Upsala J Med Sci 106: 161–174, 2001

Microencapsulation of islets of Langerhans: impact of cellular overgrowth

Mini review based on a doctoral thesis

Aileen King

Department of Medical Cell Biology, Uppsala University, Sweden

INTRODUCTION

The β-cells within the islets of Langerhans produce and secrete insulin, a hormone essential for normal glucose homeostasis. Type 1 diabetes is characterised by a deficiency in insulin, caused by the destruction of the β-cells. The aetiology of type 1 diabetes is not known, although there is evidence that most cases are due to an autoimmune disorder [4]. The current treatment of choice for type 1 diabetic patients is insulin therapy. However, many patients develop secondary complications such as nephropathy, neuropathy and cardiovascular problems. Intense insulin therapy decreases the risk for such complications but increases the risk of hypoglycaemic episodes [92]. Whole pancreas transplantation can normalise blood glucose homeostasis in diabetic patients [86]. Moreover, complications are reduced and the quality of life of the patient is improved [50]. However, this process involves an extensive surgical procedure and it is therefore more desirable to transplant only the islets of Langerhans.

The success rate of islet transplantation has, however, been low [6] until Shapiro *et al* in Edmonton succeeded in restoring insulin independence in almost 100% of patients transplanted [72,77]. There were several improvements in the Edmonton protocol that probably contributed to the high success rate. The patients received a large number of islets (on average 11,500/kg bw) compared to previous studies, the cold ischaemia time was limited, the islets were not cultured and perhaps most importantly, cyclosporin and steroids were excluded from the immunosuppression regimen. Moreover, the patients transplanted were generally free from severe secondary complications and the relatively good health of these patients may also have contributed to the success. That study proved that islet transplantation could consistently reverse hyperglycaemia in diabetic patients, although the long-term success remains to be shown. However, despite the success of the Edmonton protocol, islet transplantation is still only available to patients where the benefits of restored glucose metabolism outweigh the risks of immunosuppression. Immunosuppression has been linked to increased susceptibility to infections, increased risk of malignancy and general toxicity [32,62,68]. Moreover, some immunosuppressive drugs have been found to induce insulin resistance and/or are toxic to islets of Langerhans [1,41]. Indeed, a follow-up study from Edmonton indicated that the

patients suffered from various side effects from the immunosuppression [72]. The risks associated with such side effects presently exclude patients with stable diabetes from treatment with islet transplantation. It is therefore evident that it would be beneficial to perform islet transplantation without the need of immunosuppressive drugs.

Encapsulation of cells

Encapsulation of cells provides the means of transplanting cells in the absence of immunosuppressive drugs. The principle of encapsulation is that the transplanted cells are contained within an artificial compartment separated from the immune system. Thus, the capsule should protect the cells from potential damage caused by antibodies, complement and immune cells. However, small molecules such as nutrients should be able to freely enter the capsules and waste products and hormones such as insulin should be easily released. There are three main types of encapsulation [7,18,51,63], namely a vascular shunt system, macroencapsulation and microencapsulation. In a vascular shunt system, the islet graft surrounds a shunt so that the graft is in close vicinity to the blood. This allows the graft to be well nourished, although the risk of thrombosis together with the risks associated with the surgical procedure have prevented this type of encapsulated graft being investigated in humans [17,56].

Macroencapsulation involves the encapsulation of the whole islet graft within a hollow fibre or diffusion chamber. These types of capsules have been particularly prone to inducing a fibrotic response [17,93,96]. Moreover, the diffusion to the islets in the centre of such devices is limited and islets tend to become necrotic due to lack of oxygen and nutrients [17,87,88].

In microencapsulation, each islet is individually encapsulated (Fig. 1). This offers advantages over other types of encapsulation. An important point is that the risk of

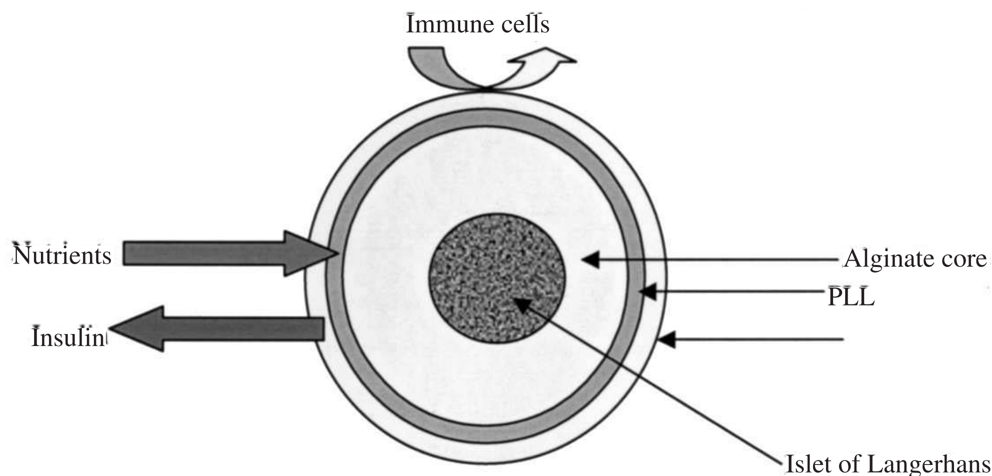

Fig. 1. Structure of an alginate microcapsule. Immune cellsare too large to penetrate the barrier whereas nutrients and insulin can easily diffuse in an out of the capsule.

breakage is spread over a large number of capsules, and thus if one capsule breaks the whole graft is not lost. Another advantage in comparison with macroencapsulation is that the distance of diffusion is decreased. Moreover, the spherical shape of the microcapsules is optimal in reducing a foreign body reaction, due to the absence of corners [83].

Microencapsulation

Chang first described microencapsulation in 1964 [8] and this technique was used by Lim and Sun in 1980 to encapsulate islets of Langerhans [55]. Microencapsulation has been used experimentally with a variety of cell types including PC12 cells [103] for the treatment of Parkinson's disease, hepatocytes [53] for the treatment of liver disease and parathyroid tissue [34] for hypoparathyroidism. Moreover, this technique has been used for the encapsulation of genetically modified cells producing, for example, factor IX for the treatment of haemophilia B [38] or growth hormone for the treatment of dwarfism [9]. However, the most common application is probably in the microencapsulation of islets of Langerhans, which has been widely studied [12,24,97].

Production of microcapsules

The most common material used for microencapsulation is alginate, although other materials have been used such as hydroxyethyl methacrylate methyl methacrylate (HEMA-MMA) [54] and polyethylene glycol (PEG) [67]. Alginate is an intercellular matrix polysaccharide in brown algae and is produced as an extracellular coating by some types of bacteria [60]. It is non-toxic and its gelling properties make it ideal for the immobilisation of cells under mild conditions. The polysaccharide consists of regions of mannuronic acid (M-blocks), regions of guluronic acid (G blocks) and regions of mixed sequence (MG-blocks). The ratio and sequence of these uronic acids differ depending on the source of the alginate and can determine the properties of the alginate [84].

To form spherical beads, islets are suspended in liquid alginate and then pushed through a nozzle to form a droplet of alginate around the islet (Fig. 2). The beads of alginate formed fall into a solution containing divalent cations such as calcium, which gels the alginate beads. An electrostatic potential is created between the needle of the droplet generator and the $CaCl_2$ solution, thus pulling down the beads rapidly before large droplets form. This allows the production of uniformly sized beads, the size of which can be regulated by the voltage. As well as acting as an electrode, a metal rod submerged in the $CaCl_2$ solution controls the level of the liquid by use of a vacuum. This is achieved through a hole in the hollow rod that is at the solution surface. Thus, when the volume of the solution increases as beads accumulate, the excess liquid is removed. This ensures that the distance between the needle and the solution surface is constant, both within and between experiments.

After the alginate beads have gelled for at least 5 min, the beads are washed in saline. To decrease the porosity of the alginate bead and to increase the strength of the capsule, a layer of poly-L-lysine (PLL) is added. This is done by shaking the beads in a solution of PLL for 5–10 min. However, the positively charged PLL facil-

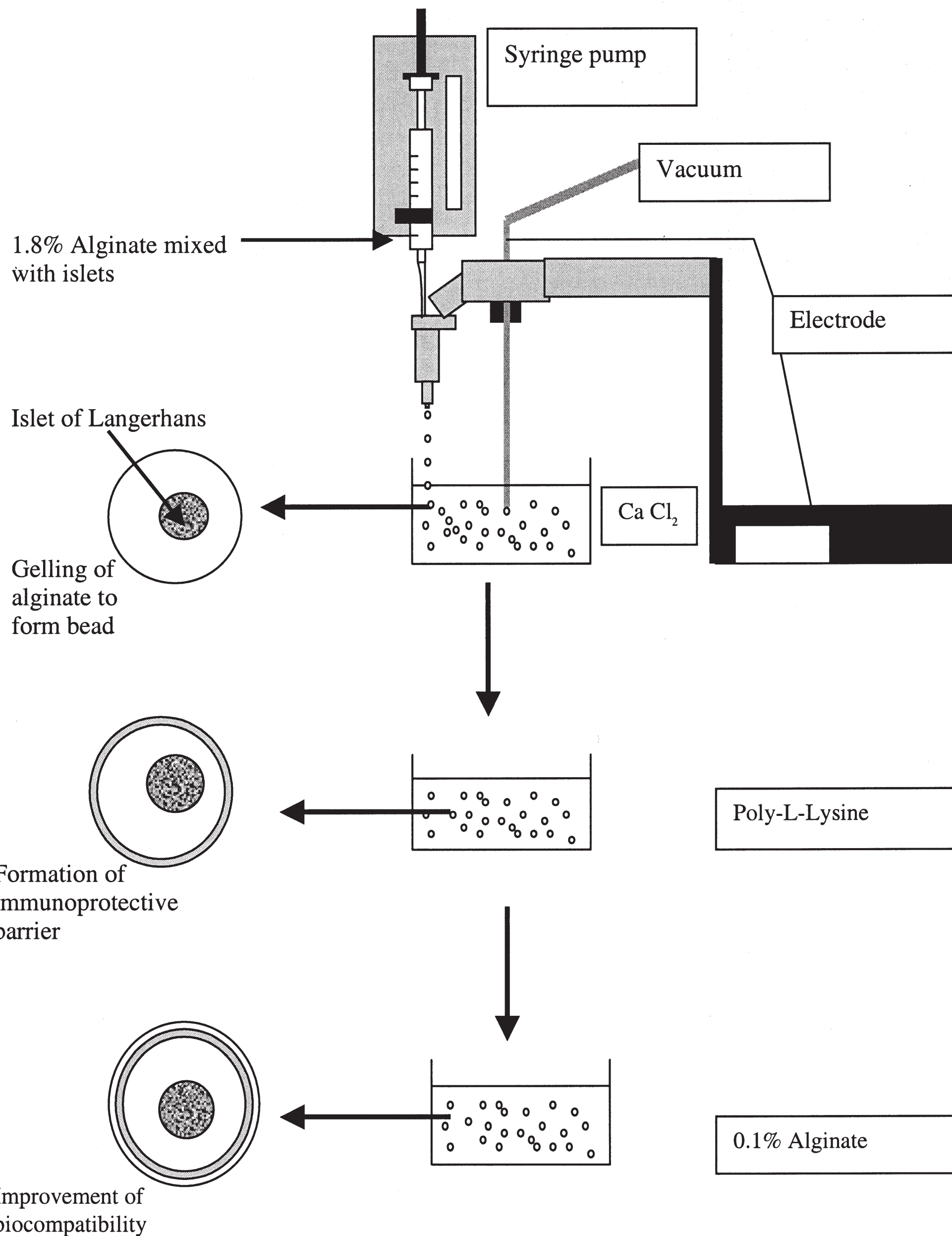

Fig. 2. Production of alginate/poly-L-lysine/alginate capsules.

itates adhesion of host cells to the surface of the capsule and thus a final layer of alginate is added to shield the PLL and improve biocompatibility. Capsules produced in this manner are approximately 500 mm in diameter. Capsules containing islets are then picked from the empty capsules formed and cultured in the same way as naked islets or transplanted.

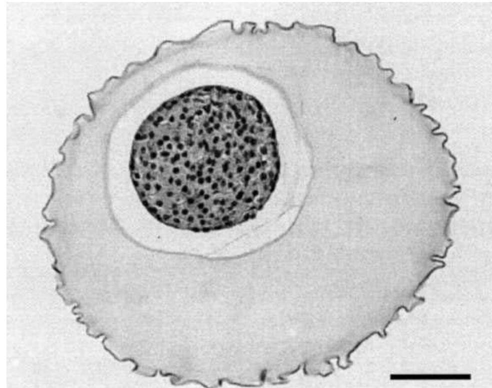

Fig. 3. Micrograph of a microencapsulated C57BL/6 islet one week after microencapsulation. Haematoxylin and eosin staining. The capsule is deformed by the histological processing. Bar is approximately 100 μm.

In vitro function of microencapsulated islets of Langerhans

For successful transplantation of encapsulated islets, it is essential that the islets function properly. Thus, islets should not be detrimentally affected by the microencapsulation process or the presence of the capsule. Indeed, it has been shown that microencapsulated mouse islets have similar glucose oxidation rates and insulin release rates as naked islets the day after microencapsulation [46]. Moreover, the histology of the microencapsulated islets appears normal after 1 week culture within the capsule (Fig. 3).

However, the insulin secretion of rat islets seems to be slightly suppressed after microencapsulation [44,91], although one study has showed that this initial suppression of the insulin secretion of microencapsulated rat islets is reversed after 6 days culture [75].

In a perifusion system, microencapsulated rat islets have been shown to respond to high glucose challenges as quickly as naked islets [14]. However, the absolute amount of insulin released was decreased compared with naked islets. This may have been due to the use of citrate to liquefy the centre of the capsules. Indeed, it has since been shown that the use of citrate severely inhibits insulin secretion from islets [30].

The composition of the alginate does not affect the viability or function of cultured microencapsulated islets [14]. However, it has been suggested that PLL in capsules may be toxic to encapsulated Jurkat cells [85], although no such studies have been carried out with islets.

The viability of microencapsulated cells is dependent on the supply of nutrients and protection from adverse components of the host's immune system. The supply of nutrients is unlikely to be a problem during culture, unless the porosity of the capsule is too low. However, the supply of nutrients *in vivo* could be affected by a variety of factors including implantation site and the host response to the capsules.

The peritoneal cavity as an implantation site

Due to the large volume of microencapsulated islet grafts required to reverse hyperglycaemia, the peritoneal cavity has to be used as a transplantation site. Interestingly, this was the first site used for experimental islet transplantation [5]. It is easily accessible and thus the surgical risk is minimised for implantation of capsules. Although the peritoneal cavity is not the physiological route for insulin delivery, studies using insulin pumps have shown that blood glucose concentrations are lowered by intraperitoneal delivery of insulin [95]. The peritoneal cavity is, however, a rather harsh environment compared to the other more commonly used sites for islet transplantation, such as the intraportal site or beneath the kidney capsule. These sites are richly vascularised, as are endogenous islets [40] but there is a low oxygen tension in the peritoneal cavity [43,65]. Moreover, the peritoneal cavity is rich in macrophages [35], which is the most active cell type in the host response to biomaterials. Indeed, it has been shown that islet transplantation to the peritoneal cavity requires an increased islet mass [79].

Host responses to capsules

The host inflammatory reaction is a normal response to the implantation of a biomedical device. However, it is important that this reaction does not interfere with the function of the microencapsulated islets. The reaction usually starts with adsorption of proteins on to the surface of the biomedical polymer, the most important being fibrinogen [90]. Macrophages may then adhere to the surface and produce cytokines such as IL-1β, TNF-α and TGF-β, which further activate macrophages and fibroblasts [3,33,39,71,90]. Empty microcapsules induce a foreign body reaction with a cellular overgrowth, which is metabolically active as indicated by the glucose oxidation rates [45,46]. Indeed, the glucose oxidation rates of the cellular overgrowth on 10 empty capsules are often in the range of that for 10 naked islets. It can thus be speculated that the cellular overgrowth could act as a metabolic barrier as well as a physical barrier to nutrient diffusion. The host response to the capsules can be affected by the composition of the capsule and biological factors produced by the encapsulated cells and/or the recipient.

Effect of capsule composition on cellular overgrowth

When lower concentrations of PLL (0.05%) are used in capsules, biocompatibility is improved [45]. Moreover, the cellular overgrowth on capsules is markedly reduced when PLL is omitted from the capsule [45]. Indeed, the integrity of the capsules is important, as any exposure of PLL in the capsule increases the cellular reaction against the capsule [25,99]. With regard to the type of alginate used in the capsule, it has been reported that alginate with a high mannuronic acid content is more effective in eliciting an immune response [81,99], whereas others have claimed that alginate with a high mannuronic acid content is less immunogeneic [22]. It has been suggested that these discrepancies may depend on the purity of the alginate [97]. Differences in comparing alginate *per se* [81] or intact capsules [22] may also account for differences reported in these studies.

Biological responses affecting the cellular overgrowth on capsules

It has been shown in several studies that macrophages are the main cell type involved in the response against capsules [29,78]. Indeed, even when capsules are transplanted to nude mice and rats, the response is intensive [14,75,98], indicating that T-cells are not involved in this process.

The presence of cells within capsules increases the cellular overgrowth on the capsules [37,46,78,98,102,104]. This may be due to shedding of cellular proteins from the encapsulated islets evoking an immune response [31], or the release of chemokines from encapsulated cells. Indeed, it has been suggested that cells within islets may produce cytokines such as IL-1 [100], which could increase the inflammatory response. Insulin released from islets could also affect, for example, the metabolism and function of macrophages [19].

The role of nitric oxide (NO) in the host response to capsules has been investigated by studying the cellular overgrowth on capsules implanted to knockout mice lacking iNOS [45]. NO is an effector molecule in macrophages and has previously been detected in macrophages in foreign body inflammation [61]. Interestingly, there was a tendency for an increased foreign body reaction to capsules transplanted in the mice lacking iNOS. Furthermore, iNOS-deficient mice have shown more severe trinitrobenzene-induced colitis, indicating that NO can play a protective role in this model of inflammation [59]. Indeed, NO can act as either a proinflammatory or anti-inflammatory molecule [11] and in this model of cellular overgrowth on implanted capsules it seems to act as an anti-inflammatory molecule. This may be due to the anti-adhesive properties of NO [36,48]. However, NO has been shown to be detrimental to microencapsulated islets co-cultured with macrophages [101] and from this viewpoint, NO should be avoided. It has been previously reported that cellular overgrowth differs between species and is increased in animal models of diabetes such as the BB rat [15]. It has thus been suggested that cytokines may be involved in this process. Moreover, it has been shown that microcapsules can induce IL-1 production from macrophages in vitro [69]. Indeed, there are differences in the host response to capsules depending on the mouse strain used [45]. C57BL/6 mice have a more severe host reaction to capsules than BALB/c mice. Furthermore, C57BL/6 mice also have an increased mRNA expression of IL-1β and TNF-α in their peritoneal macrophages after capsule implantation compared with BALB/c mice [45], suggesting a role of these cytokines in the reaction against the capsules.

Consequences of cellular overgrowth on the capsule

A foreign body reaction and the resulting cellular overgrowth on the capsule may be detrimental for encapsulated islets for two reasons. First, it may cause a physical and metabolic barrier to effective nutrient diffusion into the capsule. Second, the islets may be exposed to macrophage-derived cytokines, for example interleukin-1β (IL-1β) and tumour necrosis factor (TNF-α), which are known to be detrimental to the function of the islet.

Macrophages and fibroblasts account for the majority of cells found in the cellular

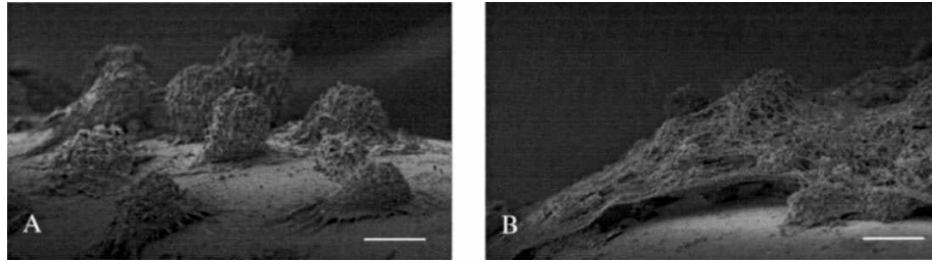

Fig. 4. Cells attached to the surface of capsules (A) and fibre production by cells on the capsule (B). Scanning Electron Micrographs of empty capsules retrieved 1 week after intraperitoneal implantation to C57BL/6 mice. The bars are 10 μm.

overgrowth on capsules [78,99]. Both of these cell types have a high metabolic rate with high rates of glucose utilisation [64,70]. These cells attach to the surface of the capsule (Fig. 4A) and it can be assumed that the nutrient diffusion to the islets within the capsules is decreased due to the presence of these cells. Moreover, fibroblasts produce different types of fibres (Fig. 4B), which could act as a physical barrier to nutrient diffusion.

The cytokines IL-1β and TNF-α are produced in activated macrophages. The expression of these cytokines has been shown to occur in macrophages in contact with biomaterials [47,94]. Moreover, these cytokines have been implicated in the pathogenesis of type 1 diabetes and have been shown to inhibit the function of pancreatic islets [57,58,76]. Experimental evidence has also suggested a role for these cytokines in rejection of allografts [73,74]. One effect of these cytokines on β-cells is the induction of inducible nitric oxide synthase (iNOS) and the subsequent production of NO [82]. NO has several detrimental effects on the islets including inhibition of the mitochondrial enzyme aconitase and induction of DNA damage [26], although some of the suppressive effects of IL-1β on islets of Langerhans are independent of NO [2,28]. Nevertheless, NO has been shown to suppress encapsulated islets co-cultured with macrophages [101] and it is therefore evident that cytokine and/or NO production in the vicinity of the islets is highly undesirable.

We have shown that microencapsulated rat islets are functionally suppressed after exposure to the macrophage derived cytokines IL-1β and TNF-α, indicating the permeability of the capsule used to these cytokines [44]. At high concentrations of IL-1β (10 U/ml and 25 U/ml), microencapsulated islets were more suppressed than naked islets exposed to the same activities of the cytokines. After a 48 h exposure to 25 U/ml IL-1β, neither microencapsulated islets nor naked islets were able to recover their function after 6 days. However, after a 48 h exposure to 2.5 U/ml IL-1β, both microencapsulated and naked islets were able to regain approximately 90 % of their function after a 6-day recovery period. These results showed that the alginate/PLL/alginate capsules used did not confer protection against the effects of cytokines. However, microencapsulated islets can recover after a short-term exposure to low concentrations of IL-1β.

Other studies have shown contradictory results with regard to the permeabili-

ty of capsules to cytokines. This is probably due to different types of capsules in those studies. Cole *et al* also found that IL-1β and TNF-α were able to penetrate alginate/PLL/alginate capsules [16]. Tai *et al* claimed that alginate/PLL/alginate capsules protected islets against functional suppression induced by IL-1 and TNF [89]. A high concentration of PLL (0.5%) was used in that study, which would markedly decrease the porosity of the capsule. It should be pointed out in this context that decreasing the porosity of the capsule may reduce its permeability to nutrients. In a study by Kulseng *et al*, IL-1β but not TNF-α penetrated the capsule [49]. The exclusion of TNF-α may be due to a larger capsule being used, which is more stable to swelling and thus the porosity is not changed.

With regard to the permeability of cytokines, the three dimensional structure of the compound should be considered. TNF-α forms conical shaped trimers with a molecular weight of approximately 55 kD [42,80]. The three dimensional structure of this protein may therefore impede its penetration into the capsule. It could therefore be feasible to exclude TNF-α from capsules, whereas preventing the penetration of IL-1β may be more problematic. IL-1β is a small protein with a molecular weight of 17.5 kD. It is likely that if the porosity of the capsule were reduced to prevent the penetration of IL-1β, insulin diffusion would probably be hindered. Thus, it would be more desirable to prevent cellular overgrowth on capsules and consequently inhibit the production of cytokines in the vicinity of the microencapsulated islets.

In vivo function of microencapsulated islets

Several studies have shown that hyperglycaemia in rodents can be reversed by implantation of microencapsulated islets, although the long-term function of such grafts has been limited [13,20,66]. Some studies have linked the failure of the graft with cellular overgrowth of the capsules [12,16,29]. However, it has also been suggested that grafts can fail due to an imbalance in the birth to death ratio of the b-cells [23,97]. Indeed, it is evident that the microencapsulation of islets and/or transplantation to the peritoneal cavity increases the number of islets required to reverse hyperglycaemia in rodents [13,79]. The insulin response from microencapsulated islets transplanted intraperitoneally in mice tends to be somewhat delayed after a meal challenge, although this delay does not prevent the normalisation of blood glucose concentrations [91].

In studies from our own laboratory, there is a clear correlation between the success of encapsulated islets in reversing hyperglycaemia and the capsules' biocompatibility. Islets in capsules that had previously been shown to have poor biocompatibility failed to reverse hyperglycaemia for more than a few days, whereas islets within capsules with better biocompatibility were functional for several weeks (unpublished results). Indeed, in grafts that had failed, cellular overgrowth on the capsule surface was seen (Fig. 5).

In a recent study by Duvivier-Kali *et al* [27], a simple barium-alginate bead protected islets in an allograft and allowed function for over 1 year. It had previously

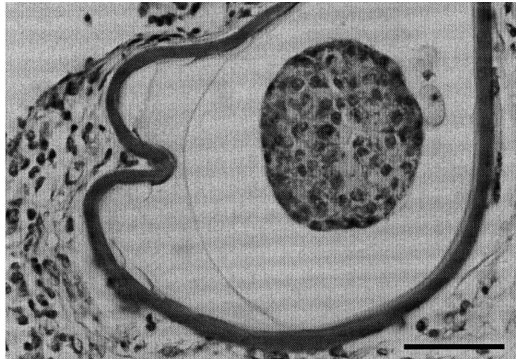

Fig. 5. Micrograph of a microencapsulated BALB/c islet four weeks after implantation to a diabetic C57BL/6 mouse. Haematoxylin and eosin staining. The capsule is deformed by the histological processing. Bar is approximately 100 μm.

been assumed that PLL was required to reduce porosity to an appropriate level to prevent allograft rejection. However, it is evident from that study that a simple barium alginate bead prevents cell-mediated rejection. Moreover, the improvement in biocompatibility of the capsules by omitting PLL has most likely also played an important role in the success of this model. It should be noted, however, that the capsules used were rather large (approximately 1 mm) and it remains to be seen whether the same results could be achieved with smaller capsules.

Concluding remarks

It is obvious from the present and previous studies that good biocompatibility is an essential property of alginate/PLL/alginate capsules for successful use in transplantation of islets. The exclusion of immune cells is probably adequate to avoid immune rejection in allogeneic transplants. For xenogeneic transplantation it is, however, likely that antibodies and complement have to be excluded from the capsules. It has been argued that small capsules should be used to decrease graft volume and diffusion distances [10], although such capsules are associated with an increased risk of protruding islets [21]. The use of smaller capsules should make it possible to implant the capsules intraportally. Indeed, recent studies have investigated implanting smaller capsules into the liver [52], which may be a more suitable site than the peritoneal cavity. However, the safety of this site has to be more thoroughly investigated before it can be considered as an alternative. It is evident that scientific investigations in the field of microencapsulation of islets have provided new, vital information in the pursuit of the end goal: transplantation of microencapsulated islets to diabetes patients.

ACKNOWLEDGEMENTS

Our own studies referred to in this review were supported by grants from BIOMed 5 of the European Community (QLRT-1999-00034), the Swedish Research Coun-

cil (72X-109, 72X-9237), the Swedish Diabetes Association, the Novo-Nordisk Insulin Fund, the Family Ernfors Fund, and the Juvenile Diabetes Foundation International and the Wallenberg Foundation. Excellent technical assistance was provided by Ing-Britt Hallgren, Astrid Nordin and Eva Törnelius. Thanks to Prof. Arne Andersson and Prof. Stellan Sandler for their expert advice on this manuscript.

REFERENCES

1. Andersson, A., Borg, H., Hallberg, A., Hellerström, C., Sandler, S. & Schnell, A.: Long-term effects of cyclosporin A on cultured mouse pancreatic islets: Diabetologia 27 Suppl:66–69, 1984.
2. Andersson, A. K., Flodström, M. & Sandler, S.: Cytokine-induced inhibition of insulin release from mouse pancreatic beta-cells deficient in inducible nitric oxide synthase: Biochem Biophys Res Commun 281:396–403, 2001.
3. Babensee, J. E., Anderson, J. M., McIntire, L. V. & Mikos, A. G.: Host response to tissue engineered devices: Adv Drug Deliv Rev 33:111–139, 1998.
4. Bach, J. F.: Insulin-dependent diabetes mellitus as an autoimmune disease: Endocr Rev 15:516–542, 1994.
5. Ballinger, W. & Lacy, P. E.: Transplantation of intact pancreatic islets in rats.: Surgery 72:175–186, 1972.
6. Brendel, M., Hering, B., Schultz, A. & Bretzel, R. G.: International islet transplant registry.: Islet transplantation registry Newsletter 8:1:3–18, 2001.
7. Chaikof, E.: Engineering and material considerations in islet cell transplantation: Annu Rev Biomed Eng 1:103–127, 1999.
8. Chang, T. M.: Semipermeable microcapsules: Science 146:524–525, 1964.
9. Cheng, W. T., Chen, B. C., Chiou, S. T. & Chen, C. M.: Use of nonautologous microencapsulated fibroblasts in growth hormone gene therapy to improve growth of midget swine: Hum Gene Ther 9:1995–2003, 1998.
10. Chicheportiche, D. & Reach, G.: In vitro kinetics of insulin release by microencapsulated rat islets: effect of the size of the microcapsules: Diabetologia 31:54–57, 1988.
11. Clancy, R. M., Amin, A. R. & Abramson, S. B.: The role of nitric oxide in inflammation and immunity: Arthritis Rheum 41:1141–1151, 1998.
12. Clayton, H. A., James, R. F. & London, N. J.: Islet microencapsulation: a review: Acta Diabetol 30:181–189, 1993.
13. Clayton, H. A., London, N. J., Bell, P. R. & James, R. F.: The transplantation of encapsulated islets of Langerhans into the peritoneal cavity of the biobreeding rat: Transplantation 54:558–560, 1992.
14. Clayton, H. A., London, N. J., Colloby, P. S., Bell, P. R. & James, R. F.: A study of the effect of capsule composition on the viability of cultured alginate/poly-l-lysine–encapsulated rat islets: Diabetes Res 14:127–132, 1990.
15. Clayton, H., London, N., Colloby, P. & Bell, P.: The effect of capsule composition on the biocompatibility of alginate-poly-lysine capsules.: J Microencapsulation 8:221–233, 1991.
16. Cole, D. R., Waterfall, M., McIntyre, M. & Baird, J. D.: Microencapsulated islet grafts in the BB/E rat: a possible role for cytokines in graft failure: Diabetologia 35:231–237, 1992.
17. Colton, C. K.: Implantable biohybrid artificial organs: Cell Transplant 4:415–436, 1995.
18. Colton, C. K. & Avgoustiniatos, E. S.: Bioengineering in development of the hybrid artificial pancreas: J Biomech Eng 113:152–170, 1991.
19. Costa Rosa, L. F., Safi, D. A., Cury, Y. & Curi, R.: The effect of insulin on macrophage metabolism and function: Cell Biochem Funct 14:33–42, 1996.
20. Darquy, S., Chicheportiche, D., Capron, F., Boitard, C. & Reach, G.: Comparative study of microencapsulated rat islets implanted in different diabetic models in mice: Horm Metab Res Suppl 25:209–213, 1990.
21. De Vos, P., De Haan, B., Pater, J. & Van Schilfgaarde, R.: Association between capsule diameter, adequacy of encapsulation, and survival of microencapsulated rat islet allografts: Transplantation 62:893–899, 1996.
22. De Vos, P., De Haan, B. & Van Schilfgaarde, R.: Effect of the alginate composition on the biocompatibility of alginate-polylysine microcapsules: Biomaterials 18:273–278, 1997.
23. De Vos, P., Van Straaten, J. F., Nieuwenhuizen, A. G., de Groot, M., Ploeg, R. J., De Haan, B. J. & Van Schilfgaarde, R.: Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth?: Diabetes 48:1381–1388, 1999.
24. De Vos, P., Wolters, G. H., Fritschy, W. M. & Van Schilfgaarde, R.: Obstacles in the application of microencapsulation in islet transplantation: Int J Artif Organs 16:205–212, 1993.
25. De Vos, P., Wolters, G. H. & Van Schilfgaarde, R.: Possible relationship between fibrotic overgrowth of alginate- polylysine-alginate microencapsulated pancreatic islets and the microcapsule integrity: Transplant Proc 26:782–783, 1994.
26. Delaney, C. A. & Eizirik, D. L.: Intracellular targets for nitric oxide toxicity to pancreatic beta- cells: Braz J Med Biol Res 29:569–579, 1996.

27. Duvivier-Kali, V. F., Omer, A., Parent, R. J., O'Neil, J. J. & Weir, G. C.: Complete protection of islets against allorejection and autoimmunity by a simple barium-alginate membrane: Diabetes 50:1698–1705, 2001.
28. Eizirik, D. L., Sandler, S., Welsh, N., Cetkovic-Cvrlje, M., Nieman, A., Geller,D.A., Pipeleers,D.G., Bendtzen,K. & Hellerstrom,C.: Cytokines suppress human islet function irrespective of their effects on nitric oxide generation: J Clin Invest 93:1968–1974, 1994.
29. Fritschy, W. M., De Vos, P., Groen, H., Klatter, F. A., Pasma, A., Wolters, G. H. & Van Schilfgaarde, R.: The capsular overgrowth on microencapsulated pancreatic islet grafts in streptozotocin and autoimmune diabetic rats: Transpl Int 7:264–271, 1994.
30. Fritschy, W. M., Wolters, G. H. & Van Schilfgaarde, R.: Effect of alginate-polylysine-alginate microencapsulation on in vitro insulin release from rat pancreatic islets: Diabetes 40:37–43, 1991.
31. Gray, D.: Encapsulated islet cells: the role of direct and indirect presentation and the relevance to xenotransplantation and autoimmune recurrence.: Brit Med Bull 53:777, 1997.
32. Gummert, J. F., Ikonen, T. & Morris, R. E.: Newer immunosuppressive drugs: a review: J Am Soc Nephrol 10:1366–1380, 1999.
33. Hanker, J. S. & Giammara, B. L.: Biomaterials and biomedical devices: Science 242:885–892, 1988.
34. Hasse, C., Bohrer, T., Barth, P., Stinner, B., Cohen, R., Cramer, H., Zimmermann, U. & Rothmund, M.: Parathyroid xenotransplantation without immunosuppression in experimental hypoparathyroidism: long-term In vivo function following microencapsulation with a clinically suitable alginate: World J Surg 24:1361–1366, 2000.
35. Heel, K. A. & Hall, J. C.: Peritoneal defences and peritoneum-associated lymphoid tissue: Br J Surg 83:1031–1036, 1996.
36. Hickey, M. J., Sharkey, K. A., Sihota, E. G., Reinhardt, P. H., MacMicking, J. D., Nathan, C. & Kubes, P.: Inducible nitric oxide synthase-deficient mice have enhanced leukocyte- endothelium interactions in endotoxemia: FASEB J 11:955–964, 1997.
37. Horcher, A., Zekorn, T., Siebers, U., Klock, G., Frank, H., Houben, R., Bretzel, R. G., Zimmermann, U. & Federlin, K.: Transplantation of microencapsulated islets in rats: evidence for induction of fibrotic overgrowth by islet alloantigens released from microcapsules: Transplant Proc 26:784–786, 1994.
38. Hortelano, G., Xu, N., Vandenberg, A., Solera, J., Chang, P. L. & Ofosu, F. A.: Persistent delivery of factor IX in mice: gene therapy for hemophilia using implantable microcapsules: Hum Gene Ther 10:1281–1288, 1999.
39. Hunt, J. A., McLaughlin, P .J. & Flanagan, B. F.: Techniques to investigate cellular and molecular interactions in the host response to implanted biomaterials: Biomaterials 18:1449–1459, 1997.
40. Jansson, L.: The regulation of pancreatic islet blood flow: Diabetes Metab Rev 10:407–416, 1994.
41. Jindal, R. M.: Posttransplant diabetes mellitus–a review: Transplantation 58:1289–1298, 1994.
42. Jones, E. Y., Stuart, D. I. & Walker, N. P.: Structure of tumour necrosis factor: Nature 338:225–228, 1989.
43. Kaur, S., Cortiella, J. & Vacanti, C. A.: Identifying a site for maximum delivery of oxygen to transplanted cells: Tissue Eng 6:229–232, 2000.
44. King, A., Andersson, A. & Sandler, S.: Cytokine-induced functional suppression of microencapsulated rat pancreatic islets in vitro: Transplantation 70:380–383, 2000.
45. King, A., Sandler, S. & Andersson, A.: The effect of host factors and capsule composition on the cellular overgrowth on implanted alginate capsules: J Biomed Mater Res 57:374–383, 2001.
46. King, A., Sandler, S., Andersson, A., Hellerstrom, C., Kulseng, B. & Skjak-Braek, G.: Glucose metabolism in vitro of cultured and transplanted mouse pancreatic islets microencapsulated by means of a high-voltage electrostatic field: Diabetes Care 22 Suppl 2:B121–B126, 1999.
47. Kishida, A., Kato, S., Ohmura, K., Sugimura, K. & Akashi, M.: Evaluation of biological responses to polymeric biomaterials by RT-PCR analysis. I. Study of IL-1 beta mRNA expression: Biomaterials 17:1301–1305, 1996.
48. Kubes, P., Suzuki, M. & Granger, D.N.: Nitric oxide: an endogenous modulator of leukocyte adhesion: Proc Natl Acad Sci U S A 88:4651–4655, 1991.
49. Kulseng, B., Thu, B., Espevik, T. & Skjak-Braek, G.: Alginate polylysine microcapsules as immune barrier: permeability of cytokines and immunoglobulins over the capsule membrane: Cell Transplant 6:387–394, 1997.
50. Landgraf, R.: Impact of pancreas transplantation on diabetic secondary complications and quality of life: Diabetologia 39:1415–1424, 1996.
51. Lanza, R. P., Sullivan, S. J. & Chick, W. L.: Perspectives in diabetes. Islet transplantation with immunoisolation: Diabetes 41:1503–1510, 1992.
52. Leblond, F. A., Simard, G., Henley, N., Rocheleau, B., Huet, P. M. & Halle, J. P.: Studies on smaller (approximately 315 microM) microcapsules: IV. Feasibility and safety of intrahepatic implantations of small alginate poly-L-lysine microcapsules: Cell Transplant 8:327–337, 1999.
53. Legallais, C. & Doré, D. E.: Bioartificial livers (BAL): current technological aspects and future developments: J Membrane Sci 181:81–95, 2001.
54. Li, R. H.: Materials for immunoisolated cell transplantation: Adv Drug Deliv Rev 33:87–109, 1998.
55. Lim, F. & Sun, A. M.: Microencapsulated islets as bioartificial endocrine pancreas: Science 210:908–910, 1980.
56. Maki, T., Otsu, I., O'Neil, J. J., Dunleavy, K., Mullon, C. J., Solomon, B. A. & Monaco, A. P.: Treatment of diabetes by xenogeneic islets without immunosuppression. Use of a vascularized bioartificial pancreas: Diabetes 45:342–347, 1996.
57. Mandrup-Poulsen, T.: The role of interleukin-1 in the pathogenesis of IDDM: Diabetologia 39:1005–1029, 1996.

58. Mandrup-Poulsen, T., Bendtzen, K., Dinarello, C. A. & Nerup, J.: Human tumor necrosis factor potentiates human interleukin 1-mediated rat pancreatic beta-cell cytotoxicity: J Immunol 139:4077–4082, 1987.
59. Mashimo, H. & Goyal, R. K.: Lessons from genetically engineered animal models. IV. Nitric oxide synthase gene knockout mice: Am J Physiol 277:G745–G750, 1999.
60. Moe, S., Draget, K., Skjåk-Braek, G. & Smidsrod, O.: Alginates: 245–286, 1995.
61. Moilanen, E., Moilanen, T., Knowles, R., Charles, I., Kadoya, Y., al Saffar, N., Revell, P. A. & Moncada, S.: Nitric oxide synthase is expressed in human macrophages during foreign body inflammation: Am J Pathol 150:881–887, 1997.
62. Mor, E., Yussim, A., Chodoff, L. & Schwartz, M. E.: New immunosuppressive agents for maintenance therapy in organ transplantation. Focus on adverse effects: Biodrugs 8:469–488, 1997.
63. Mullen, Y., Maruyama, M. & Smith, C. V.: Current progress and perspectives in immunoisolated islet transplantation: J Hepatobiliary Pancreat Surg 7:347–357, 2000.
64. Newsholme, P., Gordon, S. & Newsholme, E. A.: Rates of utilization and fates of glucose, glutamine, pyruvate, fatty acids and ketone bodies by mouse macrophages: Biochem J 242:631–636, 1987.
65. Noth, U., Grohn, P., Jork, A., Zimmermann, U., Haase, A. & Lutz, J.: 19F-MRI in vivo determination of the partial oxygen pressure in perfluorocarbon-loaded alginate capsules implanted into the peritoneal cavity and different tissues: Magn Reson Med 42:1039–1047, 1999.
66. O'Shea, G. M. & Sun, A. M.: Encapsulation of rat islets of Langerhans prolongs xenograft survival in diabetic mice: Diabetes 35:943–946, 1986.
67. Panza, J. L., Wagner, W. R., Rilo, H. L., Rao, R. H., Beckman, E. J. & Russell, A. J.: Treatment of rat pancreatic islets with reactive PEG: Biomaterials 21:1155–1164, 2000.
68. Penn, I.: Post-transplant malignancy: the role of immunosuppression: Drug Saf 23:101–113, 2000.
69. Pueyo, M. E., Darquy, S., Capron, F. & Reach, G.: In vitro activation of human macrophages by alginate-polylysine microcapsules: J Biomater Sci Polym Ed 5:197–203, 1993.
70. Ramchand, C. N., Gliddon, A. E., Clark, A. E. & Hemmings, G. P.: Glucose oxidation and monoamine oxidase activity from the fibroblasts of schizophrenic patients and controls: Life Sci 56:1639–1646, 1995.
71. Rihova, B.: Immunocompatibility and biocompatibility of cell delivery systems: Adv Drug Deliv Rev 42:65–80, 2000.
72. Ryan, E. A., Lakey, J. R., Rajotte, R. V., Korbutt, G. S., Kin, T., Imes, S., Rabinovitch, A., Elliott, J. F., Bigam, D., Kneteman, N. M., Warnock, G. L., Larsen, I. & Shapiro, A. M.: Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol: Diabetes 50:710–719, 2001.
73. Sabatine, M. S., Laufer, T., Glimcher, L. H., Widmer, M., Winn, H. & Auchincloss, H., Jr.: Delayed rejection of soluble tumor necrosis factor receptor-secreting tumor allografts: Transplantation 65:113–120, 1998.
74. Sandberg, J. O., Eizirik,D.L., Sandler,S., Tracey,D.E. & Andersson,A.: Treatment with an interleukin-1 receptor antagonist protein prolongs mouse islet allograft survival: Diabetes 42:1845–1851, 1993.
75. Sandler, S., Andersson, A., Eizirik, D. L., Hellerstrom, C., Espevik, T., Kulseng, B., Thu, B., Pipeleers, D. G. & Skjak-Braek, G.: Assessment of insulin secretion in vitro from microencapsulated fetal porcine islet-like cell clusters and rat, mouse, and human pancreatic islets: Transplantation 63:1712–1718, 1997.
76. Sandler, S., Eizirik, D. L., Svensson, C., Strandell, E., Welsh, M. & Welsh, N.: Biochemical and molecular actions of interleukin-1 on pancreatic b-cells: Autoimmunity 10:241–253, 1991.
77. Shapiro, A. M., Lakey, J. R., Ryan, E. A., Korbutt, G. S., Toth, E., Warnock, G. L., Kneteman,N. M. & Rajotte, R. V.: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen: N Engl J Med 343:230–238, 2000.
78. Siebers, U., Horcher, A., Brandhorst, H., Brandhorst, D., Hering, B., Federlin, K., Bretzel, R. G. & Zekorn, T.: Analysis of the cellular reaction towards microencapsulated xenogeneic islets after intraperitoneal transplantation: J Mol Med 77:215–218, 1999.
79. Siebers, U., Horcher, A., Bretzel, R. G., Klock, G., Zimmermann, U., Federlin, K. & Zekorn, T.: Transplantation of free and microencapsulated islets in rats: evidence for the requirement of an increased islet mass for transplantation into the peritoneal site: Int J Artif Organs 16:96–99, 1993.
80. Smith, R. A. & Baglioni, C.: The active form of tumor necrosis factor is a trimer: J Biol Chem 262:6951–6954, 1987.
81. Soon-Shiong, P., Otterlie, M., Skjak-Braek, G., Smidsrod, O., Heintz, R., Lanza, R. P. & Espevik, T.: An immunologic basis for the fibrotic reaction to implanted microcapsules: Transplant Proc 23:758–759, 1991.
82. Southern, C., Schulster, D. & Green, I. C.: Inhibition of insulin secretion by interleukin-1 beta and tumour necrosis factor-alpha via an L-arginine-dependent nitric oxide generating mechanism: FEBS Lett 276:42–44, 1990.
83. Spector, M., Cease, C. & Tong-Li, X.: The local tissue response to biomaterials: Critical Reviews in Biocompatibility 5:269–295, 1989.
84. Strand, B. L., Mörch, Y. A. & Skjåk-Braek, G.: Alginate as immbolization matrix for cells: Minerva biotecnologica 12:223–233, 2001.
85. Strand, B. L., Ryan, L., In't Veld, P., Kulseng B., Rokstad, A. M., Skjåk-Braek, G. & Espevik, T.: Poly-L-lysine induces fibrosis on alginate microcapsules via the induction of cytokines: Cell Transplantation 10:263–275, 2001.
86. Sutherland, D. E., Gruessner, R. W. & Gruessner, A. C.: Pancreas transplantation for treatment of diabetes mellitus: World J Surg 25:487–496, 2001.

87. Suzuki, K., Bonner-Weir, S., Hollister-Lock, J., Colton, C. K. & Weir, G. C.: Number and volume of islets transplanted in immunobarrier devices: Cell Transplant 7:47–52, 1998.
88. Suzuki, K., Bonner-Weir, S., Trivedi, N., Yoon, K. H., Hollister-Lock, J., Colton, C. K. & Weir, G. C.: Function and survival of macroencapsulated syngeneic islets transplanted into streptozocin-diabetic mice: Transplantation 66:21–28, 1998.
89. Tai, I., Vacek, I. & Sun, A.: The alginate-poly-L-lysine-alginate membrane: Evidence of a protective effect on microencapsulated islets of Langerhans following exposure to cytokines: Xenotransplantation 2:37–45, 1995.
90. Tang, L. & Eaton, J. W.: Natural responses to unnatural materials: A molecular mechanism for foreign body reactions: Mol Med 5:351–358, 1999.
91. Tatarkiewicz, K., Garcia, M., Omer, A., Van Schilfgaarde, R., Weir, G. C. & De Vos, P.: C-peptide responses after meal challenge in mice transplanted with microencapsulated rat islets: Diabetologia 44:646–653, 2001.
92. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus.: N Engl J Med 329:977–986, 1993.
93. Theodorou, N. A., Vrbova, H., Tyhurst, M. & Howell, S. L.: Problems in the use of polycarbonate diffusion chambers for syngeneic pancreatic islet transplantation in rats: Diabetologia 18:313–317, 1980.
94. Thomas, A., Harding, K. G. & Moore, K.: Alginates from wound dressings activate human macrophages to secrete tumour necrosis factor-alpha: Biomaterials 21:1797–1802, 2000.
95. Udelsman, R., Boyne, M.S., Loman, K. E. & Saudek, C. D.: Intraperitoneal delivery of insulin via mechanical pump: surgical implications: Langenbecks Arch Surg 385:367–372, 2000.
96. Uludag, H., De Vos, P. & Tresco, P. A.: Technology of mammalian cell encapsulation: Adv Drug Deliv Rev 42:29–64, 2000.
97. Van Schilfgaarde, R. & De Vos, P.: Factors influencing the properties and performance of microcapsules for immunoprotection of pancreatic islets: J Mol Med 77:199–205, 1999.
98. Vandenbossche, G. M., Bracke, M. E., Cuvelier, C. A., Bortier, H. E., Mareel, M. M. & Remon, J. P.: Host reaction against alginate-polylysine microcapsules containing living cells: J Pharm Pharmacol 45:121–125, 1993.
99. Vandenbossche, G. M., Bracke, M. E., Cuvelier, C. A., Bortier, H. E., Mareel, M. M. & Remon, J. P.: Host reaction against empty alginate-polylysine microcapsules. Influence of preparation procedure: J Pharm Pharmacol 45:115–120, 1993.
100. Welsh, M., Welsh, N., Bendtzen, K., Mares, J., Strandell, E., Oberg, C. & Sandler, S.: Comparison of mRNA contents of interleukin-1 beta and nitric oxide synthase in pancreatic islets isolated from female and male nonobese diabetic mice: Diabetologia 38:153–160, 1995.
101. Wiegand, F., Kroncke, K. D. & Kolb-Bachofen, V.: Macrophage-generated nitric oxide as cytotoxic factor in destruction of alginate-encapsulated islets. Protection by arginine analogs and/or coencapsulated erythrocytes: Transplantation 56:1206–1212, 1993.
102. Wijsman, J., Atkison, P., Mazaheri, R., Garcia, B., Paul, T., Vose, J., O'Shea, G. & Stiller, C.: Histological and immunopathological analysis of recovered encapsulated allogeneic islets from transplanted diabetic BB/W rats: Transplantation 54:588–592, 1992.
103. Winn, S. R., Tresco, P. A., Zielinski, B., Greene, L. A., Jaeger, C. B. & Aebischer, P.: Behavioral recovery following intrastriatal implantation of microencapsulated PC12 cells: Exp Neurol 113:322–329, 1991.
104. Zekorn, T., Endl, U., Horcher, A., Siebers, U., Bretzel, B. & Federlin, K.: Evidence for an antigen-release induced cellular immune response against alginate-polylysine encapsulated islets: Xenotransplantation 2:116–119, 1995.

Corresponding author: Aileen King,
Department of Medical Cell Biology,
Box 571,
Biomedicum
SE 751 23 Uppsala,
Sweden
Telephone: +46 18 4714925
Fax: +46 18 556401
E-mail: aileen.king@medcellbiol.uu.se