Tissue responses against immunoisolating alginate-PLL capsules in the immediate posttransplant period

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Abstract: Alginate-polylysine (PLL) capsules are commonly applied for immunoisolation of living cells for the treatment of a wide variety of diseases. Large-scale application of the technique, however, is hampered by insufficient biocompatibility of the capsules with failure of the grafts as a consequence. Most studies addressing biocompatibility issues of alginate-PLL capsules have focused on the degree of overgrowth on the capsules after graft failure and not on the reaction against the capsules in the immediate posttransplant period. Therefore, capsules were implanted in the peritoneal cavity of rats and retrieved 1, 5, and 7 days later for histological examination and X-ray photoelectron spectroscopy analysis for evaluation of chemical changes at the capsule surface. After implantation, the nitrogen signal increased from 5% on day 0, to 8.6% on day 7, illustrating protein adsorption on the capsule's surface. This increase in protein content of the membrane was accompanied by an increase in the percentage of overgrown capsules from 0.5 ± 0.3% on day 1 to 3.3 ± 1.6% on day 7. The cellular overgrowth was composed of monocytes/macrophages, granulocytes, fibroblasts, erythrocytes, multinucleated giant cells, and basophils. This overgrowth was not statical as generally assumed but rather dynamic as illustrated by our observation that at day 1 after implantation we mainly found monocytes/macrophages and granulocytes that on later time points were substituted by fibroblasts. As the inflammatory reaction predictably interfere with survival of encapsulated cells, efforts should be made to suppress activities or recruitment of inflammatory cells. These efforts may be temporary rather than permanent because most inflammatory cells have disappeared after 2 weeks of implantation. © 2002 Wiley Periodicals, Inc. J Biomed Mater Res 62: 430–437, 2002

Key words: alginate; poly-L-lysine; encapsulation; X-ray photoelectron spectroscopy; protein adsorption

INTRODUCTION

Immunoisolation is a technology in which tissue is encapsulated in semipermeable membranes to protect donor cells against antibodies and cytotoxic cells of the host immune system. This encapsulation allows for successful transplantation of cells in the absence of immunosuppression. Also, it allows for transplantation of cells from nonhuman origin, i.e., xenografts or genetically engineered cells,[1–4] which overcomes the obstacle of limited supply of donor tissue. Because of these benefits, the feasibility of transplanting cells in immunoisolating membranes is under study for the treatment of a wide variety of endocrine diseases such as diabetes mellitus,[5] central nervous system insufficiencies,[6] anemia,[7] dwarfism,[3] Hemophilia B,[8] pituitary,[9] kidney inadequacies,[10] and liver[11] failure.

A commonly used procedure for immunoisolation is microencapsulation of tissues in alginate-poly-L-lysine (PLL)-based capsules as originally described by Lim and Sun.[5] During recent years, important advances have been made with this technology. The first allotransplantations in humans with encapsulated parathyroid cells and islets have been successfully performed.[12] Although this illustrated the principle applicability of the alginate-encapsulation technique, a fundamental barrier has to be overcome since graft survival varies considerably from several days to months and was in one study even 2 years.[13] This variation in success rate is usually attributed to differences in biocompatibility of the applied capsules.

Previous investigations on biocompatibility issues of microcapsules have mainly focused on the degree

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of overgrowth of capsule grafts after failure of the graft.[1,14–19] These studies have shown that a portion of 2–10% of the capsules is overgrown by fibroblast and macrophages. This cellular response forms a metabolic barrier to nutrient diffusion with ischemia and necrosis of the tissue as a consequence. This loss of 2–10% of islets cannot explain, however, the failure of the graft. Therefore, other mechanisms not related to necrosis of overgrown encapsulated tissue should be considered responsible for failure of the graft.

It has been shown that the reaction against capsules is complete within 7 days after implantation.[20] The characteristics of this reaction against capsules in the first 7 days are not known but essential for understanding and deleting biocompatibility issues related to implantation of immunoisolating capsules. Therefore, the present study was undertaken to assess the degree and composition of the cellular overgrowth at day 1, 5, and 7 after implantation of capsules in the peritoneal cavity of rats. Also, we studied the protein adsorption on capsules after implantation since this may be an essential part of the reaction against capsules.

MATERIALS AND METHODS

Design of the study

Microcapsules were implanted in the peritoneal cavity, i.e., the usual transplantation site for an encapsulated islet graft, of AO-rats. Only highly purified alginates were applied in order to exclude that contaminating components were the cause of an inflammatory response. The capsules were inspected before and after implantation in order to confirm that the majority of the capsules was intact. The capsules were retrieved at days 1, 5, and 7. The capsules were divided in two portions. The first portion was processed for X-ray photoelectron spectroscopy (XPS) to study the protein adsorption on the capsule's surface. The other portion was processed for histology to study the degree of overgrowth and the composition of the overgrowth.

Alginate and purification procedure

Sodium alginates with an intermediate-G content (Keltone LV) were obtained from Kelco International (London, UK). Crude sodium alginate was dissolved at 4°C in a 1 m*M* sodium EGTA solution to a 1% solution for intermediate-G alginate and to a 0.25% solution for high-G alginate under constant stirring. Subsequently the solutions were filtered over, successively, 5.0, 1.2, 0.8, and 0.45 μm filters (Schleicher & Schuell, Dassel, Germany). During this filtration step, all visible aggregates were removed.

Next, the pH of the solution was lowered to 3.5 by addition of 2*N* HCL + 20 m*M* NaCl. The solution was kept on ice to prevent hydrolysis of alginate. The next step was slow lowering of the pH from 3.5 to 1.5 and was associated with gradual precipitation of alginate as alginic acid.[21] Routinely, the solutions were brought at a pH of 2.0 and subsequently filtered over a Buchner funnel (pore size, 1.5 mm) to wash out nonprecipitated contaminants. To extend the washout of nonprecipitated contaminants, the precipitate was brought in 0.01*N* HCL + 20 m*M* NaCl, vigorously shaken, and filtered again over the Buchner funnel. This washing procedure was performed three times.

Then, proteins were removed by extraction with chloroform/butanol.[22] The alginic acid was suspended in 100 mL of 0.01*N* HCl + 20 m*M* NaCl and supplemented with 20 mL chloroform and 5 mL 1-butanol. The mixture was vigorously shaken for 30 min and filtered over the Buchner funnel. This chloroform/butanol extraction was performed three times. Next, the alginic acid was brought in water and slowly dissolved by gradually raising the pH to 7.0 by slow addition of 0.5*N* NaOH + 20 m*M* NaCl over a period of at least 1 h. The alginate solution obtained was subjected to a chloroform/butanol extraction to remove those proteins that can only be dissolved in chloroform/butanol at neutral pH.[22] The solution was vigorously shaken in a mixture of chloroform (20 mL at each 100 mL alginate solution) and 1-butanol (5 mL at each 100 mL alginate solution) for 30 min. The mixture was centrifuged for 3–5 min at 3000 rpm, which induced the formation of a separate chloroform/butanol phase, which was removed by aspiration. The extraction was repeated once.

The last step was precipitation of the alginate with ethanol.[23,24] To each 100 mL of alginate solution, we added 200 mL absolute ethanol. After an incubation period of 10 min all alginate had precipitated. The alginate was filtered over the Buchner funnel and washed two times with absolute ethanol. Subsequently, the alginate was washed three times with ethylether. Finally, the alginate was freeze-dried overnight.

Endotoxin content of purified alginate samples was assessed by a commercial Limulus-lysate assay[25] following the protocol of the E-toxate kit recommended by Sigma. The alginate samples purified by this procedure was always close to zero and never higher than 0.01 units/mL.

Encapsulation

Purified alginates were dissolved at 4°C in Krebs-Ringer-Hepes (KRH) with an appropriate osmolarity to a solution with a viscosity of 4 cps. The viscosity of an alginate solution is determined by the concentration of alginate, but different alginates have different viscosities. This implies for the intermediate-G solution a concentration of 3% and for the high-G a 2% solution to obtain a viscosity of 4 cps. Before application the solutions were sterilized by 0.2 μm filtration.

The alginate solution was converted into droplets using an air-driven droplet generator as previously described.[26] Polylysine-alginate encapsulation was performed as described elsewhere.[27] Briefly, the alginate droplets were transformed to alginate beads by gelling in a 100 m*M* $CaCl_2$ (10 m*M* HEPES, 2 m*M* KCl) solution for at least 5 min.

Subsequently, the Ca-alginate beads were suspended for 1 min in Krebs-Ringer-Hepes buffer containing 2.5 m*M* $CaCl_2$. A PLL membrane was formed by suspending the alginate beads in 0.1% PLL solution for 10 min (poly-L-lysine-HCl, M_w: 22,000, Sigma). Nonbound PLL was removed by three successive washings during 3 min with Ca^{2+}-free KRH containing 135 m*M* NaCl. The outer alginate layer was subsequently applied by 5 minutes' incubation in 10× diluted alginate solution. The diameters of capsules and beads were measured with a dissection microscope (Bausch and Lomb BVB-125, and 31-33-66) equipped with an ocular micrometer with an accuracy of 25 μm. The capsules had a diameter of 600–700 μm. The same microscope was used for inspection of the capsules before implantation.

Implantation and explantation of empty capsules

Male inbred Albino Oxford (AO/G) rats served as recipients of alginate-PLL capsules and were obtained from the Central Animal Laboratory of Groningen. Their body weights ranged from 300 to 350 g. NIH guidelines for the care and use of laboratory animals were observed.

Capsules were injected into the peritoneal cavity with a 16G cannula via a small incision (3 mm) in the linea alba. The abdomen was closed with a two-layer suture. The implanted volume was always 2.0 mL, as assessed in a syringe with appropriate measure.

The microcapsules were retrieved at days 1, 5, and 7 after implantation by peritoneal lavage. Peritoneal lavage at days 1 and 5 was performed by infusing 5 mL KRH through a 3-mm midline incision into the peritoneal cavity and subsequent aspiration of the KRH containing the capsules.

On day 7 the animals were subjected to laparotomy to inspect the peritoneal cavity. Microcapsules were either freely floating and nonadherent or adherent to the surface of abdominal organs. First, nonadherent microcapsules were retrieved by peritoneal lavage and brought into a syringe with appropriate measures for quantification of the retrieval rate.[28] Subsequently, the microcapsules adherent to the surface of abdominal organs were excised and processed for histology.

All surgical procedures were performed under halothane anesthesia.

X-ray photoelectron spectroscopy

For measuring protein adsorption, samples of fresh capsules and capsules retrieved by lavage at days 1, 5, and 7 after implantation were washed three times with ultrapure water and gradually lyophilized (Leybold Herecuis, Combitron CMI). Because the XPS spectroscope only identifies elements at the surface of the capsules, it is a prerequisite that the membranes of the capsules are intact and not broken. Therefore, before applying XPS, we confirmed the integrity of the surfaces and membranes by scanning electron microscopy.

Samples of lyophilized beads or capsules with intact capsule membranes were fixed on a sample holder. The sample holder was inserted into the chamber of an X-ray photoelectron spectrometer (Surface Science Instruments, S-probe, Mountain View, CA). An aluminum anode was used for generation of X-rays (10 kV, 22 mA) at a spot size of 250 × 1000 μm. During the measurements, the pressure in the spectrometer was approximately 10^{-7} Pa. First, scans were collected over the binding energy range of 1–1100 eV at low resolution (150 eV pass energy). Next, we recorded at high resolution (50 eV pass energy) C_{1s}, N_{1s}, and O_{1s} peaks over a 20-eV binding energy range. The protein content of the capsule's surface was expressed as a percentage of the total C, N, and O content of the membrane. Pure lyophilized PLL was measured after bringing it on the sample holder in the same fashion as described above.

Experiments were repeated four times to exclude variations between different encapsulation sessions.

Microscopy

To assess the integrity of capsules before implantation, samples of capsules were meticulously inspected for the presence of irregularities or broken parts in the capsule membranes by using a dissection microscope.

To detect physical imperfections and to assess the composition and degree of overgrowth after implantation, samples of adherent capsules recovered by excision and nonadherent capsules were fixed in precooled 2% paraformaldehyde, buffered with 0.05*M* phosphate in saline (pH 7.4), and processed for glycol methacrylate (GMA) embedding.[29] Sections were prepared at 2 μm and stained with Romanovsky-Giemsa stain and applied for detecting imperfections in the capsule membrane and for quantifying the composition of the overgrowth and determining the number of capsules with and without overgrowth. Different cell types in the overgrowth were assessed by identifying cells in the capsular overgrowth with the morphological characteristics of monocytes/macrophages, lymphocytes, granulocytes, fibroblasts, basophiles, erythrocytes, and multinucleated giant cells. To confirm the adequacy of this approach, portions of adherent and nonadherent capsules were frozen in precooled isopropane, sectioned at 5 μm, and processed for immunohistochemical staining and quantification of the different cell types as previously described. The monoclonal antibodies used were as follows: ED1 and ED2 against monocytes and macrophages,[31] HIS-40 against IgM bearing B-lymphocytes,[32] and R73 against $CD3^+$ bearing T-lymphocytes.[33] In control sections we used PBS instead of the first stage monoclonal antibody. Quantification of these cell types after immunocytochemistry was compared with the assessments on the basis of morphological markers and always gave similar results.

The degree of capsular overgrowth 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.

Statistical analysis

Results are expressed as mean ± standard error of the mean. Statistical comparisons were made with the Mann

Whitney U test. $p < 0.05$ was considered statistically significant.

RESULTS

At each time point, i.e., on days 1, 5, and 7 after implantation, we washed out 25–40% of the capsule graft from the rat peritoneal cavity. After the three lavages, the total retrieval rate of the capsules varied between 91 and 95%, indicating that the majority of the capsules could readily be washed out and was freely floating in the peritoneal cavity.

Implantation of biomaterials is associated with the release of a large number of bioactive proteins that can be adsorbed by the biomaterials and facilitate the tissue responses against the materials by allowing adhesion of cells.[34–37] To investigate whether bioactive proteins are released and adsorbed after implantation of alginate-PLL capsules, we applied XPS on the capsule's surface.

Table I shows the elementary composition of capsules before and after implantation. Before implantation, on day 0, capsules are composed of 5.0 ± 1.2% nitrogen. From this assessment we can calculate the PLL content of the membrane, by dividing the N/C ratio of PLL through the N/C ratio of the capsule membrane. From this calculation it follows that the capsule membranes are composed of 56% PLL. After implantation, the nitrogen signal does not stay 5.0% but gradually increases to 8.6% at day 7 (Fig. 1), illustrating protein release in the vicinity of capsules and adsorption of those proteins by the capsule's surface. This elevation in nitrogen signal was only statistical significant at day 5 ($p < 0.05$) and day 7 ($p < 0.05$).

The other elementary changes on the capsule's surface after implantation was a gradual increase in the C-signal and a gradual decrease in the O-signal. These changes only reached statistical significance at day 7 (both $p < 0.05$). We found no new elements after implantation.

The percentage of freely floating capsules with cellular overgrowth increased during prolonged stay in the peritoneal cavity from 0.5 ± 0.3% on day 1 to 3.3 ± 1.6% on day 7 (Table II). In some cases we observed the overgrowth infiltrating a localized defect in imperfect capsules. Before transplantation, we observed all batches of microcapsules to contain some imperfect capsules, such as capsules with strains or capsules with a broken membrane. The number of imperfect capsules was small and represented not more than ~3% of the total number of capsules. This fraction of capsules with physical inadequacies must be considered to be responsible for the observed overgrowth of a portion of the capsules.[1,30,38]

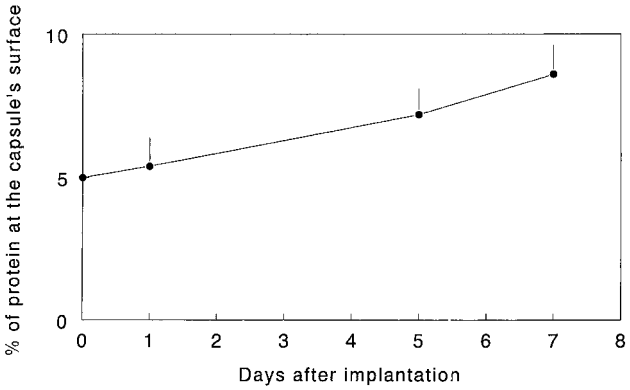

Figure 1. Protein content of the capsule surface before and after implantation, as determined from the N/C ratio as measured by XPS. The nitrogen content was measured by XPS. The n-values are identical to the elemental surface composition of alginate-PLL capsules given in Table I.

The overgrowth on capsules recovered by peritoneal lavage was composed of monocytes/macrophages, granulocytes, fibroblasts, erythrocytes, multinucleated giant cells, and basophiles. This observation was done in GMA-embedded sections, and it was confirmed by applying immunocytochemistry on frozen sections. As in previous studies with empty capsules,[30,38] we found no immune-cell elements such as B- or T-lymphocytes.

As shown in Figure 2, at day 1 the overgrowth was for its majority composed of macrophages and granulocytes. At day 5, we observed a first increase in the number of fibroblasts in the infiltrate ($p < 0.05$), which was associated with a decrease in the number of granulocytes. By day 7, the infiltrate was for a considerable fraction of 32 ± 15% composed of fibroblasts. This increase in the fraction of fibroblasts was accom-

TABLE I
Elemental Surface Composition of Alginate-PLL Capsules (n = 4) and an Analysis of Pure PLL (Representative Sample is Shown)

	C (%)	N (%)	O (%)	Others (Na, K, Si) (%)
Day 0	56.2 ± 0.6	5.0 ± 1.2	34.3 ± 1.8	4.5 ± 0.8
Day 1	57.8 ± 3.6	5.4 ± 1.0	30.0 ± 3.9	6.8 ± 1.0
Day 5	58.9 ± 1.7	7.2 ± 0.9	30.1 ± 1.2	3.8 ± 0.4
Day 7	61.6 ± 2.6	8.6 ± 1.0	26.3 ± 2.7	3.5 ± 0.5
PLL	67.2	10.7	17.9	4.2

TABLE II
Percentage of Freely Floating Alginate-PLL Capsules with Overgrowth at 1, 5, and 7 days after Implantation in the Peritoneal Cavity of AO Rats

Days after Implantation	n	% Overgrowth
1	4	0.5 ± 0.3
5	4	1.1 ± 0.4
7	4	3.3 ± 1.6

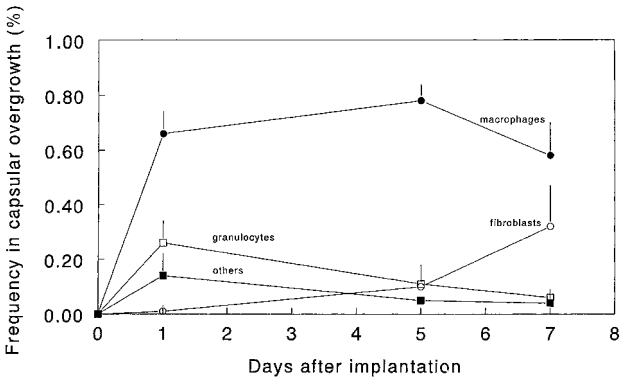

Figure 2. Frequency of granulocytes (□), macrophages (●), fibroblasts (○), and others (■) in the cellular overgrowth on alginate-PLL capsules on days 1, 5, and 7 after implantation. Others are the total number of erythrocytes, multinucleated giant cells, and basophiles at the different time point. Values represent mean ± standard error of the mean of four experiments.

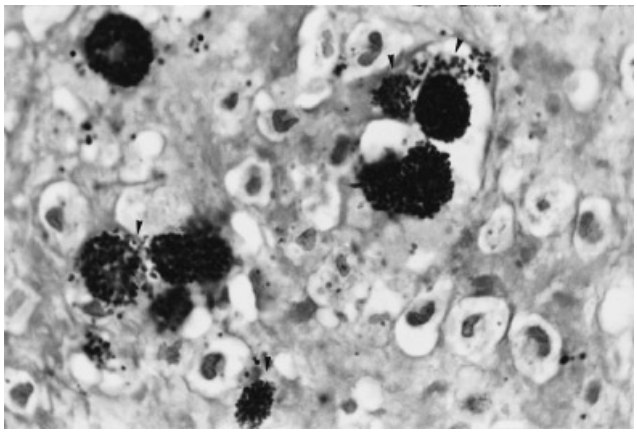

Figure 3. Basophils in the cellular overgrowth of alginate-PLL capsules at 1 day after implantation in the peritoneal cavity of AO rats. Note that the basophils marked by an arrow secretes its granula into the surrounding tissue. (GMA-embedded histological section, Romanowsky-Giemsa staining, original magnification ×250).

panied by a decrease in the fraction of macrophages and by a further decrease of granulocytes, which were by day 7 only present in low numbers.

The other cell-types, i.e., erythrocytes, multinucleated giant cells, and basophiles were present in low numbers but were pertinent for the sequence of events in the tissue response against capsules. Erythrocytes were observed in and around the capsules at day 1 and not on days 5 and 7. The observation that erythrocytes were only present at day 1, i.e., immediately after implantation, suggests that the erythrocytes have been introduced during or after trauma associated with the surgical implantation of the capsules. The large numbers of erythrocytes at day 1 were responsible for the relative high numbers of "others" at day 1 in Figure 2.

Multinucleated giant cells were absent in slices of capsules retrieved at day 1 and present in low numbers in slices of capsules retrieved at days 5 and 7. Multinucleated giant cells were mainly observed in and around capsules containing localized defects, suggesting that these cells are involved in breakdown of imperfect capsules.

At all time points, we found basophiles in the sections. These basophils were activated as suggested by the observation that large numbers of basophils were expelling its granulla into the surrounding tissue (Fig. 3). These basophils were found in the cellular infiltrate on overgrown capsules or in cell clusters containing large numbers of inflammatory cells freely floating between nonovergrown capsules (Fig. 4).

The cell clusters without capsules need some further consideration because they were freely floating in the peritoneal cavity and were observed in high numbers at days 1 and 5 after implantation. The cell clusters were for its majority composed of activated basophils, macrophages, and granulocytes. The cell clusters were not observed in sections of capsules retrieved at day 7, illustrating that the presence and formation of cell clusters was a temporary phenomenon essential for the reaction in the first few days after implantation.

DISCUSSION

This is the first study analyzing and demonstrating inflammatory reactions against immunoisolating capsules in the first days after implantation. It shows that monocytes/macrophages, granulocytes, fibroblasts, erythrocytes, multinucleated giant cells, and basophiles are involved in the reaction against capsules in a time-dependent fashion. Also, we show that the

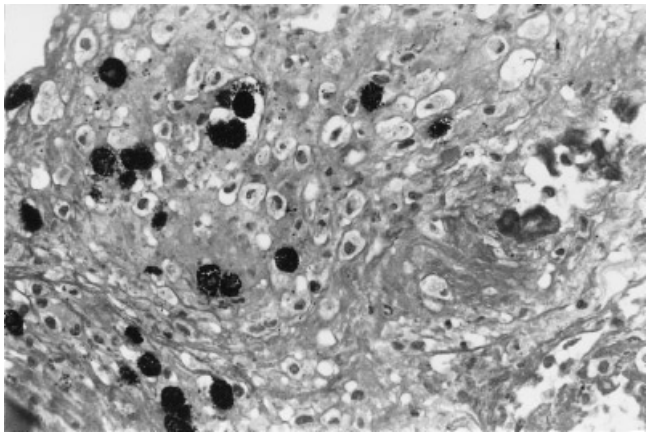

Figure 4. Cell cluster without capsules in the peritoneal cavity at day 5 after implantation in AO rats. These clusters were only observed at days 1 and 5 after implantation and are composed of basophils, macrophages, and granulocytes. (GMA-embedded histological section, Romanowsky-Giemsa staining, original magnification ×150).

quantitative protein deposition on the capsule's surface follows the profile of cellular overgrowth of capsules, suggesting that protein adsorption is essential for cellular overgrowth of the capsules.

The very first step in the reaction is probably not related to the implantation of capsules but to the required surgical procedure for implantation. Although it was only minor surgery, the procedure was associated with bleedings in the peritoneal cavity as illustrated by the observation of erythrocytes in the peritoneal cavity at the first day after implantation. The bleedings and subsequent tissue repair is associated with the introduction of serum factors in the peritoneal cavity and release of bioactive proteins such as trombin and fibronectin.[34] These factors will have chemotactic effects on inflammatory cells which will migrate to the peritoneal site.[39]

The reaction will be reinforced by the presence of the foreign biomaterials in the peritoneal cavity because the implantation of foreign materials is associated with an inflammatory reaction.[34–36,39,40] In general, the host response starts with release of bioactive proteins such as fibrinogen[41] in the implantation site of the foreign materials. These proteins are subsequently adsorbed by the foreign polymers. We clearly demonstrated in the present study that this type of response also occurs after implantation of alginate-PLL capsules because we found a substantial increase in the protein content of the capsule's surface after implantation in the peritoneal cavity. The release of proteins will facilitate the response because inflammatory cells may now adhere to the surface and produce cytokines such as IL-1β, TNF-α, and TGF-β, which further activate inflammatory cells in the vicinity of the foreign materials.[34,35,42,43]

The site of adherence of inflammatory cells on alginate-PLL capsules is probably not the whole capsule surface but only the imperfections on the capsule as suggested by our recurrent histological observations[14,28,38,44] of cellular overgrowth at sites where the capsules were broken. This is also the site where we observed the multinucleated giant cells. Multinucleated giant cells are most likely formed by fusion of macrophages[45–47] as a result of insufficient biodegradability of the capsule materials.[46–50]

The observation of high numbers of basophils in the cellular infiltrate suggests that basophils play an important role in the host response against capsules. Basophiles are strong producers of bioactive proteins and cytokines such as histamine,[51–55] monocyte and macrophage chemoattractant proteins (MIP and MCP),[52,56] IL-4,[55,57,58] and IL-13.[55,59–61] These cytokines are well known to have chemotactic and activating effects on inflammatory cells such as granulocytes, macrophages, and fibroblasts. This suggestion is supported by Tang et al.,[56] showing that histamine is primarily responsible for recruitment of inflammatory cells to the peritoneum after implantation of biomaterials. It is quite plausible that basophils are mainly responsible for the fast and high recruitment of cells to the capsules after intraperitoneal implantation.

Basophils were never observed when overgrown capsules were studied in the second week of implantation[15] or at later time points.[14,28] Also, granulocytes were only temporary present in the cellular overgrowth since Fritschy et al.[15] showed that at 2 weeks after implantation the cellular overgrowth of capsules was only composed of activating macrophages and fibroblasts, whereas granulocytes were totally absent. When studied at 2 months postimplant,[14] we showed that the overgrowth was reduced to a strictly fibrotic response because we only found fibroblasts and connective tissue, whereas macrophages had totally disappeared from the overgrowth. This illustrates that the tissue response against capsules is not a static process but a rather dynamic event with involvement of varying cell types at different time points.

Recently, it has been shown with microencapsulated pancreatic islet grafts, that in capsules retrieved by peritoneal lavage 40% of the initially grafted islets had disappeared during the first weeks after transplantation.[28] This disappearance of islet tissue could not be explained by overgrowth and necrosis of the encapsulated islets or by insufficient retrieval of capsules because <10% of the capsules was overgrown with macrophages and fibroblasts and because >90% of the capsules was retrieved by lavage.[28] Our present study provides new insight in the causes of this loss of pancreatic islets. As demonstrated, grafting of capsules is associated with severe tissue responses in the first week after implantation because high numbers of granulocytes, of macrophages, and of basophils were observed in the immediate vicinity of the capsules. These cells are known to secrete NO, TNF-α, and IL-1β, which predictably interferes with survival of islets because these cytokines can diffuse into the capsules[62] and have been shown to be potent stimulators of islet-cell death.[63–67] These reactions are expectedly more pronounced with islet-containing capsules because cells in the capsules may reinforce the tissue reaction against capsules.

There are several approaches to overcome the problems associated with tissue responses against alginate-PLL capsules. First, responses can be reduced by removing imperfect capsules before implantation.[30,68] Expectedly, however, this will only bring about a modest reduction in the tissue response because imperfections on capsules will also develop after exposure to shear forces in the implantation site.[1,69] and because the response is for its majority initiated by surgery and implantation of foreign materials. Alternatively, conceivable approaches are designing and testing pharmacological treatment strategies to suppress activities or recruitment of inflammatory cells.

These treatment strategies may be temporary rather than permanent because most inflammatory cells have disappeared after 2 weeks of implantation. These strategies will serve as a pertinent basis for future clinical application of microencapsulated tissue.

References

1. De Vos P, Van Schilfgaarde R. Biocompatibility issues. In Kühtreiber WM, Lanza RP, Chick WL, editors. Cell encapsulation technology and therapeutics. Boston, Birkhäuser; 1999. p 63–79.
2. Al-Hendy A, Hortelano G, Tannenbaum GS, Chang PL. Correction of the growth defect in dwarf mice with nonautologous microencapsulated myoblasts—An alternate approach to somatic gene therapy. Hum Gene Ther 1995;6:165–175.
3. Chang PL, Shen N, Westcott AJ. Delivery of recombinant gene products with microencapsulated cells in vivo. Hum Gene Ther 1993;4:433–440.
4. Kintsurashvili E, Zhou D, Wheeler MB, Vacek I, Sun AM. Genetic engineering of glucose-stimulated insulin secretion in Chinese hamster ovary cells. Artif Cells Blood Substit Immobil Biotechnol 1998;26:329–340.
5. Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. Science 1980;210:908–910.
6. Aebischer P, Goddard M, Signore AP, Timpson RL. Functional recovery in hemiparkinsonian primates transplanted with polymer-encapsulated PC12 cells. Exp Neurol 1994;126:151–158.
7. Koo J, Chang TSM. Secretion of erythropoietin from microencapsulated rat kidney cells. Int J Artif Organs 1993;16:557–560.
8. Liu HW, Ofosu FA, Chang PL. Expression of human factor IX by microencapsulated recombinant fibroblasts. Hum Gene Ther 1993;4:291–301.
9. Aebischer P, Russell PC, Christenson L, Panol G, Monchik JM, Galletti PM. A bioartificial parathyroid. ASAIO Trans 1986;32: 134–137.
10. Cieslinski DA, Humes HD. Tissue engineering of a bioartificial kidney. Biotechnol Bioeng 1994;43:678–681.
11. Wong H, Chang TM. Bioartificial liver: implanted artificial cells microencapsulated living hepatocytes increases survival of liver failure rats. Int J Artif Organs 1986;9:335–336.
12. Hasse C, Klöck G, Schlosser A, Zimmermann U, Rothmund M. Parathyroid allotransplantation without immunosuppression. Lancet 1997;350:1296–1297.
13. O'Shea GM, Goosen MFA, Sun AM. Prolonged survival of transplanted islets of Langerhans encapsulated in a biocompatible membrane. Biochim Biophys Acta 1984;804:133–136.
14. De Vos P, De Haan BJ, Wolters GHJ, Strubbe JH, Van Schilfgaarde R. Improved biocompatibility but limited graft survival after purification of alginate for microencapsulation of pancreatic islets. Diabetologia 1997;40:262–270.
15. Fritschy WM, De Vos P, Groen H, Klatter FA, Pasma A, Wolters GH, Van Schilfgaarde R. The capsular overgrowth on microencapsulated pancreatic islet grafts in streptozotocin and autoimmune diabetic rats. Transpl Int 1994;7:264–271.
16. Horcher A, Zekorn T, Siebers U, Klöck G, Frank H, Houben R, Bretzel RG, Zimmermann U, Federlin K. Transplantation of microencapsulated islets in rats: Evidence for induction of fibrotic overgrowth by islet alloantigens released from microcapsules. Transplant Proc 1994;26:784–786.
17. Klöck G, Frank H, Houben R, Zekorn T, Horcher A, Siebers U, Wöhrle M, Federlin K, Zimmermann U. Production of purified alginates suitable for use in immunoisolated transplantation. Appl Microbiol Biotechnol 1994;40:638–643.
18. Fan MY, Lum ZP, Fu XW, Levesque L, Tai IT, Sun AM. Reversal of diabetes in BB-rat by transplantation of encapsulated pancreatic islets. Diabetes 1990;39:519–522.
19. Lum ZP, Tai IT, Krestow M, Norton J, Vacek I, Sun AM. Prolonged reversal of diabetic state in NOD mice by xenografts of microencapsulated rat islets. Diabetes 1991;40:1511–1516.
20. 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 2001;57:374–383.
21. Haug A, Larsen B. The solubility of alginate at low pH. Acta Chem Scand 1963;17:1653–1662.
22. Staub AM. Removal of proteins. In: Whistler RL, Bemiller JN, Wolfrom ML, editors. Methods in carbohydrate chemistry. New York: Academic Press; 1965. p 163–168.
23. Haug A. Alginic acid. Isolation and fractionation with potassium chloride and manganous ions. In: Whistler RL, Bemiller JN, Wolfron ML, editors. Methods in carbohydrate chemistry. New York: Academic Press; 1965. p 69–72.
24. Haug A, Myklestad S, Larsen B, Smidsrod O. Correlation between chemical structure and physical properties of alginates. Acta Chem Scand 1967;21:768–778.
25. Scheer R. Pyrogene and limulus test. Pharm Unserer Zeit 1988; 5:137–146.
26. Wolters GHJ, Fritschy WM, Gerrits D, Van Schilfgaarde R. A versatile alginate droplet generator applicable for microencapsulation of pancreatic islets. J Appl Biomater 1992;3:281–286.
27. Fritschy WM, Wolters GH, Van Schilfgaarde R. Effect of alginate-polylysine-alginate microencapsulation on in vitro insulin release from rat pancreatic islets. Diabetes 1991;40:37–43.
28. De Vos P, van Straaten JF, Nieuwenhuizen AG, de Groot M, Ploeg RJ, De Haan BJ, Van Schilfgaarde R. Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? Diabetes 1999;48:1381–1388.
29. Fritschy WM, Gerrits PO, Wolters GH, Pasma A, Van Schilfgaarde R. Glycol methacrylate embedding of alginate-polylysine microencapsulated pancreatic islets. Biotech Histochem 1995;70:188–193.
30. De Vos P, De Haan BJ, Pater J, Van Schilfgaarde R. Association between capsule diameter, adequacy of encapsulation, and survival of microencapsulated rat islet allografts. Transplantation 1996;62:893–899.
31. Dijkstra CD, Dopp EA, Joling P, Kraal G. The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 1985;54:589–599.
32. Deenen GJ, Hunt SV, Opstelten D. A stathmokinetic study of B lymphocytopoiesis in rat bone marrow: proliferation of cells containing cytoplasmic mu-chains, terminal deoxynucleotidyl transferase and carrying HIS24 antigen. J Immunol 1987;139: 702–710.
33. Huning T, Wallny HJ, Hartly J, Lawetsky A, Tiefenthaler G. A monoclonal antibody to a constant region of the rat TCR that induces T-cell activation. J Exp Med 1989;169:73–78.
34. Tang L, Eaton JW. Natural responses to unnatural materials: A molecular mechanism for foreign body reactions. Mol Med 1999;5:351–358.
35. Rihova B. Immunocompatibility and biocompatibility of cell delivery systems. Adv Drug Deliv Rev 2000;42:65–80.
36. Hu WJ, Eaton JW, Tang L. Molecular basis of biomaterial-mediated foreign body reactions. Blood 2001;98:1231–1238.
37. Tang L, Eaton JW. Inflammatory responses to biomaterials. Am J Clin Pathol 1995;103:466–471.
38. De Vos P, De Haan B, Van Schilfgaarde R. Effect of the alginate composition on the biocompatibility of alginate-polylysine microcapsules. Biomaterials 1997;18:273–278.
39. Tang L, Jennings TA, Eaton JW. Mast cells mediate acute inflammatory responses to implanted biomaterials. Proc Natl Acad Sci USA 1998;95:8841–8846.

40. Tang L, Ugarova TP, Plow EF, Eaton JW. Molecular determinants of acute inflammatory responses to biomaterials. J Clin Invest 1996;97:1329–1334.
41. Tang L, Eaton JW. Fibrin(ogen) mediates acute inflammatory responses to biomaterials. J Exp Med 1993;178:2147–2156.
42. Babensee JE, Anderson JM, McIntire LV, Mikos AG. Host response to tissue engineered devices. Adv Drug Deliv Rev 1998; 33:111–139.
43. Hunt JA, McLaughlin PJ, Flanagan BF. Techniques to investigate cellular and molecular interactions in the host response to implanted biomaterials. Biomaterials 1997;18:1449–1459.
44. Tatarkiewicz K, Garcia M, Omer A, Van Schilfgaarde R, Weir GC, De Vos P. C-peptide responses after meal challenge in mice transplanted with microencapsulated rat islets. Diabetologia 2001;44:646–653.
45. Seitzer U, Haas H, Gerdes J. A human in vitro granuloma model for the investigation of multinucleated giant cell and granuloma formation. Histol Histopathol 2001;16:645–653.
46. Dersot JM, Colombier ML, Lafont J, Baroukh B, Septier D, Saffar JL. Multinucleated giant cells elicited around hydroxyapatite particles implanted in craniotomy defects are not osteoclasts. Anat Rec 1995;242:166–176.
47. Blottiere HM, Daculsi G, Anegon I, Pouezat JA, Nelson PN, Passuti N. Utilization of activated U937 monocytic cells as a model to evaluate biocompatibility and biodegradation of synthetic calcium phosphate. Biomaterials 1995;16:497–503.
48. Piattelli A, Scarano A, Coraggio F, Matarasso S. Early tissue reactions to polylactic acid resorbable membranes: a histological and histochemical study in rabbit. Biomaterials 1998;19: 889–896.
49. Wang W, Ferguson DJ, Quinn JM, Simpson AH, Athanasou NA. Biomaterial particle phagocytosis by bone-resorbing osteoclasts. J Bone Joint Surg Br 1997;79:849–856.
50. Piattelli A, Scarano A, Corigliano M, Piattelli M. Presence of multinucleated giant cells around machined, sandblasted and plasma-sprayed titanium implants: a histological and histochemical time-course study in rabbit. Biomaterials 1996;17: 2053–2058.
51. Falcone FH, Haas H, Gibbs BF. The human basophil: a new appreciation of its role in immune responses. Blood 2000;96: 4028–4038.
52. Kobayashi H, Ishizuka T, Okayama Y. Human mast cells and basophils as sources of cytokines. Clin Exp Allergy 2000;30: 1205–1212.
53. Huntley JF. Mast cells and basophils: a review of their heterogeneity and function. J Comp Pathol 1992;107:349–372.
54. Schedle A, Samorapoompichit P, Fureder W, Rausch-Fan XH, Franz A, Sperr WR, Sperr W, Slavicek R, Simak S, Klepetko W, Ellinger A, Ghannadan M, Baghestanian M, Valent P. Metal ion-induced toxic histamine release from human basophils and mast cells. J Biomed Mater Res 1998;39:560–567.
55. Schroeder JT, MacGlashan D-WJ, Lichtenstein LM. Human basophils: mediator release and cytokine production. Adv Immunol 2001;77.
56. Tang L, Jennings TA, Eaton JW. Mast cells mediate acute inflammatory responses to implanted biomaterials. Proc Natl Acad Sci USA 1998;95:8841–8846.
57. Brunner T, Heusser CH, Dahinden CA. Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. J Exp Med 1993;177:605–611.
58. Arock M, Merle-Beral H, Dugas B, Ouaaz F, Le-Goff L, Vouldoukis I, Mencia-Huerta JM, Schmitt C, Leblond-Missenard V, Debre P. IL-4 release by human leukemic and activated normal basophils. J Immunol 1993;151:1441–1447.
59. Wedemeyer J, Galli SJ. Mast cells and basophils in acquired immunity. Br Med Bull 2000;56:936–955.
60. Li H, Sim TC, Alam R. IL-13 released by and localized in human basophils. J Immunol 1996;156:4833–4838.
61. Falcone FH, Haas H, Gibbs BF. The human basophil: a new appreciation of its role in immune responses. Blood 2000;96: 4028–4038.
62. 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 1997;6:387–394.
63. Fernandez Alvarez J, Tomas C, Casamitjana R, Gomis R. Nuclear response of pancreatic islets to interleukin-1 beta. Mol Cell Endocrinol 1994;103:49–55.
64. Mandrup Poulsen T, Spinas GA, Prowse SJ, Hansen BS, Jorgensen DW, Bendtzen K, Nielsen JH, Nerup J. Islet cytotoxicity of interleukin 1. Influence of culture conditions and islet donor characteristics. Diabetes 1987;36:641–647.
65. Palmer JP, Helqvist S, Spinas GA, Molvig J, Mandrup Poulsen T, Andersen HU, Nerup J. Interaction of beta-cell activity and IL-1 concentration and exposure time in isolated rat islets of Langerhans. Diabetes 1989;38:1211–1216.
66. Rabinovitch A, Suarez Pinzon WL, Shi Y, Morgan AR, Bleackley RC. DNA fragmentation is an early event in cytokine-induced islet beta-cell destruction. Diabetologia 1994;37:733–738.
67. Spinas GA, Mandrup Poulsen T, Molvig J, Baek L, Bendtzen K, Dinarello CA, Nerup J. Low concentrations of interleukin-1 stimulate and high concentrations inhibit insulin release from isolated rat islets of Langerhans. Acta Endocrinol Copenh 1986; 113:551–558.
68. De Vos P, De Haan BJ, Wolters GHJ, Van Schilfgaarde R. Factors influencing the adequacy of microencapsulation of rat pancreatic islets. Transplantation 1996;62:888–893.
69. De Vos P, Wolters GH, Fritschy WM, Van Schilfgaarde R. Obstacles in the application of microencapsulation in islet transplantation. Int J Artif Organs 1993;16:205–212.