COMMUNICATION TO THE EDITOR

Entrapment of Dispersed Pancreatic Islet Cells in CultiSpher-S Macroporous Gelatin Microcarriers: Preparation, In Vitro Characterization, and Microencapsulation

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Abstract: Immunoprotection of pancreatic islets for successful allo- or xenotransplantation without chronic immunosuppression is an attractive, but still elusive, approach for curing type 1 diabetes. It was recently shown that, even in the absence of fibrotic overgrowth, other factors, mainly insufficient nutrition to the core of the islets, represent a major barrier for long-term survival of intraperitoneal microencapsulated islet grafts. The use of dispersed cells might contribute to solve this problem due to the conceivably easier nutritional support to the cells. In the present study, purified bovine islets, prepared by collagenase digestion and density gradient purification, and dispersed bovine islet cells, obtained by trypsin and DNAsi (viability > 90%), were entrapped into either 2% (w/v) sodium alginate (commonly used for encapsulation purposes) or (dispersed islet cells only) macroporous gelatin microcarriers (CulthiSpher-S, commonly used for the production of biologicals by animal cells). Insulin release studies in response to glucose were performed within 1 week and after 1 month from preparation of the varying systems and showed no capability of dispersed bovine islet cells within sodium alginate microcapsules to sense glucose concentration changes. On the contrary, bovine islet cells entrapped in CulthiSpher-S microcarriers showed maintained capacity of increasing insulin secretion upon enhanced glucose concentration challenge. In this case, insulin release was approximately 60% of that from intact bovine islets within sodium alginate microcapsules. MTT and hematoxylineosin staining of islet cell-containing microcarriers showed the presence of viable and metabolically active cells throughout the study period. This encouraging functional data prompted us to test whether the microcarriers could be immunoisolated for potential use in transplantation. The microcarriers were embedded within 3% sodium alginate, which was then covered with a poly-L-lysine layer and a final outer alginate layer. Maintained insulin secretion function of this system was observed, which raises the possibility of using microencapsulated CulthiSpher-S microcarriers, containing dispersed pancreatic islet cells, in experimental transplantation studies. © 2001 John Wiley & Sons, Inc. *Biotechnol Bioeng* **75**: 741–744, 2001.

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INTRODUCTION

Pancreatic islet transplantation is an attractive approach for the cure of diabetes mellitus (Sutherland et al., 1996). Among the factors still limiting a wide clinical application of this procedure, immunorejection of isolated islet grafts plays a major role (Weir and Bonner-Weir, 1997). A possible solution to this problem is the separation of implanted cells from the immunological environment of the host by means of biocompatible, selectively permeable membranes (immunoisolation) (Calafiore, 1997; Lanza et al., 1999). Experimental work has demonstrated that although transplanting pancreatic islets into diabetic recipients is feasible in both allo- and xenotransplantation models, success in this field remains elusive. Recently, it was shown that, even in the absence of fibrotic overgrowth, microencapsulated islet grafts fail after implantation into the peritoneal cavity, mainly due to necrosis (De Vos et al., 1999). It is conceivable

that reduced oxygenation of the immunoisolated islets is a major mechanism in this phenomenon (De Vos et al., 1999). To possibly overcome this latter problem, the use of dispersed islet cells or small islet cell aggregates might be attractive, due to the facilitated oxygen and nutritional support. However, since dispersed islet cells do not seem to maintain preserved functional intergrity (Gorus et al., 1984; Kiekens et al., 1992), it is mandatory to develop systems able to support islet cell function. In this regard, the use of microcarrier technology for the growth of surface-dependent cells has gained much interest in several fields (Maurer et al., 1999; Mitteregger et al., 1999). In the present work, we entrapped dispersed bovine pancreatic islet cells within macroporous gelatin microcarriers or sodium alginate and tested their in vitro survival and function. The potential use of these systems in immunoisolation studies was also preliminarily investigated.

MATERIALS AND METHODS

Preparation of Bovine Islets and Bovine Dispersed Islet Cells

Bovine pancreatic islets were isolated by collagenase digestion and density gradient purification, as previously described (Marchetti et al., 1995). After about 7 days from isolation, approximately 10,000 islets were suspended in calcium-free Krebs Ringer Bicarbonate solution, containing 1 mmol/L EGTA. Dispersed islet cells were obtained by adding trypsin (100 µg/mL) and Dnasi (8 µg/mL), at 37°C. The samples were checked every 2 min, and the digestion was stopped by adding cold Krebs Ringer Bicarbonate solution, when mostly single cells or small cell aggregates (three to five cells) were detected. On average, digestions lasted approximately 10 min. The dispersed cells were washed carefully with culture medium by centrifugation at 3000 g for 2 min at 4°C and finally cultured at 37°C.

Preparation of CultiSpher-S Microcarriers

CultiSpher-S microcarriers (Percell Biolytica, Astorp, Sweden) are macroporous gelatine beads in which the anchorage-dependent cells have the possibility to utilize the interior surface. For their preparation, dry microcarriers were swollen and hydrated in calcium and magnesium-free PBS (50 mL/g of dry CultiSpher-S) for approximately 1 h at room temperature. Without removing the PBS, the microcarriers were sterilized by autoclaving (121°C, 15 min, 15 psi). PBS was removed by suction, and new PBS was added. Then rehydrated microcarriers (size varying from approximately 200- to 600-µm diameter) were washed twice with culture medium and mixed with bovine islet dispersed cells (1/1000) in 50 mL of prewarmed medium, and kept at 37°C. The cells in the supernatant were counted every 24 h for 7 days and weekly thereafter, up to 1 month.

Preparation of Sodium Alginate Beads

The microcapsules used in the present study were prepared as previously detailed (De Vos et al., 1999). Sodium alginate (Sigma Chemicals, St. Louis, MO) was solubilized in sterile water (2% w/v). Intact islets or dispersed cells were suspended in the alginate solution (approximately 200,000 cells/25 µL alginate solution), and the mixture was loaded into an insulin syringe with a cut needle. By means of a droplet generator (De Vos et al., 1999) the mixture was extruded in a 100 mM CaCl$_2$ solution. The formation of poly-L-lysine (PLL) membrane was induced by suspending the beads in a 0.1% PLL solution. An outer alginate layer was applied by suspending the capsules in a 0.3% alginate solution. The beads (size varying from approximately 600- to 800-µm diameter) were washed again with sterile water and kept in culture medium at 37°C up to 1 month.

Survival and Functional Studies

Cell survival was evaluated by MTT (3-4,5-dimethyl-thiazol-2-yll-5diphenyltetrazolium bromide) staining, hematoxylin-eosin staining and insulin secretion studies. MTT is cleaved by an enzyme in the respiration chain in the mitochondria of living cells, generating MTT formazan, which is a dark blue, highly visible product. The coloring was dissolved in calcium magnesium-free PBS to a final concentration of 5 mg/mL and mixed with the samples to be evaluated. The mixture was incubated for 45 min at 37°C. The viable cells within the microcarriers were evaluated on an inverted light microscope.

Hematoxylin-eosin staining was performed according to standard histological procedures.

Insulin secretion was evaluated by static incubation, as previously described (Marchetti et al., 1995; Pupilli et al., 1999). After a 45-min preincubation period at 37°C in Krebs Ringer Hepes solution, 0.5% albumin, containing 3.3 mmol/L glucose, islet cells were incubated at 37°C for 60 min in the Krebs Ringer Hepes solution, with 3.3 mmol/L glucose or 16.7 mmol/L glucose. At the end of the incubation time, aliquots of the fluid were retrieved for insulin radioimmunoassay.

Immunoisolation of the Microcarriers

To test whether the CultiSpher-S microcarriers could be potentially used in immunoisolation studies, in some experiments islet cells containing microcarriers were immobilized in microcapsules fabricated from crude alginate (3%) and poly-L-lysine (PLL). A mixture of microcarriers and alginate (2000 microcarriers/mL of alginate) was extruded by using an air-driven droplet generator (De Vos et al., 1999) into a 100 mM CaCl$_2$ solution. The formation of poly-L-lysine (PLL) membrane was induced by suspending the beads in a 0.1% PLL solution. An outer alginate layer was applied by
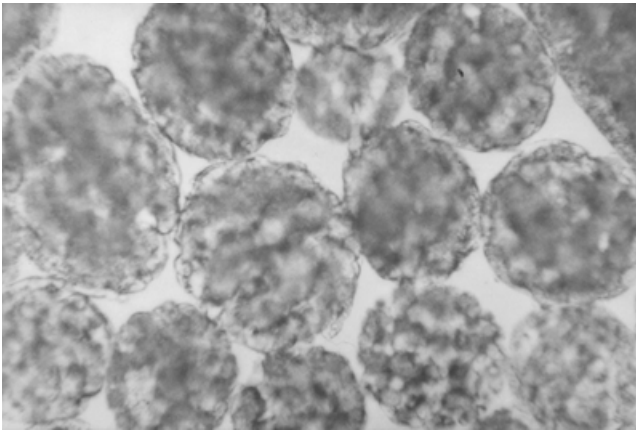
Figure 1. Inverted light microscopy of empty microcarriers.

Figure 3. Hematoxylin-eosin staining of islet cell cluster within microcarriers.

suspending the capsules in a 0.3% alginate solution. Finally, the microcapsules were washed with Krebs Ringer Hepes solution and placed in culture medium. The details of the procedure have been given previously (De Vos et al., 1999).

RESULTS

The procedure of islet dispersion was quite effective, and most bovine islets resulted disaggregated into single cells or small cell aggregates. Viability was >90%, as estimated by the trypan blue procedure. The inverted light microscopy of empty microcarriers is given in Figure 1. When the microcarriers were coincubated with dispersed cells, the number of these latter in the supernatant decreased progressively up to 3 days from the beginning of coincubation, with a nadir of $48 \pm 8\%$ (mean $\pm$ SD of five experiments). MTT staining (Fig. 2) and hematoxylin-eosin staining (Fig. 3) revealed the presence of viable cells up to 1 month from the preparation. Also in the case of dispersed islet cells within sodium alginate microcapsules, viable cells could be detected up to 1 month.

Insulin release studies in response to glucose were performed within 1 week and after 1 month from
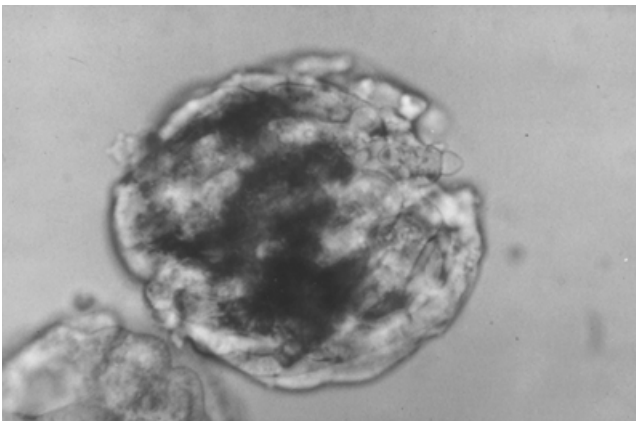

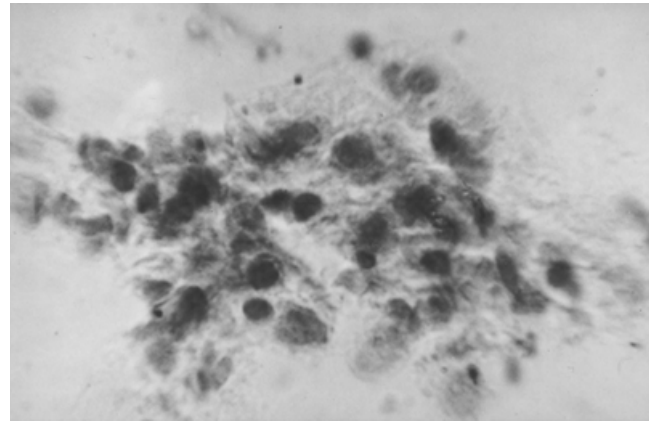**Figure 2.** MTT staining of islet cells within microcarriers.

preparation of the varying systems, and the results are given in Table I. These experiments showed no capability of dispersed bovine islet cells within sodium alginate microcapsules to sense glucose concentration changes. On the contrary, bovine islet cells entrapped in CulthiSpher-S microcarriers showed maintained capacity of increasing insulin secretion upon enhanced glucose concentration challenge. In this case, glucose-stimulated insulin release was approximately 60% of that from intact bovine islets within sodium alginate microcapsules.

Since a major problem in cell transplantation is graft rejection, we preliminarily applied an immunoisolation technique very frequently used in islet transplantation studies (microencapsulation with sodium alginate/PLL) to test whether this procedure can be used with islet cell-bearing microcarriers. The appearance of microcarriers within the sodium alginate-PLL microcapsules is given in Figure 4. When challenged with 16.7 mmol/L glucose according to the procedure described above, insulin release from the microencapsulated microcarriers was similar to that of nonmicroencapsulated microcarriers

Table I. Insulin secretion results from islet cells within CultiSpher-S microcarriers (MC) or sodium alginate microcapsules (SAmc), and from intact islets within sodium alginate microcapsules (Islmc) within 1 week and at 1 month from preparation. Replicates were 10–12 for any given experimental point.

	Insulin release (μU/mL)	
	3.3 mmol/glucose	16.7 mmol/L glucose
Within 1 week		
MC	13.3 ± 1.3	25.6 ± 8.4*
SAmc	6.1 ± 1.5	5.6 ± 0.7
Islmc	16.4 ± 2.3	40.2 ± 9.3#
At 1 month		
MC	14.3 ± 6.2	23.4 ± 12.2*
SAmc	8.9 ± 2.5	9.8 ± 5.8
Islmc	15.3 ± 3.1	42.3 ± 8.9#

[a] $P < 0.05$ versus MC, 3.3 mmol/L G and SAmc, 16.7 mmol/L glucose.
[b] $P < 0.05$ versus Islmc, 3.3 mmol/L glucose and MC, 16.7 mmol/L glucose and $P < 0.01$ versus Samc, 16.7 mmol/L glucose.
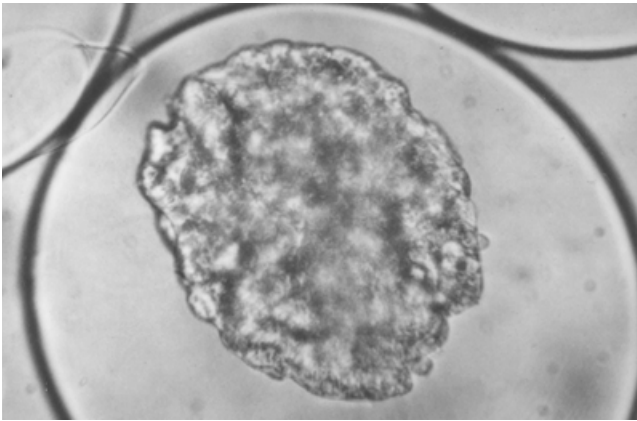
Figure 4. Inverted light microscopy of microcarrier within sodium alginate microcapsule.

both at 1 week (19.7 $\pm$ 4.5 μU/mL; n = 4 10) and at 1 month (21.7 $\pm$ 4.9; n = 4 10) from the preparation.

DISCUSSION

This study provides evidence that dispersed islet cells, prepared from large mammal (bovine) pancreatic islets, can maintain prolonged survival and function when entrapped within macroporous, gelatin-based microcarriers (Culti-Spher-S). This support appears to be more suitable than sodium alginate to preserve islet cell function (insulin release in response to glucose). Since it is well known that islet-derived single cells do not sense glucose stimulation (Kiekens et al., 1992), it is possible (although not investigated in the present report) that some cell-to-cell contact is regained within the microcarriers. Indeed, it was previously shown by using beta-cell lines or rat islet cells that close cell-to-cell contact improves functionality (Malaisse et al., 1999; Hauge-Evans et al., 1999). We also observed that the microcarriers containing the islet cells could be successfully immunoisolated. Although preliminary, this finding seems relevant, since it provides evidence that the system consisting of dispersed islet cells-microcarriers-sodium alginate-PLL could be used in in vivo experiments to possibly prevent graft rejection. In this regard, sodium alginate-PLL microcapsules have been and are widely used in intact islet transplantation studies (De Vos et al., 1999); however, microencapsulated intact islets undergo necrosis even in the absence of fibrotic overgrowth, suggesting reduced oxygenation, in particular in the center of the islets. The use of dispersed cells could conceivably facilitate oxygen and nutrient support to the cells.

A problem with the procedure that we have described is that only a percentage (approximately 50%) of the dispersed islet cells enters the microcarriers. In addition, islet cells within microcarriers release an amount of insulin which is about 60% of that secreted by intact islets within microcapsules. This means that we have to start with an amount of islets that is at least twofold the mass

considered sufficient for a given in vitro or in vivo experiment and suggests that additional effort have to be made to improve the final yield. On the other hand, using large mammals islets, as we have done in the present study with bovine islets, can minimize this problem and possibly allow the optimization of the procedure.

In conclusion, these results show that CultiSpher-S microcarriers can be used for supporting the survival and function of dispersed islet cells derived from large mammal islets; in addition, the possibility of using microencapsulated CulthiSpher-S microcarriers, containing pancreatic islet cells, in experimental transplantation studies is proposed.

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