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Recent Progress in Immunoisolated Cell Therapy

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Abstract Biohybrid implants represent a new class of medical device in which living cells, supported in a hydrogel matrix, and surrounded by a semipermiable membrane, produce and deliver therapeutic reagents to specific sites within a host. First proposed in the mid-1970s for diabetes, this treatment modality has progressed rapidly in the past four years and is now being investigated not just for endocrine disorders but also for alleviation of chronic pain, treatment of neurodegenerative disorders, and delivery of neurotrophic factors to sites within the blood brain barrier, and as a practical alternative to conventional ex vivo. © 1994 Wiley-Liss, Inc.

Key words: immunoisolation therapy, transplantation, cell therapy, therapeutic gene products, encapsulation, diabetes, neurodegenerative disorders, Parkinson's, Alzheimers

Although antecedents extend back 60 years [Biseeglie, 1933] serious investigative interest in immunoisolation therapy began in the mid 1970s when chemically induced diabetes was successfully treated in rodents using islet grafts enclosed within synthetic membrane barriers [Chick et al., 1977; Tze and Chen, 1977; Lim and Sun, 1980]. Efforts to develop a practical and medically relevant artificial endocrine pancreas have continued to expand ever since. [Lacy et al., 1991; Lanza et al., 1992a,b; Sullivan 1991; Soon-Siong et al., 1992; Dionne et al., 1993]. Starting in the mid 1980s, Aebischer, his students, and collaborators began to apply this same basic technology to the treatment of neurodegenerative disorders of the central nervous system (CNS) [Aebischer et al., 1988, 1991a,b; Jaeger et al., 1991; Emerich et al., 1992; Winn et al., 1992]. More recently, progress has been evidenced in such diverse areas as the treatment of chronic pain [Sagen, 1992; Sagen et al., 1993] and the delivery of blood components such as factor IX [Liu et al., 1993] and Erythropoietin [Koo and Chang, 1993]. Critical to these efforts has been the transplantation not just of primary cell grafts but also of dividing cell lines and genetically engineered cells. This multiplicity of applications has attracted a growing industrial and venture-based investment and a consequent expansion of development activity. The literature is well summarized in still-current reviews [Colton and Avgoustiniatos, 1991; Christenson et al., 1992a; Aebischer et al., 1992]. In this paper, we chronicle and critically examine the technological basis for the expansion of cell therapy from its origins as a novel artificial organ therapy for diabetes to its current position as a rapidly evolving platform technology for the delivery of a broad portfolio of therapeutic gene products.

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IMMUNOISOLATION TECHNOLOGY

The basic concept of immunoisolation is straightforward: living cells are surrounded by a semipermeable barrier which permits bidirectional passage of small molecules (nutrients, oxygen, secretogogues, and bioactive cell secretions) while restricting transport of larger molecules and host immunocytes. Several different approaches to immunoisolation have been proposed and evaluated. Vascular devices were prominent in the early literature and in initial canine studies of diabetes, but most current effort is focused on cylindrical or planar diffusion chambers (macrocapsules) or on dispersions of spherical beads (microcapsules). These geometries are illustrated in Figure 1. Diffusion chambers are macroscopic implants formed by sealing the peripheries of preformed thermoplastic semipermeable membranes. To minimize transport distance between grafted cells and host, the diameter of the fiber or gap thickness of the disk is maintained below about 1.0 mm. A representative cylindrical implant is shown in

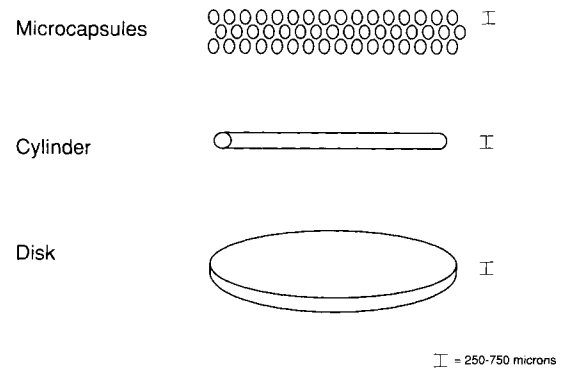

Fig. 1. Illustration of various implant geometries. Microcapsules are small spherical beads; internal volume is less than about 0.1 microliters, and a large number are typically required to deliver a therapeutically active dosage of cell product. Macrocapsules may be in the form of cylinders or disks. Tubes contain approximately 2–50 microliters and are useful for local delivery of highly potent agents within the CNS. Disks have volumes up to 1 milliliter and are most often considered in regard to diabetes applications.

Figure 2. Diffusion chambers are sturdy devices which are implanted surgically and can be retrieved if necessary or desirable. In contrast to diffusion chambers, microcapsules are usually prepared from water-swollen polymers shaped into small beads with internal diameters of 250–750 microns. A clinical dose requires several hundred to several thousand individual microspheres and can be easily administered to the patient as a suspension in intravenous fluid. The principle advantages of microcapsules are their optimal ratio of surface area to volume and the ease of administration. However, they cannot be retrieved, and cell viability in vivo and biocompatibility have proven both variable and investigator dependent [Soon-Siong et al., 1991].

Either primary postmitotic, or dividing cells may be encapsulated. Primary cells are isolated from the gland of a donor animal by enzymatic digestion, purified, and maintained in tissue culture until encapsulation. Primary cells are favoured for initial evaluation of a therapeutic approach, or in those applications where regulated release or multiple cell-secretory products are required. Dividing cells (i.e., cells which will continue to propagate indefinitely because of an induced or chance mutation) are also suitable for encapsulation. These grow to an upper limit of cell density in the capsule, typically 20–50% of capsule volume, which appears to be governed both by contact inhibition and metabolic limitations. As is evident in the rightmost panel in Figure 4, encapsulated cells often display mitotic figures. Cell debris is also observed upon histologic examination. For some applications, dividing cells are subcloned from established cell lines known to secrete a useful product—for example, PC-12 cells which synthesize and secrete dopamine. Alternatively, cells may be conventional producer lines Chinese Hamster Ovary [CHO], epithelial fibroblasts such as Baby Hamster Kidney (BHK), or other fibroblast lines [Hoffman et al., 1993] which have been genetically engineered to manufacture a particular gene product. The advantages of dividing cells come into prominence when commercial production is contemplated: they are easier and may be cheaper to produce and control for quality than cells obtained from livestock herds. Xenogeneic cells, even if dividing and even in CNS sites [Nicholas and Arnason, 1992], will be rejected by the host's xenoimmuno defense mechanisms in the unlikely event that they escape from the capsule.

Allografts are more easily and successfully transplanted than xenografts [Auchincloss, 1988; Nicholas, 1992; Platt, 1992]. Nevertheless, several difficulties attend their use in encapsulated cell therapy. The supply of transplantable human tissue is constrained by the number of cadavers suitable and available for transplantation, currently around 5,000 per year in the USA [Garland and Lysaght, 1993]. Human based cell lines avoid the supply problem but are less safe in the event of cell escape from a capsule than their xenogeneic counterparts, precisely because they are less immunogeneic. Therapeutic use of human fetal tissue is fraught with ethical and societal issues [Hurd, 1992; Smith, 1990]. Conditional immortalization or in vitro expansion of human cells represent promising future approaches.

Cells may be allowed to float freely within a capsule but are often supported on a three-dimensional hydrogel matrix or some other form of internal scaffold. These may be prepared from naturally occurring polysaccharides (alginate, agar, or chitosan) and typically contain over 95% water. Matrices serve multiple purposes. They minimize gravimetric settling of cells and provide a suitable surface for anchorage dependent cells. Chemical composition can be selected or modified to favour the growth of a desired cellular type over other strains. By hindering cell aggregation, they also prevent central necrosis. In our hands, under certain circumstances matrices improve both the viability and bio-

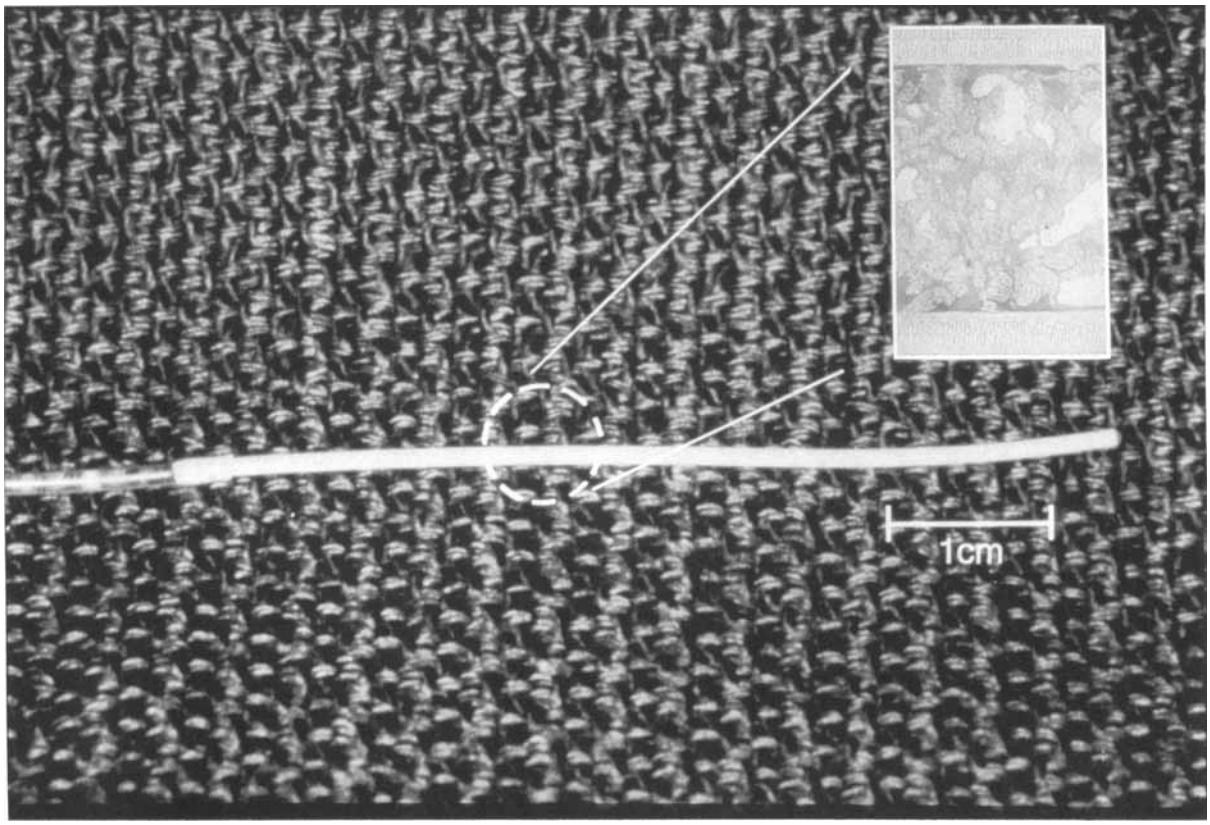

Fig. 2. Photograph of implant. The illustrated device, designed for intrathecal implant, is about 1 mm in diameter and contains an active region of 4 cm bordered at left by a silicone tether. The inset at top is a longitudenal section of bovine adrenal cells with the membrane visible at the edge. Both the device and cells are photographed from a device explanted from a sheep after 1 month implantation.

chemical functionality of cells they support. Moreover, the permeability of matrices may be low enough to provide a redundant layer of immunoisolation beyond that offered by the membrane. Research efforts are currently under way to replace the naturally occurring matrix materials with synthetic analogues, possibly with biochemically active oligopeptide derivatives.

As the critical interface between graft and host, the membrane represents the enabling component of immunoisolation [Colton and Avgoustiniatos, 1991; Christenson, 1992]. Membrane barriers are fabricated either from weak polyelectrolytes, typically polylysine-alginate, or from engineering thermoplastics, such as polysulfone, poly (acrylonitrile-vinyl chloride), or polyolefins. Weak polyelectrolytes can be employed in microcapsules and are generated by the interfacial coacervation of two oppositely charged, water-soluble polymers. The fabrication technique is straightforward [Chang, 1964; Lim and Sun, 1980]: droplets containing cells in a 1–3% solution of a charged polymer (e.g., polyalginate) are precipitated in a bath of an oppositely charged polymer (e.g., polylysine) at equivalent concentration. Transport properties of the resultant film are governed by the solids content and molecular weight of the constituent polyelectrolytes [Goosen et al., 1985; Sugamori and Sefton, 1989]. In contrast, membranes from engineering thermoplastics can be prepared by phase inversion, usually controlled precipitation in water of a solution of polymer in a water-miscible solvent [Michaels, 1971]. Transport properties are governed by the conditions of fabrication and formulation [Pusch and Walch, 1982]. The resultant membrane structures are then formed into capsules by peripheral sealing [Aebischer et al., 1986; Lacy et al., 1991]. Regardless of type, membranes must confer two basic characteristics on the implants they surround: biocompatibility and immunoisolation. An implant is biocompatible to the extent that the body's response to it does not compromise its function and that it does not adversely affect the

host. Achievement of biocompatibility in a polymeric implant is far from trivial since the normal "foreign body response," a nonspecific but highly inflammatory reaction mediated by macrophages and fibroblasts, would form a "secondary membrane" around the capsule and compromise the diffusive transport of material from host to graft and vice versa. As recently reviewed [Christenson et al., 1991a], biocompatibility is most often achieved by a careful manipulation of composition, purity, geometry, handling, surface morphology, and surgical techniques to yield an implant and implant site which is sufficiently inoffensive to the host to remain below the threshold of stimulation required to provoke macrophage activation and fibroblast deposition; specific requirements and techniques, and the interaction between them, vary with implant site. This approach has proven very successful in a number of implant sites; state of the art results are illustrated in Figure 3. Less frequently but also successfully, biocompatibility can be obtained by texturing the surface of the implant to encourage the growth of a host vascular network at the implant surface [Brauker et al., 1992]; such a layer essentially provides communication between the capsule and the host and allows the implant to function even in the presence of a secondary but more distal inflammatory or fibroblastic response. This strategy no longer requires membranes and materials which do not engender a foreign body response and consequently permits somewhat greater latitude in setting membrane transport requirements. Disadvantages are a more complex retrieval process and the need to place the implant into soft tissue, epidural fat pads, or other acceptable sites to provoke such vascularization.

Early investigators in the field constructed a simple paradigm for immunoisolation: a membrane was selected whose pores were suitably sized to pass molecules below about 50,000 MW and to reject larger molecules; this would allow transport of necessary or benign molecules and prevent transport of host immunocytes. Things are, in practice, a good deal more complex. First, and as highlighted by Colton, membranes are not "ideally semipermeable," and there will always be a finite transport of molecules above the nominal molecular weight cutoff [Colton and Avgoustiniatos, 1991]. Thus encapsulated cells will almost always be exposed to a constant, long-term, low-level challenge of IgG, complement, and IgM. Although such compounds are clearly known to be cytotoxic at physiologic concentrations, their biologic activity and half-life at concentrations likely to be encountered within a capsule is unknown. Nor are experimental outcomes easily interpreted or generalized: any observed cell destruction is as likely to result from metabolic deprivation or faulty device construction as from immune attack. What is found

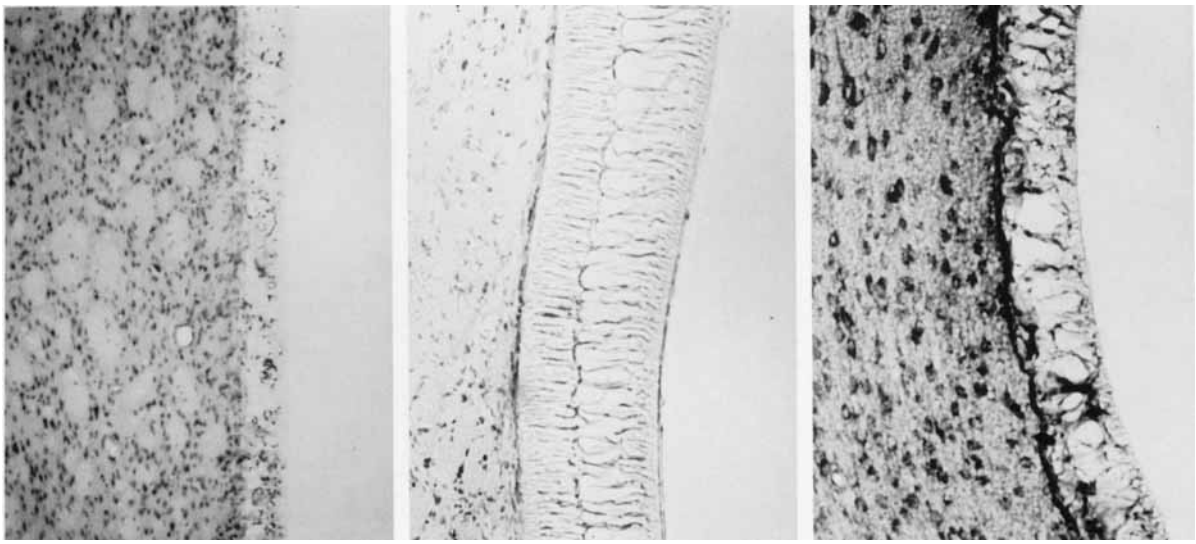

Fig. 3. Representative implant biocompatibility of capsules fabricated from acrylic copolymer containing an alginate matrix and implanted, left to right, in neuroparenchymal tissue of rats (1 month implant; H & E), guinea pig (3 months implant; H&E), and primate (7 months implant; GFAP). Magnifications, left to right: ×10, ×20, ×10.

with one target cell, graft-host species combination, or site of implant may not pertain to another. Against increased recognition of the complexity of the underlying science, we have adopted several operating premises which have resulted in satisfactory performance in ongoing studies for upwards of 6–12 months in existing animal models. First of all, allografts are far simpler to immunoisolate than xenografts. Secondly, survival of encapsulated cells is relatively easy to achieve in certain species combinations (e.g., rat to mouse), but success in these models is not necessarily predictive of success in large animals. Thirdly, immune and cellular response to implanted capsules in large animal models (dogs and primates) is more vigorous in some sites (subcutaneous, intraperitoneal) than in others (neuroparencyhymal, intrathecal). Allografts appear to survive in a capsule which merely excludes host cell contact even if it permits transport of immunologically active host proteins. Thus microporous membranes or even cross-linked hydrogels may provide adequate barriers for allograft cell survival. In contrast, xenografts will not remain viable in a capsule which is freely permeable to host proteins but can survive if protected by a membrane which restricts the transport of large proteins. The precise degree to which macromolecule transport must be restricted to permit xenograft survival is not well established and even controversial between groups: in our hands, anisotropic membranes have been consistently fabricated which provide long-term immunoprotection for most cells in most locations in most hosts. Figure 4 presents representative results of several successful cross-species transplantations. Fortunately, in most instances, xenoprotective membranes will also restrict the rate of transport of shed antigens to levels below that required any observable inflammatory or immunologic response.

THERAPEUTIC APPLICATIONS

Table I summarizes the literature of recent progress in the development of therapeutic applications for encapsulated cell therapy as well as the reported current status of clinical and preclinical testing in a variety of applications. The reported status is taken from abstracts and presentations and thus should be regarded as preliminary and is considerably more advanced than what is reported in the most recent cited publications.

Chronic pain is likely to represent the first application to reach clinical trials with human relevant xenogeneic cells and a full-sized device; such trials were initiated during 1993. Small animal studies for this application have been performed by Sagen and Aebischer [Sagen, 1992;

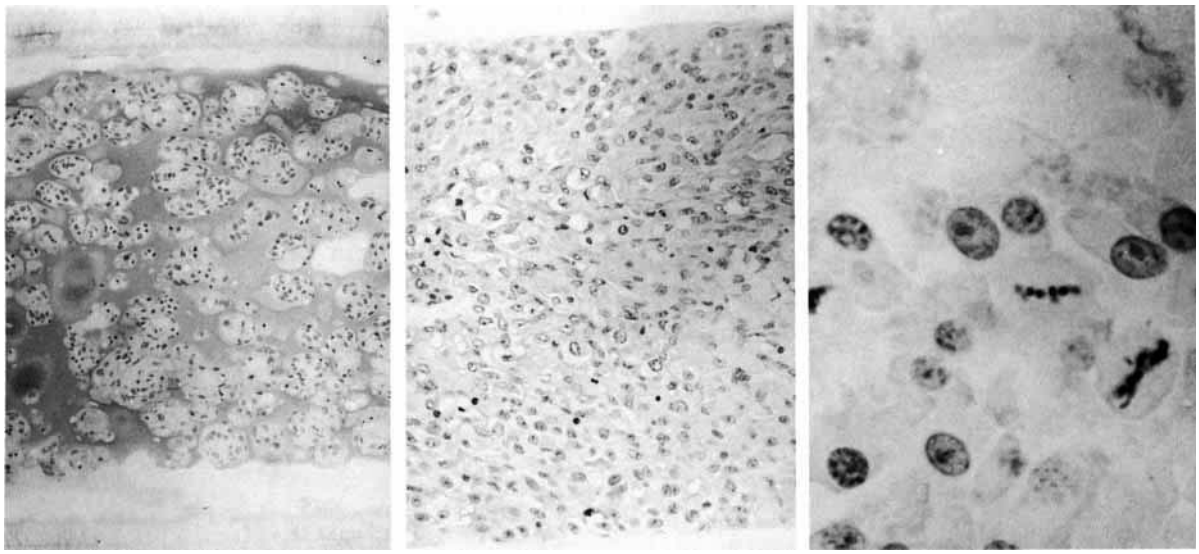

Fig. 4. Survival of encapsulated cells in xenograft models. Left to right: bovine adrenal chromaffin cells recovered from sheep intrathecal space after 30 days (H&E; note also membrane), BHK cells implanted in primate parenchyma for 30 days (H&E; membrane borders just visibleat edges), and PC-12 cells also implanted in primate parenchyma for 30 days (H&E, note extensive presence of mitotic figures). Magnifications, left to right: ×10, ×10, ×100.

TABLE I. Development Status of Principle Applications for Encapsulated Cell Therapy

Application	Recommended reference (October 1992)	Reported development status	Secretory products	Cells
Chronic pain	Sagan, 1992	Large animal studies Xenografts	Catecholamines Metenkephalins	Bovine adrenal chromaffin cells
Parkinson's	Aebischer et al., 1991a Tresco et al., 1992a,b Emerich et al., 1992	Subhuman primates	Dopamine	PC-12 cell line
Diabetes	Lanza et al., 1992a,b Lacy et al., 1991 Soon Siong et al., 1993	Mostly canine and small clinical allografts	Insulin	Islets
Hemophilia B	Liu et al., 1993	Canine	Factor IX	Rodent fibroblast cell lines
Alzheimers	Hoffman et al., 1993	Rodents Fimbria Fornix lesion	Recombinant nerve growth factor	Rodent fibroblastic cell lines
Amyotrophic lateral sclerosis	Unpublished	Rodents	Recombinant neurotrophic factors	Rodent fibroblastic cell lines
Huntington's	Emerich and Sanberg, 1992	Rodents Excitatory lesions	Dopamine/recombinant NGF	PC-12/rodent fibroblasts
Dwarfism	Chang et al., 1993	Rodents	Recombinant human growth factors	Murine fibroblasts
Anemia	Koo and Chang, 1993	In vitro	Erythropoietin	Kidney cell isolates

Sagen et al., 1993], who employed rodent adrenal chromaffin cells to release catecholamines and metenkaphalins into the spinal fluid. Large-animal preclinical trials were conducted by an investigative team headed by Aebischer, Goddard, and Christenson. In their studies, adrenal chromaffin cells were sourced from calves, and the implant configuration was similar to that shown in Figure 2.

Parkinson's disease is a neurodegenerative disease in which movement disorder results from a deficiency of the neurotransmitter dopamine in the striatum of the brain. Rodent models of Parkinson's have been successfully treated by neural implantation of capsules containing a line of PC-12 cells (derived from rats) which constitutively release dopamine. [Aebischer et al., 1991a,b,c; Emerich et al., 1992; Christenson 1992b; Tresco 1992a,b]. Aebischer has also reported amelioration psychometric symptoms in subhuman primate models based upon chemically induced dopamine deficiencies. The human-scaled implant is about the size of a toothpick, albeit far more compliant, and is introduced into the host's striatum by stereotaxic surgery. Human Parkinson's is a very complex neurodegenerative syndrome, and clinical trials, which are currently in the planning stages, will be required to determine if the human forms of the disease are as responsive to encapsulated cell therapy as are rodent and primate models.

As noted earlier, the use of immunoisolated islets to reverse hyperglycemia in mice following chemical lesioning of their beta cells was first described in the mid 1970s and has by now become almost routine [Lacy et al., 1991; Gerasimidi-Vazeou et al., 1992; Lanza et al., 1992a]. Donor cells for rodent recipients are typically sourced from the rat. The model has been extended to autoimmune forms of rodent diabetes, can achieve normal insulin secretory dynamics, and provides a basis for sophisticated dose-response protocols. Success in canine models has been more elusive. First of all, large animal

models require several hundred thousand purified islets and thus demand extreme proficiency at mass islet isolation, usually of the very difficult porcine islet. Secondly, devices have to be designed to contain and support nearly half a gram of tissue, compared to fractions of milligrams for Parkinson's and pain. The best results in large animals have been obtained by the investigative group headed up by Dr. William Chick and W.R. Grace using an intravascular device with no immunosuppression in pancreatectomized dogs [Sullivan et al., 1991] and diffusion chambers [Lanza et al., 1992b]. Most of his reported experiments were conducted with canine islets (allografts), but bovine and porcine islets seemed to function as well. Soon-Siong has reported reversal of diabetes using microcapsules in canine allografts and transient immunosuppression in "spontaneously diabetic dogs." [Soon-Siong et al., 1992] Scharp and Lacy have initiated clinical trials in which subclinical dosages of islets were implanted into normal, type I diabetics and type II diabetics for short periods, but the results of these trials are not yet available. Soon-Siong has announced a reduction in insulin requirements for an immunosuppressed human diabetic recipient of a large dose of microencapsulated human islets. However, human islets can be successfully implanted into immunosuppressed patients, in some cases resulting in complete reduction of insulin requirements, without any encapsulation [Scharp et al., 1991]. Development of a successful artificial pancreas to treat human diabetes with nonhuman islets remains one of the most challenging problems in biomedical engineering today.

Several other diseases are being approached at the rodent level. Emerich has described the treatment of an exictotoxin lesion model of Huntington's disease in rodents using dopamine-releasing chromaffin cells [Emerich et al., 1992]. Hoffman has successfully delivered recombinant NGF in vivo [Hoffman et al., 1993]. P. Chang has demonstrated the release of human growth factors from microencapsulated cells in vivo [Chang et al., 1993]. Her group has published and the Baxter group under B. Johnston have described the encapsulation of cells capable of releasing recombinant factor IX in vivo [Liu et al., 1993]. T. Chang of McGill recently described the release of erythropoietin from encapsulated rat cells as a potential treatment of anemia [Koo and Chang 1993]. Scientists at CytoTherapeutics have developed an array of implants capable of sustained, site-specific delivery of neurotrophic factors to local target sites within the central nervous system. Cell therapy may thus hasten the clinical development and enhance the practical utility of this powerful new class of therapeutic agents whose clinical utility has thus far been limited by their inability to cross the blood-brain barrier and thus to be administered as other pharmaceutical compounds.

A common question is how long it will take for this therapy to proceed from its current status to widespread availability in commercial medical practice. Answers to such queries are fraught with uncertainty, but a consensus of most workers in the field is that, provided progress continues to accelerate, remaining regulatory and product development barriers to commercialization should be complete within a time frame of 4–6 years.

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