Multicompartimental microcapsules for cell entrapment

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Introduction

Recent advances in cell biology, encapsulation technology and biomaterials sciences have led to the proposal that xenotransplantation procedures combined with an adapted immunoprotective strategy, may provide a solution to the shortage of human allografts. Cell encapsulation is a well known technique for the entrapment of viable cells within a semi-permeable polymeric membrane. These membranes are usually formulated to permit small molecules such as nutrients to freely permeate into the microcapsule while blocking larger molecules and cells of the host immune system. Encapsulation allows the transplant of xenogenic or allogenic cells into a host to repair or replace damaged or diseased tissue without the need of immunosupressive regimens (Uludag H. et al. 2000) that are often characterized by a number of adverse effects (Rabkin J.M. et al. 2000). Most cell encapsulation devices are usually composed of polymers such as alginate, carrageenans, collagen, chitosan, cellulose and agar/agarose (Park J.K. et al. 2000). Among the polymers tested so far, alginate has been and will continue to be one of the most important encapsulating material. Alginate cross-linked with Ca or Ba ions has been used successfully to encapsulate cells and to maintain their function in tissue culture (Draget K.I. et al. 1997 and De Vos P. et al. 2006). Xenogeneic and allogeneic ECM has been used as a bioscaffold for different tissue engineering applications (Badylak 2004). The extracellular matrix (ECM) is a complex mixture of structural and functional proteins, glycoproteins, and proteoglycans arranged in a unique, tissue specific three-dimensional ultrastructure. All ECMs share the common features of providing structural support and serving as a reservoir of growth factors. For this reason, ECM proteins have been utilized extensively in an effort to modify the physical, mechanical, or immunogenic properties of bioscaffolds. The present paper is intended to describe the optimization, by a factorial design approach, of a method based on the application of an "air-monojet" instrument for the encapsulation of Sertoli cells (SC) into alginate/EMC microcapsules (multicompartimental microcapsules). Special attention will be paid to fix the experimental set up resulting in the formation of uniform spherical smooth microcapsules.

Materials and method

The polymer used for microparticle preparation was ultrapure sodium alginate NAG from Kelco Biopolymers (UK). A 2% (w/v) aqueous solution of NAG has the following characteristics: MW 120,000/190,000 kd; content of M 61% and G 39%, viscosity 100/130 (Brookfield 25° C, 60 rpm), pH 6.4-8.0, endotoxin level 39 EU/g, as provided by the manufacturer. Before use, NAG solutions were successively filtered by a stainless steel pressure holder from Sartorius (Germany) equipped with 0.8, 0.45 and 0.22 μm cellulose acetate membranes. The cation used for the ionic gelation was calcium chloride diydrate from Sigma-Aldrich (Germany). All other chemicals were from Fluka and were of the highest purity available Monodisperse alginate beads were prepared using an encapsulation device based on air driven nozzle and the experimental procedure elsewhere described (Luca G. et al. 2007). Different calcium alginate microcapsules were produced by changing the reported experimental parameters following an experimental design and statistical

analysis: the atomizing air flow (air flow), the alginate pumping rate ("pump") and the distance between the nozzle and the surface of the gelling bath ("height") (see Table 1).

Parameter	Meaning	Range
Air flow	Frequency of the vibration of the nozzle	3.8-4.6 (l/min)
Pump	Polymer pumping rate	0.46-0.52 (ml/min)
Height	Distance between the nozzle and the gelling surface of the gelling bath	3.2-4.0 (cm)

Table 1. Production of alginate microcapsules by "air-monojet" encapsulation procedure: the investigated experimental parameters and their range of variation.

The morphology of alginate microbeads was evaluated by optical-stereomicroscopy (Nikon SMZ 1500 stereo microscope, Tokio, Japan). Microcapsule size and size distribution (by number) were determined by photomicrograph analyses (Eclipsenet version 1.16.5, Laboratory Imaging s.r.o. for Nikon B.V.). In the technique, bead samples, immediately after preparation and at intervals after storage under different conditions, were applied to a microscope slide and examined microscopically. A sample of 100–300 beads was examined, and the mean size was determined. For studying the effect and the influence of different experimental parameters (e.i. air flow, pump and height) on the dimensional and morphological characteristics of alginate microcapsules, a randomized central composite face-centered design (CCF) consisting of 16 runs was used. The parameters were varied as reported in the experimental matrix (see Table 1). The experimental design and the evaluation of the experiments were performed by the PC software MODDE 8.0 (Umetrics AB, Sweden), followed by multiple linear regression (MLR) algorithms. SC were isolated from testis of prepubertal neonatal Large-White piglets as previously described[2]. The culture was maintained at 37 °C in 5% CO_2-95% air in HAM F12. At 24 h of culture, the cells were treated with 10 mM tris (hydroxymethyl) aminometane hydrochloride buffer in order to eliminate any residual germinal cells. These pretreated isolated SC cell cultures were washed and maintained in the supplemented HAM-F12 medium. The in vitro cultured SC were added to a sodium alginate solution containing EMC powder resulting in a final concentration of 1.6% (w/v). Afterwards, this suspension was pumped to the encapsulator system by a peristaltic pump and separated into equal sized droplets by the atomizing nozzle. The generated microdroplets were allowed to fall into an isotonic calcium chloride solution (1.2%; w/v). After gelification (5 minutes) of the microdroplets in the calcium containing gelling bath, the microbeads were washed twice with an isotonic solution. Viability was assessed by staining the preparations with ethidium bromide (Sigma) and fluorescein diacetate (FDA). Cells were visualized under fluorescence microscope (Nikon, Optiphot-2, Nikon Corporation, Tokyo, Japan) using the filter block for fluorescein. Dead cells were stained in red, while viable cells appeared in green.

Results and Discussion

In a preliminary phase of this study, a "design of the experiments" (DoE) optimisation and screening of the experimental parameters were performed. Indeed, this approach was chosen in order to evaluate which factor(s) could more deeply affect the production of the alginate microcapsules in terms of general geometry, surface characteristics and dimension.
The DoE offers a rational approach that enhances the value of the research, reducing the number of experiments and providing much more information about the effects of different variables and their

possible interactions. For performing the factorial design test, the parameters air flow, pump and height were chosen as variables and tested at three levels.
At the end of the complete DoE study we were able to identify a set of experimental parameters resulting in the production of microcapsules with optimal characteristic for cell trasplantation protocols, at least from a morphological point of view, such as, spherical shape, smooth surface, very narrow size distribution, absence of tails and coalescences.

From the analysis of the results, it was evident that the best alginate microcapsules were those prepared with the following experimental parameters: air flow 4.2 L/min, pump rate 0.49 mL/min and height 3.6 cm (see Fig. 1).

Once obtained alginate microcapsules with a satisfactory morphology through the application of DoE approach, we started a new series of experiments aimed to the encapsulation of SC and ECM into alginate microcapsules. SC and EMC were homogeneously distributed within the microcapsules, with the latter showing no appreciable morphological defects, such as "tails", partial fusion or partial protrusion of the encapsulated cells (see Fig. 2). The fluorescence photomicrographs reported in Fig. 2 showed the viability of encapsulated SC, after 24 hours of cell culture, demonstrating that the cells were >90% viable after the encapsulation.

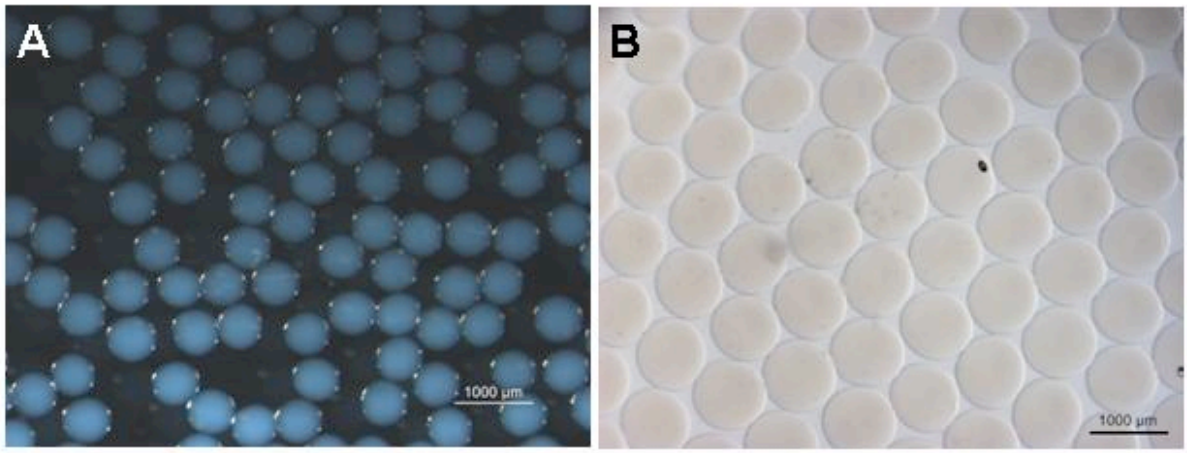

Fig 1. Optical photomicrographs of empty alginate microbeads prepared by "air-monojet" instrument with the optimized parameters: air flow 4.2 L/min, pump rate 0.49 mL/min and height 3.6 cm.

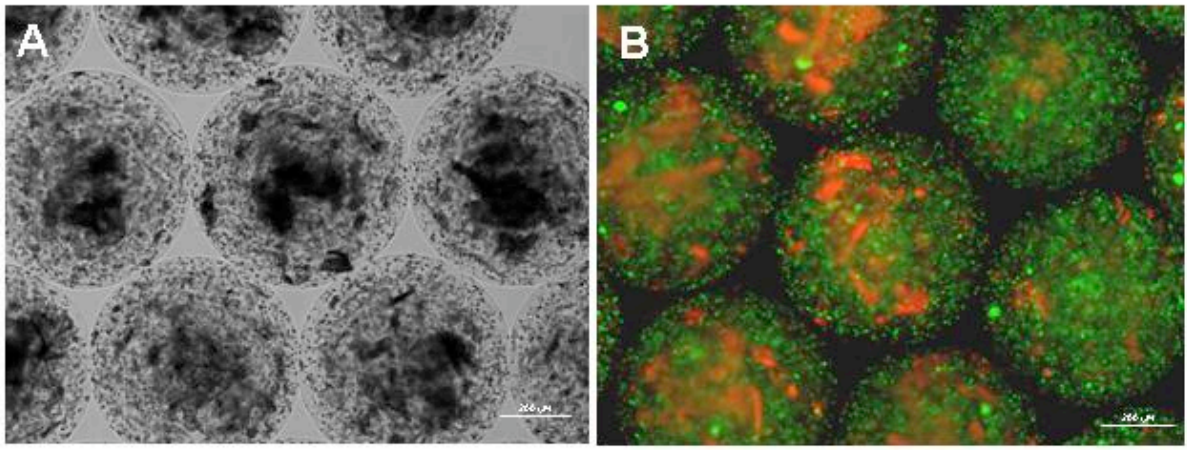

Fig. 2 Optical photomicrographs of SC and ECM encapsulating alginate microcapsules (A) and fluorescence photomicrographs of SC and ECM encapsulating alginate microcapsules after staining with ethidium bromide and fluorescein-diacetate for cell viability (B).

Conclusions

Among the different procedures of encapsulation viable cells, the “air-monojet” encapsulation procedure appears to be one of the most promising for the production of microcapsules in large scale GPL conditions. After a DoE studys we were able to optimize the entire process of enveloping SC an EMC in AG-based microcapsules with no loss of their functional and morphological properties.

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