ogous structures and thus apparently have evolved their receptor binding activities independently. The one thing that appears common to these structures is the high helical content and the use of residues in α helices for receptor binding.

Note added in proof: The secondary structure and topology of the cytokine interleukin-4 have been determined by nuclear magnetic resonance studies and it appears to have the same fold as GM-CSF (*32*).

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Maintenance of Normoglycemia in Diabetic Mice by Subcutaneous Xenografts of Encapsulated Islets

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The goal of islet transplantation in human diabetes is to maintain the islet grafts in the recipients without the use of immunosuppression. One approach is to encapsulate the donor islets in permselective membranes. Hollow fibers fabricated from an acrylic copolymer were used to encapsulate small numbers of rat islets that were immobilized in an alginate hydrogel for transplantation in diabetic mice. The fibers were biocompatible, prevented rejection, and maintained normoglycemia when transplanted intraperitoneally; hyperglycemia returned when the fibers were removed at 60 days. Normoglycemia was also maintained by subcutaneous implants that had an appropriately constructed outer surface on the fibers.

SERIOUS COMPLICATIONS INVOLVING the eyes, kidneys, and cardiovascular and peripheral nervous systems occur in individuals with diabetes mellitus. Although controversial, these diabetic complications are apparently related to the inability of exogenous insulin therapy to maintain the blood sugar within normal limits at all times. The goals of islet transplantation in the treatment of diabetes are to transplant islets early in the course of the disease in order to achieve continuous normoglycemia and to maintain the islet grafts without the use of immunosuppressive drugs. Human islet transplants in diabetic individuals who receive immunosuppressive drugs for maintenance of kidney transplants can produce normoglycemia in the recipients without exogenous insulin (*1*). The ideal immunoisolatory device for transplantation of islets in diabetic individuals would be biocompatible, maintain the viability and normal function of the islets, prevent cellular immune rejection, exclude antibodies to islet cells, maintain normoglycemia in the recipients, contain sufficient numbers of islets, be implanted either intraperitoneally or subcutaneously, and be easily and safely retrieved if desired.

Islet allograft rejection has been prevented without immunosuppression by isolating the islets from the immune system of the recipients with permselective membranes. Alginate (a naturally occurring linear polysaccharide extracted from kelp containing D-mannuronic and L-guluronic acid monomeric units) was cross-linked with Ca^{2+} to form a hydrogel. Encapsulation of individual islets in microspheres of alginate hydrogel with a coating of poly(L-lysine) produced a slight prolongation of intraperitoneal implants of encapsulated rat islet allografts (*2*). The capsules were insufficiently stable and biocompatible, a large volume of encapsulated islets was needed to achieve normoglycemia, and all of the encapsulated material could not be removed at the end of the experiments (*3*). Modifications in the encapsulation procedure have improved the biocompatibility of the membranes by covering the poly(L-lysine) envelope with an outer layer of alginate (*4*). An intravascular device that separates the islets from the blood stream by permselective membranes was developed (*5*), but blood coagulated in the vascular lumen. An improved intravascular device maintained normoglycemia for several months in diabetic canines that had been implanted with two devices that contained canine islets (*6*).

Hollow fibers formed of poly(acrylonitrile-*co*-vinyl chloride) (Amicon XM50) have been used for encapsulation of neonatal mouse islet cells before transplantation into diabetic hamsters and for encapsulation and transplantation of human insulinoma cells into rats (*7*). The encapsulated islet tissue was transplanted into the abdominal cavity, and in each of the two studies prolongation of survival of the islet xenografts was obtained in a few animals. Studies of the biocompatibility of these fibers have revealed a slight glial reaction when implanted into the brain of rats and only one to two layers of fibroblasts surrounded by collagen in mice when implanted intraperitoneally (*8*).

We studied two types of acrylic copolymer hollow fibers to determine their efficacy in achieving normoglycemia when used for encapsulation of Wistar-Furth rat islets. Type 1 and type 2 fibers were identical except for their outer surface wall. Type 1 had a totally fenestrated outer wall, whereas type 2 fibers had a smooth outer surface (Fig. 1). Type 1 fibers were formed by the usual technique of extruding the fibers di-

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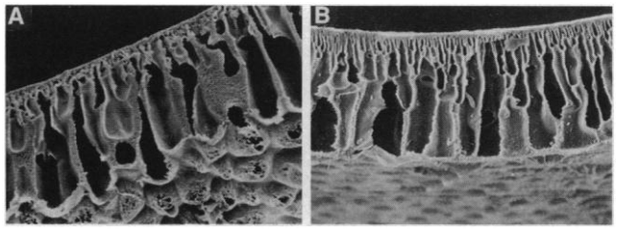

Fig. 1. Partial cross-sectional view by scanning electron microscopy of acrylic copolymer type 1 (**A**) and type 2 (**B**) fibers. Magnification, ×400. The internal diameter is 600 μm, and the outer diameter is 730 μm. Both fibers consist of a nonpermselective outer surface, a trabecular wall structure for strength, and an inner permselective skin with a nominal molecular mass cutoff of 50 kD. Rejection coefficient ± SD of blue dextran (2000 kD) is 0.99 ± 0.05; bovine serum albumin (67 kD) is 0.95 ± 0.05; α-chymotrypsinogen (24.5 kD) is 0.45 ± 0.1; cytochrome c (12.5 kD) is 0.20 ± 0.1; and vitamin B_{12} (1.35 kD) is 0.05 ± 0.05.

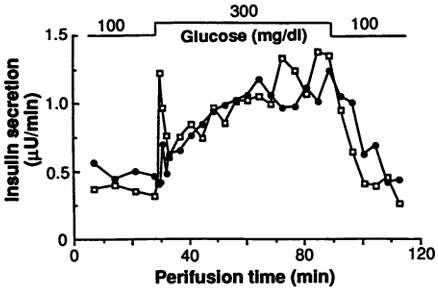

Fig. 2. In vitro perifusion comparing insulin secretion of rat islets immobilized in alginate gel and encapsulated in type 1 acrylic copolymer fibers (●) to unencapsulated islets (□). Freshly isolated islets were suspended in 1% alginate solution and injected into a hollow fiber at a final density of 70 islets per centimeter. Unencapsulated islet controls were hand-picked from the same isolation batch. Perifusion media was Dulbecco's modified Eagle's medium (Sigma) containing 10% newborn calf serum and glucose [either 100 (basal) or 300 (stimulated) mg/dl]. At a perifusate flow rate of 0.5 ml/min, the inherent lag of the perifusion system was <30 s.

rectly into water, whereas the type 2 fibers were extruded into a humidified atmosphere (*9*). Rat islets were isolated from male Wistar-Furth rats (250 to 300 g) by the collagenase technique, separated on a Ficoll gradient, and hand-picked with the aid of a dissecting microscope (*10*). In initial studies, 1000 rat islets were injected into the type 1 fibers, and the ends were sealed with a solution of the polymer (*11*). The fibers were placed intraperitoneally into noninsulin-treated, nonimmunosuppressed, male C57BL/6J mice made diabetic by the intravenous injection of streptozotocin (165 mg per kilogram of body mass) into the tail vein. Nonfasting plasma glucose concentrations were monitored three times weekly. The xenografts produced normoglycemia in the recipients for a period of 7 to 14 days, and then the mice became hyperglycemic again. Although we initially suspected that rejection had occurred because unencapsulated control rat islets that were transplanted intraperitoneally were rejected in 7 days, we found from histologic examination of the fibers that the individual islets had aggregated into large clumps, with only a thin rim of viable islet cells around the periphery of clumps of necrotic islet cells.

Because the loss of function of the islets was not a result of immune rejection but of aggregation of the islets, a search was made for a freely permeable matrix that could hold the islets apart in the fibers. Sodium alginate is compatible with islets (*2*), and we therefore used this hydrogel to separate encapsulated islets without interfering with the transport of insulin or nutrients. The effect of encapsulating and immobilizing the islets in alginate (*12*) on the kinetic response of insulin secretion from the enclosed islets was measured in a rapidly responding in vitro perifusion system (Fig. 2). The pattern of insulin secretion from the encapsulated islets was almost identical to that of unencapsulated control islets. First-phase insulin release of encapsulated islets was maintained, and there was at most a 1- to 2-min delay in the return to basal release after stimulation. Second-phase release was quantitatively the same for both encapsulated and unencapsulated islets.

Encapsulated rat islets (500 or 1000) immobilized in alginate gel were then transplanted intraperitoneally or subcutaneously into 2-cm type 1 or type 2 fibers (*13*) in mice made diabetic by the injection of streptozotocin (Table 1). Nonfasting plasma glucose concentrations were determined three times weekly; the diabetic recipients had concentrations >400 mg/dl before transplantation. The loading density was 70 islets per centi-

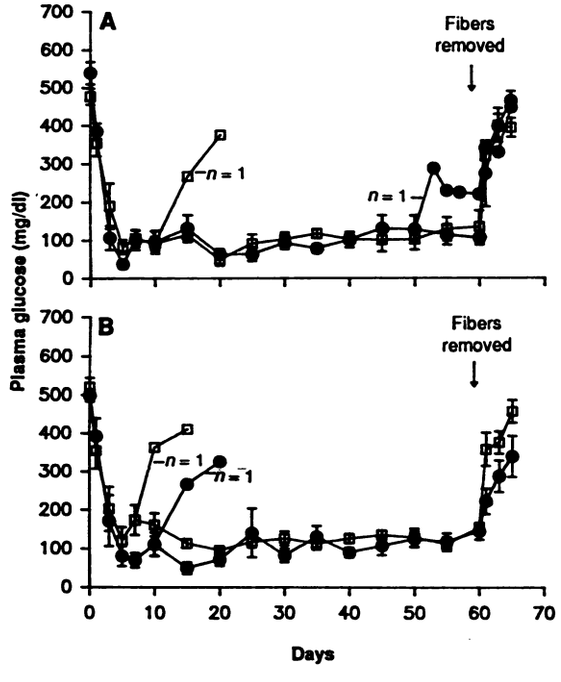

Fig. 3. Nonfasting plasma glucose concentrations ± SD of from five to nine diabetic mice after implantation of type 2 acrylic copolymer hollow fibers containing rat islets immobilized in alginate. The fibers contained either 1000 islets (**A**) or 500 islets (**B**) implanted either intraperitoneally (●) or subcutaneously (□). The recipients remained normoglycemic until the fibers were removed at 60 days. One recipient (indicated) in each of the four groups became hyperglycemic before 60 days. The individual values for days of normoglycemia are shown in Table 1.

Table 1. Duration of normoglycemia of rat islet xenografts in acrylic copolymer hollow fibers after transplantation into diabetic mice. Return to a diabetic state was considered to have occurred when the nonfasting plasma glucose concentrations exceeded 200 mg/dl. In the duration column, we have indicated multiple mice by a multiplication sign. For example, in the first entry, one mouse had normoglycemia for 27 days, one for 38 days, and seven for >60 days. IP, intraperitoneal; SC, subcutaneous.

Site	Islets	Duration (days)	Normoglycemic at 60 days (%)
		Type 1 fibers	
IP	1000	27, 38, >60 × 7	78
IP	500	>60 × 6	100
SC	1000	0, 10, 13, 20, 42, >60 × 3	38
SC	500	0, 8, 10, 10, 11, 48	0
		Type 2 fibers	
IP	1000	53, >60 × 4	80
IP	500	15, >60 × 5	83
SC	1000	15, >60 × 5	83
SC	500	10, >60 × 5	83

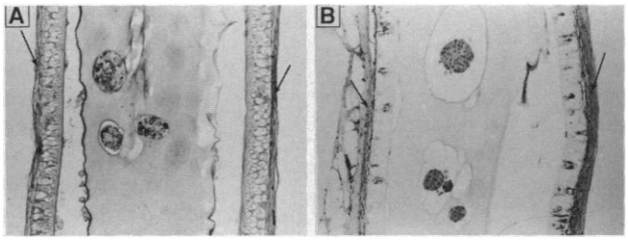

Fig. 4. Photomicrographs of rat islets embedded in alginate in type 2 acrylic copolymer hollow fibers at 60 days after implantation. The recipients were normoglycemic at the time of removal of the fibers. (**A**) The fiber had been implanted intraperitoneally, and the section was stained with aldehyde fuchsin, which stained β granules in the β cells, and counterstained with light-green, which stained a thin layer of collagen on the surface of the fiber (arrows). Magnification, ×41. (**B**) The fiber had been implanted subcutaneously, and the section was stained with hematoxylin and eosin. A thin layer of collagen is present on the surface of the fiber (arrows). Normal loose subcutaneous tissue with fat cells blends with the few layers of fibroblasts and collagen on the surface of the fibers. Scattered foci of mononuclear cells are present in the wall of the fiber because of the occasional openings in the outer skin of the membranes. Magnification, ×41.

meter for 1000 islets and 35 islets per centimeter for 500 islets. The intraperitoneal type 1 fibers induced and maintained normoglycemia for >60 days in seven of nine recipients that received 1000 islets and in all of the recipients receiving 500 islets. The fibers were removed at 60 days, and all of the normoglycemic animals became diabetic again. None of the recipients of subcutaneous type 1 implants of 500 islets remained normoglycemic for 60 days, and only three of eight recipients of 1000 islets were normoglycemic for >60 days (Table 1). Removal of the fibers from these three recipients returned the mice to a diabetic state.

Transplants of rat islets in the type 2 fibers produced and maintained normoglycemia in the recipient mice in >80% of either the intraperitoneal or subcutaneous sites with either 1000 or 500 islets (Table 1). The recipients became hyperglycemic again when the fibers were removed at 60 days from either the intraperitoneal or subcutaneous site (Fig. 3). Histologic examination of the type 2 fibers implanted either intraperitoneally or subcutaneously revealed that they were biocompatible. Only a thin layer of collagen and fibroblasts was present on the surface, and the islets were intact with a normal degree of β granulation (Fig. 4). The walls of the fibers contained only scattered foci of mononuclear cells and fibroblasts because few openings were present in the outer surface. The type 1 fiber had the same degree of biocompatibility as type 2, with only a thin layer of collagen on the outer surface in both the intraperitoneal and subcutaneous sites.

The type 1 fibers with either 500 or 1000 islets maintained normoglycemia in the recipients when the fibers were implanted intraperitoneally but not with 500 islets implanted subcutaneously (Table 1). Because the biocompatibility of both fibers appeared the same, the failure of type 1 fibers in the subcutaneous site was probably due to the greater bioburden of cells throughout the walls of the fibers and possibly because of lower oxygen tension in the subcutaneous site than is present intraperitoneally.

Subcutaneous implants of low numbers of xenogeneic rat islets in type 2 acrylic copolymer fibers were not rejected and maintained normoglycemia. The successful subcutaneous transplantation of xenografts was encouraging because isografts of free rat islets implanted subcutaneously did not achieve normoglycemia, whereas a comparable number of islets implanted in the liver as isografts did (*14*). This methodology fulfills several of the criteria for an immunoisolatory device for possible use in human diabetics, including biocompatibility, prevention of rejection, ability to function either intraperitoneally or subcutaneously, and ease of complete retrieval.

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12. Sodium alginate (Kelton HV, Kelco, NJ) was dissolved (2.5% w/v) in 0.9% NaCl, boiled for 5 min, and diluted to 1% w/v with Hanks' solution without Ca (Gibco). The islets (100) were aggregated and formed into a droplet as described (*11*). Alginate (1%, 5 μl) was added to the drop of islets, mixed, and injected into a type 1 fiber (1.5 cm) as described (*11*). The loaded fiber was removed from the tapered PE-90 tubing and placed in $CaCl_2$ (2% w/v) for 6 min to cross-link the alginate. The ends were sealed as described (*11*), and the fiber was placed in the perifusion system [K. E. Dionne, C. K. Colton, M. L. Yarmush, *Biotechnol. Prog.* **7**, 359 (1991)].
13. The 1% alginate solution was prepared as described (*12*), except that the stock solution (2.5%) was diluted to 1% with CMRL 1066 containing 10% fetal calf serum. We used serum to assure that some protein would initially be in the alginate matrix around the islets. Either 1000 or 500 islets were concentrated into a droplet, and 1% alginate (50 μl) was mixed with the islets. The islet suspension was then injected into type 1 or type 2 fibers (2 cm–long sections), the alginate was cross-linked in 2% $CaCl_2$, and the fiber ends were sealed as described (*11, 12*). Seven fibers (2 cm long each) for either 500 or 1000 islets were prepared for each transplant and inserted individually into the peritoneal cavity or into seven individual tracks made with a probe in the subcutaneous tissue of the abdominal wall perpendicular to a midline incision. At 60 days after transplantation, the fibers were easily removed with small forceps from either the intraperitoneal or subcutaneous site. Intraperitoneal fibers either remained as individual fibers or formed a clump of three to four fibers. The subcutaneous fibers remained in the individual subcutaneous tracks and were easily removed by pulling on the end of the fiber with small forceps. The fibers were fixed in Bouin's solution and processed for light microscopy.
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