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Xenotransplantation of microencapsulated pancreatic islets contained in a vascular prosthesis: preliminary results

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Abstract. Porcine and human pancreatic islets were microencapsulated in an alginate-polylysine biomembrane and put in a chamber of a new vascular prosthesis composed of an inner tubing of Dacron mesh and an outer tubing of expanded polytetrafluorethylene material. The vascular prosthesis was anastomized between the iliac artery and the contralateral vein of diabetic dogs. The recipients did not receive any immunosuppressive therapy. Function of porcine and human islets was monitored by measuring serum glucose levels and human C-peptide concentrations. After transplantation, serum glucose levels were maintained at values lower than 200 mg/dl, and C-peptide concentrations were between 0.8 and 3.2 ng/ml. Injected insulin requirements decreased by 50%–60%. Four to 8 weeks after transplantation, histologic examination showed well-preserved and functioning islets in the majority of intact microcapsules. Fibrin and inflammatory cells were not observed in the chamber. These data suggest long-term survival and function of microencapsulated pancreatic islets in the vascular prosthesis.

Key words: Xenotransplantation, islet – Islet transplantation – Pancreatic islet, xenotransplantation

Transplantation of insulin-producing islets of Langerhans is an alternate form of therapy for patients with insulin-dependent diabetes mellitus since insulin therapy alone may not prevent the complications of diabetes [10]. The goal of islet transplantation is the early prevention of diabetic complications or, at least, the stabilization of those that are already present [17].

Major obstacles to clinical islet transplantation have included the difficulty of isolating a sufficient number of islets and the management of graft rejection [17]. The rejection of islets may be prevented by immunoisolation using the procedure developed by Lim and Sun [12]. This procedure involves microencapsulation, which utilizes an alginate-polylysine membrane that is permeable to glucose and insulin [11–14] but not to lymphocytes and immunoglobulins [11–14, 18]. In addition, an alginate-polylysine membrane protects the cells against the cytotoxic effect of anti-islet antibodies present in the sera of diabetic patients [7]. Although the microcapsules should be biocompatible, the occurrence of an inflammatory reaction around them has been described [15]. It has been hypothesized that the outer membrane of the microcapsules activates the alternate pathway of complement, which, in turn , may induce macrophages to release interleukin; this could promote fibrosis [6, 8]. Consequently, there is a need for an artificial nonthrombogenic device to hold the microcapsules, one which is also compatible with long-term islet survival and does not initiate a fibroblastic response or alter insulin and glucose transfer.

Materials and methods

Pancreatic islet isolation

Porcine (6–8-month-old) and human (15–27-year-old) pancreatic islets were isolated by appropriate enzymatic digestion, without any mechanical disruption of the pancreas, according to methods previously described [4, 5]. The collected tissue suspension, comprised of 90% islets and 10% nonendocrine cells, was purified by centrifugation against ficoll density gradients. The estimated purity of the islets was between 95% and 98%.

Pancreatic islet microencapsulation

After 24 h of culture, porcine and human pancreatic islets were enveloped in algin-polyaminoacid microcapsules according to the methods previously described [2, 3]. Each microcapsule was 700 micra in diameter and contained an average of four to six islets.

Vascular device

The vascular device was a double-walled prosthesis consisting of two tubings: an inner tubing made of knitted Dacron mesh (externally supported with polypropylene wire) and an outer tubing of ex-

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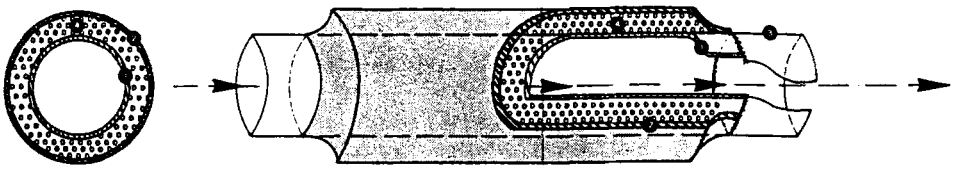


Fig. 1. The Dacron mesh *(1)* connected to the PTFE prostheses *(3)*, which are both anastomosed to the vessels. The circulating blood comes into contact with the inner surface of the Dacron mesh. The PTFE material *(2)* surrounds the Dacron mesh, thus forming a closed compartment or chamber that can be filled with microspheres *(4)*

panded polytetrafluoroethylene (PTFE) material (Fig. 1). The Dacron mesh was an open structure, 6 mm in diameter and 30 cm in length, with a porosity between 20 and 100 micra. The PTFE material was 10 mm in diameter and 30 cm in length and its extremities were attached to the outer surface of the Dacron mesh (Fig. 2). Consequently, there was a chamber between the Dacron mesh and the PTFE material that could be filled with microspheres (Fig. 1). The Dacron mesh extremities, connected to the PTFE prostheses (6 mm in diameter and 3 cm in length), were anastomose to the vessels (Figs. 1, 2).

Animal experiments

Mixed breed dogs of either sex, weighing 10–20 kg and aged 8–54 months, were used as recipients in the present study. Diabetes mellitus was chemically induced with alloxan (75 mg/kg) at least 30 days before the transplantation and each dog received a daily dose of 1 IU/kg insulin subcutaneously.

Preoperatively, 1 g cefatriaxone was administered IV. The dogs were anesthetized with an infusion of sodium pentobarbital (10 mg/kg) IV. Surgical procedures were performed under sterile conditions.

An iliac artery and its contralateral vein were exposed. The dogs received 70 IU/kg sodium heparin IV. After 2 min, the iliac vein was clamped using vascular clamps, and a continuous suture was placed between the vein and the prosthesis. When the anastomosis was completed, the clamp was released and the final segment of the prosthesis was clamped. The contralateral iliac artery was then clamped and anastomosed. Before the release of all clamps, the chamber of the prosthesis was filled with a concentration of microspheres (15 ml of microspheres per 10 cm of graft) using a 14-gauge cannula and a 50 ml syringe. After all of the clamps were released and an adequate hemostasis obtained, the incisions were closed in a routine manner.

Postoperatively, the dogs received insulin as necessary. Each dog was given 125 mg ticlopedine per os, daily, as a platelet antiaggregant agent for the duration of the study. During the 1st postoperative week, 1 mg cefatriaxone IV was administered. Each dog was examined with a duplex scanner twice a week.

Three experimental groups were examined. Group 1 consisted of seven dogs implanted with prostheses containing approximately 0.8 ml of porcine islets, group 2 was made up of three dogs implanted with prostheses containing 0.5 ml of human islets, and group 3 included five dogs implanted with prostheses containing empty microcapsules.

Histology

The implants were retrieved surgically and the vessels re-anastomosed. Histologic studies were performed 2, 15, 30, and 60 days after implantation.

Immediately prior to transplantation and after implant removal, the viability of the microencapsulated pancreatic islets was assessed by staining them with ethidium bromide and fluorescein diacetate under a fluorescence microscope.

The prostheses were fixed in 10% formaldehyde and subsequently stained with hematoxylin-eosin. The microencapsulated islets were stained for insulin with aldehyde fuchsin.

Metabolic studies

Fasting and postprandial serum glucose levels were determined daily in each group of dogs before and after transplantation (pre-Tx and post-Tx). In group 2, C-peptide concentrations were measured by radioimmunoassay (C-PEP-PR, CIS Bioindustries).

Statistical analysis

The statistical significance of the data was determined by Student's *t*-test. All values were expressed as a mean ± SEM of the number of observations indicated.

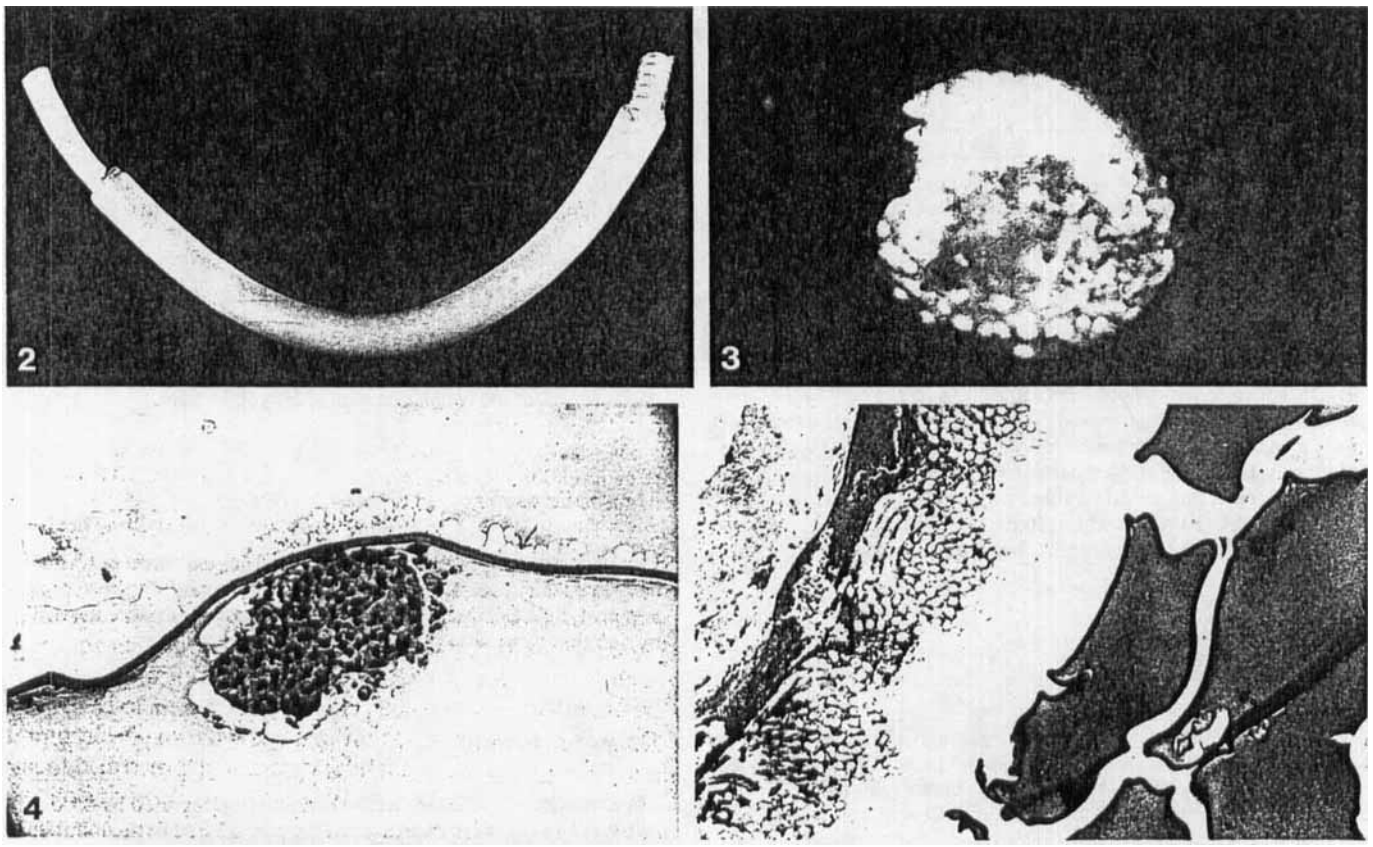
Results

Two dogs in group 1 presented infectious complications and were excluded from the study. The inner tubings (Dacron mesh) of the vascular devices were patent in all of the other dogs when the prostheses were removed for the histologic studies.

Histology

Histologic findings in groups 1 and 2 were identical. Microscopic studies revealed a chamber filled with a majority of intact microcapsules containing well-preserved islets.

The islets were shown to be viable by microscopic examination after being stained with ethidium bromide and fluorescein diacetate or with aldehyde fuchsin (Figs. 3, 4). No fibrin and/or inflammatory cells were observed in this space. The inner mesh showed traces of fibrin around the Dacron fibers (Fig. 5) and the prostheses retrieved at 60 days postimplantation showed a monolayer of endothelial-like cells on the inner surface. The outer mesh did not show any important changes, only collagen fibers around the PTFE and some neovascularization (Fig. 6). In group 3, there were no histologic differences between the prostheses filled with empty microcapsules and those filled with microencapsulated pancreatic islets.



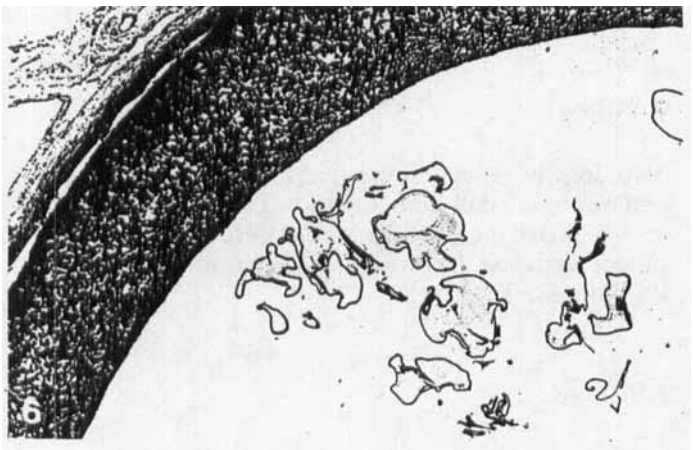


Fig. 2. The vascular device is a double-walled prosthesis consisting of two tubings: the inner tubing is a knitted Dacron mesh, externally supported, and the outer tubing is of PTFE material. The PTFE prostheses, connected to the Dacron mesh, are anastomosed to the vessels

Fig. 3. A pancreatic human islet, removed after 30 days from a dog in group 2, is shown to be viable after staining with ethidium bromide and fluorescein diacetate under a fluorescence microscope (× 400)

Fig. 4. A microencapsulated porcine islet removed after 60 days from a dog in group 1 stained positively for beta cell granuli. The microcapsule wall *(arrow)* is well-preserved and without fibrin on its surface (aldehyde fuchsin × 200)

Fig. 5. Inner mesh with traces of fibrin around the synthetic fibers. The chamber is filled with well-preserved microcapsules. No inflammatory cells and/or fibrin (H&E, × 400)

Fig. 6. PTFE mesh surrounded by collagen fibers with a small amount of neovascularization (H&E, × 200)

Metabolic studies

In group 1, transplantation of microencapsulated porcine islets reduced elevated, preoperative, fasting serum glucose levels to values lower than 200 mg/dl for periods of more than 8 weeks. In addition, the pronounced hyperglycemic response to the ingestion of a mixed meal (noted preoperatively) improved considerably at 3 and 6 days post-transplantation (Fig. 7). Insulin requirements decreased by about 50%–60%.

In group 2, pre-Tx C-peptide concentrations were undetectable both basally and in response to the ingestion of a mixed meal. After transplantation, C-peptide levels increased and were between 0.8 and 3.2 ng/ml, while serum glucose levels were maintained at less than 200 mg/dl for

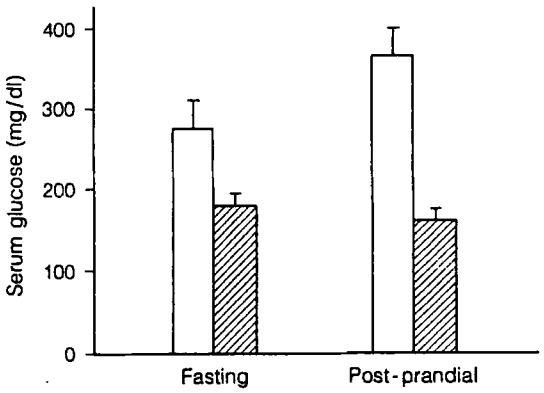


Fig. 7. Fasting and post-prandial serum glucose levels before (□) and after (▨) transplantation in group 1. $P < 0.01$

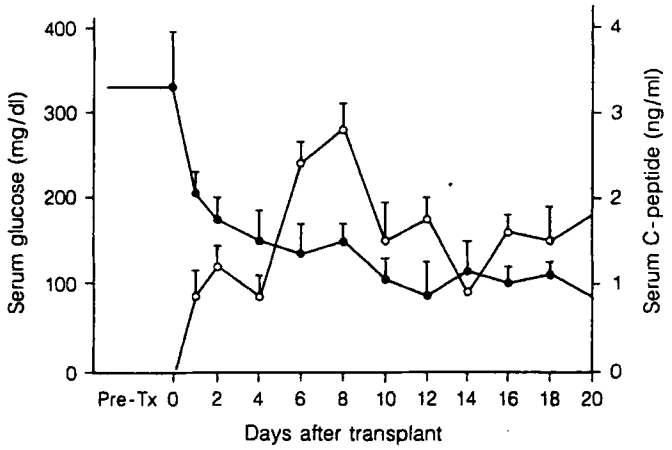


Fig. 8. Serum C-peptide (–O–) and serum glucose (——) in group 2 post-Tx. C-peptide peaks correspond to drops in serum glucose level

more than 2 weeks (Fig. 8). Insulin requirements decreased by about 60 %. One dog in group 2 did not receive insulin for 7 days and serum glucose levels were maintained at less than 180 mg/dl. The dogs in groups 1 and 2 showed an increase in serum glucose levels and pre-Tx insulin requirements after the vascular devices were retrieved.

In group 3, the implants of empty microcapsules did not produce any therapeutic effects in the recipients.

Discussion

Microencapsulated pancreatic islets have been associated with the long-term survival and function of the islet xenograft without any immunosuppression in the recipients [4, 5, 13, 14]. The applicability of this approach to a highly diabetic mammalian has been impeded by the lack of appropriate sites for the microcapsule implants [16]. To circumvent this obstacle, we developed a closed-loop insulin delivery system that consists of microencapsulated pancreatic islets inside an artificial vascular device. This device is a double-walled vascular prosthesis in which the blood of the recipient comes into contact with the inner surface of a Dacron mesh tubing, and in which microencapsulated islets are placed in a closed compartment between the outer surface of the Dacron mesh and a PTFE tubing. The porosity of the Dacron mesh and the absence of fibrin in the chamber allow the transfer of insulin and glucose from the blood to the microencapsulated islets. At the same time, the very low porosity of the PTFE material (the outer tubing of the vascular prosthesis) shelters the microencapsulated islets from any fibrotic reaction in the perigraft tissues.

The vascular device utilized in the present study can be easily placed in the body of large animals and permits direct blood perfusion of microencapsulated islets, which were viable and stained positively for beta-cell granuli at the time of graft retrieval. Moreover, the detection of C-peptide for 20 consecutive days in group 2 recipients proved that the human islet xenograft was responsive to glucose after implantation in diabetic dogs. The C-peptide assay utilized in the present study was specific for human C-peptide and did not crossreact with canine C-peptide.

In vitro perfusion studies (unpublished data) showed that the microencapsulated islets contained in the vascular prosthesis responded well to glucose challenge, producing insulin with normal biphasic release kinetics.

In vivo, the dogs in groups 1 and 2 showed a considerable improvement in pre-Tx hyperglycemia, both basally and after a mixed meal; injected insulin was temporarily withdrawn from a dog in group 2 and greatly reduced in all of the other animals. In the group 3 recipients, we did not observe a decrease in glycemia or insulin requirements, and in the dogs in groups 1 and 2, hyperglycemia and pre-Tx insulin requirements were evident when the vascular prostheses were removed.

These data seem to demonstrate that microencapsulated pancreatic islets contained in the prosthesis are capable of partially reversing the diabetic state. In fact, the amelioration of glucose homeostasis could not have been due to regenerated native beta cells since these regenerative phenomena usually occur during the first 6 days after the injection of alloxan and we transplanted the dogs after 30 days.

Unfortunately, altough the islets were vital and functioning, complete glucose homeostasis was not achieved because the tissue mass was not sufficient to assure complete and prolonged reversal of diabetes in the grafted dogs [1, 17]. Moreover, the insulin produced by the microencapsulated pancreatic islets contained in the vascular device had to pass through the systemic circulation before reaching the main target organ, the liver [9].

Clearly, a larger number of islets and further development of this vascular device will be necessary in order to obtain a more stable glucose homeostasis and long-term duration of islet function.

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