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Bioartificial Pancreas: Evaluation of Crucial Barriers to Clinical Application

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1. Introduction

The pancreas is a dual-function organ featuring both endocrine and exocrine tissue. Endocrine functionality is provided by approximately one million cell clusters called the islets of Langerhans. Islets consist of four main cell types, 1) α cells: secrete glucagon (increases glucose in blood); β cells: secrete insulin (decreases glucose in blood); δ cells: secrete somatostatin (regulates α and β cells) and PP cells: secrete pancreatic polypeptide. Thus, the islet plays a diverse role in glucose metabolism and blood glucose homeostasis.

Diabetes mellitus, which results from an insufficiency or total lack of insulin, affects 350 million people worldwide (Serup et al., 2001). Diabetes is classified into two main types: Type 1 diabetes (sometimes called juvenile-onset or insulin dependent diabetes) is usually associated with a complete lack of insulin brought about by autoimmune destruction of the insulin producing beta cells (Eisenbarth et al., 1992; Mathis et al., 2001). Recurrence of Type 1 diabetes after pancreas transplantation between identical twins has been described and is a hallmark of autoimmune disease (recurrent autoimmunity occurs) (Sibley et al., 1985). The inciting events for autoimmune (Type 1) diabetes are unknown, but possibly there are viral or environmental triggers that act upon a genetically susceptible population. Type 2 diabetes (often called adult-onset diabetes) is generally non-insulin dependent (though clinical features of Type 1 and Type 2 diabetes may overlap) and arises from peripheral resistance to insulin and a relative insufficiency of insulin, resulting in an initial attempt by the beta cells to compensate with release of higher than normal amounts of insulin. As Type 2 diabetes progresses, β cells become desensitized to persistently high glucose concentrations, and normal responses to glucose signaling are lost (Costa et al., 2002). In late Type 2 diabetes the islets become hyalinized, beta cell dropout occurs, and there is a state of insulinopenia that can be clinically difficult to distinguish from Type 1 diabetes. Although late stage Type 2 diabetic subjects often require insulin in high doses due to peripheral insulin resistance (Holman & Turner, 1995), Type 1 diabetic patients may also develop significant insulin resistance. A bioartificial pancreas capable of supplying a sufficient amount of insulin could effectively treat both Type 1 and Type 2 diabetes.

2. Therapeutic options for Type 1 diabetes

Prior to the discovery of insulin by Banting and Best (1921), effective treatment of diabetes mellitus was limited to dietary manipulation. Many thought that the ability to administer insulin exogenously would prove to cure diabetes, but the long-term imperfections in glycemic control present even with state of the art insulin management results in the so called secondary complications of diabetes (diabetic nephropathy, retinopathy, neuropathy, and vascular disease) and diminishes life expectancy and quality of life in many patients. The discovery of insulin converted an often rapidly fatal disease to a chronic condition requiring life-long treatment. Current treatment for diabetes, both Type 1 and Type 2, includes exogenous insulin therapy and endocrine replacement by transplantation. Both of these clinical approaches have considerable inherent drawbacks. A theoretical alternative that is being tested in animal and pre-clinical models is the bioartifical pancreas.

2.1 Exogenous insulin treatment

Exogenous insulin administration to control blood glucose has been the standard therapy since the discovery of insulin. In this therapy, the amount of carbohydrates consumed is estimated by measuring food, and this is used to determine the amount of insulin necessary to cover the meal. The calculation is based on a simple open-loop model based on past success. Calculated insulin is then adjusted based on pre-meal blood glucose measurement, such that, insulin bolus is increased for high blood glucose or delayed for low-blood glucose. Insulin is injected or infused subcutaneously and enters the blood stream in approximately 15 min. Then blood glucose can be tested again and adjusted by additional insulin bolus or eating more carbohydrates, until balance is achieved. However, exogenous insulin administration, even via pump, is unable to match the fine control of glucose by the endocrine pancreas. The exaggerated glycemic excursions associated with insulin administration impacts health and quality of life. The poor control of blood glucose levels with this therapy leads to severe secondary complications such as retinopathy, neuropathy, nephropathy, and cardiovascular diseases (Kort et al., 2011; Opara et al., 2010). According to the Diabetes Control and Complications Trial (DCCT, 1993), strict control of blood glucose reduces the risk of developing diabetes-related complications, but may result in an increased incidence of hypoglycemia.

2.2 Pancreas transplantation

Kelly and Lillehei performed the first clinical pancreas transplant at the University of Minnesota in 1966. Currently, pancreas transplantation is the only option therapeutically available that reproducibly achieves normoglycemia. Pancreas transplantation re-establishes endogenous insulin secretion that is responsive to normal feedback regulation. Since 1966, more than 30,000 pancreas transplants have been performed worldwide. According to the 2009 Scientific Registry of Transplant Recipients (SRTR), the 1-year rate of graft survival is 86% when a pancreas and a kidney were transplanted together (SPK), 82% when pancreas is transplanted after kidney (PAK) and 75% when pancreas is transplanted alone (PTA). In that year, 848, 258, and 104 transplants were done in those categories. Most pancreatic grafts are from cadaver donors, though transplantation of a segment of the pancreas donated by a living donor has also been reported (Reynoso et al., 2010). Transplantation, however, requires major

surgery and dependence on lifelong immunosuppression to prevent rejection. Most pancreas transplants are performed with immunosuppression induction therapy (usually monoclonal or polyclonal T-cell depleting antibody) and maintenance immunosuppression with a calcineurin inhibitor (cyclosporine or tacrolimus), an antimetabolite (mycophenolic acid) plus or minus coticosteroids (Robertson et al., 2000; Sutherland et al., 2001). Because of the limited availability of human pancreases and the need for immunosuppression, relatively few pancreas transplants are done compared to the entire diabetic population. Improvements in surgical technique or immunotherapy are unlikely to make whole organ pancreas transplantation available to the majority of patients with diabetes.

2.3 Islet transplantation

Islet transplantation promises to be a cure at least as effective as pancreas transplantation, while being much less invasive. The efficiency of islet recovery from the whole organ pancreas and the susceptibility of allogeneic islet to immune attack (both alloimmunity and autoimmunity) are the two major barriers to successful islet transplantation. There are approximately 1 million islets in an adult human pancreas. However, only half or fewer of these are successfully isolated on a consistent basis. Thus, islet transplantation usually requires islets isolated from two or more donor pancreases. Because islet isolation requires manipulation of human tissue, the process must be carried out in a good manufacturing process (GMP) facility, which adds to the expense of the procedure. Islets are transplanted by transfusion into the portal vein and embolization into the liver. The transplanted islets engraft in the distal portal triad (Figure 1).
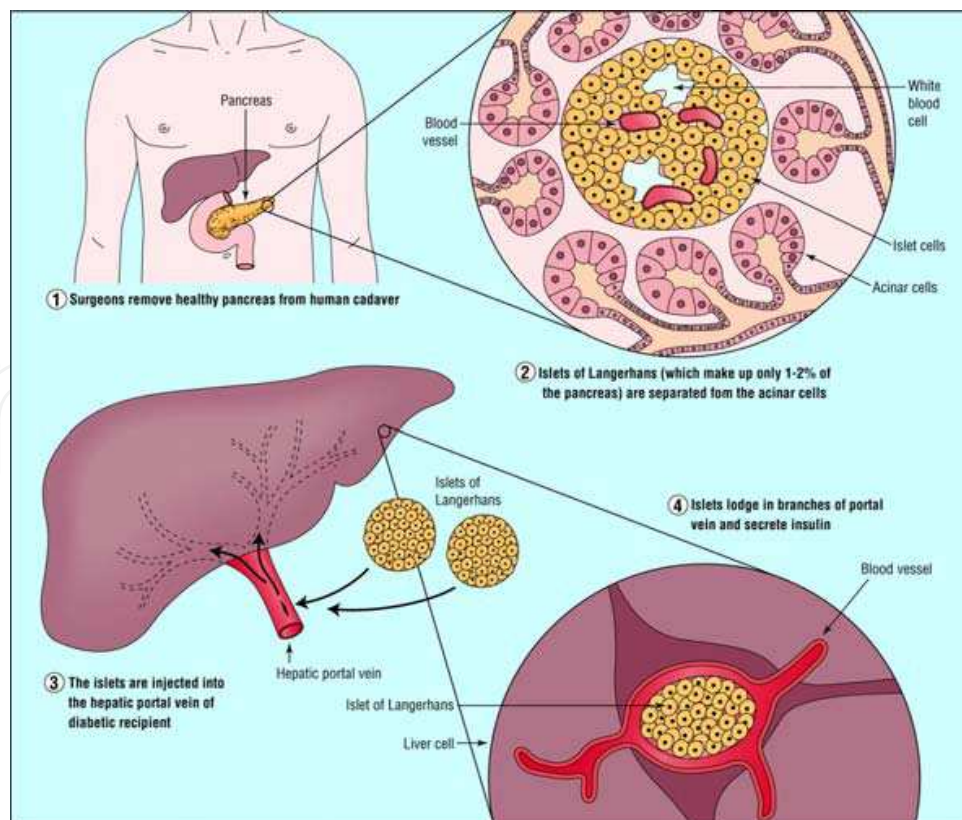

Fig. 1. Illustration of islet transplantation (Serup et al., 2001)(with permission).

Allogeneic human islets have been successfully transplanted using the Edmonton immunosuppression (steroid free) protocol (Shapiro et al., 2000). In investigations with this protocol, glycemic control has been restored for extended periods of greater than 5 years, but at the expense of immunosuppression of the transplant recipient. The necessary life long adherence to an immunosuppression drug regimen is inconvenient and associated with side effects and complications of over-immunosuppression.

Transplantation of the islets isolated from the same individual is referred to as autologous or autotransplantation, transplantation from one individual to another of the same species is referred to as allogenic or allotransplantation, and transplantation from a different species is referred to as xenogenic or xenotransplantation. Autotransplantation of islets is generally done for nondiabetic patients who require a pancreatectomy for benign disease of the pancreas (Farney et al., 1991) As of April 2008, allogeneic islet transplantation had been applied to 325 patients worldwide (CITR 2008). Only limited numbers of xenoislet transplants have been performed in humans and would appropriately be considered experimental.

2.4 Artificial pancreas

In the pancreas, the insulin is released in proportional response to actual blood glucose levels. The insulin gets released into the portal vein, where it predominately flows toward the liver, which is the major organ to store glycogen and about 50% of secreted insulin gets used in the liver. Also, the insulin release is pulsatile which helps to maintain the insulin sensitivity of the hepatic tissue. Owing to severe shortage of human pancreas and the shortcomings of insulin therapy, a lot of effort has been made to develop an artificial pancreas. The artificial pancreas is a technological development to enable Type 1 diabetic patients to automatically control their blood glucose, acting in essence like a healthy pancreas. The goals of the artificial pancreas are: 1) To improve presently popular but inefficient insulin therapy to attain a better glycemic control, thus avoiding the complication due to blood glucose fluctuations, and 2) To mimic normal stimulation of the liver by the pancreas and to normalize carbohydrate and lipid metabolism.

There are various approaches to the artificial pancreas:

1. Medical equipment approach: An insulin pump under closed loop control utilizing real-time data from a continuous blood glucose sensor.
2. Gene therapy approach: Therapeutic infection of a diabetic person by a genetically engineered virus causing a DNA transformation of few intestinal cells to become insulin-producing cells. It has even been suggested to tackle the cause of beta cell destruction itself hence curing the patients before full and irreversible β cells destruction (Rothman et al., 2005). While novel and potentially able to treat diabetes, this approach is still in infancy with a lot of unanswered questions.
3. Bioengineering approach: Development of microcapsules or biocompatible sheet of encapsulated islets. When implanted, these would behave as the native pancreas itself. This approach is the main focus of this chapter, as it has promise to be a good alternative to pancreas transplantation.

3. Bioartificial pancreas

Bioengineering approach to the artificial pancreas is to implant islet, which would secrete insulin, amylin and glucagon in response to host blood glucose without any external

interference. Severe shortage of human islets and the associated need for immunosuppression have led to a lot of interest to overcome these barriers using the bioartificial pancreas approach. In Type 1 diabetes, the afflicted individual has pre-existing antibodies and immune cells against β-cell surface epitopes and insulin (Jaeger et al., 2000) and hence a simple islet transplant is not tenable. Therefore, the islets would require a protective coating to preserve their viability and function prior to transplantation. With this approach allo- and xenotransplants can be done safely, thus overcoming the shortage of islets and rejection problem while serving to restore the pancreatic endocrine function. This approach not only benefits the longevity of transplant but also relieves patients from the burden of immunosuppressant drugs.

3.1 Islet isolation

Isolation of pure islets without inflicting any significant damage to islets is key to successful islet transplantation. A critical balance of composition, process and duration of collagenase digestion is required for isolating islets with integrity, viability and high purity with a significant yield. This overall process has tremendous impact on the clinical outcome of islet transplants (Lakey et al., 2002). The pancreas is digested with combined collagenase and protease action, which disintegrates the intercellular matrix of collagen, releasing islets. These islets are isolated, purified, tested for viability, and sometimes cultured before being transplanted in the patient. Collagenase digestion disrupts islet-exocrine tissue adhesive contacts (Wolters et al., 1992). Thus, shorter duration or lower concentration of collagenase would lead to incomplete purification of islets from exocrine tissue, leading to reduced yield on purification. On the other hand, extended duration of incubation or higher concentration of collagenase would adversely affect the islet cell-cell adhesion, leading to loss of islet integrity and viability. Intra-islet cell-cell adhesion is protease-sensitive, while extra-islet cell-matrix adhesion is collagenase sensitive. In the pig, very little periinsular capsule is present and the structural integration of the porcine islet in the exocrine pancreas is almost exclusively cell-cell adhesion. In canine, the islets are almost exclusively encapsulated with very little exocrine-endocrine cell-cell contact. In rodent and human, the situation is intermediate with a tendency towards predominance of cell-matrix adhesion. The presence of protease in the collagenase preparations has been reported to reduce the yield and quality of isolated islets in rats (Vos-Scheperkeuter et al., 1997), however it is more efficient for the isolation of pig islets (Deijnen et al., 1992). Figure 2 shows the effect of transplanted islet mass on short- and long-term glycemic normalization in a rat.

3.2 Biomaterials

Islets are encapsulated in a protective coating for immunoisolation, hence it is very important that not only are the biomaterials used biocompatible, they should also be permeable for hormonal, nutrient and oxygen exchange. Biocompatibility of these devices is mostly assessed for fibrosis at the site of implantation. Use of a smooth outer surface and hydrogels further improves biocompatibility of these devices through absence of interfacial tension, thus reducing protein adsorption and cell adhesion.

Polyacrylonitrile and polyvinylchloride copolymer has been examined for the construction of microcapillaries used with intravascular macrocapsules. Extravascular macrocapsules have been made with various biomaterials, including nitrocellulose acetate, 2-hydroxyethyl

methacrylate (HEMA), acrylonitrile, polyacrylonitrile and polyvinylchloride copolymer, sodium methallylsulfonate, and alginate (Narang & Mahato, 2006). Hydrogels are very attractive for making microcapsules as they provide higher permeability for low molecular weight nutrients and metabolites. Furthermore, the soft and pliable features of the gel reduce the mechanical or frictional irritations to surrounding tissue (de Vos et al., 2002). The most commonly applied materials for microencapsulation are alginate (Lim & Sun, 1980), chitosan (Zielinski & Aebischer, 1994), agarose (Iwata et al., 1989), cellulose (Risbud & Bhonde, 2001), poly(hydroxyethylmetacrylate-methyl methacrylate) (HEMA-MMA) (Dawson et al., 1987), copolymers of acrylonitrile (Kessler et al., 1991), and polyethylene glycol (PEG) (Cruise et al., 1999).
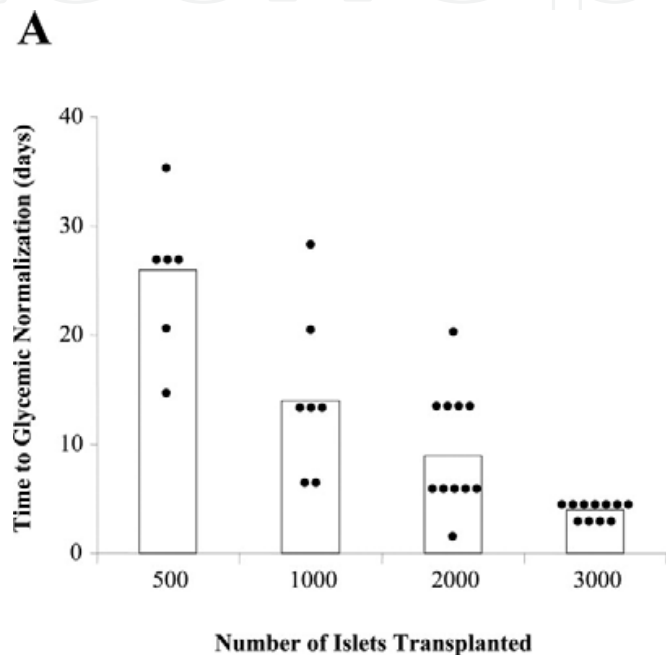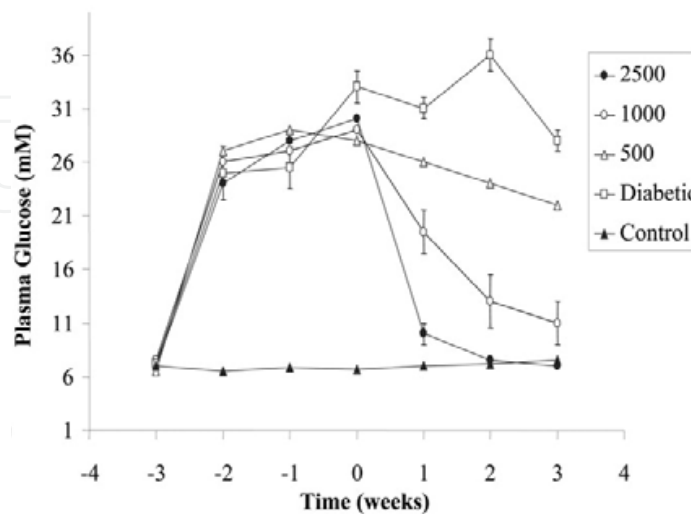

Fig. 2. Effect of transplanted islet mass on short- and long-term glycemic normalization in a rat (Finegood et al., 1992; Bell et al., 1994)(with permission).

3.2.1 Alginate

Alginate is the most studied hydrogel for islet encapsulation, as it provides some major advantages over other encapsulation alternatives. Alginate molecules are linear block co-polymers of β-d-mannuronic (M) and a-l-guluronic acids (G). It forms a gel in the presence of divalent ions like Ca^{2+} and Ba^{2+}. Recent findings have shown that divalent ions crosslink not only G blocks but also blocks of alternating M and G (M-G blocks) (Donati et al., 2005). Mainly calcium is used for gelling, as barium is known to be toxic and concerns have been raised about patients' safety if it is used as the cross-linking agent.

Alginate is one of the few materials that allow islet encapsulation at physiological conditions. The encapsulation can be done at room or body temperature, at physiological pH, and in isotonic solutions. Islets have been shown to more readily and adequately survive when being enveloped in alginate capsules (Sandler et al., 1997). It does not interfere with cellular function of the islets (Fritschy et al., 1991a,b; Haan et al., 2003). It has been shown that alginate capsules provide a microenvironment facilitating functional survival of islets probably because the three-dimensional matrix provides mechanical support for the islets and prevents clumping and fusion of the free islets, thus preserving its organization. Alginate-based capsules have been shown to be stable for years in both animals and human (Soon-Shiong et al., 1994; Sun et al., 1996). Also, since alginates are negatively charged, the attachment of immune cells to the microcapsule is limited due to the negative charge on the cell surface.

3.3 Islet encapsulation

Presently, life-long immunosuppression with drugs is required for islet transplantation, but immunosuppression can be obviated by immunoisolating the islets in a semipermeable membrane to protect them from the host immune system. Immunoisolation by encapsulation would not only allow for successful transplantation of allogeneic islets without immunosuppression (Lim & Sun, 1980) but also transplantation of islets from non-human origin (Omer et al., 2003; Zimmermann et al., 2005).

Islet encapsulation is done in aqueous dispersion with low agitation, in the presence of iso-osmotic salt, glucose and oxygen in the media under physiological pH with a preferably short encapsulation time. Usually the formed beads are post-coated with a cationic poly(amino acid), e.g., PLL or PLO, to provide perm-selectivity and improve capsule integrity (Chaikof, 1999). This is followed by a surface coating of low viscosity alginate, resulting in a microcapsule morphology that presents encapsulated islet(s) in a liquid layer of alginate, followed by PLL/PLO coating and gel layer of alginate on exterior, thus creating an alginate-PLL/PLO-alginate construct known as APA microcapsules. Figure 3 shows encapsulated islets in alginate microcapsules.

Islet encapsulation has been done mainly with one of these techniques: 1) Interfacial precipitation, 2) Phase Inversion, and 3) Polyelectrolyte coacervation. Phase inversion has been used for macrocapsules and would be discussed in that section below while interfacial precipitation would be discussed in the microcapsules section. Polyelectrolyte coacervation is a modification of alginate-calcium interfacial precipitation system, in which complexation of oppositely charged polymers leads to formation of a hydrogel membrane encapsulating the islets (Chaikof, 1999). Encapsulated islets have shown improved graft function and survival in animals compared with unencapsulated islets. Sun et al. reported that the transplantation of

encapsulated porcine islets in spontaneously diabetic monkeys induced normoglycemia without immunosuppression for more than 800 days (Sun et al., 1996). Schneider et al. showed survival of encapsulated human and rat xenografts in mice for 7 months without a semi-permeable coating (Schneider et al., 2005). However, generally alginate microcapsules without perm-selectivity have not been shown to be viable in clinical trials (Tuch et al., 2009).
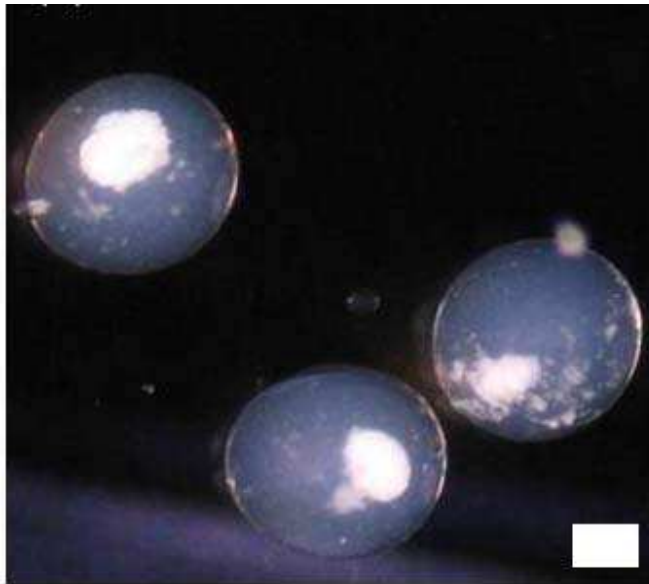

Fig. 3. Encapsulated islets in an alginate microcapsule. Scale=100 μm.

3.3.1 Macroencapsulation

There are two types of macrocapsule systems, based on their transplant location; extravascular and intravascular. Intravascular macrocapsules are based on the principle of "dialysis cartridges". Islets are seeded between hollow fibers that get perfused by blood flow. These hollow fibers are enclosed within a larger tube and implanted into the vessels of the host by vascular anastomoses. These devices have been successful in inducing normoglycemia in various diabetic animal models including rats, dogs and monkeys (Maki et al., 1993; Sun et al., 1977). The use of these devices however requires intense systemic anticoagulation (due to direct contact of foreign material with blood) and thus has the potential for fatal thrombus formation.

Extravascular macrocapsule devices in contrast have the advantage that biocompatibility issues do not pose a serious risk to patient. They have been designed in both flat sheet membrane and hollow fiber forms (Zekorn et al., 1995). A semi-permeable membrane around the sheet allows diffusion of nutrients and secreted hormones but not macrophages. They are usually coated with hydrogels to achieve a smooth outer surface to improve biocompatibility. Initial studies with extravascular macrocapsules have encapsulated multiple islets in one or several large capsules. Islets aggregated in large clumps and these studies were not successful, due to necrosis at the center of the clumps (Lacy et al., 1991). Later, this problem was addressed by solitude immobilization of islets in a matrix before encapsulation. Promising results were observed in animal studies, with survival rate of islets in the device up to 200 days after implantation in the peritoneal cavity of rat but human studies have not been very encouraging (Jain et al., 1999; Scharp et al., 1994).

The major drawback of macrocapsules is relative low surface to volume ratio, which interferes with optimal diffusion of nutrients and oxygen. For adequate nutrients and oxygen the islet density in the macrocapsules is kept quite low (usually 5-10% volume). This makes the macrocapsules rather impractical as large devices have to be implanted to provide sufficient masses of islets and these devices cannot be implanted in conventional transplantation sites (Suylichem et al., 1992). Low surface to volume ratio also interferes with glucose regulation due to slow exchange of glucose and insulin.

3.3.2 Microencapsulation

In most tissues, it has been shown that maximum diffusion distance for effective oxygen and nutrient diffusion from blood capillary to cells is about 200 µm. Absence of this convection inside a capsule induces a nutrient-gradient from the capsule surface to center of islet. Present insights suggest microcapsules as preferable system over macrocapsules due to their high surface to volume ratio for fast exchange of hormones and nutrients. Microencapsulation uses the interfacial precipitation predominantly, where a polyanionic polymer (alginate) gels with a divalent cation (Ca^{2+}, Ba^{2+}). Islets are suspended in an alginate solution and its droplets are generated either by air jet spray method (Wolters et al., 1991), electrostatic generators (Hallé et al., 1994; Hsu et al., 1994), submerged oscillating coaxial extrusion nozzles (Dawson et al., 1987), conformal coatings (Desmangles et al., 2001), and spinning disk atomization (Senuma et al., 2000). Of these methods, the air jet spray method, which uses a two-channel air droplet microencapsulator, is the most commonly used. Two-channel air droplet microencapsulators operate by allowing the alginate cell suspension to drip through an inner channel of the device while the outer channel uses an air jacket to shear off the alginate droplet. Using this method, the diameters of the inner and outer channels, the flow rate of the alginate, and air pressure of the outer channel can be adjusted to vary the microcapsule size (Wolters et al., 1991). In order to prevent hypoxic damage to islet cells, microencapsulation must be done relatively quickly around 4°C.

One major limitation to the current encapsulation devices is that they are incapable of efficiently encapsulating large numbers of islets in a reasonable amount of time. This may result in hypoxic stress and loss of functionality to islets in larger scaled up experiments (de Vos et al., 1997; Opara et al., 2010). A recently proposed alternative microencapsulation method utilizes multichannel air jacket microfluidic devices. These devices have the advantage of rapidly encapsulating large numbers of islets into microcapsules, at speeds in excess of 8 times conventional methods, without effecting the functionality of the islets. Additionally, this microfluidic approach can be easily scaled up to increase production rates, and can be cost effectively produced using rapid prototyping technology (Tendulkar et al., 2011). A reduction in capsule size would benefit the islet and also exponentially decrease the total transplant volume. Therefore, much work has been done with various new technologies to make beads as small as 185 µm (diameter), which is about four times smaller than conventional beads (800 µm). The smaller the diameter of the capsules the better the diffusion of nutrients to the islets, and Omer et al. demonstrated that capsules with a diameter of 600±100 µm showed improved stability in vivo over larger capsules with diameters of 1000±100 µm (Omer et al., 2005). However, there is another factor, with reduction in capsule size the number of capsules containing partially protruding islets also proportionally increases, and this in turn increases the number of capsules affected by an inflammatory response. Decreasing the islet density in alginate can solve this problem. It has

been shown that each capsule size has an optimal islet density. Usually this is associated with a slight increase in empty capsules but minimizing protruding islets is of upmost priority. In many cases, the inner alginate bead will be either completely or partially liquefied by the removal of calcium ions with calcium quenching reagents such as sodium citrate, which allows for improved diffusion in the microbeads (Darrabie et al., 2005). Another consideration is the morphology of the microcapsules used for encapsulated islets. Spherical microcapsules are necessary for long term functionality; irregularities or imperfections in the microcapsules can cause an immune response and result in loss of islet functionality (Hobbs et al., 2001).

3.4 Immune barrier

Uncoated non-permselective alginate microbeads have been reported to have a high permeability (>600 kD). Uptake studies with IgG (150 kD) and thyroglobulin (669 kD) suggested they were able to get into these uncoated microbeads. Similarly, uncoated alginate microbeads implanted in the peritoneum were positive for both IgG and C3 component after only 1 week (Lanza et al., 1995). So, small molecules from macrophages and T-cells to smaller cytokine molecules such as IL-1β, TNF-α, and IFN-γ can easily penetrate into the microcapsules and can damage or destroy the encapsulated islets (van Schilfgaarde & de Vos, 1999). To provide immunoisolation for the microcapsules, it is essential to apply a permeability barrier between the encapsulated islets and host immune system. Applying a polyamino acid layer, followed by an additional outer coating of alginate, typically creates this barrier. The positively charged polyamino acid molecules will readily bind to the negatively charged alginate molecules forming a complex membrane (Bystrický et al., 1990; Thu et al., 1996), which significantly reduces the pore size of the microcapsule, and prevents immune cells from entering the microcapsule (Hallé et al., 1994; King et al., 1987; Kulseng et al., 1997). In order to prevent interactions of non-bound polyamines to host tissue, a thin second layer of alginate is added. This polyamino barrier acts as a shell, providing mechanical stability to the microcapsule, allowing for the liquefaction of the inner alginate (Darrabie et al., 2001). The thickness of this barrier can be varied through incubation time and concentration (Gugerli et al., 2002). The most researched perm-selective biomaterial is poly-L-Lysine (PLL) which was the first material used to generate this barrier, however more recent research has shown that poly-L-ornithine had markedly reduced immune response and has been shown to provide more mechanical support to the microcapsules (Lim & Sun, 1980; Darrabie et al., 2001).

In general, we can distinguish the host reaction towards encapsulated islets into two types: 1) Inflammatory reaction against the capsule material. With the present technology these reactions can be successfully prevented by applying purification steps to the materials to be used, and 2) Host response against the allogenic or xenogenic cell-derived bioactive factors or antigens that leak out of the capsules (de Vos et al., 2002). It results in overgrowth by macrophages and lymphocytes on a small portion (~10%) of the capsules and in a humoral immune response against the encapsulated tissue. It has long been known that islets secrete cytokines upon stress (Cardozo et al., 2003). Encapsulated islets have been shown to produce the cytokines MCP-1, MIP, nitric oxide (NO) and IL-6 under stress (stress induced by adding IL-1β and TNF-α), and these cytokines are well known to contribute to the recruitment and activation of inflammatory cells (de-Groot et al., 2001). Also, it has been demonstrated that activated macrophages on the 2-10% microcapsules with overgrowth do secrete the cytokines

IL-1β and TNF-α when cultured with encapsulated islets but not with empty capsules (Vos et al., 2004). This activation of inflammatory cells results in the production of cytokines, which are deleterious not only to the islet cells in the overgrown capsules but also the islets in the vast majority of transplanted, clean, and non-overgrown capsules. Figure 4 shows the manipulation of alginate microcapsules permeability to control cytokine action.
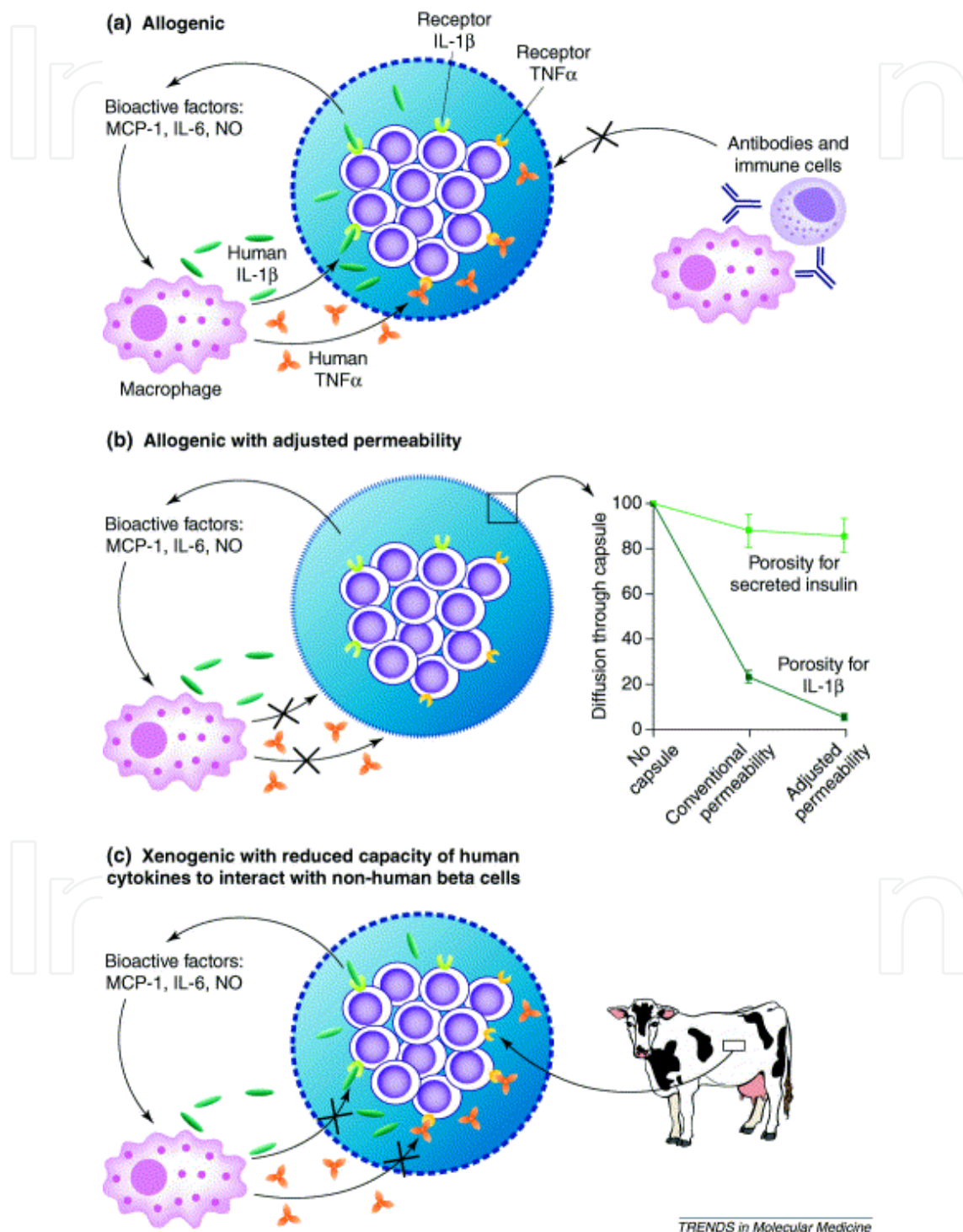

Fig. 4. Manipulation of microcapsules permeability to control cytokine action (de Vos & Marchetti, 2002)(with permission).

3.4.1 Poly-L-Lysine (PLL)

Since the introduction of the concept of microencapsulation of islets, PLL has been routinely used as the selectively-permeable membrane for microcapsules. It makes xenotransplantation of islets a feasible option and provides mechanical stability features required for application in large animals and human. After gelation of the beads in calcium, the beads are suspended in polycation solutions such as PLL, which increases membrane integrity via electrostatic association with the anionic alginate.

Many studies have shown the effectiveness of APA microcapsules in small animals (Chen et al., 1994; Fan et al., 1990; Fritschy et al., 1991a; Lim & Sun, 1980; Lum et al., 1992; O'Shea & Sun, 1986; O'Shea et al., 1984; Sun et al., 1984), large animals (Kendall et al., 2001; Soon-Shiong et al., 1992; Sun et al., 1996; Wang et al., 2008), and humans (Calafiore et al., 2006; Elliott et al., 2000; Sun et al., 1996). However, unbound PLL has been shown to cause an inflammatory response and result in fibrotic over growth over microcapsules (Strand et al., 2001; Thu et al., 1996b). This fibrotic overgrowth can severely affect islet functionality and viability by significantly reducing the rate of diffusion into the capsule (de Vos et al., 2006). Recent studies which have examined the alginate–PLL interface have found that the PLL does not form a distinct membrane separate from the alginate as originally thought, rather the PLL is found within 30 µm of the alginate (Strand et al., 2003; Thu et al., 1996a). These results indicate that even in capsule systems which use an outer alginate core, PLL is still present to some extent on the surface of the capsules. This could increase the chance of triggering an inflammatory reaction and fibrotic overgrowth (Clayton et al., 1991; King et al., 1987; Strand et al., 2001). An additional problem caused by the PLL layer is an increase in surface roughness, and one study by de Vos et al. (1999) showed that implanted alginate-PLL microcapsules made with ultra-pure alginate still caused significant fibrotic overgrowth. Also, atomic force microscopy indicated that these capsules had an increased surface roughness due to the PLL molecules (Bunger et al., 2003). However when PLL is properly bound, the implanted capsules can avoid an inflammatory response.

3.4.2 Poly-L-Ornithine (PLO)

One well-investigated alternative to PLL is PLO. Like PLL, PLO is a positively charged polyamine which, when applied to alginate microcapsules, forms a semi-permeable membrane which significantly reduces the porosity of the microcapsules, allowing for immuno-isolation without impairing oxygen and nutrient diffusion. Unlike PLL, PLO has been shown to evoke less of an immune response and has been shown to have improved mechanical properties (Brunetti et al., 1991; Calafiore et al., 1997; Calafiore et al., 2004; Kizilel et al., 2005). When compared to alginate-PLL microcapsules, alginate-PLO microcapsules have been shown to better resist swelling and bursting under osmotic stress (Darrabie et al., 2005). Bead swelling is an important factor to take into consideration because it can lead to increased in pore size, permeability, and shear stress, which leads to decreased islet viability (Thu et al., 1996a). It has been hypothesized that the improved mechanical properties of alginate-PLO microcapsules over alginate-PLL microcapsules is due to the improved bonding of PLO to alginate owing to the shorter monomer structure of PLO (Darrabie et al., 2005; Inaki et al., 1997). Also, while PLL seems to bind to M-G sequences, PLO has been shown to prefer M-M sequences (Calafiore et al., 2006). Long-term studies, where empty alginate-PLO microcapsules were injected intraperitoneally in

rodents, dogs, or pigs have always resulted in retrieval of intact and overgrowth-free microcapsules up to one year post-implant (de Vos et al., 2006).

3.5 Transplantation site

It is necessary for clinical application to find a site where encapsulated islets are in close contact with the blood stream. Unfortunately, it is difficult to find such a site since it should combine the capacity to bear a large graft volume in the immediate vicinity of blood vessels. Implantation of islets is most commonly done intraperitoneally, as it offers the advantages of laparoscopic implantation or through injection, and allows ample room to implant numerous microcapsules (Calafiore et al., 2006; Elliott et al., 2000). However, there are several disadvantages to this site. The most significant disadvantage is that microcapsules that are implanted intraperitoneally are vulnerable to an immune response from intra-peritoneal T-cells and macrophages (de Groot et al., 2004; de Vos et al., 1999; de Vos et al., 2003; Safley et al., 2005), and have less access to the vasculature (de Vos and Marchetti, 2002; de Vos et al., 1999). This results in an increased likelihood of fibrotic growth over encapsulated islets, a loss of graft functionality, and a delay in insulin uptake into the blood circulation (de Vos et al., 1996). Consequently, alternative transplantation sites have been investigated, including transplanting into the liver (Toso et al., 2005), kidney capsule (Dufrane et al., 2006a), subcutaneously (Dufrane et al., 2006b), and into an omentum pouch (Kin et al., 2003; Kobayashi et al., 2006; Moya et al., 2010(a,b); Opara et al., 2010). In a study conducted by Toso et al., microcapsules were injected into the portal veins of rats; however, the results of the study showed that immunosuppressants are necessary to prevent fibrotic overgrowth (Toso et al., 2005), and the risk of hepatic thrombosis makes this approach impractical. Studies by Dufrane et al. investigated implant sites such as subcutaneous and the kidney capsule, and the results indicated that encapsulated islets implanted in these two sites had less cellular overgrowth compared to encapsulated islets implanted intraperitoneally. Additional studies demonstrated the functionality of encapsulated islets implanted within the kidney capsule of primates (Dufrane et al., 2006a); however, for clinical applications the limited space within this site may be a problem (de Vos et al., 2002). Studies have also investigated using the omentum as pouch for implanting encapsulated islets, which, like the kidney capsules, offers a well vascularized site for transplantation but has more space for microcapsules and is easier to access (Kin et al., 2003).

3.5.1 Omentum pouch

The use of an omentum pouch as a site for islet transplantation has also been investigated, and early studies (Ferguson & Scothorne, 1977; Yasunami et al., 1983) demonstrated the ability of unencapsulated islet grafts to survive within an omental pouch. More recent research has shown that encapsulated islets implanted into the omentum pouch have increased periods of functionality than encapsulated islets implanted intraperitoneally (Aomatsu et al., 1999; Kobayashi et al., 2006). In a study by Aomatsu et al. diabetic Balb/c mice were implanted with 1000 agarose microencapsulated islets in both the peritoneal cavity and omentum pouch; the results indicated that graft functionality was significantly longer in the omentum pouch (27.1±5.5 days) than the peritoneal cavity (12.5±12.5 days) (Aomatsu et al., 1999). In one long-term study, agarose microencapsulated islets were implanted into the omentum pouch of diabetic NOD mice and evaluated up to 400 days post transplantation. Results from this study indicated that the islets were able to maintain

normoglycemia for up to 100 days; however, a portion of islets evaluated at 400 days showed signs of central necrosis (Kobayashi et al., 2006). Omentum pouches are generally created by exposing the greater omentum and running a suture along the perimeter of the omental pouch; capsules are then placed in the center of the omental tissue, and the suture is then pulled up and tied, creating the pouch (Kin et al., 2003). Figure 5 shows encapsulated islets in an omentum pouch created in a Lewis rat (Opara et al., 2010).
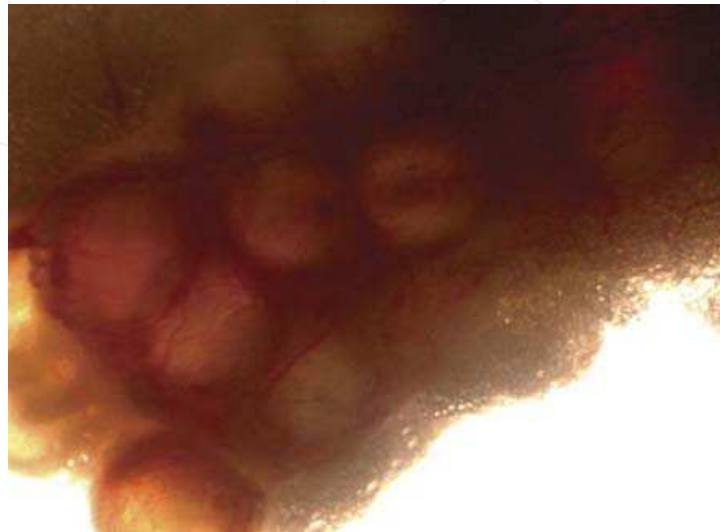

Fig. 5. Alginate microcapsules implanted within an omentum pouch and retrieved after 1 week in vivo (Opara et al., 2010).

3.6 Vascularization

While considerable research has improved the quality of the microcapsules and found suitable implantation sites, there is still a limit to long-term islet survival (de Vos et al., 2004; Opara et al., 2010). A major cause for the long-term failure of grafts is lack of appropriate vascularization to deliver oxygen and nutrients. Islets require functional vasculature to support normal function, as evident by the fact that they receive up to 10-12% of pancreatic blood flow to the pancreas while they account for only about 1-2% of the organ mass (Lifson et al., 1980; Jansson & Hellerstrom, 1983). Deficiencies in vascularization results in hypoxia causing islet dysfunction and death, and, in cases of implanted microencapsulated islets, severe damage has been caused by hypoxia within three days of implantation (Davalli et al., 1996). One approach to this problem is to redesign the microcapsule to provide for the controlled release of angiogenic proteins, such as growth factor fibroblast growth factor 1 (FGF-1) as recently suggested (Opara et al., 2010).

3.6.1 Angiogenesis

During the islet isolation, the vascular supply is disrupted causing rapid endothelial fragmentation and compromises perfusion to the core of islets. Thus, it is very crucial to revascularize the islets for their post-transplantation survival and function (Brissova et al., 2004). Successful islet grafts have been observed to regenerate the microvasculature in about 10-14 days post-transplantation (Beger et al., 1998; Furuya et al., 2003; Menger et al., 1994; Merchant et al., 1997; Vajkoczy et al., 1995). The proportion of islets that restore their

original vasculature determines the long term graft survival and function. In encapsulated islets, islets depend on the diffusion of oxygen and nutrients from the periphery. Lower oxygen and nutrient supply leads to hypoxia and eventual cell death in the inner core of islets which is predominantly comprised of insulin-secreting β-cells (Vasir et al., 1998). Research has shown that the application of angiogenic growth factors such as vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and FGF-1 improved graft functionality of naked islets as a result of increased angiogenesis (Lai et al., 2005; Miao et al., 2005; Rivas-Carrillo et al., 2006). However, bolus administration of growth factors has been shown to result in abnormal and unsustainable vasculature formation. It has also been shown that sustained release of FGF-1, however, allows for normal blood vessel growth, but this growth can actually be limited to the site of implantation (Moya et al., 2009). Using this information, studies have shown that microbeads can be used for a controlled localized delivery of FGF-1 for improved angiogenesis (Moya et al., 2010a). Additionally methods for generating alginate-PLO-alginate microbeads for sustained release of FGF-1 have been investigated (Khanna et al., 2010) and have been shown to cause significant angiogenesis over controls when implanted into the omentum pouches of rats for two weeks (Opara et al., 2011).

3.6.2 Antioxidants

Another concern for the survival of encapsulated islets is the protection from oxidative stress in vivo. Islets are exposed to oxidative stress during the isolation process and after implantation when exposed to activated immune cells, which release free radicals. β cells in particular, are highly vulnerable to oxidative stress (Rabinovitch & Suarez-Pinzon, 1998) and encapsulated islets have been shown to be damaged as a result of oxidative stress (Wiegand et al., 1993). The application of free radical scavengers is an established solution to this problem. Oxidative stress is caused when the amount of oxygen free radicals present within a tissue or cell exceeds the ability of that tissue or cell to neutralize these free radicals (Opara, 2006). These oxygen radicals are most commonly superoxide oxygen (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and peroxynitrite ($ONOO^-$) and are generally referred to as reactive oxygen species (ROS) (Opara, 2002). This process can occur intracellularly and extracellularly as well as from exogenous sources. Intracellular sources of ROS include normal cellular metabolism, and extracellular ROS are generated from the immune system during the destruction of foreign bodies (Opara, 2006). Several studies have found that the addition of traditional antioxidants such as the free radical scavenger Catalase, or Trolox (a water soluble derivative of vitamin E) have improved islet functionality following isolation (Stiegler et al., 2010) and during encapsulation. Other studies have investigated the use of hemoglobin as an antioxidant for encapsulated islets (Chae et al., 2004).

3.7 Functionality studies

The technique of microencapsulation of islets prior to transplantation has shown promise in both large animal trials and pilot clinical trials (Table 1). Multiple canine and primate studies have been conducted and have demonstrated the ability of encapsulated islets to maintain insulin independence (Dufrane et al., 2006a,b; Kendall et al., 2001; Soon-Shiong et al., 1992; Wang et al., 2008). A study conducted by Sun Y. et al. demonstrated the ability of encapsulated islet xenografts to reverse diabetes for periods of time greater than 800 days (Sun et al., 1996). A more recent study by Dufrane et al. demonstrated the ability of

encapsulated islets to survive and produce insulin in the kidney capsule of *Cynomolgus macacus* for up to six months (Dufrane et al., 2006a).

Several pilot clinical studies (Calafiore et al., 2006; Elliott et al., 2007) have been conducted on humans. While these trials have failed to establish long-term insulin independence in any of the subjects, they have established that the implantation of viable encapsulated islets can stabilize blood glucose levels and reduce the required amount of exogenous insulin required. In a study by Soon-Shiong et al., a long-term Type 1 diabetic patient was implanted with 15,000 encapsulated islet equivalents per kilogram body weight and evaluated for up to 9 months post-transplantation. In this study average blood glucose levels were maintained at 135mg/dl, and daily insulin requirements decreased from 0.69±0.01 U/kg to 0 U/kg, and hyperglycemic episodes (>200 mg/dl) decreased from 11.7% to 6.14% at nine months. Furthermore, the patient's quality of life was evaluated and shown to have greatly improved over the duration of the study (Soon-Shiong et al., 1994). Another human study by Calafiore et al. evaluated two individuals 60 days after receiving encapsulated allografts. Although insulin independence was not attained, there was a significant reduction in the daily insulin requirements as well as a significant reduction in the number of hypoglycemic events (Calafiore et al., 2006). A third human study by Elliot RB et al., evaluated the effectiveness of porcine xenograft encapsulated islets up to 9.5 years after implantation. In this study, immediately after implantation, the daily insulin dosage was reduced by 30% and C-peptide was present in urine samples up to 14 months post transplantation. Retrieval of the capsules 9.5 years later revealed that the islets were still capable of producing insulin, however the levels of insulin were significantly reduced and C-peptide could not be measured (Elliott et al., 2007).

Study	Model	Type of Graft	Islet Equivalents (IEQ/Kg body weight)	Duration of insulin independence
(Soon-Shiong et al., 1992)	Canine	Allograft		63-107 days
(Sun et al., 1996)	Primate	Xenograft (porcine)	7,500-17,500	120-803 days
(Dufrane et al., 2006a)	Primate	Xenograft (porcine)	15,000	
(Wang et al., 2008)	Canine	Allograft	55,270-87,031	50-214 days
(Kendall et al., 2001)	Primate	Xenograft (porcine)	15,000	up to 9 months
(Soon-Shiong et al., 1994)	Human	Allograft	15,000	9 months
(Calafiore et al., 2006)	Human	Allograft	400,000-600,000 total IEQ	
(Elliott et al., 2007)	Human	Xenograft (porcine)	15,000	

Table 1. Summary of large animal and human studies. With the exception of Dufrane et al. (2006a), where encapsulated islets were implanted within the kidney capsule, all studies implanted encapsulated islets intra-peritonealy with either PLL or PLO barriers.

In addition to these small pilot trials, larger clinical trials are underway in New Zealand and Russia by Living Cell Technologies Limited (LCT). LCT is currently undergoing phase I and II clinical trials on DIABECELL® which are encapsulated neonatal porcine islets that are injected into the peritoneal cavity via laparoscopy at doses of 10,000-20,000 islet equivalents/kg. Currently the short-term and long-term safety and effectiveness as well as proper dosage are being evaluated (Zukerman, 2010).

4. Conclusion

In spite of the simplicity of the concept of microencapsulation and the urgent need for alternatives to immunosuppression in transplantation, the progress in the field during the past decades has not met the high expectations. Causal factors for this situation include insufficient knowledge of the microcapsule structure, immunoisolation technology and biomaterial properties, and their combined effect relative to biocompatibility. Most of the prematurely failed grafts had one or more flaws such as unpurified alginate, surface exposure of PLL, rough external surface, low surface to volume ratio, and other issues. Recent studies have indicated that it is important to have absolutely no overgrowth on the transplanted microcapsules to ensure its longevity. Another limiting factor in the progress of the microencapsulated islet technology has been the dearth of high-throughput devices for making encapsulated islets. When dealing with high numbers of islets (~500,000 islets, as expected in human studies), at present capabilities, it would take a multitude of hours to encapsulate this number of islets and islet viability would suffers immensely. Recent developments in encapsulation devices allow scaling up the process such that encapsulation time can be exponentially reduced from hundreds of hours to less an hour (Tendulkar et al., 2011).

Barriers to Microcapsule Transplantation	Strategies for Clinical Success
Loss of viability during islet isolation and encapsulation	Improved enzymatic blends and process control to maximize pure islet yield
	Culture media for long-term in-vitro culture and encapsulation
	Scalable microfluidic encapsulation devices
Failure to revascularize on transplantation	Better vascularized implant site
	Co-encapsulation with angiogenic factors to promote revascularization
	Co-encapsulation with antioxidants to counter oxidative stress
Inflammatory reaction	Good hydrogel coating
	Immunoisolation barriers with no surface exposure
Inadequate islet mass	Xenotransplants
	Stem cell and gene therapy based approaches

Table 2. Barriers to clinical success of encapsulated islet transplantation and counter-strategies.

It is also very important to have sufficient islet mass to achieve normoglycemia for long-term graft survival and function (Rickels et al., 2005). When inadequate numbers of islets are transplanted, increased metabolic demand and persistent hyperglycemia may lead to islet apoptosis and hence graft failure (Leahy et al., 1992; Rossetti et al., 1990). Since the life span of a β cell is approximately 3 months (Finegood et al., 1992), success in encapsulated islet graft studies over a long period suggests that regeneration of islet cells occurs in capsules. Indeed, β cell replication has been shown to be 10-fold higher in encapsulated islets compared to the native pancreas (van Schilfgaarde & de Vos, 1998). Inadequate islet supply issues are being addressed by regeneration therapy and xenotransplantation. Various strategies are being explored to improve islet graft outcomes, including immunoisolation using semipermeable biocompatible polymeric capsules, induction of immune tolerance, enhanced vascularization of transplant sites, and reduction of oxidative stress induced by the islet isolation process using antioxidants, as well as the use of adequate number of islets. Furthermore, synergistic application of more than one strategy maybe required to improve the success of islet transplantation. Table 2 summarizes the crucial barriers and strategies to achieve clinical success.

5. Acknowledgements

6. References

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Edited by Dr. Gurch Randhawa

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