20

# Perspectives of Islet Cell Transplantation as a Therapeutic Approach for Diabetes Mellitus

Prabha D. Nair and Neena Aloysious
*Division of Tissue Engineering & Regeneration Technologies,*
*Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum*
*India*

## 1. Introduction

Diabetes mellitus, the metabolic disorder is rapidly on the rise, becoming one of the main threats to human health and imposing large socio-economic burden on the society in the 21st century (Dall et al.; 2010). International Diabetes Federation in 2011 estimated that over 300 million people around the world have diabetes and is expected to rise to 500 million within next 20 years. The global prevalence of diabetes is shifting significantly from the developed countries to the developing countries. Current therapy for diabetes involves oral antidiabetic drugs and insulin administration; these approaches do not mimic the pulsatile insulin secretory patterns of native β islets for the regulation of glucose in real-time nor provide tight control of blood glucose to avoid late complications of the disease. Whole pancreas transplantation holds promise towards a cure for diabetes, but this procedure requires major surgery and lifelong immunosuppression to prevent graft rejection. Transplantation of islet cells isolated from a donor pancreas has been shown to control glucose levels successfully. Being less invasive, it is a better alternative to pancreas transplantation yet scarcity of donors, maintenance of islet functions such as cell growth and survival *in vitro*, and concern over the adverse effect of life long immunosuppressants used to prevent graft rejection precludes the benefits of islet transplantation from becoming universally acceptable.

One approach to overcome these obstacles of immune rejection is islet encapsulation (Kizilel et al., 2005; Mikos et al; 1994) that uses an immuno-protective biomaterial to create a permselective membrane around a group of islet cells. Transplanted islet cells are separated from the immunological system of host by means of an artificial selectively permeable membrane which allows passage of metabolites and nutrients, while excluding based on size, the larger proteins and cells of the immune system. Thus, encapsulation is designed to limit, and ideally eliminate, an immunological response to the non-host islet cells. Isolation of the islet cells from the human immune system may also make xeno-transplants such as porcine islets, stem cells derived insulin producing cells possible, eliminating the supply problem that exists and the usage of immune suppressive drugs.

Current research is directed towards exploration of alternative sources of pancreatic islet cells. Pancreatic β- cell lines, embryonic stem cells (ESC), adult progenitor cells (APC), and regenerating native islet cells, generation of β cells by therapeutic cloning and

differentiation of islets from pancreatic duct cells as well as stem cells are being explored for their potential to serve as β-cell sources. Large numbers of cells can be generated from β cell lines, although their unrestricted proliferation is also a serious concern in the context of cellular therapies. Unlike stem and progenitor cells, β islet cells have limited ability to multiply under normal conditions, although investigations into factors that stimulate β-cell regeneration have yielded promising results. Therapeutic molecules capable of increasing β-cell mass in vivo may eliminate the need for invasive surgical procedures. However, the extent of adult β cell regenerative capacity is unclear. Ex-vivo expansion of islets is indispensable prior to transplantation regardless of the source of islets. Monolayer culture of islets fail to maintain the three dimensional shape and integrity of islets resulting in consequent apoptosis and necrosis. *In vitro* culturing of pancreatic islets result in the loss of extracellular matrix (ECM), basement membrane and introduction of free radicals in isolation procedure, that cause islet cells to loose their three dimensional (3D) structure, function and ultimately undergo apoptosis (Parakevas et al., 1997). These problems could be alleviated by using tissue engineering principles and culturing pancreatic islets in 3 dimensional (3D) scaffold which can help in maintaining cell-cell, and cell-matrix interactions.

## 2. Islet transplantation

Islet cell transplantation is an effectual treatment for improving glycemic condition in diabetic patients thereby reducing the late complications of disease (Shapiro, 2003). The procedure involves isolating islets from a deceased organ donor, purifying, processing and infusing them into diabetic patients.

In the early 1970's, Dr. Clyde Barker at the University of Pennsylvania and the late Paul Lacy at the Washington University in St. Louis were the pioneers in exploring the concept of islet transplantation as a means to cure diabetes. In 1972, Ballinger and Lacy reported amelioration of diabetes in islet recipient rats (Ballinger & Lacy, 1972). In 1973, Reckard and Barker were the first to show that islet transplantation could completely and permanently restore normoglycemia in rodent models of chemically induced diabetes (Reckard & Barker, 1973).

Human islets isolation procedure is more complex than rodent islets isolation (Gray et al., 1984). Ricordi's automated isolation method had given hope for the production of abundant islets for the clinical use (Ricordi et al., 1989). Scharp et al. performed the islets transplantation under immunosuppression in diabetes patients and patients were insulin independent at the period of 22 days (Scharp et al., 1990). This was followed by several other cases, but success rates continued to be low (International Islet Transplant Registry). In 1999, Bretzel et al reported a markedly improved 3-month islet graft function rate of at least 75% in 24 consecutive patients (Bretzel et al., 1999). In the 1-year follow-up of 37 patients, 24% had achieved insulin independence (Bretzel et al., 2000). Between 1999 and 2005 about 650 patients were treated worldwide (Bretzel et al., 2007).Unfortunately, long-term results did not prove that promising.

The first successful islet allograft was reported in 1990 with steroid free immunosuppressant tacrolimus (Tzakis et al., 1990). The success rate of islet transplantation became outstanding after the Edmonton trial in 2000, which described successful intraportal alloislet transplantation, defined as insulin independence, in 7 consecutive patients with hyperlabile diabetes and frequent episodes of hypoglycemia (Shapiro et al., 2000). The success was

partly ascribed to the usage of a steroid free immunosuppressive regimen which was a new combination of immunosuppressive drugs, consisting of sirolimus, tacrolimus and daclizumab, excluding the diabetogenic glucocorticoids and large numbers of donor islets (Shapiro et al., 2006).

The short-term results of islet transplantation with Edmonton trial were promising, with insulin independence in approximately 80% of patients at 1 year post-transplant but the proportion of insulin independent recipients declined after the first year post-transplant. Five year follow-up study after transplantation revealed that only 10% of the patients remained insulin independent (Ryan et al., 2005; Shapiro et al., 2006). The suggested reasons attributing for this decline include alloimmune rejection, autoimmune recurrence, and/or toxicity of immunosuppressive medications (Monti et al., 2008). However, about 80% were still C-peptide positive, indicating functioning grafts. Now, a slightly modified Edmonton protocol is used worldwide with reproducible results (Shapiro AM et al., 2006).

Though islet transplantation research has made significant progress, concern over toxicity as well as cost of immunosuppressive therapy still remains. Insulin independence and long term graft survival were achieved for more than three years through a modified immunosuppressive protocol (Bellin et al., 2008) even so the requirement of multiple donors to obtain 10000 islet equivalents per kilogram of patient's weight remains unsolved. Although insulin independence remains the ultimate goal, today, stabilization of glucose levels and avoidance of hypoglycemia are considered to be the main indications for islet transplantation.

## 3. Alternate sources of pancreatic β cells

The scarcity of donor pancreas for islet transplantation is a major obstacle to the widespread use of islet transplantation which urged the focus towards alternate sources of β cells for future transplants. Several alternative means have been suggested which include use of xenogenic islets and immortalized beta cell lines (Narushima et al., 2005). Recent advances in the field of stem cell differentiation and regeneration therapy have focused on new ways to generate insulin-producing beta cells that can be used for transplantation. Several candidate cells have been identified including embryonic stem cells (ESC) and adult stem cells or progenitor cells residing in the pancreas or other organs. The differentiated beta cells have shown to regenerate by replication, which opens the possibility to generate novel beta cells *in vitro* and / or *in vivo* from pre-existing beta cells. Additionally, there are reports that show the successful use of liver cells, endocrine cells from the gut, and bone marrow derived stem cells as source to generate islets by cell transdifferentiation.

### 3.1 Xenogenic islets

In a xenogenic approach, islets from different species are used for transplantation purpose. Porcine islets serve as a potential source in view of the fact that porcine insulin differ from human insulin by 1 amino acid. Neonatal porcine islets were also induced to mature endocrine phenotype under *in vitro* and *in vivo* conditions (Korbutt et al., 1997). Xenogenic tissues induce more vigorous rejection than that of allogenic tissue; hence immunosuppressant dosage should be high enough to prevent graft rejection. Alternately, the cells of xeno origin can be immunoisolated by encapsulation technology to separate the transplanted cells from host immune system which will be discussed later in this review.

Risks associated with transfer of endogenous virus from porcine cells to human genome (Patience et al., 1997; Van der Laan et al., 2000) and the public concerns regarding the use of animal organs for transplantation are major factors impeding the success of this approach in clinical applications.

### 3.2 Stem cells to treat diabetes

Stem cells are non specialized cells which have the ability to self regenerate and differentiate into specialized cell types depending on the niche or external signaling cues (Smith, 2006). Stem cells offer a limitless supply source for islets as well reduces the graft rejection problems (Street et al., 2004). Ideally stem cells used for cell based therapy should meet the following criteria (Gimble, 2003) : It should be available in abundant quantities (millions to billions of cells), harvest procedure should be less invasive, have multilineage differentiation potential and could be efficiently transplanted to the host.

#### 3.2.1 Embryonic Stem Cells (ESC)

Embryonic stem cells which are derived from the inner cell mass of pre-implantation blastocysts have gained the attention of researchers due to its pluripotent nature. Human embryonic stem cells (hES) hold promise for research and clinical applications. hES have some unique abilities as compared to all sources of adult cells: 1) the expansion of ESC in the undifferentiated state is nearly unlimited; and 2) ESC can give rise to all cell types including pancreatic insulin-producing beta cells. Attempts of directed differentiation of hES to cardiomyocytes (Klug et al., 1996), and neurons (Li et al., 1998) have been reported. Many studies have reported the differentiation of mouse and human embryonic stem cells to islet like clusters (Segev et al., 2004; Vaca et al., 2006) either by modifying the culture conditions or by genetic manipulation. Lineage tracing experiments revealed that beta cells are derived from the embryonic endoderm followed by a sequential and transient activation of specific transcription factors like Pdx1, NeuroD / Beta 2, Isl1, Nkx6.1, Nkx2.2, MafA, Pax4, and Pax6. Most published protocols aim to mimic these differentiation factors for the stepwise development of the endo and exocrine pancreas during the embryonic phase. In one approach ESC were induced to generate embryoid bodies under *in vitro* culture and the nestin positive cells were selected to differentiate towards β cell lineage by culturing in serum free conditions (Lumelsky et al., 2001). Manipulation of the culture conditions with various growth supplements like insulin, transferrin, selenium and fibronectin (ITSFn), B27,bFGF and nicotinamide resulted in regulated secretion of insulin. Phosphoinositide kinase inhibitors have been reported to promote the differentiation of larger numbers of ESC towards functional β cells (Hori et al., 2002). Genetic approach involves the incorporation of a reporter selector gene whose expression controlled by an insulin promoter. Here mouse embryonic stem cells were cultured in manipulated culture conditions to develop into embryoid bodies which were then differentiated to insulin producing cells. Insulin containing population which exhibited *in vitro* regulated hormone secretion was selected for transplantation into diabetic mice. Genetically engineered insulin producing cells maintained glucose homeostasis *in vivo* in mouse models but 40% of animals reversed to hyperglycemic condition after 12 weeks (Soria et al., 2000). ESC culture under serum-free conditions or only low-level fetal calf serum together with stage specific differentiation factors results in temporal expression of pancreatic lineage genes. The final differentiated cells do express insulin secretory granules / C peptide in about 2 – 8 % of the

total cell population and secrete insulin / C-peptide after glucose challenge (D'Amour et al., 2006; Baharvand et al., 2006; Jiang et al., 2007). Feeder free conditions were developed for the differentiation of embryonic stem cells to insulin like clusters which allowed single species propagation of ESC thus avoiding possible zoonotic infection of cells evident by the increased expression of Pdx 1 and reduced expression of Oct-3/4 (Assady et al., 2001). The activation of Pax-4 and Pdx 1 gene expression in embryonic stem cells upregulated the genes involved in the differentiation towards pancreatic endocrine lineage. Pax-4 expression led to an increase in nestin positive cells and insulin producing beta cells but not glucagon producing alpha cells (Moritoh et al., 2003). The development pathway for induction of ESC to insulin producing cells involves series of events which include generation of endoderm lineage cells followed by precursors of pancreatic endocrine lineage cells, and finally the insulin-secreting cells. D'Amour et al., developed a five stage protocol for differentiation of hES to pancreatic endocrine hormone expressing cells through a series of endodermal intermediates resembling those that occur during pancreatic development *in vivo* (D'Amour et al., 2006). Controversial findings also have been reported regarding the differentiation of ESC to pancreatic beta cells.

Insulin immunoreactivity was demonstrated to occur as the consequence of insulin uptake from medium (Rajagopal, J., 2003). Few authors proved that nestin positive cells tend to differentiate towards neuronal lineage rather than pancreatic lineage (Sipione et al., 2004). Kania et al explained the cause for the controversial results in generation of pancreatic cells. It was suggested that insulin producing cells derived from embryonic stem cells without applying lineage selection is dependent on the differentiation factors and protocols (Kania et al., 2004).

Though hES are versatile cells, ethical concerns on the use of human hES, and chances of teratoma formation (Fujikawa et al., 2005) limits their usage. Direct transplantation of embryonic stem cells has reported to culminate in teratoma formation (Nussbaum et al., 2007) from contaminating undifferentiated ESCs. Safe transplantation of hES could be attempted by viral vector mediated transfection *in vitro*, yet the risks associated with cytomegalovirus promoters in transfection cannot be ruled out.

### 3.2.2 Adult Stem Cells

The potential use of adult stem cells offers the advantage of an autologous model whereby a patient's own cells can be used, thereby circumventing immune rejection. Adult stem cells (ASC) are multipotent cells capable of self renewal. They have been reported to be present in almost every tissue like bone marrow, blood, heart, liver, pancreas, adipose tissue and could be transplanted directly without genetic modification or pre-treatments. They exhibit high degree of genomic stability during culture conditions. ASC lack tissue specific characteristics but it could be differentiated to specialized cell types under the influence of appropriate signaling cues (Barry & Murphy, 2004). The stem cell microenvironment plays an important role in its differentiation to committed cells (Galli et al., 2000; Zhao et al., 2002). The potential of adult human stem cells from various sources to differentiate to insulin producing cells have been explored by various research groups. The relative ease of isolating adult stem cells and their expansion makes it an ideal source for cell based therapy.

#### 3.2.2.1 Pancreatic stem cells

Pancreatic progenitor/stem cells which are closely related with beta cell lineage represent an attractive source for generation of beta cells (Serup et al., 2001). Human pancreatic ductal

cells and islet stem cells have been expanded and differentiated to islet like clusters capable of producing insulin *in vitro* which were capable of reversing of diabetes in non obese diabetic mice thus normalizing blood glucose levels for more than 3 months (Ramiya et al., 2000). Nestin positive cell derived islet cell clusters expressed pancreatic endocrine markers like Glut2, glucagon, and homeodomain transcription factor PDX-1 as well as pancreatic exocrine genes (Zulewski et al., 2001). Glucagon like peptide -1, an intestinal hormone was shown to stimulate the neogenesis of beta cells by inducing the expression of various transcription factors involved in beta cell development (Abraham et al., 2002). Exocrine pancreatic tissue (Baeyens et al., 2005) and neurogenin 3 positive cells (Gu et al., 2002) could also serve as alternative source for beta cells. Even though pancreatic cells seem to be the better source than embryonic stem cells, the fraction of precursor cells isolated from pancreas is very less and heterogenous. Furthermore the harvest procedure from pancreas is also invasive thus limiting this source being applicable in clinical purposes.

#### 3.2.2.2 Bone marrow stem cells (BMSC)

Bone marrow stem cells were induced to differentiate to mature endocrine pancreatic lineage *in vitro* (Y. Sun et al., 2007). The *in vitro* differentiation of human bone marrow stem cells (hBMSC) to endocrine pancreatic cell types were investigated by genetic manipulation using adenovirus coding for mouse transcription factors involved in the early phase of endocrine developmental pathway (Karnielli et al., 2007). The results suggested that bone marrow stem cells shifted towards pancreatic endocrine phenotype with expression of insulin and other transcription factors involved in β cell development. Enhanced green fluorescent protein (GFP) system based genetic approach was utilized to study the differentiation of BMSC to islet like cells. BMSC from transgenic (GFP) male mice were transplanted into sublethally irradiated female mice. After 4 weeks 1.7-3% bone marrow derived GFP positive cells were found only in the pancreatic islets which ruled out the *in vivo* occurrence of cell fusion. The results indicated that bone marrow derived cells activated insulin gene expression after entering pancreatic islet niche (Ianus et al., 2003). However controversial finding was also reported suggesting that bone marrow derived stem cells cannot differentiate to beta cells without the influence of differentiation factors (Lechner et al., 2004) and favorable microenvironment (Moriscot et al., 2005). Recently Phadnis et al evaluated the fate of transplanted epithelialised human bone marrow stem cells under the kidney capsule of pancreatomized NOD/SCID mice (Phadnis et al., 2011).The results suggested that regenerating pancreas secreted paracrine factors and provided the niche which induced the differentiation of hBMSC to mature islet like aggregates capable of secreting insulin.

#### 3.2.2.3 Adipose stem cells

Human subcutaneous adipose tissue, abundant and easily accessible serves as a potential source of adult mesenchymal stem cells. The harvest procedure by lipoaspiration / liposuction is less invasive. Adipose stem cells have been reported to exhibit an increased *in vitro* proliferative potential than bone marrow stromal cells (De Ugarte et al., 2003). Adipose stem cells release cytokines TGF-β and IL-10 which are responsible for its immunomodulatory properties (Puissant et al., 2005). The immunosuppressive property of adipose stem cell has been exploited for the treatment of severe graft versus host disease (Yanez et al., 2006). The differentiation potential of these cells to pancreatic endocrine cells have been investigated by several research groups. Human adipose stem cells induced to

islet like cells in serum free differentiation medium for 3 days exhibited an upregulation of pancreatic developmental transcription factors like Isl-1, Ngn3 along with islet hormones such as insulin, glucagon and somatostatin (Timper et al., 2006). A novel protocol using taurine designed for islet differentiation generated 47-51% C- peptide positive cells when compared to reports where the yield was only 2-8% (Jiang et al., 2007).

#### 3.2.2.4 Progenitor cells and stem cells from other tissues

The mechanisms involved in the generation of new beta cells in postnatal life remains controversial. Lineage tracing experiments suggest that after partial pancreatectomy, the majority of novel beta cells derive from pre-existing beta cells by beta cell proliferation rather than stem cell differentiation (Dor et al., 2004; Georgia and Bhushan, 2004). These findings raise new hope that it may be possible to use beta cells as source for the *in vitro* cell expansion in cell therapy. For example, it has been shown that human beta cells can transiently dedifferentiate to fibroblast-like cells, which can proliferate and redifferentiate into insulin-positive cells (Gershengorn et al., 2004; Lechner et al., 2005). However, direct evidence that this process can be used to produce fully mature beta cells for transplantation is missing, thus far.

It is important to note that the above mentioned experiments do not exclude the existence of pancreatic stem cells. There is convincing evidence that under specific experimental conditions adult stem cells, which reside in the pancreatic ducts, within islets, or exocrine tissue, can develop into beta cells. Bonner-Weir and colleagues as well as other groups provide evidence in several studies that murine and human stem cells or progenitor cells reside in the epithelium of the small pancreatic ducts, expressing the marker cytokeratin-19 (CK19). The ductal cells can expand and give rise to novel beta cells after 90% pancreatectomy or treatment with streptozotocin (Bonner-Weir et al., 1993; Wang et al., 1995; Bonner-Weir S et al., 2003; Gao et al., 2003). This suggests that still unknown cellular or soluble factors are needed to induce terminal differentiation into the endocrine fate. The identification of these critical factors is one focus of current research.

Amnion epithelial cells (Hou et al., 2008; Kadam et al., 2010a), and stem cells from liver (Yang et al., 2002), wharton's jelly of umbilical cord (Chao et al., 2008), umbilical cord blood (Phuc et al., 2010), placenta (Kadam et al., 2010b), gall bladder (Sahu et al., 2009) etc were also differentiated to insulin producing cells. In every case the differentiated islets expressed islet specific proteins and were capable of secreting insulin in response to glucose stimulation, albeit less than native islets. A better understanding of the events that control stem cell commitment and the signaling pathways for differentiation to pancreatic lineage is required to improve the culture conditions for *in vitro* generation of islet like clusters.

## 4. Tissue engineering based strategies

Tissue engineering applies the principles of cell transplantation, material science, and engineering towards the development of biologic substitutes that can restore and maintain normal function. The success of tissue engineering relies on the development of a suitable scaffold, cell source and defined culture conditions. Tissue engineering strategies employed for islet transplantation could be categorized into use of scaffolds for simulation of the native ECM and immunoisolation via encapsulation of islets.

The biomaterial chosen to synthesize the scaffold should be biocompatible and biodegradable (Mohan & Nair, 2005). The scaffold should reproduce an extracellular

environment for supporting cell function (Dufour et al., 2005). Neither the scaffold nor its degradation products should be toxic to host. Biocompatibility of chosen biomaterial plays an important role in controlling the rate of protein adsorption and fibrosis. The scaffold should provide three dimensional space for neotisssue formation and the mechanical strength should match the native tissue to withstand *in vivo* forces. It should be highly porous and pores should have interconnectivity to facilitate tissue ingrowth, diffusion of nutrients, oxygen and metabolites (Blomeier et al., 2006). Scaffolds should provide an initial support for the islet grafts during the early post transplant period, enabling the development of a capillary bed and recovery of extracellular matrix interactions (Stock & Vacanti, 2009). Polymers such as polylactic acid, polyglycolic acid, polylactide-co-polyglycolide (PLGA) (Mao et al., 2009), polyvinyl alcohol – polycaprolactone (Mohan et al., 2010) were extensively used for scaffold fabrication purpose for the tissue engineering of various tissues.

### 4.1 Extracellular matrix - Mimicking nature

A major limitation with two dimensional cultures is the lack of microenvironment indispensable for appropriate spatial organization and function of cells which in turn is provided by extracellular matrix (ECM) in native tissue. The ECM of native islets is mainly constituted by type IV collagen, laminin although fibronectin, collagen I, collagen V also have been detected (Stendahl et al., 2009). The effect of ECM proteins on adhesion and proliferation of rat islets have been studied. The results indicated that there were strong interactions between islets and ECM proteins via integrins (Chen et al., 2008). ECM protein coated scaffolds have shown improved graft efficacy at implanted site (Salvay et al., 2008).

Earlier reports of islets cultured under two dimensional conditions exhibited low survival rate and reduced insulin secretion (Paraskevas et al., 1997). Progress in survivability of islets and increased insulin secretion has been achieved by adopting 3D culture conditions. Polyglycolic acid scaffolds coated with lysine were shown to promote islet cell adhesion and survival. The control cells grown in 2D culture underwent apoptosis by day 7 due to accumulation of metabolites and shortage of nutrients (Chun et al., 2008).

Pancreatic islets cultured on agarose cryogel sponge (Bloch et al., 2005) were reported to secrete 15 fold higher insulin at 3mM glucose than islets cultured on polystyrene cell culture dish but failed to respond to stimulation at 16.7mM glucose. The failure was due to limited oxygen and nutrient diffusion across agarose cryogels. Adequate oxygen is a critical parameter to islet cell function and survival (Sweet et al., 2002). The decreased insulin response of pancreatic islets cultured on scaffold for prolonged period due to inefficient oxygen transfer were also reported (Cui et al., 2001).

A novel 3D culture system comprising a semi-interpenetrating polymer network of gelatin and polyvinyl pyrrolidone was designed in our laboratory for pancreatic islets and stem cells to promote their survival and function. The porous nature of the scaffold facilitated efficient nutrient and metabolite exchange. Islets seeded on this scaffold maintained their morphology for more than 30 days whereas control islets cultured on cell culture dish underwent apoptosis by 7th day. The test islets secreted insulin on stimulation with glucose which was comparable to that of freshly isolated mouse islets (Muthyala et al.,2010).

Zhao et al demonstrated the use of three dimensional self assembling peptide nanofiber hydrogel scaffold for islet culture. The peptides formed two beta sheet structures with hydrophilic and hydrophobic surfaces in aqueous solution. The hydrophobic moiety

facilitated its self assembly in water and the nanofiber structures were flexible for fabrication to different geometrical shapes that allowed for efficient nutrient and metabolite transfer. The nanofiber scaffold simulated the microenvironment *in vitro* as in native condition which accounted for improved islet viability and function (Zhao et al., 2010).

### 4.2 Immunoisolation strategies for islet transplantation

The principle behind immunoisolation is protection of islets from host immune system using a selectively permeable membrane as a barrier. Low molecular weight substances which include nutrients, oxygen, secretory molecules and cell signaling molecules freely diffuse through the membrane, but passage of immune cells and its products which have high molecular weight is prevented. Immunoisolation mechanism encourages the use of allogenic/ xenogenic sources of islets for transplantation and holds promise towards use of autologous stem cell derived islets in type I diabetic patients. Immunoisolation mechanism includes macroencapsulation and microencapsulation (Narang & Mahato, 2006) of cells.

#### 4.2.1 Microencapsulation

Microencapsulation is the encapsulation of single islets or small groups of islets. These capsules are usually spherical in shape (Chang, 1964). Microcapsules offer the advantage of increased oxygen and nutrient transport due to the large surface area to volume ratio. Microcapsules are advantageous due to several reasons like greater surface to volume ratio, and ease of implantation. The spherical shapes owe to better diffusion capacity and are mechanically stable. The primary drawback of microencapsulation is the difficulty in removing the implants if necessary. Moreover the implantation could be achieved by simple injection procedure (De Vos et al., 2002). Porcine islets microencapsulated in alginate-polylysine-alginate transplanted to diabetic monkeys could achieve normoglycemia without immunosuppression for more than 800 days (Y. Sun et al., 1996). Human and rat islets encapsulated in alginate gels when transplanted in mice survived for 7 months (Schneider et al., 2005). Xenogenic islets immobilized in microcapsules fabricated from alginate –PLL when implanted into peritoneum of non immunosuppressed diabetic rats remained in excellent condition for more than 40 weeks (Lanza et al., 1999). Despite of these advantages some authors have reported reduced functionality of microencapsulated islets in response to glucose challenge (Sandler et al., 1997).

#### 4.2.2 Macroencapsulation

Macroencapsules contain a large mass of islet cells within a diffusion chamber, which are usually formed from spun coat membranes or spun drawn hollow fibers. The advantages of macrocapsules are they could be easily retrieved when required and can be shaped in required geometries such as tubes or discs. Two approaches such as intravascular and extravascular have been tried out in macroencapsulation. Intravascular approach utilizes the principle of perfusion chambers which consists of microporous tubular structures perfused with blood and enclosed within another tube. Islets were seeded in the space between the hollow fibers and the device is anastomised to the host vasculature (Chick et al, 1975). Polyacrylonitrile and polyvinylchloride copolymers have been chosen as materials for creating artificial microcapillaries. Results from implantation of intravascular macrocapsules of islets have shown restoration of normoglycemia in various animal models (AM. Sun et al., 1977). Due to the direct contact of device with the blood, intense anticoagulation is required

to prevent thrombus formation, consequently the material chosen should be highly blood compatible and thromboresistant. These concerns have shifted the attention towards extravascular devices.

Extravascular devices are based on the principle of diffusion chambers which does not require anastomosis to host vasculature. The geometry could be planar in the form of flat or hollow fiber model (Scharp et al., 1984). This approach does not pose severe biocompatibility issues and risks to the patient as that of intravascular devices. Extravascular devices can be implanted to different sites such as peritoneal cavity (Lanza et al., 1999), subcutaneously or under kidney capsules (Siebers et al., 1990) with minimal surgical risks. Most commonly used biomaterials for macrocapsule fabrication are nitrocellulose acetate, 2-hydroxyethyl methacrylate (HEMA), acrylonitrile, polyacrylonitrile and polyvinylchloride copolymer, and alginate.

The biocompatibility of immunoisolation membrane depends on several factors like geometry of the device, implantation site and material chosen. Hollow fiber geometry is preferred because of its reduced surface area of contact with the host per islet and reduced foreign body response. Higher density of islet cells often results in reduced viability and necrosis at the center due to nutrient limitation. Smooth outer surface and hydrogels have been reported to improve the biocompatibility by the absence of interfacial tension, thus reducing protein adsorption, cell adhesion and fibrosis (Burczak et al., 1996). Nair et al studied the effect of degree of hydrophilicity on tissue response of polyurethane interpenetrating networks (IPN) (Nair et al., 1992).The results indicated that an increase in hydrophilicity of polyurethane –polyvinyl pyrrolidone IPN's elicited an inert tissue response.

George et al., (2002), Nair (2009, Indian Patent 230740) demonstrated the use of non porous polyurethane membranes and porous polyurethane IPN macrocapsules as islet immunoisolative matrices. Islet cell morphology remained intact and insulin secretion ability was also retained within the immunoislation membranes. Membranes allowed diffusion of glucose and insulin while retained transplant rejection factors like antibodies, immunoglobulins and immune cells. Reduced protein adsorption and cell adhesion on polyurethane membranes contributed to improve the biocompatibility which made them ideal for immunoisolation. The IPN macrocapsules also served as an *in vivo* bioreactor cum immunoisolation device permitting immature islet like clusters derived from a variety of stem cell sources to mature completely and control glycemic levels of streptozotocin induced diabetic animal models without immunosuppression for periods upto 3 months. (Kadam, 2010a; Phadnis, 2011; Muthyala, 2011;; Kadam, 2010 b).Hybrid systems involving macro and microencapsulation have also been fabricated and analyzed for its efficiency in immunoisolation. Chitosan/gelatin hydrogel system was used as an immunoisolative matrix to protect the microencapsulated islet cells from recipient's immune system in xenotransplantation. Mouse insulinoma /agarose microspheres macroencapsulated in chitosan/gelatin hydrogel reversed diabetes in rats. The study suggests that this could be applied as a cell carrier for injectable bioartificial pancreas after certain modifications (Yang et al., 2008).

### 4.3 Combined approach of tissue engineering and immunoisolation

Muthyala et al (2011) employed a combination approach utilising the properties of scaffold to mimic the native ECM and macroencapsulation for immunoisolation to protect the islets

from immune cell responses. Pancreatic progenitor derived islets were seeded on gelatin-polyvinylpyrrolidone scaffolds and further macroencapsulated in a polyurethane–polyvinylpyrrolidone semi IPN macrocapsule. The construct when implanted into peritoneal cavity of diabetic rats normalized glycemic condition all through the study period of 3 months. Animals implanted with tissue engineered islets without macroencapsulation showed no reversal of hyperglycemia and died within 15-20 days due to infiltration of host immune cells. Hence the combination approach was found to be very effective in achieving euglycemia by maintaining islet survival and functionality as well as protecting the cells from host immune attack.

## 5. Site for transplantation

The optimal site should be chosen for transplanting islets inorder to meet its high energy requirement and metabolic rate (Hardy et al., 2010). The implantation site has effect on hypoxic conditions which determines islet survival. Safety considerations have been raised regarding the optimal site for transplantation so as to improve islet engraftment and survival (Dufrane et al., 2006; Pillegi et al., 2001). Graft vascularization is an important criterion in islet survival and function (Jansson & Carlsson, 2002). Although immunoisolation prevents the integration of host blood vessels with transplanted islets effective diffusion of nutrients and oxygen can occur within 200µm distances hence highly vascularised sites should be chosen for transplantation. Islet transplantation into prevascularized sites dramatically improves graft survival and function relative to transplantation into non-modified tissue (Balamurugan et al., 2003). Vascularization can be introduced in graft by incorporation of angiogenic growth factors like VEGF (Stendahl et al., 2008) or endothelial cells (Miki et al., 2006). Insulin independence have been achieved by intra-portal islet transplantation in diabetic patients (Shapiro et al., 2005), however liver could not be considered as an optimal site since islets in liver will be exposed to high nutrient concentration and other factors that are toxic and may result in impairment of beta cells(Hiller et al., 1991; Wilson & Chaikof, 2008). Peritoneal cavity has also been tried for implantation of islets which requires 200%-400% more islets (Siebers et al., 1993). Subcutaneous site (Pillegi et al., 2006) have been chosen in diabetic athymic mice for transplantation of islets cultured on plasma –fibroblast gel scaffold (Perez-Basterrechea et al., 2009). Normoglycemia was achieved over 60 day period and vascularization was observed in and around islets. Kidney subcapsular spaces have also been chosen as implantation site to improve biocompatibility of tissue engineered constructs. Islets cultured on biodegradable polymer scaffold transplanted to omental pouch of diabetic dogs resulted in achievement of euglycemia upto 152 days till graft was taken out (Kin et al., 2008).

## 6. Conclusions

Curative therapy for diabetes mellitus mainly implies replacement of functional insulin-producing pancreatic cells, with pancreas or islet-cell transplants. Shortage of donor organs spurs research into alternative means of generating cells from islet expansion, encapsulated islet xenografts, human islet cell-lines, and stem cells. Embryonic and adult stem cells are potential sources for cell replacement and merit further scientific investigation. The expense of the benefit of cell transplantation is the need for immunosuppressive treatment of the recipient, with all its potential risks. Biocompatible macrocapsules for transplantation of

islets and islet-like cell clusters differentiated from stem cells help overcome the immune rejection without Immunosuppressive drug therapy. A tissue engineering approach aims to mimic the natural extracellular matrix environment for supporting the transplanted islet cells without sacrificing form and function. A combination approach of tissue engineering, immunoisolation and most appropriately differentiated islet may propel clinical trials involving engineered strategies for cell replacement in diabetic patients, in the not too distant future.

## 7. Acknowledgment

Authors are grateful to Director, SCTIMST and Head BMT Wing for permissions to publish this work and funding from the DBT, Govt. of India. N. Aloysious also acknowledges the CSIR for the JRF fellowship.

## 8. References

Abraham, EJ.; Leech, CA.; Lin, JC.; Zulewski, H. & Habener, JF. (2002). Insulinotropic Hormone Glucagon-Like Peptide-1 Differentiation of Human Pancreatic Islet-Derived Progenitor Cells into Insulin-Producing Cells. *Endocrinology*, Vol.143, No.8, pp. 3152-3161

Assady, S.; Maor, G.; Amit, M.; Itskovitz-Eldor, J.; Skorecki, KL. & Tzukerman, M. (2001). Insulin production by human embryonic stem cells. *Diabetes*, Vol.50, No. 8, pp. 1691–1697

Baeyens, L.; De Breuck, S.; Lardon, J.; Mfopou, JK.; Rooman, I. & Bouwens, L. (2005). *In vitro* generation of insulin-producing beta cells from adult exocrine pancreatic cells. *Diabetologia*, Vol.48, No.1, pp. 49–57

Baharvand, H.; Jafary, H.; Massumi, M. & Ashtiani, SK. (2006). Generation of insulin secreting cells from human embryonic stem cells. *Development, Growth & Differentiation*, Vol.48, No.5, pp. 323-32

Balamurugan, AN.; Gu, Y.; Tabata, Y.; Miyamoto, M.; Cui, W.; Hori, H.; Satake, A.; Nagata, N.; Wang, W. & Inoue, K. (2003). Bioartificial pancreas transplantation at prevascularized intermuscular space: effect of angio-genesis induction on islet survival. *Pancreas*, Vol.26, No.3, pp. 279–285

Ballilnger, WF. & Lacy PE. (1972). Transplantation of intact pancreatic islets in rats. *Surgery*,Vol. 72, No.2, pp. 175-186

Barry FP. & Murphy, JM. (2004). Mesenchymal stem cells: clinical applications and biological characterization. *The International Journal of Biochemistry & Cell Biology*, Vol.36, No.4, pp. 568–584

Bellin, MD.; Kandaswamy, R.; Parkey, J.; Zhang, HJ.; Liu, B.; Ihm, SH.; Ansite, JD.; Witson, J.; Bansal-Pakala, P.; Balamurugan, AN.; Papas, K.; Sutherland, DE.; Moran, A. & Hering, BJ. (2008). Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *American Journal of Transplantation*, Vol. 8, No.11, pp. 2463-2470

Bloch, K.; Lozinsky, VI.; Galaev, IY.; Yavriyanz, K.; Vorobeychik, M.; Azarov, D.; Damshkaln, LG.; Mattiasson, B. & Vardi, P. (2005). Functional activity of insulinoma cells (INS-1E) and pancreatic islets cultured in agarose cryogel sponges. *Journal of Biomedical Materials Research Part A*, Vol.75, No.4, pp. 802–809

Blomeier, H.; Zhang, X.; Rives, C.; Brissova, M.; Hughes, E.; Baker, M.; Powers, AC.; Kaufman, DB.; Shea LD. & Lowe, WL Jr. (2006). Polymer Scaffolds as Synthetic Microenvironments for Extrahepatic Islet Transplantation. *Transplantation*, Vol.82, No.4, pp. 452–459

Bonner-Weir, S.; Baxter, LA.; Schuppin, GT. & Smith, FE. (1993). A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development . *Diabetes*, Vol. 42, No.12, pp. 1715 – 1720

Bonner-Weir, S.; Taneja, M.; Weir, GC.; Tatarkiewicz, K.; Song, KH.; Sharma, A. & O ' Neil, JJ. (2000). *In vitro* cultivation of human islets from expanded ductal tissue. *Proceedings of the National Academy of Sciences* USA , Vol.97, Vol.14, pp. 7999 – 8004

Bretzel, RG. (2000). Current status and perspectives in clinical islet transplantation. *Journal of Hepato-Biliary-Pancreatic Surgery*, Vol.7, No.4, pp. 370-373

Bretzel, RG.; Brandhorst, D.; Brandhorst, H.; et al. (1999). Improved survival of intraportal pancreatic islet cell allografts in patients with type-1 diabetes mellitus by refined peritransplant management. *Journal of Molecular Medicine*, Vol.77, No.1, pp. 1432-1440

Bretzel, RG.; Jahr, H.; Eckhard, M.; Martin, I,.; Winter, D. & Brendel, MD. (2007). Islet cell transplantation today. *Langenbeck's Archives of Surgery*, Vol.392, No.3, pp. 239-253

Burczak, K.; Gamian, E. & Kochman, A. (1996). Long-term in vivo performance and biocompatibility of poly(viny1 alcohol) hydrogel macrocapsules for hybrid type artificial pancreas. *Biomaterials*, Vol. 17, No. 24, pp. 2351-2356

Chandra, VGS.; Phadnis, S.; Nair, PD. & Bhonde, RR. (2009). Generation of Pancreatic Hormone-Expressing Islet-Like Cell Aggregates from Murine Adipose Tissue-Derived Stem Cells. *Stem Cells*, Vol.27, No. 8, pp. 1941–1953

Chang TM. Semipermeable microcapsules. (1964). *Science*, Vol.146, pp. 524-525

Chao, KC.; Chao, KF.; Fu, YS. & Liu, SH. (2008). Islet-Like Clusters Derived from Mesenchymal Stem Cells in Wharton's Jelly of the Human Umbilical Cord for Transplantation to Control Type 1 Diabetes. *Plos One*, Vol.3, No.1, e1451

Chen, G.; Kawazoe, N. & Tateishi, T. (2008). Effects of ECM Proteins and Cationic Polymers on the Adhesion and Proliferation of Rat Islet Cells. *The Open Biotechnology Journal*, Vol.2, pp. 133-137

Chick, WL.; Like, AA. & Lauris, V. (1975). Beta cell. culture on synthetic capillaries:an artificial endocrine pancreas. *Science*, Vol.187, No.4179, pp. 847-849

Chun, S.; Huang, Y.; Xie, WJ.; Hou, Y.; Huang, RP.; Song, YM.; Liu, XM.; Zheng, W.; Shi, Y. & Song, CF. (2008). Adhesive Growth of Pancreatic Islet Cells on a Polyglycolic Acid Fibrous Scaffold. *Transplantation Proceedings*, Vol.40, No.5, pp. 1658–1663

Cui, W.; Kim, DH.; Imamura, M.; Hyon, SH. & Inoue, K. (2001). Tissue engineered pancreatic islets: culturing rat islets in the chitosan sponge. *Cell Transplantation*, Vol.10, No.4-5, pp. 499 –502

Dall, TM.; Zhang, Y.; Chen, YJ.; Quick, WW.; Yang, WG. & Fogli J. (2010). The economic burden of diabetes. *Health Affairs*, Vol.29, No.2, pp. 297–303

D'Amour, KA.; Bang, AG.; Eliazer, S.; Kelly, OG.; Agulnick, AD.; Smart, NG.; Moorman, MA.; Kroon, E.; Carpenter, MK. & Baetge, EE. (2006). Production of pancreatic hormone– expressing endocrine cells from human embryonic stem cells. *Nature Biotechnology*, Vol.24, No.11, pp. 1392-1401

De Ugarte, DA.; Morizono, K.; Elbarbary, A.; Alfonso, Z.; Zuk, PA.; Zhu, M.; Dragoo, JL.; Ashjian, P.; Thomas, B.; Benhaim, P.; Chen,I.; Fraser, J. & Hedrick, M.H. (2003).

Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs*, Vol.174, No.3, pp. 101–109

De Vos, P.; Hamel, AF. & Tatarkiewicz, K. (2002). Considerations for successful transplantation of encapsulated pancreatic islets. *Diabetologia*, Vol.45, No.2, pp. 159-173

Dor, Y.; Brown, J.; Martinez, OI. & Melton, DA . (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation . *Nature*, Vol.429, No.6987, pp. 41 – 46

Dufour, JM.; Rajotte, RV.; Zimmerman, M.; Rezania, A.; Kin, T.; Dixon, DE. & Korbutt, GS. (2005). Development of an ectopic site for islet transplantation, using biodegradable scaffolds. *Tissue Engineering*, Vol.11, No.9/10, pp. 1323-1331

Dufrane, D.; Steenberghe, M.; Goebbels, RM.; Saliez, A.; Guiot, Y. & Gianello, P. (2006). The influence of implantation site on the biocompatibility and survival of alginate encapsulated pig islets in rats. *Biomaterials*, Vol.27, No.17, pp. 3201-3208

Fujikawa, T.; Oh, SH.; Pi, L.; Hatch, HM.; Shupe, T. & Petersen, BE. (2005). Teratoma formation leads to failure of treatment for type I diabetes using embryonic stem cell-derived insulin-producing cells. *The American Journal of Pathology*, Vol.166, No.6, pp. 1781–1791

Galli, R,.;Borello, U.; Gritti, A.; Minasi, MG.; Bjornson, C.; Coletta, M.; Mora, M.; De Angelis, MG.; Fiocco, R.; Cossu, G. & Vescovi, AL. (2000). Skeletal myogenic potential of human and mouse neural stem cells. *Nature Neuroscience*, Vol.3, No.10, pp. 986–991

Gao, R.; Ustinov, J.; Pulkkinen, MA.; Lundin, K.; Korsgren, O. & Otonkoski, T. (2003). Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture . *Diabetes*, Vol.52, No.8, pp. 2007 – 2015

George, S.; Nair, PD.; Risbud, MV. & Bhonde, RR. (2002). Nonporous Polyurethane Membranes as Islet Immunoisolation Matrices - Biocompatibility Studies. *Journal of Biomaterial Applications*, Vol. 16, No. 4, pp. 327-340

Georgia, S. & Bhushan, A . (2004). Beta cell replication is the primary mechanism for maintaining postnatal beta cell mass . *Journal of Clinical Investigation*, Vol.114, No.7, pp. 963 – 968

Gershengorn, MC.; Hardikar, AA.; Wei, C.; Geras-Raaka, E.; Marcus-Samuels, M. & Raaka, MB. (2004). Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells . *Science*, Vol.306, No.5705, pp. 2261 – 2264

Gimble JM. (2003). Adipose tissue-derived therapeutics. *Expert Opinion on Biological Therapy*, Vol.3, No.5, pp. 705-713

Gray, DW.; McShane, P.; Grant, A. & Morris, PJ. (1984). A method for isolation of islets of Langerhans from the human pancreas. *Diabetes*, Vol.33, No.11, pp. 1055-61

Gu, G.; Dubauskaite, J. & Melton, DA. (2002). Direct evidence for the pancreatic lineage: Ngn3+ cells are islet progenitors and are distinct from duct progenitors. *Development*, Vol.129, No.10, pp. 2447–2457

Hardy, MA.; Witkowski, P.; Sondermeijer, H. & Harris, P. (2010). The Long Road to Pancreatic Islet Transplantation. *World Jounal of Surgery*, Vol.34, No.4, pp. 625–627

Hiller, W.F.; Klempnauer, J.; Luck, R. & Steiniger, B. (1991). Progressive deterioration of endocrine function after intraportal but not kidney subcapsular rat islet transplantation. *Diabetes*, Vol.40, No.1, pp. 134-140

Hori, Y.; Rulifson, IC.; Tsai, BC.; Heit, JJ.; Cahoy, JD. & Kim, SK. (2002). Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. *Proceedings of the National Academy of Sciences of the USA*, Vol.99, No.25, pp. 16105–16110

Hou, Y.; Huang, Q.; Liu, T. & Guo, L. (2008). Human amnion epithelial cells can be induced to differentiate into functional insulin-producing cells. *Acta Biochimica et Biophysica Sinica*, Vol.40, No.9, pp. 830-839

Ianus, A.; Holz, GG.; Theise, ND. & Hussain, MA. (2003). In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *The Journal of Clinical Investigation*, Vol.111, No.6, pp. 843-850

Jansson, L. & Carlsson, PO. (2002). Graft vascular function after transplantation of pancreatic islets. *Diabetologia*, Vol.45, No.6, pp. 749-763

Jiang, J.; Au, M.; Lu, K.; Eshpeter, A.; Korbutt, G.; Fisk, G. & Majumdar, AS. (2007). Generation of insulin producing islet like clusters from human embryonic stem cells. *Stem cells*, Vol.25, No.8, pp. 1940-1953

Kadam, S.; Muthyala, S.; Nair, P. & Bhonde, R. (2010a). Reversal of experimental diabetes in mice by transplantation of neo-islets generated from human amnion--derived mesenchymal stromal cells using immuno-isolatory macrocapsules. *Cytotherapy*, Vol.12, No.8, pp. 982-991

Kadam, S.; Muthyala, S.; Nair, P. & Bhonde, R. (2010b). Human Placenta-Derived Mesenchymal Stem Cells and Islet-Like Cell Clusters Generated From These Cells as Novel Sources for Stem Cell Therapy in Diabetes. *The Review of Diabetic Studies*, Vol.7, No.2, pp. 168-182

Kania, G.; Blyszczuk, P. & Wobus, AM. (2004). The generation of insulin-producing cells from embryonic stem cells - a discussion of controversial findings. *International Journal of Developmental Biology*, Vol.48, No.10, pp. 1061-1064

Karnieli, O.; Izhar-Prato, Y.; Bulvik, S. & Efrat, S. (2007). Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem cells*, Vol.25, No.11, pp. 2837-2844

Kin, T.; O'Neil, JJ.; Pawlick, R.; Korbutt, GS.; Shapiro, AM. & Lakey, JR. (2008). The Use of an Approved Biodegradable Polymer Scaffold as a Solid Support System for Improvement of Islet Engraftment. *Artificial Organs*, Vol.32, No.12, pp. 990-993

Kizilel, S.; Garfinkel, M. & Opara, E. (2005). The bioartificial pancreas: progress and challenges. *Diabetes Technology & Therapeutics*, Vol.7, No.6, pp. 968-985

Klug, MG.; Soonpaa, MH.; Koh, GY. & Field, LJ. (1996). Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. The *Journal of Clinical Investigation*, Vol.98, No.1, pp. 216–224

Korbutt, GS.; Ao, Z.; Flashner, M. & Rajotte, RV. (1997). Neonatal porcine islets as a possible source of tissue for humans and microencapsulation improves the metabolic response of islet graft post transplantation. *Annals of the New York Academy of Sciences*, Vol. 831, No. 1, pp. 294-303

Lanza, RP.; A M Beyer, AM. & Chick, WL. (1999). Xenogenic humoral responses to islets transplanted in biohybrid diffusion chambers. *Transplantation*, Vol. 57, No.9, pp. 1371-1375

Lechner, A.; Nolan, AL.; Blacken, RA. & Habener, JF. (2005). Redifferentiation of insulin secreting cells after *in vitro* expansion of adult human pancreatic islet tissue. *Biochemical and Biophysical Research Communications*, Vol 327, No.2, pp. 581 – 588

Lechner, A.; Yang, YG.; Blacken, RA.; Wang, L.; Nolan, AL. & Habener, JF. (2004). No evidence for significant transdifferentiation of bone marrow into pancreatic beta-cells in vivo. *Diabetes*, Vol.53, No.3, pp. 616-623

Li, M.; Pevny, L.; Lovell-Badge, R. & Smith, A. (1998). Generation of purified neural precursors from embryonic stem cells by lineage selection. *Current Biology*, Vol.8, No.17, pp. 971–974

Lumelsky, N.; Blondel, O.; Laeng, P.; Velasco, I.; Ravin, R. & McKay, R. (2001). Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science*, Vol. 292, No.5520, pp. 1389–1394

Mao, GH.; Chen, GA.; Bai, HY.; Song, TR. & Wang, YX. (2009). The reversal of hyperglycaemia in diabetic mice using PLGA scaffolds seeded with islet-like cells derived from human embryonic stem cells. *Biomaterials*, Vol.30, No.9, pp. 1706-1714

Miki, A.; Rivas-Carrillo, JD.; Navarro-Alvarez, N.; Soto-Gutierrez, A.; Chen, Y.;Tanaka, K.; Narushima, M.; Tabata, Y.; Okitsu, T.; Noguchi, H.; Matsumoto, S.; Tanaka, N. & Kobayashi, N. (2006). Maintenance of neovascularization at the implantation site of an artificial device by bFGF and endothelial cell transplant. *Cell Transplantation*, Vol.15, No.10, pp. 893–901

Mikos, A. G., Papadaki, M. G., Kouvroukoglou, S., Ishaug, S. L. and Thomson, R. C. (1994), Mini-review: Islet transplantation to create a bioartificial pancreas. Biotechnology and Bioengineering, 43: 673–677Lavik, E. & Langer, R. (2004). Tissue engineering: current state and perspectives. *Applied Microbiolology and Biotechnology*, Vol.65, No.1, pp. 1–8

Mohan, N. & Nair, PD. (2005). Novel Porous, Polysaccharide Scaffolds for Tissue Engineering Applications. *Trends* in *Biomaterials* & *Artificial Organs*. Vol.18, No.2, pp. 219-224

Mohan, N.; Nair, PD. & Tabata, Y. (2010). Growth factor-mediated effects on chondrogenic differentiation of mesenchymal stem cells in 3D semi-IPN poly(vinyl alcohol)–poly(caprolactone) scaffolds. *Journal of Biomedical Materials Research Part A*, Vol.94, No.1, pp. 146–159

Monti, P.; Scirpoli, M.; Maffi, P.; Ghidoli, N.; De Taddeo, F.; Bertuzzi, F.; Piemonti, L.; Falcone, M.; Secchi, A. & Bonifacio, E. (2008). Islet transplantation in patients with autoimmune diabetes induces homeostatic cytokines that expand autoreactive memory T cells. *The Journal of Clinical Investigation*, Vol.118, No.5, pp. 1806-1814

Moriscot, C.; de Fraipont, F.; Richard, MJ.; Marchand, M.; Savatier, P.; Bosco, D.; Favrot, M. & Benhamou, PY. (2005). Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation *in vitro*. *Stem Cells*, Vol.23, No.4, pp. 594–603

Moritoh, Y.; Yamato, E.; Yasui, Y.; Miyazaki, S. & Miyazaki, J. (2003). Analysis of Insulin-Producing Cells During *In vitro* Differentiation From Feeder-Free Embryonic Stem Cells. *Diabetes*, Vol.52, No.5, pp. 1163-1168

Muthyala, S.; Bhonde, RR. & Nair, PD. (2010). Cytocompatibility studies of mouse pancreatic islets on gelatin - PVP semi IPN scaffolds *in vitro*: Potential implication towards pancreatic tissue engineering. *Islets*, Vol.2, No.6. pp. 357-366

Nair, PD.; Mohanty, M.; Rathinam, K.; Jayabalan, M. & V.N. Krishnamurthy, VN. (1992). Studies on the effect of degree of hydrophilicity on tissue response of Polyurethane interpenetrating polymer networks. *Biomaterials*, Vol. 13, No.8, pp. 537-542.

Nair, PD. (2009) A process for the preparation of a biocompatible polymeric composition of an inter-penetrating polymeric network (IPN), Indian patent 230740

Narang, AS. & Mahato, RI. (2006). Biological and Biomaterial Approaches for Improved Islet Transplantation. *Pharmacological Reviews*, Vol.58, No.2, pp. 194–243

Narushima, M.; Kobayashi, N.; Okitsu, T.; Tanaka, Y.; Li, SA.; Chen, Y.; Miki, A.; Tanaka, K.; Nakaji, S.; Takei, K.; Gutierrez, AS.; Rivas-Carrillo, JD.; Navarro-Alvarez, N.; Jun, HS.; Westerman, KA,.; Noguchi, H.; Lakey, JR.; Leboulch, P.; Tanaka, N. & Yoon, JW. (2005). A human beta cell line for transplantation therapy to control type 1 diabetes. *Nature Biotechnology*, Vol. 23, No.10, pp. 1274 - 1282

Nussbaum, J.; Minami, E.; Laflamme, MA.; Virag, JA.; Ware, CB.; Masino, A.; Muskheli, V.; Pabon, L.; Reinecke, H. & Murry, CE. (2007). Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *The FASEB Journal*, Vol.21, No.7, pp. 1345–1357

Paraskevas, S.; Duguid, WP.; Maysinger, D.; Feldman, L.; Agapitos, D. & Rosenberg, L. (1997). Apoptosis Occurs in Freshly Isolated Human Islets Under Standard Culture Conditions. *Transplantation Proceedings*, Vol.29, No.1-2, pp.750-752

Patience, C.; Takeuchi, Y. & Weiss, RA. (1997). Infection of human cells by an endogenous retrovirus of pigs. *Nature Medicine*, Vol.3, No.3, pp. 282-286

Perez-Basterrechea, M.; Briones, RM.; Alvarez-Viejo, M.; Garcia-Perez, E.; Esteban. MM.; Garcia, V.; Obaya, AJ.; Barneo, L.; Meana, A. & Otero J. (2009). Plasma–Fibroblast Gel as Scaffold for Islet Transplantation. *Tissue Engineering: Part A*, Vol.15, No. 3, pp. 569-577

Phadnis, SM.; Joglekar, MV.; Dalvi, MP.; Muthyala, S.; Nair, PD.; Ghaskadbi, SM.; Bhonde RR. & Hardikar, AA. (2011). Human bone marrow-derived mesenchymal cells differentiate and mature into endocrine pancreatic lineage in vivo. *Cytotherapy*, Vol.13, No.3, pp. 279-293

Phuc, PV.; Nhung, TH.; Loan, DT.; Chung, DC. & Ngoc, PK. (2010). Differentiating of banked human umbilical cord blood-derived mesenchymal stem cells into insulin-secreting cells. *In vitro Cellular & Developmental Biology*, Vol.47, No.1, pp. 54-63

Pileggi, A.; Molano, RD.; Ricordi, C.; Zahr, E.; Collins, J.; Valdes, R. & Inverardi, L. (2006). Reversal of diabetes by pancreatic islet transplantation into a subcutaneous, neovascularized device.*Transplantation*, No.81, pp. 1318–1324

Pileggi, A.; Ricordi, C.; Alessiani, M. & Inverardi, L. (2001). Factors influencing islet of Langerhans graft function and monitoring. *Clinica Chimica Acta*. Vol.310, No.1, pp. 3-16

Puissant, B.; Barreau, C.; Bourin, P.; Clavel, C.; Corre, J.; Bousquet, C.; Taureau, C.; Cousin, B.; Abbal, M.; Laharrague, P.; Penicaud, L.; Casteilla, L. & Blancher, A. (2005). Immunomodulatory effect of human adipose tissue-derived adult stem cells: Comparison with bone marrow mesenchymal stem cells. *British Journal of Haematology,* Vol.129, No.1, pp. 118-129

Rajagopal, J.; Anderson, WJ.; Kume, S.; Martinez, OI. & Melton, DA. (2003). Insulin staining of ES cell progeny from insulin uptake. *Science*, Vol.299, No.5605, pp. 363

Ramiya, VK.; Maraist, M.; Arfors, KE.; Schatz, DA.; Peck, AB. & Cornelius, JG. (2000). Reversal of insulin-dependent diabetes using islet cells generated *in vitro* from pancreatic stem cells. *Nature Medicine*, Vol.6, No.3, pp. 278 –282

Reckard, CR. & Barker, CF. (1973). Transplantation of isolated pancreatic islets across strong and weak histocompatibility barriers. *Transplantation Proceedings*. Vol.5, No.1, pp. 761-763

Ricordi, C.; Lacy, PE.; Finke, EH.; Olack, BJ. & Scharp, DW. (1989). Automated method for isolation of human pancreatic islets. *Diabetes*, Vol.37, No.4, pp. 140-142

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*, Vol.54, No.7, pp. 2060-2069

Sahu, S.; Joglekar, MV.; Dumbre, R.; Phadnis, SM.; Tosh, D. & Hardikar, AA. (2009). Islet-like cell clusters occur naturally in human gall bladder and are retained in diabetic conditions. *Journal of Cellular and Molecular Medicine*, Vol.13, No.5, pp. 999-1000

Salvay, DM.; Rives, CB.; Zhang, X.; Chen, F.; Kaufman, DB.; Lowe, WL Jr. & Shea, LD. (2008). Extracellular Matrix Protein-Coated Scaffolds Promote the Reversal of Diabetes After Extrahepatic Islet Transplantation. *Transplantation*, Vol.85, No.10, pp. 1456–1464

Sandler, S.; Andersson, A.; Eizirik, DL.; Hellerstrom, C.; Espevik, T.; Kulseng, B.; Thu, B.; Pipeleers, DG. & Skjak-Braek, G. (1997). Assessment of insulin secretion *in vitro* from microencapsulated fetal porcine islet like clusters and rat, mouse and human pancreatic islets. *Transplantation*, Vol.63, No.12, pp. 1712-1718

Scharp, DW.; Lacy PE.; Santiago, J, et al. (1990). Insulin independence after islet transplantation into type I diabetic patient. *Diabetes*, Vol.39, No.4, pp. 515-518

Scharp, DW.; Mason, NS. & Sparks, RE. (1984). Islet Immunoisolation:the use of hybrid artificial organs to prevent islet tissue rejection. *World Journal of Surgery*, Vol. 8, No. 2, pp. 221-229

Schneider, S.; Feilen, PJ.; Brunnenmeier, F.; Minnemann, T.; Zimmermann, H.; Zimmermann, U. & Weber, MM. (2005). Long-term graft function of adult rat and human islets encapsulated in novel alginate-based microcapsules after transplantation in immunocompetent diabetic mice. *Diabetes*, Vol. 54, No. 3, pp. 687–693

Segev, H.; Fishman, B.; Ziskind, A.; Shulman, M. & Itskovitz-Eldor, J. (2004). Differentiation of human embryonic stem cells into insulin-producing clusters. *Stem Cells*, Vol.22, No. 3, pp. 265–274

Serup, P.; Madsen, OD. & Mandrup-Poulsen, T. (2001). Islet and stem cell transplantation for treating diabetes. *British Medical Journal*, Vol.322, No.7277, pp. 29 –32

Shapiro, AM.; Gallant, HL.; Hao, EG.; Lakey, JR.; McCready, T.; Rajotte, RV.; Yatscoff, RW. & Kneteman, NM. (2005). The portal immunosuppressive storm: relevance to islet transplantation? *Therapeutic Drug Monitoring*, Vol.27, No.1, pp. 35-37

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. *The New England Journal of Medicine*. Vol. 355, No.13, pp. 1318-1330

Shapiro, AMJ. (2003). Islet Transplants and Impact on Secondary Diabetic Complications: Does C-Peptide Protect the Kidney?. *Journal of the American Society of Nephrology*, Vol. 14, No.8, pp. 2214–2216

Shapiro, AMJ.; Lakey, JRT.; Ryan, EA.; Korbutt, GS.; Toth, EL.; Warnock, GL.; Kneteman, NM. & Rajotte, RV. (2000). Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *The New England Journal of Medicine*. Vol.343, No.4, pp. 230-238

Siebers, U.; Horcher, A.; Bretzel, RG.; Klock, G.; Zimmermann, U.; Federlin, K. & Zekorn, T. (1993). Transplantation of free and microencapsulated islets in rats: evidence for the requirement of an increased islet mass for transplantation into the peritoneal site. The *International Journal of Artificial Organs*, Vol.16, No.2, pp. 96-99

Siebers, U.; Zekorn, T. & Bretzel, RG. (1990). Histocompatibility of semipermeable membranes for implantable diffusion devices (bioartificial pancreas). *Transplantation Proceedings*, Vol. 22, No. 2, pp. 834-835

Sipione, S.; Eshpeter, A.; Lyon, JG.; Korbutt, GS. & Bleackley, RC. (2004). Insulin expressing cells from differentiated embryonic stem cells are not beta cells. *Diabetologia*, Vol.47, No.3, pp. 499-508

Smith A. (2006). A glossary for stem-cell biology. *Nature*, Vol.441, No.7097, pp. 1060

Soria, B.; Roche, E.; Berna, G.; Leon-Quinto, T.; Reig, JA. & Martin F. (2000). Insulin secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes*, Vol.49, No.2, pp. 157–162

Stendahl, JC.; Kaufman, DB. & Stupp, SI. (2009). Extracellular Matrix in Pancreatic Islets:Relevance to Scaffold Design and Transplantation. *Cell Transplantation*, Vol.18, No.1, pp. 1–12

Stendahl, JC.; Wang, LJ.; Chow, LW.; Kaufman, DB. & Stupp, SI. (2008). Growth factor delivery from self-assembling nanofibers to facilitate islet transplantation. *Transplantation*, Vol.86, No.3, pp. 478–481

Stock, UA.; and Vacanti, JP. (2009). Tissue engineering: current state and prospects. *Annual Review of Medicine*, Vol.52, No.1, pp. 443-451

Street, CN.; Sipione, S.; Helms, L.; Binette, T.; Rajotte, RV.; Bleackley, RC. & Korbutt, GS. (2004). Stem cell-based approaches to solving the problem of tissue supply for islet transplantation in type 1 diabetes. *The International Journal of Biochemistry & Cell Biology*, Vol.36, No.4, pp. 667–683

Sun, AM.; Parisius, W.; Healy, GM.; Vacek, I. & Macmorine, HG. (1977). The use, in diabetic rats and monkeys, of artificial capillary units containing cultured islets of Langerhans (artificial endocrine pancreas). *Diabetes*, Vol. 26, No.12, pp. 1136–1139

Sun, Y.; Chen, L.; Hou, XG.; Hou, WK.; Dong, JJ.; Sun,L.; Tang, KX.; Wang, B.; Song, J.; Li, H. & Wang, KX. (2007). Differentiation of bone marrow-derived mesenchymal stem cells from diabetic patients into insulin-producing cells *in vitro*. *Chinese Medical Journal*, Vol.120, No.9, pp. 771–776

Sun, Y.; Ma, X.; Zhou, D.; Vacek, I. & Sun, AM. (1996). Normalization of diabetes in spontaneously diabetic cynomologus monkeys by xenografts of microencapsulated porcine islets without immunosuppression. *The Journal of Clinical Investigation*, Vol. 98, No. 6, pp. 1417–1422

Sweet, IR.; Khalil, G.; Wallen, AR.; Steedman, M.; Schenkman, KA.; Reems, JA.; Kahn, SE. & Callis, JB. (2002). Continuous measurement of oxygen consumption by pancreatic islets. *Diabetes Technology & Therapeutics*, Vol.4, No.5, pp. 661– 672

Timper, K.; Seboek, D.; Eberhardt, M.; Linscheid, P.; Christ-Crain, M.; Keller, U.; Muller, B. & Zulewski H. (2006). Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochemical and Biophysical Research Communications*, Vol.341, No.4, pp. 1135-1140

Tzakis, AG.; Ricordi, C.; Alejandro, R.; Zeng, Y.; Fung, JJ.; Todo, S.; Demetris, AJ.; Mintz, DH. & Starzl, TE. (1990). Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. *Lancet*, Vol.336, No.8712, pp. 402-405

Vaca, P.; Martin, F.; Vegara-Meseguer, JM.; Rovira, JM.; Berna, G. & Soria, B. (2006). Induction of differentiation of embryonic stem cells into insulin-secreting cells by fetal soluble factors. *Stem Cells*, Vol. 24, No.2, pp. 258–265

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*, Vol.407, No.6800, pp. 90-94

Wang, RN.; Kloppel, G & Bouwens, L. (1995). Duct - to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats . *Diabetologia*, Vol. 38, No.12, pp. 1405 – 1411

Wilson, JT. & Chaikof, EL. (2008). Thrombosis and Inflammation in Intraportal Islet Transplantation: A Review of Pathophysiology and Emerging Therapeutics. *Journal of Diabetes Science* and *Technology*. Vol.2, No.5, pp. 746-759

Yanez, R.; Lamana, ML.; Garcia-Castro, J.; Colmenero, I.; Ramirez, M. & Bueren, JA. (2006). Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. *Stem Cells*, Vol.24, No.11, pp. 2582–2591

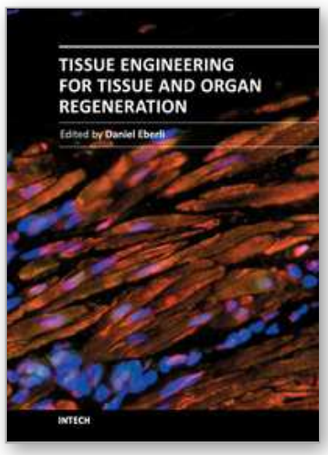
Yang, KC.; Wu, CC.; Lin, FH.; Qi, Z.; Kuo, TF.; Cheng. YH.; Chen, MP. and Sumi, S. (2008). Chitosan/gelatin hydrogel as immunoisolative matrix for injectable bioartificial pancreas. *Xenotransplantation*, Vol.15, No.6, pp. 407–416

Yang, L.; Li, S.; Hatch, H.; Ahrens, K.; Cornelius, JG.; Petersen, BE. & Peck, AB. (2002). *In vitro* trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone producing cells. *Proceedings of the National Academy of Sciences*, Vol.99, No.12, pp. 8078–8083

Zhao, LR.; Duran, WM.; Reyes, M.; Keene, CD.; Verfaillie, CM. & Low, WC. (2002). Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischaemic brain of rats. *Experimental Neurology*, Vol.174, No.1, pp. 11–20

Zhao, M.; Song, C.; Zhang, W.; Hou, Y.; Huang, R.; Song, Y.; Xie, W.; Shi, Y. & Song, C. (2010). The three-dimensional nanofiber scaffold culture condition improves viability and function of islets. *Journal of Biomedical Materials Research Part A*, Vol.94, No.3, pp. 667–672

Zulewski, H.; Abraham, EJ.; Gerlach, MJ.; Daniel, PB.; Moritz, W.; Muller, B.; Vallejo, M.; Thomas, MK. & Habener, JF. (2001). Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes*, Vol.50, No.3, pp. 521–533.




**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.

**How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Prabha D. Nair and Neena Aloysious (2011). Perspectives of Islet Cell Transplantation as a Therapeutic Approach for Diabetes Mellitus, Tissue Engineering for Tissue and Organ Regeneration, Prof. Daniel Eberli (Ed.), ISBN: 978-953-307-688-1, InTech, Available from: http://www.intechopen.com/books/tissue-engineering-for-tissue-and-organ-regeneration/perspectives-of-islet-cell-transplantation-as-a-therapeutic-approach-for-diabetes-mellitus

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