SiO_2 membrane biocompatibility: a critical issue for long term islet transplantation

C. MAGAGNA[1], M. ROSSI[1], P. BELLAVITE[1], G. CARTURAN[2], S.BONINSEGNA[3], R. DAL MONTE[3], R. DAL TOSO[3]

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[1] Department of Surgery, University of Verona, Italy
[2] Department of Materials Engineering, University of Trento, Italy
[3] I.R.B. Istituto di Ricerche Biotecnologiche s.r.l., Altavilla Vicentina, Italy

Introduction

The Biosil (1) process has been demonstrated to be a suitable approach for the encapsulation of pancreatic islets (2), besides other function cells such as hepatocytes (3), fibroblasts (4), Jurkat cells, and their aggregates (5). The silica layer, is constructed directly on the cell surface, providing mechanical stability (6), cell viability and immunological protection (7). These features are quite promising in the endeavor to reach a definite protocol for cell encapsulation to be used for cell therapy in substitution of full organ transplantation. In this perspective, biocompatibility of the encapsulating material is a crucial issue, as it ensures the long term function of implanted cells.

The calcium-alginate process for immobilization of cells is quite versatile allowing the treatment of important cell masses. However, the material presents some limitations particularly the with time stability and poor mechanical stiffness. We reported the possible combination of the traditional Ca-alginate encapsulation with the Biosil processing (5): alginate droplets, are invested by a gas flow of silcon alkoxides at the moment of formation and dropping so that the siliceous membrane is homogeneously distributed on the surface of the alginate beads. This new technology appears quite promising since function cells are known to maintain function and viability into a Ca-alginate network. These advantages are added to the above mentioned properties of the siliceous membrane (8). Accordingly, the study of the biocompatibility has been studied on Ca-alginate beads coated by the Biosil membrane. This feature has been tested in comparison with other encapsulating material and matrices for cell immobilization.

For a new material, the definition of its biocompatibility is a very demanding task and various tests at differing sensitivity levels have been advanced, such as blood clotting, complement activation, hemolysis, platelet aggregation and neutrophil granulocyte (PMN) activation. However, while in vivo experimentation is generally accepted as the best proof of biocompatibility, (particularly for materials used and stored in a host

organism), **this paper also studies the PMN activation by the implanted material as a discriminating factor between biocompatible and non biocompatible materials**. Adhesion of platelets and PMNs to artificial materials such as the wells of cell culture plates (with and without coating with various proteins) is associated with a cell activation, of different magnitudes according to the nature of the surface and the presence or absence of other soluble activator or priming molecules (10, 11). Previous studies showed that this cell function is modified in several physiopathological conditions and even under pharmacological treatment (12-18).

As previously indicated, our interest for encapsulation of cells by a siliceous membrane is oriented towards cell transplantation: PMN activation with the consequent inflammatory process and deposition of fibrous materials may suppress the advantage of immobilization resulting the exclusion of transplanted function cells from contacts with the host organism.

Accordingly, we studied the activation of PMN adhesion by our silica coated beads, and performed a series of in vivo experiments to ascertain formation of fibrotic layers.

Materials and Methods

Biosil coated Ca-alginate microsphere production: Alginate microspheres were produced by the conventional air-jet extrusion. The alginate solution (Keltone LVCR, 1.5% w/V Na- alginate in 0.9% NaCl solution) was loaded into a sterile syringe barrel and the piston is guided by a uniformly driven pushing device. A sterile needle (0.25 mm internal diameter, 0.5 mm external diameter) was connected to the syringe and placed into a coaxial air-jet extrusion nozzle (0.65 mm diameter). Sterile air-flux ranging from 0.4 L/minute to 1.5 L/minute was bubbled into a solution of $CH_3SiH(OEt)_2$ and $Si(OEt)_4$. The organosilane saturated air flux entered the air-jet extrusion nozzle, providing the siliceous coating and the dripping-off of the alginate drop from the needle tip. Microspheres, dropped into 100 mM calcium chloride solution pH 7,2, were kept in this solution for 10 minutes. The microspheres were then washed two times in HBSS. Microspheres were incubated overnight in cell culture medium. The mean microsphere diameter was of approximately 250 μm.

In vitro tests: Human peripheral blood polymorphonucleate (PMN) cells were separated with a two step Percoll gradient (62% and 73%) and centrifugation at 500xg for 20 minutes at 4°C. Following hemolysis of erythrocytes, PMN cells were suspended in Hanks-buffered solution at $5x10^6$ cells/ml. PMN cells (0,5 mL) were incubated at 37 °C with an equal volume of Biosil-coated alginate microspheres or microcarrier collagen-coated dextran microspheres, with periodic (10 min) stirring. Incubation was prolonged up to 120 minutes. After incubation the microspheres were separated from the unbound cells by a low-speed centrifugation for 2 minutes and washed three times with Hanks-buffered solution. PMN cell adhesion to microspheres was determined by the assay of the constitutive PMN acid phophatase, released in the solution after lysis with Triton-X100 (10, 12-13). Activated PMN cells were quantified with reference to a standard curve of acid phosphatase of a known number of PMN cells of the same donor. Where indicated, opsonization of microspheres by complement fragments was performed by mixing equal

volumes of microspheres suspension and human serum for 30 minutes at 37 °C.

In vivo tests: Balb/c mice (25g, from Charles River) were intraperitonealy injected with either control or BIOSIL coated Ca-alginate microspheres (approximately 10,000 spheres per mouse in 0,6 mL of DMEM culture medium). The mice were kept under normal housing conditions for 8 weeks and, then, microspheres were harvested for microscopical observation.

Results and Discussion

In vitro tests: PMN adhesion was studied as a function of the concentration of microspheres consituted of collagen/dextran and of Ca-alginate beads coated by a 0.2 μm thickness of siliceous material. The data are set forth in Figure 1, where the percent adhesion after extensive washing is used as a quantitative parameter of the strength of interaction between microsphere surface and cells.

Figure 1. Neutrophil adhesion to different doses of microspheres

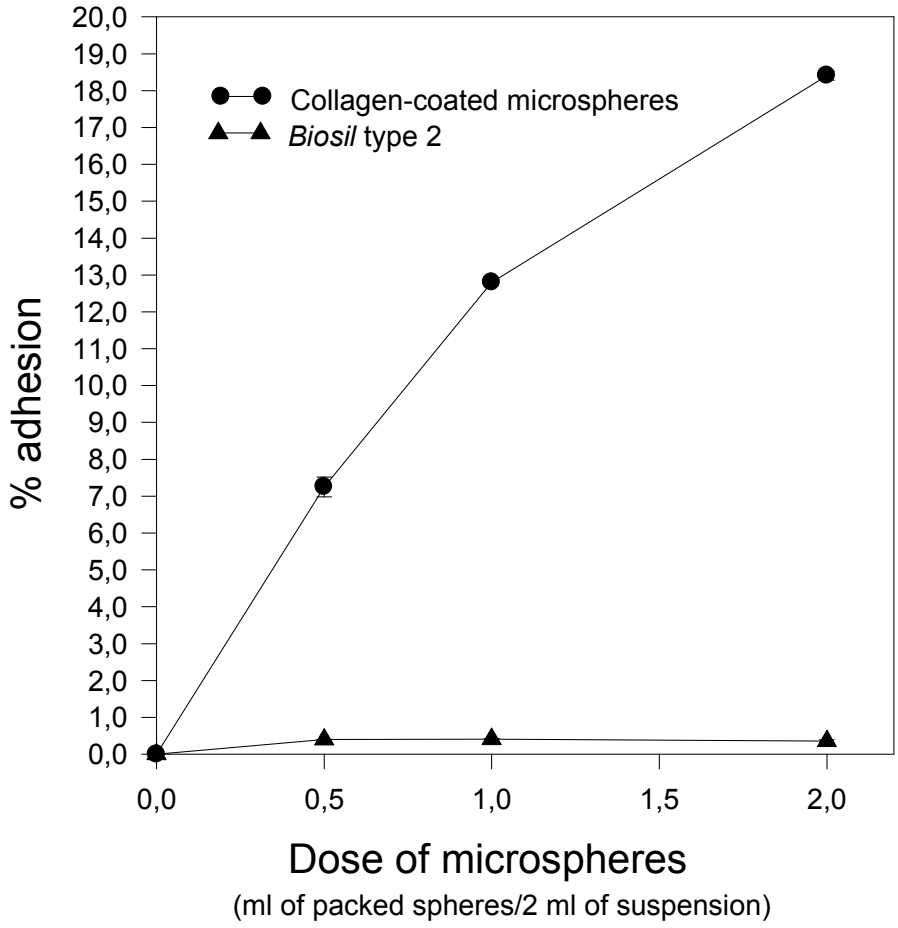
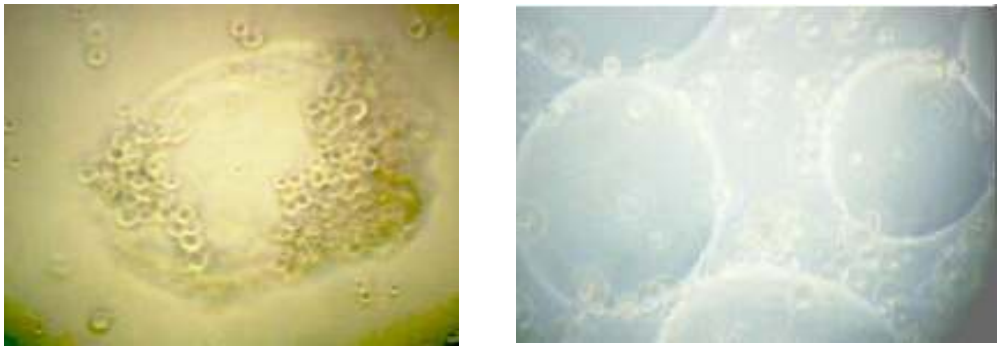


Figure 3. Adhesion of peritoneal cells to microspheres



A) Non coated Ca-alginate B) Biosil coated Ca-alginate

These data indicate a negligible PMN adhesion to by silica coated beads in contrast to the almost linear increase of percent adhesion in the case of collagen/dextran microspheres. This important PMN test for biocompatibility also studies the adhesion as a function of time. Figure 2 shows PMN test comparing the collagen/dextran and the Ca-alginate+silica microspheres.

No difference was found in the PMN adhesion to Biosil microspheres when these microcarriers were pre-treated with human serum for 30 min at 37 °C (data not reported).

Therefore, according to the PNM activation test, It can be concluded that our capsules are fully biocompatible.

In vivo tests

Biocompatibility test were carried out by implantation of Ca-alginate beads coated or not by silica implanted intraperitonealy in Balb/c mice. The implanted material was extracted after 2 and 8 weeks. A representative experiment showing the feature of extracted microspheres is presented in Figure 3.

The non coated beads recovered after 8 weeks are fully populated by peritonealcytes, in contrast to the silica coated microspheres which show the greater part of the surface to be uncolonized.

Therefore, the ensemble of evidence presented here substantiates the total biocompatibility of the Biosil membrane. The results also confirm our indirect observation on the use of pancreatic islet encapsulation and grafting in diabetic mice (9).

References

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