The Bioartificial Pancreas: Progress and Challenges

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ABSTRACT

Diabetes remains a devastating disease, with tremendous cost in terms of human suffering and healthcare expenditures. A bioartificial pancreas has the potential as a promising approach to preventing or reversing complications associated with this disease. Bioartificial pancreatic constructs are based on encapsulation of islet cells with a semipermeable membrane so that cells can be protected from the host’s immune system. Encapsulation of islet cells eliminates the requirement of immunosuppressive drugs, and offers a possible solution to the shortage of donors as it may allow the use of animal islets or insulin-producing cells engineered from stem cells. During the past 2 decades, several major approaches for immunoprotection of islets have been studied. The microencapsulation approach is quite promising because of its improved diffusion capacity, and technical ease of transplantation. It has the potential for providing an effective long-term treatment or cure of Type 1 diabetes.

INTRODUCTION

DIABETES MELLITUS represents a major public health problem, as it is the most frequent endocrine disease in industrialized countries. According to the latest estimates, the number of people with this disease worldwide is 177 million, and this figure will double by the year 2025. The complications of diabetes are associated with multiple medical problems related to ophthalmic, renal, neurological, cerebrovascular, cardiovascular, and peripheral vascular disease. The economic burden of diabetes is related to its management, to the treatment of its secondary complications, and to resultant productivity loss. While one estimate shows that the cost of treating diabetes in the United States in 1997 was $44 billion, another study has shown that it cost about $100 billion to treat diabetes and its complications in this country in 1992.

Type 1 diabetes, also previously known as insulin-dependent diabetes mellitus, occurs when pancreatic islet cells are unable to produce insulin. Consequently, the blood glucose concentration becomes high while tissues are starving for metabolic fuel. In Type 2 diabetes, previously referred to as non-insulin-dependent diabetes, the body continues to make at least some insulin, but is unable to respond properly to the action of insulin produced by the pancreas. The Diabetes Control and Complications Trial showed that an improved metabolic control was achieved using intensive insulin treatment in Type 1 diabetes patients. However, the aggressive management of dia-
Advances in transplantation and in immunosuppression have made pancreas transplantation another treatment option for Type 1 diabetes. The main objective for pancreas transplantation is to achieve normal level of glucose in the blood and to free the patient from exogenous insulin requirements based on multiple finger stick glucose measurements. Even though successful pancreas transplantation provides normal glucose homeostasis, the requirement of lifelong immunosuppression makes it unclear whether transplantation is advantageous over continued insulin treatment. Also, because of the need for immunosuppression, most pancreas transplantsations have been done simultaneously with a kidney transplant in patients who have advanced nephropathy. In contrast to pancreas transplantation, islet transplantation requires no major surgery. Also, islet transplantation offers an alternative to exogenous insulin treatment, as it can result in better glycemic control and potentially avoids surgical complications associated with whole-organ pancreas transplantation. It is known that islet cell transplantation can prevent, and, in some cases, reverse existing complications of diabetes probably because of the role played by C-peptide. However, major challenges remain to be addressed before islet transplantation can be used routinely and applied more widely to patients with diabetes. One major barrier is the shortage of human pancreas as a source of islet cells. Also, lifelong use of immunosuppressive drugs to overcome the rejection of transplanted tissue poses a significant risk to patients, as the use of glucocorticoids and cyclosporine have dose-dependent deleterious effects on glucose homeostasis and β-cell function, and result in increased incidences of infection and cancer. The introduction of the steroid-free immunosuppressive drug regimen has resulted in long-term graft survival of islets with concomitant control of blood glucose levels. However, this immunosuppressive drug regimen is also not without risks to graft recipients. The routine application of islet transplantation would be tremendously enhanced by an unlimited supply of donor tissue, a standard implantation procedure, and the ability to maintain the transplanted tissue without the requirement of immunosuppressive drugs. To achieve these goals, immunosolation (encapsulation) of islet cells has been proposed to protect cells from attack by the host immune system. Using immunosolation of the islets, chronic administration of immunosuppressive drugs can be theoretically eliminated or minimized, as transplanted cells are separated from the host immune system by a biocompatible and semipermeable membrane. Moreover, immunosolation could open up the possibility of using islets harvested from animals, or insulin-producing cells engineered from stem cells without the requirement of immunosuppression of transplant recipients. Porcine and bovine insulin amino acid sequences have considerable sequence homology with human insulin, and therefore pigs and cows are considered attractive as options for xenogeneic donors.
centration of essential minerals. In addition, the release of insulin from the peritoneal cavity to the blood stream is delayed compared with hormones released to the portal vein. Also, peritoneal macrophages have a high toxicity against encapsulated islets.

Several researchers have also explored the portal vein of the liver as an alternative site for transplantation of immunoisolated islets. Based on its double vascularization (hepatic artery and portal vein), the intraportal location has a high oxygen and nutrient supply, which could be beneficial for the performance and the longevity of microencapsulated cells. Also, the liver is considered to be an immunologically privileged site. But, even more clinically relevant, is the use of percutaneous transhepatic catheterization, which provides relatively simple, inexpensive, and non-surgical access to the liver. In addition, The International Islet Registry reports more C-peptide-positive cases (more than 0.5 ng/mL) 1 year after transplantation into the portal vein than from any other site.

This article reviews the recent progress and challenges remaining for the successful development of a bioartificial pancreas, and discusses the requirements to bring this technology closer to clinical application.

HISTORICAL DEVELOPMENT

The pancreatic origin of diabetes was discovered in 1889 by Mering and Minkowski, when surgical removal of the pancreas caused dogs to develop diabetes. In 1913, Murlin and Kramer prepared extracts of bovine pancreas to lower the blood glucose in a dog with diabetes. However, the benefit of the extract was explained by the presence of lactate. Finally, in 1922, Banting and Best developed a method for preparing a pancreatic extract, and a few years later, injection of the active factor in this extract, insulin, became the main therapy for Type 1 diabetes.

Among these treatments, the bioartificial pancreas avoids the use of immunosuppressive drugs while providing moment-to-moment glucose homeostasis consistent with a well-functioning native pancreas. It is important in this context to distinguish between the terms "artificial pancreas" (sometimes called "mechanical artificial pancreas") and "bioartificial pancreas."

The mechanical artificial pancreas consists of a glucose sensor, an insulin pump, and a computer, which determines the rate of insulin delivery. The sensor may be implanted in the vena cava, and may require periodic replacement. The development of a glucose biosensor has been challenged by the difficulty of creating a sensitive, stable, and accurate sensor, despite the capability of a mechanical artificial pancreas to regulate glucose levels. An error in sensing, computation, or delivery could result in insulin overdosing, which could be potentially risky with life-threatening hypoglycemia, an unacceptable potential side effect of an artificial pancreas. It is also very difficult to produce a mechanical artificial pancreas that responds quickly enough to changes in blood sugar (islets respond in less than 10 min).

In contrast, a bioartificial pancreas is a device that substitutes for the endocrine portion of the pancreas. Devices in this category contain synthetic materials and functional islets encapsulated within a semipermeable membrane to protect cells from the host immune response. The semipermeable membrane permits exchange of nutrients, glucose, and insulin with the host, but excludes the diffusion of immunoglobulins, complement, and white blood cells. The device may be implanted into a vascularized site or into the peritoneal cavity.

The idea of using ultrathin polymer membrane microcapsules was proposed by Chang in 1964 for the immunoprotection of transplanted cells. Nearly 2 decades later, the concept of bioencapsulation was successfully shown to maintain glucose homeostasis in rats with diabetes using encapsulated allogeneic islets. When alginate microcapsules containing islets were implanted in rats, normal blood glucose levels were achieved for 13 weeks. The microcapsule used in that study consisted of an inner alginate core surrounded by a polyllysine.
membrane, which was then surrounded by an outer polyethyleneimine coating. In later studies, the outer layer was replaced by the more biocompatible alginate coating because of an inflammatory reaction induced by polyethyleneimine. Since then, a variety of studies have been performed to understand the requirements for successful transplantation of encapsulated islet cells, but there is still a need for convincing evidence of success of encapsulated islet transplantation in either non-human primates or humans.

**IMPEDEMENTS TO THE PROGRESS OF CELL ENCAPSULATION TECHNOLOGY**

Fibrotic overgrowth of the implanted microcapsules is one of the major obstacles to progress in cell encapsulation technology. Invariably, the materials used for encapsulation are not completely inert, and can induce foreign body and inflammatory reactions. As a result of this fibrous tissue overgrowth, the diffusion of nutrients, oxygen, hormones, and waste products through the capsule is diminished, and encapsulated islets are destroyed because of hypoxia, starvation, and the secretion of nitric oxide by the stimulated macrophages. This fibrotic reaction to implanted microcapsules had previously been associated with commercially available alginate with high mannuronic acid content, which activates macrophages in vivo, resulting in fibroblast proliferation and eventual overgrowth. However, later studies did not support that notion. It has also been shown that polylysine may cause a fibrotic response through the induction of cytokines.

Another important component of capsular design is permselectivity. Most capsule designs are based on the presumption that the effectiveness of immunosolation of a polymer capsule is closely related to the pore size of the capsule membrane. However, pore sizes of polymer membranes are not homogeneous by nature, and consist of a broad spectrum of sizes. Total immunoprotection of encapsulated cells can only be provided if the polymer membrane does not have any pores larger than the antibody complement component. This fact has been largely ignored by researchers and has resulted in inconsistent findings in the field.

**Mechanism of immunologic attack of allografts and xenografts**

With regard to specific immunologic attack of encapsulated cell implants, there are differences in the immune protection requirement of allografts versus xenografts. An allograft is a graft between genetically different individuals within the same species, while a xenograft is a graft between individuals from different species. Allograft rejection occurs as result of activation of cellular immunity by interactions of host T cells with a graft, while humoral immunity, including antibodies and complement proteins, is responsible for the rejection of xenografts. In the direct pathway of antigen presentation, the T cell receptor recognizes T cells presented by donor-type antigen-presenting cells along with Class I or II major histocompatibility complex (MHC) molecules. The idea of immunoisolation using a polymer membrane is to separate allogeneic or xenogeneic tissue from the immune system of the recipient. Therefore, microencapsulation of islets within effective permselective membranes would prevent contact with immunoglobulin, complement components, and inflammatory cells. However, there is also a potential immune response towards antigens shed by encapsulated allogeneic or xenogeneic cells. Such antigens could be therapeutic agent themselves, cell surface molecules, or cell components (phospholipids, DNA), including those released upon cell death. Shedding of antigens from encapsulated cells would initiate a molecular tissue response around the implant, which could affect the viability and function of the encapsulated cells. As a result of this immunological reaction, these shedded antigens may be internalized, processed, and presented in association with host Class II MHC molecules (macrophages and dendritic cells) to host CD4+ helper T cells (Fig. 1). The recognition of antigens through this indirect pathway may lead to the activation of T helper cells, which then secrete cytokines and regulate cell-mediated immune response and inflammation. Small molecules, such as reactive oxy-
gen species and cytokines, may also pass through the polymer membrane and damage the transplanted tissue.

Owing to the complexity of the immune response mechanism, it is important to understand the mechanism of immune protection, and considerable efforts have been made to investigate this issue. Chen et al. developed cell lines that have resistance to cytokines and oxygen radical-induced damage. Recently, macrophage depletion has also been used as a tool to protect xenografts from immune destruction. It has been found that depletion of peritoneal macrophages with clodronate liposomes improved the survival of macroencapsulated porcine neonatal pancreatic cell clusters. Safley et al. evaluated the cellular immune response in non-obese diabetic (NOD) mice after two different pathways of T cell co-stimulation were blocked. Alginate–poly-L-lysine-encapsulated porcine islet xenografts were transplanted intraperitoneally in NOD mice treated with CTLA4-immunoglobulin to block CD28/B7 and with anti-CD154 monoclonal antibody to inhibit CD40/CD40–ligand interactions. It was concluded that blocking two different pathways of T cell co-stimulation inhibited T cell-dependent inflammatory responses, and significantly prolonged the survival of encapsulated islet xenografts.

Another approach to improving the survival of microencapsulated islets can be to use local immunosuppression. Studies have shown that preimplantation of an immunosolating device improved the survival of an encapsulated islet graft and reduced fibroblast outgrowth. Functional capsules may also be designed to release the immunosuppressive agent in a controlled manner. Local immunosuppression induced using this approach may prevent the occurrence of an undesirable reaction around the implant. Inflammatory reaction in response to the implantation of cell-free capsules involves a sequence of events similar to a foreign body reaction, starting with an acute inflammatory response and leading to a chronic inflammatory response or granulation tissue development and fibrous capsule development. The intensity and duration of each of these responses are dependent upon several factors, such as the extent of injury created in the implantation, biomaterial chemical composition, surface free energy, surface charge, porosity, surface roughness, and implant size and shape. The biocompatibility of the material is determined by the extent and deviation from the optimal wound healing conditions.

Sources of donor islets

Despite all of the advances in islet transplantation and in encapsulation technology, some challenges must be still overcome before the bioartificial pancreas can be broadly applied. The most significant of these challenges is the shortage of donor islets. This shortage is the motivation for researchers to find alternative sources of insulin-producing cells. Promising approaches for resolving this problem are differentiation of stem cells into cells with β-cell-like characteristics and genetic engineering of adult cells for secretion of recombinant insulin. Based on similarities between mechanisms that control the development of both the adult pancreas and the central nervous system, methods promoting neural differentiation of embryonic stem cells have been adapted by researchers to derive insulin-producing cells.

Hori et al. investigated the possibility of directing neural progenitors to develop into glu-
cose-responsive insulin-producing cells using inductive signals involved in normal pathways of islet development. Compared with other methods for insulin-producing cell development from human stem cells, the method developed by this group produced insulin at the highest levels yet achieved from an expandable, human stem cell-derived tissue. However, the method developed in the study still needs to be improved, perhaps through the addition of glucagon-like peptide-1, transforming growth factor-β ligands, or other factors to potentiate β-cell maturation, growth, and insulin secretion.

Gene therapy has also been suggested as a treatment of insulin-dependent diabetes mellitus. This strategy can be applied by preventing the autoimmune destruction of β-cells, by regenerating β-cells, or by engineering insulin-secreting surrogate β-cells. Sapir et al. recently suggested the potential use of adult human liver as an alternate tissue for autologous β-cell-replacement therapy. Using pancreatic and duodenal homeobox gene 1 (PDX-1) and soluble factors, they managed to induce a comprehensive developmental shift of adult human liver cells into functional insulin-producing cells. According to the findings of that study, PDX-1-treated human liver cells expressed insulin, stored it in defined granules, and secreted the hormone in a glucose-regulated manner. When these cells were implanted under the renal capsule of immunodeficient mice with diabetes, the cells ameliorated hyperglycemia for prolonged periods of time. The technique offers both the potential of a cell-replacement therapy for patients with diabetes and allows the patient to be the donor of his or her own insulin-producing tissue.

Preservation of encapsulated cell systems

The last challenge for a bioartificial pancreas to become a routine clinical application is the long-term preservation of encapsulated cell systems to maintain a product inventory, and in order to meet end-user demands. One of the current strategies for overcoming the problem of cryopreservation of tissue is the reduction of cryoprotectant [dimethyl sulfoxide (DMSO)] concentration or complete replacement by equally powerful substances. Recent work in Dr. Opara’s laboratory has demonstrated that cryopreservation of islet cells cultured overnight in the presence of 10% polyvinylpyrrolidone yielded higher intact islet recovery compared with islets frozen in the presence of DMSO and glycerol. The lower islet cell integrity and function were explained on the basis of hypothesis that low-molecular-weight compounds, such as DMSO and glycerol, permeate the cell and interact hydrophobically with intracellular proteins, which results in perturbed cytoskeletal architecture of the frozen cells and diminished islet cell integrity and function.

The other promising technical improvement of the cryopreservation technology is the reduction of total sample volume. Miniaturization of the cryosubstrates from 1 mL to microliters reduces temperature gradients in cryosubstrates, which makes it possible to achieve higher freezing rates and more homogeneous freezing of the sample. Islets cryopreserved with this strategy were highly functional even with the lower DMSO concentrations. Misler et al. studied stimulus–secretion coupling in whole islets as well as single β-cells from carefully selected cryopreserved and thawed human islets of Langerhans. They found that without using any of the recent advances in cryopreservation technique, cryopreserved and thawed human islets, which were selected based on their smooth surface and diameter, respond to glucose in a calcium- and metabolic-dependent fashion. In another study by von Mach et al., viability of pancreatic islets after cryopreservation was correlated negatively with their size, and suppression of insulin release was not observed for islets <300 μm.

DIFFERENT FORMS OF BIOARTIFICIAL PANCREAS

Various configurations have been studied for the purpose of immunoisolation of islets. These
include biohybrid vascular devices, extravascular chambers, and encapsulation. Encapsulation is the technique of islet immunoisolation using biopolymeric spheres of different sizes. Currently, results in rodents and dogs with diabetes indicate that spherical micro- and macrocapsules appear to have the highest therapeutic potential. A microcapsule typically contains only a few hundred cells or a single islet, and to provide a therapeutic dosage, tens of hundreds of thousands of cells are required. In contrast, macrocapsules employ much larger depots to contain the full therapeutic dosage in one or a few implants.

Macrocapsules are shaped as cylinders or planes, and typically one dimension is in the range of 2–6 mm (Fig. 2a). One advantage of the implantation of macrocapsules is the ease of retrieval in case of complications. However, despite reports of reduced risks in the implantation of macrocapsules, it has been shown that macroencapsulated islets have significantly impaired insulin secretion because of necrosis in the center of aggregated islet clumps as a result of diffusion limitations of nutrients and oxygen. Encouraging results have been obtained by immobilization of the islets in a matrix before final macroencapsulation. Limited diffusion of nutrients, as well as slow exchange of glucose and insulin, occurs as a result of relative large surface-to-volume ratio of these devices. To ensure sufficient nutrient and oxygen diffusion to the cells, islet cell density within the macrocapsules is kept to around 5–10% of the volume fraction. This approach, however, requires the implantation of large devices in order to provide adequate masses of insulin-producing cells, and large grafts are not practical and cannot be transplanted in conventional sites.

In contrast, microencapsulation offers an optimal volume-to-surface ratio for fast exchange of hormones and nutrients without a large device. The technique involves enclosure of one or two islets within small spheres, which have diameters of less than 1 mm (Fig. 2b). Also, because of reduced volume of the islet transplants, and in contrast to macroencapsulated islets, microcapsules can theoretically be transplanted in delicate sites, such as the portal vein. The transplantation of microcapsules into the peritoneal cavity, an outpatient-based procedure, makes it particularly attractive to patients and doctors. Interestingly, intraperitoneally implanted microencapsulated islets have shown promising results in large animal models as well as in limited clinical trials.

**TECHNIQUES IN MICROENCAPSULATION**

Materials used for microencapsulation

Various materials have been used as biopolymeric coats in islet microencapsulation. These have included alginate, agarose, tissue-engineered chondrocytes, polyacrylates, and poly(ethylene glycol) (PEG).

Alginate–polylysine-based microencapsulation of islets, first described by Lim and Sun, has been found not to interfere with cellular function, and these microcapsules have been shown to be stable for years in small and large animals as well as in human beings. Alginate is a linear polysaccharide extracted from seaweed. It is composed of three types of 1→4-linked polyuronic acid blocks containing poly-L-guluronic acid segments (G blocks), poly-D-mannuronic acid segments (M blocks), and segments of alternating L-guluronic and D-mannuronic acid residues, and it has gel-forming properties in the presence of most polyvalent cations. Impurities, such as monomers, catalysts, and initiators present in synthetic polymers, may contribute to the failure of the encapsulated islet im-
plants. Other impurities, such as pyrogens and mitogens, can be found in polymers derived from natural substances, such as seaweed. Up to 90% of the impurities can be removed by electrophoresis or by the Klock extraction purification procedure. Conflicting reports exist on whether alginate with high M blocks or high G blocks results in increased fibrosis. It has been shown, however, that the inflammatory response to alginate becomes less with purification, irrespective of the composition used.

The immunoisolating permselectivity of alginate microcapsules can be achieved by soaking the initial alginate beads in 0.05–0.1% polylysine dissolved in normal saline for 6–20 min. This occurs as a result of the binding of negatively charged carboxyl groups on the alginate to positively charged amino groups on the amino acid polymer. The droplets are again rinsed with normal saline, followed by the addition of an outer alginate layer by soaking in a lower concentration of 0.06–0.25% sodium alginate for 4 min. It is extremely important that the outer coating of alginate on top of the polylysine membrane is complete. In the case of incomplete cover of the polylysine coating and when an impure alginate is used for the outer coating, microcapsules may become completely covered by cell overgrowths within 1 week of transplantation because of the inflammatory reactions attributable to polylysine and the impurities in the alginate. To dissolve the intracapsular sodium alginate by chelation of the cross-linking calcium, the resulting microcapsules are treated with 55 mM sodium citrate for 7 min. The hollow microcapsules containing a few islets are then washed with saline (Fig. 3). It appears that liquefaction of the inner alginate core of microcapsules is required for optimal function of the enclosed islets. One successful transplantation of encapsulated islets performed with this technique was reported by a team of investigators at Duke University. The encapsulated islets were made with an outer alginate coating and inner poly-L-ornithine layer for permselectivity. To liquefy the inner alginate core of each bead a salt treatment was used. After transplantation, encapsulated islet cells were shown to keep a baboon with diabetes from requiring exogenous insulin for more than 9 months.

For a capsule to be stable in vivo it has to be water insoluble, as capsule dissolution will elicit a continuing inflammatory response and will ultimately expose the transplanted cells to the host. Both alginate and polylysine are water-soluble polymers and are both expected to elicit an inflammatory or foreign body response. However, findings indicate that the polyelectrolyte complex formed during encapsulation is biocompatible and less soluble in water than the individual components. Differences in biocompatibility were also noted between empty and cell-containing capsules in NOD mice, which could be due to the difficulty of producing reproducible cell-containing capsules. For better biocompatibility, one group has suggested the use of high guluronic acid-containing alginates, while another group modified alginate–polylysine capsules with PEG. An alternative approach has been chosen to obtain stronger and more biocompatible microcapsules using synthetic polyacrylates such as the water-insoluble synthetic copolymer hydroxymethyl methacrylate-methyl methacrylate. This research group has selected the acrylate monomers because of their diversity and has shown that mammalian cells...
may be microencapsulated in uncharged and polyelectrolyte polymer, in polyelectrolyte complexes, and inside a cohesive precipitate from a destabilized emulsion, without loss of viability.

Polymerization of acrylamide monomer on islet cells encapsulated in agarose microspheres has also been reported. However, when polymerization is in direct contact with tissue, this can generate excessive local heating and cytotoxicity. One of the most potent complement-interacting polymers, poly(styrene sulfonic acid), was selected for the purpose of consuming cytolytic complement activity and mixed with agarose for the preparation of microbeads to protect xenogeneic islets in mice with diabetes from the humoral immune reaction. Antibodies and complement proteins play a major role in the rejection of xenografts. Binding of antibodies to the antigens on the xenogeneic cell surface cannot alone damage the islet cell. For destruction of the xenogeneic cells to occur, complement activation by antigen–antibody complexes present on the cell surface is required.Various polymers bearing hydroxyl groups such as regenerated cellulose, cellulose acetate, and poly(ethylene-co-vinyl alcohol) activate the complement system through the alternative pathway, whereas other polymers bearing sulfonic acid or sulfate groups strongly interact with complement proteins and decrease the cytolytic complement activity.

As previously noted, a fibrotic reaction after implantation would limit the diffusion of essential nutrients or metabolites, leading to reduced viability or impaired functional activity of the encapsulated cells. Several studies have reported the usefulness of coating membrane surfaces with poly(ethylene oxide) (PEO) in order to reduce protein adsorption and associated fibrotic reaction. This was achieved by exposing the poly(acrylonitrile-vinyl chloride) membrane surface to amine-terminated PEO after acid hydrolysis of the nitrile group to a carboxylic acid. Membrane surfaces were also exposed to PEO-succinimide after base reduction to an amine. The in vivo response proved that a smaller number of macrophages and foreign body giant cells were present on the PEO-grafted membrane surface without any change in transport properties. The other application to modify the membrane surface was achieved by synthesizing a graft copolymer having poly-L-lysine as the backbone and the monomethoxy PEG as pendant chains. Microcapsules with sodium alginate were formed using this polycationic copolymer, which demonstrated reduced protein adsorption, complement binding, and cell adhesion in vitro compared with materials with unmodified poly-L-lysine.

That artificial materials used for immunosolation purposes are not completely inert and can induce foreign body and inflammatory reactions have led researchers to study tissue-engineered capsules of rat chondrocytes. Pollok et al. proposed the encapsulation of islets with a layer of chondrocytes and their matrix to prevent immunorecognition and destruction of transplanted allogeneic or xenogeneic islets. This investigation demonstrated the membrane’s ability as an immunosolation barrier by utilizing the immunoprivileged properties of the chondrocyte matrix. This approach might offer a therapeutic advantage for patients with diabetes, using the patient’s own chondrocytes from a cartilage biopsy specimen as the encapsulation material.

PEG is another polymer that has been utilized for the purpose of encapsulating islets. In order to obtain stable and biocompatible gels without any cytotoxicity generation, Pathak et al. and Cruise et al. reported rapid photopolymerization of water-soluble PEG-based macromers in direct contact with islet cells. PEG is biocompatible, nontoxic, non-immunogenic, and hydrophilic and can be chemically cross-linked into hydrogels for a variety of applications. Diffusion profiles of proteins through PEG diacrylate hydrogels showed that gels formed with the proper formulation were capable of being immunoprotective. The semi-permeable properties of cross-linked PEG hydrogels made by interfacial photopolymerization of PEG diacrylate were also studied by Cruise et al. by forming hydrogels upon poly(vinylidene fluoride) microporous filters. This approach allowed them to study the effect of molecular weight and concentration of the PEG diacrylate on the diffusivity of biological molecules.
Techniques used for droplet generation

The other major consideration in microencapsulation technology is how to get the cells into the individual polymer capsules. Techniques to form such a physical barrier include air-jet syringe pump droplet generator, interfacial photopolymerization, and selective withdrawal.

One of the main devices used for microencapsulation of islets with alginate–polylysine is the air-jet syringe pump droplet generator.\textsuperscript{157} The device consists of an air jacket surrounding an alginate nozzle through which alginate solution is injected.\textsuperscript{158,159} Islets are suspended in a solution of 1.4–3% sodium alginate, and spherical droplets of this suspension are formed by an air-jet syringe pump generator. As alginate droplets are forced out of the end of the needle by the syringe pump, the droplets are pulled off by the shear forces set up by the flowing air stream. The size of the spherical droplets is controlled by adjusting the flow rate of the air jacket. The spherical droplets are collected in a funnel containing HEPES acid buffer supplemented with 1.1% CaCl\textsubscript{2} solution, which transformed alginate droplets into rigid beads by gelation.\textsuperscript{110} Excess fluid is drained with the aid of a filtering device consisting of a nylon mesh and a stopcock at the exit end of the funnel, and the droplets are washed in normal saline.

As noted earlier, an air jet-syringe pump extrusion method generates gel droplets containing entrapped islets from the suspension of the islets in aqueous sodium alginate solution. However, there are various constraints concerning the use of this procedure to produce microcapsule diameters of less than 700 \textmu m.\textsuperscript{155} To form perfectly spherical capsules, the viscosity of the gel-forming liquid must be greater than 30 cp. Also, to prevent the blockage of the needle by the islets, the minimum internal diameter of the needle must be greater than 300 \textmu m. Finally, the volumetric airflow rate must remain below 2,000 mL/min in order to produce capsules of uniform diameter. This procedure was improved by employing an electrostatic droplet generator to form uniform, smooth, and perfectly spherical microcapsules having a diameter about 150–500 \textmu m.\textsuperscript{160}

A droplet, which is charged with high static voltage, is suspended from a needle and attracted to a collecting vessel with opposing polarity. Once the voltage threshold potential is passed, the droplet moves from the needle to the collecting vessel. Droplets with predetermined sizes may be repeatedly generated as the voltage pulse height, pulse frequency and length, and extrusion rate of the droplets are adjustable. As shown in Figure 4, droplets are formed as the plunger is driven by the syringe pump and expelled towards a collecting vessel containing a hardening solution, which may be aqueous calcium chloride in the case of an aqueous droplet forming liquid containing sodium alginate. Negative polarity was attached to the needle, while positive polarity was attached to the metal ring. As the voltage applied during droplet formation is a static voltage, the viability of the encapsulated islets is not compromised.

Biomolecules such as proteins and cells can also be deposited as patterned droplets onto surfaces for the development of cell-based biosensing devices for applications such as high-throughput drug screening.\textsuperscript{161} There are numerous techniques for microfabrication of patterned polymer surfaces for protein delivery. Lithographic techniques and microcontact printing have been most widely used to generate patterned droplets of cells.\textsuperscript{162–168} PEG hy-
drogel is the most commonly used material for cell patterning purposes. In the case of surface-initiated photopolymerization of PEG, the technique involves immobilization of initiator onto a substrate surface using a stamp such as poly(dimethyl siloxane), followed by polymerization of precursor solution. One recent study focused on the cellular delivery based on micro- and nanotechnology. To provide an immunoisolating microenvironment for islet cells, nanoporous biocapsules were bulk and surface micromachined to present uniform and well-controlled pore sizes. The designs of the membranes with defined nanopores were fabricated using a thermally grown silicon oxide sandwiched between two structural layers of silicon.

Another technique used to generate droplets of encapsulated islet cells was proposed by Cruise et al. using interfacial photopolymerization. The technique involves physical adsorption of the initiator eosin Y on the islet cell surface, which then allows polymerization to occur on the islet–prepolymer interface. The interfacial photoinitiation process employed with this technique results in conformal coating of cross-linked PEG-based hydrogel on the islet cell surface. The process of selective withdrawal is another novel method reported recently for the purpose of coating cell clusters, such as islet cells. Briefly, the process involves the insertion of a tube into a container such that its tip is suspended at a specific height above an interface separating two immiscible fluids (Fig. 5). In the case of a low fluid withdrawal rate, only the upper fluid (oil) is withdrawn through the tube. Increasing the fluid flow rate or decreasing the height of the tip above the interface results in a transition where the lower liquid (water) is incorporated in a thin spout along with the oil. The technique was demonstrated through encapsulation of a poppy seed in a poly(styrenesulfonic acid). The particles were coated with the prepolymer solution, which has styrene sulfonic acid sodium salt and triethylene glycol diacrylate, and mixed with the initiator eosin Y and co-initiator triethanolamine used for the purpose of initiating polymerization. After the selective withdrawal step, the coated particles were collected and photopolymerized with a halogen lamp. The technique could also be used to encapsulate cells through surface initiated photopolymerization of PEG. The photoinitiator eosin Y could be immobilized on the islet cell surface through covalent attachment, and after the selective withdrawal process, cells could be collected and illuminated for 2–3 min with an argon ion laser in order to cross-link the coat around the cells.

CONCLUSIONS

The successful development of a bioartificial pancreas involves several considerations. Two major obstacles in islet transplantation are the limited supply of islet cells and the use of immunosuppressive drugs to prevent transplant rejection. It is hoped that the use of encapsulated islets as a form of bioartificial pancreas would overcome these obstacles. Three potential sources of islet cell tissue, including human or allogeneic cells, porcine or xenogenic cells, and engineered cells, are currently under investigation. Among these sources, human islet cells would be the least immunogenic; however, there is a shortage of pancreata retrieved from human cadaveric donors, and these cells have a limited secretory capacity and life span. The use of porcine islet cells could be a better option, because these are readily available, and there is an unlimited supply of donors. However, one major concern about the use of islets harvested from pigs has been the possibility of transmittance of porcine endogenous retroviruses (PERVs) to humans during transplantation. This concept arose from studies on the
infection of humans and immunodeficient mice after pig cell xenotransplantation. There are studies, however, showing no possibility of transmission of PERVs from pigs to human recipients of islet xenografts. Indeed, one recent study showed that the porcine genome harbors a limited number of infectious PERV sequences, which suggests that many breeds of pig fail to produce PERVs capable of infecting human cells even in laboratory testing. This raises the possibility of using special breeds of pig for an unlimited supply of islet cells for encapsulation and use as a bioartificial pancreas in clinical xenotransplantation.

The other attractive approach to generate unlimited supply of islets is the use of embryonic stem cells, which have the ability to differentiate into a variety of cell lineages in vitro. Recent studies in small animals showing successful transplantation of pancreatic stem cells suggest that this approach could provide an unlimited source of allogeneic insulin-producing tissue in the future.

The maintenance of cell viability is another important concern in the development of a successful bioartificial pancreas. When cells are encapsulated and implanted in an environment without a natural circulation, lack of an adequate supply of oxygen and nutrients results in cell necrosis, making implantation unsuccessful. To improve long-term islet survival and function, vascularization of transplanted islets has been proposed. An ideal architecture for implantation would be to promote vascularization around encapsulated cells using a novel approach. Recently, a technique to form covalently bonded multilayers of PEG hydrogels has been developed. This approach may be used to encapsulate cells with bilayers such that outer layer would promote vascularization around the inner layer.

There are other areas of urgent need for the successful development of a bioartificial pancreas through islet cell microencapsulation. All current techniques for cell microencapsulation are slow in producing desirable microcapsules, requiring days to generate enough encapsulated islets for one transplantation. An ideal procedure for routine use of encapsulated islets would require the development of massively parallel concepts in microencapsulation, which would generate sufficient quantities of microcapsules for multiple transplantsations in a matter of minutes. In the absence of a technique based on such a principle, there is a need for an adequate procedure for long-term storage of encapsulated cells to enhance their use for transplantation. It is also highly desirable to develop rapid in vitro techniques for determination of the functional viability of encapsulated islets prior to transplantation.

In summary, a potential cure for Type 1 diabetes could be the use of bioartificial pancreatic constructs based on insulin-secreting cells (allogeneic or xenogeneic islet cells) that are immunoisolated with the microencapsulation technique. Additionally, enhanced survival of the graft might be supported with a novel approach to induce neovascularization, which could make this technology a clinical reality in the near future. This will in turn serve as an important progress in cell therapy for the treatment of diabetes as well as other diseases, such as cancer, hemophilia, liver failure, and Parkinson's disease.

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