We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

19

Regenerative Medicine and Tissue Engineering for the Treatment of Diabetes

Matsumoto S, SoRelle JA and Shimoda M
Baylor Research Institute / Baylor University / Baylor University Medical Center
USA

1. Introduction

Diabetes mellitus is a disease of insulin insufficiency, which causes hyperglycemia and has both acute and chronic complications. Acute complications consist of hyperglycemic ketoacidosis and hypoglycemic episodes. Chronic complications consist of micro- and macro-angiopathies. Micro-angiopathy leads to diabetic nephropathy, neuropathy and retinopathy; macro-angiopathy leads to brain infarction, brain hemorrhage and cardiac infarction. Both acute and chronic complications significantly deteriorate the quality of life of diabetic patients and are sometimes fetal.

More than 23.7 million people suffer from diabetes in the USA today and that number will reach 44.1 million in 2034 (Huang et al., 2009). It has been demonstrated that the loss of beta-cell mass is approximately 95% in type 1 diabetic patients, 65% in type 2 diabetic patients, and even 50% in metabolic syndrome patients (Butler et al., 2003, 2007; Meier et al., 2005, 2008).

From the treatment viewpoint, diabetes is categorized into non-insulin dependent diabetes mellitus (NIDDM) and insulin dependent diabetes mellitus (IDDM). The standard therapy for the IDDM is insulin injection (Table 1). However, in the advanced phase, those patients are not able to control blood glucose levels by insulin injection. Beta cell replacement therapies including whole pancreas transplantation and islet cell transplantation are currently applied clinically for such patients (Matsumoto, 2010a) (Table 1). Unfortunately, there are more than one million IDDM patients in the United States and the number of cadaveric organs available is approximately 7,000 in each year. There is a clear donor shortage and regenerative medicine and/or tissue engineering for creating insulin-producing cells are critical to overcome this issue.

Diabetes is an excellent candidate for regenerative medicine and tissue engineering because only the beta cell with or without alpha cells is necessary to be generated for improving glycemic control. Since islet cell transplantation has already been proven to be an effective treatment for diabetic patients, generating insulin-producing cells is guaranteed for clinical effectiveness.

Indeed, tissue engineering using pig islet cells has already been clinically attempted as a bio-artificial islet transplantation for the treatment of IDDM patients (Table 1). In addition, some approaches including direct signal delivery to pancreas and neural relay of signal from liver to pancreas have been established for beta cell regeneration experimentally (Table 1).

Category	Treatment	Clinical application	Donor sources	Need for Anti-rejection drugs	Prevention of acute and chronic complications
Insulin therapy	Insulin injection	Standard therapy	N.A.	No	No
Beta cell replacement	Pancreas transplant	Standard therapy	Cadaveric or living donor	Yes	Yes
Beta cell replacement	Islet transplant	Semi-standard therapy	Cadaveric or living donor	Yes	Yes
Bio-artificial islet	Xeno islet transplant	Under clinical trial	Pig	No	Possible
Bio-artificial islet	Generated islet transplant	Not clinically available	Stem cells (ES cells, iPS cells, pancreatic stem cells)	Yes or No	Unknown
Beta cell regeneration	Direct signal delivery	Not clinically available	N.A.	Yes for type 1 diabetes	Unknown
Beta cell regeneration	Neural relay of signal	Not clinically available	N.A.	Yes for type 1 diabetes	Unknown

Table 1. Current and future treatments for insulin dependent diabetes mellitus (IDDM). Allogeneic islet transplantation for the treatment of type 1 diabetes is considered as the standard therapy in some countries. Of note, bio-artificial islet transplantation using pig islets has been already initiated for the treatment of type 1 diabetic patients. ES cells: Embryonic stem cells, iPS cells: induced pluripotent stem cells, N.A.: not applicable

Thus this field is one of the most advanced areas for regenerative medicine and tissue engineering.

In this book chapter we describe the current status of regenerative medicine and tissue engineering for creating insulin-producing cells and clinical application or the path to the clinical application of those technologies.

2. Regenerative medicine of beta cells

Regenerative medicine of beta cells consists of two major categories. The one is a replacement/implantation of alternative cell sources instead of human islets from cadaver donors. Such cell sources include embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, or other systemic stem cells. They are expanded and differentiated to insulin producing cells *in vitro*, and implanted into a diabetic patient. However, although several straightforward protocols were established, both the efficiency of *in vitro* programming and the function of derived-beta cells remain unsatisfactory. In addition, safety concerns due to inherent risks of neoplasm originating from residual stem cells remain a major hurdle (Borowiak & Melton, 2009; Ricordi & Edlund, 2008; McKnight et al., 2010). To avoid the risks of neoplasm of beta cell generation from stem cells, beta cell transdifferentiation from exocrine tissues has been performed (Minami et al., 2005). Impressively, simple culture of exocrine tissue with EGF and nicotinamide enabled the transdifferentiation of exocrine tissue to beta cells. Additionally, exocrine tissues from type 1 diabetic mice model were able to trans-differentiate into beta cells (Okuno et al., 2007). However, the efficacy was not effective yet to generate enough beta cells to reverse diabetes, and this approach requires human exocrine tissue.

Another medical approach is bona fide regeneration of islet cells/beta cells in a patient. Because there is a slow rate of beta cell turnover in the human pancreas even after injury, regenerative medicine is focusing on stimulating either beta cell replication or neogenesis. Finding a molecular intervention that can be safely used *in vivo* seems challenging but not impossible. There are ways in which neogenesis might be stimulated to expand beta cell mass using agents such as exendin-4, gastrin and epidermal growth factor (Bonner-Weir & Weir, 2005). Another method is differentiation/transdifferentiation in which either existing stem cells or differentiated cells can be programmed/reprogrammed to change their identity. To achieve this goal, most studies have used a gene induction method with a viral vector. It was demonstrated that pancreatic acinar cells might be reprogrammed in mice with injections into the pancreas of adenoviruses expressing three transcription factors, pancreatic duodenal homeobox-1 (PDX-1), musculoaponeurotic fibrosarcoma oncogene homolog A and neurogenin-3 (Zhou et al., 2008). However, gene delivery with viral vectors has shown adverse effects, which have been related to enhancer-mediated mutagenesis of genomic DNA (Hacein-Bey-Abina S et al., 2003) or immunological responses to viral proteins (Manno et al., 2006). Before these permanent or long-term side effects are fully understood and resolved, the safety of using viral vectors must be established.

In this chapter, for bona fide beta cell regeneration, we will introduce two unique methods. The first method is gene delivery using ultrasound targeted micro-bubble destruction (UTMD) technology. UTMD technology allows us to deliver genes specifically into the pancreas by using ultrasound without using viral vectors (Chen et al., 2006, 2010). The other method is activating neural relay mechanism to stimulate beta cell regeneration and insulin secretion in naïve pancreas (Imai et al., 2010). The initial signal is activated in the liver and the signal relay to pancreas via neural system. The unique signal in the liver can actually stimulate beta cell regeneration and insulin secretion in the naïve pancreas.

2.1 Ultrasound targeted micro-bubble destruction (UTMD) for gene delivery to regenerate beta cells

2.1.1 Development of UTMD for gene delivery

In order to obtain high gene expression after gene delivery *in vivo* without viral vectors, we have established an ultrasound-mediated gene transfer method named Ultrasound Targeted Microbubble Destruction (UTMD) and achieved efficient gene transfer of plasmid DNA (pDNA) *in vivo* (Chen S et al., 2006, 2010; Korpanty G et al., 2005). Delivery of pDNA does not transport toxic or immunogenic viral protein or polymer particles. UTMD is known to be a novel and potential gene delivery method *in vivo*. The mechanism of gene delivery is as follows: the microbubbles consist of lipid shell and perfluorocarbon gas on the inside. The plasmid gene to be delivered resides in the shell. After infusing the microbubbles with pDNA intravenously, they are detected in the target organ by echography. Under ultrasound exposure, the microbubbles burst and the energy creates transient pores in membranes of surrounding cells, and pDNAs are inserted into the cells (Figure 1).

UTMD has many of the desired characteristics of gene therapy including low toxicity, low immunogenicity, potential for repeated application, organ specificity and broad applicability to acoustically accessible organs.

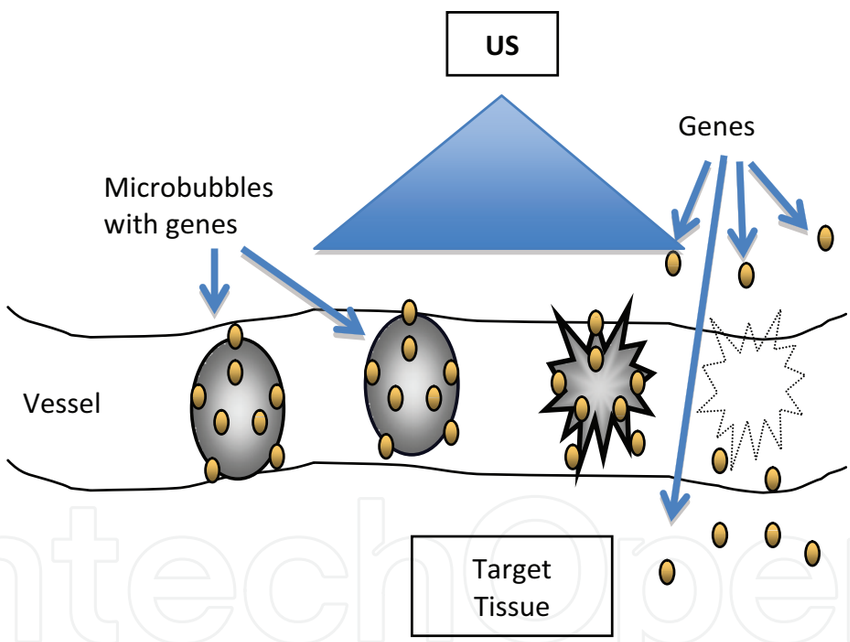


Fig. 1. The mechanism of Ultrasound Targeted Microbubble Destruction (UTMD) for gene delivery. In vessels, microbubbles can be destroyed with high mechanical index ultrasound in the target organ, and the released genes (pDNAs) pass through the vasculature, thus releasing the genes (pDNAs) into the surrounding tissue. The microbubble destruction can only happen under ultrasound

Using this technology, plasmids containing rat insulin 1 promoter (RIP)-human insulin and RIP-hexokinase I were successfully delivered to the islets of adult rats (Chen S et al., 2006). Delivery of RIP-human insulin plasmid resulted in clear increases in circulating human C-peptide and decreased blood glucose levels. Delivery of RIP-hexokinase plasmid resulted in a clear increase in hexokinase I protein expression in islets. Furthermore, delivery of RIP-

NeuroD1 by UTMD technology into streptozotocin induced diabetic rats resulted in promotion of islet regeneration in the naïve pancreas with the return of normal glucose, insulin and C-peptide levels (Chen S et al., 2010). Thus, an exciting new possibility has emerged with this technology. Much work is now underway to determine the potential clinical applications of this *in vivo* gene induction used alone or in combination with other regeneration techniques.

2.1.2 Path to clinical application of beta cell regeneration by UTMD

Even though UTMD for gene delivery is promising for beta cell regeneration, there are several issues that remain to be solved before clinical application. First of all, the safety of this technology needs to be confirmed using a large animal model. Microbubbles have been clinically used as contrast agents for ultrasound; however, the material of microbubbles for UTMD is modified for gene delivery. Therefore, it is necessary to assure the microbubbles for UTMD is safe. Furthermore, since the ability of beta cell regeneration in rodent is much higher in human or large animal (Noguchi et al., 2009b, 2010), it is important to confirm the efficacy of beta cell regeneration in large animal. The next issue is the long-term effect of beta cell regeneration by gene delivery with UTMD. In the rodent model, normoglycemia were maintained for up to 3 months (Chen et al., 2010). Identifying the mechanisms of failure to maintain long-term insulin independence is important. On the other hand, the UTMD technology is relatively easy to be applied. Therefore repeating this technology is feasible. In such case, the effect and safety of repeated UTMD need to be assessed.
When applying the UTMD method for type 1 diabetic patients, it is necessary to prevent autoimmune recurrence. Therefore, immunosuppressive drugs might be necessary to prevent the immunological rejection of regenerated beta cells.

2.2 Neural relay for beta cell regeneration in naïve pancreas

The concept of neural relay is very unique. Beta cell proliferative activity changes dynamically to meet systemic needs throughout life. One condition in which beta cell proliferation is enhanced is obesity-related insulin resistance. However, the mechanism underlying this compensatory beta cell response is not well understood.
Recently, Katagiri et al. have identified a neuronal relay, originating in the liver, which enhances both insulin secretion and pancreatic beta cell proliferation for the possible mechanism of obesity-related insulin resistance (Katagiri et al., 2009). Blockade of this neural relay in murine obesity models inhibited pancreatic islet expansion during obesity development, showing this inter-organ communication system to be physiologically involved in compensatory beta cell proliferation. They demonstrated that proliferation of pre-existing beta cells contributes to a beta cell increment by neural relay mechanism. Therefore, this neural relay system is not only for explaining the mechanism of obesity-related insulin resistance but also might be applicable for beta cell regeneration in the naïve pancreas for the treatment of diabetes.

2.2.1 Discovery of neural relay

Metabolism in different organs and tissues works in a coordinated manner. This coordinated metabolism requires inter-organ/tissues communication, therefore the communication among organs and tissues are critical for maintaining normal metabolism (Katagiri et al., 2009). It has been demonstrated that humoral factors including hormones

and cytokines play major roles. However, a number of studies have shown that unexpected metabolic phenotypes also make a contribution suggesting the presence of currently unknown metabolic communication systems.

Recently, it was demonstrated that neuronal signaling plays important roles in inter-organ metabolic communication (Yamada et al., 2006). Obesity induces insulin hypersecretion and pancreatic beta cell hyperplasia in response to insulin resistance. These compensatory responses of pancreatic beta cells prevent hyperglycemia; however, this causes hyperinsulinemia which is involved in the pathogenesis of the metabolic syndrome. To elucidate the mechanisms of these compensatory mechanisms, Katagiri et al. activated several proteins which are known to be activated in the livers of obese and lean mice. They discovered that extracellular signal-regulated kinase (ERK) plays an important role in compensatory pancreatic beta cell responses. To activate ERK in the liver, they used adenoviral gene transduction system and discovered that liver-selective ERK activation induced insulin hypersecretion and pancreatic beta cell proliferation (Fujishiro et al., 2003). These pancreatic effects of hepatic ERK activation were inhibited by either splanchnic afferent blockade with pancreatic vagus dissection or midbrain transection. This result indicated that a neuronal relay system from liver to the pancreas consists of the afferent splanchnic nerve, the central nervous system and efferent vagus (Figure 2).

Furthermore, blockage of the neuronal relay at several levels in murine obesity model inhibited pancreatic islet expansion during obesity development indicating that the neural relay played an important role in the inter-organ mechanism in compensatory beta cell responses.

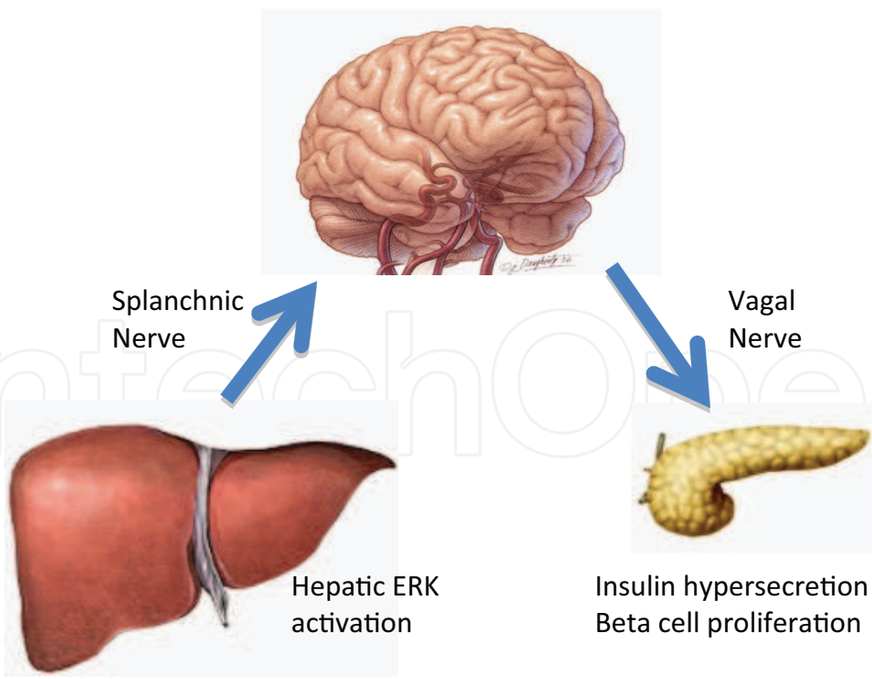


Fig. 2. The concept of neural relay for beta cell regeneration. The signal of hepatic ERK activation reaches the brain via the splanchnic nerve. Then the signal reaches the pancreas via the vagal nerve. This signal stimulates insulin hypersecretion and beta cell proliferation in the pancreas

2.2.2 Potential application of neural relay for beta cell regeneration

Regeneration of beta cells in the original pancreas could be an ideal cure for type 1 diabetic patients. Since the neural relay system can stimulate beta cell proliferation and insulin secretion, this system might be used for beta cell regeneration.

Indeed, it has been demonstrated that liver-selective activation of extracellular signal-regulated kinase (ERK) using an adenoviral gene transduction system resulted in an increase in beta cell mass and normalization of serum glucose levels in streptozotocin (STZ) induced diabetic mice and Akita diabetic mice (Imai et al., 2008). A bromodeoxyuridine (BrdU) staining study demonstrated that beta cell proliferation was the mechanism for increasing beta cell mass by neural relay (Imai et al., 2008). Therefore, stimulating the ERK pathway is a promising idea for beta cell regeneration in the naïve pancreas. However, it should be noted that they used only the STZ model and Akita diabetic mice model. STZ model is a model of insulin dependent diabetes mellitus, however, no autoimmune mechanism is involved. Therefore, although the neural relay works on the STZ model it is still unknown whether this method can be effective on the type 1 diabetic patients with autoimmune disease. In addition, the STZ model cannot completely eliminate beta cells therefore beta cell proliferation was possible. On the contrary, type 1 diabetic patients without insulin secretory ability have completely lost their beta cells. Therefore, it might be impossible to induce beta cell proliferation because no beta cells remain. In order to apply neural relay technology for type 1 diabetes, it is necessary to confirm the efficacy using NOD mice which is an autoimmune induced type 1 diabetes model. Additionally, it is necessary to examine the minimum number of remaining beta cells which will be proliferated to reverse diabetes. If a significant amount of beta cells are necessary, this method can only be applied for the patients who still have insulin secretory ability. Akita diabetic mouse is a model of type 2 diabetes. In general, patients with type 2 diabetes do not require insulin injection; therefore the indication of neural relay for type 2 diabetes should be limited. However, currently, insulin therapy is applied for type 2 diabetic patients in order to save functional beta cell mass. Therefore, neural relay therapy might be an excellent option for type 2 diabetic patients with insulin therapy.

The advantage of this method is that no stem cells such as embryonic stem (ES) cells (Thomson et al., 1998) or induced pluripotent stem (iPS) cells (Takahashi et al., 2007) or pancreatic stem cells are necessary. Therefore, the notorious problem of carcinogenesis of those stem cell derived beta cells is no longer an issue. In addition, *ex vivo* manipulation to create beta cells from stem cells is not necessary.

The possible disadvantage of this method is hepatic injury by activation of hepatic ERK. Also, the adenoviral system may not be appropriate for clinical trials because of the risk of viral infection. UTMD system for activation of hepatic ERK might be useful approach to avoid using viral transfection. Identifying the efficient activation of hepatic ERK should be the key for beta cell regeneration. When ERK stimulation for beta cell regeneration will be applied for type 1 diabetic patients, the prevention autoimmune recurrence and immunosuppressive drugs might be necessary. Furthermore, in advanced type 1 diabetes, all beta cells are destroyed as mentioned above. Therefore, it might be impossible to proliferate islets because no original islets exist. In this case, a combination of beta cell generation from pancreatic stem cells and neural relay might be useful.

3. Bio-artificial islets using pig islets

Islet transplantation using the bio-artificial islets created from alternative sources instead of human islets is very attractive to overcome the issue of severe donor shortage for the treatment of diabetes.

Very importantly, two clinical trials using bio-artificial islets consisting of piglet islets have been already clinically performed with promising results. The first trial was performed in Mexico using neonatal pig islets combined with Sertoli cells for the treatment of pediatric type 1 diabetic patients (Valdes-Gonzales et al., 2005a, 2007). Islets and Sertoli cells were put into a chamber. The Sertoli cells protected islets from immunological attacks. The other series were performed by New Zealand group for the treatment of adult type 1 diabetic patients with severe hypoglycemic episodes (Elliott et al., 2011). The New Zealand group used microencapsulated pig islets and those bio-artificial islets were transplanted into the abdominal cavity.

Impressively, both groups achieved insulin independence after transplantation in some cases without use of immunosuppressive drugs.

3.1 Bio-artificial islets with Sertoli cells

3.1.1 Preparation of bio-artificial islets with Sertoli cells and transplantation

Valdes-Gonzalez et al. performed bio-artificial islet transplantation into twelve pediatric type 1 diabetic patients (Valdes-Gonzalez et al., 2005a). Their bio-artificial islet consists of collagen-generating devices, islets from neonatal pigs and Sertoli cells. The collagen-generating devices were not considered as immune-isolation devices. Islets were isolated from male 7-10 days old piglets. The animals were bred in New Zealand in a specific pathogen-free environment in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care. After pancreas retrieval, pancreases were digested using collagenase for islet isolation (Elliott et al., 2000; Valdes-Gonzalez et al., 2005b). Islets were placed in RPMI-1640, 2% human serum albumin, 0.12% nicotinamide and 1.5mg/l ciproxine at room temperature and centrifuged at 1000 rpm for 20 min. The average islet yield was 290,730 islet equivalents (IEQ: 1IEQ=1 of 150 μm islet). The purity of islets was assessed with dithizone staining and was greater than 85% in all cases. The viability of islets assessed by acridine orange/propidium iodide staining was more than 85% in all cases. Isolated Sertoli cell-enriched testicular cells were placed in DMEM media with 0.12% nicotinamide and 1.5mg/l ciproxine. All cell preparations underwent full microbiologic screening both in New Zealand and again at the time of transplantation. Cells from ten neonatal pigs were used for each transplant. Sertoli cells and islets were mixed together immediately prior to transplantation.

Two devices were implanted subcutaneously in the upper anterior wall of the patient's abdomen under general anesthesia. The devices were left in place for two months to allow formation of vascularized collagen tissue that completely surrounded and penetrated the device.

Both islets and Sertoli cells were isolated in New Zealand and sent at room temperature in culture media to Mexico. Islets and Sertoli cells were cultured for one day before transplantation. The transplant procedure was carried out by infusing 250,000 islets with 30-100 Sertoli cells per islet. The number of islets per body weight ranged from approximately 14,000 to 21,000IEQ/kg. From 6 to 9 months later, all patients except one,

received a second islet and Sertoli cell transplant into previously implanted new devices. No immunosuppressive drug was administered at any point.

3.1.2 Clinical outcomes

Immediately upon entering the study, the patients followed a diet and exercise regimen standard for diabetic patients, with periodic weight and height measurements. The patients were instructed to record blood glucose determination seven times a day (pre- and postprandial, and 3 am). Eleven age and disease matched control group was subjected for 10 months to exactly the same exhaustive endocrine monitoring, and diet and exercise program without receiving a transplant.

In the transplanted patients following the first and more markedly after the second transplant, cluster analysis revealed that two distinct insulin requirement patterns appeared. Half of the patients had a 50% or greater reduction in their insulin requirements, and the other six patients showed a slight increase. This increase in these patients seen corresponded with that that seen in the control group. Half of the patients significantly reduced amount of insulin compared to the control group from the first month post transplant onwards. Two patients achieved insulin independence. The first one was a 15 year old female who had exogenous insulin requirements of 61 U/day before transplant and HbA1c was 13.4%. After the first transplant, she reduced her insulin requirements by 73% and after the second transplant she began to have intermittent period of 3-5 days, alternating between periods of no insulin injections, followed by periods of 1-2 U/day. This pattern last for 3 months, and HbA1c reached 9.6%. The second patient was a 16-year-old female who had exogenous insulin requirement of 55 U/day and HbA1c was 12%. Six months after the first transplant, the patient showed a 6 week reduction to 1-3 U/day and HbA1c was 6.8%. After the second transplant she was totally free of insulin for two consecutive months and her HbA1c was 6.5-7.8%. Interestingly all patients improved glycemic control after transplantation irrespective of the amount of insulin reduction. Long-term follow-up of those patients revealed that all patients have positive porcine C-peptides in urine (Valdes-Gonzalez et al., 2010).

In terms of safety, routine microbiological screening of all patients and close family have been consistently negative, although two patients exhibited transient chimerism as evidenced by porcine DNA thru polymerase chain reaction (PCR). No complication related to the surgery or to the presence of the cells has occurred at any time.

3.2 Bio-artificial islets using micro-encapsulation technology

3.2.1 Preparation of bio-artificial islets using micro-encapsulation technology and transplantation

Elliott et al. performed bio-artificial islet transplantation into adult type 1 diabetic patients with severe hypoglycemic episodes (Elliot et al., 2011). Their bio-artificial islets were made of micro-encapsulated islets from neonatal pigs. This group used the same islet isolation procedures using the same herd with Valdes-Gonzalez. The islets were micro-encapsulated using alginate. The capsule allows entering nutrients and glucose inside the capsule and passing insulin outside the capsule (Figure 3). Meanwhile, the capsule blocks antibodies resulting in protecting the islet from immunological attack (Figure 3).

The encapsulation process was a modification of the method described by Calafiore (Calafiore et al., 2006). Encapsulation material was started with raw pharmaceutical grade

alginate powder. The alginate powder was dissolved in sterile pyrogen-free deionized water over 24 to 36 hours in the dark at room temperature and 3% NaCl was added. The solution underwent multiple sequential passages through methylcellulose and polyester filters to ensure sterility. The final 1.6% solution was stored in the dark room at 4°C to avoid alginate depolymerization. The islet tissue pellet, usually amounting to a few tenths of a milliliter, was thoroughly mixed with the 1.6% alginate solution. The alginate/islet proportion was adjusted so that one capsule would contain one islet, with fewer than 5% empty capsules. The suspension was extruded through a microdroplet generator, combining air shears with mechanical pressure: the alginate droplets were collected in 1.2% $CaCl_2$ immediately turning into gel micro-beads. They were sequentially overcoated with poly-L-ornithine and an outer alginate layer.

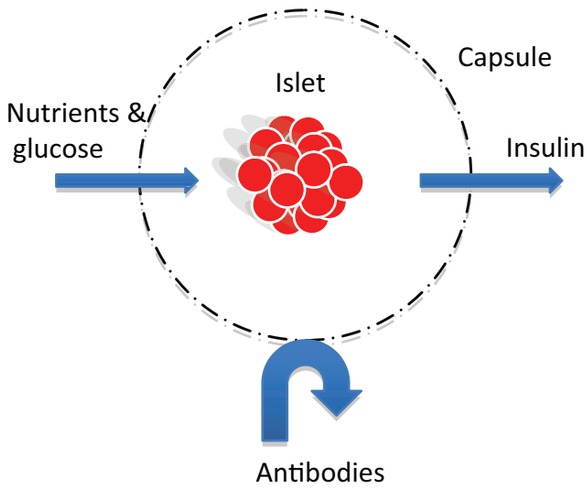


Fig. 3. The concept of microencapsulation of an islet. Nutrients, glucose and insulin can pass through the capsule, but antibodies cannot enter into the capsule

Bio-artificial islets consisted of microencaplusted neonatal pig islets were transplanted into the peritoneal cavity via a laparoscope under general anesthesia. Four patients received 10,000 IEQ/kg body weight islets and the other four patients received 15,000IEQ/kg body weight islets.

3.2.2 Clinical outcomes

In order to perform clinical trials, they gained regulatory approval from the relevant authorities after prolonged national and international consultation (Elliott et al., 2011). A national consensus on the bioethical issues was conducted and a separate national consultation on the acceptability of the science was also conducted. Approval from Medsafe the relevant department of the Ministry of Health was obtained. Eight adult patients with longstanding proven type 1 diabetes who met all inclusion and exclusion criteria, were selected on the basis of severe recurrent hypoglycemia usually with hypoglycemic unawareness.

To date, most patients have shown modest reduction in insulin dose commencing about four weeks after transplantation with reduction in HbA1c levels. Most outstanding has

been the reduction in severe hypoglycemic episodes and reduction or abolition of unaware hypoglycemia. For example, the first patient had an average of 4 episodes of unaware hypoglycemia per week, which was completely diminished after 8 weeks of transplantation. Transitory insulin independence of several months duration has been seen.
In terms of safety, evidence of xenosis in the xenotransplant recipients has been diligently sought but not found. This is reasonable given the credentials of the source herd used. No serious adverse events related to the surgery or to the presence of the cells have occurred at any time.

3.3 Future direction of bio-artificial islets

Since year 2000 after the publication of the Edmonton protocol, allogeneic islet transplantation has become popular as the treatment of type 1 diabetes (Shapiro et al., 2000). Using the Edmonton protocol, type 1 diabetic patients who had severe hypoglycemic episodes became insulin independent and free from hypoglycemic episodes after allogenic islet transplantation (Shapiro et al., 2000). The allogeneic islet transplantation has been expanded using non-heart beating donors (Markmann et al., 2003; Matsumoto et al., 2006b) and even living donor (Matsumoto et al., 2005, 2006a). However, the drawbacks of the Edmonton protocol include necessity of multiple donor organs, unstable islet isolation results, necessity of immunosuppressive drugs, difficulty of maintaining long-term insulin independence and severe shortage of donor organs (Ryan et al., 2005; Shapiro et al., 2006). Currently, we introduced new pancreas preservation (Matsumoto et al., 2002a, 2002b, 2010b) and islet isolation strategies (Noguchi et al., 2009a; Shimoda et al., 2010) and immunosuppressive therapy to improve the efficacy of islet isolation (Matsumoto et al., 2011). Now, we have a very stable islet isolation method, in addition, a single donor pancreas is enough to achieve insulin independence (Matsumoto et al., 2011). Our preliminary data demonstrated that super-high dose islet transplantation could lead to long-term insulin independence after allogeneic islet transplantation (Matsumoto et al., 2010c). However, the severe donor shortage can be never be solved by allogeneic islet transplantation alone. Bio-artificial islets using porcine islets can solve the issue of donor shortage. In addition, both bio-artificial islets with encapsulated islets and islets with Sertoli cells do not require immunosuppressive drugs. This is huge benefit of bio-artificial islet transplantation because one of the major issues of allogeneic islet transplantation is the side effects and cost of immunosuppression (Hatanaka et al., 2010).
Currently, bio-artificial islets were transplanted into the abdominal cavity or under skin. These transplant sites have unique advantages. In the case of allogeneic islet transplantation, islets were transplanted into liver. Multiple infusions of isolated islets into liver cause portal hypertension. Therefore allogenic islet transplantation has the limitation of the numbers of transplantation. In the case of bio-artificial islet transplantation, there is no risk for portal hypertension. Therefore, there is no limitation of number of transplantation. These comparisons were summarized in table 2.
Impressively, pigs in New Zealand have been maintained in a clean, non-pathogenic environment and seem suitable for clinical use. Both clinical trials of bio-artificial islets used these pigs. Expansion of the herd of pigs should be the key to enhancing the bio-artificial islet project. Islet isolation from neonatal piglets is relatively easy and stable; this is important advantage for commercialization.

	Standard Allogeneic Islet Transplantation	Advanced Allogeneic Islet Transplantation	Bio-artificial Islet Transplantation
Primary Endpoints	Preventing hypoglycemia	Insulin free	Preventing hypoglycemia
Secondary Endpoints	Insulin free Preventing diabetic complications	Preventing diabetic complications	Insulin free Preventing diabetic complications
Donor numbers	2 or more human pancreases	1 human pancreas	10 piglets pancreases
Stability of islet isolation	Not stable	Stable	Stable
Transplant site	Liver	Liver	Intra-peritoneum Under skin
Immunosuppression	Necessary	Necessary	Not necessary
Attaining insulin independence	Most likely	Most likely	Possible
Long-term insulin free	Difficult	Possible	Difficult
Long-term function	Possible	Most likely	Possible
Re-transplant	Up to 3 or 4 times	Up to 3 or 4 times	No limitation

Table 2. Comparison among the standard allogeneic islet transplantation, the advanced allogeneic islet transplantation and bio-artificial islet transplantation. Of note, bio-artificial islet transplantation has several important advantages including using alternative source, no immunosuppressive drugs and no limitation of re-transplantation

As shown Elliott et al., the bio-artificial islets can eliminate hypoglycemic unawareness, and this is the one of the major goals of allogeneic islet transplantation. Therefore, the patients

with hypoglycemic unawareness will be suitable candidates for bio-artificial islet transplantation instead of allogeneic islet transplantation in future.
More importantly, allogeneic islet transplantation can reduce or eliminate diabetic secondary complications such as diabetic nephropathy, retinopathy and neuropathy (Thompson et al., 2011). Especially, if the bio-artificial islet transplantation can also reduce or eliminate such diabetic secondary complications, this treatment will be very valuable because the real problems of diabetes are the secondary complications.
Cost effectiveness is an issue of bio-artificial islets, because maintenance of clean pigs is expensive. The system to maintain cleanness of a huge herd of pigs needs to be developed to overcome the cost issue. The major concern of bio-artificial islet transplantation is zoonosis. Especially, creating a new viral disease by xeno-transplantation must be avoided. Infection of porcine endogenous retrovirus after xeno-transplantation into immune compromised mice demonstrated the risk of the combination of immunosuppression and xeno-transplantation (van der Laan LJ et al., 2000). Therefore current bio-artificial islet transplantations have been performed without immunosuppression.
Acceptance of pig islets by patients is an emotional and highly debated issue for xeno-transplantation. Our survey of type 1 diabetic patients revealed that more than 60% of type 1 diabetic patients were willingly to accept pig islets if the treatment was effective (Hatanaka et al., 2010).

4. Conclusions

We introduced two unique gene therapies UMTD and neural relay for beta cell regenerations. Both methods are not clinically applied yet; however due to their relatively safe feature, we believe those methods can be clinically used in near future. We also described the clinical applications of bio-artificial islets using neonatal porcine islets with promising results. Most importantly, so far there are no severe adverse events. Although the results of bio-artificial islet transplantation are not as effective as allogeneic islet transplantation there are many aspects including islet isolation methods (Shimoda et al., 2011a, 2011b), islet culture methods, transplantation sites and patients' treatments, which can be improved.
Currently, diabetes is considered a non-curable disease therefore current treatments are focusing on improving quality of life and preventing diabetic complications (Takita 2011, Hatanaka 2011). However, we believe that the bio-artificial islets and/or gene therapy for beta cell regeneration will cure a majority of both types of diabetes in the future.

5. Acknowledgement

This work is partially supported by Juvenile Diabetes Research Foundation (JDRF).

6. References

Bonner-Weir S, Taneja M, Weir GC, Tatarkiewickz K, Song KH, Sharma A, & O'Neil JJ. (2000) In vitro cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci USA*. 97: 7999-8004.

Bonner-Weir S, & Sharma A. (2002) Pancreatic stem cells. *J Pathol* 197: 519-6.

Bonner-Weir S, & Weir GC. (2005) New sources of pancreatic beta-cells. *Nat Biotechnol.* 23:857-61.

Borowiak M & D.A. & Melton DA. (2009) How to make beta cells?, Curr Opin Cell Biol 21: 727–732.

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, & Butler PC. (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102-10.

Butler PC, Meier JJ, Butler AE, Bhushan A. (2007) The replication of β cells in normal physiology, in disease and for therapy. *Nature Clinical Practice Endocrinologu & Metabolism* 3: 758-68.

Chen S, Ding JH, Bekeredjian R, Yang BZ, Shohet RV, Johnston SA, Hohmeir HE, Newgard CB, & Grayburn PA. (2006) Efficient gene delivery to pancreatic islets with ultrasonic microbubble destruction technology. *Proc Natl Acad Sci USA*, 103: 8469-74.

Chen S, Shimoda M, Wang MY, Ding J, Noguchi H, Matsumoto S, & Grayburn PA. (2010). Regeneration of pancreatic islets in vivo by ultrasound-targeted gene therapy. *Gene Ther*, 17: 1411-20.

Elliott RB, Escobar L, Garkavenko O, Croxoson MC, Schroeder BA, McGregor M, Ferguson G, Beck Man N & Ferguson S. (2000) No evidence of infection with porcine endogenous retrovirus in recipients of encaplulated porcine islet xenografts Cell Transplant 9: 895-901.

Fujishiro M, Gotoh Y, Katagiri H, Sakoda H, Ogihara T, Anai M, Onishi Y, Ono H, Abe M, Shojima N, Fukushima Y, Kikuchi M, Oka Y, & Asano T. (2003). Three mitogen-activated protein kinases inhibit insulin signaling by different mechanisms in 3T3-L1 adipocytes. *Mol Endocrinol.* 17:487-97.

Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulffraat N, McIntyre E, Radford I, Villeval JL, Fraser CC, Cavazzana-Calvo M, & Fischer A. (2003) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med 348:255-6.*

Hatanaka N, Takita M, Yamaguchi T, Kami M, & Matsumoto S. (2010) Interests in beta-cell replacement therapies among Japanese patients with type 1 diabetes. *Diab Res Clin Pract* 89: e5-8.

Hatanaka N, Takita M, Yamaguchi T, Kami M, Matsumoto S. (2011) Development of a novel scale to assess the quality of life in type 1 diabetic patients for beta cell replacement therapy. *Diabetology International* in press

Huang ES., Basu A, O'Grady M, & Capretta JC. (2009) Projecting the Future Diabetes Population Size and Related Costs for the U.S. *Diabetes Care* 32: 2225-9.

Imai J, Oka Y, & Katagiri H. (2010) Identification of a novel mechanism regulating β-cell mass: Neuronal relay from the liver to pancreatic β-cells. *Islets* 1: 75-7.

Imai J, Katagiri H, Yamada T, Ishigaki Y, Suzuki T, Kudo H, Uno K, hasegawa Y, Gao J, Kaneko K, Ishihara H, Niijima A, Nakazato M, Asano T, Minokoshi Y, & Oka Y. (2008) Regulation of pancreatic beta cell mass by neuronal signals from the liver. *Science* 322: 1250-4.

Katagiri H, Imai J, & Oka Y. (2009) Neural relay from the liver induces proliferation of pancreatic beta cells. A path to regenerative medicine using the self-renewal capabilities. *Communicative & Integrative Biology* 2: 425-7.

Korpanty G, Chen S, Shohet RV, Ding J, Yang B, Frenkel PA, & Grayburn PA. (2005) Targeting of VEGF-mediated angiogenesis to rat myocardium using ultrasonic destruction of microbubbles. *Gene Ther. 12:1305-12.*

Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, Ozelo MC, Hoots K, Blatt P, Konkle B, Dake M, Kaye R, Razavi M, Zajko A, Zehnder J, Rustagi PK, Nakai H, Chew A, Leonard D, Wright JF, Lessard RR, Sommer JM, Tigges M, Sabatino D, Luk A, Jiang H, Mingozzi F, Couto L, Ertl HC, High KA, & Kay MA. (2006) Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med 12:342-7.*

Markmann JF, Deng S, Desai NM, Huang X, Velidedeoglu E, Frank A, Liu C, Brayman KL, Lian MM, Wolf B, Bell E, Vitamaniuk M, Doliba N, Matschinsky F, Markmann E, Barker CF, & Naji A. (2003) The use of non-heart-beating donors for isolated pancreatic islet transplantation. Transplantation.75: 1423-9.

Matsumoto S, Rigley T, Qualley S, Kuroda Y, Reems J, & Stevens RB. (2002a) Efficacy of the oxygen-charged static two-layer method for short-term pancreas preservation and islet isolation from nonhuman primate and human pancreata. *Cell Transplant* 11: 769-77.

Matsumoto S, Qualley S, Goel S, Hagman D, Sweet I, Poitout V, Strong DM, Robertson RP, Reems J. (2002b) Effect of the two-layer (University of Wisconsin solution-perfluorochemical plus O2) method of pancreas preservation on human islet isolation, as assessed by the Edmonton Isolation Protocol. *Transplantation* 74: 1414-19

Matsumoto S, Okitsu T, Iwanaga Y, Noguchi H, Nagata H, Yonekawa Y, Yamada Y, Fukuda K, Tsukiyama K, Suzuki H, Kawasaki Y, Shimodaira M, Matsuoka K, Shibata T, Kasai Y, Maekawa T, Shapiro J, & Tanaka K. (2005) Insulin independence after living-donor distal pancreatectomy and islet allotransplantation. *Lancet* 365: 1642-4.

Matsumoto S, Okitsu T, Iwanaga Y, Noguchi H, Nagata H, Yonekawa Y, Liu X, Kamiya H, Ueda M, Hatanaka N, Kobayashi N, Yamada Y, Miyakawa S, Seino Y, Shapiro AM, & Tanaka K. (2006a) Follow-up study of the first successful living donor islet transplantation. Transplantation. 82: 1629-33.

Matsumoto S, Okitsu T, Iwanaga Y, Noguchi H, Nagata H, Yonekawa Y, Yamada Y, Fukuda K, Shibata T, Kasai Y, Maekawa T, Wada H, Nakamura T, & Tanaka K. (2006b) Successful islet transplantation from non-heart-beating donor pancreata using modified Ricordi islet isolation method. *Transplantation* 82: 460-5.

Matsumoto S. (2010a). Islet cell transplantation for type 1 diabetic patients. *J Diabetes*, 2: 16-22.

Matsumoto S, Noguchi H, Shimoda M, Ikemoto, Naziruddin B, Jackson A, Tamura Y, Olsen G, Fujita Y, Chujo D, Takita M, Kobayashi N, Onaca N, & Levy MF. (2010b) Seven consecutive successful clinical islet isolations with pancreatic ductal injection. *Cell Transplant* 19: 291-7.

Matsumoto S, Noguchi H, Takita M, Shimoda M, Tamura Y, Olsen G, Chujo D, Sugimoto K, Itoh T, Naziruddin B, Onaca N, & Levy MF. (2010c) Super high dose islet transplantation is associated with high SUITO index and prolonged insulin independence: A case report. *Transplant Proc* 42: 2156-58.

Matsumoto S, Takita M, Chaussabel D, Noguchi H, Shimoda M, Sugimoto K, Itoh T, Chujo D, SoRelle J, Onaca N, Naziruddin B, & Levy MF. (2011) Improving efficacy of clinical islet transplantation with iodixanol based islet purification, thymoglobulin induction and blockage of IL-1 beta and TNF-alpha. *Cell Transplant* in press

Meier JJ, Bhushan A, Butler AE, Rizza RA, & Butler PC. (2005) Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia* 48:2221-2228

Meier JJ. (2008) Beta cell mass in diabetes: a realistic therapeutic target? *Diabetologia* 51: 703-713

Minami K, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, Kawaguchi M, Ishizuka N, Iwanaga T, & Seino S. (2005) Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *Proc Natl Acad Sci U S A.* 102: 15116-21.

Noguchi H, Ikemoto T, Naziruddin B, Jackson A, Shimoda M, Fujita Y, Chujo D, Takita M, Kobayashi N, Onaca N, Levy MF, & Matsumoto S. (2009a) Iodixanol-controlled density gradient during islet purification improves recovery rate in human islet isolation. Transplantation. 87: 1629-35.

Noguchi H, Oishi K, Ueda M, Yukawa H, Hayashi S, Kobayashi N, Levy M, & Matsumoto S. (2009b) Establishment of mouse pancreatic stem cell line. *Cell Transplant* 18: 563-71.

Noguchi H, Naziruddin B, Jackson A, Shimoda M, Ikemoto T, Fujita Y, Chujo D, Takita M, Kobayashi N, Onaca N, Hayashi S, Levy M, & Matsumoto S. (2010) Characterization of human pancreatic progenitor cells. *Cell Transplant* 19; 879-86.

Okuno M, Minami K, Okumachi A, Miyawaki K, Yokoi N, Toyokuni S, & Seino S. (2006) Generation of insulin-secreting cells from pancreatic acinar cells of animal models of type 1 diabetes. *Am J Physiol Endocrinol Metab.* 292: E158-65.

Ricordi R & Edlund H. (2008) Toward a renewable source of pancreatic beta-cells, Nat Biotechnol 26: 397–8.

Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, & Shapiro AM. (2005) Five-year follow-up after clinical islet transplantation. *Diabetes* 54: 2060-9.

Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, & Rajotte RV. (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343: 230-8.

Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbutt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, & Lakey JR. (2006) International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 355: 1313-30.

Shimoda M, Noguchi H, Naziruddin B, Fujita Y, Chujo D, Takita M, Peng H, Tamura Y, Olsen GS, Sugimoto K, Itoh T, Onaca N, Levy MF, Grayburn PA, & Matsumoto S.

(2010) Improved method of human islet isolation for young donors. *Transplant Proc* 42: 2024-6.

Shimoda M, Noguchi H, Fujita Y, Takita M, Ikemoto T, Chujo D, Naziruddin B, Levy MF, Kobayashi N, Grayburn PA, & Matsumoto S. (2011) Improvement of porcine islet isolation by Aralast inhibition of trypsin with pancreatic ductal preservation method. *Cell Transplant* in press

Shimoda M, Noguchi H, Fujita Y, Takita M, Ikemoto T, Chujo D, Naziruddin B, Levy MF, Kobayashi N, Grayburn PA, & Matsumoto S. (2011) Islet purification method using large bottle effectively achieves high islet yield from pig pancreas. *Cell Transplant in press*

Takahashi K, Tanae K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, & Yamanaka S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861-72.

Takita M, Matsumoto S, Qin H, Noguchi H, Shimoda M, Itoh T, Sugimoto K, Naziruddin B, Onaca N, & Levy M. (2011) Secretory Unit of Islet Transplant Objects (SUITO) Index can predict severity of hypoglycemic episodes in clinical islet cell transplantation. *Cell Transplant* in press

Thompson DM, Meloche M, Ao Z, Paty B, Keown P, Shapiro RJ, Ho S, Worsley D, Fung M, Meneilly G, Begg I, Al Mehthel M, Kondi J, Harris C, Fensom B, Kozak SE, Tong SO, Trinh M, & Warnock GL. (2011) Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy. *Transplantation* 91: 373-8.

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshal VS & Jones JM. (1998) Embryonic stem cell lines derived from human blastocytes. *Science* 282: 1145-7.

Valdés-González RA, Dorantes LM, Garibay-Nieto GN, Bracho-Blanchet E, Mendez AJ, Dávila-Pérez R, Elliott RB, Terán L, & White DJ. (2005a). Xenotransplantation of porcine neonatal islets of Lanberhans and Sertoli cells: a 4-year study. *Eur J Endocrinol.* 153: 419-27.

Valdés-González RA, Silva-Torres ML, Ramírez-González B, Terán L, Ormsby CE, & Ayala-Sumuano JT. (2005b) Improved method for isolation of porcine neonatal pancreatic cell clusters. *Xenotransplantation* 12: 240-4.

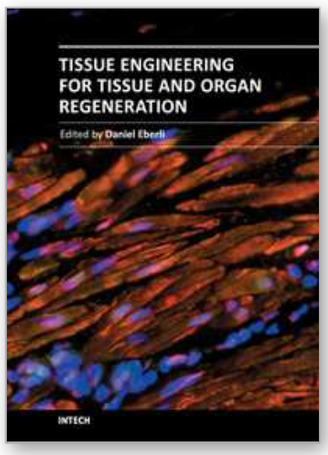
Valdés-González RA, White DJ, Dorantes LM, Terán L, Garibay-Nieto GN, Bracho-Blanchet E, Dávila-Pérez R, Evia-Viscarra L, Ormsby CE, Ayala-Sumuano JT, Silva-Torres ML, & Ramírez-González B. (2007). Three-yr follow-up of a type 1 diabetes mellitus patient with an islet xenotransplant. *Clin Transplant,* 21: 352-7.

Valdes-Gonzalez R, Rodriguez-Ventura AL, White DJ, Bracho-Blanchet E, Castillo A, Ramírez-González B, López-Santos MG, León-Mancilla BH, & Dorantes LM. (2010) Long-term follow of patients with type 1 diabetes transplanted with neonatal pig islets. *Clin Exp Immunol*. 162: 537-42.

van der Laan LJ, Lockey C, Griffeth BC, Frasier FS, Wilson CA, Onions DE, Hering BJ, Long Z, Otto E, Torbett BE, & Salomon DR. (2000) Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. *Nature.* 407(6800): 90-4.

Yamada T, Katagiri H, Ishigaki Y, Ogihara T, Imai J, Uno K, Hasegawa Y, Gao J, Ishihara H, Niijima A, Mano H, Aburatani H, Asano T, & Oka Y. (2006). Signals from intra-abdominal fat modulate insulin and leptin sensitivity through different mechanisms: neuronal involvement in food-intake regulation. *Cell Metab* 3: 223-9.

Zhou Q, Brown J, Kanarek A, Rajagopal J, & Melton DA. (2008) In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* 455: 627-32.



Tissue Engineering for Tissue and Organ Regeneration

Edited by Prof. Daniel Eberli

ISBN 978-953-307-688-1
Hard cover, 454 pages
Publisher InTech
Published online 17, August, 2011
Published in print edition August, 2011

Tissue Engineering may offer new treatment alternatives for organ replacement or repair deteriorated organs. Among the clinical applications of Tissue Engineering are the production of artificial skin for burn patients, tissue engineered trachea, cartilage for knee-replacement procedures, urinary bladder replacement, urethra substitutes and cellular therapies for the treatment of urinary incontinence. The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues reconstructed from readily available biopsy material induce only minimal or no immunogenicity when reimplanted in the patient. This book is aimed at anyone interested in the application of Tissue Engineering in different organ systems. It offers insights into a wide variety of strategies applying the principles of Tissue Engineering to tissue and organ regeneration.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen