

Reviews

Considerations for successful transplantation of encapsulated pancreatic islets

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Abstract

Encapsulation of pancreatic islets allows for transplantation in the absence of immunosuppression. The technology is based on the principle that transplanted tissue is protected for the host immune system by an artificial membrane. Encapsulation offers a solution to the shortage of donors in clinical islet transplantation because it allows animal islets or insulin-producing cells engineered from stem cells to be used. During the past two decades three major approaches to encapsulation have been studied. These include intravascular macrocapsules, which are anastomosed to the vascular system as AV shunt; extravascular macrocapsules, which are mostly diffusion chambers transplanted at different sites; and extravascular microcapsules transplanted in the peritoneal cavity. The advantages and pitfalls of these three approaches are discussed and compared in the light of their applicability to clinical islet transplantation. All systems

have been shown to be successful in preclinical studies but not all approaches meet the technical or physiological requirements for application in human beings. The extravascular approach has advantages over the intravascular because since it is associated with less complications such as thrombosis and infection. Microcapsules, due to their spatial characteristics, have a better diffusion capacity than macrocapsules. Recent progress in biocompatibility of microcapsules has brought this technology close to clinical application. Critical issues such as limitations in the functional performance and survival are being discussed. The latest results show that both issues can be solved by the transplantation of microencapsulated islets close to blood vessels in prevascularized solid supports. [Diabetologia (2002) 45: 159–173]

Keywords Transplantation, encapsulated pancreatic islets.

A major challenge in the treatment of Type I (insulin-dependent) diabetes mellitus is the prevention of late complications, and thereby improvement of the quality of patient life. There is no dispute that euglycaemia

is of essential importance to reach that goal. Euglycaemia can be achieved by insulin treatment and the results of the Diabetes control and complications trial clearly show that insulin treatment delays the onset and reduces the progression of diabetic complications [1]. Intensified treatment, however, is not a simple chore because it requires multiple daily injections, frequent monitoring, dosage adaptations and, thus, patient compliance. It is also associated with frequent episodes of severe hypoglycaemia and with glycaemic unawareness. A different approach to euglycaemia is to provide the diabetic patient with an endogenous rather than an exogenous source of insulin by the transplantation of the endocrine pancreatic tissue. Pancreatic organ transplantation is now an es-

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Abbreviations: a-FGF Acidic-fibroblast growth factors; ePTFE expanded polytetrafluoroethylene; G, guluronic acid; HEMA, 2-hydroxyethyl methacrylate; M, mannuronic acid; PAN-PVC, poly acrylonitrile and polyvinylchloride copolymer; PLL, poly-L-Lysine

established mode of treatment with 15000 cases worldwide [2, 3]. Results have substantially improved during the past two decades and the current patient and graft survival rates almost equal those of kidney transplantation. A successful pancreas transplant provides almost normal glucose homeostasis but it requires lifelong immunosuppressive medication. It is still not clear whether the advantages of a pancreas transplant over continued insulin treatment outweighs the disadvantage of obligatory immunosuppression. Most transplant centres still restrict themselves to combined pancreas and kidney transplantation in diabetic patients with end-stage renal failure [2, 4]. Islet transplantation, in contrast to pancreas transplantation, requires no major surgery. Moreover, successful islet transplantation without immunosuppression might be achieved by relatively simple methods as immunoisolation by encapsulation. Such an approach utilizes a semipermeable membrane which forms a mechanical barrier separating the graft from the host antibodies and immune cells but allows for the diffusion of glucose, insulin, nutrients and islet waste products.

Macroencapsulation of pancreatic islets

The two major encapsulation systems are macroencapsulation and microencapsulation. In the first one, the macrodevices contain many islets in one immunoisolating membrane. The intravascular device is usually composed of a microporous tube with blood flow through its lumen and with a housing on its outside containing the implanted tissue [5, 6]. The device is implanted into the vessels of the host by vascular anastomoses. The concept of extravascular devices does not require vascular anastomoses because it is based on the principle of diffusion chambers [7]. The geometry could be planar in the form of a flat, circular double layer or tube-like as a so-called hollow fibre [7].

The most intensively studied intravascular device is the modified diffusion chamber of Chick et al [8]. It is technically advanced and has been tested extensively in small [9] as well as in large animals [9, 10]. The original device was composed of a number of small diameter artificial capillaries contained by one large diameter tube. The artificial capillaries were composed of polyacrylonitrile and polyvinylchloride copolymer (PAN-PVC) ultrafiltration capillaries [11], and the remaining lumen of the large diameter tube, i.e. the outside of the artificial capillaries, was loaded with hormone producing cells. The design allows close contact between the islets and blood which are separated only by the microporous walls of the capillaries. These devices were found to induce normoglycaemia in diabetic rats [9], dogs [10] and monkeys [9] but required systemic anticoagulation. The

duration of this normoglycaemia was usually restricted to several hours and successes of a somewhat longer duration were exceptional. Clotting of the blood in the lumen of these small diameter artificial capillaries proved to be a major obstacle, in spite of anticoagulant medication in massive doses. This thrombus formation was an early sign of insufficient biocompatibility and has led to the use of tubular membranes with larger diameters in the hope of minimising or eliminating clot formation in the absence of systemic anticoagulation. The present device is composed of a single, coiled and tubular membrane with an internal diameter of 5–6 mm. The membrane has been modified but is still composed of PAN-PVC with a nominal molecular weight cutoff of 50 kD. This approach was found to be rather successful because these devices implanted as high flow arteriovenous fistulas could remain patent for periods of 7 weeks in the absence of systemic anticoagulant therapy [12]. This success can be explained in part by the high flow rates through the device which prevents adhesion of cells to the membranes or collection of those cells in the immediate vicinity [13]. However, high flow arteriovenous fistulas are not without risk and much longer patency rates are required for effective applicability. Obviously, more thromboresistant materials are required for this type of device.

Although the intravascular devices have shown some degree of success, the problems mentioned above should be solved if clinical application is considered. Even then, the complications associated with any type of vascular prosthetic surgery – such as thrombosis, either primary or secondary to intimal hyperplasia at the venous anastomosis, defects of the device, or infection – remain a serious threat. This is a major drawback for application in large numbers of diabetic patients.

The surgical risks are much lower with extravascular than with the intravascular devices. Biocompatibility problems are usually deleterious only to the function of the encapsulated tissue and have no or only minimal risk for the recipient. The relative safety is an important advantage of extravascular over intravascular devices but the interactions of tissue-material are of similar complexity. These biocompatibility problems are usually related to toxicity and activation of non-specific foreign body reactions resulting in fibrotic overgrowth with subsequent necrosis of the encapsulated tissue.

Macrocapsules can be implanted with minimal surgery in different sites such as the peritoneal cavity [14–17], the subcutaneous site [18–24], or the renal capsule [25]. They can also be readily retrieved and produced in a relatively simple way.

Many different biomaterials have been applied for the production of macrocapsules. The most commonly applied are nitro-cellulose acetate [26], 2-hydroxyethyl methacrylate (HEMA) [27], acrylonitrile and

sodium-methallylsulfonate [28], and alginate [29]. The hollow fibre geometry is usually preferred over the planar membranes for their smaller foreign body response [30]. Most studies on hollow fibres use fibres made of PAN-PVC [13, 31], similar to those used in intravascular devices. They have been produced with a smooth and fenestrated outer skin with the same spongy matrix as applied in the intravascular. The design with the smooth outer skin proved to be the most successful because it provokes much less fibrosis than the rough fenestrated surface which allows host tissue to grow into the spongy matrix. Many modifications of this concept have been proposed in order to further improve the biocompatibility. One of those was the coating of the membranes with poly(ethyleneoxide) to reduce protein adsorption [32].

Initial studies with macrocapsules were not very successful. This was not so much the consequence of fibrotic overgrowth but rather of aggregation of the encapsulated tissue into large clumps [18]. Extensive necrosis occurred in the centre of the clumps as a result of diffusion limitations for nutrients. This problem was readily solved by preventing contact between the encapsulated tissue elements through permanent solitude immobilization in a matrix such as collagen [33, 34], Ca-alginate [18], or chitosan [11]. Usually in PAN-PVC fibres islets are immobilized in alginate. The islet density is kept quite low and never exceeds 5–10% of the volume fraction because viability has been found to be substantially reduced with higher densities. When transplanted in *BB*-rats, islets encapsulated in fibres were found to induce normoglycaemia for up to 8 months but glucose tolerance remained disturbed and decreased rapidly with time, in spite of prolonged normoglycaemia [31, 35]. A major factor is that hollow fibres, as a consequence of their shape, tend to break when forced to bend under physiological stress [11, 36]. Another factor is the low number of islets implanted which could be insufficient for achieving long-term graft survival. The use of higher numbers, however, is impractical because enormous lengths of fibres would be required as a consequence of the low seeding density of the membranes. A modification was to use tubes with a wider lumen of several millimetres. This, however, is associated with a substantial increase in diffusion distance, which enhances rather than reduces problems such as necrosis as a consequence of insufficient nutrient supply, and accumulation of waste materials. Some success with these devices has been reported in diabetic rats, but extreme amounts of islets, ie. 30 000 islets/kg, were required to maintain normoglycaemia for only a few months [36]. In addition, after intraperitoneal implantation, the membranes still provoked a foreign body reaction which resulted in overgrowth by a thin, but avascular fibrotic cellular infiltrate, which implies further limitations in the diffusion capacity and in the life span of the islets.

During the past few years, an interesting trend has been the growing number of groups applying hydrogels for macroencapsulation. Hydrogels provide a number of features which are advantageous for the biocompatibility of the membranes. Firstly, as a consequence of the hydrophilic nature of the material, there is almost no interfacial tension with surrounding fluids and tissues which minimises the protein adsorption and cell adhesion. Furthermore, the soft and pliable features of the gel reduce the mechanical or frictional irritations to surrounding tissue. And, finally, they provide a high degree of permeability for low molecular weight nutrients and metabolites, which is required for the optimal functioning of living cells.

Many hydrogels have been applied, such as gels prepared from alginate [37–39], agarose [17, 40], HEMA [27, 41] and a copolymer of acrylonitrile and sodium-methallyl sulphonate, AN69 [28]. Primary attention has been focussed on the hydrogel membrane AN69, which induced only minimal fibrosis in the peritoneal cavity of rats [42, 43] but had low permeability for insulin [42, 43]. Recently, Corona discharge has been introduced to obtain a membrane with a more hydrophobic surface [42, 43]. Fewer molecules adhered to the surface of such membranes, improving not only the permeability for insulin but also its long-term biocompatibility. One year after implantation in rats, only a few macrophages were found on the membranes' surface. Moreover, encouraging results were reported by Jain et al who demonstrated functional porcine islets transplanted in agarose macrobeads almost 200 days after intraperitoneal transplantation into *BB* rats [28].

Microencapsulation of pancreatic islets

Microencapsulation is the technique by which each islet is enveloped in its own, spherical semipermeable membrane. Several arguments favour microcapsules over macrocapsules. Their spherical shape offers better diffusion capacity because of a better surface-to-volume ratio. Microcapsules cannot be easily disrupted, are mechanically stable, do not require complex or expensive manufacturing procedure, and can be implanted into the patient by a simple injection procedure.

Reportedly, the clinical experience is still restricted to one recipient [44] but several research groups are concentrating on transplantation of microencapsulated islets in the experimental setting [45] [22, 23, 46, 47, 47–68]. Although successful encapsulated islet grafts do induce normoglycaemia in streptozotocin diabetic rodents recipients, some authors have reported reduced functional performance in response to glucose challenge [59, 69–72].

For several years, we have concentrated on microencapsulation and we have directed our efforts to

a stepwise analysis of the factors influencing performance of the microencapsulated grafts. To this end, we have been using only one technique of alginate-polylysine microencapsulation as initially introduced by Lim and Sun [73], instead of testing other materials in a trial and error approach. Our aim is to define the conditions which must be met for successful transplantation of microencapsulated islets as a feasible treatment of Type I diabetes mellitus.

Technical considerations for the production of microcapsules

Microcapsules are almost exclusively produced from hydrogels, and Table 1 lists the most popular biomaterials applied in this technique. A major distinction exists between water soluble polymers such as alginates and water insoluble polymers such as HEMA-MMA. Water insoluble polymers are preferred by a number of groups [41, 74–76] because they are assumed to be more stable than water soluble polymers after implantation. However, a major obstacle in using water insoluble polymers for encapsulation of cells is the use of an organic solvent, which usually interferes with cellular function [41, 77].

We, like others [62, 67, 68, 78–80], have concentrated on alginate-based capsules because they have consistently been found not to interfere with cellular function. Despite their solubility in aqueous solutions, they have been shown to be stable for years in small and large animals and recently also in human beings. The most commonly used alginate-based capsules are formed by the alginate-polylysine system. The technique is based on entrapment of individual islets in an alginate droplet which is transformed into a rigid bead by gelification in a divalent cation solution, mostly rich in Ca^{2+} . Alginate-molecules are composed of mannuronic (M) and guluronic acids (G). In the first step of the microencapsulation process (ie. the gelification) the alginate-molecules are connected by Ca^{2+} through binding of consecutive blocks of G-molecules on each of both molecules. After gelification the beads are coated with a polylysine membrane by suspending the beads in a poly-L-lysine (PLL) solution. During this step, PLL binds to mixed sequences of G and M in the alginate molecules [81, 82]. This induces the formation of complexes at the capsule surface consisting of α -helical PLL surrounded by superhelically orientated polysaccharide chains [82]. The presence of these complexes decreases the porosity of the membrane. By varying the molecular weight and the concentration of the polylysine, and the incubation time the porosity of the capsule membrane can be modulated [58, 83–88]. Usually, 10 min incubation in 0.1% polylysine with a molecular weight of 22 kDa is sufficient to form an immunoprotective membrane. However, binding of polylysine

Table 1. Main components of capsules proposed for microencapsulation of pancreatic islets

Main component of the capsule	Source	Initially proposed by
Alginate	Alga	Lim and Sun 1980 [73]
Chitosan	Alga	Zielinski 1994 [131]
Agarose	Alga	Iwata et al. 1989 [60]
Poly(hydroxyethylmetacrylate-methyl methacrylate)(HEMA-MMA)	Synthetic	Dawson et al. 1987 [132]
Copolymers of acrylonitrile (AN69)	Synthetic	Kessler et al. 1991 [28]
Polyethyleneglycol (PEG)	Synthetic	Cruise et al. [48]

does not only depend upon the incubation time and the molecular weight of the polylysine but also on the type and concentration of alginate [87, 89] as well as on the temperature of an incubation [86, 87, 89]. In a final step to provide biocompatibility, the capsules are suspended in a solution of alginate or other negatively charged molecules [86, 89] to bind all positively charged polylysine residues still present at the capsule surface.

Another very important aspect of adequate biocompatibility, which requires a lot of experience in microencapsulation process, is a smooth and mechanically stable microcapsule. In our laboratory we have observed that high rather than low viscosity alginates produce smooth beads with no obvious tails or strains. Moreover, we have found that after implantation, alginates with low-G concentrations had a tendency to swell with subsequent breakage of the PLL membrane followed by cellular overgrowth of the capsules. Therefore, for our studies we have chosen the alginate with an intermediate G-concentration.

The stability of a capsule is determined by stability of a membrane and stability of an alginate core. The stability of a membrane can be controlled by the PLL-step because shorter incubation time, lower PLL concentrations, and lower PLL molecular weight than described above, were associated with an increase of the number of capsules with broken membranes. Additionally, incubation at 4 °C instead of room temperature led to less stable microcapsules. Finally, we did not apply EGTA or citrate [77] to liquify the inner core of the capsule. The reason for this modification of the original method [73] was that many capsules were observed to lose their integrity during the treatment. In spite of all the improvements in the stability of the capsules, tissue reactions still continued to interfere with longevity of the graft survival. We have found that other factors, not directly related to the production procedure, were responsible for the tissue reaction as signalling insufficient biocompatibility of microcapsules.

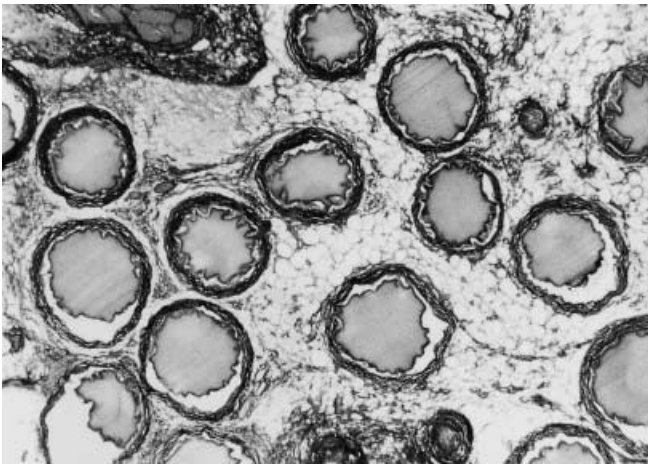


Fig. 1. Alginate-polylysine capsules after provoking a foreign body reaction in the peritoneal cavity of an AO rat. At 1 month after implantation the capsules adhere to the omentum and overgrown by fibroblasts. (GMA-embedded section, syrius red staining, original magnification $\times 50$)

The biocompatibility issue I: chemistry of the capsule

Failure of microencapsulated islet grafts is usually interpreted as the consequence of insufficient biocompatibility, which induces a non-specific foreign body reaction against the microcapsules and results in progressive fibrotic overgrowth of the capsules (Fig. 1). This overgrowth interferes with adequate nutrition of the islets and consequently causes islet cell death.

We have tested the hypothesis that the lack of biocompatibility is caused by impurities contaminating the alginate. Alginates are crude products extracted from alga and contain several substances which are known to provoke inflammation [59, 87, 90–92]. Therefore, we designed the purification procedure, which is basically composed of a filtration step, an extraction step, and a dialysis step. The procedure is associated with a loss of 25% to 40% of the alginate but it had no influence on its chemical composition [59, 93].

Empty capsules prepared of either crude or purified alginate were implanted in the peritoneal cavity of normoglycaemic AO/G rats and retrieved by peritoneal lavage at varying time points after implantation. Crude alginate capsules were always overgrown by fibrotic tissue. In contrast, 80% to 100% of the purified capsules could routinely be retrieved up to 12 months after implantation, and less than 10% of those retrieved capsules showed fibrotic overgrowth (Fig. 2). Similarly to empty capsules, capsules containing islets and prepared from purified alginate were found to be adequately biocompatible. After allotransplantation and islet transplantation, the majority was found freely floating in the peritoneal cavity and 80% to 100% of capsules could be recovered and less than 10% of retrieved microcapsules showed signs of fibrotic overgrowth.

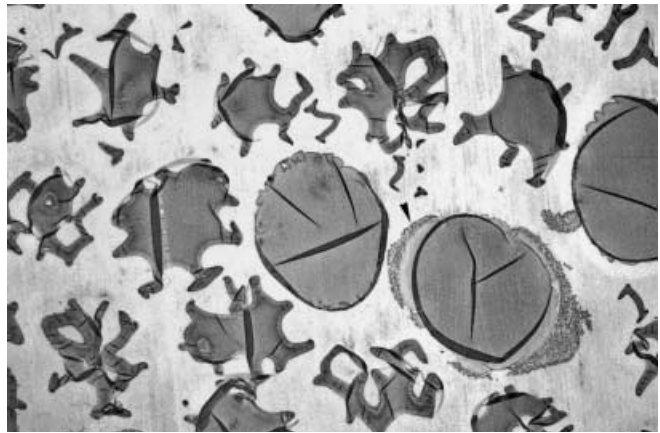


Fig. 2. Alginate-polylysine capsules prepared from purified alginates. Capsules were retrieved at 12 months after implantation in the peritoneal cavity of AO rats. The arrow indicates one of the few capsules with fibrotic overgrowth (GMA-embedded section, Romansky-Giemsa stain, original magnification $\times 50$). Capsules are deformed as the consequence of inhomogeneous water extraction during processing for histology

We have also studied the biocompatibility of alginates composed of different proportions of guluronic acid blocks (G) to mannuronic acid blocks (M). Empty capsules with varying G/M ratio were implanted into the peritoneal cavity. We found that the biocompatibility of alginate-polylysine capsules strongly depends on the alginate G-content. Empty capsules prepared from high-G alginate provoked a severe inflammatory response, while capsules prepared from alginates with an intermediate G-content remained free of overgrowth. This could be due to different binding properties of polylysine to high-G and intermediate-G alginates [90]. When inadequately bound to alginate, polylysine can be a strong initiator of fibrosis. This was shown by others [94] and by us [90] when comparing the biocompatibility of high-G alginate-polylysine capsules and high-G alginate beads in the absence of polylysine. The high-G beads remained free of any obvious overgrowth while all of high-G alginate-polylysine capsules were overgrown by several layers of fibroblast within the first month of implantation in AO-rats.

In summary, our findings show that purification of alginate is associated with a clear-cut and long-lasting improvement in biocompatibility. They also show that the majority but not all capsules stay free of fibrotic overgrowth. A small minority of the capsules shows signs of bioincompatibility in spite of the fact that all are composed of the same material. Apparently, factors other than the purity and composition of the material play a role. Such factors might be associated with the mechanical aspects of the production process and are discussed in the following section.

The biocompatibility issue II: adequacy of encapsulation

The observation that only a small percentage of the capsules show cellular overgrowth after implantation suggests that physical imperfections of individual capsules might be responsible for inducing insufficient biocompatibility. Such physical defects imply inadequate encapsulation of individual islets, and thereby inadequate immune protection as well as insufficient biocompatibility.

In order to further analyse the issue of individual capsule inadequacies we first have designed an assay to identify individual islets which had been inadequately encapsulated. The assay is based on a binding of lectin and FITC labelled with RCA-I (Vector Laboratories, Burlingame, UK), and allows for quantification of the percentage of inadequately encapsulated islets under varying experimental conditions. Lectin, which has a high affinity for pancreatic islets, has a high molecular weight (120 kD), which prevents the lectin molecules from the diffusion through the pores of an adequate microcapsule. Inadequately encapsulated islets are identified by positive fluorescence (Fig. 3), and the overall adequacy of an islet encapsulation procedure can be quantified by expressing the number of inadequate capsules as the percentage of the total number of capsules containing islets [58, 95].

When this assay was applied after encapsulation of Wistar rat islets using a 3% solution of Keltone LV to produce microcapsules with the usual 800 μm diameter, $6.3 \pm 0.2\%$ of the capsules was found to be inadequate. Interestingly, when the droplet generator was set to produce smaller capsules with a diameter of 500 μm , the percentage of inadequate capsules rose approximately fourfold to $24.2 \pm 1.5\%$. When tested in vivo by transplanting Lewis islets encapsulated in either larger or smaller capsules into streptozotocin diabetic AO/G rats, inadequate encapsulation was found to carry substantial significance because normoglycaemia and prolonged graft survival was obtained with the larger but not with the smaller capsules [95]. These observations are rather disturbing, because small capsules reduce diffusion distances as well as the total graft volume. The retrieval rate with peritoneal lavage was more than 80% with the larger capsules but only 5.5% with the smaller capsules. Of those retrieved capsules, only 12% of the larger capsules but 65% of the smaller capsules showed fibrotic overgrowth. Clearly, inadequate encapsulation enhances fibrotic overgrowth and reduces the chance of graft survival. The cellular overgrowth is composed of fibroproliferative connective tissue, ED-1 and ED-2 positive macrophages, T-cells but no beta cells, and occasionally some multinucleated giant cells. This composition suggests that two distinct causes are simultaneously responsible for the ob-

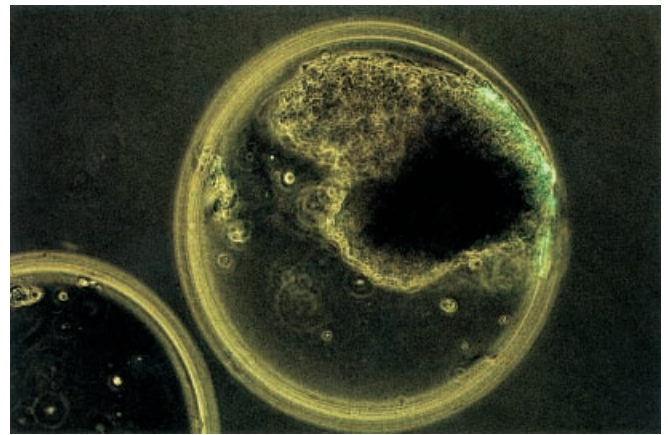


Fig. 3. Inadequate encapsulation of pancreatic islets. The islet is incompletely encapsulated. Islet cells protruding from the capsule are specifically labelled by the FITC-labelled lectin RCA-I. (original magnification $\times 100$). Reproduced from De Vos et al. [58] with permission of the publisher

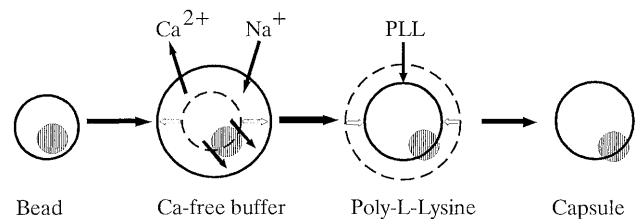


Fig. 4. Schematic presentation of the process of swelling and shrinkage during the encapsulation procedure. After gelification, the calcium-alginate beads are washed with Ca-free HRH buffer, causing partial displacement of Ca by Na. This process initiates the formation of an anionic interface which interacts with PLL and becomes semipermeable. The exchange of Ca for Na also induces an increase of the alginate bead diameter. As a consequence, the islets tend to be displaced towards the periphery of the alginate bead. During the subsequent step of PLL binding, shrinkage occurs while islets maintain their peripheral location and consequently tend to readily protrude from their capsules. Reproduced from De Vos et al. [58] with permission of the publisher

served reaction. One is that the islet, by its incomplete protection, induces an allograft reaction. The other is that the inadequate capsule itself, by its broken integrity, induces a foreign body reaction.

Factors other than diameters could also influence the adequacy of the microcapsules. When Manugel (55% G-content) instead of Keltone LV (45% G-content) alginate is used to produce the smaller type of 500 μm capsules, the percentage of inadequate capsules decreases from 24% to 12%. The different results with Keltone LV and Manugel alginates are related to differences in swelling and subsequent shrinkage properties during the consecutive steps of the encapsulation procedure [58] (Fig. 4). The higher G/M ratio of Manugel is associated with less swelling, which is associated with a reduced chance for islets to

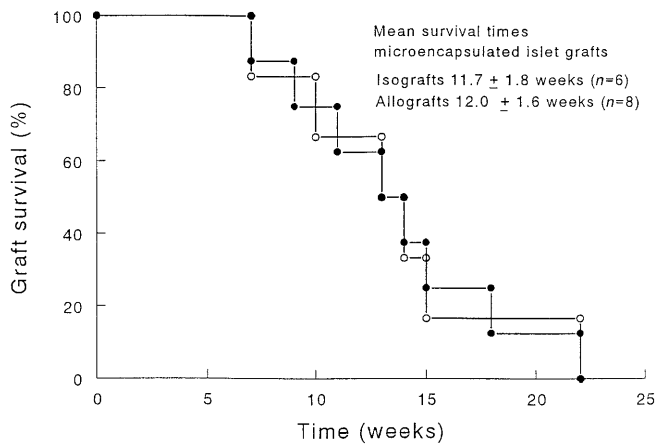


Fig. 5. Survival of encapsulated islet isografts ($n = 6$, open symbols) and allografts ($n = 8$, closed symbols) in streptozotocin diabetic AO-rats. Note the similar if not identical survival times

protrude and, consequently, inadequate encapsulation. Apparently, alginates with a high G/M ratio should be applied to reduce the percentage of inadequate capsules.

In conclusion, to provide complete immunoprotection and optimal biocompatibility, application of purified and fully biocompatible material is not enough; the capsule production process itself should result in mechanically adequate microcapsules. This is an important consideration because variable factors like the capsule diameter and the type of alginate were shown to influence these mechanics.

Functional performance of microencapsulated islet grafts

Intraperitoneal transplantation of a microencapsulated islet graft restored normoglycaemia in streptozotocin diabetic rat recipients within 1 week after transplantation. This normalization of blood glucose concentrations was associated with an evident metabolic improvement as illustrated by a normalization of body weight gain and by a dramatic reduction in daily urine production. Regrettably, however, graft functional survival times were limited and hyperglycaemia returned between 5 and 16 weeks after transplantation [59, 95]. These results were confirmed in later experiments with survival times of 6 to 20 weeks, which were similar if not identical in isografts and allografts [59] (Fig. 5). This latter observation proves that rejection was not the cause of graft failure. Graft failure could not be explained either by fibrotic overgrowth and subsequent cell death because this was observed in only a small minority of capsules as described above. It could also not have been caused by insufficient viability of isolated islets because isogenically transplanted naked, non-encap-

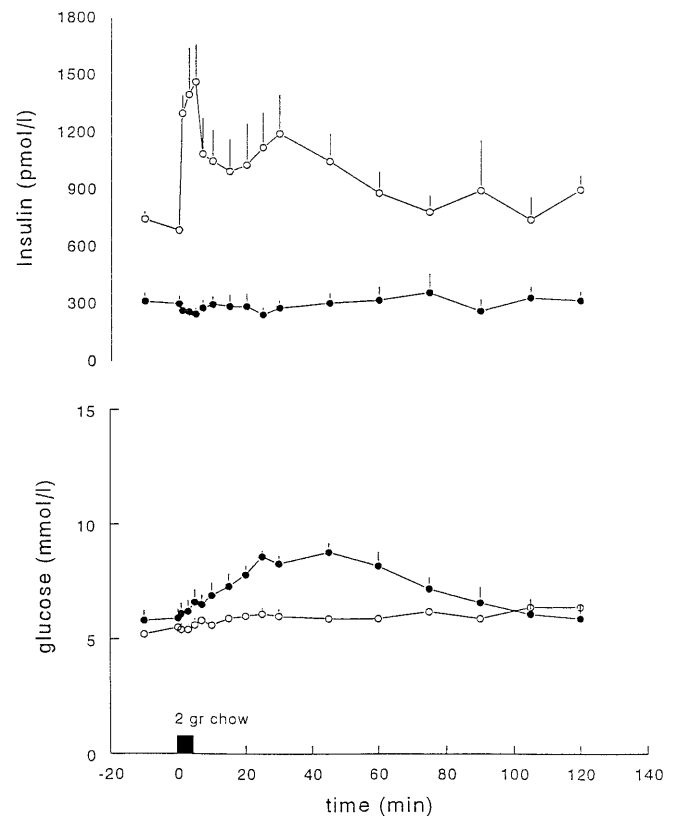


Fig. 6. Blood glucose and plasma insulin levels after spontaneous ingestion of a meal ($n = 5$) in AO-recipients of microencapsulated islet allografts (closed symbols) and in normal control rats (open symbols). The animals were trained to consume a meal of 2 gram chow within 5 mins. Values represent means \pm SEM

sulated islets survived after intraperitoneal implantation [96].

When functioning grafts were tested by oral or intravenous glucose challenge, glucose tolerance was found to be rather adequate as indicated by normal HBA_{c1} levels and maximum glucose levels of 8.3 mmol/l after the consumption of a glucose-rich meal but a rise in systemic insulin was never observed (Fig. 6).

We have further substantiated this observation experimentally by assessing portal and systemic insulin responses and glucose concentrations after gradual infusion of low amounts of insulin into the peritoneal cavity, thereby mimicking the gradual release of insulin from the capsules of an intraperitoneal graft. We found that the dose-dependent rise of insulin and decrease of glucose levels with intraperitoneal insulin infusion were strongly delayed and reduced as well as prolonged in comparison to intraportal insulin infusion [70, 97] (Fig. 7).

In the subsequent experiments on function of intraperitoneally transplanted microencapsulated islets, we assessed C-peptide in the systemic circulation instead of insulin. C-peptide is released in equimolar

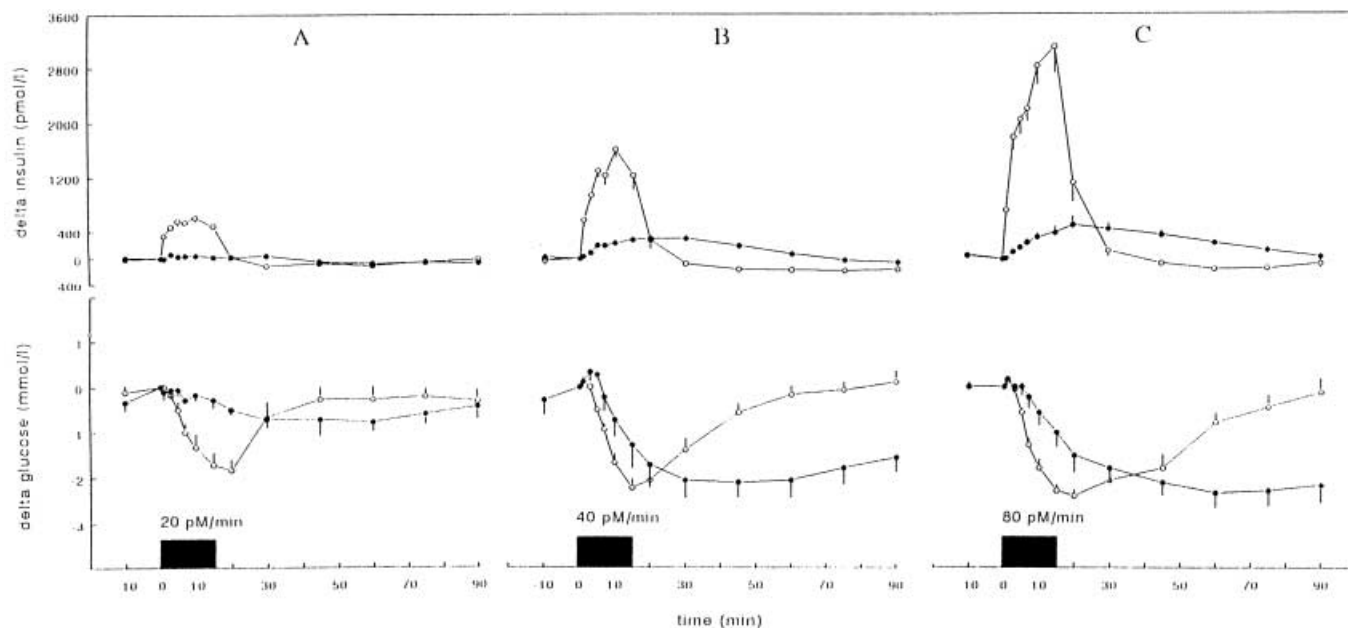


Fig. 7. Effect of intraperitoneal (●) and intraportal (○) infusion of different concentrations of insulin on plasma insulin and glucose concentrations. Insulin was infused in a dose of $20 \text{ pmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ (A, $n = 6$), $40 \text{ pmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ (B, $n = 5$), and $80 \text{ pmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ (C, $n = 7$), during 15 min. The dose of $20 \text{ pmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ induces a rise in systemic insulin concentrations after portal infusion similar to that observed in AO-rats consuming a meal. Values are means \pm SEM of levels expressed as delta levels with the $t = 0$ level as the zero reference. Reproduced from De Vos et al [70] with permission of the publisher

concentrations with insulin, is not readily absorbed by the abdominal organs and does not undergo hepatic extraction. With this approach, we have found a glucose-induced response from the encapsulated islets as shown by an increase of C-peptide in systemic circulation when diabetic mice were subjected to meal challenge [71]. The increase, however, was delayed when compared to normal non-diabetic control mice (Fig. 8 A). Generally, when compared to the normal control mice, the C-peptide levels in plasma were considerably lower in the mice transplanted with either naked or encapsulated islets. In accordance with this lower C-peptide production, we observed a reduced body growth rate in the mice transplanted with either naked or encapsulated islets. Surprisingly, glucose clearance was about the same as that of mice transplanted with naked islets (Fig. 8 B). Similar delay and relatively low response of C-peptide during intravenous glucose tolerance test were reported in NOD mice transplanted with microencapsulated islets [68]. One potential explanation of that phenomenon is that the peritoneal cavity is known to be less accommodating to transplanted islets. It has been shown that naked syngeneic islets

transplanted under the kidney capsule or into the portal vein of diabetic rats were much more efficient in normalising glucose concentrations than when transplanted into the peritoneal cavity [96, 98, 99]. Additionally, islets in microcapsules in the absence of the vascularization of a graft, could be exposed to relative hypoxia [11, 100].

A vascularized transplantation site for microencapsulated islets

The absence of revascularization of the encapsulated islets interferes with both the functional performance and the longevity of the grafts. Apparently, a site where encapsulated islets are in close contact with the blood stream is obligatory for clinical application. Unfortunately, it is difficult to find such a site because it should combine the capacity to bear a large graft volume in the immediate vicinity of blood vessels. Reported sites allowing for successful islet transplantation such as the liver [101–104], the spleen [101, 105–107], and the renal capsule [20, 108, 109] do not meet these requirements because they can never carry the volumes of more than 100 ml of capsules required for transplantation in human beings [44, 87, 110]. To overcome this obstacle, we recently introduced the concept of an intraperitoneally implanted solid support system for pancreatic islets [111]. This site allows for implantation of high numbers of islets, which can readily be retrieved and, theoretically, can be designed to be highly vascularized.

We developed a solid support system of expanded PTFE (ePTFE) because ePTFE have been shown to be biocompatible and to become neovascularized after implantation in the peritoneal cavity. Initially, we

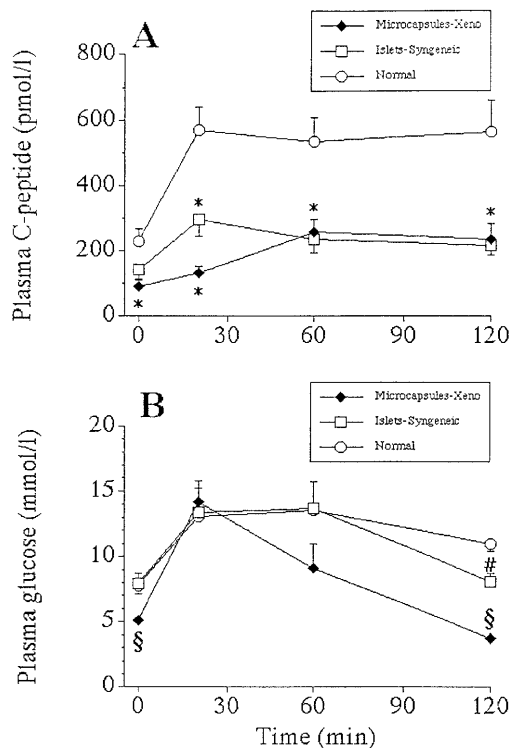


Fig. 8 (A, B). Meal challenge performed 9 weeks after intraperitoneal transplantation of either microencapsulated xenogeneic rat islets ($n = 7$) or syngeneic non-encapsulated mouse islets ($n = 5$) into STZ diabetic B6AF₁ mice. **A** Plasma C-peptide concentrations and **B** plasma glucose concentrations during a meal challenge. As controls, age-matched normal non-diabetic B6AF₁ mice were used ($n = 9$). Data are presented as means \pm SEM; * $p < 0.01$ microcapsules and syngeneic vs normal, $\beta p < 0.02$ microcapsules vs normal and syngeneic, # $p < 0.001$ syngeneic vs normal. Reproduced from Tatar-kiewicz et al. [71] with permission of the publisher

applied solid supports coated with collagen type IV (ie. the collagen type predominantly present in the basal membrane) and acidic-fibroblast growth factors (a-FGF) to facilitate the ingrowth of blood vessels [111–113]. These supports were always implanted 4 weeks before the introduction of the islets to prevent islet dysfunction as the consequence of inflammatory reaction against the ePTFE – a reaction which is usually complete within 4 weeks [111]. Before implanting encapsulated islets, we tested the efficacy of these ePTFE solid support systems as a transplantation site for naked, non-encapsulated islet grafts by comparing the functional performance of islet iso-grafts implanted in the solid supports to those implanted in the unmodified peritoneal cavity. These experiments indicate that the solid supports were much more efficacious than the unmodified peritoneal cavity because when we implanted 10 μ l of islet tissue in the solid support we found that all rat recipients became normoglycaemic while only 40% with transplantation of the islets in the unmodified peritoneal cavity did [111].

In the subsequent histological evaluation of the solid supports, we assessed the degree of vascularization of the grafts and compared it with the vascularization of the liver. The liver was chosen because it is an organ allowing for successful transplantation of islets both in experimental animals and in human beings [101, 109, 114, 115]. Unfortunately, we found that the degree of vascularization of the solid supports was far behind that of the liver. Another observation illustrating the inadequate vascularization of the supports was that transplantation of an suboptimal non-encapsulated islet volume of 5 μ l, ie. half the volume of the islet tissue present in the pancreas of a control rat, was associated with normoglycaemia in 60% of the recipients while it was 100% after grafting in the liver [111].

In our subsequent studies on vascularization of solid supports, we focussed on VEGF-165 [116–118], which is considered to have the greatest potential as an angiogenic stimulus when compared to other angiogenic growth factors such as acidic-fibroblast growth factor, basic-fibroblast growth factor, platelet-derived growth factor, epidermal growth factor, epidermal growth factor, alpha and beta transforming growth factor, interleukines (IL-8 and TNF) or prostaglandines.

Our approach to stimulate vascularization by administration of angiogenic factors is shown in Figure 9. Firstly, angiogenic stimuli degrade the basal membrane and components of the extracellular matrix around capillaries in the immediate vicinity of the solid support. Subsequently, the endothelial cells start to proliferate and migrate into the site of release of the angiogenic factors. Finally, the endothelial cells form tubular structures after which they differentiate into cells aligning full mature blood vessels. The whole sequence of events leading to the formation of blood vessels is under control of a wide variety of angiogenic growth factors which are endogenously produced by cells in the immediate vicinity of the solid supports.

Figure 10 shows our present experimental approach to stimulate vascularization of the solid supports applicable for encapsulated islets. These supports were prepared from flat sheets of ePTFE which were coated with collagen type IV and subsequently implanted in the peritoneal cavity in the immediate vicinity of the liver in order to promote portal drainage of insulin. The supports were infused with a solution of saline containing VEGF-165 and heparin (to stabilize the VEGF).

After 4 weeks of infusion with VEGF-165, we found, macroscopically, much more blood vessels running over and into the supports when compared to injections of aFGF. Subsequent staining with RECA-1 (ie. a specific rat endothelial cell marker), showed a degree of vascularization of 66 ± 8 vessels/ mm^2 in VEGF-165 treated supports (at a dose of 80

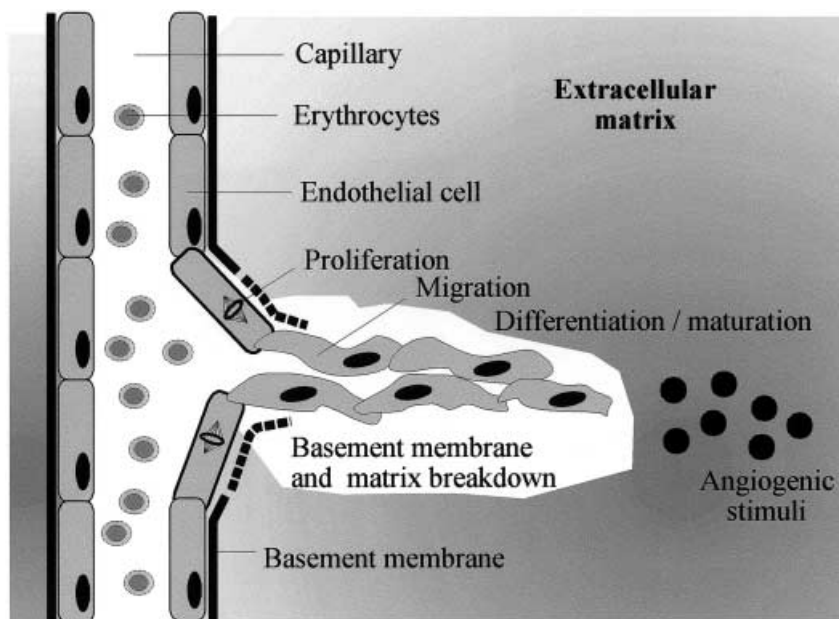


Fig. 9. The process of angiogenesis after exogenous administration of angiogenic stimuli. Basal membrane and matrix degradation facilitates the migration of proliferation endothelial cells to the angiogenic stimulus

ng/day) which was similar to the 72 ± 7 vessels/ mm^2 found in the liver. Control supports, i.e. supports infused with saline and heparin in the absence of VEGF-165, showed a lower degree of vascularization of 44 ± 12 vessels/ mm^2 .

At present we are exploring the possibility of repeated replacement of encapsulated islet grafts from VEGF-165-treated supports because this could be mandatory for clinical application in case of limited function of encapsulated grafts. This is done by injecting encapsulated islets into the supports in a solution of alginate with a high-M content. These high-M alginates chelify in the human body and therefore can keep the capsules at its place in the solid support. If replacement is required the high-M alginates can easily be solved by the administration of Ca-gelators such as EGTA. Liquification of the solution allows aspiration of the alginate in combination with the encapsulated islets in a procedure, which requires only minor surgery.

Longevity of microencapsulated islets

As follows from the preceding sections, minor modifications in the encapsulation procedure could have an important impact on the capsule's biocompatibility and thus on the functional outcome of the graft. However, the factors contributing to the quality of microcapsules are not standardized. As a conse-

quence, there are many different encapsulation procedures, each resulting in capsules with different porosities and with specific chemical and mechanical characteristics. Obviously, these differences contribute to the enormous variations in reported success rates of encapsulated islet allografts and xenografts.

It has been assumed that indefinite survival would be achieved with islet-containing microcapsules which elicit a minimal foreign body reaction [78, 87, 119]. However, even when the foreign body reaction affected only an insignificant number of capsules [59, 91], long-term survival of the encapsulated islet grafts in rats was not achieved. This phenomenon of graft failure in the absence of overgrowth of the capsules has been reported before [120–122] and is usually explained by exhaustion of the graft as a consequence of a combination of a too high glycaemic load on an insufficient number of transplanted encapsulated islets. In our studies in rats, however, neither the volume of the graft nor the glycaemic load on the islets caused graft failure. We found that after transplantation of a sufficient islet volume of $10 \mu\text{l}$, the functional mass of the graft decreased rapidly not only in diabetic but also in normoglycaemic recipients.

Some dispute exist as to the effect of the ongoing activity of the immunological rejection process on the survival of immunoprotected islets. It has been shown that cytotoxic antibodies can be formed against the islets in spite of the presence of a capsule around the tissue [16, 29, 123, 124]. It has also been shown that cytokines produced during the rejection process, such as IL- 1β can pass through the capsule membrane [125]. In our in vivo studies, we found no effect of the histoincompatibility of the encapsulated islets on the graft survival because isograft had identical survival times as allografts [59]. These findings

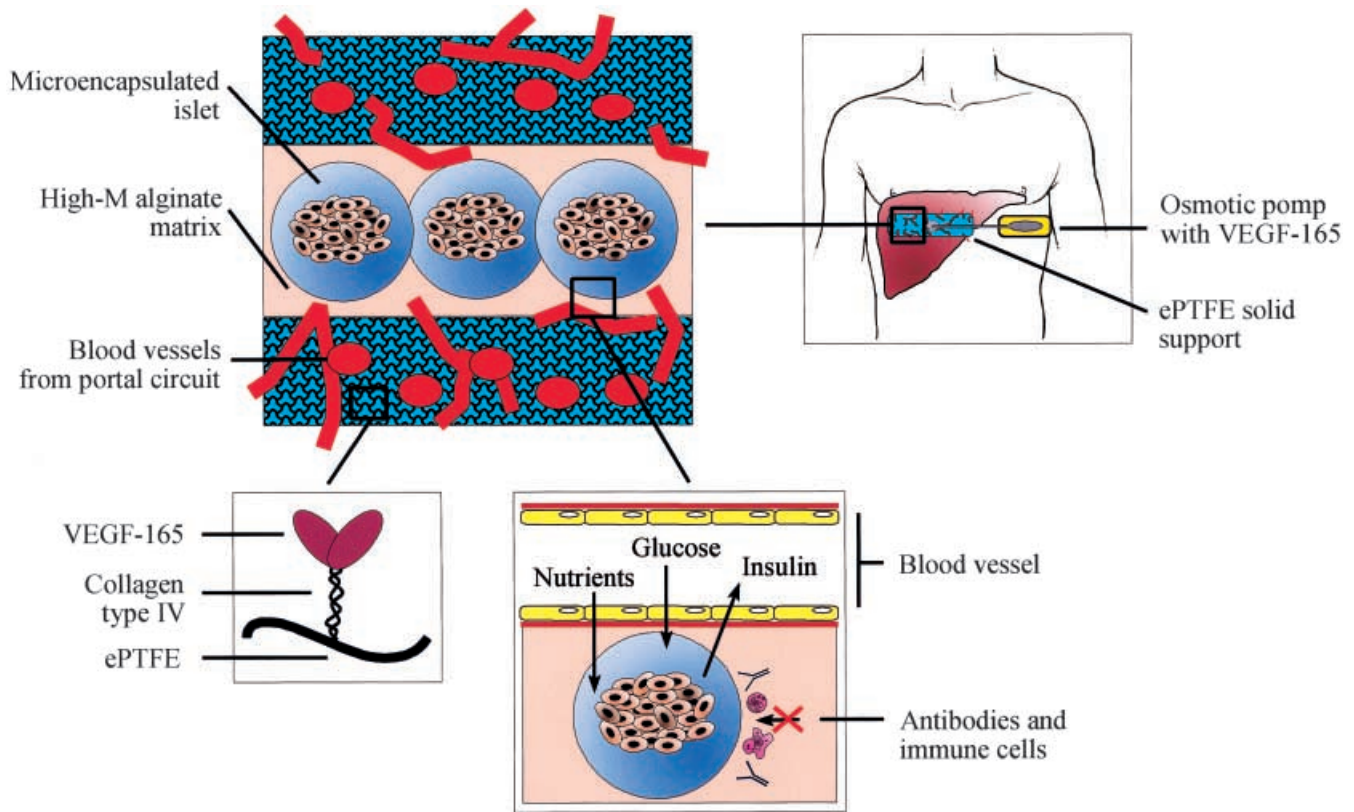


Fig. 10. Schematic presentation of the principle of grafting of encapsulated islets in prevascularized solid support systems. ePTFE solid supports are coated with collagen type IV and subsequently implanted in the peritoneal cavity. The solid supports are infused with an ALZET osmotic pump for 4 weeks with VEGF-165 in order to stimulate the ingrowth of blood vessels. The collagen type IV functions as a matrix to prevent the VEGF-165 from immediate diffusion into the surroundings. After 4 weeks, the osmotic pump is removed and alginate-PLL encapsulated islets are infused in a solution of high-M alginate (which forms a gel in the presence of the physiological Ca²⁺-concentration) to keep the encapsulated islets at their place in the solid support

suggest that capsules can adequately protect against the allograft reactions initiated by the encapsulated islets after transplantation.

Our results suggest that the long-term graft survival might depend on the access of encapsulated tissue to blood supply and, consequently, on sufficient supply of nutrition and/or growth factors delivered to the encapsulated islets. Although this issue is not covered by the current definition [126], it should be considered to be a biocompatibility problem because long-term survival of the tissue is required for this specific application but, regrettably, is not compatible with the presence of the biomaterial around the tissue.

Concluding remarks

It is clear that important advances have been made in immunoisolation of pancreatic islets during the last few decades. This view is corroborated by the results of the restricted experience obtained in human beings [21, 44, 127]. Very few reports from clinics on transplantation of encapsulated islets might be explained by not only scientific reasons. Limited availability of human islets, recent concerns about using pig tissue in humans, and extremely high expenses probably contribute to sluggish progress in clinical application of microencapsulation approach. In view of the recent successful transplantation of pancreatic stem cells [128–130], one might expect unlimited sources of allogeneic insulin-producing tissue in the near future. Additionally, enhanced survival of grafts supported by induced neovascularization might result in encapsulation technology which will be the ultimate solution to cure Type I diabetes.

Sources. This review is based on the relevant literature published in the English language during the period 1980–2001, and seminal prior contributions. The sources available to the authors were integrated with sources identified through PubMed searches for “encapsulation of pancreatic islets” and “vascularization and transplantation of microencapsulated islets”.

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