Encapsulated Islet Cell Therapy for the Treatment of Diabetes: Intraperitoneal Injection of Islets

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Since conventional insulin therapy has failed to achieve tight glucose control, an alternative treatment is urgently needed to treat diabetes. The findings of the Diabetes Control and Complication Trial[1] conclusively establish that improved glycemic control delays the onset and slows the progression of neuropathy, nephropathy, and retinopathy in insulin-dependent diabetic patients. The disease leads inexorably to one or more of the secondary complications, including renal failure, blindness, coronary and peripheral vascular occlusive disease. The challenge physicians face is to intervene before these secondary complications take their toll. Transplantation of encapsulated human islets to reverse diabetes by a minimally invasive or minor surgical procedure, without the risk of high-dose or life-long immunosuppression will be described here. In the near future, the shortage of donor human tissue will be overcome either by the use of encapsulated xenograft (porcine) islets or proliferated human islets. Thus, encapsulated islet therapy may become possible for the millions of diabetic patients who may benefit from islet cell transplantation.

Unfortunately, the previous results of unencapsulated islet transplantation in Type I diabetic patients have been disappointing. Despite the use of high-dose immunosuppressive regimens, rejection remains a major obstacle. An alternative to overcome the issue of rejection is immunoprotection of the islets from the host's immune system by encapsulating these insulin-secreting cells in a semi-permeable membrane.

Investigators have long sought to reverse insulin-dependent diabetes by a simple injection of immunoprotective insulin-secreting cells without immunosuppression therapy.[2-6] The idea of preventing the immune system from gaining access to cells by means of encapsulation evolved more than 35 years ago[7] when a diffusion-chamber device was explored, and since that time numerous immunoisolation systems have been described.[8-21] Significant challenges are faced regarding clinical application of these devices, including fragility, limited surface area, and in the case of vascularized devices

and chamber systems, the need for a major surgical procedure, with the risk of thrombosis and infection.

The microencapsulation technology obviates many of these technical issues and provides a safe, simple technique for implanting immuno-isolated cells into various sites. The principal reason encapsulated islet transplants have failed is the lack of a biocompatible and mechanically stable, immunoprotective system which also allows sufficient oxygenation of the enclosed insulin-secreting cells and provides adequate in vivo kinetics of insulin diffusion in response to a glycemic signal.

RECENT ADVANCES IN ALGINATE-BASED MICROCAPSULES

Over a decade has passed since Lim and Sun[6] described the reversal of diabetes in rats by alginate-based encapsulated islets. Since that time there have been, until recently,[21-23] no reports of successful studies of the large animal model nor any attempts in Type I diabetic patients. The progress of this technology has been limited by the lack of understanding of the fundamental mechanism(s) affecting biocompatibility of the alginate capsule membrane.

We have recently elucidated that some of the factors affecting long-term viability of alginate-based encapsulated islets include the following:

1. The immunostimulatory capacity of the alginate material.
2. The mechanical integrity of the microcapsule.
3. The chemical stability of the alginate membrane.
4. The propensity for the outer capsule membrane for cell adherence.

These factors are affected in turn by the sequential structure, chemical composition, and molecular size of the alginate polymer, as well as by the kinetics of the gel-formation process. The scope of this article does not allow in-depth detailed description of these issues.

REVERSAL OF DIABETES IN SPONTANEOUS DIABETIC DOGS

A blinded controlled study of microencapsulated canine islets compared to free, unencapsulated islets was undertaken in spontaneous diabetic dogs.[22,23] A total of 10 insulin-dependent, spontaneous diabetic dogs (insulin requirement 1 to 4 units/kg per day, absence of cumulating C-peptide and diabetic K-values of 0.6 ± 0.6) were enrolled into this study. The first four animals all received encapsulated islets. Subsequently, the remaining six dogs were randomized to receive either free or encapsulated islets. The form of therapy instituted was withheld from the veterinarians monitoring the recipients post-transplant (blind controlled).

Islets were isolated from canine donors by the standard collagenase digestion technique and purified using a two-phase aqueous separation solution.[24] Encapsulated or free islets were implanted by a simple injection via a 2-cm incision in the abdominal cavity. Serum glucose levels were determined daily, and at day 14 an intravenous glucose tolerance test (IVCTT) was performed on all recipients. In all seven encapsulated islet recipients, euglycemia was achieved within 24 hours (serum glucose falling from 304 ± 117 to 116 ± 72 mg/dL). IVGTT was performed 14 days after islet transplant demonstrated normalization of K-values changing from a pretransplant level of 0.6 ± 0.4 to 2.6 ± 0.6 mg/dL. All animals receiving encapsulated islets remained euglycemic, free of the need for exogenous insulin, for a period of 63 to 172 days, with a median insulin-independence for 105 days. In contrast, the recipients receiving free islets and cyclosporine (CsA) rejected their graft within seven days of implantation.

Of the seven dogs receiving encapsulated islets in the initial trial,[22] five were available for follow-up for two years, four of which received retransplantation. Three dogs received a second transplant after loss of insulin independence; at the time of the second transplant, they still had ongoing islet function from the first transplant. One dog received a third injection of encapsulated islets.

In this follow-up study,[23] the anti-inflammatory dose of CsA was discontinued 30 days after transplantation in all five dogs. Thus, these recipients were followed for a range of 110 to >600 days without receiving immunosuppressive agents.

In the four dogs receiving a second injection of encapsulated islets, the duration of insulin independence from the first transplant (125 ± 33 days; range: 95 to 172 days) did not differ significantly from the duration of insulin independence following the second injection (102 ± 20 days; range, 83 to 130 days). The recipient that received a third injection demonstrated insulin independence for 172, 83, and 138 days following the first, second, and third transplant, respectively.

Tight glycemic control was noted in all five recipients as evidenced by significant improvements in hemoglobin A1c (HbA1c) levels, K-values, and C-peptide release. Long-term islet function was demonstrated in each of the five recipients receiving encapsulated islets. Graft survival was 641, >228, 550, and >269 days in the dogs receiving multiple implants, and for as long as 726 days in the recipient receiving a single transplant. We chose a strict definition of positive C-peptide secretion as evidence of ongoing graft survival, even though this unfavorably skews our data when compared to the definition of graft survival reported by others in the literature who used reduction of insulin dose as the measure for ongoing islet function. By the latter criteria, ongoing islet survival would be equal to or greater than 780, >619, 720, >269 and 760 days respectively in these five dogs.

Insight into the potential duration of long-term graft survival after a single injection was provided by the recipient who received only a single injection and was followed for over two years. To date this recipient has not been retransplanted and has demonstrated continuous islet function for >726 days from a single encapsulated islet transplant as evidenced by ongoing basal C-peptide secretion (0.18 pmol/mL) on that date. On the basis of these preclinical data, as well as controlled studies demonstrating absence of acute toxicity or mutagenicity of the purified high G-alginate material, we initiated the first human clinical trial to assess the safety and efficacy of intraperitoneally transplanted encapsulated human islets in Type I diabetic patients.

PHASE I/II HUMAN CLINICAL TRIALS

Governmental regulatory (FDA) and institutional review board (IRB) approvals were obtained to initiate these trials in patients who are currently candidates for whole organ pancreas transplantation, i.e., insulin-dependent diabetic patients with functioning renal grafts. Eligibility criteria included the following: Type I diabetic patients 18 to 50 years of age with evidence of insulin dependence for more than 5 years; a functioning stable kidney transplant; glucagon-stimulated C-peptide <0.2 ng/mL; and absence of severe coronary artery disease.

Patient Pretransplant Status

Our first patient[25] to receive encapsulated islets was a 38-year-old white male with insulin-dependent diabetes for 30 years, requiring a mean ± SE of 0.7 ± 0.01 units of insulin/kg/day amounting to 45 to 50 units of insulin daily. The patient suffered from severe complications of the disease including a seven-year history of lower extremity peripheral neuropathy (daily, sharp, shooting pains of the left lower foot with progressive sensory loss), ulcerations of the left foot, retinopathy, left eye vitrectomy, and end-stage renal failure resulting in a living-related kidney transplant. The patient's renal function was stable (serum creatinine 1.0 mg/dL) on low-dose maintenance immunosuppression of Cyclosporine and Imuran 50 mg daily. In the nine-month post-islet-transplant period, the patient's OsA level ranged from 59 to 197 ng/mL.

Transplant Procedure

Through a 2-cm midline incision, encapsulated islets were injected directly into the peritoneal cavity. Based on our preclinical data, a full therapeutic dose would be 20,000 islets per kg. The patient received a subtherapeutic dose of 10,000 islets at the first procedure, followed by a supplemental dose of 5,000 islets six months later as part of a dose escalation study.

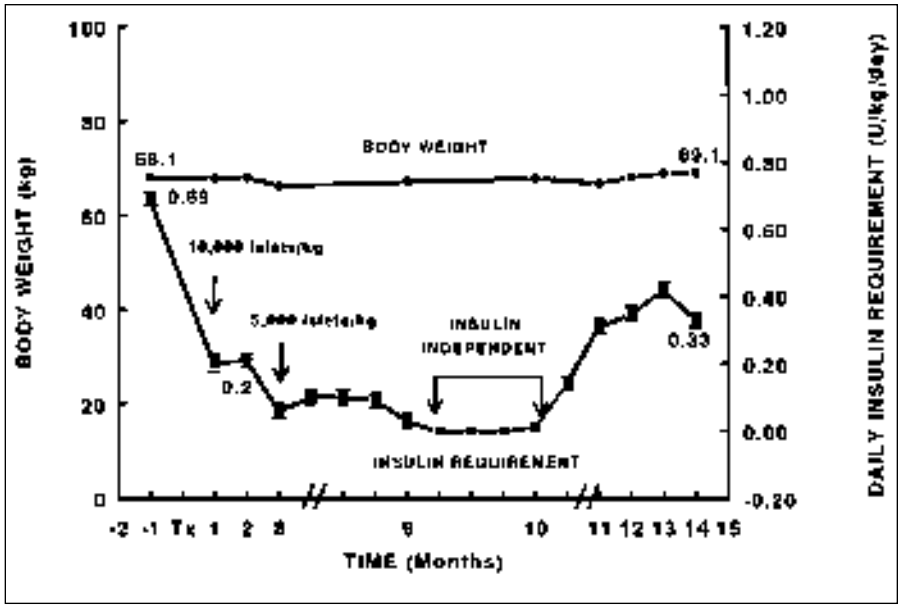

Figure 1. Indices demonstrating improved glycemic control and insulin requirement post-encapsulated islet transplantation in patient 1.

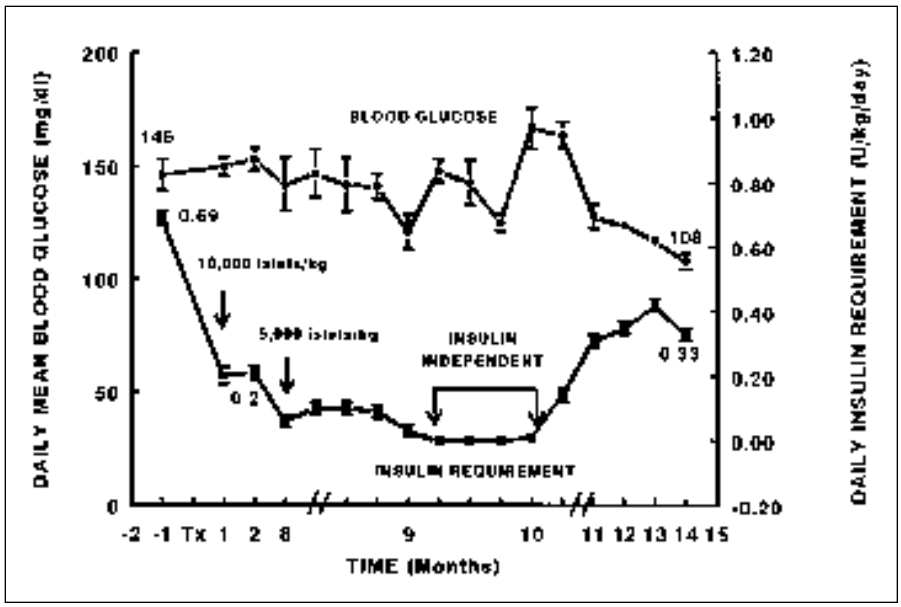

Figure 2. Blood sugar levels on days 416 and 417 while on no insulin therapy demonstrating long-term islet function.

Safety

To date, the patient has had no adverse effects. Despite the significant reduction of insulin, his body weight has increased from 68.1 to 72.7 kg (Table 1).

Efficacy

Islet Function. The patient demonstrated ongoing insulin secretion from the intraperitoneally transplanted encapsulated islets for over 20 months. Following injection of the initial subclinical dose of 10,000 islets per kg, insulin secretion from the transplanted cells was noted within 24 hours of transplantation. On the second postoperative day, all exogenous insulin was discontinued. The patient maintained normoglycemia and tolerated breakfast and lunch without requiring any insulin therapy. Occasional hyperglycemic episodes were noted postprandially and the patient was placed on a minimal dose of insulin to maintain normoglycemia. He maintained stable daily mean blood glucose levels ranging from 129 ± 3 to 150 ± 5 mg/dL, with less lability relative to his pretransplant levels while on a significantly ($p<0.001$) reduced dose of insulin of approximately 0.2 units/kg/day for a period of six months (Fig. 2, Table 1).

Following the dose escalation of a further 5,000 islets per kg in the seventh month, the patient's insulin requirements dropped even further to .07 units/kg/day, and he achieved insulin independence in the ninth month (Fig. 2, Table 1). The patient maintained insulin-independence for the entire 30-day period, with a daily mean blood glucose of 134 ± 4.2 mg% (114 observations) and M-value of 1.16. During this period of insulin independence, he demonstrated no evidence of ketosis and maintained his body weight (67.3 kg) (Fig. 1).

Fasting pro-insulin levels were high (1.24 ng/mL), suggesting stress from the subclinical dose of islets, and it was decided to supplement the patient with NPH insulin on a daily basis. He was thus returned to exogenous insulin therapy and for 20 months demonstrated ongoing islet function with tight glycemic control (Table 1). Significantly, in the face of this tight glycemic control, the patient reported no episodes of symptomatic

Table 1. INDICES DEMONSTRATING IMPROVED GLYCEMIC CONTROL AND INSULIN REQUIREMENT POST ENCAPSULATED ISLET TRANSPLANTATION

		Post Tx									
	Pre-Tx	1 mo	2 mo.	3 mo	4 mo	5 mo	6 mo	7 mo.	8 mo.	9 mo	10 mo.
Blood Glucose Evaluation											
# of observations	69	201	182	154	114	120	117	131	120	114	116
Daily Mean ± SE (mg/dl)	146±7.1	150±3.62*	150±4.01*	141±4.18*	150±4.95*	131±4.10*	129±2.61*	132±4.79*	144±4.52*	134±4.20*	154±5.5*
< 50 mg/dl episodes (%)	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.00	0.00
> 200 mg/dl episodes (%)	11.7	15.42	16.48	4.55	15.79	5.00	1.71	4.52	10.00	6.14	22.41
M-Value (Standard=120 mg/dl)	4.05	3.71	4.03	1.72	3.69	0.65	0.23	0.45	3.04	1.16	0.18
Body Weight (Kg)	68.1	68	68.1	69.1	67.7	69.5	67.5	62.3	56.6	67.3	68.1
Daily Insulin Requirement											
Daily Mean ± SE (Units/kg/day)	0.59±0.01	0.21±0.01***	0.21±0.01***	0.22±0.01***	0.27±0.01***	0.23±0.01***	0.23±0.01***	0.22±0.01***	0.61±0.01***	0.60±0.00***	0.64±0.00***
Metabolic Parameters											
Fasting Pro-Insulin (ng/ml)	<0.04	0.92	--	0.68	0.36	--	0.28	0.80	0.59	1.24	
Fasting C-Peptide (ng/ml)	0.1	0.70	0.40	0.30	0.60		0 h	0 hr	1.0	--	--
Hemoglobin A1c (% TL HB)	9.3	8.20	8.20	7.40	7.60	--	7.8	7.90	7.8	7.9	--
Glycosylated Albumin (% TL ALB.)	10.6	5.20	4.40	4.40	4.20	--	5.1	5.30	5.1	5.5	
Renal Function											
Creatinine	1.1	1.0		1.2	--	--	1.3	1.1	1.1	1.0	--
BUN	19	17	--	22.5	--	--	25	23	22.1	23	

	Post Tx									
	11 mo	12 mo.	13 mo.	14 mo	15 mo	16 mo	17 mo	18 mo	19 mo	20 mo
Blood Glucose Evaluation										
# of observations	117	120	116	111	65	116	120	120	120	120
Daily Mean ± SE (mg/dl)	127±4.94***	134±1.69**	137±1.59**	108±3.50**	108±4.69**	119±3.74**	116±4.0**	127±3.8**	128±2.8**	124±3.9**
< 50 mg/dl episodes (%)	0.9	0.00	0.00	1.8	0	1.72	0.8	0.8	0.00	0.00
> 200 mg/dl episodes (%)	7.6	0.00	0.86	0.00	0	2.59	3.33	3.33	2.5	1.67
M-Value (Standard=120 mg/dl)	0.35	0.22	0.53	3.97	2.56	2.21	--	--	--	-3.88
Body Weight (Kg)	67	68.2	69.1	69.1	69.5	72.7	72.7	72.7	72.7	72.7
Daily Insulin Requirement										
Daily Mean ± SE (Units/kg/day)	0.51±0.00***	0.53±0.00***	0.62±0.00***	0.59±0.03***	0.25±0.05***	0.44±0.01***	0.51±0.02***	0.57±0.02***	0.59±0.00***	0.59±0.00***
Metabolic Parameters										
Fasting Pro-Insulin (ng/ml)	0.33	1.06	2.30	1.63			--	0.45	--	--
Fasting C-Peptide (ng/ml)	0.6	0.5	0.2	0.1	0.3	--	--	--	--	<0.1
Hemoglobin A1c (% TL HB)	7.1	8.1	7.9	8.4		7 h			--	8.1
Glycosylated Albumin (% TL ALB.)	4.2	5.6	5.1	3.0	--	--	--	--	--	--
Renal Function										
Creatinine	1.0	1.0	1.1						--	1.1
BUN	22.7	19.6	27.6	--	--	--	--	--	--	21.2

* P > 0.05. Not significantly different from Pre-Tx.
** P < 0.05, Significantly different from Pre-Tx.
*** P < 0.001. Significantly different from Pre-Tx.

hypoglycemia, in sharp contrast to the one to two episodes of insulin reactions noted routinely on a weekly basis prior to the transplant.

During the long-term follow-up period, the patient found that there were occasions when he had to remove himself from exogenous insulin in order to maintain normoglycemia. For example, on days 416 and 417, the patient's mean blood glucose levels were 79 and 106 mg% while on zero exogenous insulin, confirming long-term islet function from the encapsulated islet transplant (Table 2). In the 20th month, his daily mean blood sugar was 120 ± 3.9 mg% (120 observations), with a normal M-value of –3.97 (Table 1).

C-peptide and Pro-insulin Secretion. Basal C-peptide secretion increased, concomitant with the drop in insulin requirement, from a pretransplant level of <0.1 ng/mL to a posttransplant fasting level of 1.0 ng/mL at the eighth month (Fig. 3, Table 1), confirming substained insulin secretion from the encapsulated islets. The patient demonstrated a greater than 100-fold improvement in M-value at six months (0.20) compared to the pretransplant level of 4.35, indicating significantly less glycemic lability over 24-month periods since the transplant. Over the 20-month period, M-values continue to improve with a normal level of –3.88 noted in the 20th month (Fig. 4).

These improvements of glycemic control over an extended time period were corroborated by improvements in both glycosylated serum albumin and glycosylated hemoglobin levels. Glycosylated serum albumin decreased from 10.6% pretransplant to 5.1% at 6 months, 5.1% at 14 months and 3.0% at 20 months, while glycosylated hemoglobin levels fell from 9.3% to 8.1% at 20 months (Fig. 4).

Peripheral Neuropathy

The patient reported subjective improvements in his lower extremity peripheral neuropathy symptoms. The sharp, shooting pains in his left lower foot, which occurred constantly on a daily basis pretransplant, abated in the posttransplant period. EMG studies (Fig. 5) confirmed improvement in axonal nerve function by demonstration of continued bilateral increases of amplitude of peroneal motor latencies at 3 months, 6 months, and 11 months posttransplant (from 45 m.v. to 194 m.v. at 11 months on the left, and from 200 m.v. to 425 m.v. at 11 months on the right). The left foot ulcer which required approximately 3 months to heal pretransplant recurred on week 10 posttransplant. This foot ulcer healed completely within seven days, possibly as a consequence of his improved glycemic control.

Renal Function

Throughout the 20-month period, the patient's renal function has remained stable with serum creatine levels ranging from 0.7 to 1.1 mg/dL.

Quality of Life

A quality of life questionnaire was completed by the patient pretransplant, and again at three and six months posttransplant. The patient reported significant improvement in various aspects of quality of life including an increased energy level, an ability to walk further,

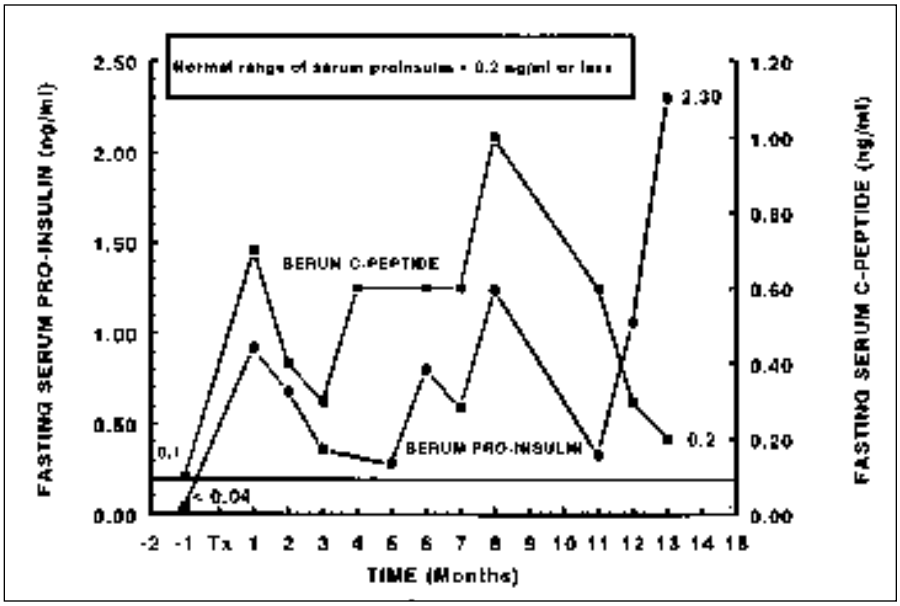

Figure 3. Serum C-Peptide and pro-insulin levels posttransplantation demonstrating ongoing islet function.

Table 2. Blood sugar levels on days 416 and 417 while on no insulin therapy demonstrating long-term islet function

Time	Day 415	Insulin	Day 416	Insulin	Day 417	Insulin
0700	65 mg%	6 units	49 mg%	0 units	83 mg%	0 units
1300	76 mg%	---	72 mg%	---	120 mg%	---
1900	53 mg%	4 units	90 mg%	---	122 mg%	---
2300	69 mg%	---	102 mg%	---	100 mg%	---
	x = 66 mg%	10 units	x = 79 mg%	0 units	x = 106 mg%	0 units

and a general feeling of improved health. He has recently secured full-time employment, an accomplishment he was not able to achieve due to his diabetes for almost a decade prior to his transplant.

THE FUTURE

Ongoing studies in our laboratory are addressing two important issues: long-term mechanical stability of the microcapsule formulation and sourcing of sufficient islets to treat the large number of patients who may benefit from encapsulated islet transplantation. With regard to capsule formulation, a photopolymerizable, covalent crosslinkable alginate microcapsule appears promising with demonstration of biocompatibility and successful xenograft (dog to rat, dog to mouse) transplantation without immunosuppression. Increasing the source of insulin-secreting cells is being addressed by exploring the use of porcine islets, as well as the possibility of proliferating beta cells. STI

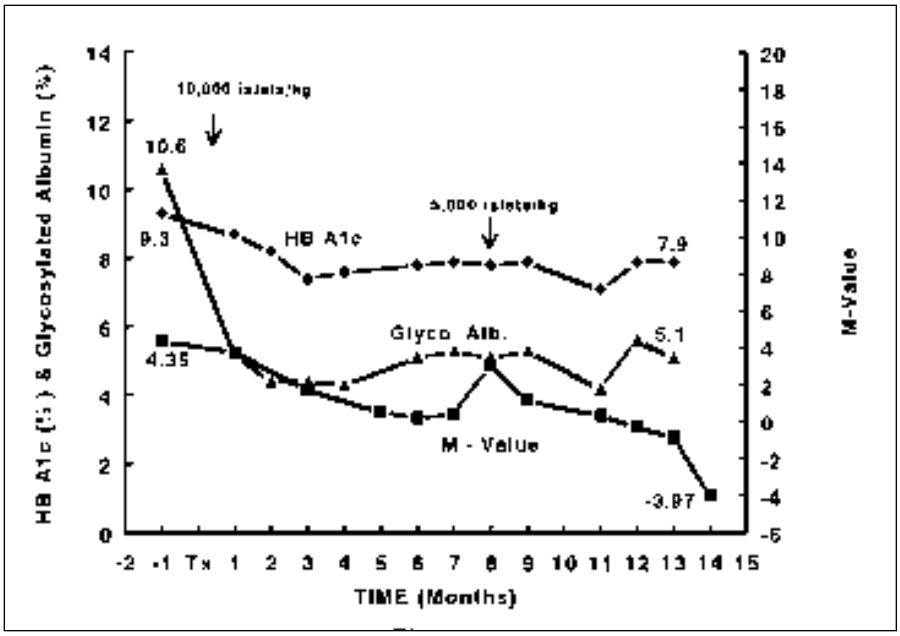

Figure 4. Glycemic parameters (HbA1c, glyco-albumin, M-value) all demonstrating significant improvements following transplantation.

REFERENCES

1. The Diabetes Control and Complication Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications is insulin-dependent diabetes mellitus. N Engl J of Med l993; 329: 977-986.
2. O'Shea GM, Goosen MFA, Sun AM. Prolonged survival of transplanted islets of langerhans encapsulated in a biocompatible membrane. Biochem Biophys Acta 1984; 804:133.
3. Darquy S, Reach G. Immuno-isolation of pancreatic B cells by microencapsulation. an in vitro study. Diabetologia 1985;28:776.
4. Sun AM, O'Shea GM, Goosen MFA. Injectable microencapsulated islets as a bioartificial endocrine pancreas. Appl Biochem Biotechnol 1984;10:87.
5. Sun AM, O'Shea GM. Microencapsulation 0ú living cells–a long term delivery system. J Controlled Release 1985;2:137.
6. Lim F, Sun AM. Microencapsulated islets as a bioartificial endocrine pancreas. Science 1980;210:908.
7. Prehn RT, Weaver JM, Algire GH. The diffusion-chamber technique applied to a study of the nature of homograft resistance. J Nat Cancer Inst 1954;15:509-17.
8. Albisser AM, Leibel BS, Ewart G, et al. An artificial endocrine pancreas. Diabetes 1974;23:389-396.
9. Strautz RL. Studies of hereditary-obese mice (obob) after implantation of pancreatic islets in Millipore filter capsules. Diabetologia 1970;6:306-312.

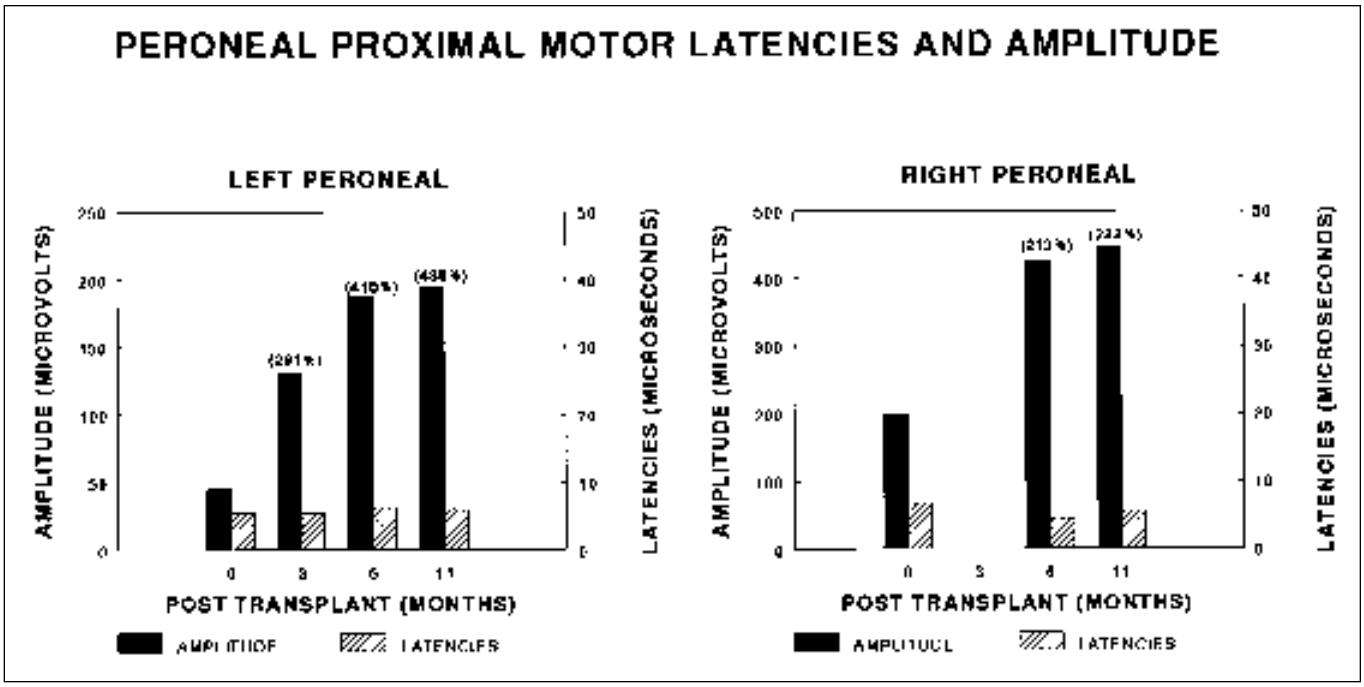

Figure 5. Nerve conduction studies demonstrating significant improvement of nerve function posttransplant.

10. Theodorou NA, Vrbova H, Tyhurst M, et al. An assessment of diffusion chambers for use in pancreatic islet cell transplantation. Transplantation 1979;27:350-352.
11. Gates RI, Lazarus NR. Reversal of streptozotocon-induced diabetes in rats by intraperitoneal implantation of encapsulated neonatal rabbit pancreatic tissue. Lancet 1977; 11:1257-59.
12. Woodward CL. Diabetes Care 1982; 5:278-281.
13. Archer J, Kaye R, Mutter G. Control of streptozotocin diabetes in Chinese hamsters by cultured mouse islet cells without immunosuppression: a preliminary report. J Surg Res 1980;28:77-85.
14. Altman JJ, Manoux A, Callard P, et al. Successful pancreatic xenoqrafts using semipermeable membranes. Artif Organs 1981;5 Suppl :776-779.
15. Tze WJ, Wong FC, Chen LM, et al. Implantable artificial endocrine pancreas unit used to restore normoglycemia in the diabetic rat. Nature 1976;264:466.
16. Chick WL, Perna J, Lauris W, et al. Artificial pancreas using living beta cells: effects on glucose homeostasis in diabetic rats. Science 1977;197:780-782.
17. Sun AM, Parisius W, Healy G, et al. The use, in diabetic rats and monkeys, of artificial capillary units containing cultured islets of Langerhans artificial endocrine pancreas. Diabetes 1977;26:1136-39.
18. Tze WJ, Tai EC, Davis HR. Studies with implantable artificial capillary units containing rat islets on diabetic dogs. Diabetologia 1980;19:541-45.
19. Reach G, Poussier P, Sausse A, et al. Immuno-isolation systems. Horm Metab Res 1982;12 (Suppl):177-79.
20. Reach G, Poussier P, Sausse A, et al. Immuno-isolation systems. Functional evaluation of a bioartificial pancreas using isolated islets perfused with blood ultrafiltrate. Diabetes 1981;30:296-301.
21. Sullivan SJ, Borland KM, Mahoney MD, et al. Biohybrid artificial pancreas: long term implantation studies in diabetic, pancreatectomized dogs. Science 1991; 252 :718.
22. Soon-Shiong P, Feldman E, Nelson R, et al. Successful reversal of spontaneous diabetes in dogs by intraperitoneal microencapsulated islets. Transplantation 1992; 54:769-774.
23. Soon-Shiong P, Feldman E, Nelson R, et al. Long-term reversal of diabetes by injection of immunoprotected islet cells. PNAS 1993;90:5843-5847.
24. Lanza RP, Heintz R, Merideth N, et al. Large-scale canine and human islet isolation using a physiological islet purification solution. Diabetes 1990;391:309A.
25. Soon-Shiong P, Heintz R, Merideth N, et al. Insulin independence in a Type I diabetic patient after encapsulated islet transplantation. Lancet 1994;343(April 16).