

Smad7 in inflammatory cells can dampen their responses to TGF- β produced at the site of chronic inflammation. Thus, Smad7 could be a key molecule for the mechanisms of chronic inflammation in these disorders, and downregulation of Smad7 expression (e.g. by the use of antisense Smad7 oligonucleotides) might restore normal control of TGF- β signaling in inflammatory cells and be beneficial for the treatment of chronic inflammatory disorders associated with high TGF- β production and its resistance.

The observed reduced expression of Smad7 in scleroderma should encourage us to analyze the transcriptional regulation of the promoter region of the Smad7 gene in more detail; for example, by searching for single nucleotide polymorphisms (SNPs) that might affect Smad7 transcription in patients with scleroderma. Such studies might also provide clues as to why certain population are susceptible to tissue fibrosis or scar formation after skin injury. In addition, exploring mechanisms of Smad7 degradation should be another important area of investigation for scleroderma study.

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Techniques & Applications

Encapsulation of pancreatic islets for transplantation in diabetes: the untouchable islets

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The aim of encapsulation of pancreatic islets is to transplant in the absence of immunosuppression. It is based on the principle that transplanted tissue is protected from the host immune system by an artificial membrane. Encapsulation allows for application of insulin-secreting cells of animal or other surrogate sources, to overcome human islet shortage. The advantages and pitfalls of the approaches developed so far are discussed and compared, together with some recent progress, in view of applicability in clinical islet transplantation.

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Recent successes [1] have increased the optimism and interest in clinical application of pancreatic islet transplantation in type 1 diabetes on a large scale. Unfortunately, present approaches involve the use of high-dose and strict immunosuppressive protocols, which is associated with serious side-effects. Therefore, it is still doubtful whether clinical islet transplantation in combination with immunosuppression will ever be a sound alternative to insulin therapy for

the majority of diabetic patients [2]. By encapsulation (i.e. immunoisolation) of the islets, chronic administration of immunosuppressants can be eliminated, as the hostile host-immune system cannot reach the physically protected pancreatic islet cells.

Immunoisolation is based on the principle that transplanted cells are separated from the host immune system by a biocompatible, semipermeable membrane [2,3]. The membrane does not allow the entry of inflammatory cells and large molecules such as antibodies,

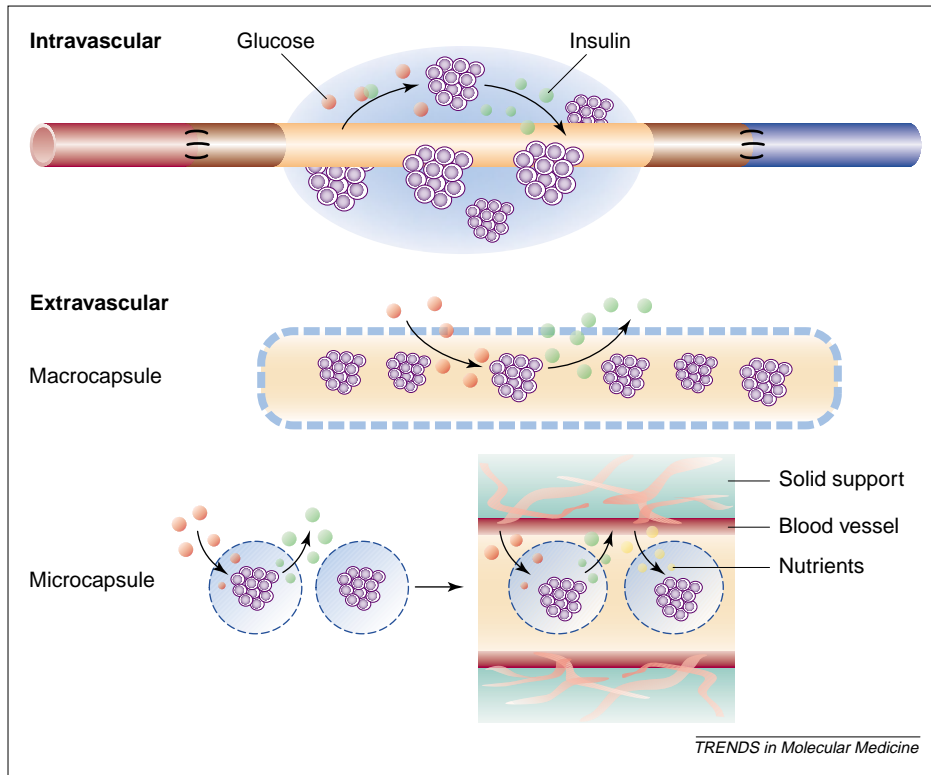


Fig. 1. Immunisolating devices. In the intravascular device, islets can be distributed in a chamber surrounding a selectively permeable membrane. The device is implanted as a shunt in the vascular system. In the extravascular approach, islets are immunisolated within membrane-diffusion chambers or enveloped in microcapsules and implanted, without direct vascular connection in the peritoneal cavity or subcutaneous site. A recent development is that microcapsules are implanted in a prevascularized solid-support system where exchange of glucose and insulin is optimal and nutrition of the islets is improved. This is beneficial for both the function and longevity of the microencapsulated islets. Also, the site allows for retrieval of the graft.

but it does allow the exchange of low-molecular-weight substances such as nutrients, hormones, and therapeutic agents. Most attention in the area of immunoisolation has focused on encapsulation of pancreatic islets for transplantation into diabetic patients, mainly because the technology solves two major obstacles in clinical application of pancreatic islet transplantation: (1) immunoisolation allows for transplantation of islets in the absence of immunosuppression, and (2) it permits grafting of xenogenic islets, and therefore overcomes the logistical problems associated with limited supply of human pancreases [1].

Immunoisolation approaches

Immunisolating devices can be categorized into three types: intravascular macrocapsules, which are connected as a shunt to the systemic circulation; extravascular macrocapsules, which are transplanted subcutaneously or intraperitoneally; and extravascular microcapsules containing individual

islets, which are mostly transplanted in the peritoneal cavity (Fig. 1, Table 1).

The intravascular devices provide the advantage that the encapsulated islets are in close contact with the blood stream, which allows for fast exchange of glucose and insulin and, therefore, fast correction of changes in blood-glucose levels. Unfortunately, this type of device, as any other type of vascular prostheses, carries serious threats, such as thrombosis, either primary or secondary to intimal hyperplasia at the venous anastomosis, or infections. Furthermore, animal studies have shown that to prevent blood clotting and cell adhesion in the device, it is

required to use both large lumen and high flow-rates through the device, in the presence of anti-coagulation therapy. Obviously, this is not without risk for the patient. The drawbacks of the present generation of devices makes it doubtful that intravascular devices can serve in diabetic patients as a sound alternative for insulin therapy.

Risks for the recipients are much lower with extravascular devices, which can be implanted with minimal surgery, and can be readily retrieved in case of complications. Initial experiments with extravascular macrocapsules in which groups of islets were enveloped in one or several large capsules were not very successful. This was mainly because of aggregation of the islets into large clumps, which caused necrosis in the center of the clumps as a consequence of diffusion limitations for nutrients [4]. This problem was solved by permanent solitude immobilization of the islets in a matrix before final encapsulation in the macrocapsules. Encouraging results have been subsequently observed in experimental animals, with survival rates of islets in the devices up to 200 days after implantation in the peritoneal cavity of rats [5]. Also, some degree of success has been shown after subcutaneous implantation in humans [6].

A drawback of macrocapsules is the relative large surface-to-volume ratio of the devices, which interferes with optimal diffusion of nutrients. To guarantee adequate feeding of the cells, islet density of the macrocapsules is kept quite low and never exceeds 5–10% of the volume fraction. As a consequence, large devices have to be implanted to provide sufficient masses of insulin-producing islets. These large graft volumes are impractical and cannot be implanted in conventional sites for transplantation of islets such as the liver, kidney capsule, or spleen [7]. Even the relatively large space in the peritoneal cavity does not suffice the large volume

Table 1. Advantages and limitations of different immunoisolation approaches

System	Advantages	Limitations
Intravascular macrocapsule	Close contact with bloodstream	Thrombosis Infections Major implantation surgery
Extravascular macrocapsule	Minor implantation surgery Device can be retrieved	Large surface-to-volume ratio Low seeding density of cells
Extravascular microcapsule	Minor implantation surgery Optimal surface-to-volume ratio Implantation in several sites	Transplantation near blood vessels required for optimal longevity and function

required for long-term function of an islet graft in macrocapsules. Also, the relatively large surface-to-volume ratio of the macrocapsules interferes with adequate regulation of the glucose levels, as exchange of glucose and insulin occurs rather slowly.

Present insights point towards microcapsules as the preferable system for transplantation in diabetes as they offer an optimal volume-to-surface ratio for fast exchange of hormones and nutrients. Also, microcapsules, in contrast to macrocapsules, can be transplanted in sites where they are in close contact with the blood stream, which is beneficial for their performance as well as the longevity of the islets in the capsules. To facilitate the contact between microencapsulated islets and the bloodstream, we have recently designed a solid-support system of expanded polytetrafluoroethylene (PTFE) for transplantation of encapsulated islets. This site becomes highly vascularized after implantation in the peritoneal cavity [8] and is readily accessible following only minor surgery. As a consequence the capsule's graft can be repeatedly replaced in case of failure of the islet tissue (Fig. 1).

Novel insight in factors determining the success of encapsulated islet grafts
Microencapsulated islets have shown some success in a clinical trial [9] and in preclinical studies in large mammals [10,11]. These studies show survival of microencapsulated islet grafts *in vivo* for periods varying between one and 12 months. Although this illustrates the clinical applicability of microencapsulated islets, it also demonstrates a common and pertinent problem, as graft survival was always temporary and never permanent. A major cause for this limited survival is the host reaction against the encapsulated islet grafts. In general, we can distinguish the host reaction towards encapsulated islets into two types. The first and most dramatic is the inflammatory reaction against the materials for preparation of the capsules [3,12]. This bioincompatibility results in a host reaction that interferes with adequate nutrition of the encapsulated cells, and necrosis of the tissue occurs as a consequence [12]. With the present technology these reactions can be successfully prevented by applying purification steps to the materials to be used [12].

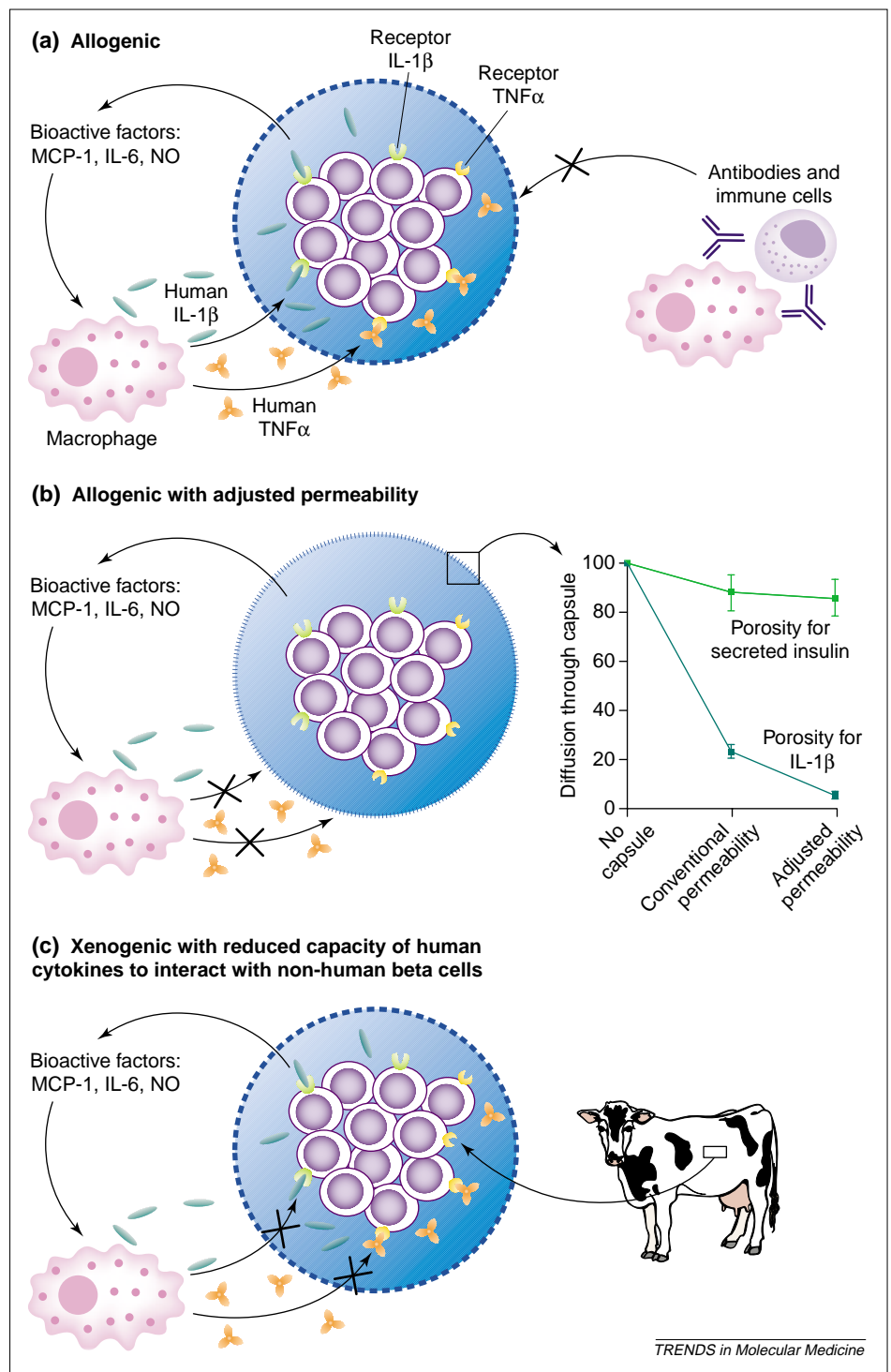


Fig. 2. Options to overcome deleterious effects of diffusion of cytotoxic cytokines and chemokines into the immunisolating capsules after transplantation. (a) Islets produce bioactive factors that diffuse out of the capsules and activate inflammatory cells such as macrophages in the vicinity of the microencapsulated islets. The cytokines secreted by the macrophages diffuse into the capsules and induce massive cell death in the allogenic human islet cells. (b) By adjusting the porosity of the capsules for interleukin (IL)-1 β and for secreted insulin, permeability can be lowered so that cytokines cannot pass the membrane, whereas the porosity of the capsules for insulin remains unaffected. (c) In case of xenotransplantation, we found that cytokines of human origin are less deleterious for islets of animal origin such as bovine islets, possibly as a result of reduced interaction at the receptor level. Abbreviations: NO, nitric oxide; MCP-1, macrophage-colony-promoting factor 1; TNF α , tumor necrosis factor α .

The second type of host reaction has only recently been identified, and is the host response against the allogenic or xenogenic cell-derived bioactive factors or

antigens that leak out of the capsules. This reaction is less acute and more difficult to prevent. It results in overgrowth by macrophages and lymphocytes on a small

portion (~10%) of the capsules [13] and in a humoral immune response against the encapsulated tissue [14]. Recently, we have found that this reaction is reinforced by the islets in the capsules. Islets are capable of producing bioactive factors such as macrophage-colony-promoting factor 1 (MCP-1), interleukin (IL)-6, and nitric oxide (NO) [15], which activate inflammatory cells (such as tissue macrophages) in the vicinity or macrophages on the overgrown capsules. This activation of inflammatory cells results in the production of cytokines, which are deleterious not only for the islet cells in the overgrown capsules but also for the islets in the vast majority of transplanted, clean, and non-overgrown capsules (Fig. 2a).

As conventional immunisolating capsules cannot prevent the entry of most cytokines and chemokines [16], the clinical application of immunoisolation is still a matter of some dispute. Recently, as a result of combined efforts from our groups, we found that cytokines released by the host immune system might not represent an insurmountable weakness for clinical application of immunoisolated islet transplantation (Fig. 2). We found that by decreasing the conventional permeability of the membrane, we could reduce the porosity of the membrane for IL-1 β (i.e. a small cytotoxic cytokine) from 26% to <4% (Fig. 2b), whereas 90% of the secreted insulin could still readily diffuse out of the capsules [17]. This reduction in permeability can be done with standard laboratory equipment such as an increase in polymer concentration and, by that, the islets are fully protected against the attacks from the host immune system.

Also, we found evidence that cytokines might not interfere with islet function in case of xenografting of encapsulated islets in humans. We have observed that following exposure to a combination of human cytotoxic cytokines of human origin, a marked decrease in functional survival and a high percentage of apoptotic cells could be found in human islets but not in bovine islets [18]. Preliminary data show that this is due, at least in part, to the fact that xenogenic islet cells are less capable of binding and taking up human cytokines. This implies that, at least in some combinations, even when capsules are applied that are permeable for cytokines, the function and

survival of xenogenic islet-sources will be less affected (Fig. 2c). Obviously, selection of appropriate virus-free xenogenic islet sources and testing of the susceptibility of these sources for human cytokines is required.

Concluding remarks

In the past decade, it has become increasingly clear that problems associated with immunoisolation of pancreatic islets can be overcome by a step-wise approach of the questions involved. Now that immunoisolation has become a reproducible procedure in small mammals, it is mandatory to scale-up the procedure and to address potential obstacles for clinical application. This includes human biocompatibility testing and adjustments of the system to the human diabetic immune system. However, recent insights and technological advances in immunoisolation, in combination with the possibility of yielding islets from animal pancreases [19] or the creation of islets by stem-cell technology [20], justifies some optimism about application of grafting immunoisolated insulin-secreting cells to diabetic patients.

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