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# Transplantation of islets using microencapsulation: studies in diabetic rodents and dogs

**Abstract** Studies involving the transplantation of human islets in Type I diabetics 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 which must be overcome before this approach can be used in large numbers of patients. The currently limited supply of adult human pancreatic glands, and the fact that chronic immunosuppression is required to successfully transplant islets into patients, indicate that techniques must be further developed and refined for allo- and xenografting of isolated islets from human and animal sources to diabetic patients. An increasing body of evidence using microencapsulation techniques strongly suggests that this will be achieved during the next few years. Data from our laboratory in rodents and dogs indicate that these systems can function for extended periods of time. In one study, insulin independence was achieved in spontaneously diabetic dogs by islet microencapsulation inside uncoated alginate gel spheres ($M_r$ exclusion >600 kD). No synthetic materials or membrane coatings were employed in this study. Spheres containing canine islets were implanted into the peritoneum of 4 diabetic dogs. The animals received low-dose CsA (levels below readable limits by HPLC at 3 weeks). Implantation of these spheres completely supplanted exogenous insulin therapy in the dogs for 60 to >175 days. Blood glucose concentration averaged 122±4 mg/dl for these animals during the first 2 months. The glycosylated hemoglobin ($Hb_{AIC}$) levels during this period dropped from 6.7±0.5% to 4.2±0.2% ($P$<0.001). IVGTT K-values at 1 and 2 months postimplantation were 1.6±0.1 ($P$<0.002) and 1.9±0.1 ($P$<0.001), respectively compared with 0.71±0.3 before implantation. In a second group of studies, bovine islets were immobilized inside a new type of selectively permeable "microreactor" ($M_r$ exclusion <150 kD) and implanted into the peritoneum of 33 STZ-induced diabetic rats without any immunosuppression. Diabetes was promptly reversed, and normoglycemia maintained for periods of several weeks to months. Immunohistochemical staining of microreactors recovered from these animals revealed well-granulated β-cells consistent with functionally active insulin synthesis and secretion. To test further the secretory function of the islets, some of the explanted microreactors were incubated in media containing either basal or stimulatory concentrations of glucose. The islets responded with an approximately 3- to 5-fold average increase above basal insulin secretion. These results are encouraging, and may have important implications in assessing the potential role of these microencapsulation systems as therapy for human insulin-dependent diabetes.

**Key words** Islets of Langerhans · Diabetes · Encapsulation · Immunoisolation · Xenotransplantation

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## Introduction

Over the past few decades, a number of encapsulation systems have been studied in which transplanted cells can be separated from the immune system of the host by a selectively permeable barrier [1–6]. Low-molecular-weight substances such as nutrients, electrolytes, oxygen, bioactive secretory products, and cellular waste products can diffuse across the membrane while immunocytes and other immune effector mechanisms are excluded. Results in diabetic rodents and dogs indicate that these immunoisolation devices significantly improve glucose homeostasis and can retain islet function for more than a year with little or no immunosuppressive and/or antiinflammatory drug therapy [2]. However, problems such as fragility, limited surface area, and, in the case of vascular approaches, the surgery required for implantation or shunt connection limit the usefulness of these devices. Moreover, the placement of plastic materials in the peritoneum can lead to an interstitial acute and/or chronic inflammatory reaction and development of granulation tissue, intestinal adhesions, and abscess formation [7, 8]. Furthermore, it is uncertain whether these implants will require localization and removal. Surgical excision could also be necessary if the implants become fibroencapsulated. Consequently, we have investigated the use of two microencapsulation systems (one for allografts and one for xenografts) that are bi-

ocompatible, and that in the long-term, are resorbed and the products excreted. This report describes studies of canine and bovine islets encapsulated within these systems and implanted into the peritoneum of diabetic rats and dogs.

## Materials and methods

*Islet isolation, encapsulation, and implantation.* Islets were prepared from either adult mongrel dogs or bovine calves (0–2 weeks old) by a modification of the method of Warnock and Rajotte [9] as previously described by our laboratory [10]. Pancreatic tissue was dissociated using a collagenase digestion procedure and the islets separated from exocrine tissue on a discontinuous Ficoll density gradient. Isolated islets (>90% purity) were then cultured for 1 day either in M199/Earle's medium supplemented with 10% (vol/vol) fetal bovine serum, 20 Mm HEPES, 100 mg/dl glucose, and 400 IU/ml penicillin (canine islets), or in α-MEM plus 10% heat-inactivated horse serum (bovine islets) in a humidified atmosphere of 5% $CO_2$/95% air at 37°C. The canine islets were then encapsulated within uncoated alginate microreactors ($M_r$ exclusion of >600 kD) as previously described [11], whereas the bovine islets were encapsulated within microreactors fabricated using a permselective barrier system ($M_r$ exclusion of <150 kD) [12]. Both groups of microreactors measured approximately 800–1200 μm in diameter.

*Recipients.* Streptozotocin diabetic rats. Adult male Lewis rats (Charles River, Wilmington, MA) weighing 250 to 300 g were used as implant recipients. Diabetes was induced by a single injection of streptozotocin (38–42 mg/kg body weight) into the tail vein 10 to 14 days prior to implantation. Recipient animals were anesthetized with ketamine/xylazine (5.0–7.5 μl/g i.p.). Bovine islet containing microreactors were introduced into the peritoneal cavity through a 16 gauge catheter. Nonfasting plasma glucose concentrations were measured by tail bleedings using the glucose oxidase method (Beckman Glucose Analyzer 2); determinations were performed three times weekly for 30 days, and then weekly for the duration of each study. Failure of the implants was considered to have occurred when plasma glucose concentrations exceeded 200 mg/dl on two consecutive determinations.

Canines. Four spontaneously diabetic dogs with exogenous insulin requirements of less than 20 Units/day were used as implant recipients. All of the animals enrolled in the study were pets with clinical features similar to human type I diabetes. Surgeries were performed as previously described [12]. Beginning 2 days prior to implantation, each dog received cyclosporin (10 mg/kg/day PO) for 2 weeks; then 5 mg/kg/day PO. Fasting blood glucose concentrations, C-peptide levels, glycosylated hemoglobin ($Hb_{AIC}$) levels, and cyclosporin levels were measured periodically after implantation. Intravenous glucose tolerance tests (IVGTT) were performed before surgery and monthly after microreactor implantation. Fifty % (wt/vol) D-glucose (0.5 g/kg body weight) was infused i.v., and C-peptide levels and blood glucose concentrations were measured before and at 5, 10, 20, 30, 45, and 60 min after the glucose injection. K-values (decline in glucose levels, % per minute) were calculated according to standard methods [13].

*In vitro glucose-insulin kinetics.* In vitro perifusion was carried out to evaluate the function and kinetic performance of bovine-containing microreactors explanted from diabetic rats after 54 days. The microreactors were perifused with M199 containing glucose at concentrations of 50, 300, and 50 mg/dl for 20–60 min at each concentration as previously described [10]. The flow rate was 0.5 ml/min and the perifusate was collected with a microfraction collector (Gilson Model 203, Middleton, WI). The samples were frozen for subsequent insulin assay using a standard radioimmunoassay protocol.

*Histology.* The engrafted islet implants were fixed in Bouin's solution, dehydrated, and embedded in paraffin. Five micron sections were prepared and stained with hematoxylin/eosin and for the presence of insulin using immunoperoxidase techniques as previously reported from our laboratory [10].

*Statistical analysis.* Data are presented as mean ±SEM and compared using the unpaired Student's t-test or one-way analysis of variance (ANOVA). Differences were considered significant at $P<0.05$.

## Results

### Streptozotocin diabetic rats

Diabetes was reversed in the animals within 24 h after microreactor implantation, and normoglycemia maintained for periods of several weeks to months (Fig. 1). The implanted reactors were generally found intact and free "floating" in the peritoneal cavity. The external surfaces of the spheres were mostly free of fibrosis and host cell adherence. Viable insulin-containing β-cells were observed, consistent with functionally active hormone synthesis and secretion. In some of the animals, the viability of the explanted bovine islets was actually greater than that of control islets that were maintained in vitro for the duration of the study.

In addition to demonstrating long-term islet viability and function, in vitro studies were also carried out to test the function and kinetic performance of the microreactors.

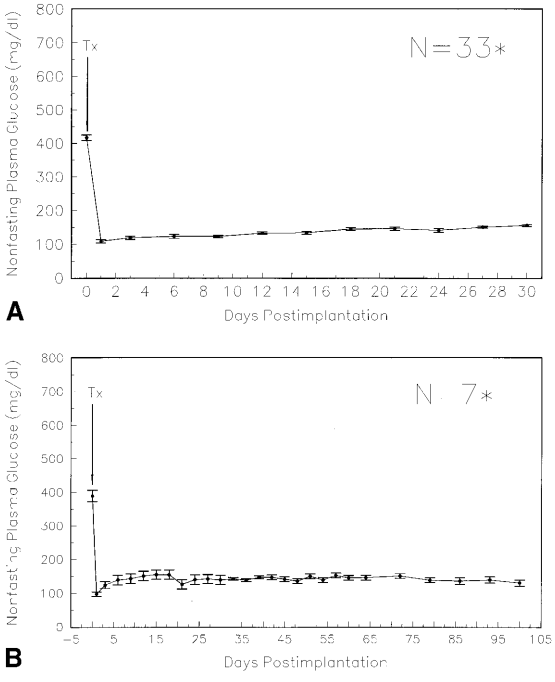


**Fig. 1A, B** Nonfasting plasma glucose levels (mean±SEM) in diabetic rats (**A**, *n*=33; **B**, *n*=7) that received intraperitoneal implants of encapsulated bovine islets. No immunosuppression was used in these studies. These data were extracted from a series of twelve experiments in which various variables were studied, including islet density, dosage and size and composition of microreactor

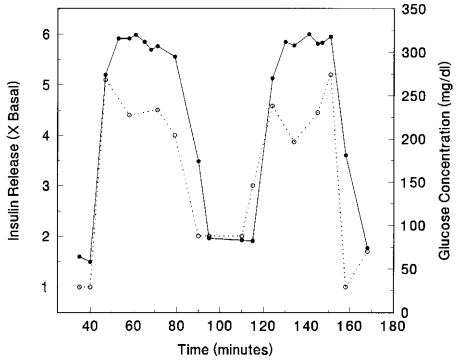


**Fig. 2** Insulin (o) secretory response to glucose (o) by perifused alginate-encapsulated bovine islets retrieved 54 days after implantation into the peritoneum of a rat. There was no delay before insulin concentration in the perfusate began to increase. These data suggest that uncoated calcium alginate beads respond rapidly to fluctuations in glucose concentrations

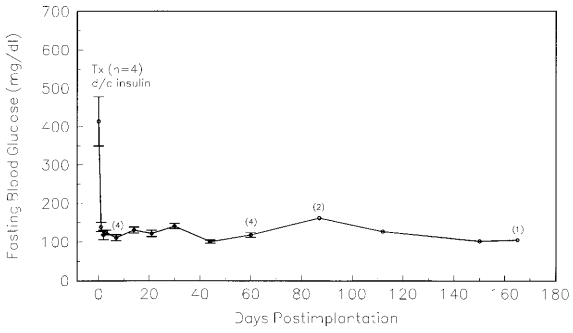


**Fig. 4** Fasting blood glucose concentrations (mean±SEM) in four spontaneously diabetic dogs that received intraperitoneal implants of alginate-encapsulated canine islets. The animals were administered subtherapeutic doses of CsA therapy

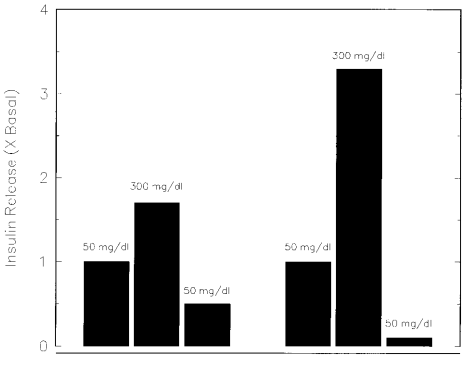


**Fig. 3** Secretory function of encapsulated bovine islets retrieved 6 weeks after implantation into the peritoneum of a rat. The microreactors were incubated in either basal (50 mg/dl) or stimulatory (300 mg/dl) glucose for 24 h

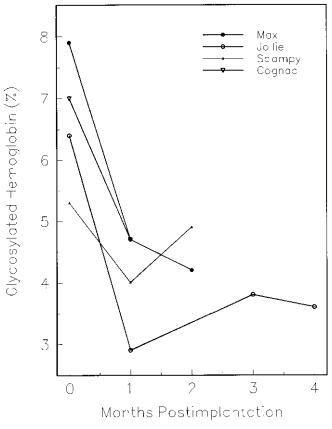


**Fig. 5** Glycosylated hemoglobin levels in four spontaneously diabetic dogs before and after microreactor implantation

The in vitro insulin secretory response of the encapsulated bovine islets retrieved from rats after 54 days is shown in Fig. 2. An approximately 4- to 5-fold increase from the basal insulin secretion was observed. The secretory response of the encapsulated islets was sustained for 1 h of glucose stimulation (300 mg/dl) and returned to basal levels after perifusion with the low-glucose solution (50 mg/dl). A second glucose challenge resulted in a similar insulin secretory response with virtually no delay before the insulin concentration in the perifusate began to increase. In addition to demonstrating islet viability and function nearly two months after xenoimplantation, these finding suggests that islets encapsulated by this procedure can respond in a physiological fashion to fluctuations in concentrations of glucose.

To test further the secretory function of the explanted microreactors, two implant recipients were sacrificed with functioning grafts six weeks after implantation. The explanted spheres were incubated in either basal [2.8 Mm (50 mg/dl) or stimulatory 16.8 Mm (300 mg/dl)] glucose for 24 h. In both experiments in which this test was performed, the islets responded with an approximately 1- to 3-fold increase above basal insulin secretion (Fig. 3).

## Spontaneously diabetic dogs

Implantation of alginate-encapsulated canine islets completely supplanted exogenous insulin therapy in the dogs for 60 to >175 days (Fig. 4). All of the dogs developed self-limiting hypoglycemia during the first 24 h postimplantation. However, no other adverse effects were observed. Blood glucose concentrations averaged 122±4 mg/dl for these animals during the first two months. The glycosylated hemoglobin ($Hb_{AIC}$) levels during this period dropped from 6.7±0.5% to 4.2±0.2% ($P$<0.001) (Fig. 5). IVGTT K-values at 1 and 2 months postimplantation were 1.6±0.1 ($P$<0.002) and 1.9±0.1 ($P$<0.001), respectively compared with 0.71±0.3 before implantation (Fig. 6A). Figure 6B shows the C-peptide response over the various time points of the IVGTT in one of the dogs before and one and two months after microreactor implantation. C-

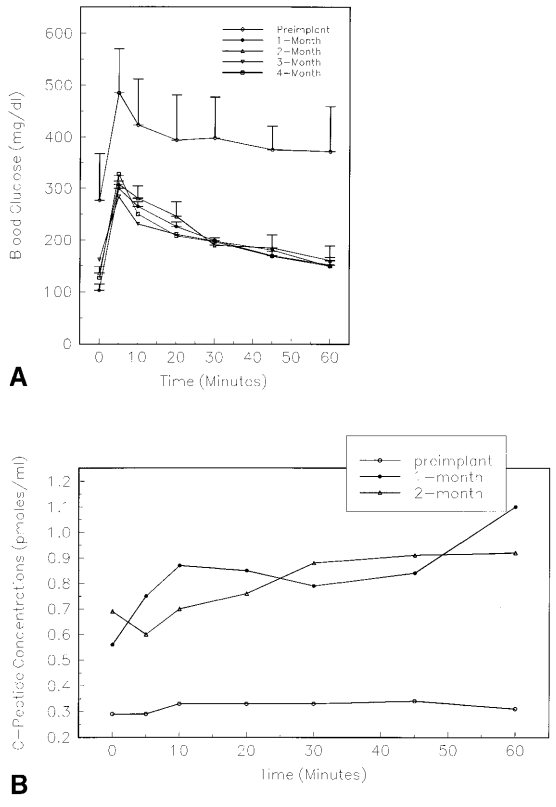


**Fig. 6** **A** Intravenous glucose tolerance test (IVGTT) K-values in four spontaneously diabetic dogs before and after microreactor implantation. **B** C-peptide response over the various time points of the IVGTT in a spontaneously diabetic dog before and after microreactor implantation

peptide levels were significantly elevated compared with preimplantation values.

## Discussion

The development of safe and effective techniques for immunoisolating islet tissue from humans and animals would markedly facilitate the treatment of diabetes on a wide scale. Over the past fifteen years, several methods for immunoisolating islets have been investigated. These include both intra- and extra-vascular devices which employ synthetic, selectively permeable membranes. Using the alginate-poly-L-lysine (PLL) technique adapted from Lim and Sun [14], several groups have shown prolonged survival of islet allografts and concordant (rodent-to-rodent, i.e. rat- or hamster-to-mouse) islet xenografts. Although prolongation of survival of discordant islet xenografts has also been achieved with the alginate-PLL technique, these results have usually been obtained in mice. However, the mouse is known to have puny immune system, and similar results have been obtained in diabetic mice using uncoated alginate microspheres, that is, using alginate droplets which have undergone gelation in $CaCl_2$, but which have not been coated with a synthetic PLL membrane [11].

In the present experiment, uncoated alginate spheres containing canine islets were also found to reverse diabetes in spontaneously diabetic dogs for 60 to more than 175 days. Although low-dose CsA was also administered, by 3 weeks postimplantation the levels of the drug were below the detectable limits of HPLC (i.e. <30 ng/ml). Soon-Shiong et al. [15] have also reported successful long-term implantations of microencapsulated allografts in larger animals. They treated spontaneous diabetes in dogs that were administered CsA. However, these microspheres were PLL-coated. The implants maintained euglycemia for 63 to 172 days, comparable to the results obtained in the present study without the use of a synthetic PLL membrane.

Naturally, a major goal of encapsulated islet transplantation is to eliminate the need for immunosuppression altogether. In the present study, a new type of encapsulation system was successfully tested that allows transplantation of islets across a wide species barrier without immunosuppression. These selectively permeable 'microreactors' are fabricated from biodegradable polymers which are slowly absorbed and excreted from the body. The microreactors can simply be injected into the peritoneum using a syringe and needle. Moreover, the rate of microreactor degradation can be adjusted to correspond to the functional longevity of the encapsulated islets. These microreactors were successfully tested using bovine islet xenografts in diabetic rats for periods of several weeks without any immunosuppression. These results, together with more recent data generated using porcine islets, indicate that long-term survival of discordant islet xenografts can be achieved in both rodents and dogs without immunosuppressive drugs using microreactors fabricated from biodegradable materials.

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