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Towards engineering a novel transplantation site for pancreatic islets

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Chapter 8

General discussion

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Every year approximately 70,000 people are newly diagnosed with type 1 diabetes mellitus [1] indicating the worldwide prevalence is still growing. Type 1 diabetes is characterized by the destruction of pancreatic beta-cells and this results in impaired glucose homeostasis. As insulin injections do not prevent the development of secondary complications such as cardiovascular diseases, nephropathy, and retinopathy, other treatment options are needed to restore the glucose homeostasis [2]. Pancreatic islet transplantation via infusion of islets into the portal vein is a promising treatment option [3]. However, it is still associated with low long-term success rates [4-6]. The liver does not provide an optimal environment for islet engraftment [7], and alternative sites also seem to be insufficient [8,9]. Therefore, an artificial transplantation site for islets is desired. A three-dimensional (3D) scaffold as artificial transplantation site is expected to facilitate revascularization to improve oxygenation, nutrient access, glucose sensing, and insulin release shortly after islet transplantation. In this thesis we described the design and performance of a 3D polymer scaffold for islet transplantation. Our approach should improve long-term success rates of islet transplantation as treatment of type 1 diabetic patients.

Importance of standard isolation procedures

In **chapter 2** we describe the impact of the use of different enzyme preparations in islet isolation on the longevity of encapsulated rat islet grafts. Seemingly minor differences in enzyme activities in collagenase lot numbers have functional, ultrastructural, and immunological consequences for the success of encapsulated islet grafts. Therefore, improving long-term success rates of islet transplantation starts with optimizing the isolation procedure including the usage of a competent collagenase. The importance of a standardized isolation procedure is also confirmed by the different responses of human and rat islets to the different polymers used in this thesis (**chapter 3 and 4**). Due to large variability in human islet quality, it was impossible to determine the magnitude of the polymer effects on human islets, whereas these

effects were clearly demonstrated on rat islets. Although, other variables will also contribute to the variations in human islet quality, the use of an optimal collagenase will contribute to improved viability and yield.

Importance of biomaterial selection

The principal applicability of an artificial transplantation site for pancreatic islets has been shown [10-16]. However, the results were very variable due to degradation of the material, foreign-body responses, fibrotic reactions, or diffusion issues of oxygen and nutrients through the material towards the islet graft [9,17,18]. Remarkably, in none of the studies the direct effect of the biomaterials on functional islet survival was studied which may be another factor contributing to the variations in success. We feel that the success of our poly (D,L-lactide-co- ϵ -caprolactone) (PDLLCL) scaffold is due to the stepwise exclusion of the polymers with negative effects on islet function and by determining the minimal required pre-transplant period for dampening of responses against the biomaterials as well as allowing ingrowth of vasculature to fasten revascularization of the islets and prevent long periods of ischemia (**chapter 3 and 4**). This latter suggestion is supported by the findings of Pepper *et al.* who demonstrated that prevascularization plays an important role in using the subcutaneous site for islet transplantation [19]. Pepper and colleagues implanted a biomaterial to induce vascularization and removed the biomaterial before introduction of the islets. We concentrate on a concept in which the biomaterial is also used as a retrievable vehicle as this is to our opinion needed for safe and effective clinical treatment as not only islets from cadaveric donors but also islets from stem-cell sources are proposed for treatment of diabetes [20]. The need for explantation or re-implantation and the risks posed by potential teratoma formation [21], malignant transformation [22], or disturbed hormone release [23], suggest an implantation design that allows immobilization of the cells and a concept that is retrievable or slowly degrades.

During our stepwise selection procedure, poly(ethylene oxide terephthalate)/polybutylene terephthalate (PEOT/PBT) block copolymer and polysulfone showed to hamper functional islet survival (**chapter 3 and 4**). Basal insulin levels were disturbed, elevated levels of dead cells, and increased clumping of islets was observed when cultured on these polymers. Furthermore, increased levels of danger associated molecular patterns were observed indicating that these polymers are not suitable for islet transplantation. In conflict with these results, PEOT/PBT and polysulfone have been suggested for islet transplantation in other studies [11,24,25]. A PEOT/PBT microwell system was tested *in vitro* with human islets, preventing islets from clumping and retaining islet function [11]. In this study from Buitinga *et al.* large donor variability was observed as well. It is possible that repeating this experiment with rat islets will corroborate our results with PEOT/PBT. However, no *in vivo* experiments were published with PEOT/PBT. Subcutaneous islet transplantation in polysulfone hollow fibers resulted in normoglycemia in a diabetic rat model [25]. However, twenty days after transplantation islet function was lost due to poor vascularization and the occurrence of a foreign body response. Our data supports the slow vascularization of polysulfone and the occurrence of an inflammatory reaction after subcutaneous implantation. To our knowledge, we showed for the first time the applicability of PDLLCL as scaffold material for subcutaneous islet transplantation.

As hydrophilicity of a polymer might influence functional islet survival, we investigated a hydrophilic and a hydrophobic PDLLCL polymer. The functional survival of both human and rat islets was not altered by the hydrophilicity of PDLLCL, whereas for the other polymers the function or survival did change when altering the hydrophilicity. Besides hydrophilicity, other polymer surface characteristics such as surface energy, roughness, and stiffness [26,27] can play a role in cell behavior. The influence of these PDLLCL surface characteristics on cellular outgrowth, survival, and function of islet cells should be further investigated.

Kidney versus skin, relevant comparison?

We validated our prevascularized PDLCL scaffold in a diabetic rat model (**chapter 5**) and assessed the efficacy of our scaffold (**chapter 6**). We found that the subcutaneous scaffold was a slightly less efficacious transplantation site than the kidney capsule. Our observations are consistent with previous studies of subcutaneous scaffolds in rodents [19,28]. Tatarkiewicz *et al.* showed also differences in efficacy of the kidney capsule compared with a subcutaneous scaffold. Rat islet transplantation under the kidney capsule resulted in normoglycemia within a week in diabetic mouse model whereas transplantation into a subcutaneous scaffold resulted in normoglycemia within three weeks [28]. The blood supply of islets in the kidney capsule and in a subcutaneous site differ, as kidneys receive 20% of the total cardiac output, whereas the skin receives 7.5% [29]. In addition, the blood flow in the skin has been shown to decrease in type 1 diabetic patients [30]. Especially during the glucose tolerance tests these blood flow differences will cause altered glucose clearance patterns. Therefore, we consider if it is realistic to compare the efficacy of these two sites. These sites will never give completely the same results due to the physiological differences. In addition, islet transplantation under the kidney capsule in large animals and humans never resulted in long-term insulin independence. As the overall health in both animal groups improved and glucose tolerance, although different, was adequate, we feel we can safely conclude that an islet graft under the skin in our scaffold is fully functional by regulating glucose metabolism.

The results of Tatarkiewicz *et al.* were obtained by transplanting 1200 islets into a scaffold, which was preimplanted two weeks before infusion of the islets [28]. In contrary, transplantation of 1200 islets in our scaffold, preimplanted four weeks before transplantation, resulted in normoglycemia three times faster, showing the importance of monitoring the minimal required pre-transplant period for ingrowth of vasculature (**chapter 4**) and the hastening of normoglycemia by a well-formed vascular network. However, we cannot assume that vascularization occurs in a similar degree in patients suffering for a long time from type

1 diabetes. It is known that vascularization is impaired in diabetes [31,32]. Therefore, further investigation of the vascularization of our scaffold in along-term diabetic model is warranted.

Improvement growth factor delivery

To enhance the efficacy of our artificial transplantation site we investigated the addition of growth factors. A commonly used method to apply growth factors is the coupling of growth factors to heparin-coated materials [33-36]. Heparin is known to bind many of the vascular growth factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). Implantation results in sustained delivery of the growth factors over an extended period to induce a stable vascular network and prevent the potential safety concerns associated with bolus delivery of growth factors [37-40]. Polyganics B.V. provided heparinized PDLCL and we determined *in vitro* the release pattern of VEGF from the heparinized PDLCL. Besides that heparin is difficult to bind to PDLCL, and therefore resulted in a low efficiency of VEGF binding and fast release of VEGF, a glucose-stimulated insulin secretion test showed that heparinized PDLCL negatively influenced human islet functionality. This indicates that heparinized PDLCL is not suitable for an islet scaffold.

As binding of growth factor directly to PDLCL itself was not possible, we proposed a new strategy for vascularization of an islet transplantation site. We applied the delivery of growth factors by liposomes (**chapter 7**). Liposomes are a bilayer phospholipid system that can entrap drugs and serve as drug delivery system [41]. Liposomes can be easily applied via injections and these can be repeated to maintain a certain concentration of growth factor for a longer period. Here, we demonstrated the ability of loading PC / cholesterol liposomes with acidic FGF (aFGF) and showing a gradual release of aFGF over 7 days. Although liposomes are extensively studied as drug delivery system, not much is published about aFGF containing liposomes. For enhancement of vascularization with liposomes, the majority of studies use

VEGF [42-44]. We showed that injections with aFGF liposomes do improve the immediate restoration of blood glucose levels by enhanced vascularization but do not facilitate the long-term engraftment of transplanted islets in our subcutaneous scaffold. The injections and the degradation of the liposomes seem to be detrimental for long-term success. Therefore, the gradual release of aFGF from the liposomes should be improved to a release of four weeks to decrease the number of injections or another delivery system for growth factors should be investigated. Montazeri *et al.* used carbodiimide chemistry to covalently bind heparin to fibrin; subsequently growth factors such as VEGF can be coupled to the heparin to produce a sustained delivery system [45]. As our current scaffold already contains fibrin, such a strategy could be easily applied. Another option could be binding of aFGF to the tubing material that is used to keep the islet channels patent during the preimplantation period. There will be no interaction between islets and this tubing material as it is removed before islet transplantation. Therefore, islet interaction with this material does not have to be investigated, research can focus on establishing gradual release of aFGF from this material. This strategy has been applied for treatment of chronic myocardial ischemia [46].

Weidling *et al.* developed a technology by which vascularization can be observed in real time and in a non-invasive way in an animal model [47]. With this technology the partial oxygen pressure at the site of transplantation can be studied. Oxygen sensitive microparticles can be added to the scaffold to directly measure the partial oxygen pressure within the subcutaneous implanted scaffold. An electro-optical probe can detect the emitted light from the microparticles, which is related to the partial oxygen pressure. By using this technology, we obtain insight into the degree of vascularization within our scaffold. In future research, we like to apply this technology for testing the different strategies as mentioned above or other growth factor cocktails to further improve vascularization of the subcutaneous polymer scaffold.

Future perspectives

Before this concept can be applied in humans, several improvements need to be made to overcome the size differences between animals and humans. Evidently, it is easier to achieve successful islet transplantation in smaller diabetic animals, such as rats and mice, compared to larger animals or humans. We showed the ability of our scaffold to achieve normoglycemia in mice and rats, but there are several issues to encounter when moving to larger animal models. As shortly explained in **chapter 6**, the scaffold can be scaled up to the size of a credit card (approximately 85 mm x 55 mm x 10 mm) that can easily be implanted under the skin of a human recipient. The vascularization process of a larger scaffold will be different from the vascularization of our SD card-sized scaffold (approximately 10 mm x 15 mm x 5 mm). Vascularization of a larger scaffold is even more important than that of the SD card-sized scaffolds, as diffusion distance of oxygen and nutrients to the core of the scaffold is larger. Therefore, to prevent loss of islets by ischemia when moving to larger animal models or humans, further research should focus on adequate vascularization of the credit card-sized scaffold.

To further improve the 3D support structure of our scaffold by mimicking the pancreatic microenvironment, extracellular matrix (ECM) components can be incorporated in the scaffold. ECM components are known to play an important role in islet biology including development, morphology, differentiation, intracellular signaling, gene expression, adhesion and migration, proliferation, secretion, and survival [48,49]. However, after islet isolation the ECM is damaged. Previous studies have shown that addition of ECM components, such as collagen IV, fibronectin, or laminin derived recognition sequences, to a scaffold improved islet function and viability [50-53].

Besides the lack of a sufficient transplantation site, the low availability and suboptimal quality of cadaveric donor pancreata for islet isolation also contribute to the low long-term success rates. Therefore, an alternative cell source is needed to improve the application for

cell transplantation in type 1 diabetes mellitus. During the past few years, the applicability of porcine islets [54], human embryonic stem cells [55], human induced pluripotent stem cell derived pancreatic progenitor cells [56], or other beta-islet cell preparations as cellular therapeutic has been shown. Our PDLLCL scaffold may be applicable not only for free islets but also for the alternative cell sources. Pancreatic islets are sensitive and vulnerable cells. We showed that these cells maintain functional and survive in our scaffold, which is a good indication for other cell types, such as stem cells. Furthermore, to exclude the use of immunosuppressant drugs, the scaffold can be used to transplant cells encapsulated in immunoprotective membranes. Or similar to the delivery of aFGF, a drug delivery system can be developed to incorporate immunosuppressant drugs locally in the scaffold to prevent the detrimental effects of systemic application of these drugs.

Engineering of a novel transplantation site is immensely important for the successful treatment of type 1 diabetic patients. We designed a 3D polymer scaffold that can be easily implanted under the skin, where it provides a transplantation site for islets and can cure diabetes in rodent models. However, we are aware that the relevance of our results to the clinic remains to be determined as we used immunodeficient rodent models.

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Summary

The worldwide prevalence of type 1 diabetes mellitus is still growing. Type 1 diabetes is characterized by the destruction of pancreatic beta-cells and results in impaired glucose homeostasis. Insulin injections prevent sudden death but do not prevent the development of secondary complications such as cardiovascular diseases, nephropathy, and retinopathy. Also, frequent hypoglycemia associated with intensive insulin therapy interferes with the quality of life of the patients. The side effects of insulin therapy can be prevented by pancreatic islet transplantation via infusion of islets into the portal vein. Despite its advantages, transplantation is still associated with too low long-term success rates to merit large scale application. The reasons for graft failure are considered to be multifactorial, but the portal vein as infusion route and engraftment in the liver are considered to be major contributors.

In **chapter 1**, we reviewed the issues associated with the liver as islet transplantation site such as high concentrations of immunosuppressive drugs, large numbers of natural killer T cells, and the instant blood-mediated inflammatory reaction. These issues result in loss of a large number of islets immediately after transplantation. Islets are also exposed in the liver to relative low oxygen and nutrient concentrations caused by lower vascularization degree in the liver compared to the native pancreas, resulting in impaired function and cell death. Several alternative sites in the human body were investigated for efficacy as transplantation site for islets, but none accommodate sufficient islet engraftment. Therefore, we propose in chapter 1 an artificial transplantation site for islets. A three-dimensional (3D) scaffold as artificial transplantation site is expected to facilitate revascularization to improve oxygenation, nutrient access, glucose sensing, and insulin release shortly after islet transplantation. This ultimately could lead to improved long-term success rates of islet transplantation as treatment of type 1 diabetic patients.

Optimal biocompatible biomaterials are needed for creating an a 3D scaffold as

artificial transplantation site. Biocompatibility studies on polymers for islet transplantation are usually focusing on tissue responses against the biomaterials and the influence hereof on survival of pancreatic islets. The compatibility of materials with islet survival and conditional aspects of the islet-tissue in the biomaterials has gained minor attention. To determine the influence of the functional condition of the islets that are in contact with biomaterials on the survival of pancreatic grafts, we describe in **chapter 2** the impact of the use of different enzyme preparations in islet isolation on the longevity of encapsulated rat islet grafts. Seemingly minor differences in enzyme activities in collagenase lot numbers resulted in profound statistical significant higher production of the chemokines IP-10 and Gro-alpha, lower amounts of polarized insulin-producing beta-cells, and a two-fold shorter survival period after islet transplantation. This showed the importance of optimizing the islet isolation procedure for improving the long-term success rates of islet transplantation.

Polymer characteristics, such as the polymer chemistry and hydrophilicity, can influence functional islet survival. A technology platform was developed to efficiently test the effects of poly(D,L-lactide-co- ϵ -caprolactone) (PDLLCL), poly(ethylene oxide terephthalate)/polybutylene terephthalate (PEOT/PBT) block copolymer, and polysulfone. In **chapter 3**, we demonstrated that the type of polymer influenced the functional survival of human islets. In islets cultured on PDLLCL the glucagon-producing alpha-cells and insulin-producing beta-cells contained more hormone granules than in islets in contact with PEOT/PBT or polysulfone. This was studied with ultrastructural analysis by electron microscopy during 7 days of culture. PDLLCL was also associated with statistical significant lower release of double-stranded DNA (dsDNA, a so called danger-associate molecular pattern (DAMP)) from islets on PDLLCL when compared to the other polymers. DAMPs support undesired immune responses. Hydrophilicity of the polymers did not influence dsDNA release. Islets on PDLLCL also showed less cellular outgrowth. These outgrowing cells were mainly fibroblast and some beta-cells undergoing epithelial to mesenchymal cell transition. None of the

polymers influenced the glucose-stimulated insulin secretion. As PDLLCL was associated with less release of DAMPs, it is a promising candidate for creating a scaffold for human islets. However, donor variability made it impossible to determine the magnitude of the polymer effects on islet function.

To determine the effects of the polymers on islets in the absence of large human donor variability, we repeated in **chapter 4** the studies with rat islets of standardized age, sex, and gene pool. Culture on PEOT/PBT and polysulfone profoundly disturbed function of islets, and induced severe tissue responses *in vivo*. Modification of their hydrophilicity did not change the suitability of the polymers. PDLLCL was the only polymer that promoted functional survival of rat islets *in vitro* and was associated with minor tissue-reactions after 28 days. Rat islets were transplanted in the PDLLCL scaffold in a diabetic rat model. Before islet seeding, the scaffold was allowed to engraft for 28 days to allow the tissue response to dampen and to allow blood vessel growth into the device. Islet transplantation into the scaffold resulted in normoglycemia within 3 days and for the duration of the study period of 16 weeks. PDLLCL seems to be a suitable scaffold candidate for the treatment of type 1 diabetes.

The primary aim of **chapter 5** was to study the quality of metabolic control of an allogenic rat islet graft transplanted into a subcutaneous placed PDLLCL scaffold in a diabetic rat model. The glucose clearance of this prevascularized and replaceable scaffold was compared with an islet graft transplanted under the kidney capsule. Scaffolds were subcutaneously implanted four weeks before transplantation to allow vascularization. Normoglycemia (nonfasting blood glucose < 10 mmol/L) was restored within 1 week in both the kidney capsule and the scaffold group. In both groups, normoglycemia was maintained till the end of the study. However, nonfasting blood glucose levels were more tightly regulated in the kidney capsule group as more fluctuations were observed in the scaffold group. The oral glucose tolerance test, which was performed between 8 and 10 weeks after transplantation, showed that glucose was cleared more efficient in recipients of an islet grafts under the kidney

capsule as glucose was metabolized with a prompt increase of plasma insulin levels compared to recipients under de skin, but no differences were found in islet survival between both groups. Remarkably, this difference in insulin regulation resulted in more weight gain in recipients of an islet graft in the scaffolds. Our data demonstrate that a subcutaneous, prevascularized PDLCL scaffold can obtain metabolic control after allogenic rat islet transplantation in a diabetic rat model. The quality of this metabolic control seemed to be slightly less efficient than after transplantation under the kidney capsule. This difference is probably the consequence of differences in speed and amount of blood supply to islets under the skin and under the kidney capsules. However, the observed efficacy is still acceptable suggesting that our scaffold can be used to create transplantation site for pancreatic islets.

In the rat studies we applied a dose of 10 μ l of islet tissue, which is the equivalent of the endocrine volume of the rat pancreas. In **chapter 6** we tested the efficacy of the PDLCL scaffold by transplanting three dosages of rat islets, i.e. 400, 800, and 1200, into the scaffold in diabetic nude mice. Islet transplantation under the kidney capsule served as control. Transplantation of 800 and 1200 islets into the scaffold reversed diabetes in respectively 80 and 100% of the mice within 6.8 – 18.5 days post transplant. With the marginal dosage of 400 islets 20% of the mice became normoglycemic. The glucose tolerance test showed major improvement of the glucose clearance in the scaffold groups compared to diabetic controls. However, the kidney capsule was slightly more efficacious. All mice receiving 800 and 1200 islets became normoglycemic within 2.5 days and glucose tolerance was statistical significantly improved with respect to the diabetic controls. Transplantation of 400 islets under the kidney capsule reversed diabetes in 40% of the mice. Our findings demonstrate that the prevascularized PDLCL scaffold protects viability and function of islets in the subcutaneous site. Transplantation of 800 islets into the scaffold already results in normoglycemia.

A conceivable way to further improve the efficacy of the PDLCL scaffold is by stimulating vascularization in order to reduce the distance between the engrafted islets and

blood vessels facilitating faster ingrowth into the islets. In **chapter 7** we address this by developing a growth factor delivery system to improve the vascularization of our subcutaneous polymer scaffold before islet transplantation. The angiogenic capacity of the proteins platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), acidic fibroblast growth factor (aFGF), and basic FGF (bFGF) were compared in a tube formation assay and the release kinetics and loading capacity of different liposome compositions were tested. By this stepwise approach, aFGF and L- α -phosphatidylcholine / cholesterol liposomes were selected to improve vascularization of our scaffold. Two dosages of growth factor liposomes, i.e. 0.5 and 1.0 μg aFGF, were administered for a month after which efficacy of the subcutaneous scaffold as transplantation site for an islet graft was compared to untreated scaffolds. After transplantation of 800 islets in the aFGF treated scaffolds, we observed enhanced efficacy in obtaining normoglycemia in the immediate post-transplant period compared to the untreated scaffolds. However, on the long-term blood glucose levels of the aFGF treated animals started to increase to diabetic levels, which did not happen in the untreated scaffolds. These results suggest that injections with aFGF liposomes do improve the immediate restoration of blood glucose levels but do not facilitate the long-term engraftment of transplanted islets in our subcutaneous scaffold.

In this thesis, we have shown the importance of a stepwise selection procedure to test the compatibility of materials with islet survival and conditional aspects of the islet-tissue in the biomaterials. We designed a 3D PDLCL scaffold that can be easily implanted under the skin, where it provides a transplantation site for islets and can cure diabetes in rodent models. Ultimately, the novel islet transplantation site might contribute to higher success rates and more widely application of islet-transplantation for the treatment of diabetes.

Nederlandse samenvatting

Wereldwijd neemt het aantal patiënten met type 1 diabetes nog altijd toe. Type 1 diabetes ontstaat door een auto-immuun reactie gericht tegen de beta cellen in de alveesklier, dit resulteert in een verstoorde glucose huishouding doordat de beta cellen niet meer in staat zijn om insuline te produceren. Deze patiënten worden behandeld met insuline injecties, echter wordt intensieve insuline therapie geassocieerd met frequente periodes van hypoglykemie. Hierdoor wordt de kwaliteit van leven van deze patiënten aangetast en wordt de ontwikkeling van secundaire complicaties zoals cardiovasculaire ziektes, nefropathie en retinopathie niet voorkomen. Deze bijwerkingen van intensieve insuline therapie kunnen worden voorkomen door transplantatie van de eilandjes van Langerhans via infusie in de poort ader. Ondanks de voordelen van transplantatie is het succes op lange termijn laag waardoor het nog niet op grote schaal kan worden toegepast. Veel eilandjes overleven de transplantatie niet doordat ze in de lever bloot gesteld worden aan allerlei condities. Dit suggereert dat de lever niet geschikt is als transplantatie plaats.

In **hoofdstuk 1**, beschrijven we de condities in de lever zoals de hoge concentratie van immunosuppressieve medicijnen, grote aantallen natural killer T cellen, en de instant blood-mediated inflammatory reaction. Daarnaast bevat de lever een relatieve lage dichtheid aan bloedvaten in vergelijking met de alveesklier, hierdoor worden de eilandjes bloot gesteld aan relatief lage hoeveelheden zuurstof en andere voedingsstoffen. Al deze factoren zorgen voor het verlies van grote aantallen eilandjes onmiddellijk na transplantatie. Verschillende alternatieve transplantatie plaatsen in het menselijk lichaam zijn onderzocht, maar er lijkt geen plaats te zijn die voldoet voor succesvolle eilandjes transplantatie. Daarom beschrijven wij een artificiële transplantatie plaats voor eilandjes van Langerhans. Een driedimensionale (3D) scaffold als artificiële transplantatie plaats zal de ingroei van bloedvaten naar de eilandjes ondersteunen waardoor de zuurstof toevoer, beschikbaarheid van voedingsstoffen, glucose

gevoeligheid, en de insuline afgifte direct naar transplantatie verbeterd wordt. Uiteindelijk zal dit leiden tot verbeterde lange termijn succes van eilandjes transplantatie als behandeling van type 1 diabetes.

Optimale, compatibele biomaterialen zijn nodig voor het creëren van een 3D scaffold als artificiële transplantatie plaats. Biocompatibiliteit studies over polymeren voor eilandjes transplantatie focussen meestal op de weefsel reactie tegen het biomateriaal en het effect hiervan op de overleving van eilandjes van Langerhans. Weinig onderzoek wordt gedaan naar het effect van het biomateriaal direct op eilandjes overleving en de conditie van eilandjes zelf. In **hoofdstuk 2** beschrijven we het effect van het gebruik van verschillende enzym preparaties tijdens eilandjes isolatie op de overleving van een ingekapselde transplantaat. Ogenschijnlijke kleine verschillen tussen de collagenases in enzym activiteit zorgt al voor een statistisch significant hogere productie van de chemokines IP-10 en Gro-alpha, beta cellen bevatten minder insuline en een halvering van de overlevingsperiode na transplantatie. Dit geeft duidelijk het belang aan van een geoptimaliseerde isolatie procedure voor verbetering van de lange termijn overleving van eilandjes na transplantatie.

De eigenschappen van een polymeer, zoals de chemische samenstelling en hydrofiliteit, kunnen de functionele overleving van eilandjes beïnvloeden. Een technologie platform was ontwikkeld om efficiënt de effecten van poly(D,L-lactide-co-ε-caprolactone) (PDLLCL), poly(ethylene oxide terephthalate)/polybutylene terephthalate (PEOT/PBT) block copolymeer en polysulfone te testen. In **hoofdstuk 3** tonen wij aan dat het type polymer invloed heeft op de functionele overleving van humane eilandjes. De glucagon producerende alpha cellen en de insuline producerende beta cellen van eilandjes gekweekt op PDLLCL bevatten meer hormoon granules dan eilandjes gekweekt op PEOT/PBT of polysulfone. Deze ultra structuren zijn geanalyseerd met behulp van elektronen microscopie na 7 dagen kweken. Verder werd PDLLCL geassocieerd met een statistisch significant lagere secretie van dubbelstrengs DNA (dsDNA, een zogenoemd danger-associate molecular pattern (DAMP))

in vergelijking met de andere polymeren. DAMPs ondersteunen ongewilde immuun reacties. De hydrofilititeit van de polymeren had geen invloed op de secretie van dsDNA. Verder was er minder cel uitgroei wanneer eilandjes gekweekt werden op PDLCL. Deze uitgroeiende cellen werden getypeerd als voornamelijk fibroblasten en een aantal beta cellen die epitheliaal naar mesenchymaal cel transitie onder gingen. Geen van de polymeren had een effect op de glucose gestimuleerde insuline secretie. PDLCL is een veel belovende kandidaat voor het creëren van een scaffold voor humane eilandjes omdat PDLCL geassocieerd werd met verminderde DAMP secretie. Echter zorgde de grote variatie tussen de donoren ervoor dat het onmogelijk was om de omvang van het effect op de functie van eilandjes te bepalen.

In **hoofdstuk 4** hebben we de experimenten herhaald met ratten eilandjes. Deze ratten waren gestandaardiseerd voor leeftijd, geslacht en genen om de effecten van de polymeren op de eilandjes te kunnen bepalen zonder de aanwezigheid van de grote donor variatie. Het kweken van eilandjes op PEOT/PBT en polysulfone resulteerde in sterk verstoorde eilandjes functie en *in vivo* in een heftige vreemd lichaamsreactie. Modificatie van de hydrofilititeit veranderde niks aan deze resultaten. PDLCL was het enige polymeer dat functionele overleving van ratten eilandjes ondersteunde *in vitro* en ook werd geassocieerd met een minimale weefsel reactie na 28 dagen van implantatie. Vervolgens hebben we ratten eilandjes getransplanteerd in een PDLCL scaffold in een ratten model met geïnduceerde diabetes. De scaffold werd subcutaan geplaatst 28 dagen voordat de eilandjes werden getransplanteerd om de weefsel reactie te laten afzwakken en bloedvaten de kans te geven om de scaffold in te groeien. Eilandjes transplantatie in deze scaffold resulteerde in normoglykemie binnen 3 dagen en dit werd behouden tot het einde van de studie 16 weken later. PDLCL lijkt een geschikte kandidaat voor een scaffold ter behandeling van type 1 diabetes.

De kwaliteit van de metabole controle door allogene ratten eilandjes getransplanteerd in een subcutane PDLCL scaffold in een ratten model met geïnduceerde diabetes werd onderzocht in **hoofdstuk 5**. De glucose opname snelheid door de eilandjes in de

gevasculariseerde en vervangbare scaffold werd vergeleken met eilandjes getransplanteerd onder het nierkapsel. Ook hier werden de scaffolds subcutaan geïmplantéerd vier weken voor transplantatie om de ingroei van bloedvaten te faciliteren. Binnen 1 week waren de dieren van zowel het nierkapsel als de scaffold groepen normoglykemisch (bloed glucose < 10 mmol/L). In beide groepen werd normoglykemie behouden tot het eind van de studie. Echter waren de bloed glucose levels in de nierkapsel groep preciezer gereguleerd aangezien in de scaffold groep veel meer fluctuaties in bloed glucose werd gemeten. Tussen 8 en 10 weken na transplantatie werd de functie van het transplantaat getest met behulp van een orale glucose tolerantie test. Uit de test bleek dat de glucose opname in de nierkapsel groep efficiënter was dan in de scaffold groep. De nierkapsel groep liet een snelle toename in insuline in het bloedplasma zien ten opzichte van de scaffold groep. Door deze toename werd glucose snel gemetaboliseerd. Er werden geen verschillen gevonden tussen beide groepen in de overleving van eilandjes. Opmerkelijk was dat het verschil in insuline regulatie resulteerde in meer gewichtstoename in de dieren die eilandjes ontvingen in de scaffold. Onze data laat zien dat een subcutaan, gevasculariseerde PDLCL scaffold zorgt voor metabole controle na allogene ratten eilandjes transplantatie in een ratten model met geïnduceerde diabetes. De kwaliteit van de metabole controle in de scaffold groep is wel iets minder efficiënt dan na transplantatie onder het nierkapsel. Dit verschil kan verklaart worden door de verschillen in snelheid en hoeveelheid bloed toevoer naar eilandjes onder de huid en onder het nierkapsel. Ondanks het verschil in efficiëntie kan er geconcludeerd worden dat onze scaffold gebruikt kan worden als transplantatie plaats voor eilandjes van Langerhans.

In de bovenstaande ratten studies hebben we gebruik gemaakt van 10 µl eilandjes weefsel, dit is het equivalent van het endocriene volume van een ratten alveesklier. In **hoofdstuk 6** hebben we de efficiëntie van de PDLCL scaffold onderzocht door drie verschillende doseringen ratten eilandjes, namelijk 400, 800 en 1200 eilandjes, te transplanteren in naakte muizen met diabetes. Waar 1200 eilandjes het equivalent is van

het endocriene volume van een muis alveesklier en transplantatie van eilandjes onder het nierkapsel fungeerde als controle. Transplantatie van 800 en 1200 eilandjes in de scaffold resulteerde in normoglykemie in respectievelijk 80 en 100% van de muizen in 6.8 – 18.5 dagen na transplantatie. Met de marginale dosering van 400 eilandjes werd 20% van de muizen normoglykemisch. De glucose tolerantie test liet een enorme verbetering van de glucose opname zien in de scaffold groep in vergelijking met controles met diabetes. Echter, bleek transplantatie onder het nierkapsel efficiënter. Alle muizen die 800 en 1200 eilandjes ontvingen onder het nierkapsel werden normoglykemisch binnen 2.5 dag en de glucose tolerantie was statistisch significant verbeterd ten opzichte van controles met diabetes. Transplantatie van 400 eilandjes onder het nierkapsel resulteerde in 40% van de muizen in normoglykemie. Onze bevindingen laten zien dat de gevasculariseerde, subcutane PDLCL scaffold eilandjes overleving en functie beschermd. Transplantatie van 800 eilandjes in de scaffold resulteert al in normoglykemie.

Stimulatie van de vascularisatie, zodat de afstand tussen de eilandjes en bloedvaten verkleind wordt voor verbetering van de toevoer van nutriënten, kan er voor zorgen dat de effectiviteit van de PDLCL scaffold wordt verhoogt. In **hoofdstuk 7** wordt de ontwikkeling van een groei factor afleveringssysteem beschreven om de vascularisatie van onze subcutane scaffold te verbeteren voordat eilandjes transplantatie plaats vindt. De capaciteit om bloedvaten groei te stimuleren van platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), acidic fibroblast growth factor (aFGF) en basic FGF (bFGF) zijn vergeleken in een tube formation assay en het afgifte patroon en laad vermogen van verschillende liposoom composities werd getest. Doormiddel van deze stapsgewijze aanpak, zijn aFGF en L- α -phosphatidylcholine / cholesterol liposomen geselecteerd voor verbetering van de vascularisatie van onze scaffold. Twee doseringen van groei factor liposomen, 0.5 en 1.0 μ g aFGF, zijn wekelijks gedurende één maand toegediend waarna de effectiviteit van de subcutane scaffold als transplantatie plaats voor eilandjes vergeleken is met onbehandelde

scaffolds. We observeerde een verhoogde effectiviteit in het behalen van normoglykemia onmiddellijk na transplantatie van 800 eilandjes in de aFGF behandelde scaffolds. Echter, op lange termijn namen de bloed glucose levels van de aFGF behandelde dieren weer toe tot diabete levels, wat niet gebeurde in de onbehandelde scaffold groep. Deze resultaten suggereren dat injecties met aFGF liposomen onmiddellijk de bloed glucose levels herstellen maar dat deze injecties niet het succes op lange termijn faciliteren.

In dit proefschrift laten we zien hoe belangrijk een stapsgewijze selectie procedure is voor het testen van materialen voor de overleving en functioneren van eilandjes van Langerhans. Wij hebben een 3D PDLCL scaffold ontwikkeld welke gemakkelijk onder de huid geplaatst kan worden, waar het de transplantatie van eilandjes ondersteunt en zorgt voor genezing van diabetes in dier modellen. De ontwikkeling van een nieuwe transplantatie plaats voor eilandjes draagt bij aan wereldwijde, succesvolle behandeling van type 1 diabetes.

Curriculum vitae

The author of this thesis, Alexandra Maria Smink was born on the 7th of June 1989 in Oosterwolde, The Netherlands. She completed her high school education at the Stellingwerf College (Oosterwolde) in 2007. That same year she started studying Biology at the University of Groningen. She obtained her bachelor's degree with the major Biomedical Sciences in 2010. Thereafter, she continued with her master in Biomedical Sciences. During her master she conducted two research projects. The first project was at the Pathology department of the University Medical Center Groningen (UMCG) under the supervision of Prof. JL Hillebrands. She studied the role of vascular progenitor cells in the development of macrovascular disease in type 2 diabetes mellitus. The author received funding from the Dutch Kidney Foundation (Kolff student researcher), the University of Groningen (Marco Polo fund), and the Groninger Universiteitsfonds to perform a second research project at the Immunology and Stem Cell Laboratory of Monash University (Melbourne, Australia). Under the supervision of Prof. S. Ricardo she investigated the capacity of induced pluripotent stem cell derived podocyte progenitors to participate in kidney development. In 2012 she obtained her master's degree and started with her PhD project. This resulted in this thesis about engineering an artificial transplantation site for the transplantation of pancreatic islets to treat type 1 diabetes mellitus under the supervision of Prof. P. de Vos at the Medical Biology department of the UMCG. The research was in collaboration with academic research groups, biotechnology companies, and the Dutch Diabetes Research Foundation, all united in the Diabetes Cell Therapy Initiative (DCTI). The author received further funding from the Jan Kornelis de Cock-stichting (January 2016) and a JDRF short-term fellowship for discovery consortia grant (March 2015).

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