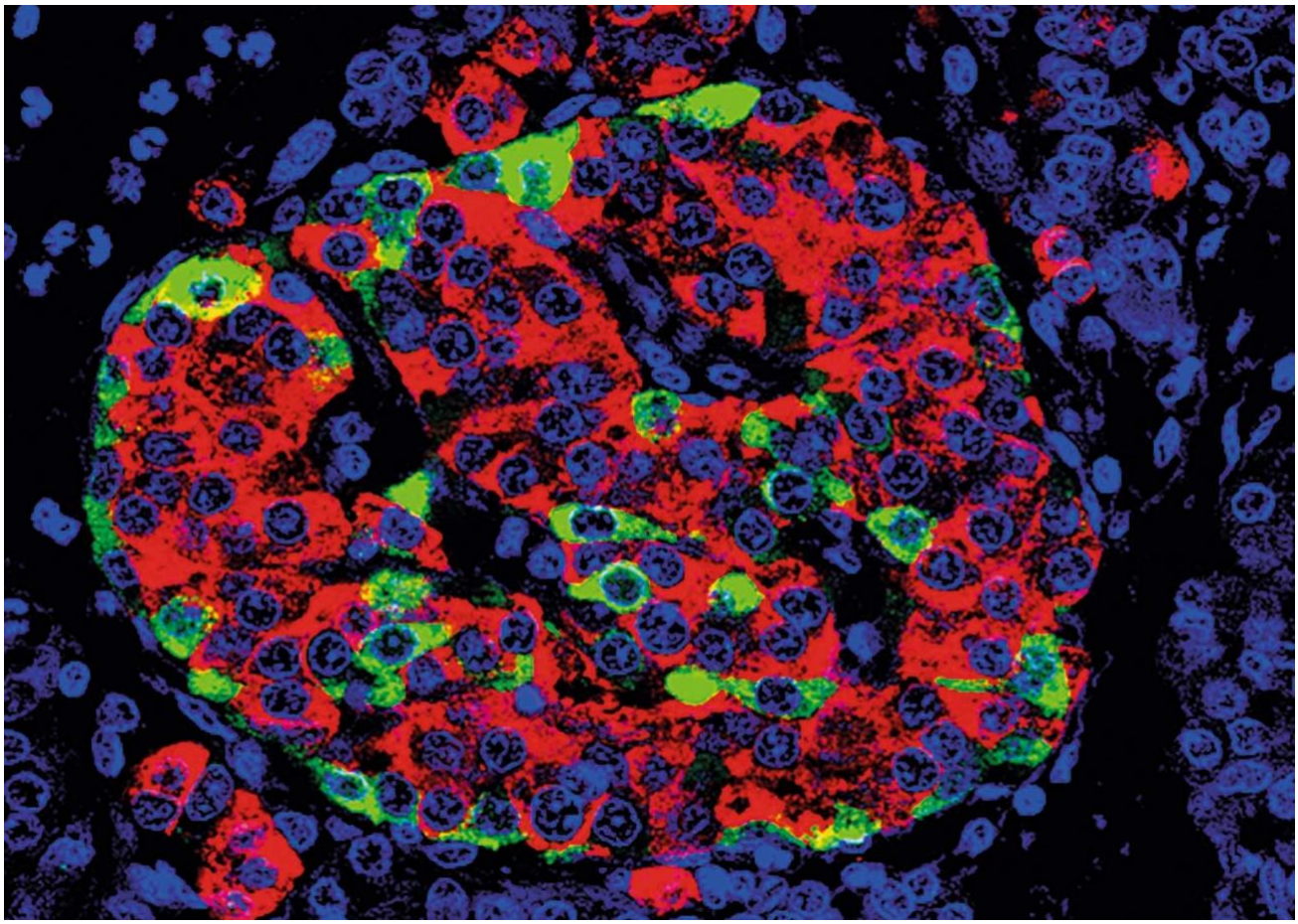
Pre-clinical research models for regenerative therapies in diabetes mellitus type one

A review and comparison of three current regenerative research models



Current research models for regenerative therapies in diabetes mellitus type one

Figure 1. *An immuno-fluorescent staining of an islet of Langerhans of the pancreas, containing insulin producing cell clusters.*[1]

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Abstract

In diabetes type 1, the yet incurable variant of diabetes mellitus, patients are unable to overcome the auto-immune nature of the disease. Donor islet transplantation was a breakthrough in the treatment of these patients, but this therapy has shown to be far from optimal. Therefore, regenerative models are currently exploited for their utility as new diabetes therapies. In this thesis, three regenerative research models on diabetes type 1 are reviewed and compared. All three models show the capability of developing insulin-producing cells in vitro and in vivo. Pluripotent stem cell models could allow patient-derived cells to develop into functional beta-cells, but their safety and stability in vivo is questionable. Organoids are superior to stem cell models regarding maturation, cell-matrix interactions and genomic stability that is suggested in adult-stem cell derived organoids. However, just like in stem cell models, in vivo acceptance of the graft is not guaranteed, since immune-system reactions towards the implanted cells are highly likeable to appear. Another research model is the bio-artificial pancreas, designed to escape an immune-system attack towards insulin-producing cells. This improvement makes the bio-artificial pancreas the most promising model, even though all three research models manage to control diabetes in mice in vivo. However, inadequate production of insulin by cultured cells, vascularization of cells for sufficient oxygen flow and the human immune-system reaction towards (encapsulated) cells remain recurrent problems in all three research models. With these problems solved, the discussed regenerative therapies could be used to improve the quality of life in diabetes type 1 patients significantly.

Inhoudsopgave

Introduction

The pancreas has both exocrine and endocrine functions, involved in digestion and the secretion of hormones respectively.[2] The digestive capabilities of the pancreas were first observed in the 19th century by Claude Bernard, where after Paul Langerhans in 1869 first described the islet of Langerhans.[3] These islets contain endocrine cells, which secrete hormones that regulate pancreatic function itself, gastrointestinal motility and carbohydrate mechanism.[2,4] Among these hormones are insulin and glucagon. Insulin from bèta-cells is responsible for lowering the blood glucose level, while glucagon secreted by alpha-cells increases it. Insulin and glucagon are closely regulated in response to ingested food.[2,4]

Insulin is formed and released by beta-cells as a response to food ingestion, when gastro-intestinal hormones are released and the plasma glucose level rises. [2] It works on the insulin receptor to promote glucose uptake and storage by the increasement of glucose uptake channels on the membrane, mainly in liver and muscle cells.[2,5] Insulin is therefore important for the glucose homeostasis and for reducing the hunger and increasing metabolism.[2,5]

In diabetes mellitus, a common metabolic imbalance in these pancreas hormones[4], the bèta-cells of the pancreas are destructed, causing an insufficient level of insulin secretion.[2] This disease knows two pathological types. Type 1 diabetes is a result of an auto-immune destruction of the insulin-producing cells, where auto-antibodies and an inflammatory infiltrate has infiltrated the islets of Langerhans.[6] The bèta-cells undergo destruction and there is insufficient secretion of insulin, disturbing the uptake of glucose from the plasma.[2,6] In type 2, pancreatic bèta-cells gradually become less functional due to insulin insensitivity, mostly caused by high glucose intake and unhealthy lifestyle. This can cause bèta-cell dysfunction and insufficient secretion of insulin, as in diabetes type 1.[2,7] Hyperglycemia is the result of insufficient glucose uptake[5] and can cause serious effects, mostly on the cardiovascular system.[8] The current treatment for diabetes type 1 and 2 is focused on controlling the hyperglycemia by administrating exogenous insulin.[9] However, diabetes 1 patients are unable to overcome the autoimmune nature of the disease.[6] In diabetes type 1, exogenous insulin is often combined with other drugs, for example insulin analogues or immune-suppressing drugs. Unfortunately, these therapies are not always successful in controlling the disease and its complications.[6] Besides that, type 1 diabetes has not proven to be fully responsive to all the therapeutic interventions, immune-suppressing drugs are only partly successful in controlling the disease[6] and can induce many side effects.[10,11] Another difficulty with diabetes type 1 is that the pathogenesis is known to be heterogenous.[6,12] Thereby, the pattern and aggression of auto-immune mediated beta-cell attack can vary between patients[13], and spontaneous periods of remission are sometimes seen.[14,15]

Because of the current incurability of diabetes type 1, a lot of research has been conducted to examine the pathology of diabetes to eventually find a cure for this disease.[16] A breakthrough in this research was the transplantation of Langerhans islets from a donor into diabetic rats, where the transplanted islets contributed on partial remission of the diabetes.[17] Unfortunately, research on these transplantations in humans lead to the conclusion that only low numbers of the transplanted allografts were functional and only a few of the participants achieved insulin independence for longer than 1 year.[18] Besides that, recipients had to face the risk of the transplantation surgery and endure immuno-suppression for prevention of rejection of the donor-islets.[18–20] These immunosuppressive drugs could also be harmful to the patient, possibly leading to renal nephropathies.[18,21] Moreover, the transplanted islets could also activate an additional auto-immune response, leading to reduced functionality of

these transplanted islets. The transplanted islets are attacked in a similar way as the individuals own islets, thereby leading to a recurrent diabetes and its symptoms.[22] An additional problem is the shortage of donor islets, with only a few thousand donors a year.[20] Besides that, not all the harvested donor islets are fully functional and usable for transplantation.[21]

Transplantation of donor-islets has been a step forward in regenerative research for diabetes type 1, but is far from optimal, for the many reasons stated above.[18–20] For this reason, research is now focusing on developing other regenerative therapeutic methods in patients with this type of diabetes. The evolvement of pluripotent stem cell techniques led to more prospects on individual therapy, using the patient's own cells to develop beta-cells could avoid an immune-attack since these stem cells are not recognized as foreign body cells. Organoids are another regenerative research model[23]. These are tissue-resembling structures, formed due to self-organization of cells in a 3D culture.[19,24] Next to organoids, encapsulation of pancreatic cells can yield a so-called bio-artificial pancreas, as seen in figure 2.[25] These three new regenerative therapies could improve the management of diabetes, restoring the patient's insulin metabolism and avoiding the need of immunosuppressive therapy. Therefore, it is relevant to compare these models and state their impact on diabetes research. In this thesis, three pre-clinical regenerative research models for diabetes type 1 will be reviewed and compared, thereby visualizing the current progress of regenerative medicine research on diabetes type 1.

Research models on regenerative medicine in the pancreas

Stem cell models

There are several types of stem cells, but mainly two types of them have been used in regenerative diabetes research.[26] Embryonic pluripotent stem cells (hESC), derived from the inner cell mass of the human or animal blastocyst embryo, can differentiate into all cell types in the body.[27,28] Human induced pluripotent stem cells (hiPSC) are generated from adult somatic cells using integrating viral vectors or other genetic methods to incorporate pluripotency factors.[27] HiPSC are similar to human embryonic stem cells in morphology, proliferation and gene expression.[27] They are able to grow out to all three germ layer cells and express similar pluripotency factors as hESC.[29]

Both hESC and hiPSC 2D cultures can differentiate into pancreatic cells in vitro by a five- or six-step protocol, with stepwise adding of differentiation-stimulating factors to the culture.[26,30,31] The most important factors are activins, promoting development to endoderm tissue.[32] After activin removal, the endoderm foregut cells develop into pancreatic cells. After this cell-stage is reached, cells are often able to secrete hormones.[33,34] Immunohistochemistry can then demonstrate if human beta-cells are secreting only insulin and no other pancreatic hormones, indicating maturity of the beta-cells. Immature beta-cells are still able to develop multiple hormones and don't show a halo sign on electron microscopy imaging.[34]

The benefit of *embryonic stem cell* derived pancreatic cells is that they can be generated without genetic modification, unlike to induced pluripotent stem cells, and in large batches.[26] In vitro generation of hESC-derived beta-cells following the protocol above, yielded hormone secreting cells. Maturity was not always seen in vitro[30,34] but in vivo transplantation eventually lead to maturation of most of the beta-cells.[26,30] The in vivo transplantation of hESC in mice

was often executed by placing the cells beneath the kidney capsula[26,35], or by the deposition of cells on a matrix and the placement of this matrix in adipocyte tissue.[34] The in vivo insulin production managed to assist the mice in controlling their glucose levels, suggesting a possible intervention against the diabetes.[26,35] However, the insulin production remained lower than in normal human islet-cells.[26] This may be due to lack of supportive cells like mesenchymal or endothelial cells, which are present in the Langerhans islets around the beta-cells.[26] HESC have also shown the possibility of development to non-islet cells, which could lead to impure islet-formation and induction of necrosis and fibrosis nearby the implant. These non-islet cells included teratoma cells.[29,36] The formation of teratomas has earlier been showed spontaneously in immune-deficient mice, where embryonal stem cells lead to these non-malignant tumors in vivo in mice.[37] A difficulty of human embryonic stem cells is that the use of them bring up ethical problems, because of their origin from blastocyst embryos.[28] Besides that, because hESC are foreign cells, a similar graft rejection as in donor islet transplantation could arise, leading to the need of immunosuppressive drugs in patients.[38]

Instead of harvesting cells from blastocyst, *induced pluripotent stem cells* can be grown out of adult somatic cells.[27] The most successful protocol for these hiPSC-derived pancreatic beta-cells has not been found yet. The development of beta-cells producing insulin seemed to have succeeded, with cells expressing factors of insulin-producing cells.[29,31,39] However, variation was also seen between beta-cells from one patient, suggesting gene silencing or activation in some cells.[31,39] The evidence for the successful development of beta-cells in these papers was also not entirely solid, since morphology of beta-cells to assess their maturity sometimes remained unexamined.[29,39] More recent iPSC protocols lead to more solid evidence for the development of more functional beta-cells, where maturity was also seen on electron microscopy in most but not all cells.[26,40,41] The cells were not identical to adult beta-cells[41] but present in higher numbers and more efficient in glucose-stimulated secretion of peptide C, a protein released in the production pathway[5] of insulin.[40] In vitro as well as in vivo response to glucose was observed[26,41] and the beta-cells also responded to insulin releasing drugs.[41] The in vivo transplantation site was located, similar as in hESC, under the kidney capsula.[26,29,41]
Using this protocol, iPS cells could be used as a source to obtain beta-cells from a patient-derived somatic cell[40], even from for example hair cells or urine samples, making the accessibility more patient-friendly and less ethical controversial than hESC.[42,43] When derived from the patient's somatic cells, the genome characteristics of the developed beta-cell are almost similar to the genome of the patients and a foreign body immune-attack would probably not occur following a transplantation.[44] A problem of iPSC transplantation could however be the auto-immune character of type 1 diabetes initiating an immune attack, destroying the transplanted cells, because of their autologous phenotype.[44] In allogenous islet transplantation, auto-immunity was seen towards the implanted islets, reducing their functionality.[45,46] However, the implanted cells did still manage to produce insulin, even though progressive loss of islets was observed.[45] This could suggest the possibility of cell transplant being functional for a certain amount of time, even though auto-immunity towards these cells is present. Besides the auto-immune character of diabetes being a problem, the culture system is not maximally efficient. In vitro cell cultures only show less than 30% secreting beta-cells.[26,41] Besides that, the percentages of insulin-positive cells between every culture protocol varies, suggesting the heterogeneity of hPSCs and the low efficiency of some of the culture procedures.[44,47]

Both hESC[26,30,34,35] and hiPSC[26,41] have shown to successfully produce insulin in mice, as seen in figure 3. Translation of this research to human individuals might however be difficult, since human and rodent beta-cells[48] as well as their immune systems[49] have shown differences. Maturation remains an issue in both pluripotent stem cell research models.[29,30,39] Even using the latest and most efficient protocol, not all developed cells gained maturity or cells remained relative immature to adult beta cells.[35] Cell-cell interactions between these cells and the beta-cells could be beneficial for the maturation of beta-cells[50,51], but the exact mechanisms involved in the improvement of maturation of beta-cells in vivo are unknown.[23] Besides that, the produced stem-cell derived pancreatic cells still differ in gene expression compared to normal human pancreatic cells.[26] This could be due to the culture medium, since culture-induced genomic alterations have been observed in hiPSC and hESC. The effects of these alterations in human stem cells have to be characterized and monitored in developed adult-cells.[52,53]

Stem cells are thereby difficult to generate and there is not one protocol suitable for every hPSC yet,[54] and genomic changes can accumulate in these cells, possibly leading to abnormalities.[52] In iPSC these genomic changes can be minimalized by using non-integrative factors to induce pluripotency.[55] What is also lacking in these 2D stem cell systems are surrounding cells, such as mesenchymal cells, present in normal pancreas tissue.[23] The auto-immune character of type 1 diabetes remains a difficult factor in stem cell research, with the possibility of destruction of autologous hiPSC.[44] Besides that, in hESC transplantation, normal immune-system could reject hESC grafts because of the non-autologous character of these cells.[38] The avoidance of an immune-system attack is suspected to facilitate the use of these stem cells, and research on this has been reported already, where the functionality of encapsulated hESC-derived beta-progenitor cells is assessed in humans.[56] In my expectation, stem cells could contribute to the research in diabetes therapy, only if their stability, safety and effectivity in vivo proves efficient in long-term experiments.

Organoid models

Organoids are tissue resembling structures[24] that can be generated from induced pluripotent stem cells, embryonic stem cells or adult stem-cells.[23,57] Multiple cell-types, organ-like morphology and functioning similar as the organ are key features of organoids, generated by a similar development pattern as in the real organ.[24] Self-organization of cells is a key feature and this can be generated by cells themselves by expressing several factors or cell-fate features that determine the end-stage of the cell.[24] The possibility of growing from 2D cultures into 3D tissues enables self-organization of the tissue, as seen in epithelial cells developing tubular-formed structures or villus structures similar as in the intestine.[57,58] To facilitate self-organization, cells forming organoids require an environment that allows 3D growth, such as extra-cellular matrix hydrogels.[24] Addition of laminin into the ECM matrix gel allows even more stability of this self-organization.[59,60] The use of this hydrogels led to the finding that pancreatic adult cells could grow and differentiate into hormone-secreting cell cultures, which are also able to secrete insulin.[59,60] In vitro research on organoids has been done[60–62] as well as in vivo research, via transplantation of organoids into the kidney capsule[63–65], in the pancreas[43], in the peritoneum[66] or into the testicular fat pad.[67,68]

The 3D organogenesis from *embryonic stem cells* relies mainly on FGF and Notch signaling, similar like in normal embryonal development.[62] Maturity was first not seen in the organoid

pancreas cells and mostly duct cells were present in the organoids, in vivo developing into a few endocrine cells.[62] In more recent protocols, organoids from hESC showed the in vitro capacity of insulin secretion, also as a reaction on glucose sensation.[68,69] In vivo, potential to reverse hyperglycemia in mice was shown[68], although this sometimes lasted only a short amount of time and cells still remained relatively immature.[68] The influence of an incomplete micro-environment was suspected to be the reason for immaturity and less functional insulin-secretion in organoids opposed to normal human beta-cells.[68] In the last few years multiple hESC clusters have been developed to solve the incomplete micro-environment by the addition of extra mesenchymal hESC-cultures.[66,70] In these experiments, the hESC-derived organoids were suggested to be functional regarding the glucose-induced insulin-release and their morphology and their expressing patterns, both in vitro[70] and in vivo.[66]

Organoids from *induced pluripotent stem cells* have also been generated.[43] The multipotent nature of cells within these organoids, similar as in embryonal stem cells, allows generation of self-organizing niches, which also seem to produce insulin.[43,71,72] Spontaneous in vivo 3D growth has been observed, and iPSC injected into the kidney grew out to an organoid-like structure.[73] After vivo observation, beta-cells were observed and glucose levels were seen responding to insulin secretion.[71,73] Unexpected vascularization of the organoids was seen in one investigation, but the in vivo functionality of these organoids has to be exploited before any further applications.[73] The same benefits or disadvantages as in iPSC treatment apply in these organoids, since the use of autonomous cells can be helpful to diminish the immune-reaction towards the transplant, but there is also a safety risk due to genetic manipulation. The problem of beta-cell maturation remained, as in the earlier discussed stem cell models. This is a recurrent problem in hiPSC-derived organoids, with cells sometimes resembling fetal cells instead of differentiated cells.[74]

Organoids from *adult stem cells* have also been generated. Normally, not much proliferation is seen in adult pancreatic tissue, but when damage arises in duct cells, Wnt factor signalling-pathways initiate repair and therefore cell proliferation.[65,75] Progenitor cells, located in these pancreatic ducts, are then activated and they give rise to all islet cell types including beta-cells.[76] The pancreas tail progenitor cells in the ducts of the pancreas also contain Lgr5, a stem cell factor also found in intestinal stem cells.[77,78] This suggest that these cells are the adult-stem cell self-renewing part of the pancreas, but not much information on these progenitor cells has been obtained yet.[65] Mainly the ductal cells from these Lgr5 progenitor cells maintained growth for several months in vitro and in vivo and also gave rise to endocrine cells capable of producing insulin.[60,61,65] Since ductal cells can give rise to endocrine cells[79], even in vivo[80], the proliferation of exclusively ductal cells might not indicate the amount of endocrine cells generated.[76] However, with this Lgr5 progenitor-derived endocrine cells, no experiments were conducted on glucose-stimulated insulin-release.[60,61,65] In later protocols, ductal tree-like structures were seen forming from adult stem cells. Differentiation of cells to insulin-producing cells was seen, but the number of insulin-producing cells was low and could not obtain hyperglycemia control in vivo in mice.[64]
The advantage of these adult-stem cell derived organoids is that they are already from pancreatic origin. Since autologous human pancreas islets are often co-transplanted with ductal cells without adverse effects[64,81], the body is possibly more receptive to these cells than to hESC or hiPSC.[64] Another benefit of these stem cells is the independence of reprogramming. With organoids from adult stem cells, long-term in vitro and in vivo expansion and genomic

stability is suggested.[63,82] The protocols in these organoids did however not yet obtain cells with optimal insulin-production[64] and are complicated.[63] Also, the results above were obtained with a few samples of donor pancreatic tissue in immunodeficient mice. For a better translation to humans, adult stem cells of many different samples of pancreas tissue have to be observed. Thereby, beta cells from type 1 diabetes patients might be difficult to obtain, since beta-cell mass continues to decrease during the disease[83], probably including stem cell populations.[23] Also, diseased tissue may eventually lead to divergent organoid formation.[84]

The organoid research on pancreatic endocrine cells is still at its start, but some relevant experiments have proven the development of insulin-secreting cells[60,64–66,70,73], suggesting the possibility to eventually lead to a new source of beta-cell transplantation[24,60]. The benefit of organoids is that they expand rapidly, as seen in the formation of ductal structures in vivo after a few months.[64,65] Organoids are also relatively simple to maintain and expand[85], and they allow cell-cell or cell-matrix interactions, which could be beneficial for the maturation and differentiation of beta-cells [50,51] and cell growth and stability.[85] Yet, organoids often differ in shape, there is no uniformity between produced organoids, and size and the blood-supply of the structures in vivo is known to be difficult.[23,84,85] Vascularization is moreover a well-known shortcoming of organoids, due to their incomplete micro-environment.[74,84,85] Improving this micro-environment with additional mesenchymal-hESC cultures could improve vascularization[74], and is suggested to be beneficial for the cell culture forming the organoid.[66,70] Because pluripotent stem cells are used in these organoids, immune-rejection might also be a problem in these organoids.[38,44,86] In all the in vivo investigations, mice were therefore immunodeficient, to obtain results without the host immune-reaction and possible graft-rejection of the organoids. Therefore, immunosuppressant might still be necessary in organoid therapy.[38] The problem of the auto-immunity remains a difficulty in these organoids, similar as in stem cell models, since the immune-system could elicit an attack towards the transplanted beta-cells.[44,45] The benefit of organoids over the stem cell models is the however the long-term culture preservation and functionality and the biological structure that resembles the normal organ structure.[23,87] The matrix that is used in most of the organoid models above, is animal-derived. Therefore, the effects of this matrix type in human tissue have to be assessed.[84] Not all organoid models have yielded functional insulin-producing clusters, but they are nevertheless an improvement on the stem cell models earlier discussed.

The bio-artificial pancreas

The principle of the bio-artificial pancreas is the encapsulation of islets or functional beta-cells, shown in figure 2.[25,88] This was discovered to provide isolation from the immune-system a long time ago, thereby dodging graft rejection in patients,[89] but allowing nutrients and oxygen diffusion and produced insulin to diffuse out.[88,89] Since the bio-artificial pancreas isolates cells from the immune-system, the potential of this research model possibly exceeds the potential of the other models that do not protect insulin-producing cells against the auto-immune character[6] of the disease.

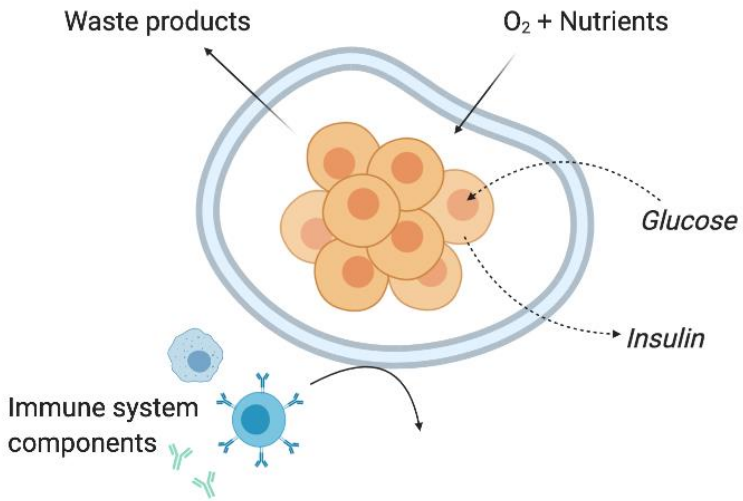

***Figure 2**. The principles of the bio-artificial pancreas. The encapsulation is permeable for oxygen and nutrients, but shields the endocrine cells inside from the immune-system. Glucose sensation can provoke insulin-production through the encapsulation.*

Many forms of encapsulation have been described[25,90–92], but the most relevant division in these encapsulation techniques is the formation of macro-capsules or the formation of microcapsules.[25,88] *Macro-capsules* consist out of multiple cells within a large encapsulation.[25,88] They can be intravascular, connected to the patients cardiovascular system, or extravascular.[25] Intravascular macro-capsules seem beneficial for the blood supply of all the encapsulated cells, but thrombosis in the blood vessel attachment location is a common problem.[88,93] Extravascular macro-capsules are not connected to the vascular system of the patients and therefore less perfused. Both intravascular and extravascular macro-encapsulations can be implanted between the skin and subcutaneous tissue or intraperitoneally.[88] The main problem in macro-capsules is their relatively large volume, causing difficulties in oxygen flow throughout the capsule and to the beta-cells inside.[25]
Micro-encapsulations are smaller, and the size and shape can influence the way they behave inside the body.[94,95] However, since their smaller size, vascularization is suggested to be more difficult.[25,96] Their size permits more transplantation sites, such as the skin or omentum.[88,97] The oldest and micro-capsulation material is an alginate capsule[89], besides other natural polymers such as agarose or collagen.[25] Non-natural polymers, such as poly-tetra-fluoroethylene (PTFE)[98] and polyvinyl alcohol (PVA)[99] are also used as encapsulation materials.

Finally, the bio-artificial pancreas can be made with different cell types or cell clusters. Human donor islets are mostly used, but since the shortage on grafts from donors[20,21], other options evolved. Porcine islets have been used in bio-artificial pancreas models[100], as well as pluripotent stem cells[101] or mesenchymal stem cells.[25,88] Safety concerns of these pluripotent stem cells, such as teratoma formation[37], are still present.[88] Thereby, the relative immaturity of these stem cells[30], could have effects on the amount of glucose-induced insulin-secretion.[88]

Since these islet encapsulation methods could mean a breakthrough in diabetes research, many encapsulation methods are explored.[25] I will discuss the three, in my opinion, most relevant bio-artificial pancreas therapeutic devices, some of whom are already in a clinical trial phase. One of the most used micro-encapsulation materials is alginate.[25,90] Alginate can be extracted from seaweed[102], is easy to fabricate[25] and can be used in different compositions, such as incorporated in hydrogel[25,88] or in small droplets.[102] Using an alginate micro-encapsulation of hESC derived insulin-producing cells, diabetic mice were independent of

immunosuppression after transplantation and their glycemic control was restored during the transplantation period.[101] In primate,s the micro-encapsulation with alginates was suggested to be successful for several months[103], but there was an also immune-reaction observed against the implanted graft. This is known as a remaining issue with alginate encapsulation[91,96,102,103], and cytokines are suspected to be responsible for this immune-attack.[91,92,103] Despite these immune-responses sometimes observed, research in mice on alginate encapsulation lead to a pilot study in type 1 diabetic patients[104] and a clinical trial.[105] In the clinical trial the safety of these alginate encapsulations seemed acceptable so far. However, the functionality was not as predicted, since the use of insulin injections was not fully eliminated in the patients.[105]

Another promising encapsulation is the TheraCyte system. This macro-encapsulation consists out of two (non-natural) PTFE membranes, with the outer membrane as a neovascularization stimulant.[25,106,107] The TheraCyte has proven to obtain normoglycemia in rodents in vivo[106,108] and the outer membrane could improve the vascularization of the device, which is a remaining problem in all forms of encapsulation.[25] Since it is known that hypoxic beta-cells will die or stop producing insulin[92,109], an improvement of the encapsulation model was suspected with higher oxygen levels reaching the beta-cells.[110] A bio-artificial pancreas improving this oxygen supply is the beta-air device, a bioreactor containing pancreas-cells with alginate sheets that receive oxygen supply.[25,111] The functionality of this beta-air device has been suggested in studies in pigs and rodents[111–113], where the immune-reaction against the device seemed minimal and glucose-induced insulin-release seemed sufficient. However, long-term effects remained unknown and, even though they are not highly cited, some investigations state that hyperoxia can also be toxic for transplanted beta-cells or insulin-release.[114–116] Following the animal studies, the beta-air device was transplanted to a human individual,[113] where no dangerous host-immune-reactions were observed, but contrary to the animal models the diabetes was not controlled. Since islet structures looked healthy, the reason of the low effectivity of insulin production is not yet known.[113]

The benefits of encapsulation are that pancreatic tissue or endocrine cells inside the encapsulation are guarded from an immune-mediated graft-rejection (figure 2), on the contrary of the earlier discussed stem cell models and organoids.[25,92,113] Several encapsulation-techniques have proven not to provoke an immune-response[104,105,113], but the implantation of the encapsulated islets is known to create a basal immune-response, which could recruit inflammatory cells and enhance an immune response.[102,117] Therefore, the encapsulation needs to effectively separate the endocrine cells inside from this response.[88] Important in this basal immune response phase, is diminishing the amount of cells protruding through the encapsulation, since this has been observed in various bio-artificial pancreas models.[88,94,118] Many protruding cells could lead to an immune-response against islet-cells inside the encapsulation[88], similar as in normal islet transplantation.

Despite some encapsulation-techniques have reached the clinical trial stage already, none of them is of certain safety or functionality to be used in clinical practice yet.[25] This has to do with the fact that in vivo successes in rodents are sometimes difficult to translate to humans.[105,113] Also, the transplantation site that is recommended varies in the literature[103,104,106,108,111], mainly due to vascularization problems that sometimes occur in encapsulations.[88,109] Although the kidney capsule is the most used transplantation site, other

sites like the peritoneum have also suggested to be successful[104], and blood vessel presence at the transplantation site seems to be the most important factor for transplant success.[109] Encapsulation of stem cells might have the potential to eventually become a non-donor[25,88], but the risks of stem cells have to be taken into account, as well as the possible graft-reaction to these allogenous stem cells (as seen in pigs).[103] The best cell-source for the bio-artificial pancreas is yet to be determined, and long-term stability and safety has not been studied enough[119]. Nevertheless, clinical trials are already conducted and for some techniques these trials seem to have beneficial predictions on the safety.[56,105,113] In my perspective, the bio-artificial pancreas is the most promising research model suggested in this thesis, having the benefit of avoiding an immune-system mediated graft rejection of the insulin-producing cells in vivo. This is the biggest problem in the other discussed research models is that eventual insulin-producing beta-cells will continue to be under attack by the immune-system, since diabetes type 1 is an auto-immune disease.[6,44]

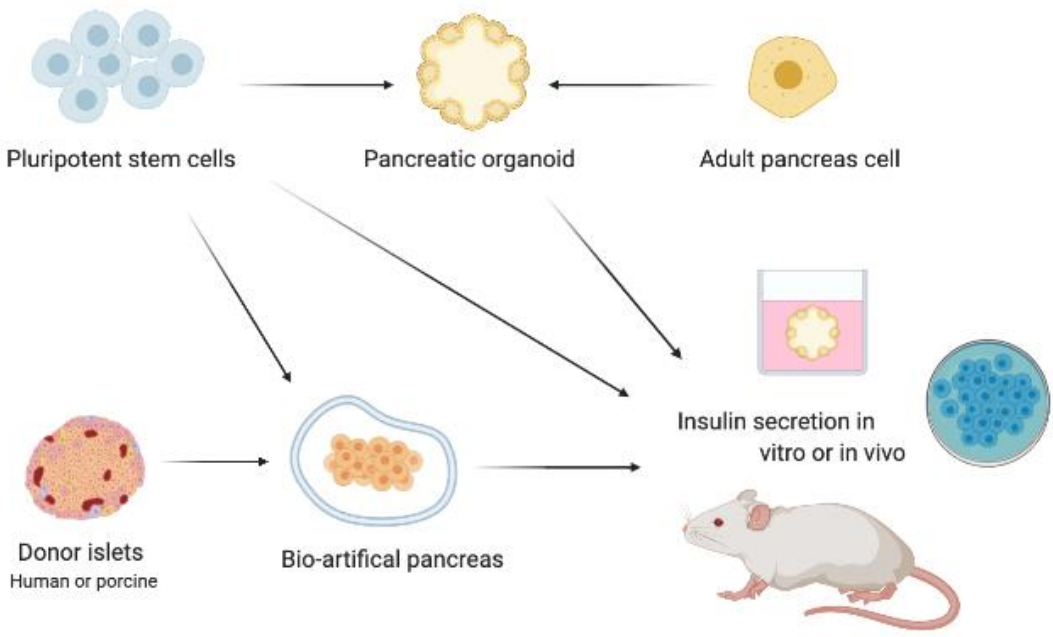

Figure 3. *A schematic representation of all three discussed regenerative medicine models in diabetes type 1. The stem cells are capable of insulin secreting cells in vitro and in vivo, or could be grown to an organoid or a bio-artificial pancreas system, also capable of insulin-production in vitro or in vivo.*

Discussion and conclusion

A quality of life improving, safe and immuno-suppressive independent is needed in the treatment of diabetes type 1.[120] The three discussed regenerative therapy models have all yielded insulin production in vitro and sometimes in vivo, schematically shown in figure 3. The bio-artificial pancreas, as seen in figure 2, shows major improvement on both the stem cell research model and the organoids, since the isolation of insulin-producing cells has suggested protection of cells from the immune-system, so that a graft-rejection of the implanted cells is prevented.[88] This is the major hurdle to overcome in diabetes type 1, since the disease is auto-immune mediated.[6] The other research models might yield insulin-producing cells, but auto-immunity against implanted cells from these models could result in destruction of these beta-cells.[45,46] This does not have to result in graft failure, since insulin production can be seen continuing during the auto-immune response to transplanted islet-cells.[45] However, immune-suppressing drugs might still be of need in the stem cell or organoid model, and in the bio-artificial pancreas model this might not be necessary.

Various types of the bio-artificial pancreas are currently in clinical trial stages[56,105], already suggesting positive outcomes regarding the safety in vivo. However, the insulin-production in some of these clinical trials was insufficient and patients still needed exogenous insulin injections.[56,105] Therefore, insulin-production of cells must be optimized and maturation problems need to be prevented by exploring the most important factors for beta-cell maturation in further research. Important to state is also that the encapsulation in the bio-artificial pancreas does not completely shield the cells inside from the external environment.[88] In several experiments, external protrusion of cells occurs, leading to immune-responses against cells inside the encapsulation[94,118]. Improvements are needed in preventing this protrusion of cells and leakage of epitopes[88], preventing immune-responses against cells inside the encapsulation, for example the use of less permeable materials for encapsulation.
Not yet mentioned, but important for eventual transplantation surgery is that a cell cluster or bio-artificial pancreas is easy implantable and safe for years in vivo, providing less surgery discomfort to the patient, in comparison to islet transplantation.[121,122] Besides that, the device has to be easily accessible to eventually replace it.[91,95] The transplantation site is suggested to important for this accessibility, since some micro-encapsulations implanted in the peritoneal cavity could not be retrieved after transplantation.[91,95]

Combinations of the discussed research models could yield more efficient and safer insulin-producing transplants. In my opinion, the optimal regenerative therapy would be an encapsulated organoid from adult-stem cells from the patient himself. Since alginate has already proven to be relatively safe in the clinical phase[105], encapsulation with this natural polymer could be safe and functional. The adult-stem cell-derived organoids have safety benefits over other pluripotent-stem cell derived organoids, for example independency of genetic reprogramming to become self-regenerative. Thereby, long-term in vivo expansion and stability is suggested[63], including genomic stability.[82] However, these adult-stem cell derived organoids did also not reach optimal insulin-production yet and further investigations have to unravel the cell or 3D models with the best insulin-production and the best stability and safety in vivo. The complicating factor in this area of diabetes 1 research is that the pathogenesis is complicated and not fully understood.[6,123] The pathogenesis is thereby heterogenous, meaning it variates between patients[6,12], complicating the design of a uniform therapy, since immune-responses to beta-cells or insulin-production by cells may vary.[6,12,13]

Whilst the in my opinion most relevant investigations have been discussed here, other investigations might have yielded other or even improved results. Because of the relatively young age of the area of research, some articles were obtained from less impactful journals. Also, next to the discussed models, other pancreatic regenerative therapies have been investigated. These include pseudo-islets, immortalized beta-cell lines forming 3D islet-like structures, other formed 3D cell line types, or other encapsulation materials or devices.[23,88] These regenerative research models have not been included and might also be of great potential to develop a new therapy for diabetes type 1.

Future perspectives

Although a lifelong therapy is far away, the extension of the implant lifetime could attribute to the clinical benefits of a regenerative intervention.[25] The fact that adult stem cells are suggested to have genetic stability and that a form of alginate encapsulation has already proven to be safe in clinical trials are hopeful indicators for the future developments in this area or research. If the problems in the research models discussed could be overcome, a new regenerative therapy could restore the hyperglycemia in patients with diabetes type 1 and therefore avoid the need of exogenous insulin or donor transplantations accompanied by immuno-suppressive therapy.

The therapeutic contribution of all three research models is not their only utility. The field of regenerative research has also contributed to clarification of the pathogenesis of diabetes type 1. By research on regenerative models, light was shed on impact of in vivo factors for maturity of beta-cells[23], the role of Wnt signalling after pancreatic damage[65] and novel aspects of pancreatic development.[62] Organoids have the greatest potential in other research areas, since they can establish 3D cultures of pancreatic cells, establishing an organ-like structure.[24] They could be designed from induced pluripotent or adult pancreatic-cells of diabetic type 1 patients, contributing to the exploration of pancreas biology, tissue homeostasis and pathophysiology in these patients.[63,84] Combined with co-cultures of micro-environments including blood vessels, stromal cells and immune cells, the organoid research model would improve, also mimicking the natural environment in the body.[84,85] Pancreatic organoids could also play a role in drug screening, since human tissue drug responses could be observed due to the fact that these organoids resemble the human organ of interest.[24,75,85] This could avoid the need of animal models[24] and contribute to eventually personalized medicine approaches in diabetes type 1.[75,85]

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