ASSOCIATION BETWEEN CAPSULE DIAMETER, ADEQUACY OF ENCAPSULATION, AND SURVIVAL OF MICROENCAPSULATED RAT ISLET ALLOGRAFTS

De Vos, Paul²,³; De Haan, Bart²; Pater, Jenny⁴; Van Schilfgaarde, Reinout²

Author Information
Surgical Research Laboratory, Department of Surgery, and Department of Histology and Cell Biology, University of Groningen, Groningen, The Netherlands

¹This work was supported by a grant from the Diabetes Research Fund of The Netherlands.
²Surgical Research Laboratory, Department of Surgery, University of Groningen.
³Address correspondence to: P. De Vos, Surgical Research Laboratory, Department of Surgery, University of Groningen, Bloemsingel 1, 9713 BZ Groningen, The Netherlands.
⁴Department of Histology and Cell Biology, University of Groningen.
Received 2 January 1996.
Accepted 8 May 1996.

Abstract
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 using small capsules. However, reduction of the diameter of the capsules holds a certain risk, because 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 that are insufficiently immunoprotected as a consequence of inadequate, and particularly incomplete, encapsulation. 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 diameter (700-800 µm) and small diameter (400-500 µm) capsules. With large-capsule islet grafts, all recipients (n=5) became normoglycemic for 7-16 weeks, whereas with small-capsule 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 floating freely in the peritoneal cavity without any cell adhesion, whereas 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 of both an allograft reaction and a foreign body reaction.
We conclude that successful use of capsules with small diameters requires further study to determine which factors in the encapsulation procedure should be modified to reduce the number of inadequate small capsules.

Alginate-polylysine microencapsulation allows for longterm function of islet allografts (1-4) and xenografts (5, 6) in diabetic animal recipients and looks promising for future application in humans (7). As a consequence of its large volume, however, a microencapsulated islet graft can be implanted only into the peritoneal cavity.

This is a drawback for several reasons. First, the abdominal cavity is not the most efficient transplantation site in terms of diffusion kinetics of glucose (8) and insulin (9). Intra-abdominal grafts are therefore, predictably, associated with less than optimal glucose tolerance (10). Also, removal of an encapsulated islet graft from the abdominal cavity is not readily achieved since capsules are floating freely or localized diffusely. This is a pertinent consideration, since survival times of encapsulated islet grafts still have their limitations, and secondary or even multiple transplant procedures can be required with removal of the previous, voluminous, graft. Thus, we would prefer to transplant the encapsulated islets to a site that is better vascularized than the unmodified peritoneal cavity and that more easily permits removal of the implant in case of graft failure. However, placing the graft under the kidney capsule (11), in an omental pouch (12), or in a prevascularized solid support system (13) is not feasible with the usual capsule diameter of 800 µm, which is associated with a graft volume that cannot be accommodated by these sites. Thus, we would prefer smaller capsule diameters.

Additional considerations to prefer smaller capsule diameters are that better glucose-insulin kinetics over the capsule membrane may be expected (14), as well as better conditions for optimal islet nutrition (15), as has been shown in several in vitro studies.

The capsule diameter usually chosen is approximately 800 µm. Capsules as small as 500 µm can be readily produced with our adaptable droplet generator (16), but their production holds a risk. The location of an islet within an alginate bead is presumably determined by chance, which implies that an islet may be located in the center but also in the periphery of the bead, and thereby may even be found to protrude from the bead (17). Consequently, with smaller capsules, the chance increases for protruding, and thus inadequately encapsulated, islets.

The present study investigates the effect of reducing the capsule diameter from 800 µm to 500 µm. To this end, we first designed an assay for identifying the individual capsules inadequately encapsulating an islet. This assay was subsequently applied for comparing both diameters in terms of adequacy of encapsulating rat islets. Finally, the differences were tested in vivo by transplantation studies in the rat.

**MATERIALS AND METHODS**
Animals and induction of diabetes. Male, highly inbred albino Oxford (AO/G) rats were obtained from the Central Animal Laboratory of Groningen. Body weight of graft recipients, before the induction of diabetes, ranged from 290 to 320 g. Diabetes was induced by injection of 75-90 mg/kg of streptozotocin (Zanosar, Upjohn Co., Kalamazoo, MI) via the tail vein. A second injection of 70-90 mg/kg of streptozotocin was administered when, at 10-14 days after the first injection, the blood glucose level was less than 20 mmol/L. Only animals with severe weight loss, polyuria, polydipsia, and fasting blood glucose levels exceeding 20 mmol/L over a period of at least 4 weeks were used as recipients. At the time of peritoneal lavage a biopsy was taken from the tail of the native pancreas, fixed in Bouin’s solution for paraffin processing, sectioned at 5 µm, and stained with aldehyde fuchsine. In all instances, the absence of [beta] cells was confirmed when defined as less than 5% of normal controls.

Donor islets were isolated from highly inbred Lewis rats (Harlan CPB, Zeist, The Netherlands) weighing 300-350 g. Islets for in vitro assessment of inadequate encapsulation were isolated from the pancreas of male Wistar rats (Harlan CPB) weighing 300-350 g.

Islet isolation. Islets were isolated as described previously (18). Briefly, under ether anesthesia, the abdomen was opened and the common bile duct was cannulated under nonsterile conditions. The donor pancreas was distended with 10 ml of sterile Krebs-Ringer-Hepes (KRH*) buffer containing 10% bovine serum albumin. Subsequently, the pancreas was excised and put into a laminar flow cabinet. All further procedures were performed under sterile conditions.

The pancreas was chopped, digested using a two-stage incubation of 20 min at 37 °C with successively 1.0 mg/ml and 0.7 mg/ml collagenase (type XI, Sigma, St. Louis, MO). Islets were separated from exocrine tissue using a discontinuous dextran gradient and further purified by handpicking.

The total islet volume obtained by the isolation procedure was determined by measuring the diameters (11) of islets in a 4% aliquot of the islet suspension. Subsequently, the total volume was calculated, assuming the islets to be perfect spheres. In each experiment, we killed four to five donors from which we isolated 3500-4200 islets, which corresponds to 10-15 µl of islet tissue.

The islets were cultured in nontreated Petri dishes (Greiner, Alphen a/d Rijn, The Netherlands) in portions of 100 islets/25 cm² for 19 to 44 hr in CMRL 1066 (containing 10% fetal calf serum [Life Technologies, Paisley, Scotland], 8.3 mmol/L glucose, 10 mmol/L Hepes, and 1% penicillin/streptomycin) at 37 °C in humidified air containing 5% CO₂. After this culture period, the total islet volume was assessed again.

The diameter of the islets was measured with a dissection microscope with a fluorescent illuminator (Bausch and Lomb BVB-125, and 31-33-66) equipped with an ocular micrometer with an accuracy of 25 µm.

Islet microencapsulation. Before encapsulation, the islets were washed three to five times with RPMI containing 10% fetal calf serum. After removal of the medium, the islets were suspended in sterile, filtered(0.2-µm filtration) purified 3% alginate solution (Keltone LV, Kelco International, London, UK).
The alginate-islets suspension was converted into droplets using an air-driven droplet generator, as described previously (16). Briefly, alginate droplets are formed by pulling (with 0.2 bar) the alginate-islets suspension through a blunted 25-gauge needle. The needle is surrounded in the droplet generator by an air mantle. By increasing the velocity of the airstream in this mantle, we decrease the diameter of the droplets. For the formation of 400- to 500-µm capsules, we used an airstream velocity of 25-30 cm H\textsubscript{2}O. For the 700- to 800-µm capsules, we used an airstream velocity of 11-13 cm H\textsubscript{2}O. The setting of our droplet generator, including the level of airstream velocity for producing 500-µm capsules, does not interfere with the viability of the encapsulated islets (19).

For islet encapsulation in 700- to 800-µm capsules, we used a suspension of 1500-2200 islets/ml alginate. After droplet formation of this alginate-islets suspension, we observed that approximately 50% of the capsules were empty, whereas the islet-containing capsules surrounded one and occasionally two islets. Since a large capsule (750 µm) contains, as calculated, 2.5 times the volume of alginate present in a small capsule (500µm), we can produce 2.5 times more small capsules than large capsules using the same amount of alginate. Theoretically, therefore, we can produce from the amount of alginate present in a large capsule with one islet, one small capsule with an islet and 1.5 capsules without an islet. Consequently, the portion of empty capsules will increase in small-capsule preparations. Since islet-containing capsules are associated with more fibrotic overgrowth (20), sound comparison of the percentage of capsules with pericapsular infiltrate in small-capsule preparations (400-500 µm) and large-capsule preparations (700-800 µm) is only possible when the ratio of empty to islet-containing capsules is equal in both preparations. Therefore, for small-capsule preparations, we increased the number of islets per milliliter of alginate 2.5 times and used a suspension of 4000-4500 islets/ml alginate. Polylysinealginate encapsulation was performed as described elsewhere (19).

Identification of inadequate capsules. The adequacy of encapsulation was assessed by identifying the capsules that provide inadequate immunoisolation of the islet. The adequacy of encapsulation was quantified by expressing the number of inadequate capsules as the percentage of the total number of islet-containing capsules.

To identify inadequate capsules, a lectin binding assay was developed to specifically label the inadequately encapsulated islets. A suitable lectin was selected from a panel of fluorescein isothiocyanate-labeled lectins available as a test kit (Vector Laboratories, Burlingame, UK). The stock solutions of the lectins were diluted 100-fold in KRH and incubated for 45 min with the pancreatic islets. Table 1 lists the reactivity of the lectins with isolated pancreatic islets. Only the lectin ricinus communis agglutinin I (RCA-I) (Vector) combined a high affinity for pancreatic islets with a high molecular mass (120 kDa), which prevented the lectin diffusing into the microcapsules.
Table 1. Fluorescence intensity of isolated pancreatic islets labeled with fluorescein isothiocyanate-labeled lectins

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Molecular mass (KDa)</th>
<th>Fluorescence$^{a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concanavalin A</td>
<td>100</td>
<td>+++</td>
</tr>
<tr>
<td>Dolichos biflorus agglutinin</td>
<td>120</td>
<td>–</td>
</tr>
<tr>
<td>Peanut agglutinin</td>
<td>110</td>
<td>±</td>
</tr>
<tr>
<td>RCA-I</td>
<td>120</td>
<td>+++</td>
</tr>
<tr>
<td>Soybean agglutinin</td>
<td>120</td>
<td>+</td>
</tr>
<tr>
<td>Ulex europaeus agglutinin</td>
<td>63</td>
<td>±</td>
</tr>
<tr>
<td>Wheat germ agglutinin</td>
<td>36</td>
<td>+</td>
</tr>
</tbody>
</table>

$^{a}$ Symbols: ++++, very bright homogeneous fluorescence; ++, bright homogeneous fluorescence; +, homogeneous fluorescence; ±, some fluorescent spots; –, no fluorescence.

Aliquots of 300-500 microencapsulated islets were incubated with 0.5 ml of RCA-I solution (0.42 nM RCA-I) in KRH for 45 min at 4 °C. Subsequently, the microcapsules were washed four times with KRH to remove unbound RCA-I and stored at 4 °C.

Positive fluorescence was assessed by using a fluorescence microscope (Olympus TMT-2) equipped with inverted phase contrast. This inversion was required to focus on the islet, which, as a consequence of its eccentric location within the capsule, usually caused some rotation of the capsule until gravity maintained the islet at the lowest point. Microscopic examination was always performed to confirm that the fluorescence was caused by labeling of islet cells and not by nonspecific labeling.

Transplantation and retrieval of microencapsulated islets. With the rats under ether anesthesia, the microencapsulated islets were injected into the peritoneal cavity with a 16-gauge cannula via a small incision (3 mm) in the linea alba. The abdomen was closed with a two-layer suture. The implanted volumes for the capsules with a diameter of 400-500 µm varied between 0.4 and 0.6 ml, and for capsules with a diameter of 700-800 µm, volumes varied between 2.0 and 2.5 ml. Blood glucose was determined at regular time intervals using blood sampled from the tail vein. The glucose concentration was determined with glucose test tapes (Reflolux, Boehringer Mannheim, Germany). Recipients with a blood glucose level less than 8.4 mmol/L were considered normoglycemic.

Microcapsules were retrieved at 4 weeks after transplantation in cases of persistent hyperglycemia or within 2 weeks after the recurrence of hyperglycemia in cases with temporary normoglycemia. Microcapsules were either floating freely and nonadherent, or adhered to the surface of intra-abdominal organs. First, nonadherent microcapsules were retrieved by peritoneal lavage, which was performed by infusing 20 ml of KRH through a 3-cm midline incision into the peritoneal cavity and subsequent flushing with additional KRH two or three times above a 50-ml centrifuge tube. From this tube, the microcapsules were put into a 10-ml measuring cylinder (for a volume of 2.0-2.5 ml) or a 1-ml syringe (for volumes smaller than 1 ml) to assess the retrieved volumes. Subsequently, the microcapsules that adhered to the surface of abdominal organs were excised and processed for histology.
Histology and assessment of capsular overgrowth. Nonadherent capsules recovered by peritoneal lavage were always divided into three portions. One portion was fixed in precooled 2% paraformaldehyde, buffered with 0.05 M phosphate, and processed for glycol methacrylate embedding (21, 22). Sections were prepared at 2 µm and stained with Romanovsky-Giemsa stain. This portion was used to assess the degree of capsular overgrowth, which was quantified by expressing the number of recovered capsules with overgrowth as the percentage of the total number of recovered capsules for each individual animal. A second portion was fixed in Bouin’s solution for paraffin processing, sectioned at 5 µm, and stained with aldehyde fuchsin in order to determine the presence or absence of viable[beta] cells. A third portion of the recovered capsules was frozen in precooled 2-methylbutane, sectioned at 5 µm, and processed for immunohistochemical staining, as described previously (1). The monoclonal antibodies used were: ED1 and ED2 against monocytes, and a subset of macrophages (23), HIS-40 against IgM-bearing B lymphocytes (24), and R73 against CD3+–bearing T lymphocytes (25). After several washing steps, the sections were incubated with appropriate horseradish-peroxidase-conjugated second-step reagents. Peroxidase activity was demonstrated by applying 3,3’-diaminobenzidine tetra-HCl containing hydrogen peroxidase. In control sections, we used phosphate-buffered saline instead of the first-stage monoclonal antibody.

Processing for methacrylate embedding and Romanovsky-Giemsa staining and for immunohistochemical staining as specified above was also applied to samples of adherent capsules recovered by excision.

Statistical analysis. Statistical evaluation was performed using the paired and unpaired two-tailed Student’s \( t \) test when appropriate. Values are expressed as mean ± SEM. Only \( P<0.05 \) was considered to be significantly different.

RESULTS

The lectin binding assay was applied to quantify the percentage of inadequate capsules in preparations of Wistar islets encapsulated in either small (500 µm) or large (800 µm) capsules. As graphically illustrated in Figure 1, this percentage was 24.2±1.5% with small-capsule preparations, and only 6.3±1.2% with large-capsule preparations. Many of the inadequate capsules were observed to contain more than one islet, up to three islets per capsule with the large diameter capsules.
Figure 1. Percentage of capsules with inadequately encapsulated islets. Pancreatic islets encapsulated in an alginate-polylysine microcapsule of 500µm or 800 µm were suspended for 45 min in KRH containing fluorescein isothiocyanate-labeled RCA-I. Values represent mean ± SEM of seven experiments.

To determine the in vivo significance of inadequate encapsulation, Lewis islets were encapsulated in either small (400-500 µm) or large (700-800µm) capsules and allogenically transplanted into streptozotocin-diabetic AO rats.

With small-capsule islet grafts, only one of seven recipients became normoglycemic (Fig. 2A). This normoglycemia was reached at the 10th postoperative day after a gradual decrease of blood glucose levels, and maintained for the first 8 weeks after transplantation. This animal was the only recipient with more than 5% vital [beta] cells in the native pancreas, which may have contributed to the normoglycemia as observed in this single recipient. The other six recipients had no significant numbers of vital[beta] cells in the native pancreas. They showed not more than a moderate and temporary decrease in non-fasting blood glucose levels during the first week, after which they returned to the preoperative levels of hyperglycemia.
Figure 2. Non-fasting blood glucose concentrations in streptozotocin-diabetic AO rats after implantation of islet allografts microencapsulated in capsules of (A) 400-500 µm (n=7) and (B) 700-800µm (n=5). The dashed line (-----) indicates recurrence of hyperglycemia in individual animals. Values represent mean ± SEM.
In contrast, with large-capsule islet grafts, all five recipients became normoglycemic (Fig. 2B). This normoglycemia was reached within 5 days and it was maintained for 7 to 16 weeks.

The in vivo significance of inadequate encapsulation was further substantiated by our finding that the vast majority of small diameter capsules was found to be adherent to the surface of intra-abdominal organs, as indirectly quantified by a retrieval rate of only 5.5±1.8% in cases with persistent hyperglycemia (Table 2). In contrast to small-capsule grafts, the majority of large capsules floated freely in the peritoneal cavity and did not adhere to abdominal organs. Consequently, a mean percentage of 82±5.7% of the capsules could be retrieved at the time of peritoneal lavage. The only small-capsule graft with temporary normoglycemia showed an intermediate 40% retrieval rate with peritoneal lavage.

<table>
<thead>
<tr>
<th>Microcapsule diameter</th>
<th>n</th>
<th>Graft failure (wks post-Tx)</th>
<th>Implantated islet volume (µl)</th>
<th>% Recovery</th>
<th>% Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>700-800 µm</td>
<td>5</td>
<td>11.5±1.4</td>
<td>11.6±0.9</td>
<td>82±5.7</td>
<td>12±5</td>
</tr>
<tr>
<td>400-500 µm</td>
<td>6</td>
<td>0</td>
<td>13.2±1.1</td>
<td>5.5±1.8</td>
<td>65±11</td>
</tr>
<tr>
<td>300-400 µm</td>
<td>1</td>
<td>8</td>
<td>11.5</td>
<td>40</td>
<td>43</td>
</tr>
</tbody>
</table>

Table 2. Weeks of graft function, implanted islet volume, recovery rates, and percentage of capsules with cell adhesion in microencapsulated rat allografts encapsulated in 400- to 500-µm and 700- to 800-µm capsules

Histologic examination of nonadherent capsules showed some cellular adhesion to be present in the recovered capsules (Table 2), composed of mostly fibroblasts with some collagen deposits (Fig. 3).

Figure 3. Alginate-polylysine microencapsulated islet allograft in capsules of 700 to 800 µm 8 weeks after implantation. The capsules are retrieved from the peritoneal cavity by peritoneal lavage. Most capsules are free of any cell adhesion. The arrow shows a capsule covered by several layers of fibroblasts (GMA-embedded histological section, Romanovsky-Giemsa stain, original magnification ×50).
To verify that the presence of islets is responsible for inducing a fibrotic reaction rather than the capsules as such, we also implanted small empty capsules into the peritoneal cavity of AO rats (n=3) and explanted them 1 month after implantation. Only a small portion of the capsules was found to be adherent (2-10%), whereas 80-90% of the capsules could be retrieved from the peritoneal cavity.

In recipients of small diameter capsule grafts with persistent hyperglycemia, the adherent capsules were retrieved and histologically examined approximately 4 weeks after transplantation. As a rule, they were found in one or two clumps adhered to the omentum, the liver, the rectum, and/or the parietal peritoneum. These capsules were infiltrated or surrounded by fibroproliferative connective tissue, ED1- and ED2-positive macrophages, and immune cell elements staining positive for CD3+ bearing T lymphocytes (Fig. 4). We observed no B lymphocytes. Occasionally, we found some multinucleated giant cells within the infiltrates (Fig. 5). The islets in the capsules were found to be necrotic, containing no vital [beta] cells.

Figure 4. Alginate-polylysine microencapsulated islet allograft in capsules of 400 to 500 µm 1 month after implantation. The capsules are adherent to the liver capsule. There were no vital [beta] cells left. The arrows indicate the presence of T cells (section of frozen material, stained for R73, original magnification ×200).
In the only recipient of a small-capule graft with temporary normoglycemia, the adherent capsules were recovered at approximately 10 weeks after transplantation. We found two clumps with capsules, one adhering to the omentum and one adhering to the liver capsule. These capsules were surrounded and infiltrated by fibroblasts and some ED1- and ED2-positive macrophages.

Different observations were made in the recipients of large diameter capsule grafts, in which the adherent capsules were retrieved at 9-18 weeks after implantation. Here, rather than clumps of adherent capsules, occasionally some large capsules were found to be adherent to the omentum. These capsules were always found to be infiltrated or surrounded by ED1- and ED2-positive macrophages and by several layers of fibroblasts. We also found some multinucleated giant cells within the infiltrates, but no T lymphocytes or B lymphocytes. We found no islets in the adherent large capsules. The islets in nonadherent large capsules were found to contain large necrotic zones with only a few vital [beta] cells left (Fig. 6).
DISCUSSION

Theoretically, encapsulation of a 200-µm islet in an 800-µm capsule is associated with a 64-fold increase in graft volume, whereas this increase is only 15.6 times with a 500-µm capsule. Obviously, reducing the diameter from 800 µm to 500 µm is quite effectively associated with a fourfold reduction of the total encapsulated graft volume. However, it is also associated with a fourfold increase in the percentage of inadequately encapsulated islets, as was documented quantitatively with our lectin binding assay.

The latter finding is not without consequence, since, in vivo, we found several indications that inadequate encapsulation initiates fibrotic overgrowth with subsequent ischemia and necrosis of the islets. First, a number of capsules always escaped from fibrotic overgrowth. This indicates that inadequacies of individual capsules are the cause of the capsular overgrowth, rather than systemic insufficiencies in the composition or structure of the capsules. Second, the occurrence of these inadequacies required the presence of islets in the capsule, since capsular overgrowth was virtually absent when empty capsules were implanted. The role of inadequate islet encapsulation in the initiation of the inflammatory response was further substantiated by the observation that the adherent small capsules were surrounded and infiltrated by immune cell elements, fibroblasts, and connective tissue characteristic of rejection of the allogeneic islets (26). The role of inadequate islet encapsulation in the induction of capsular overgrowth was further confirmed by the fact that small-capsule islet grafts were associated with much more overgrowth than large-capsule islet grafts. The large-capsule islet graft overgrowth remained restricted to only a small percentage of the capsules. This concurred with the low frequency of inadequate encapsulation with large capsule diameters when compared with the high frequency with small capsule diameters. Thus, our results indicate that inadequate capsules represent those capsules that, in vivo, induce fibrotic overgrowth and thereby contribute to graft failure.
The composition of the cellular overgrowth suggests that the inflammatory reaction provoked by inadequate encapsulation is composed of two different responses induced by two different capsule deficiencies. One part of the inflammatory reaction is an allograft response aimed against islets in inadequate capsules as indicated by the presence of immune cell elements in the capsular overgrowth. Second, however, not only the islet, but also the inadequate capsules as such contribute to the inflammatory response by inducing a foreign body reaction in addition to the allograft reaction, as documented by the presence of multinucleated giant cells in the capsular overgrowth of some capsules.

The quantitative difference in overgrowth between small diameter and large diameter capsule islet grafts suggests that the severity of the inflammatory response provoked by inadequately encapsulated islets is proportional to the number of inadequate capsules in the graft. As illustrated by transplantation of large diameter capsule islet grafts, a low number of inadequate capsules may induce a modest inflammatory response that causes some capsular overgrowth and sometimes aggregation of capsules, but that does not exclude normal graft function. In contrast, transplantation of small-capsule islet grafts with a high number of inadequate capsules induced fibrotic overgrowth of the majority of the capsules. Consequently, most of these grafts could not induce normoglycemia. This observation also indicates that the percentage of inadequate capsules may reach a certain threshold, above which the provoked inflammatory response is actually associated with failure of the graft. Under such circumstances and similar to other intraperitoneal implants, the inflammatory response is probably associated with intra-abdominal adhesions (26, 27, 28) and aggregation and fibroencapsulation of the capsules (26, 27) affecting both inadequately and adequately encapsulated islets.

Which factors eventually caused the islet grafts in the large capsules to fail remain to be determined. The necrotic appearance of these islets in the absence of capsular overgrowth suggests that insufficient nutrition is a major factor. An elevated metabolic demand of the adequately encapsulated islets as the consequence of the loss of the inadequately encapsulated islets may have been a contributing factor. Proportionally, this loss of islet tissue may well be larger than suggested by the number of inadequate capsules in relation to the total number of capsules, since capsules containing a relatively large islet or more than one islet are more likely to show inadequacies.

The present study demonstrates that a single and seemingly minor modification in the encapsulation procedure, such as a reduction in capsule diameter, may have a tremendous influence on the in vivo performance of the encapsulated islet graft. Unfortunately, reducing the capsule diameter was associated with a substantial increase in the number of inadequate capsules and, as a consequence, with graft failure. Further studies are required to determine which factors in the encapsulation procedure should be modified in order to reduce the number of inadequate small capsules.

Acknowledgments. The authors thank R.A. Wieringa for making the photographs and Tineke S. Roorda for typing the manuscript.

REFERENCES

2. Fan MY, Lum ZP, Fu XW, Levesque L, Tai IT, Sun AM. Reversal of diabetes in BB rats by transplantation of encapsulated pancreatic islets. Diabetes 1990; 39: 519. Check if we have the full text | Bibliographic Links | [Context Link]


5. Chicheportiche D, Darquy S, Lepeintre J, Capron F, Halban PA, Reach G. High performance liquid chromatography analysis of circulating insulin distinguishes between endogenous insulin production (a potential pitfall with streptozotocin diabetic rats) and islet xenograft function. Diabetologia 1990; 33: 457. Check if we have the full text | Bibliographic Links | [Context Link]


8. Velho G, Froguel P, Reach G. Determination of peritoneal glucose kinetics in rats: implications for the peritoneal implantation of close-loop insulin delivery systems. Diabetologia 1989; 32: 331. Check if we have the full text | Bibliographic Links | [Context Link]


10. Fritschy WM, Strubbe JH, Wolters GHJ, Van Schilfgaarde R. Glucose tolerance and plasma insulin response to intravenous glucose infusion and test meal in rats with microencapsulated islet allografts. Diabetologia 1991; 34: 542. Check if we have the full text | Bibliographic Links | [Context Link]


12. Yasunami Y, Lacy PE, Finke EH. A new site for islet transplantation-a peritoneal-omental pouch. Transplantation 1983; 36: 181. Ovid Full Text | Check if we have the full text | Bibliographic Links | [Context Link]


14. Chicheportiche D, Reach G. In vitro kinetics of insulin release by microencapsulated rat islets: effect of the size of the microcapsules. Diabetologia 1988; 31: 54. Check if we have the full text | Bibliographic Links | [Context Link]


18. Wolters GHJ, Van Suylichem PTR, Van Deynen JHM, Van Schiffgaarde R. Factors influencing the isolation process of islets of Langerhans. Horm Metab Res Suppl 1990; 25: 20. Check if we have the full text [Context Link]


21. Gerrits PO, Horobin RW, Wright DJ. Staining sections of watermiscible resins. J Microsc 1990; 169: 279. Check if we have the full text [Context Link]

22. Fritschy WM, Wolters GHJ, Pasma A, Van Schiffgaarde R. Glycol methacrylate embedding of alginate-polylysine microencapsulated pancreatic islets. Biotech Histochem 1995; 70: 188. Check if we have the full text [Context Link]


24. Deenen GJ, Hunt SV, Opstelten D. A stathmokinetic study of B lymphocytopoiesis in rat bone marrow: proliferation of cells containing µ-chains, terminal deoxynucleotidyl transferase and carrying HIS24 antigen. J Immunol 1987; 139: 702. Check if we have the full text [Context Link]


27. Lanza RP, Kühtreiber WM, Ecker D, Staruk JE, Chick WL. Xenotransplantation of porcine and bovine islets without immunosuppression using uncoated alginate microspheres. Transplantation 1995; 59: 1377. Ovid Full Text Check if we have the full text [Context Link]