Immunosolation is a potentially important approach to transplanting islets without need for immunosuppressive drugs. Imunosolation systems have been conceived in which the transplanted tissue is separated from the immune system of the host by an artificial barrier. These systems offer a solution to the problem of human islet procurement by permitting use of islets isolated from animal pancreases. The devices used are referred to as biohybrid artificial organs because they combine synthetic, selectively permeable membranes that block immune rejection with living transplants. Three major types of biohybrid pancreas devices have been studied. These include devices anastomosed to the vascular system as AV shunts, diffusion chambers, and microcapsules. Results in diabetic rodents and dogs indicate that biohybrid pancreas devices significantly improve glucose homeostasis and can function for more than a year. Recent progress made with this approach is discussed, and some of the remaining problems that must be resolved to bring this technology to clinical reality are addressed. Diabetes 41:1503-10, 1992

Significant progress has been achieved in the area of islet cell transplantation (1). Development of improved islet isolation techniques has led to the initiation of a new phase of clinical trials that use isolated human islet allografts. Exogenous insulin independence after islet transplantation has been achieved in a small number of diabetic patients for varying periods of time with immunosuppression (2,3). Unfortunately, use of these agents is associated with a variety of problems (4,5). Interestingly, many of these immunosuppressive drugs, including Cs (6-9) and FK-506 (10,11), have deleterious effects on islet cell function.

Ultimately, the goal of islet transplantation is to treat patients without generalized immunosuppression and early enough in the course of the disease to prevent or retard the development of complications associated with the disease. Immunosolation of islets in biohybrid devices offers a distinct advantage in this respect. The islet tissue is isolated from the immune system of the host by a selectively permeable membrane. Low-molecular-weight substances, such as nutrients, electrolytes, oxygen, and bioactive secretory products, are exchanged across the membrane, while immunocytes and other transplant rejection effector mechanisms are excluded (12,13). This approach has the potential not only to allow allogeneic transplantation without immunosuppression, but also to allow the use of xenografts. In the form of a vascular implant, the islets can be distributed in a chamber surrounding the membrane, and the device implanted as a shunt in the vascular system (14,15). Alternatively, the islets can be encapsulated (that is, immunosolated) within diffusion chambers (16-19) or microcapsules (20-22) and placed intraperitoneally (16-18), subcutaneously (23,24), or in other sites (25) (Fig. 1).

DIFFUSION CHAMBERS AND MICROCAPSULES
Numerous biohybrid devices of this type have been evaluated during the last several decades. These include disk-shaped diffusion chambers (26-30), Millipore cellulosic membranes (26-28), hollow-fiber diffusion chambers, (19,31-33) and wider-bore tubular membrane chambers (16-18,34). Small diameter spherical chambers (microcapsules) also have been studied (20-22). Membrane materials used to fabricate these devices
with giant and pseudepithelioid cells that were observed only in the pigs. The reaction consisted of several layers (<50 μm in the peritoneum to >100 μm in the abdominal muscles) of fibroblasts and collagen with polymorphonuclear leukocytes, macrophages, histiocytes, and small lymphocytes. The fenestrated outer wall of the tubular membrane was always infiltrated by collagen, fibroblasts, and macrophages.

Recent reports from our laboratory (16–18,34) described a series of experiments that use wider-bore tubular membrane diffusion chambers of 1.7–4.8 mm i.d. These studies were carried out with membranes (XM-50, nominal cutoff 50,000–80,000 M,) that had a smooth outer skin. Porcine, bovine, and canine islets placed within these chambers restored normoglycemia in STZ-induced diabetic rats for >150 days without immunosuppression (17). Only minimal tissue reactivity was observed. The external membrane surfaces were generally free of fibrotic overgrowth and exhibited only occasional host cell adherence. Encapsulated canine xenografts implanted in spontaneously diabetic BB/Wor rats also had the same success, resulting in fasting normoglycemia for ≥1 mo in all of the animals (34). IVGTT K values (decline in glucose levels, %/min) after implantation in BB/Wor rats (at 10 days) and STZ-induced diabetic rats (1 mo) were 2.3 ± 0.4 and 2.6 ± 0.2 to 3.5 ± 0.3, respectively vs. 3.1 ± 0.1 and 3.3 ± 0.1 for normal control groups. In contrast, the K values for untreated diabetic rats were 0.6 ± 0.1 and 0.9 ± 0.1. Both light and electron microscopy of long-term functioning grafts revealed well-preserved islets, with hormone-producing α-, β-, and δ-cells.

Although these wider-bore XM membranes solved many of the problems associated with diffusion chambers (such as fibrosis, abscess formation, and adhesions) (42,43), studies in large animal models (closer to humans) likely will be required before clinical trials can be contemplated. Experiments in totally pancreatectomized, severely diabetic dogs are currently in progress in our laboratory. Preliminary results are encouraging (18) and indicate that canine islet implants can provide long-term correction of hyperglycemia without the use of immunosuppressive and/or anti-inflammatory drugs. Insulin independence has been achieved for >10 wk in dogs with preimplantation insulin requirements of ≥38 U/day. Little or no fibrosis has been observed for periods as long as 30 wk (Fig. 2).

In view of these encouraging results, unsolved issues that are crucial to the clinical success of these devices must be addressed: 1) membrane breakage, 2) further improvements in glycemic control, and 3) potential limitations imposed by the size and geometry of these chambers.

**Membrane breakage.** Most of the transplants described above ultimately failed because of membrane breakage. Under stress, the tubular chambers can bend, leading to fracture of the membrane walls and subsequent destruction of the encapsulated islet tissue. By 5–7 mo postimplantation, 80–90% of the membrane chambers in dogs had broken. In addition to loss of islet viability, an acute and/or chronic inflammatory response and development...
FIG. 2. Membrane chambers retrieved from the peritoneal cavity of a diabetic dog 215 days after implantation. Note the absence of fibroencapsulation. Under stress, however, these chambers can bend, leading to fracture of the membrane wall and subsequent destruction of the encapsulated islet tissue.

of granulation tissue was observed. Whether this peritoneal tissue reaction was caused by the broken membranes themselves or by the release of alginate or islet tissue from the ruptured chambers is not clear. The tubular membranes used in most of these studies had a wall thickness of only 69–105 μm. The chambers fabricated from these membranes were relatively fragile and susceptible to breakage. A modest increase in the membrane wall thickness or a decrease in the length of the chambers may resolve this problem. Results of theoretical modeling predict that, with rapid intrinsic islet secretion kinetics, satisfactory dynamic responses can be obtained with membrane thicknesses as great as 200 μm (38,44).

Blood glucose control. The motivation for islet transplantation is to provide physiological control of blood glucose concentration. In vitro and in vivo experiments with tubular chambers have shown only moderately delayed changes (lag time <10 min) in insulin secretion in response to changes in glucose concentration. Perfusion of encapsulated canine islets with glucose elicited an approximate fourfold average increase from the basal insulin secretion (16). A delay of only 7 ± 1 min occurred before the insulin concentration in the perfusate began to increase. This response is well within a time frame compatible with closed-loop insulin delivery; pharmacokinetic modeling of glucose homeostasis in humans suggests that the lag time of the increase in insulin delivery by an artificial pancreas must be <15 min to avoid the overexcursion of postprandial blood glucose (45).

In a recent set of experiments with dogs (18), wider-bore chambers were implanted into the peritoneum of 6 totally pancreatectomized dogs, and the animals were monitored for glycemic control by FBG and postprandial blood glucose measurements and by responses to both intravenous glucose (IVGTT, 0.5 g/kg) and oral glucose (OGTT, 1 g/kg). The dogs had varying degrees of reduced insulin requirements for control of FBG levels. Implantation of the chambers completely supplanted exogenous insulin therapy in 3 animals for 51 to >90 days (each of these implants continued to maintain blood glucose control for >20 wk) (Table 1). The mean ± SE FBG concentrations averaged 4.5 ± 0.3 mM (81 ± 6 mg/dl) for these 3 animals during the 1st month. This was lower than the average FBG levels before pancreatectomy, which were 5.0 ± 0.2 mM (91 ± 3 mg/dl). The reason for these lower levels is not clear at present.

The results of IVGTTs and OGTs are shown in Fig. 3. IVGTT K values at 1 and 2 mo postimplantation were 2.7 ± 0.4 and 2.0 ± 0.5 vs. 3.5 ± 0.5 before pancreatectomy. Although this rate of glucose decline is lower than normal, it is still well above the level considered diabetic (K < 1.0). By 3 mo, however, tolerance was grossly abnormal, and the animals remained significantly hyperglycemic throughout the test, with K values impaired at
TABLE 1
Insulin requirement after implantation of diffusion chambers containing islet allografts into diabetic dogs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Pretransplantation</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Function* (days)</th>
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</thead>
<tbody>
<tr>
<td>SI75</td>
<td>28</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>3</td>
<td>7</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>&gt; 215</td>
</tr>
<tr>
<td>SI81</td>
<td>42</td>
<td>6</td>
<td>13</td>
<td>10</td>
<td>13</td>
<td>9</td>
<td>17</td>
<td>8</td>
<td>15</td>
<td>20</td>
<td>35</td>
<td>R</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>SI84</td>
<td>38</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>145</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
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<td></td>
<td></td>
<td></td>
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<td>9</td>
</tr>
</tbody>
</table>

Values are means. R, membranes removed. * >10 U/day.

<1.0. A progressive deterioration of blood glucose control also was observed during OGTT at 2, 6, and 12 wk postimplantation, with maximal glucose levels of 11.6 ± 1 (208 ± 23), 15.0 ± 2 (267 ± 44), and 23.2 mM (414 mg/dl), respectively. This deterioration of response to oral and intravenous glucose stimulation might reflect membrane chamber breakage or inadequate secretory capacity, secondary to a loss of islet cell viability and/or insulin secretory function.

Diffusion limitations. Immunoisolated islets lack intimate vascular access and must be supplied with oxygen and nutrients by diffusion from the nearest blood vessels over distances greater than those normally encountered. In wider-bore membrane chambers, the problem of cell death or dysfunction as a result of oxygen supply limitations, or accumulation of wastes or other agents, is likely to be more severe. Our observations with 4.8-mm i.d. chambers is consistent with this. Chambers retrieved from the peritoneal cavity of dogs several months after allotransplantation contained a central necrotic core. Only a rim of islets remained viable within ~0.5 mm of the inner membrane wall. Similar results were obtained with canine islet implants into rats. These findings also may explain the surprisingly large number of islets required to achieve blood glucose control. Clearly, careful attention must be paid to the diffusion distances and transport properties of the membranes.

Microcapsules

Over the past decade, several methods for microencapsulating islets have been investigated. These include the alginate-poly (L-Lys) technique adapted from Lim and Sun (20), the chitosan-alginate system of Rha (46), the polyacrylate capsules of Sefton et al. (47), and the agarose gels of Iwata et al. (48). However, problems limit the usefulness of these procedures (22), and only the alginate-poly (L-Lys) system has resulted in long-term function in larger animals. The microencapsulation system, widely used to immobilize microbial cells for industrial applications (49,50), involves extruding a mixture of cells and sodium alginate with a droplet generation device into a CaCl₂ solution.

Using this procedure to entrap islets in calcium alginate hydrogels, Lim and Sun (20) then coated the negatively charged gelled droplets with positively charged poly (L-Lys). However, these capsules were unstable and produced an inflammatory response when implanted into the peritoneal cavity of animals (51,52). Modifications in the encapsulation procedure have improved the biocompatibility of the capsules, resulting in a dramatic increase in the duration of islet allograft function in diabetic rodents to >1 yr (53). Implanted concordant (rat-to-mouse) islet xenografts produced blood glucose control for a shorter period of time. Although prolonged survival
of canine islets also has been achieved with the alginate-poly technique, these studies have been performed in mice and usually have required adjunctive treatment with immunosuppressive agents (54–56). Weber et al. (57) found that alginate-poly (l-Lys) microcapsules containing $4 \times 10^3$ to $1.2 \times 10^4$ canine islets functioned for only 11.5 ± 3 days in diabetic NOD mice. With anti-CD4 monoclonal antibody treatment, however, long-term functional survival (function > 100 days) was observed for 4 of 8 recipients.

Recently, Soon-Shiong et al. (58) showed prolongation of discordant islet xenograft function in STZ-induced diabetic rats by microencapsulation. Encapsulated canine and human islets were implanted intraperitoneally and compared with nonencapsulated islet implants. Low dose Cs therapy was instituted in both groups. Euglycemia was maintained for 43–123 days (canine) and 42–136 days (human) after implantation of the encapsulated islets. In contrast, the nonencapsulated islets achieved euglycemia for <2 days. Although these experiments are encouraging, the need for immunosuppression could severely limit the usefulness of this technology because clinical application of islet transplantation techniques on a wide scale likely will require the use of discordant islet xenografts because of limited availability of human donor tissue.

Soon-Shiong et al. (59) and Lanza et al. (60) have reported the only successful, long-term implantations of microencapsulated allografts in larger animals. They treated spontaneous and surgically induced diabetes in dogs administered subtherapeutic doses of Cs therapy. In the former study (59), the microcapsules maintained euglycemia for 63–140 days. IVGTTs performed pre- and 2 wk postimplantation revealed $K$ values of 0.59 ± 5.1 and 3.0 ± 0.74, respectively. Despite average Cs blood levels >200 µg/L, 2 dogs that received nonencapsulated islets remained euglycemic for only 8–12 days. Calafiore (61) recently achieved insulin independence in 1 of 3 ALX-induced diabetic dogs and, transiently, in 1 of 2 patients without any pharmacological immunosuppression. However, the microcapsules in that study were deposited in artificial prostheses directly anastomosed to blood vessels. Whether microencapsulated islets can maintain function without immunosuppression in the long-term requires further assessment and study.

**DEVICES IMPLANTED AS AV SHUNTS**

Immunoprotective devices that are designed to be implanted into the vascular system of the recipient have particular advantages. For example, direct access to the arterial circulation is an optimal implantation site for delivery of oxygen to the transplanted tissue. In this regard, data suggest that insulin secretion is inhibited at low oxygen tensions (62,63). In addition, it should be possible to access the cell chamber for removal and replacement of nonfunctioning islets once a device is implanted. Early studies by Chick et al. (64,65) showed that isolated islets could be cultured in capillary fiber devices. In these experiments, tissue culture medium was circulated through the lumen of the fibers, and the islets secreted insulin in response to stimulatory glucose concentrations. However, the use of these small diameter fibers (i.d. <1 mm) as vascular implants was limited to short-term, ex vivo studies because of the problems associated with clotting and/or the need for systemic anticoagulation (66–72). Experiments in which tubular membranes with an i.d. of 2.7 mm were used resulted in
the first demonstration of in vivo longevity. These larger diameter fibers remained patent as AV shunts for 7 wk in dogs that received no systemic anticoagulation (73).

An artificial pancreas device that uses a single, coiled, tubular membrane with an i.d. of 5–6 mm and a wall thickness of 120–140 μm has been investigated by our group for the past several years (14,15). This device design incorporates the XM50 membrane within an acrylic housing. The islet chamber is created by the space between the membrane and the housing. The XM50 membrane cannot be sutured, so it is connected to standard PTFE graft material of the same diameter, which extends beyond the housing, and is used for anastomosis to the vascular system.

The first phase of our in vivo evaluation of this device was designed to determine the device's long-term patency as an AV shunt. Devices without islet tissue (unseeded devices) were implanted in normal dogs by anastomosis to the iliac artery and vein with standard surgical techniques. In the first group of 12 dogs that received unseeded devices, 8 were terminated within 2 mo because of a loss of bruit. Upon removal, these devices were found to be occluded and, in 2 cases, the membrane had ruptured. In all of the devices, the thrombosis appeared to have originated at the anastomotic sites, predominantly on the venous side of the graft. Three devices remained patent for significantly longer than the loss of bruit occurring at 5.5-, 9- and 18-mo postimplant. One of these devices was ongoing with device patency now >3 yr.

In the second series of unseeded device implants, some surgical modifications were incorporated and low-dose aspirin therapy (75 mg/day) was administered. Four devices from this group were removed because of loss of patency at 9, 16, 21, and 26 mo postimplant. One device was electively removed after 18 mo for evaluation. Histological examination confirmed that the graft was totally patent. More than 34 mo after device implant, 3 dogs remain and still are followed. These results suggest that the larger i.d. of this tubular membrane is associated with a dramatic improvement in the vascular compatibility of the device.

The in vivo function of this biohybrid artificial pancreas was evaluated by implanting devices seeded with canine islet allografts into pancreatectomized dogs with a protocol that had been shown to optimize long-term insulin secretion in devices maintained in vitro (14). The first study to determine in vivo function included 12 animals that each received a single device seeded with 260 ± 24 × 10³ allogeneic canine islets. Because the average diameter of these islets was 82 μm, this corresponds to ~42,000 islet equivalents (islet equivalents are used to quantify tissue mass by standardizing to islets of 150 μm in diameter). In 6 of these dogs, the daily dose of exogenous insulin required to control FBG concentrations was significantly reduced or eliminated. These results showed that islet function could be supported by the device for months in vivo. However, even when the implant resulted in fasting normoglycemia, the response to an oral or intravenous glycemic challenge was abnormal. In addition, implantation of a single device did not eliminate the requirement for exogenous insulin in animals requiring >20 U/day before implant. These observations suggested that the capacity of a single seeded device to secrete insulin was inadequate for the more severely diabetic dogs. Consequently, in the next series of in vivo experiments, each animal received two devices.

Included in this group were 17 dogs that received a total of 320 ± 16 × 10³ allogeneic canine islets (52,000 islet equivalents) in two devices. This corresponds quite closely to the tissue volume of 44,000 islet equivalents required in the allograft studies of Warnock and Raje (74). Of these animals, 3 died from surgical complications shortly after implantation of the devices. After implantation, 3 dogs continued to require exogenous insulin. This lack of function was correlated with a device defect or thrombosis in 2 cases while both devices were patent in the 3rd animal.

Data from the remaining 11 dogs showed that implantation of two devices was able to replace exogenous insulin therapy in each of these animals for periods ranging from 1 mo to >8 mo. Most dogs in this group had a preimplant insulin requirement of >30 U/day, which demonstrates that islets implanted in the biohybrid artificial pancreas can be used to treat severe diabetes. Seven of these implants replaced exogenous insulin for an average of 170 ± 27 days. Histological evaluation of these devices after removal indicated a substantial loss of islet mass despite patent devices. No evidence of infiltration of immune cells was observed, suggesting that the membrane was indeed immunoprotective. However, the possible effects of low molecular weight humoral factors on islet viability could not be excluded. In 2 animals, the devices were removed 1 yr after implantation. In both cases, the exogenous insulin required to control FBG concentration increased by >20 U/day after device removal. In addition, ~25% of the islets remained viable after the year in vivo in the vascular device.

Three of the implants were terminated after ≤2 mo because of health complications associated with infection that appeared to originate at the vascular anastomotic site. The risk of developing infection as a result of surgery seems to be higher for the diabetic dogs because infection has not been a factor in the study of unseeded devices in normal dogs. In 1 of the dogs in this study, both devices, upon removal, were found to be occluded by a well-organized thrombus, and neither contained any surviving endocrine tissue.

Despite the observation that these allogeneic islet implants could regulate FBG levels, the response to glycemic challenge remained impaired. Clearance of glucose after oral or intravenous administration was somewhat improved compared with the preimplant diabetic animals, but was not normal. The results from islet transplantation studies, in general, have indicated that normoglycemia is difficult to attain. Ongoing studies with the biohybrid pancreas devices are designed to facilitate transplantation of additional tissue, which should improve the level of glycemic control. Our data from the implantation of devices containing xenogeneic islets are limited, but do indicate that discordant xenografts also should be
feasible. One dog that received devices containing bovine islets maintained excellent control of FBG levels for almost 2 mo without exogenous insulin. The results with porcine islets are even more preliminary, but, in 1 dog, devices containing porcine islets decreased the exogenous insulin requirement from >24 U/day to <10 U/day for ~3 mo.

These experiments indicate that the perfused biohybrid artificial pancreas can be used to treat diabetes in a large animal model. Incorporation of a single, large-diameter tubular membrane into the device design has led to long-term patency as an AV shunt. Furthermore, FBG concentrations could be controlled in pancreatectomized dogs for as long as 8 mo in the absence of exogenous insulin, and islet function has been maintained for up to 1 yr in 2 animals (Fig. 4). However, several key issues remain to be resolved and may be critical to evaluation of the therapeutic potential of this approach.

First, the underlying causes for the eventual loss of islet function need to be elucidated. Identification of contributing factors may lead to methods for extended longevity of these implanted islets. Nonetheless, it is likely that the islets will require replacement in the implanted device. Protocols for reseeding in a minimally invasive manner are currently being developed. In addition, the xenograft studies must be expanded to determine if longevity of graft function is comparable to allogeneic islets. The studies to date indicate that the immunoprotective membrane does prevent immunocyte infiltration and tissue rejection without immunosuppressive therapy. The potential effect of humoral factors are still uncertain—but are particularly relevant for xenografts.

Finally, the goal of islet transplantation is to approximate more closely physiological glucose regulation. Clearly, the glycemic control provided by the current device design is not optimal. Further optimization of device design and islet seeding protocols should facilitate transplantation of more islet tissue, thereby increasing insulin secretion. Once this has been achieved, it should be possible to determine whether normal responses to glycemic stress can be achieved under varying conditions. Despite the challenges remaining, results from recent experiments that use the pancreatectomized dog model clearly indicate that this type of perfused vascular device is a promising approach for the treatment of IDDM.

CONCLUSION

There is considerable excitement among clinicians who hope to see the introduction of a biohybrid pancreas to replace conventional insulin therapy. The transplantation of immunoisolated islets recently has been extended from rodents to larger animals. Studies have shown the ability of these devices to retain islet function for >1 yr in diabetic, totally pancreatectomized dogs. Although several unresolved issues critical to the clinical success of these devices remain, these problems should be soluble. For the patient with IDDM, the next few years hold promise of disease treatment by islet transplantation with immunosolation.

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