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TECHNICAL NOTE

A laboratory scale device for microencapsulation of genetically engineered cells into alginate beads

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The microencapsulation of recombinant cells, widely used for *in vitro* high-density cell culture, is a novel and potentially cost-effective method of *in vivo* heterologous protein delivery, where the protein producing cells are immunologically protected from tissue rejection. We report here a simple, reliable and inexpensive laboratory method to generate calcium alginate microcapsules containing genetically engineered, interleukin-2 expressing, Chinese hamster ovary (CHO) cells.

Alginate microencapsulated genetically engineered cells can be used for *in vitro* recombinant DNA derived protein production as well as for *in vivo* slow release system of a therapeutic polypeptide. Both applications require the optimisation of alginate beads generation to reproducibly obtain uniformly shaped and sized particles that contain viable producing cells. Such particles allow *in vitro* high-density cell culture and are also suitable for *in vivo* intra-tissue injection (Chang et al. 1999).

We report here a simple laboratory method to generate calcium alginate microcapsules containing recombinant human interleukin-2 (rhIL-2) producing Chinese hamster ovary (CHO) cells.

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Materials and Methods

Cells culture

Cell CHO-K1 [ATCC #: CCL 61] derived cells were grown at 37ºC in a humidified atmosphere of 95% air and 5% CO_2 in complete medium: IMDM/F12 medium (Gibco-BRL, Carlsbad, CA) supplemented with 8% fetal bovine serum (FBS, Gibco-BRL), 2 mM L-glutamine and 25 mM HEPES buffer (pH 7.4). Serial passages were made by trypsinisation (0.25% trypsin and 0.02% EDTA in PBS) of sub-confluent monolayers.

Device assembly

Air jet driven droplet generation was performed by a simple device assembled in our laboratory as depicted in Figure 1. Briefly, a sterile i.v. administration set for gravity infusion was cut with a scalpel both at the tubing 10 cm before and in the middle of the blister and connected to an air flow output filtered by a parallel pair of 0.22 mm disposable filter units (2.5 cm diameter). Piercing the blister, a regular stainless steel injection 27½-gauge needle was placed with its tip under the air tubing outlet. The needle was then connected to a peristaltic pump driving the cells suspended in the sodium alginate solution. A beaker containing $CaCl_2$ collecting solution was placed about 4-5 cm below the needle tip (Figure 1).

Since sterility is a major concern during cell culture, all the system with the exception of the air compressor pump was placed inside a laminar flow hood.

Cells encapsulation

Encapsulation of genetically engineered CHO cells producing rhIL-2 obtained in our laboratory was performed without or with poly-L-lysine coating (Sun, 1988; Ross et al. 1999). Cells were suspended in complete medium containing 1% (wt) low viscosity sodium alginate (Sigma, St. Louis, MO), at a final cell concentration of $1x10^7$ cells/ml. This cell suspension was extruded through a 27½-gauge needle at a rate of 120 ml/hr. The air flow (5-10 L/min) pointed to the tip of the needle producing small droplets that were falling into a beaker containing a 125 mM $CaCl_2$, 2.5 mM glucose and 25 mM Hepes (pH 7.2) solution. The cross-linked alginate polysaccharide polymers formed solid spherical beads containing embedded cells that were transferred to T 25 flasks containing complete medium or to a 50 ml polypropylene tube for further treatment.

For poly-L-lysine coating the beads were washed successively for 3 min with 0.55 and 0.28% $CaCl_2$ in saline, 0.9% saline, 0.1% CHES [2 (*N*-cyclohexylamino) ethane-sulfonic acid] (pH 8.2), and 1.1% $CaCl_2$. The beads were further cross-linked with 0.05% (w/v) poly-L-lysine (MW 22,000; Sigma) for 6 min, washed with 0.1% CHES, then 1.1% $CaCl_2$ and 0.9% saline, and coated with a second layer of 0.15% alginate for 4 min. The polymer in the core of the capsule was either (i) kept by washing with saline, getting radially arranged cells; or (ii) dissolved by washing for 6 min in 55 mM sodium citrate to get a centred spheroid (Figure 2). After rinsing in serum free medium, these alginate-polylysine-alginate (APA) capsules treated or not with citrate were transferred to complete medium and incubated under regular culture conditions with a culture medium replacement every 48 h.

Quantitative methods

Cell number was estimated in the hemocytometer by trypan blue exclusion staining method by gently crushing a known volume of microcapsules into a known volume of trypsine-EDTA as described above. Cell viability within capsules was measured by spectrophotometry using MTS kit (Promega, Madison, WI). The hrIL-2 microcapsules secretion into the culture medium was measured by an enzyme-linked immunosorbent assay (ELISA) kit for hrIL-2 (R&D Systems, Minneapolis, MN).

Results and Discussion

To encapsulate genetically engineered rhIL-2 secreting CHO cells obtained in our laboratory, we used the assembled system depicted in Figure 1. The air jet flow was usually set between 2 and 10 L/min and cell suspension flow between 120 and 180 ml/h. As expected, microbead diameter decreased with increasing air jet flow (2 to 10 L/min) or decreasing needle diameter (from 19 to 27½-gauge) with bead diameters ranging from 1800 (± 250) to 300 (± 100) mm, respectively.

When encapsulated at an initial density of $3\text{-}5x10^6$ cells/ml alginate, capsules containing rhIL-2 secreting CHO cells ranged from 350 to 550 mm in diameter as shown in Figure 2. While alginate and APA capsules showed the typical radially arranged cells (Figure 2a), APA/citrate capsules displayed single centred spheroids (Figure 2b). We did not observe any sign of necrosis within the APA/citrate capsules during the first 30 days of culture.

Alginate as well as APA/citrate capsules resulted resistant to standard manipulations required for laboratory scale culture. The main physical difference between these capsules was the presence of the semi-permeable membrane of APA on the surface of the APA/citrate capsules with a soluble inner filling compared to a semi-solid gel in alginate capsules. Besides the advantage of APA/citrate regarding the immune isolation from any potential humoral host immune response against cells contained into beads, both sorts of capsules displayed a 7- to 8-fold enhancement of transgene expression with respect to monolayer culture, probably due to the three-dimensional configuration of cells growing as muticellular spheroids (data not shown).

As demonstrated by the MTS assay, both alginate and APA/citrate encapsulated cells were maintained *in vitro* viable for more than 30 days post-encapsulation, producing

about 200 ng rhIL-2/million cells/day, without significant differences between groups, as determined by ELISA assay (Figure 3). In some long-term *in vitro* experiments, there was not any significant loss of rhIL-2 expression up to 60 days post-encapsulation (data not shown).

On the other hand, we performed a preliminary experiment in Balb/c mice using intraperitoneally injected alginate capsules that confirmed the *in vivo* release of the cytokine. One to three days after injection of capsules containing $1x10^6$ cells, we could detect rhIL-2 serum concentrations ranging from 100 to 300 pg/ml.

This work presents a simple, reliable and inexpensive method to microencapsulate cultured cells in the laboratory, using regular molecular biology and biochemistry laboratory equipment and disposable supplies. All the system can be assembled in few minutes and repeatedly used, while maintained under sterile conditions.

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APPENDIX

Figures

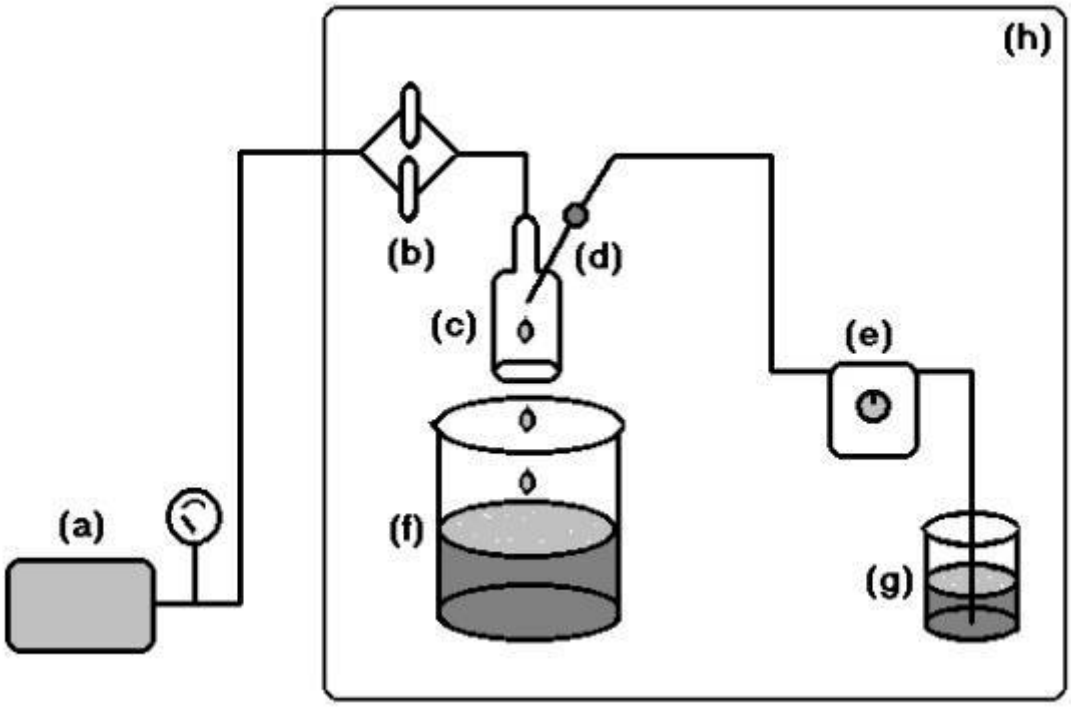

Figure 1. Schematic diagram of the microcapsule generator.

(a) air compressor,
(b) 0.22 mm filter units,
(c) half blister chamber,
(d) 27½ gauge needle,
(e) peristaltic pump,
(f) $CaCl_2$ containing solution,
(g) cell suspension containing sodium alginate,
(h) laminar flow hood.