Bioactive cell-hydrogel microcapsules for cell-based drug delivery

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Introduction

The technology of cell microencapsulation is based on the immobilization of therapeutically active cells within a polymer matrix, often alginate, surrounded by a semipermeable membrane. Such encapsulated cells are protected against immune cell- and antibody-mediated rejection and have the potential to produce an array of therapeutically active substances (Lim and Sun 1980; Lanza 1996; Orive 2003). These "living" drug delivery systems are especially interesting for controlled and continuous expression of hormones and growth factors, for the local and targeted delivery of drugs and to improve the pharmacokinetics of easily degradable peptides and proteins, which often have short half-lives in vivo. One potential impact of this drug delivery approach is that administration of immunosuppressants and implementation of strict immunosuppressive protocols can be partially or fully eliminated and therefore the serious risks associated with these drugs can also be avoided.

Typical calcium-alginate beads are sensitive to non-gelling agents such as sodium and potassium, which in physiological conditions, results in osmotic swelling of the beads, inevitably leading to increased pore size and destabilization and rupture of the polymer scaffold. Addressing some of these limitations may have a major impact on the long-term functionality of the enclosed drug-secreting cells. However, one major risk when designing strategies aimed to increase the stability of the devices is that this enhancement may sacrifice other properties such as capsule permeability, intra-cellular microenvironment and cell functionality.

Interestingly, until now, only inactive biomaterials and polymers have been used for microcapsule elaboration and all the reported approaches have considered the three dimensional capsules as inert scaffolds in which cells are physically entrapped and transported. In contrast, we investigated the possibility of designing biomimetic cell-hydrogel capsules to promote the in vivo long-term functionality of the enclosed cells and improve the mechanical stability of the capsules. To address these issues, first, we fabricated biomimetic alginates by coupling the fibronectin-derived adhesion peptide arginine glycine aspartic acid (RGD) to alginate polymer chains. An alginate-mixture providing a bimodal molecular weight distribution was used for capsule preparation with the aim of increasing the elastic modulus of the gel while minimally raising the viscosity of the pre-gelled solution. Increasing the mechanical properties of the gels by simply raising the concentration of the high molecular weight alginates typically used for cell encapsulation would also significantly raise the viscosity of the pre-gel solution, and increase cell damage during mixing (Kong 2003). Because alginate itself does not support cell attachment, the decoration of the polymer with RGD will facilitate its interaction with the integrin receptors of the enclosed cells and hypothetically prolong the survival and functionality of the latter (Alsberg 2002).

We suggest that biomimetic cell-hydrogel capsules can increase the stability of the cell-loaded capsules and provide an improved microenvironment for the enclosed cells. In the present paper, we report that appropriately designed cell-hydrogel scaffolds significantly increase the mechanical

resistance of the capsules while minimizing damage during cell encapsulation. The novel biomimetic capsules provide cell adhesion for the enclosed cells and prolong their long-term functionality and drug release. In addition, controlling the cell-dose within the biomimetic capsules enables a controlled in vitro and in vivo drug delivery.

Material and Methods

Biomimetic alginates: Low viscosity and high guluronic acid containing alginate (LVG) and medium viscosity and high guluronic acid containing alginate (MVG) were purchased from Protanal LF 20/40 (FMC Technologies). The irradiation dose was varied from 2 to 5 Mrads. Following irradiation, alginate molecules were functionalized with synthetic oligopeptides containing a sequence of (glysine)4-arginine-glysine-aspartic acid-tyrosine (G4RGDY, Commonwealth Biotechnology Inc., Richmond, VA, USA) by using previously described carbodiimide chemistry (Rowley JA, Mooney DJ 1999). The molar ratio between RGD peptides and alginate chains was kept constant at 0.016: 1. Briefly, alginate solutions were prepared with a 2-[N-morpholino]ethanesulfonic acid hydrate (MES, Sigma) buffer and sequentially mixed with N-hydroxysulfosuccinimide (sulfo-NHS, Pierce Chemical), and 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide (EDC, Sigma). Oligopeptides were added to the reaction, and the alginates were allowed to react for 24 h. The modified alginates were purified by extensive dialysis against distilled water which removed the molecules smaller than 3,500 g/mol.

Cell culture: C2C12 myoblasts derived from adult C3H mouse and genetically engineered to secrete murine EPO were kindly provided by the Institute des Neurosciences (Ecole Polytechnique Federale of Lausanne, Lausanne, Switzerland). Cells were grown in Dulbecco´s modified Eagle medium containing 10% fetal bovine serum, 2mM L-glutamine, 4.5 g/L glucose, and 1% antibiotic/antimycotic solution.

Cell encapsulation: C2C12 cells were immobilized within RGD and non-RGD microcapsules using an electrostatic droplet generator. Briefly, cells (5x106 cells/ml) were suspended in RGD-alginate mixture solution (2.75% w/v) (50:50 LVG-RGD and MVG-RGD alginate) and non-RGD alginate mixture solution (1.4% w/v) (50:50 LVG and MVG alginate). The cell suspension was extruded into calcium chloride solution and the resulting alginate beads were successively ionically linked with a 0.05% (w/v) poly-L-lysine solution and alginate solution 0.1% (w/v) for 5 min.

Implantation: Adult female Balb/c mice (Harlan Interfauna, Spain) were used as allogeneic recipients. Before implantation, microcapsules were washed several times in Hank´s balanced salt solution (HBSS). Animals were anesthetized by isoflurane inhalation and a total volume of 0.2 ml of cell-loaded RGD and non-RGD microcapsules were implanted.

Hematocrit measurement:At specific time points blood was collected by retroorbital puncture on anesthetized animals using heparinized capillaries (Deltalab, Spain). The hematocrit was measured by a standard microhematocrit method. Results are expressed as mean ± standard deviation.

Histological analysis. At day 330 after transplantation, animals were sacrificed and microcapsules were retrieved and fixed in a 4% paraformaldehyde solution in 0.1 M sodium phosphate, pH 7.2. Thereafter, serial horizontal cryostat sections (14 µm) were processed for hematoxylin-eosin staining.

Results and Discussion

Small diameter (450 μm) and uniform three dimensional (3D) microcapsules were fabricated with RGD and non-RGD alginate solutions. We hypothesized that together with the bimodal molecular weight distribution of alginate mixture, the cell-ligand interactions may be helpful to increase the mechanical properties of the matrices as cells themselves will take part in the matrix network formation (Figure 1).

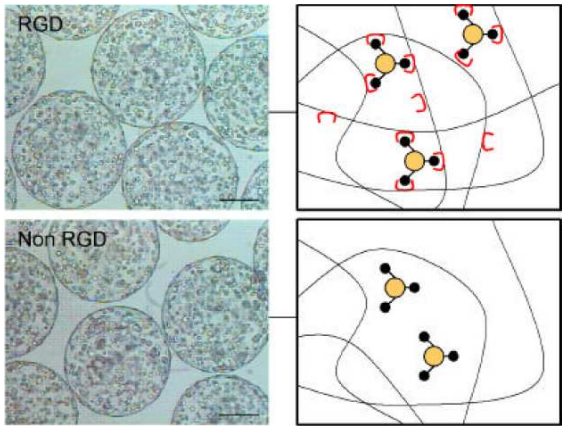

Figure 1. Schematic description of the biomimetic cell-hydrogel capsules compared to non-RGD capsules. RGD modification provides a specific mechanism for cell adhesion.

The mechanical rigidity of the RGD and non-RGD alginate microcapsules was evaluated. The swelling of RGD alginate microcapsules (1.12 ± 0.01) was significantly lower than non-RGD devices (1.16 ± 0.01) ($n = 20$, $P < 0.05$) (Figure 2a), whereas the resistance of the capsules against compression was significantly higher (49.6 ± 6.7 g/microcapsule vs. 39.1 ± 4.7 g/microcapsule; $n = 20$, $P < 0.01$) (Figure 2b). In addition, the binary gel system in either case significantly improved the mechanical resistance reported for classical single alginate-poly-L-lysine capsules, which is close to 16 g/microcapsule (Grandoso 2007).

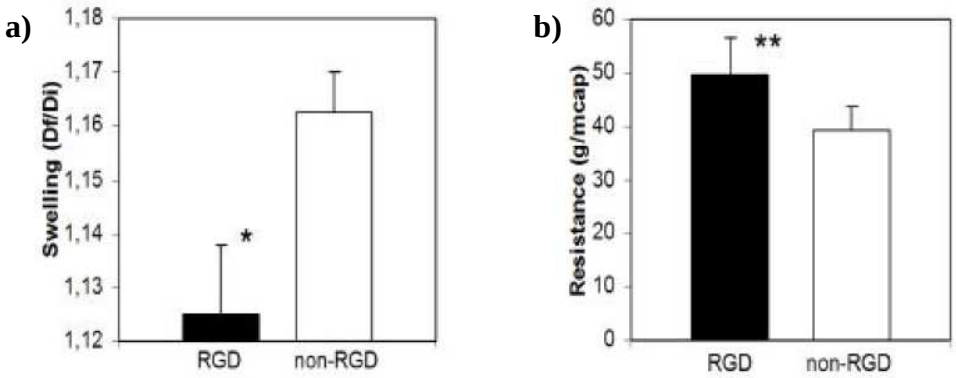

Figure 2. a) The swelling of biomimetic scaffolds was significantly lower than non-RGD devices whereas b) the resistance of the capsules against compression was significantly higher.

Both types of microcapsules were evaluated in an *in vivo* study. Results indicated that hematocrit levels of all implanted recipients (RGD and non-RGD microcapsules) were significantly increased ($P < 0.01$), as compared to the control mice. Interestingly, in the last 100 days of the study the

hematocrit level of the recipients with RGD alginate matrices was significantly higher than non-RGD devices (Figure 3a). Therefore, biomimetic cell-hydrogel capsules exhibit a more sustained functionality, maintaining the hematocrit levels close to 85% during 10 months after only one administration. To our knowledge, this is the first report describing such a sustained EPO release from encapsulated cells implanted subcutaneously and provides a three-fold increase in the efficacy of the drug delivery system compared to other previous reports from our group. The histological analysis of the capsules revealed a minimal inflammatory reaction based on a thin fibroblastic layer surrounding both RGD and non-RGD capsules (Figure 3b).

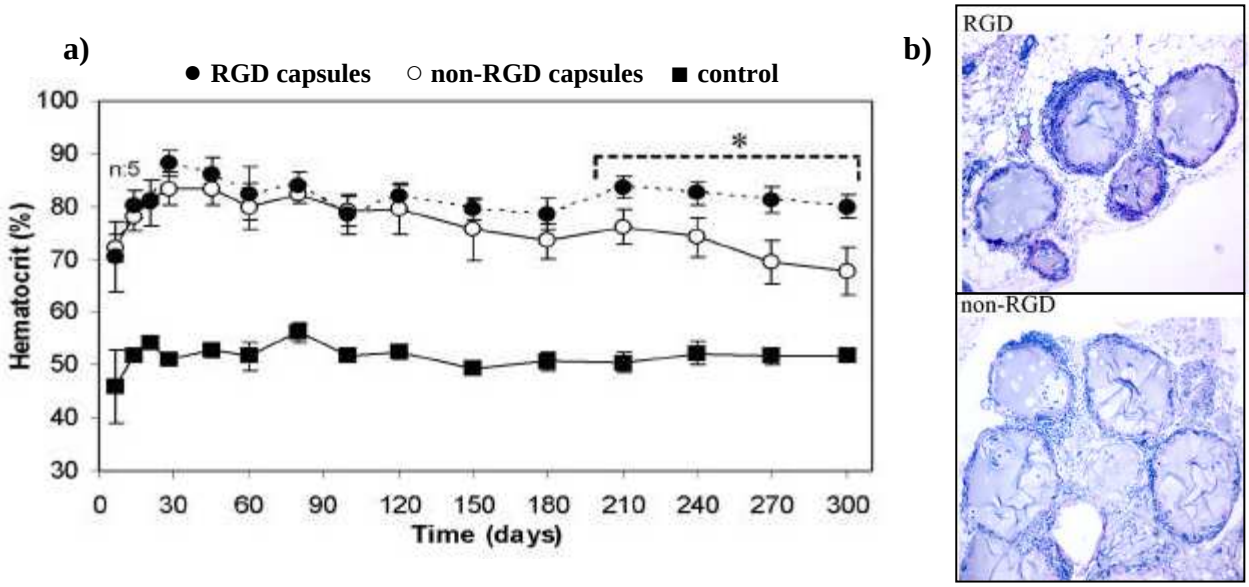

Figure 3. a) Hematocrit levels of capsule implanted mice. b) histological evaluation of the samples.

Conclusions

Biomimetic design of cell-loaded capsules can improve cell performance as cells are immobilized in a functional scaffold which mimics the natural microenvironment of the cells (Orive 2008). This approach may be broadly applicable to any therapeutic approach in which continuous and controlled drug or growth factor delivery is necessary, including deficiencies of a gene product, central nervous system diseases and pathologies demanding chronic drug treatments.

Bibliography

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