Alginate-polylysine microencapsulation of pancreatic islets. Biocompatibility and function

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The introduction (Chapter I) of this thesis provides the rationale for islet immunoisolation and transplantation. The aim of islet transplantation is to provide the diabetic patient with an endogenous source of insulin in order to delay the onset and progression of diabetic complications. In clinical trials, immunosuppressive therapy is applied to circumvent immune destruction of the islets. Effective immunosuppression, however, is associated with serious side effects which make it doubtful whether such an approach will ever be applicable in large numbers of diabetic patients as a sound alternative for insulin treatment. This is the main reason to concentrate on approaches which fundamentally differ from the conventional approach of suppressing the host's immune system. One such approach which has the potential to be developed into a widely applicable therapy for diabetics is immunoisolation of islets in a membrane permeable for glucose, insulin and nutrients but not for humoral and cellular components of the immune system. This technique allows for successful transplantation of islets derived from xenogenic, and thus unlimited, donor sources in the absence of immunosuppressive medication. A historical review of the transplantation of insulin producing cells in semipermeable membranes is presented. Presently, there are three different technical approaches for transplantation of immunoisolated islets. These approaches are: (i) immunoisolation in extravascular macrocapsules, (ii) immunoisolation in intravascular macrocapsules and (iii) immunoisolation in extravascular microcapsules. The features of these different approaches are discussed in the perspective of future application in man. Of those techniques, microencapsulation offers the best option for being developed into a technique widely applicable in diabetic patients with a fair chance of success.

A review of the results of experimental studies with microencapsulated islets is presented in the second part of the introduction (Chapter I). These results vary considerably which usually is interpreted to be the consequence of problems associated with the biocompatibility of the capsule materials. Consequently, the majority of studies on microencapsulated islets somehow seem to focus on the problem of capsule biocompatibility while other problems, such as restrictions of the production
of capsules and the metabolic control by the grafts, have gained little no attention.

In Chapter II we have reviewed the obstacles on the road to transplantation of microencapsulated islets. These obstacles can be arranged in three categories. The first regards the technical aspects of the production process. Limiting factors are the insufficient ability to produce small capsules with an adequate production rate, and insufficient insight in the factors determining the optimal chemical and mechanical properties of the capsules. The second category regards functional aspects of the microencapsulated islets, such as the limitations of the transplantation site and the absence of a physiologic insulin response of encapsulated islets to elevated blood glucose levels. The third category regards the fact that survival times of encapsulated islet grafts are still limited to several weeks or months, which mainly is explained by fibrotic overgrowth induced by biocompatibility of the capsule membrane. This overview describes these obstacles, and thereby summarizes the requirements for successful clinical application of encapsulated islet transplantation.

In Chapter III we present the rationale for the studies included in this thesis (Chapters IV-XI). In view of the obstacles mentioned in Chapter II, our aims were to identify the obstacles which presently prevent the clinical application of microencapsulated islets, and to develop strategies to overcome these obstacles. In the course of time we found that some properties of the capsules which were initially categorized as 'technical aspects of the production process' are closely related to problems initially categorized as 'functional survival of the graft'. Thus, in presenting our results we have chosen a rational sequence of chapters, rather than maintaining the strict categories. The rationale of each individual study is given below in the summary of each chapter.

Graft failure of alginate-polylysine microencapsulated islets is often interpreted as the consequence of a non-specific foreign body reaction against the microcapsules, initiated by impurities present in crude alginate. The aim of the studies presented in Chapter IV was to investigate if purification of the alginate improves the biocompatibility of alginate-polylysine microcapsules.
Alginate was purified by filtration, extraction and precipitation. Microcapsules prepared of crude or purified alginate were implanted in the peritoneal cavity of normoglycemic AO-rats and retrieved at 1, 2, 3, 6, 9, and 12 months after implantation. With crude alginate, all capsules were overgrown within one month after implantation. With purified alginate, however, the portion of capsules overgrown was usually below 10%, even at 12 months after implantation. All recipients of islet allografts in capsules prepared of purified alginate became normoglycemic within 5 days after implantation, but hyperglycemia occurred after 6 to 20 weeks. With IVGTT and OGTT, all recipients had impaired glucose tolerance and insulin responses were virtually absent. After graft failure, capsules were retrieved (80-100%) by peritoneal lavage. Histologically, the percentage of overgrown capsules was usually below 10% and maximally 31%. This small portion cannot explain the occurrence of graft failure. The immunoprotective properties of the capsules were confirmed by similar if not identical survival times of encapsulated islet allo- and isografts. Our results show that purification of the alginate improves the biocompatibility of alginate-polylysine microcapsules. Nevertheless, graft survival was still limited.

In search of other processes than overgrowth contributing to graft failure we have studied the islets in non-overgrown capsules at several time points after allotransplantation in the rat (*Chapter V*). All recipients of islet allografts became normoglycemic. Grafts were retrieved at four and eight weeks after implantation, and at 15.3 ± 2.3 weeks postimplant *i.e.* two weeks after the mean time period at which graft failure occurred. Overgrowth of capsules was complete within four weeks postimplant. It was usually restricted to less than 10% of the capsules. During the first four weeks of implantation, 40% of the initial number of islets was lost. Thereafter, we observed a decrease in function rather than in numbers of islets, as illustrated by a decline in the *ex vivo* glucose induced insulin response. At four and eight weeks postimplant, Beta-cell replication was ten-fold higher in encapsulated islets than in islets in the normal pancreas. But, these high replication rates were insufficient to prevent a progressive increase in the percentage of necrotic tissue in the islets. This necrosis mainly occurred in the center of the
islets, which indicates insufficient nutrition as a major causative factor. Our study demonstrates that not only capsular overgrowth but also an imbalance between Beta-cell birth and Beta-cell death contributes to the failure of encapsulated islet grafts. Our observations indicate that we should focus on finding or creating a transplantation site which, more than the unmodified peritoneal cavity, permits for close contact between the blood and the encapsulated islet tissue.

As a consequence of its large volume, a microencapsulated islet graft can be implanted only into the peritoneal cavity. The graft volume can be reduced by applying small capsules. However, reduction of the capsules diameter holds a certain risk as with smaller capsules more islets may be found to protrude from the capsules. We have developed a lectin binding assay which, after encapsulation, specifically labels islets or parts of islets which are insufficiently immunoprotected as a consequence of inadequate, and particularly incomplete, encapsulation (Chapter VI). With this assay we found that a reduction of the capsule diameter from 800 µm to 500 µm was associated with an increase in the percentage of inadequately encapsulated islets from 6.3 ± 1.2% to 24.2 ± 1.5%. The in vivo significance of this finding was investigated by performing allotransplantations with large (700-800 µm) and small (400-500 µm) capsule diameters. With large capsule diameter islet grafts all recipients (n = 5) became normoglycemic for 7-16 weeks, while with small capsule diameter islet grafts only one of seven recipients became normoglycemic. The in vivo significance of inadequate encapsulation was further substantiated by our finding that most large capsules were freely floating in the peritoneal cavity without any cell adhesion, while the vast majority of small capsules was found to be adherent to the surface of intra-abdominal organs and infiltrated by immune cell elements characteristic for both an allograft reaction and a foreign body reaction. We conclude that successful application of small capsule diameters requires further study to determine which factors in the encapsulation procedure should be modified in order to reduce the number of inadequate small capsules.

The observation that not all but only a portion of all alginate-polylysine microcapsules are overgrown after implantation suggests that
physical imperfections of individual capsules rather than the chemical composition of the material applied is responsible for inducing insufficient biocompatibility and thereby fibrotic overgrowth of those capsules. The study presented in Chapter VII investigates factors influencing the adequacy of encapsulation of pancreatic islets. We applied our lectin binding assay and found that the number of inadequate, and particularly incomplete, capsules is influenced by the following factors. (i) A capsule diameter of 800 μm is associated with a lower percentage of inadequate capsules than smaller (500 μm, 600 μm) or larger (1800 μm) capsules. (ii) The composition of the alginate. Alginate is composed of mannuronic acid (M) and guluronic acid (G). A high- rather than low-G content of the alginate is associated with a lower percentage of inadequate capsules. This can at least in part be explained by smaller ranges of swelling and subsequent shrinkage during the encapsulation procedure. (iii) An increase in viscosity by applying a higher alginate concentration compensates for a low-G content. This effect of increased viscosity cannot be explained by a reduced range of swelling and shrinkage during the encapsulation procedure. We conclude that one should preferably use alginates with a high-G content and a viscosity near the filtration limit in order to minimize the number of inadequate capsules.

Little is known of the influence of the differences in G-content in alginates on biocompatibility of alginate-PLL capsules. Therefore, we have investigated in vivo the effect of the G content of the alginate on the biocompatibility of the capsules (Chapter VIII). Capsules prepared of commercially available alginates with either a high- or an intermediate-G content were implanted in the peritoneal cavity of rats and retrieved one month later for histological evaluation. The fibrotic reaction was more severe against high-G alginate capsules than to intermediate-G alginate capsules which are usually applied for islet encapsulation (Chapter IV and V). The majority of the high-G capsules proved to be overgrown and adherent to the abdominal organs while with intermediate-G alginate most capsules were found freely floating in the peritoneal cavity and free of any adhesion of cells. This was not caused by the alginate as such but rather by inadequate binding of high-G alginate to PLL since in the absence of PLL, i.e. with beads instead
of capsules, no fibrotic reaction was observed. As high-G alginate has beneficial effects for islet encapsulation, efforts should be made to apply polycations which more effectively interact with high-G alginate than PLL.

Presently used single needle air-driven droplet generators are incapable of producing sufficient numbers of islet containing droplets in a sufficiently short time period to allow for successfully grafting alginate-polylysine encapsulated islets in large animals or humans. Therefore, we have designed an air-driven multineedle droplet generator, which increases the production rate by simultaneously producing multiple droplets (Chapter IX). Although we have tested a four-needle device, the construction is such that the number of needles, and thereby the production rate, can be readily extended. The production rate can be further extended by increasing the number of islets per milliliter alginate in the reservoir. When tested with 500 μm and 800 μm capsules, an increase in the number of islets per milliliter alginate was found to be associated with an increase in the number of inadequately encapsulated islets in a diameter dependent fashion. When small instead of large capsules are produced from a given volume of alginate, larger numbers of capsules are obtained but also a larger portion of inadequate capsules. With 10,000 islets per millimeter alginate, these combined effects can be calculated to result in a twofold increase in the production rate of adequate capsules when 500 μm instead of 800 μm capsules are produced. So, substantial upscaling of the production can be achieved by combining an increase in the number of needles with a decrease in the capsule diameter.

Intraperitoneal transplantation of encapsulated islets can restore normoglycemia in diabetic recipients but not normal glucose tolerance nor normal insulin responses to a physiologic stimulus. The study presented in Chapter X investigates whether the intraperitoneal implantation site as such contributes to the interference with optimal transport kinetics between the islets and the bloodstream. Insulin was infused into the peritoneal cavity of conscious and freely moving rats in doses of 20, 40 and 80 pM/min during 15 minutes, to mimic the gradual release of insulin from an encapsulated, i.e. a non-vascularized, islet graft. With 20 pM/min, we observed virtually no rise
of insulin levels and it took 30 minutes until glucose levels had dropped significantly. With 40 and 80 pM/min insulin infusions, there was a dose dependent rise of insulin and decrease of glucose levels. When compared to intraportal infusions with the same insulin dosages, however, they were strongly delayed and reduced as well as prolonged. Similar results were obtained when insulin instead of insulin was intraperitoneally infused, which indicates that the transport of insulin from the peritoneal cavity to the bloodstream is mainly by passive diffusion. With view on the clinical efficacy of the bioartificial pancreas, our findings indicate that we should focus on finding or creating a transplantation site which, more than the unmodified peritoneal cavity, permits for close contact between the bloodstream and the encapsulated islet tissue.

An intraperitoneally located and prevascularized ePTFE solid support is potentially a suitable transplantation site for encapsulated pancreatic islets, since it allows both for the implantation of a large volume islet graft in the immediate vicinity of blood vessels, and for its complete removal. The study presented in Chapter XI investigates the efficacy of solid supports for the implantation of non-encapsulated islet isografts in streptozotocin diabetic rat recipients. These solid supports were always coated with α-FGF, since we found that this growth factor enhances the neovascularization. The success rates of 5 μl (group A) and 10 μl (Group B) islet isografts in solid supports were compared to the success rates of 5 μl (Group C) and 10 μl (Group D) islet isografts implanted in the unmodified peritoneal cavity. Four of 7 rats in Group A and all 7 rats in group B became normoglycemic for at least 6 months. Only 2 of 8 rats in group C and 4 of 11 rats in group D showed normoglycemia, which lasted for at least 6 months in none of 2 in group C and 3 of 4 in group D. Because of the low success rates in groups C and D, intravenous and oral glucose testing were restricted to the successful recipients in groups A and B. Glucose tolerance was found to be proportional to the grafted islet volume but, expectedly, in both groups the glucose tolerance and the insulin responses were somewhat lower than in controls. Thus, the prevascularized ePTFE solid support rather than the unmodified peritoneal cavity is an efficacious transplantation site, potentially
suitable for encapsulated islets.

The results of our studies are discussed in the perspective of other studies in the general discussion (Chapter XII). We discuss the prerequisites for long-term survival of the encapsulated islet graft. These not only include a capsule which elicits a minimal foreign body reaction, but also a normal balance between Beta-cell birth and death in the encapsulated islets. Also, we discuss the different approaches to improve the kinetics of the glucose induced insulin responses in the recipients. We suggest that it is likely that longer graft survival times and better insulin responses than currently achieved with implantation in the unmodified peritoneal cavity can be achieved when encapsulated islets are transplanted in well vascularized sites such as the prevascularized ePTFE solid supports.