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SYMPOSIUM

Biocompatibility and Immune Acceptance of Adult Porcine Islets Transplanted Intraperitoneally in Diabetic NOD Mice in Calcium Alginate Poly-L-lysine Microcapsules versus Barium Alginate Microcapsules without Poly-L-lysine

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Abstract

Background:

If alginate microcapsules are to be used clinically for therapeutic cell transplants, capsule formulations must be designed to enhance optimal biocompatibility and immune acceptance.

Methods:

Microcapsules were generated using highly purified, endotoxin-free, ultra-low viscosity, high mannuronic acid alginate. The capsules differed with respect to gelling cation (50 m*M* barium or 100 m*M* calcium), alginate concentration (2.0% or 3.3%), alginate density (homogeneous or inhomogeneous), and the presence or absence poly-L-lysine (PLL) coating. Four types of empty capsules were implanted intraperitoneally (i.p.) in normal NOD mice, and their biocompatibility was evaluated after various time periods *in vivo*. Encapsulated adult porcine islets (APIs) were transplanted i.p. in diabetic NOD mice, and immune acceptance was evaluated by graft survival times, host cell adherence to capsule surfaces, and flow cytometric analysis of peritoneal host cells.

Results:

All empty alginate capsules were biocompatible *in vivo*, but barium-gelled alginate capsules without PLL were clearly the most biocompatible, since 99% of these empty capsules had no host cell adherence up to 9 months *in vivo*. In diabetic NOD mice, APIs functioned significantly longer in barium-alginate capsules without PLL than in calcium-alginate capsules with PLL and had strikingly less host cell adherence, although large numbers of host cells (predominantly macrophages and eosinophils) infiltrated the peritoneal cavities of recipients with APIs in both types of capsules. Addition of PLL coatings to barium-alginate capsules dramatically decreased graft survival.

continued →

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Abbreviations: (ANOVA) analysis of variance, (anti-LFA-1 mAb) anti-lymphocyte function-associated antigen-1 monoclonal antibody, (API) adult porcine islet, ($BaCl_2$) barium chloride (BG) blood glucose, ($CaCl_2$) calcium chloride, (CTLA4-Ig) cytotoxic T-lymphocyte antigen 4-immuno-globulin, (IEQ) islet equivalents, (i.p.) intraperitoneally, (NOD) non-obese diabetic, (PLL) poly-L-lysine,

Keywords: alginate, barium, porcine islets, microencapsulation, NOD mice, xenografts

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Abstract cont.

Conclusions:

Inhomogeneous barium-gelled alginate capsules without PLL are the optimal candidates for clinical trials, based on their enhanced biocompatibility and immune acceptance *in vivo*.

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Introduction

Our overall goal is to improve the long-term success of islet transplants in patients with type 1 diabetes mellitus. Our laboratory is investigating porcine islet xenografts, because of the scarcity of donated human islets. We and others have sought to protect xenogeneic donor cells from strong host immune responses by the insertion of a membrane, or coating, on or around the donor cells,[1-3] but biocompatibility has been a formidable obstacle.[4] One of the most promising immunoisolation methods to date is microencapsulation.[5] Successful allogeneic and xenogeneic islet cell transplantation has been demonstrated in non-autoimmune, chemically diabetic, rodent models using calcium-alginate-poly-L-lysine (PLL) and barium-alginate microcapsules.[6-10] Host immune responses to microencapsulated islet xenografts have been investigated in non-immunosuppressed rats[11] and in mice treated with CTLA4-Ig and anti-LFA-1 mAb.[12] Studies of microencapsulated islet grafts in a few non-human primates have been performed with claims of remarkable success[13-15]; also, several patientsreceiving microencapsulated human donor islets have exhibited short-term graft function.[16,17]

We have found that intraperitoneal, microencapsulated, neonatal porcine islet and adult porcine islet (API) xenografts restore normoglycemia in diabetic NOD recipients with excellent metabolic control.[18,19] We have documented synergy between microencapsulation of islets (rat, dog, rabbit, neonatal porcine, and adult porcine) and host immunosuppression (T cell depletion or costimulatory blockade) in diabetic NOD mice.[20-24] Our previous work has shown that encapsulated islet xenografts can correct diabetes for over 100 days in immunosuppressed diabetic mice, but eventually the grafts fail. We are investigating the mechanisms responsible for the graft failure, focusing on issues of capsule durability and immunoreactivity.

Recently, we developed a barium-gelled alginate microcapsule that protects porcine islets in diabetic NOD recipients significantly longer than calcium-gelled alginate-PLL capsules in the absence of immunosuppression. Our goal in this study was to compare these two alginate capsule formulations to identify the microcapsule with optimal biocompatibility and immune acceptance.

Methods

Mice

Eight-week-old, adult, female NOD/LtJ mice (H-2^{g7}) from Jackson Labs (Bar Harbor, ME) were housed under specific pathogen-free conditions, screened weekly for diabetes by measuring random blood glucose (BG) levels, and subsequently treated daily with insulin until transplantation.[18] Non-diabetic, female NOD/LtJ mice (7–9 months of age) were used for implantation of empty microcapsules. All procedures were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources (National Research Council, Washington, DC).

API Isolation

APIs were prepared by dissociating the pancreas with liberase (Roche Diagnostics, Indianapolis, IN) followed by density gradient purification as previously described.[25]

Encapsulation

Alginate microcapsules were prepared as previously reported[26] using highly purified, low endotoxin (49 EU/g), low viscosity (52 mPa·s), high mannuronic acid (57%) sodium alginate (FMC Biopolymer, Oslo, Norway). For calcium-gelled alginate PLL microcapsules, droplets of 2% sodium alginate in 0.9% saline were aspirated through a 22-gauge air jet encapsulation needle (pump

speed 2 ml/min, air flow 6 liters/min) and gelled in 1.1% calcium chloride ($CaCl_2$) in 9% saline, pH 7.1. The capsules were washed in 0.55% $CaCl_2$, then 0.28% $CaCl_2$, then 0.9% saline, and coated for 4–6 min with 0.5 mg/ml PLL (Sigma Aldrich Co., St. Louis, MO; MW 18,000). The coated capsules were washed in 0.1% 2-(*N*-cyclohexylamino)-ethanesulfonic acid (CHES) (Sigma Aldrich Co.), pH 8.2, followed by another saline wash. Dilute sodium alginate (0.2%) was added to cover the PLL. A second PLL layer was added by repeating the above steps with a final coating of dilute alginate. To dissolve the alginate core, the capsules were incubated with 55 m*M* sodium citrate in saline, pH 7.4, and washed three times in 0.9% saline. The resulting liquid-core, calcium-gelled alginate PLL capsules were 916 ± 25 µm in diameter (range 850–960 µm, $n = 20$).

A second type of capsule with a solid, homogeneous alginate core (having the same density throughout) was prepared exactly as above, but omitting the sodium citrate step. A third type of capsule was prepared by increasing the concentration of sodium alginate to 3.3% in 0.9% saline and gelling with 50 m*M* barium chloride ($BaCl_2$), in a solution of 10 m*M* 3-(*N*-Morpholino)propanesulfonic acid hemisodium salt (MOPS), 140 m*M* mannitol, and 0.025% Tween 20. By gelling the alginate in mannitol, instead of in a charged osmolyte such as sodium chloride, the resulting alginate bead becomes inhomogeneous with the highest gel concentration near the surface.[5] The gelled capsules were incubated at room temperature in 50 m*M* $BaCl_2$ for 5 minutes on a rotator and successively washed in 25 m*M* $BaCl_2$, 12.5 m*M* $BaCl_2$, followed by six washes with sterile saline. No PLL was added to these capsules. The diameter of the resulting barium-gelled alginate capsules was 854 ± 11 µm (range 830–880 µm, $n = 50$). Fourth, a barium-gelled capsule was prepared with the addition of a single PLL layer as described above, having a diameter of 906 ± 25 µm.

Transplantation

It has been demonstrated that the intraperitoneal site is suitable for the transplantation of microencapsulated islets.[27] Therefore, diabetic NOD mice were transplanted i.p. with 9000 islet equivalents (IEQ) of encapsulated APIs in a volume of 0.5–0.8 ml via a midline celiotomy. Sixteen mice were transplanted intraperitoneally (i.p.) with APIs in calcium-alginate-PLL microcapsules, and 24 mice were transplanted i.p. with APIs in barium-alginate microcapsules. In our experience, 9000 IEQ of islets consistently results in prompt reversal of diabetes, allowing islet function to be monitored from day 1 post-transplantation by measuring random BG levels. For testing capsule biocompatibility, non-diabetic NOD mice were implanted i.p. with empty microcapsules. Eight mice were implanted with calcium-alginate-PLL microcapsules with a liquid core, 9 mice received calcium-alginate-PLL microcapsules with a homogeneous core, 11 mice received inhomogeneous barium-gelled capsules with one layer of PLL, and 10 mice received inhomogeneous barium-gelled capsules with no PLL. Empty capsules were collected at subsequent time points and evaluated by light microscopy for host cell overgrowth and evidence of breakage.

Flow Cytometry

Flow cytometry was carried out as previously described.[19]

Statistical Analysis

All data were analyzed using the GraphPad InStat program (GraphPad Software, Inc., San Diego, CA). Graft survival data were reported as the mean days of graft function ± the standard deviation. The statistical significance of differences in quantitative variables between groups was analyzed by nonparametric unpaired (two-tailed) Mann Whitney tests. The statistical significance of differences among more than two groups was analyzed by the Tukey-Kramer multiple comparisons test (parametric analysis of variance [ANOVA]). P values ≤ .05 were considered significant.

Results

Biocompatibility of Alginate Microcapsules

The biocompatibility of calcium-gelled alginate PLL capsules either treated with sodium citrate (liquid core, $n = 9$) or not treated (homogeneous core, $n = 8$) was evaluated 1, 2, or 3 months after intraperitoneal implantation in normal NOD mice. After 1–2 months post-implantation, 10–15% of the capsules were covered with adherent host cells, but 85–90% of the capsules were clean, with no evidence of host cell adherence, regardless of citrate treatment (**Figure 1**). After 3 months, the majority of capsules in most animals were clean, although there was a strong cellular reaction to all the capsules in one animal implanted with homogeneous capsules (**Figure 1**).

Next, the biocompatibility of inhomogeneous barium-gelled alginate capsules with either one PLL layer ($n = 11$) or no PLL ($n = 10$) was evaluated after 3, 6, or 9 months *in vivo*. After 3–6 months post-implantation, 95% of barium-gelled capsules with PLL in all mice were clean, although rare capsules were covered with adherent host cells. Virtually all capsules were clean at the 9-month

time point (**Figure 2**). When the PLL coating was omitted, barium-gelled alginate capsules clearly had superior biocompatibility, since 99% of these capsules were clean with no host cell adherence after 3, 6, and 9 months *in vivo*. Only about 1% of the capsules had any evidence of host cell adherence at any time point, and host cells covered only a small fraction of their surface (**Figure 2**).

NOD mice typically have about 2 x 10^6 resident mononuclear cells in the peritoneal cavity,[19] and implantation of empty capsules did not significantly increase the number of peritoneal cells or the populations of different host cells above background level at any time point for any of the capsule formulations studied (by ANOVA, p = nonsignificant; **Table 1**). Eosinophils, which are associated with rejection of microencapsulated xenografts,[19] were not found in recipients of any of the four capsule types (data not shown). However, in a single mouse after 3 months *in vivo*, empty homogeneous calcium-alginate PLL capsules elicited 8 x 10^6 peritoneal cells, predominantly macrophages (**Table 1**). This animal also had capsules heavily coated with adherent host cells (**Figure 1**). There was a trend toward higher numbers of peritoneal macrophages in mice implanted with barium-gelled capsules compared to normal NOD mice (**Table 1**), but the infiltrating cells did not tend to attach to the barium-gelled capsules (**Figure 1**).

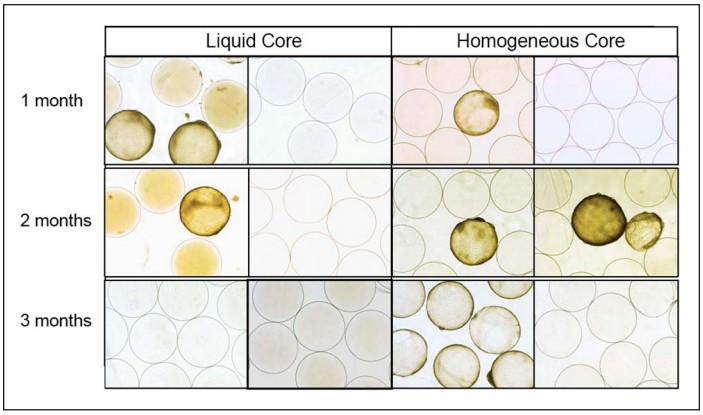

Figure 1. Empty calcium-alginate PLL capsules freshly collected from the peritoneal cavities of representative normal NOD mice at 1, 2, and 3 months post-implantation. Representative images are shown from two different mice receiving the same type of capsule at each time point (light micrograph, original magnification 40x).

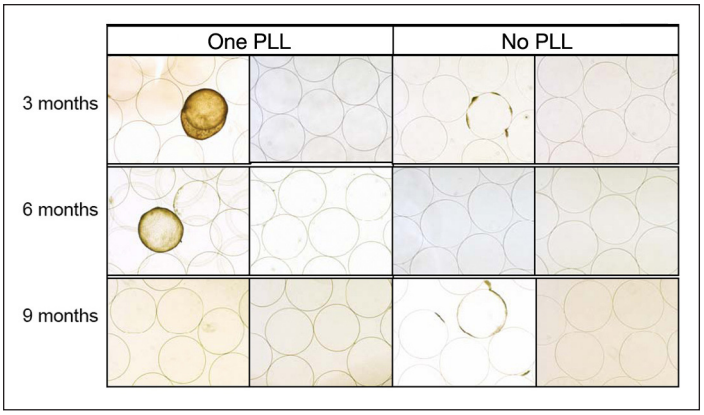

Figure 2. Empty barium-alginate microcapsules freshly collected from the peritoneal cavities of representative normal NOD mice at 3, 6, and 9 months post-implantation. Representative capsules are shown from two different mice receiving the same type of capsule at each time point (light micrograph, original magnification 40x).

Table 1.
Numbers and Phenotypes of Host Cells in the Peritoneal Cavity at Different Times after Implantation of Four Different Types of Empty Microcapsules[a]

Capsule	Months *in vivo*	(*n*)[a]	Total PECs[b]	CD3+ T cells	CD19+ B cells	Neutrophils	Macrophages
Ca++-gelled 2 PLL layers Homogeneous	1	3	1.7, 2.2, ND	0, 0, ND	1.3, ND, ND	0.2, 0.5, ND	1.1, 1.1, ND
	2	3	1.0 ± 0.5	0.05 ± 0.04	ND	0.5 ± 0.3	0.4 ± 0.2
	3	2	8[c],1	0, 0	0, 0	0, 0.1	7[c], 0.4
Ca++-gelled 2 PLL layers Liquid Core	1	3	2.6 ± 1.9	0.05 ± 0.07	ND	0.4 ± 0.4	1.4 ± 1.3
	2	3	1.0 ± 0.3	0.05 ± 0.02	ND	0.2 ± 0.2	0.5 ± 0.07
	3	3	2.2 ± 1.0	0.05 ± 0.02	0.1 ± 0.06	0.03 ± 0.02	1.9 ± 0.9
Ba++-gelled 1 PLL layer Inhomogeneous	3	4	2.5 ± 1.2	0.17 ± 0.23	0.2 ± 0.2	0.02 ± 0.03	2 ± 1
	6	4	4.9 ± 2.4	0.06 ± 0.07	0.4 ± 0.4	0.03 ± 0.04	3.8 ± 2.3
	9	3	2.1 ± 1.6	0, 0.01, ND	0.1, ND, ND	0, ND, ND	3.8, 0.6, 0.1
Ba++-gelled No PLL Inhomogeneous	3	4	4.8 ± 3.0	0.13 ± 0.19	0.5, 3.9, 0.2	0	2.1 ± 1.8
	6	3	8.6 ± 2.3	0.29 ± 0.17	2.0 ± 1.2	0	5.7 ± 1.5
	9	3	4.72 ± 3.08	0.34 ± 0.17	4.7, 0.8, 0.2	0.4 ± 0.5	2.0 ± 1.4

[a] Numbers are expressed in millions. Values are expressed as mean ± standard deviation.
[b] PECs, peritoneal cells; ND, no data
[c] 99% of the capsules in this mouse were covered with host cells (see **Figure 1**).

Effect of Encapsulation with Calcium Alginate PLL versus Barium Alginate Without PLL on Immune Acceptance of API Transplanted IP in Diabetic NOD Mice

To determine whether immune acceptance varies with the type of alginate capsule, diabetic NOD mice were transplanted i.p. with APIs encapsulated in homogeneous calcium alginate capsules with PLL (n = 29) or inhomogeneous barium alginate capsules without PLL (n = 20), and graft function was assessed by measuring random BG levels. Islets in calcium-alginate-PLL capsules functioned for 8.9 ± 2.6 days (**Figure 3**), and, at graft failure, virtually all of the capsules were opaque, clumped together by thick layers of adherent host cells (**Figure 4**). By day 11 post-transplant, large numbers of host cells (44.8 ± 18 x 10^6 cells, **Table 2**), predominantly macrophages and eosinophils, had infiltrated the peritoneal cavities of the mice with relatively low numbers of CD4⁺ T cells, but there was no increase in the numbers of CD8⁺ T cells, B cells, and neutrophils compared to mice receiving empty capsules (**Tables 1 and 2**). This same trend was found on days 15, 16, and 20 post-transplantation.

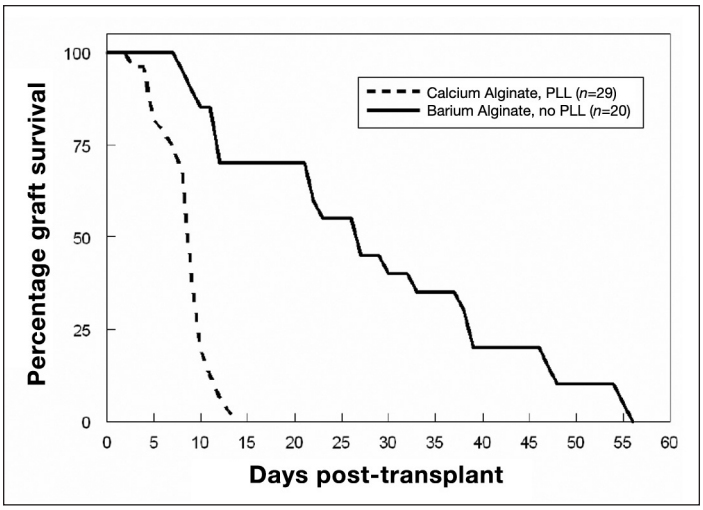

Figure 3. Survival of APIs in calcium-alginate-PLL capsules versus barium-alginate capsules without PLL transplanted i.p. in diabetic NOD mice. Twenty-nine NODs were transplanted i.p. with APIs in calcium-alginate PLL capsules (- - - - -), and 20 NODs were transplanted i.p. with APIs in barium-alginate capsules (——). The mice were allowed to survive until graft failure. Random tail-vein BG levels were monitored as evidence of graft survival. Technically successful grafts were defined as those reducing random BG <150 mg/dl for at least 3 days within the first week post-transplant. Graft failure was defined at the first of two consecutive days with random BG >250 mg/dl.

APIs in barium alginate capsules without PLL functioned for a significantly longer time, 28 ± 16 days (p < .01, n = 20), with some grafts surviving for up to 58 days (**Figure 3**). Instead of being clumped together, the majority of barium alginate capsules were free-floating in the peritoneal cavities of mice with either functioning grafts (**Figure 5**, days 3 and 7) or rejected grafts (**Figure 5**, days 12–68), and minimal host cell adherence was observed up to day 68 post-transplant, in spite of increasing total numbers of peritoneal cells with time (**Figure 5** and **Table 3**). There was little host cell infiltration during the first week post-transplant, and the peritoneal cell populations were similar to those of mice receiving empty barium-gelled capsules without PLL (**Tables 1 and 3**). However, there was a 10-fold increase in the numbers of host cells infiltrating the peritoneal cavity after day 7, predominantly macrophages and eosinophils, with relatively low numbers of CD4+ T cells (**Table 3**). Two mice with the longest surviving grafts also had the lowest numbers of infiltrating host cells (days 59 and 63,

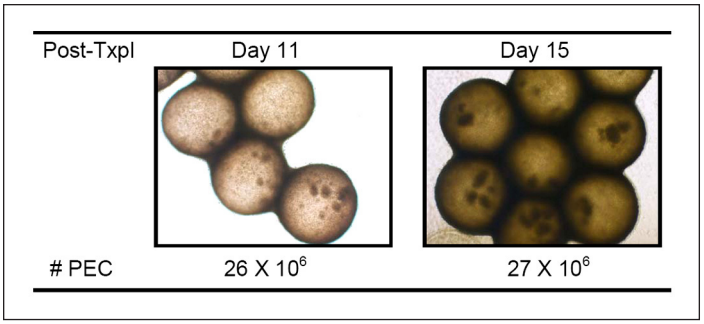

Figure 4. Representative islet-containing calcium-alginate-PLL capsules retrieved from the peritoneal cavity of rejecting diabetic NOD recipients. **(Left panel)** Capsules collected on day 11 post-transplantation (Post-Txpl) from a mouse with 26 x 10^6 total peritoneal cells (PEC). **(Right panel)** Capsules collected on day 15 from a mouse with 27 x 10^6 total peritoneal cells (light micrograph, original magnification 40x).

Table 2.
Numbers and Phenotypes of Peritoneal Cells in Diabetic NOD Mice Tansplanted i.p. with APIs in Homogeneous Calcium-Alginate-PLL Capsules and Sacrificed at Various Time Points when Grafts Were Rejected[a]

Day Post-Transplant	(n)[a]	Total PECs[b]	CD4⁺ T cells	CD8⁺ T cells	CD19⁺ B cells	PMNs[b]	Macrophages	Eos[b]
11	5	44.8 ± 18.0	1.5 ± 0.9	0.3 ± 0.3	0.5 ± 0.3	0.4 ± 0.3	29.2 ± 15.4	9.9 ± 3.6
15–16	8	34.4 ± 12.1	3.0 ± 4.2	0.2 ± 0.4	0.4 ± 0.3	0.9 ± 1.1	22.2 ± 15.4	3.9 ± 1.6
20	3	44.8 ± 18.0	0.4 ± 0.04	0.1 ± 0.1	2.1 ± 1.3	0.4 ± 0.2	37.1 ± 39.9	0.2 ± 0.3

[a] Numbers are expressed in millions. Values are expressed as mean ± standard deviation.
[b] PECs, peritoneal cells; PMNs, polymorphonuclear leukocytes; Eos, eosinophils

Table 3). Next, we asked whether adding PLL coatings to the inhomogeneous barium-gelled capsules would have an effect on immune acceptance. There was a significant decrease in function of the encapsulated APIs with the addition of one layer of PLL to barium alginate capsules (unpaired Student's *t*-test, $p < .01$, No PLL vs. one PLL; **Figure 6**). A further decrease in function was observed with the addition of 2 and 3 layers of PLL, but these differences were not significant, because of the small number of mice transplanted ($n = 3$).

Discussion

Our working hypothesis is that islet immunoisolation (microencapsulation) will promote the function of xenogeneic islets and also minimize the immunosuppressive therapy required for their long-term survival *in vivo*. The possibility of using porcine islet xenografts in patients with type 1 diabetes mellitus is promising, due to advances in porcine islet isolation and recent evidence of prolonged function of porcine islet xenografts in non-human primates treated with multidrug immunosuppressive regimens.[28,29] The success rate of microencapsulated islet transplants in animal models has been encouraging, but results have been variable, depending on a number of factors, including the type of capsule, the islet source (allogeneic versus xenogeneic), the implantation site, and the recipient (normal versus autoimmune).[7,30-32] One important factor postulated to contribute to the loss of islet viability and function after intraperitoneal implantation is insufficient biocompatibility of the capsule with overgrowth by macrophages and fibroblasts.[33] The goal of this study was to compare four different alginate capsule formulations to identify a microcapsule with optimal biocompatibility and immune acceptance.

All four types of empty capsules were relatively biocompatible and non-inflammatory in the peritoneal cavities of normal NOD mice, possibly because they

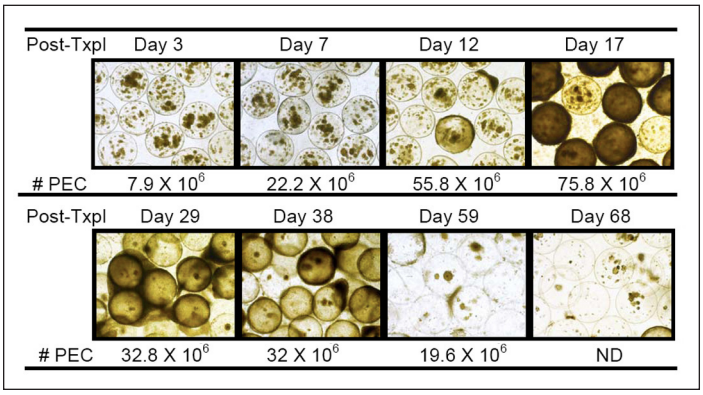

Figure 5. Representative islet-containing barium-alginate capsules retrieved from the peritoneal cavity of rejecting diabetic NOD recipients on days 3, 7, 12, 29, 38, 59, and 68 post-transplantation (Post-Txpl) (light micrograph, original magnification 40x). The total numbers of peritoneal cells (PEC) collected by lavage from each mouse are shown. ND, no data.

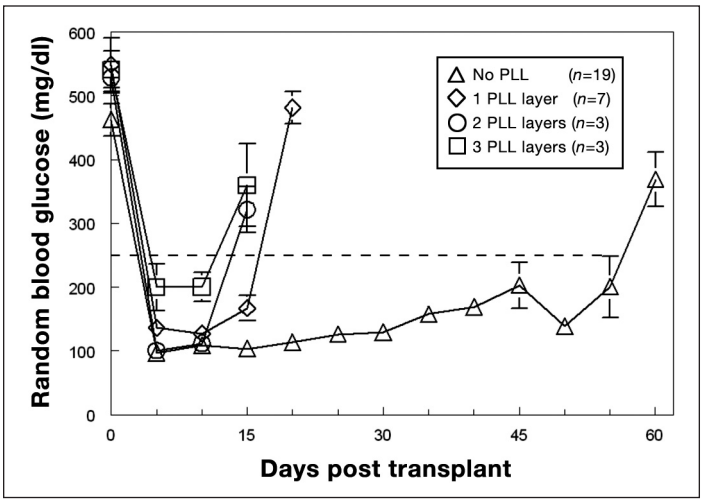

Figure 6. Random BG levels in groups of mice transplanted with APIs in barium-alginate capsules with different PLL coatings. No PLL (Δ, $n = 19$), One layer PLL (◊, $n = 7$), Two layers PLL (○, $n = 3$), or Three layers PLL (□, $n = 3$).

Table 3.
Numbers and Phenotypes of Peritoneal Cells in Diabetic NOD Mice Tansplanted i.p. with APIs in Inhomogeneous Barium-Alginate Capsules and Sacrificed at Various Time Points when Grafts Were Rejected[a]

Day Post-Transplant	(*n*)[a]	Total PECs[b]	CD4⁺ T cells	CD8⁺ T cells	CD19⁺ B cells	PMNs[b]	Macrophages	Eos[b]
3–6	4	4.6 ± 3.8	0.1 ± 0.2	< 0.1	0.79 ± 0.82	0.28 ± 0.44	2.17 ± 1.58	0.33 ± 0.41
7–12	6	41.4 ± 13.6	0.3 ± 0.4	< 0.1	2.06 ± 1.53	0.24 ± 0.46	27.2 ± 9.7	7.4 ± 6.8
15–25	4	50.6 ± 23.9	0.8 ± 0.8	< 0.1	0.66 ± 0.58	0.67 ± 0.68	30.5 ± 7.5	16.1 ± 15.3
27–36	4	44.7 ± 13.2	0.2 ± 0.2	< 0.1	0.55 ± 0.39	1.65 ± 1.57	34.2 ± 8.2	5.8 ± 4.8
36–48	4	43.9 ± 7.4	0.7 ± 0.8	< 0.1	0.75 ± 0.70	1.47 ± 2.15	33.4 ± 6.2	5.0 ± 2.5
59, 63	2	19.6, 21	0.17, 0	< 0.1	0.38, 0	0.17, 0	18.4, 18.3	0, 2.4

[a] Numbers are expressed in millions. Values are expressed as mean ± standard deviation.
[b] PECs, peritoneal cells; PMNs, polymorphonuclear leukocytes; Eos, eosinophils

were all made with the same type of low endotoxin, low viscosity, high mannuronic acid alginate, but the inhomogeneous barium-gelled alginate capsule without PLL was clearly superior with respect to host cell overgrowth. These capsules were remarkably clean up to 9 months post-implantation, in spite of the infiltration of increasing numbers of host macrophages with time (**Table 1**). We hypothesize that their apparent ability to resist overgrowth in the face of a relatively high number of surrounding host cells may be due to the strong affinity of barium for alginate,[34] a toxic effect of barium itself on the host cells,[35,36] and/or the absence of PLL, which is known to induce fibrosis on alginate microcapsules by the induction of cytokines.[4]

In diabetic NOD mice, barium-gelled alginate capsules protected API xenografts significantly longer than calcium-gelled alginate PLL capsules. The islet-containing calcium-alginate-PLL capsules were covered with host cells at the time of graft failure, suggesting cell death as a result of hypoxia or nutrient starvation. In contrast, the majority of barium-gelled alginate capsules were clean and free-floating at graft failure, suggesting a different mechanism of graft destruction, such as the action of cytokines, reactive oxygen species, or other soluble factors. To elucidate the ability of barium-gelled versus calcium-alginate-PLL capsules to protect islets from the direct effects of cytokines and other soluble factors, permeability studies are currently being performed in our laboratory to measure the rates at which radioactively labeled proteins of various sizes can diffuse into the capsules. We are also comparing host sensitization by islet xenografts transplanted in different types of capsules.

To our knowledge, this is the first report of micro-encapsulated islet xenograft survival for longer than 50 days without immunosuppression in an autoimmune animal model. Our findings suggest that barium-gelled alginate microcapsules without PLL provide exceptional protection for xenogeneic islet graft survival, because of both enhanced biocompatibility and improved immune acceptance; thus, these capsules are excellent candidates for studies of islet transplantation in non-human primates and in future clinical trials with patients with diabetes.

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