy for type 1 diabetes. Eur J Endocrinol. 2017;177(4):R159-R68.
29. 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.
30. Hardstedt M, Lindblom S, Karlsson-Parra A, Nilsson B, Korsgren O. Characterization of Innate Immunity in an Extended Whole Blood Model of Human Islet Allotransplantation. Cell Transplant. 2016;25(3):503-15.
31. 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.
32. Lau J, Carlsson PO. Low revascularization of human islets when experimentally transplanted into the liver. Transplantation. 2009;87(3):322-5.

33. 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.
34. Balboa D, Barsby T, Lithovius V, Saarimäki-Vire J, Omar-Hmeadi M, Dyachok O, et al. Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells. Nature Biotechnology. 2022;40(7):1042-55.
35. Pagliuca FW, Millman JR, Gurtler M, Segel M, Van Dervort A, Ryu JH, et al. Generation of functional human pancreatic beta cells in vitro. Cell. 2014;159(2):428-39.
36. Thomson JA, Itskovitz-Eldor J, Sharpiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic Stem Cell Lines Derived from Human Blastocysts. Science. 1998;282.
37. Reubinoff BE, Pera MF, Fong C-Y, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nature. 2000;18:399-404.
38. Balboa D, Barsby T, Lithovius V, Saarimaki-Vire J, Omar-Hmeadi M, Dyachok O, et al. Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells. Nat Biotechnol. 2022.
39. Maxwell KG, Kim MH, Gale SE, Millman JR. Differential Function and Maturation of Human Stem Cell-Derived Islets After Transplantation. Stem Cells Transl Med. 2022;11(3):322-31.
40. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol. 2014;32(11):1121-33.
41. Millman JR, Xie C, Van Dervort A, Gurtler M, Pagliuca FW, Melton DA. Generation of stem cell-derived beta-cells from patients with type 1 diabetes. Nat Commun. 2016;7:11463.
42. Toivonen S, Lundin K, Balboa D, Ustinov J, Tamminen K, Palgi J, et al. Activin A and Wnt-dependent specification of human definitive endoderm cells. Exp Cell Res. 2013;319(17):2535-44.
43. D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol. 2005;23(12):1534-41.
44. Mahaddalkar PU, Scheibner K, Pfluger S, Ansarullah, Sterr M, Beckenbauer J, et al. Generation of pancreatic beta cells from CD177(+) anterior definitive endoderm. Nat Biotechnol. 2020;38(9):1061-72.
45. Rezania A, Bruin J. E. , Riedel MJ, Mojibian M, Asadi A, Xu J, et al. Maturation of Human Embryonic Stem Cell–Derived Pancreatic Progenitors Into Functional Islets Capable of Treating Pre-existing Diabetes in Mice. Diabetes. 2012;61(8):2016-29.
46. Wesolowska-Andersen A, Jensen RR, Alcantara MP, Beer NL, Duff C, Nylander V, et al. Analysis of Differentiation Protocols Defines a Common Pancreatic Progenitor Molecular Signature and Guides Refinement of Endocrine Differentiation. Stem Cell Reports. 2020;14(1):138-53.
47. Wells JM, Melton DA. Early mouse endoderm is patterned by soluble factors from adjacent germ layers. Development. 2000;127(8):1563-72.
48. Johannesson M, Stahlberg A, Ameri J, Sand FW, Norrman K, Semb H. FGF4 and retinoic acid direct differentiation of hESCs into PDX1-expressing foregut endoderm in a time- and concentration-dependent manner. PLoS One. 2009;4(3):e4794.

49. D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol. 2006;24(11):1392-401.
50. Rezania A, Bruin JE, Xu J, Narayan K, Fox JK, O'Neil JJ, et al. Enrichment of human embryonic stem cell-derived NKX6.1-expressing pancreatic progenitor cells accelerates the maturation of insulin-secreting cells in vivo. Stem Cells. 2013;31(11):2432-42.
51. Taylor BL, Liu FF, Sander M. Nkx6.1 is essential for maintaining the functional state of pancreatic beta cells. Cell Rep. 2013;4(6):1262-75.
52. Dhawan S, Dirice E, RKulkarni RN, Bhushan A. Inhibition of TGF-b Signaling Promotes Human Pancreatic b-Cell Replication. Diabetes. 2016;65:1208-18.
53. Veres A, Faust AL, Bushnell HL, Engquist EN, Kenty JH, Harb G, et al. Charting cellular identity during human in vitro beta-cell differentiation. Nature. 2019;569(7756):368-73.
54. Jennings RE, Berry AA, Kirkwood-Wilson R, Roberts NA, Hearn T, Salisbury RJ, et al. Development of the Human Pancreas From Foregut to Endocrine Commitment. Diabetes. 2013;62(10):3514-22.
55. Bruin JE, Rezania A, Xu J, Narayan K, Fox JK, O'Neil JJ, et al. Maturation and function of human embryonic stem cell-derived pancreatic progenitors in macroencapsulation devices following transplant into mice. Diabetologia. 2013;56:1987–98.
56. A Safety, Tolerability, and Efficacy Study of VX-880 in Participants With Type 1 Diabetes - Tabular View - ClinicalTrials.gov ClinicalTrials.gov2021 [cited 22 May 2022. Available from: https://clinicaltrials.gov/ct2/show/record/NCT04786262?view=record.
57. Parkins K. Impressive early results for Vertex's potentially curative stem cell diabetes therapy Clinical Trials Arena; 2021 [Available from: https://www.clinicaltrialsarena.com/analysis/vertex-stem-cell-therapy-diabetes-cure/.
58. Vertex Announces Positive Day 90 Data for the First Patient in the Phase 1/2 Clinical Trial Dosed With VX-880, a Novel Investigational Stem Cell-Derived Therapy for the Treatment of Type 1 Diabetes [press release]. https://investors.vrtx.com/news-releases/news-release-details/vertex-announces-positive-day-90-data-first-patient-phase-122021.
59. Vertex Presents Positive, Updated VX-880 Results From Ongoing Phase 1/2 Study in Type 1 Diabetes at the European Association for the Study of Diabetes 59th Annual Meeting [press release]. https://news.vrtx.com/news-releases/news-release-details/vertex-presents-positive-updated-vx-880-results-ongoing-phase-122023.
60. Vertex Provides Pipeline and Business Updates in Advance of Upcoming Investor Meetings [press release]. https://investors.vrtx.com/news-releases/news-release-details/vertex-provides-pipeline-and-business-updates-advance-upcoming2024.
61. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol. 2008;26(4):443-52.
62. King M, Pearson T, Rossini AA, Shultz LD, Greiner DL. Humanized mice for the study of type 1 diabetes and beta cell function. Ann N Y Acad Sci. 2008;1150:46-53.
63. Walsh NC, Kenney LL, Jangalwe S, Aryee KE, Greiner DL, Brehm MA, et al. Humanized Mouse Models of Clinical Disease. Annu Rev Pathol. 2017;12:187-215.

64. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol. 2012;12(11):786-98.
65. King M, Pearson T, Shultz LD, Leif J, Bottino R, Trucco M, et al. A new Hu-PBL model for the study of human islet alloreactivity based on NOD-scid mice bearing a targeted mutation in the IL-2 receptor gamma chain gene. Clin Immunol. 2008;126(3):303-14.
66. Ishikawa F, Yasukawa M, Lyons B, Yoshida S, Miyamoto T, Yoshimoto G, et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor {gamma} chain(null) mice. Blood. 2005;106(5):1565-73.
67. Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol. 2005;174(10):6477-89.
68. Melkus MW, Estes JD, Padgett-Thomas A, Gatlin J, Denton PW, Othieno FA, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med. 2006;12(11):1316-22.
69. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. Nat Rev Immunol. 2007;7(2):118-30.
70. Normandin MD, Petersen KF, Ding YS, Lin SF, Naik S, Fowles K, et al. In vivo imaging of endogenous pancreatic beta-cell mass in healthy and type 1 diabetic subjects using 18F-fluoropropyl-dihydrotetrabenazine and PET. J Nucl Med. 2012;53(6):908-16.
71. Jahan M, Johnstrom P, Selvaraju RK, Svedberg M, Winzell MS, Bernstrom J, et al. The development of a GPR44 targeting radioligand [(11)C]AZ12204657 for in vivo assessment of beta cell mass. EJNMMI Res. 2018;8(1):113.
72. Eriksson O, Johnstrom P, Cselenyi Z, Jahan M, Selvaraju RK, Jensen-Waern M, et al. In Vivo Visualization of beta-Cells by Targeting of GPR44. Diabetes. 2018;67(2):182-92.
73. Espes D, Carlsson PO, Selvaraju RK, Rosestedt M, Cheung P, Ahlstrom H, et al. Longitudinal Assessment of (11)C-5-Hydroxytryptophan Uptake in Pancreas After Debut of Type 1 Diabetes. Diabetes. 2021;70(4):966-75.
74. Lindskog C, Korsgren O, Ponten F, Eriksson JW, Johansson L, Danielsson A. Novel pancreatic beta cell-specific proteins: antibody-based proteomics for identification of new biomarker candidates. J Proteomics. 2012;75(9):2611-20.
75. Bruin JE, Asadi A, Fox JK, Erener S, Rezania A, Kieffer TJ. Accelerated Maturation of Human Stem Cell-Derived Pancreatic Progenitor Cells into Insulin-Secreting Cells in Immunodeficient Rats Relative to Mice. Stem Cell Reports. 2015;5(6):1081-96.
76. Gravina A, Tediashvili G, Rajalingam R, Quandt Z, Deisenroth C, Schrepfer S, et al. Protection of cell therapeutics from antibody-mediated killing by CD64 overexpression. Nat Biotechnol. 2023;41(5):717-27.
77. Henry RR, Pettus J, Wilensky J, Shapiro AMJ, Senior PA, Roep B, et al. Initial Clinical Evaluation of VC-01TM Combination Product—A Stem Cell–Derived Islet Replacement for Type 1 Diabetes (T1D). Diabetes. 2018;67(Supplement_1).
78. Shapiro AMJ, Thompson D, Donner TW, Bellin MD, Hsueh W, Pettus J, et al. Insulin expression and C-peptide in type 1 diabetes subjects implanted with stem cell-derived pancreatic endoderm cells in an encapsulation device. Cell Rep Med. 2021;2(12):100466.

79. Carlsson PO, Espes D, Sedigh A, Rotem A, Zimerman B, Grinberg H, et al. Transplantation of macroencapsulated human islets within the bioartificial pancreas betaAir to patients with type 1 diabetes mellitus. Am J Transplant. 2018;18(7):1735-44.
80. Yoshihara E, O'Connor C, Gasser E, Wei Z, Oh TG, Tseng TW, et al. Immune-evasive human islet-like organoids ameliorate diabetes. Nature. 2020;586(7830):606-11.
81. Parent AV, Faleo G, Chavez J, Saxton M, Berrios DI, Kerper NR, et al. Selective deletion of human leukocyte antigens protects stem cell-derived islets from immune rejection. Cell Rep. 2021;36(7):109538.
82. Deuse T, Hu X, Gravina A, Wang D, Tediashvili G, De C, et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. Nat Biotechnol. 2019;37(3):252-8.
83. Hu X, White K, Olroyd AG, DeJesus R, Dominguez AA, Dowdle WE, et al. Hypoimmune induced pluripotent stem cells survive long term in fully immunocompetent, allogeneic rhesus macaques. Nat Biotechnol. 2023.
84. Hu X, White K, Young C, Olroyd AG, Kievit P, Connolly AJ, et al. Hypoimmune islets achieve insulin independence after allogeneic transplantation in a fully immunocompetent non-human primate. Cell Stem Cell. 2024;31(3):334-40 e5.
85. Balboa D, Saarimaki-Vire J, Borshagovski D, Survila M, Lindholm P, Galli E, et al. Insulin mutations impair beta-cell development in a patient-derived iPSC model of neonatal diabetes. Elife. 2018;7.
86. 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):3246-54.
87. Carlsson PO, Palm F, Mattsson G. Low revascularization of experimentally transplanted human pancreatic islets. J Clin Endocrinol Metab. 2002;87(12):5418-23.
88. Espes D, Singh K, Sandler S, Carlsson PO. Increased Interleukin-35 Levels in Patients With Type 1 Diabetes With Remaining C-Peptide. Diabetes Care. 2017;40(8):1090-5.
89. Ahl D, Eriksson O, Sedin J, Seignez C, Schwan E, Kreuger J, et al. Turning Up the Heat: Local Temperature Control During in vivo Imaging of Immune Cells. Front Immunol. 2019;10:2036.
90. Rydgren T, Oster E, Sandberg M, Sandler S. Administration of IL-1 trap prolongs survival of transplanted pancreatic islets to type 1 diabetic NOD mice. Cytokine. 2013;63(2):123-9.
91. Singh K, Hjort M, Thorvaldson L, Sandler S. Concomitant analysis of Helios and Neuropilin-1 as a marker to detect thymic derived regulatory T cells in naive mice. Sci Rep. 2015;5:7767.
92. Singh K, Kadesjo E, Lindroos J, Hjort M, Lundberg M, Espes D, et al. Interleukin-35 administration counteracts established murine type 1 diabetes--possible involvement of regulatory T cells. Sci Rep. 2015;5:12633.
93. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012;9(7):676-82.
94. Reinert RB, Cai Q, Hong JY, Plank JL, Aamodt K, Prasad N, et al. Vascular endothelial growth factor coordinates islet innervation via vascular scaffolding. Development. 2014;141(7):1480-91.

95. Westermark GT, Krogvold L, Dahl-Jorgensen K, Ludvigsson J. Islet amyloid in recent-onset type 1 diabetes-the DiViD study. Ups J Med Sci. 2017;122(3):201-3.
96. 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.
97. 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.
98. Westermark P, Eizirik DL, Pipeleers DG, Hellerström C, Andersson A. Rapid deposition of amyloid in human islets transplanted into nude mice. Diabetologica. 1995;38:543-9.
99. Potter KJ, Westwell-Roper CY, Klimek-Abercrombie AM, Warnock GL, Verchere CB. Death and dysfunction of transplanted beta-cells: lessons learned from type 2 diabetes? Diabetes. 2014;63(1):12-9.
100. Krogvold L, Wiberg A, Edwin B, Buanes T, Jahnsen FL, Hanssen KF, et al. Insulitis and characterisation of infiltrating T cells in surgical pancreatic tail resections from patients at onset of type 1 diabetes. Diabetologia. 2016;59:492-501.
101. Dwyer AJ, Ritz JM, Mitchell JS, Martinov T, Alkhatib M, Silva N, et al. Enhanced CD4(+) and CD8(+) T cell infiltrate within convex hull defined pancreatic islet borders as autoimmune diabetes progresses. Sci Rep. 2021;11(1):17142.
102. Gysemans C, Waer M, Laureys J, Depovere J, Pipeleers D, Bouillon R, et al. Islet xenograft destruction in the hu-PBL-severe combined immunodeficient (SCID) mouse necessitates anti-CD3 preactivation of human immune cells. Clin Exp Immunol. 2000;121(3):557-65.
103. Rongvaux A, Willinger T, Martinek J, Strowig T, Gearty SV, Teichmann LL, et al. Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol. 2014;32(4):364-72.
104. Tesovnik T, Kovac J, Pohar K, Hudoklin S, Dovc K, Bratina N, et al. Extracellular Vesicles Derived Human-miRNAs Modulate the Immune System in Type 1 Diabetes. Front Cell Dev Biol. 2020;8:202.
105. Mahmood A, Shakir A. Microbial and Viral Contamination of Animal and Stem Cell Cultures: Common Contaminants, Detection and Elimination. Journal of Stem Cell Research & Therapeutics. 2017;2(5).
106. Manukyan L, Marinaki ME, Mihranyan A. Would 20 nm Filtered Fetal Bovine Serum-Supplemented Media Support Growth of CHO and HEK-293 Cells? ACS Appl Bio Mater. 2020;3(12):8344-51.
107. Manukyan L, Padova J, Mihranyan A. Virus removal filtration of chemically defined Chinese Hamster Ovary cells medium with nanocellulose-based size exclusion filter. Biologicals. 2019;59:62-7.
108. Jiang W, Shi Y, Zhao D, Chen S, Yong J, Zhang J, et al. In vitro derivation of functional insulin-producing cells from human embryonic stem cells. Cell Res. 2007;17(4):333-44.
109. Ayatollahi M, Sanati MH, Kabir Salmani M, Geramizaeh B. Differential expression pattern of the human endoderm-specific transcription factor sox17 in various tissues and cells. Int J Organ Transplant Med. 2012;3(4):183-7.
110. Gao T, McKenna B, Li C, Reichert M, Nguyen J, Singh T, et al. Pdx1 maintains beta cell identity and function by repressing an alpha cell program. Cell Metab. 2014;19(2):259-71.

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