Long-term delivery of mEPO in mice using cell encapsulation technology

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Introduction

Somatic gene therapy is being considered in the treatment of a number of acquired chronic diseases. One interesting approach is cell encapsulation, in which engineered somatic cells are protected against immune cell- and antibody-mediated rejection by their immobilization in a polymer matrix surrounded by a semipermeable membrane. The latter regulates the bi-directional diffusion of nutrients, oxygen and waste, allowing the controlled and continuous delivery of therapeutic proteins in the absence of immunosuppression (1). In addition to sharply reducing the frequency of administrations and thus improve patient comfort, cell encapsulation strategy would improve the pharmacokinetics of easily degradable peptides and proteins, which often have short half-lives *in vivo*.

In the last few years, much effort has been focused on studying the biocompatibility of materials and capsules, designing new immunoisolation devices or just demonstrating the proof of principle of this cell-based technology. We have reported previously that careful selection and evaluation of purified alginates and cell lines and fabrication of small and uniform microcapsules are key requirements to ensure an optimal biocompatibility, long-term functionality and a suitable zero-order kinetic release of the therapeutic molecules (2). However, little research has involved the study of parameters such as the implantation site of the encapsulated cells, the feasibility of using the same approach for syngeneic or allogeneic transplantation or the implication of a well-vascularized immobilization device in order to permit close contact between the encapsulated cells and the blood stream and thus improve the long-term efficacy of the graft.

Recently, we have demonstrated that the subcutaneous route is an excellent administration way to implant the microencapsulated cells. In addition, we have observed that erythropoietin (EPO) secreting C_2C_{12} myoblast cells immobilized in alginate microcapsules implanted at this level give rise to high hematocrit levels during 100 days (3).

In this new approach, we pretend to study the long-term functionality of the encapsulated mEPO secreting cells after their administration into the subcutaneous space of Balb/c mice without implementation of immunosuppressive protocols. With the aim of addressing this issue, we have studied the viability, functionality and biocompatibility of the encapsulated EPO-secreting cells for six months.

Material and Methods

Cell culture: C2C12 myoblast cells derived from the skeletal muscle of a C3H mouse and genetically engineered to secrete murine EPO were kindly provided by the Institute des Neurosciences (Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland). Cells were grown in Dulbecco´s modified Eagle medium (DMEM) containing 10% foetal bovine serum (FBS), 2 mM L-glutamine, 4.5 g/l glucose, 100 U/mL penicillin, and 100 U/mL streptomycin. Cells were

passed every 2-3 days and maintained at 37ºC in 5% CO_2. All the components of the culture medium were purchased from Gibco BRL (Invitrogen S.A., Spain).

Cell encapsulation: We encapsulated engineered myoblasts into alginate-poly-L-lysine-alginate (APA) microcapsules prepared using an electrostatic droplet generator. Cells were suspended in 1.5% (w/v) low-viscosity high guluronic acid (LVG) alginate (FMC Biopolymer, Norway) obtaining a cell density of 2 x 10^6 cells per mL alginate. This suspension was extruded into a 55 mM calcium chloride solution and the resulted alginate beads were successively coated with poly-L-lysine 0.05% (w/v) for 5 min (MW: 15,000-30,000, Sigma, St. Louis, MO) and alginate 0.1% (w/v) for 5 min. Microcapsules were prepared at room temperature and under sterile conditions and cultured in complete medium. The mean microcapsule diameter was 485 ± 15 µm.

Characterization: Before implantation, the diameters and overall morphology of the encapsulated cells were characterized using an inverted optical microscope (Nikon TSM) equipped with a camera (Sony CCD-Iris). The in vitro viability of the entrapped cells was evaluated by the MTT assay and the morphology of the cells was evaluated by confocal microscopy. EPO was determined using a sandwich ELISA kit for human EPO (R&D Systems, Minneapolis, MN, USA).

Implantation: Adult female Balb/c mice were anesthetized by ether inhalation and microcapsules were implanted subcutaneously using a catheter (Nipro 18 gauge; Nissho Corp.). Control animals received HBSS saline solution by the same route. Blood was collected weekly by retro-orbital puncture using heparinized capillary tubes

Histological analysis. At day 180, capsules were explanted, and fixed in 4% paraformaldehyde solution in 0.1 M sodium phosphate, pH 7.2, for a minimal of two days. Thereafter, tissues were in increasing 10 to 30% sucrose/PBS and frozen in dry-ice cooled isopentane. Serial horizontal cryostat sections (14 µm) were processed for haematoxiline-eosine staining.

Results and Discussion

Figure 1 illustrates the morphology of the microcapsules

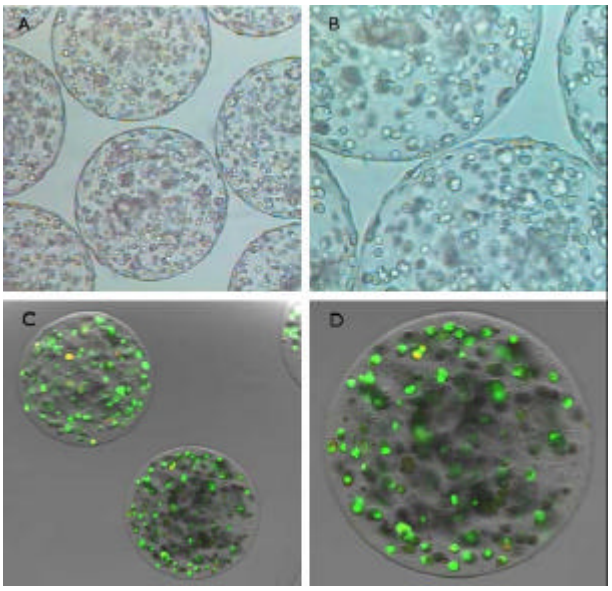

Figure 1. Morphology of microencapsulated EPO-secreting myoblasts. Optical microscopy image (A, B). Fluorescence microscopy image (C, D).

As it is observed in Figure 1, all the microcapsules had a uniform and spherical morphology without irregularities in the surface. The microphotographs indicate that cells enclosed in the alginate matrix present high viability and thus good adaptation to the polymer-scaffold.

The metabolic activity of the encapsulated cells was analyzed over the course of 21 days in an *in vitro* assay. As it can be observed in Figure 2, C_2C_{12} myoblasts showed similar viabilities over the days. A slight decrease was observed during the first week but after this period, entrapped cells maintained their viability.

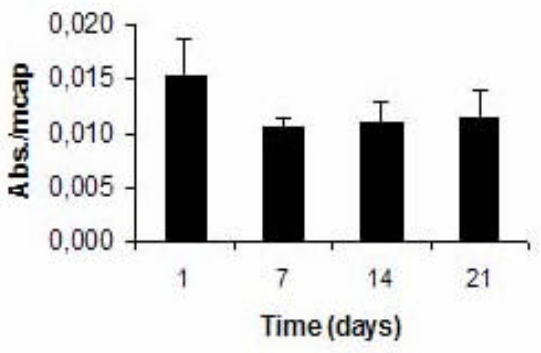

Figure 2. In vitro viability of EPO secreting C_2C_{12} myoblasts immobilized in APA microcapsules.

We measured the EPO production of 0.2 mL of polymer microcapsules loaded with EPO-secreting C_2C_{12} myoblasts to characterize the EPO dose. Results indicated encapsulated cells released 17500±600 IU EPO/24h.

Once the 0.2 mL of microcapsules were implanted in the subcutaneous tissue of allogeneic Balb/c mice, the hematocrit level of all the animals increased in comparison with the control group (receiving a saline solution). An increase in the hematocrit level was seen in the implanted recipients during the first 14 weeks post-transplantation. Although the hematocrit level decreased slightly after this period, the levels remained quite uniform (Figure 3).

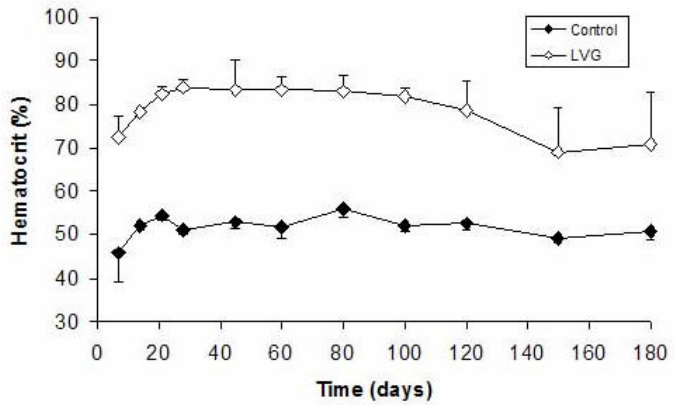

Figure 3: Hematocrit levels of Balb/c mice after implantation of encapsulated cells.

The histological analyses of the explanted microcapsules revealed a vascularization development surrounding the microcapsule aggregates (Figure 4). A weak fibroblast outgrowth was detected especially surrounding the capsules.

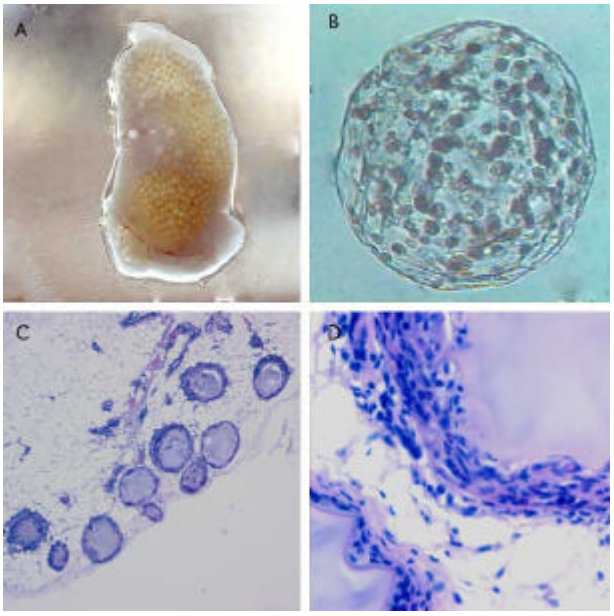

Figure 4: (A-B) Microcapsules explanted from the subcutaneous tissue 180 days post-implantation. (C-D) Histological analysis of explanted cell-containing microcapsules.

Although EPO delivery might be limited by the formation of this fibrotic layer around the microcapsules, caused by impurities of the alginates and other factors, the high EPO secretion of the encapsulated cells together with the pharmacodynamic behaviour of this molecule may lead to enough EPO plasma levels and thus a therapeutic effect.

Conclusion

In the present study, subcutaneous implantation of encapsulated EPO-secreting cells resulted in a significant increase of hematocrit levels. High and constant levels were maintained with only one shot of cell-loaded microcapsules and lacking immunosuppressive protocols.

Bibliography

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