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# Alginate-based microencapsulation of retinal pigment epithelial cell line for cell therapy

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**Abstract**

The goals of this study were to evaluate human retinal pigment epithelial cell line (ARPE-19) for cell encapsulation and to optimize the alginate-based microencapsulation. We used immortalized ARPE-19 cells and the transfected sub-line that expresses secreted alkaline phosphatase (SEAP) reporter enzyme. Alginate was cross-linked with different divalent cations ($Ca^{2+}$, $Ba^{2+}$, $Sr^{2+}$ and combination of $Ca^{2+}$ and $Ba^{2+}$), coated first with poly-L-lysine (PLL), and then with alginate. Microcapsules with different pore sizes and stability were generated. The pore size of the microcapsules was assessed by the release of encapsulated fluorescein isothiocyanate (FITC)-dextrans. The viability of the cells in the microcapsules was studied *in vitro* by assessing the secretion rates of SEAP and oxygen consumption by the cells. The best microcapsule morphology, durability and cellular viability were obtained with alginate microcapsules that were cross-linked with $Ca^{2+}$ and $Ba^{2+}$ ions and then coated with PLL and alginate. Based on FITC-dextran release these microcapsules have porous wall that enables the rapid contents release. The ARPE-19 cells maintained viability in the $Ca^{2+}$ and $Ba^{2+}$ cross-linked microcapsules for at least 110 days. The alginate microcapsules cross-linked with $Ca^{2+}$ and $Ba^{2+}$ have sufficiently large pore size for prolonged cell viability and for the release of secreted SEAP model protein (Mw 50 kDa; radius of gyration of 3 nm). ARPE-19 cells show long-term viability and protein secretion within alginate microcapsules cross-linked with $Ca^{2+}$ and $Ba^{2+}$. This combination may be useful in cell therapy.


*Keywords:* Cell encapsulation; Alginate; Protein release; Retinal pigment epithelial cells; ARPE-19

## 1. Introduction

Microencapsulated cells can be used for cell therapy (see Ref. [1]). The microcapsule walls protect the cells from the immunological reactions and maintain their viability by allowing diffusion of oxygen, nutrients, and metabolic products. The cells should release therapeutic protein over prolonged periods.

After the early study of Lim and Sun [2] alginate became the most widely used polymer for cell entrapment. The microcapsules are prepared by dispersing sodium alginate solution into water solution of cross-linking divalent ($Ca^{2+}$, $Ba^{2+}$, $Sr^{2+}$) or trivalent cations ($Al^{3+}$, $Fe^{3+}$), and thereafter they are coated by a cationic polyelectrolyte [3]. The coating slows down the swelling and degradation of the microcapsules, but it may cause immunological reactions and fibrotic growth [4–7]. The microcapsule structure is affected by the guluronic acid and mannuronic acid contents of alginate [8]. Normally the cross-linking cations distribute primarily to the microcapsule surface, but Zimmermann et al. [9] avoided this by gunning barium crystals into the alginate droplets.

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Many neurodegenerative diseases require life-long therapy and, thus, cell therapy is a promising therapeutic option. The cells should survive over prolonged periods in non-dividing state, and secrete the neurotrophic factors. *In vivo* retinal pigment epithelial (RPE) cells do not divide and they secrete growth factors to maintain the neural retina for the entire lifetime. The symptoms of Parkinson's disease were alleviated with microcapsules of RPE cells derived from post-mortem human eye [10] and from fetal stem cells [11]. The RPE cells provide dual benefit in Parkinson's disease by producing both dopamine and neurotrophic support of the basal ganglia [12]. Phase I trial of age-related macular degeneration utilizes RPE cells to produce ciliary neurotrophic growth factor (CNTF) [13]. As primary RPE cells are difficult to obtain, a cell line with similar properties may be more applicable for microcapsule therapy.

We used an engineered RPE cell line (ARPE-19; [14]) in alginate microcapsules to study the secretion of a reporter enzyme secreted alkaline phosphatase (SEAP). Immortalized ARPE-19 cells can be maintained in differentiated non-dividing state for months [14]. Various polycation-coated alginate microcapsules were tested by using different cross-linking cations ($Ca^{2+}$, $Ba^{2+}$, $Sr^{2+}$ and combination of $Ca^{2+}$ and $Ba^{2+}$). We show that alginate microcapsules cross-linked with a combination of $Ca^{2+}$ and $Ba^{2+}$ cations have optimal properties compared to the other formulations.

## 2. Materials and methods

### 2.1. Materials for microencapsulation

Microcapsule matrix polymer was sodium alginate (UP LVG, FP-303-02) from Novamatrix (Norway). Cross-linking salts were strontium chloride ($SrCl_2 \cdot 6H_2O$, Sigma-Aldrich, Germany), calcium chloride ($CaCl_2 \cdot 2H_2O$, Riedel-de-Haën, Germany), and barium chloride ($BaCl_2 \cdot 2H_2O$, Merck, Germany). poly-L-lysine (PLL) hydrobromide (22 kDa) was from Sigma, USA. Texas-Red labeled PLL (22 kDa) was a gift from Prof. Etienne Schacht (University of Ghent, Belgium). All the solutions were prepared in ultrapure water.

### 2.2. Microencapsulation of FITC-dextrans

Solutions of fluorescein isothiocyanate (FITC)-dextrans (100 μg/ml) in 1.2% sodium alginate (w/v) were prepared by suspending FITC-dextran powder (Mw 4.3, 43.2, 464 and 2000 kDa; from Sigma) in alginate solution for at least 1 week to dissolve the probe completely. FITC-dextran solution (500 μl) was dispersed through a 1 ml syringe and blunt ended cut needle (G22, Terumo, Belgium) connected to a nitrogen flow (35 ml/s). The FITC-dextran solution was sprayed into 15 ml of cross-linking cation solution (68 mM $CaCl_2$, 10 mM $SrCl_2$, 10 or 20 mM $BaCl_2$) into a vessel with a filter in the bottom. All cross-linking solutions contained 13 mM HEPES [15]. The microcapsules were washed with 13 mM HEPES after 5 min in the cross-linking solution and then PLL (0.1%, 5 ml) solution was added for 7 min and washed twice with 13 mM HEPES. A second coating of sodium alginate was then added by incubating the microcapsules in 2.5 ml of 0.125% sodium alginate for 5 min to bind all positively charged PLL still present on the capsule surface. Finally, the capsules were washed twice with 13 mM HEPES-solution.

In the case of combination of $Ca^{2+}$ and $Ba^{2+}$ cross-linkers, the microcapsules were first produced in calcium chloride (68 mM, 7.5 ml) for 3 min. After the formation of microcapsules, calcium chloride was replaced with barium chloride solution (20 mM, 7.5 ml) for 5 min. Thereafter, the microcapsule preparation was carried out as described above. The microcapsules were kept in serum-free Dulbecco's MEM/nut mix F-12 (DMEM) cell culture medium (Gibco, UK) that was supplemented with penicillin/streptomycin and L-glutamine (EuroClone, Great Britain) in incubator at +37 °C and protected from light.

The release of the FITC-dextran from alginate microcapsules was determined by periodically collecting samples from the medium. In the end of the release experiment, the microcapsules were disintegrated with 0.1% EDTA (w/v) (Merck, Germany) solution and mechanical manipulation with syringe and needle to release all encapsulated FITC-dextran.

The released FITC-dextran in the samples was determined by measuring fluorescence of the samples at the excitation and emission wavelengths of 495 and 520 nm, respectively, with Victor$^2$ multilabel counter (Wallac, Turku, Finland).

### 2.3. Sizing of microcapsules

For the evaluation of different sample sizes (capsule/batch) microcapsule batches were stained with erythrosine-B and photographed with a digital camera. The amount of microcapsules/batch was calculated from the photographs by image analysis (Image Processing Toolbox, The MathWorks, Inc.).

### 2.4. Plasmid DNA

CMV promoter-driven SEAP reporter gene with neomycin resistance gene for selection was constructed by inserting the *Hind*III–*Sal*I fragment of pSEAP2-Basic (Clontech, La Jolla, CA) into the *Hind*III–*Xho*I digested pCR3 vector (Invitrogen, Carlsbad, CA). The plasmid DNA was amplified in *Escherichia coli* (DH5α) and isolated using Qiagen column chromatography (Qiagen, Hilden, Germany). The pCMV-SEAP2/neo plasmid was verified by restriction digestions and quantified by absorbance at 260 nm.

### 2.5. Cloning of SEAP-expressing ARPE-19 cells

Human ARPE-19 cells (ATCC CRL-2302, [14]) were genetically engineered to stably express the SEAP reporter enzyme. ARPE-19 cells were seeded on 100 mm culture dishes at various densities and transfected in serum-free medium with pCMV-SEAP2/neo plasmid (10 μg/plate) and 50 kDa polyethylene imine (PEI, Sigma) at a charge ratio of +9 according to established methods [16]. The cells were selected with 0.8 mg/ml G418 (Calbiochem, La Jolla, CA). The resulting colonies were isolated with cloning rings, expanded and screened for expression of the SEAP activity in the medium using luminometric assay (The great EscAPe SEAP chemiluminescence detection kit, BD Biosciences). The cell sub-line with the highest expression of SEAP and the fastest rate of proliferation was chosen for the cell encapsulation tests and maintained in the presence of 0.4 μg/ml G418. The growth rate of the cloned cells was 14 times slower than that of normal ARPE-19 cells.

### 2.6. Cell microencapsulation

Both normal and SEAP-secreting ARPE-19 cells were cultured to about 70–80% confluence in DMEM/nut mix F12 with 10% fetal bovine serum (FBS) (Gibco BRL, Germany) and penicillin/streptomycin. Prior to the encapsulation the cells were washed once with PBS (Gibco BRL, Scotland), detached with trypsin/EDTA (T/E) (Gibco Invitrogen, Canada), and counted. After brief centrifugation the cell pellet was suspended in 1.2% sodium alginate (1.2% w/v, 500 μl). Cell alginate suspension was dispersed into the cross-linking solution and, thereafter, the cell encapsulation was conducted as described in "*Microencapsulation*