A novel approach for immunoisolation of pancreatic islets

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Introduction

During recent years, islet transplantation has been shown to be an efficacious methodology to treat diabetic patients. However, the technology of islet transplantation is only applied on minor numbers of diabetic patients due to the necessity to apply lifelong immunosuppression. This obstacle of application of immunosuppression can be overcome by immunoisolation of the tissue. Immunoisolation applies to all technologies that use semipermeable mechanical barriers to protect grafts against antibodies and immune cells but allows for diffusion of nutrients, waste products and, the therapeutic molecules.
The most intensively studied immunoisolation technology for pancreatic islets is the microencapsulation-technique in alginate-polylysine as originally described by Lim and Sun (Lim et al 1980). Over the years, many have shown the principle applicability of the alginate-encapsulation technique. Both allografts and xenografts have shown prolonged survival after implantation in chemical and autoimmune diabetic animal models.
Up to a couple of years ago survival of the grafts was limited to several weeks. This was due to severe inflammatory reactions provoked by the capsule membranes. This problem was reduced to a reaction in which not more than 2-10% of the capsules was affected by an inflammatory response and cellular overgrowth. This progress was accomplished by technical modifications to the procedure such as application of pure alginate and new types of alginate.
Unfortunately the reduction in overgrowth from 100 to only 2-10% of the capsules did not bring about the expected long-term survival of the encapsulated islet grafts (De Vos P et al. 2004). The survival rates of encapsulated islet grafts increased from several weeks to periods up to 4 months. Recently we found the reason for this limitation in survival time. Microencapsulated islet grafts always induce an up to now unrecognized inflammatory response in the immediate period after transplantation which is associated with a loss of up to 60% of the endocrine islet volume (De Vos P et al. 1997). This inflammatory response is initiated by the mandatory surgery and can be enhanced and prolonged by imperfection on capsules such as protruding cells or incompletely covered PLL molecules. It is therefore mandatory to design an encapsulation technique that is not associated with these type of imperfections.
In the present study we present a novel approach of islet encapsulation in a so called double layer system in which protruding cells and proinflammatory-PLL is not available anymore on the surface. The efficacy of this new concept was studied an xenomodel of AO encapsulated rat islets engrafted into diabetic BL6 mice.

Materials and Methods

Male inbred AO-rats weighing 300-350 gram served as donors. Male BL-6 mice rats (AO/G, the Central Animal Laboratory of Groningen, The Netherlands) weighing 25 to 31 gram were used as recipients of encapsulated islet xenografts. Diabetes was induced by injection of 190 mg/kg of streptozotocin (Sigma, The Netherlands) via peritoneal injection. Glucose concentration in blood was determined with glucose test bandelettes (Accu-Chek, Roche). A second injection of 220 mg/kg of streptozotocin was administered if the blood glucose level was lower than 20 mM at 7 to 10 days after the first injection. Only animals with severe weight loss, polyuria, polydipsia and blood glucose levels exceeding 20 mM over a period of at least ten days were used as recipients. After transplantation glucose levels were measured once a week for one year. Animals with two blood glucose measurements above 12,5 mM were considered diabetic and sacrificed.

Islets were isolated as previously described (Wolters et al. 1990). Islets were cultured before encapsulation in order to reduce contamination of the grafts with exocrine tissue, ducts or damaged cells. They were cultured in non-treated petri-dishes (Greiner, Alphen a/d Rijn, The Netherlands) in portions of 100 islets per 25 cm^2 for 19 to 21 hours in CMRL-1640 (containing 10% fetal calf serum (FCS, Gibco, Breda, The Netherlands), 8.3 mM glucose and 10 mM Hepes and 1% Penicillium/Streptomycin) at 37^oC in humidified air containing 5% CO_2. After culture, islets were washed three to five times with RPMI containing 10% FCS and and were subsequently washed three times with Ca^{2+}-free KRH containing 135 mM NaCl.

Encapsulation (conventional method)
After washing the islets were mixed with 3,3 % intermediate-G alginate (Kelco International, London, UK) in the ratio 2000 islets with 1 ml alginate. The 3,3 % alginate solution was converted into droplets using an air-driven droplet generator as previously described. Subsequently, the alginate droplets were transformed to alginate beads by gelling in a 100 mM $CaCl_2$ (10 mM HEPES, 2 mM KCl) solution for at least 5 min. After gelation the beads had a diameter of 550 µm when 800 µm capsules were produced. Subsequently, the Ca-alginate beads were suspended for 3 minutes in KRH containing 2.5 mM $CaCl_2$. A poly-L-lysine (PLL) membrane was formed by suspending the alginate beads in 0.1% PLL solution for 5 or 10 minutes (poly-L-lysine-HCl, Mw: 22,000, Sigma). Non-bound PLL was removed by three successive washings during 3 min with Ca^{2+}-free KRH containing 135 mM NaCl. The outer alginate-layer was subsequently applied by 5 minutes incubation in ten times diluted alginate solution.

Islet encapsulation (double layer method)
After washing the islets were mixed with 2,7 % intermediate-G alginate (Kelco International, London, UK) in the ratio 1000 islets with 100 µl Alginate. The alginate solution was converted into droplets using an electrostatic-bead generator. The droplet size was regulated by using the following parameters: electrostatic potential 14 kV, distance between needle and the $CaCl_2$ solution 3 cm, flow-rate of the alginate solution 2,5 ml/hour and diameter of the needle 27 G. Polylysine-alginate encapsulation was performed as described elsewhere. Briefly, the alginate droplets were transformed to alginate beads by gelling in a 100 mM $CaCl_2$ (10 mM HEPES, 2 mM KCl) solution for at least 5 min. After gelation the beads had a diameter of 275-325 µm. Subsequently, the Ca-alginate beads were suspended for 1 min in KRH containing 2.5 mM $CaCl_2$. A PLL membrane was formed by suspending the alginate beads in 0.05% PLL solution for 7 minutes. Non-bound PLL was removed by three successive washings during 3 min with Ca^{2+}-free KRH containing 135 mM NaCl. The outer alginate-layer was subsequently applied by 5 minutes incubation in ten times diluted alginate solution. Finally, the capsules had a diameter of 325-350 µm. Non-bound alginate was removed by three successive washings during 3 min with Ca^{2+}-free KRH containing 135 mM NaCl.
The small capsules were suspended in a 4 ml 1.9 % high-G (> 45% G) alginate (Kelco International, London, UK) solution for a second envelopment in beads. The alginate containing capsules were converted into droplets using an air-driven droplet generator as previously described. The droplets were allowed to gelify for 5 min in a 100 mM $CaCl_2$. After gelation the beads had a diameter of 750-800 µm. The $CaCl_2$ was removed by three successive washings during 3 min with KRH containing 2.5 mM $CaCl_2$. Prior to transplantation we removed empty capsules by handpicking.

Results and Discussion

Protruding islet cells and unbound proinflammatory PLL at the capsule surface after conventional encapsulation (figure 1) have been shown to induce an immune response that leads to overgrowth and production of deleterious cytokines which interfere with adequate islet function with failure of

the graft as a consequence. By applying our lectine binding assay we found that 2-25% percent of these capsules may contain protruding cells. The percentage of protruding cells is determined by the characteristics of the capsule's applied such as capsule size and the type of alginate (De Vos et al. 1996). High-G alginate show much lesser numbers of protruding cells than intermediate-G alginates.
The adequacy of binding of PLL is also determined by the capsule's characteristics. When high-G capsules are applied we observe more fibrotic overgrowth associated with incomplete PLL binding than when intermediate-G alginate is applied. This latter observation has been a true pitt-fall in encapsulation-research since high-G alginates provide a number of pertinent advantages over intermediate-G alginates since they are not only associated with lower numbers of protruding cells but also with a higher mechanical stability and therefore a higher durability (De Vos et al 2002).

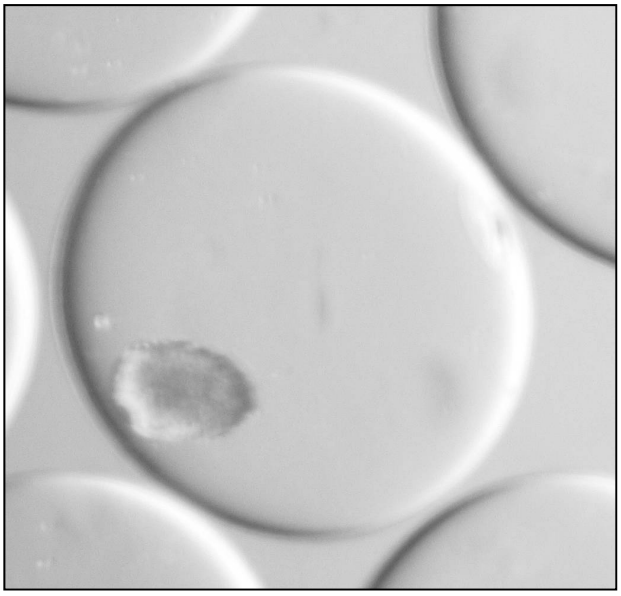

Figure 1. Traditional intermediate-alginate encapsulated islet located in the periphery of the APA capsule membrane.

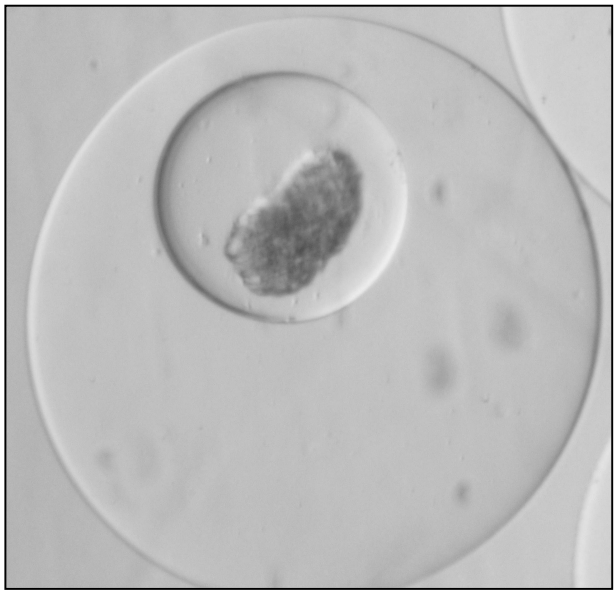

Figure 2. Double encapsulated islet in capsule of intermediate-G and poly-L-lysine membrane within high-G bead

To overcome the protrusion problem and incomplete PLL binding we decided to design a new technology in which we take advantage of the beneficial properties of the different types of alginates. We have designed a double layer system in which we first have produced an alginate-PLL capsules with application of intermediate-G alginate. This intermediate-G alginate binds efficaciously to PLL but is associated with high numbers of protruding cells. Therefore we envelope the capsule in a second layer of alginate. In this layer we apply high-G alginate that as such is not immunoprotective but covers all protruding cells and residual unbound PLL. The advantage of application of high-G is that it provides a high durability to the capsules (figure 2).

Graft function
In order to determine the efficacy of function of the double layer capsules we compared the graft survival of the double layer encapsulated islets with the conventional system in a xenotransplantation model. Therefore we engrafted AO rats in chemically induced diabetic BL6 mice. For each transplanted animal we used one rat donor from which we isolated a total of 800-1000 islets, which corresponds to 2.2-2.8 µl of islet tissue. Islets were cultured before encapsulation in order to reduce contamination of the grafts with exocrine tissue, ducts or damaged cells.

All grafts were meticulously inspected before transplantation to avoid transplantation of capsules with irregulaties. The capsule size of the single- and the double encapsulated islets ranged from 750 to 825 µm (figure 1 and 2). After removing a part of the empty capsules the remaining volume of the transplant ranged between 0.6 and 0.8 ml. All transplanted animals (n=9; 4 conventional and 5 double encapsulated) became normoglycemic within 1 week.
The maximal survival of the single encapsulated islets is 49 days (with a median of 39) in contrast to 400 days (with a median of 91) of the double encapsulated (which returned hyperglycemic 1 year after retrieval of the capsules). The double encapsulated group is still in progress; two animals are normoglycemic for respectively 15 and 55 days. The increased graft survival times is probably due the effect of prevention of protruding islets and the covering of the PLL layer.
The new method was associated with much less fibrotic overgrowth than with the conventional system. Only 1 percent were found to be affected by inflammatory cells with the new double layer system while it was 27 percent with the conventional system.

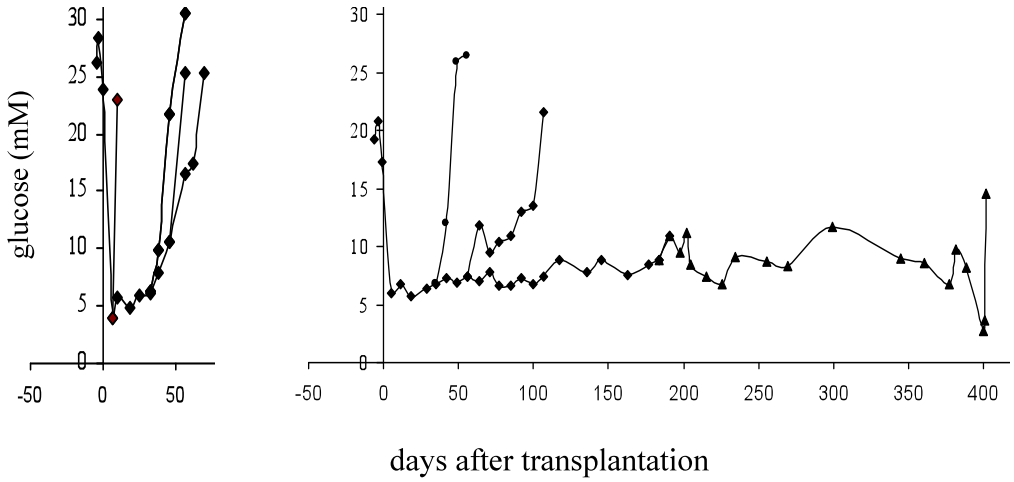

Figure 3. Encapsulated islet from AO rats transplanted in diabetic Bl-6 mice (xeno) with the convetional method (left) and the novel double encapsulation (right).

Conclusions

In the current study we present an new encapsulation method in which we reduce the number of protruding cells and unbound proinflammatory PLL. The new encapsulation methods is associated with longer graft survival times. With conventional capsules the median was 39 days while it was 91 days with the double layer system. The beneficial effects of the new capsule types can also be observed in the overgrowth rates in this xenograft model. This was 27 percent with conventional capsules while it was only 1 percent with the double layer system.

References

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