Clinical review

Transplantation of encapsulated cells and tissues


The potential applications of transplantation have grown significantly during the past decade. In addition to solid organs, the transplantation of cells and tissues that produce specific biotherapeutic substances represents an important conceptual and technologic advance. The potential economic impacts of cell transplantation as a treatment for human disease are enormous because of the fact that in the United States alone, nearly one-half trillion dollars are spent each year to care for patients who suffer tissue loss or dysfunction. More than six million patients suffer from neurodegenerative disorders such as Alzheimer’s disease, amyotrophic lateral sclerosis (ALS), and Parkinson’s disease; more than 14 million patients suffer from diabetes; and millions more suffer from immunodeficiency disorders, liver failure, hemophilia, and other diseases caused by the loss of specific vital metabolic functions. It appears likely that by the end of the decade clinical trials using encapsulated cells to treat many of these diseases will become a reality. This technology has recently been extended from rodents to large animals, and the Food and Drug Administration (FDA) has already authorized studies to evaluate the safety and biologic activity of several types of systems.

A number of encapsulation systems have been developed during the past several years in which transplanted cells can be separated from the immune system of the body by a selectively permeable membrane. The membrane allows the free exchange of nutrients, oxygen, and biotherapeutic substances between the blood or plasma and the encapsulated cells, whereas high molecular weight substances such as immunocytes, antibodies, and other transplant rejection effector mechanisms are excluded. These systems may also modulate the bidirectional diffusion of antigens, cytokines, and other immunologic moieties on the basis of the chemical characteristics of the membrane and matrix support. Encapsulated cell technology offers a solution to the problem of donor organ supply, not only by allowing the transplantation of human cells and tissues without immunosuppression but also by permitting use of tissues isolated from animals. The ability to cross species lines has the potential to expand dramatically the number of patients and the scope of diseases that can be treated successfully with transplantation.

POTENTIAL APPLICATIONS

Cellular encapsulation has broad application to treating major diseases such as diabetes and a wide range of other disorders (Table). These include the use of a variety of cells such as hepatocytes for the treatment of liver failure and enzymatic defects (both implantable and extracorporeal approaches), adrenal chromaffin cells for chronic pain, genetically engineered cells that produce clotting factor IX for hemophilia, growth hormone for dwarfism, erythropoietin for anemia, parathyroid cells for hypocalcemia, nerve growth factors for ALS, and for the treatment of Parkinson’s disease, Alzheimer’s disease, epilepsy, Huntington’s disease, spinal cord injuries, and strokes.
Table. Some disorders potentially treatable with encapsulated cell transplantation

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<tr>
<th>Disorder</th>
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<td>ALS</td>
<td>Hemophilia</td>
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<td>Alzheimer’s</td>
<td>Huntington’s</td>
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<td>Anemia</td>
<td>Hypoparathyroidism</td>
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<td>Atherosclerosis</td>
<td>Immunodeficiencies</td>
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<td>Cancer</td>
<td>Kidney failure</td>
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<td>Chronic pain</td>
<td>Liver failure</td>
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<td>Diabetes</td>
<td>Muscular dystrophy</td>
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<td>Dwarfism</td>
<td>Parkinson’s</td>
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<td>Enzymatic defects</td>
<td>Spinal cord injuries</td>
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<td>Epilepsy</td>
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Continuous delivery of factor IX would circumvent the hemorrhagic crises associated with the disease, providing a much improved and more economical therapy.

HISTORICAL PERSPECTIVE

To date, most of the research in the area of cellular encapsulation has been carried out with pancreatic islets. In patients with type I diabetes, a marked decrease occurs in the number of β cells in the pancreas. There is hope that the transplantation of islets will not only eliminate the need for daily insulin injections, but it will prove effective in preventing or retarding the development of complications associated with the disease. In fact, the restoration of normal glucose metabolism has already been achieved in several patients with diabetes by means of the transplantation of islets. Success, unfortunately, has been sporadic, and the requirement for immunosuppressive drugs exposes these patients to a wide variety of serious complications. In addition, many of these immunosuppressive drugs, including glucocorticoids and cyclosporine A, also have dose-dependent deleterious effects on glucose homeostasis and β-cell function. Nevertheless, these studies have been of significant value both in documenting the potential importance of islet transplantation as a therapeutic modality and in defining some of the problems that must be overcome before this approach can be used in large numbers of patients. The currently limited supply of human pancreata and the fact that multiple glands may be required to isolate sufficient numbers of islets to treat a single patient indicate that techniques must be further developed and refined for xenografting of isolated islets from animal sources to patients with diabetes. Such techniques must overcome both classic immune rejection of transplanted cells and tissues and autoimmune destruction of β cells known to occur in patients with type I diabetes.

Three types of encapsulation systems have been studied by our own and other groups (Fig. 1). These include devices anastomosed to the vascular system as arteriovenous shunts, diffusion chambers, and spherical cap-
Fig. 2. Successful treatment of diabetes in totally pancreatectomized dogs with encapsulated islets. First description of ability of an extravascular device to sustain normoglycemia in a large animal model without any immunosuppression. Exogenous insulin requirements (Δ) and fasting blood glucose concentrations (○) in two dogs before and after device implantation. From Diabetes 1992;41:886.

sutures. Results in diabetic animals indicate that these systems can function for up to more than 1 year. However, these data also strongly suggest that because of limitations in functional islet longevity, periodic replenishment of islets will be required in patients. In some designs this may pose significant difficulties. Use of biodegradable materials that are slowly absorbed and excreted may well help to solve this potential problem.

The modern era of biohybrid device development began approximately two decades ago with the introduction of islet-containing perfusion devices implanted as arteriovenous shunts. The original perfusion devices used bundles of capillary fibers seeded on their outside surfaces with isolated islet cells. Tissue culture medium was circulated through the lumen of the fibers, and the islets secreted insulin in response to stimulatory glucose concentrations. However, the use of these small diameter fibers as vascular implants was limited to short-term studies because of clotting. A device that used a coiled tubular membrane with an inner diameter of 5 to 6 mm was investigated by our group for several years. In vivo studies indicated that this design had excellent biocompatibility with respect to blood clotting. Four dogs that received devices without islet tissue were still living after 3 years. The function of this perfused artificial pancreas was evaluated by implanting devices seeded with canine islet allografts into severely diabetic pancreatectomized dogs by using a protocol that had been shown to optimize long-term insulin secretion in devices maintained in vitro. In two animals the devices were removed 1 year after implantation. In both cases the exogenous insulin required to control blood glucose concentrations increased by more than 20 units/day after device removal. Data from the implantation of devices containing xenogeneic islets were limited but did indicate that discordant xenografts were also feasible. One dog that received devices containing bovine islets exhibited excellent control of glucose levels for almost 2 months without exogenous insulin. The results with porcine islets substantially decreased the insulin requirement for up to 9 months.

However, a number of issues remained that appeared to limit the therapeutic potential of this approach. Perhaps most importantly, data suggested that the size and geometry of perfusion devices imposed a critical limitation on the amount of islet tissue that could be transplanted into a patient by using a single unit. At present, two devices would be required to treat a patient with an insulin requirement of approximately 30 units/day. Attempts to lengthen the tubular membrane, thereby increasing insulin secretion, failed because of clotting. In addition, the glycemic control provided by
the perfusion device design clearly was not optimal. Nevertheless, much was learned from these studies. They represent an important step toward developing simpler, more viable strategies for transplanting islets by using encapsulation.

Numerous types of diffusion chambers have also been evaluated by our own and other groups. These are typically tubular or planar designs, although the most significant progress has been achieved with cylindrical polyacrylonitrile-polyvinyl chloride (PAN-PVC) membranes that have a smooth outer skin. Porcine, bovine, and canine islets transplanted intraperitoneally within these chambers restored normoglycemia in spontaneously diabetic BB and STZ rats for periods of several weeks to more than 1 year. Although these membranes solved many of the problems associated with diffusion chambers (e.g., fibrosis, abscess formation, adhesions), studies in large animals closer to human beings will be required before clinical trials can be contemplated. Experiments in totally pancreatectomized, severely diabetic dogs have in fact already been performed in our laboratory. They indicated that canine islet implants can provide long-term correction of hyperglycemia without the use of immunosuppressive or antiinflammatory drugs. Insulin independence was achieved for more than 10 weeks in dogs with preimplantation insulin requirements of 30 to 40 units/day (Fig. 2).

In view of these encouraging results, a number of unsolved issues critical to the wide-scale clinical success of these devices must be addressed. These include long-term biocompatibility, membrane breakage, and suitability for retrieval. Experiments have demonstrated the feasibility of long-term immunosuppression of islets by artificial (PAN-PVC) membranes and the long-term biocompatibility of the membrane versus the graft and versus the recipient. These data indicate that islet implants can provide correction of hyperglycemia in dogs and rodents for up to 1 year without the use of immunosuppressive drugs. Histologic examination of the
chambers revealed that they were biocompatible. However, most of the implants ultimately failed because of membrane breakage. By 6 months after implantation most of the chambers in dogs had broken. The membranes used in these studies were relatively fragile and susceptible to breakage. An increase in the membrane wall thickness may minimize this problem. Because of limitations imposed by islet longevity, membrane chambers will also eventually require localization and removal. If necessary, the devices could be removed by means of laparoscopy. However, surgical excision might be necessary if the chambers were to become fibroencapsulated. Open surgery, of course, carries risk of infection and would involve a more extensive procedure.

MICROENCAPSULATION

During the past decade several methods for microencapsulating islets have been investigated. Microcapsules offer a number of distinct advantages over the use of other encapsulation devices, including (1) greater surface to volume ratio, (2) ease of implantation, and (3) retrievability by lavage and needle aspiration. Most of the procedures for fabricating microspheres involve extruding a mixture of cells and sodium alginate by using a droplet generation device into a CaCl$_2$ solution. The negatively charged gelled droplets can then be coated with positively charged agents such as poly-L-lysine (PLL) to form a permeselective membrane.

Small animal models. Microencapsulation has been successfully used to reverse diabetes in rodents by using islet allografts and rodent-to-rodent islet xenografts. Although prolongation of survival of canine islets has also been achieved with the alginate-PLL technique, these studies have been performed in mice and have usually required adjunctive treatment with immunosuppressive agents. Recently, prolongation of porcine and bovine xenograft survival in diabetic mice has been achieved in our laboratory without immunosuppression by using alginate microspheres without the synthetic PLL membrane. Uncoated alginate spheres containing porcine and bovine islets routinely reversed hyperglycemia after intraperitoneal injection into diabetic mice. The ability of uncoated microspheres to achieve marked prolongation of xenograft survival is surprising, because the destruction of the grafts might have been expected to occur on the basis of the presence of circulating preformed natural antibodies in the recipients that are reactive to cells of the donor. Our data suggest that proteins of the complement system would also have had access to the encapsulated islet graft. An explanation for the immunoprotective effect of the alginate spheres also needs to accommodate the fact that cytokines (molecular weight of 10 to 30 kDa), nitric oxide,
and other toxic moieties are small enough to diffuse readily into the gel matrix, yet did not induce obvious dysfunction or destruction of the islet graft.

Preliminary experiments in diabetic rats suggest that uncoated alginate spheres containing porcine and bovine islets can also reverse hyperglycemia for periods of up to 175 days. However, these results have usually required the use of larger spheres or adjunctive treatment with immunosuppressive agents. Of course, a major goal of encapsulated islet transplantation is to eliminate the need for immunosuppression altogether. To that end, we have developed a new type of encapsulation technology that allows transplantation of islets across a wide species barrier without immunosuppression. These selectively permeable "microreactors" are fabricated from biodegradable polymers that are slowly absorbed and excreted from the body. The microreactors can simply be injected under the skin or placed intraperitoneally or in other extravascular sites by using a needle and syringe. Moreover, the rate of degradation of the microreactors can be adjusted to correspond to the functional longevity of the encapsulated islets. We have successfully tested these microreactors by using discordant islet xenografts in several animal models. In one set of experiments porcine islets were immobilized in reactors and implanted into the peritoneum of nonobese mice and diabetic rats and rabbits (Fig. 3). Diabetes was reversed in the animals for periods of up to 20 weeks without any immunosuppression (Fig. 4).

Large animal model. Experiments in spontaneously diabetic dogs have also been performed in our laboratory by using canine islets encapsulated inside uncoated alginate microspheres. Although low-dose cyclosporine A was also administered, by 3 weeks after implantation the levels of the drug were below detectable limits by high-performance liquid chromatography. Implantation of the microspheres completely supplanted exogenous insulin therapy in the dogs for 60 to more than 175 days. Soon-Shiong et al. have also reported successful long-term implantations of microencapsulated allografts in larger animals. They treated spontaneous diabetes in dogs that were administered cyclosporine A. However, these microspheres were PLL-coated. The implants maintained euglycemia for 63 to 172 days, comparable to the results obtained in our laboratory without the use of a synthetic PLL membrane. More recently, we have tested our new type of microreactors in dogs by using discordant islet tissue. Bovine islets were implanted in either uncoated alginate spheres or in the new type of selectively permeable microreactors for periods of 4 to 6 weeks. No islets survived in the uncoated alginate spheres, even with the use of triple immunosuppressive therapy. However, when the islets were immobilized within the new selectively permeable microreactors, viable tissue was observed both with and without immunosuppression (Fig. 5). Immunohistochemical staining of selectively permeable microreactors recovered from these dogs revealed well-granulated α, β, and δ cells consistent with functionally active hormone synthesis and secretion. To test further the secretory function of the islets, the explanted microreactors were incubated in medium containing either basal or stimulatory concentrations of glucose. The islets responded with an approximately fourfold to sixfold average increase above basal insulin secretion. These results, together with data generated by using porcine islets transplanted into nonobese diabetic mice and diabetic rats and rabbits, indicate that long-term survival of discordant islet xenografts can be achieved in both rodents and dogs without immunosuppressive drugs by using microreactors fabricated from biodegradable materials. We hope to obtain FDA approval to bring this new approach to xenotransplantation to clinical reality within the next year.

**CLINICAL TRIALS**

Encapsulation technology will, as previously noted, be applicable to transplanting a wide variety of primary and of bioengineered cells into patients. Human clinical trials have already been initiated, not only with encapsulated allogeneic islets for the treatment of diabetes, but also with bovine adrenal chromaffin cells for the treatment of chronic pain, cells releasing recombinant ciliary neurotrophic factor (CNTF) for the treatment of ALS, and porcine hepatocytes for the treatment of liver failure.

**Diabetes.** Using encapsulated islet allografts, Soon-Shiong et al. achieved insulin independence for approximately 1 month in a patient with type 1 diabetes who was on a regimen of immunosuppressive drugs (cyclosporine A and azathioprine). However, this patient received larger "pea-sized" macrocapsules and subsequently required exogenous insulin therapy for control of blood glucose concentrations. Glycosylated serum albumin levels decreased from 10.6% before transplantation to 5.1% at 6 months, whereas glycosylated hemoglobin levels fell from 9.3% to 7.8%. Electromyography studies suggested improvement in axonal nerve function. The significance of these results is difficult to interpret, because similar or better results have been achieved in immunosuppressed patients by using allogeneic islets that were not encapsulated.66

**Chronic pain.** Preliminary clinical trials have also been initiated in Europe involving patients with end-stage cancer suffering from intractable pain. The patients received bovine chromaffin cells encapsulated inside a tethered tubular membrane chamber. The use of chromaffin cells in these studies was based on their ability to secrete natural analgesic substances such as...
The chambers were implanted either into the subarachnoid space by using a minimally invasive surgical procedure similar to a spinal tap or into the lateral ventricle of the brain. There were no serious complications, and seven of 10 patients showed a decrease in narcotic intake and improved pain scores. The FDA has now approved phase I clinical trials in the United States.

ALS. In animal models of chronic degeneration, CNTF has been shown to prevent motor neuron loss.58 In light of these results, baby hamster kidney (BHK) cells have been genetically engineered to produce CNTF. Encapsulation and subcutaneous implantation of transfected BHK cells in membrane chambers slowed down progressive motor neuropathy and reduced motoneuron death in a mouse model of ALS.59 With this approach phase I clinical trials have been initiated in Europe by using cells transfected with the gene for human CNTF. The investigators hope that low doses of CNTF will be effective when delivered to specific sites by implanting the chambers in the lumbar spine area where the motor neurons degenerate in patients with ALS.

Liver failure. As an alternative to whole liver transplantation, investigators are now attempting to develop both implantable and extracorporeal liver assist devices. Rozga et al.60 have demonstrated improved ammonia and glucose metabolism in dogs with ischemic liver failure by using a microporous hollow-fiber device seeded with porcine hepatocytes. This device was further tested in nine patients with fulminant liver failure.61 Eight of the patients either recovered completely or were bridged to orthotopic liver transplantation and survived. The results of this preliminary clinical study suggest that the treatment with porcine hepatocytes is not only safe, but that it may be a potentially beneficial therapy. A clinical trial examining the use of this treatment in a greater number of patients is currently underway.6

SCALING-UP AND MANUFACTURING

Pending the successful outcome of clinical testing, a number of issues must be addressed, including the sourcing of raw materials, the design and building of manufacturing facilities, the scale-up and optimization process, storage and distribution of the product, and quality control. The latter area is of particular concern, because the use of animal tissues in human beings carries the potential risk of infection with both recognized zoonotic pathogens and unknown xenogenic agents. A comprehensive herd and bioburden screening program will be necessary to minimize the risk of such transplant-associated zoonosis. We must assure ourselves, our regulators, and our customers that these new living drug delivery systems are every bit as safe as the tablets, capsules, and liquids that we have trusted for so many years. Because our therapeutic product is living tissue, many time-tested methods of pharmaceutical product sterilization will not be available to us. New methods, approaches, and assays must now be developed to provide the same standard of safety assurance that we now accept in more traditional therapies.

Cellular technologies that incorporate either primary animal tissue isolates or cells grown in cell culture from primary animal tissue isolates raise new manufacturing process issues. The potential risk of infection from both well-recognized and previously unidentified zoonotic pathogens must be effectively eliminated from the product. All animals raised to provide raw material for such cellular medicine products will be grown under carefully controlled conditions, including the air they breathe, the food they eat, and the human beings and animals with which they come in contact. They will be periodically screened for a comprehensive list of known zoonotic pathogens and infectious agents, and assays and tests are being developed to rule out as yet unrecognized but potentially damaging infectious agents. The cellular products that are derived from this raw material must also be screened during the manufacturing process for both infectious agents latent in the raw material and infectious agents that could be introduced during the product manufacturing process.

The scale-up of manufacturing processes to serve many of these markets that are quite large in terms of patients and patient doses presents new bioprocess engineering challenges. Although the task of culturing large numbers of cells is well established, albeit only a decade old, the task of maintaining large numbers of cells in a totally sterile and quality-assured environment from primary cell isolation to implantation in patients is just beginning. Each step of quality assurance must now be designed so that the biologic product meets required criteria for cell standardization, characterization, and function. Dosing must be redefined in terms of the ability of a given number of cells to release the desired quantity of bioactive substances instead of a simple quantification of purity and volume. Distribution of these living drug delivery systems presents a new and complex profile of dosing, shelf life, and packaging requirements. The combination of high cost levels embodied in the final product and sensitivity to time and method of distribution will demand new approaches to product inventory and distribution.

All of these challenges will be met and overcome. The tide of emerging cellular products brings the promise of dramatic improvements in quality of life and therapeutic impact for millions of patients. Although such benefits are usually weighed against their costs, many of these new therapies will also result in significant reductions in health care costs by eliminating the high
expense of treating advanced disease conditions and their associated complications.

CONCLUSION

It is apparent that the transplantation of cells and tissues with specific differentiated functions will play an important therapeutic role in medicine in the future. The transplantation of encapsulated cells has recently been extended from rodents to larger animals and is now moving from the laboratory into the marketplace. Human clinical trials have already been initiated for the treatment of diabetes with encapsulated islet allografts and for the treatment of chronic pain, ALS, and liver failure by using xenografted cells. Pending the successful outcome of clinical testing, scale-up of the manufacturing processes can begin to meet the requirements for the wide-scale general distribution and ultimate commercialization of this type of cellular therapy.

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REFERENCES