# SIMPLIFIED METHOD OF MAKING ALGINATE-POLYLYSINE MICROCAPSULES FOR HYBRIDOMA CELL CULTURE USING RPMI 1640 MEDIUM

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### Abstract
The method of making alginate-poly-L-lysine microcapsules for hybridoma cell culture can be simplified by cultivating the cells in RPMI 1640 medium. Phosphate concentration in RPMI 1640 medium is sufficiently high to dissolve the alginate gel and, thereby, can be used to eliminate the step of citrate buffer treatment required for reliquefying the interior alginate gel.

## INTRODUCTION

Among various efficient cell culture systems, the encapsulation of living cells within alginate-poly-l-lysine (PLL) membrane has received much attention as a means of improving the production of desirable cellular products. This method, which was successfully demonstrated by Lim and Moss in 1979, was primarily developed to transplant living cells without an *in vivo* rejection reaction (Lim and Sun, 1980; Lim and Moss, 1981). Damon Biotech has employed this process (ENCAPCEL) to grow a number of cell types including hybridoma cells and genetically engineered cells and has shown that microencapsulation of hybridoma cells has advantages in production and purification of monoclonal antibodies (Rupp, 1985; Posillico, 1986; Nilsson, 1987). Overall, the microencapsulated cell system has several advantages when compared to a free cell system. First, microcapsules are much larger than free cells and their use in perfusion systems is much easier. Second, direct sparging of gases into a bioreactor is permitted since cells are protected from physical shear inside microcapsules. Third, the product can be partitioned into either the microcapsules or the medium. However, the current technique of producing the microcapsules is complex and it involves many steps: the formation of alginate beads, treatment of PLL, liquefaction of the inner core of the alginate beads using citrate buffer and several washing steps (Lim and Sun, 1980; Lim and Moss, 1981; Lim, 1984; Goosen *et al.*, 1985).

In this article, we show that this method can be simplified by cultivating cells in RPMI 1640 medium which is one of the typical basal media (Adamson *et al.*, 1987).

## MATERIALS AND METHODS

### Cell line, medium and culture maintenance

The murine hybridoma used was S3H5/γ2bA2, provided by Dr. Mark Kaminski from the University of Michigan Medical Center. The antibody produced by this cell line is γ2bA anti-idiotype antibody, directed against the 38C13.

The cells were grown in RPMI 1640 (Sigma, St.Louis, MO) supplemented with 10% fetal bovine serum (Gibco Laboratories, Grand Island, NY) and 100 units of penicillin/ml and 100 μg of streptomycin/ml (Sigma).

The cells were maintained in T 25 cm$^2$ plastic cell culture flasks (Bellco Glass, Inc., Vineland, NJ) at 37°C in a $CO_2$ incubator.

### Immobilization

Hybridoma cells were entrapped in a gel matrix of calcium alginate as shown in Figure 1. This is the modified procedure described by Posillico *et al.* (1987). (1) Exponentially growing cells (cell
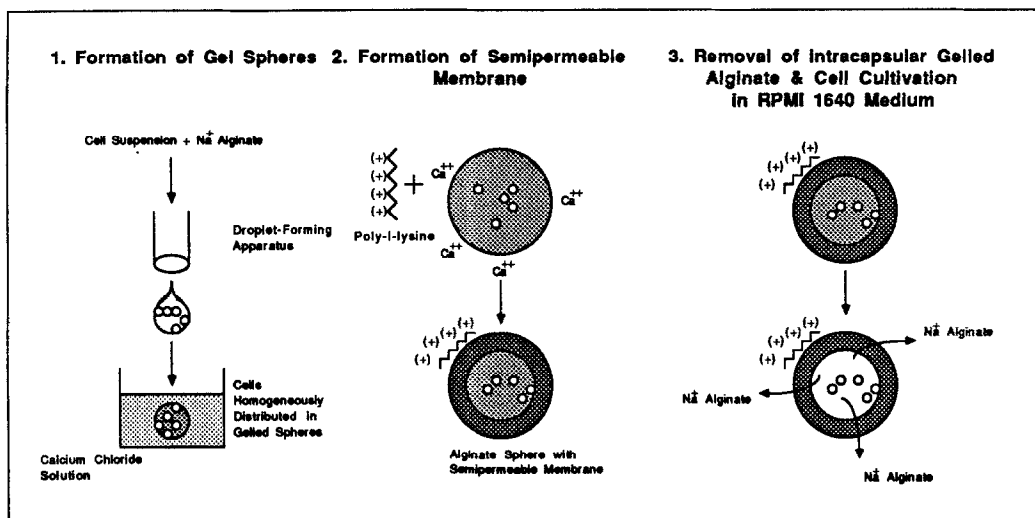
**1. Formation of Gel Spheres**  **2. Formation of Semipermeable Membrane**  **3. Removal of Intracapsular Gelled Alginate & Cell Cultivation in RPMI 1640 Medium**

Figure 1: The microencapsulation process

density$\cong$1.7·$10^6$ cells/ml) were centrfuged at 1000 rpm for 10 min. After discarding the supernatant, cells were suspended in buffered saline (0.9%(w/v)) NaCl buffered with 2.2 mM HEPES (pH 7.4 and 150mg glucose/l)) and were centrifuged again. After decanting the supernatant, the cell pellet remaining at the bottom of the centrifuge tube was then resuspended in alginate solution (1.5%w/v in 0.85% NaCl). The final cell concentration was approximately 4·$10^6$ cells/ml in 1.5% sodium alginate (Keltone LV, Kelco, Chicago, IL). A mixture of viable cells and alginate solution was packed in a syringe. (2) The mixture was extruded dropwise into 1.5%(w/v) $CaCl_2$ solution using an air-syringe droplet generator (Lim, 1984). Uniform sized gel particle (0.8-1.0 mm in diameter) were obtained in this manner. Gel-entrapped hybridoma cells were allowed to stand for 10 minutes in order to achieve proper gelation. (4) The supernatant solution was removed by decantation, and the immobilized cells were washed 3 times with buffered saline. Gel particles were resuspended in 0.05%(w/v) poly-L-lysine (PLL, molecular weight 22,000, Sigma) for 6 minutes to form the membrane. After washing the gel particles twice, the gel particle were resuspended in 0.06% sodium alginate to tie up any uncomplexed amino acids groups on the poly amino acid molecules. The supernatant solution was removed by decantation, and immobilized cells were washed three times with fresh media. Finally, the immobilized cells were transferred to 100ml spinner flasks (Bellco).

## Cell culture

Immobilized cell cultures were performed in a spinner flask at a agitation speed of 50 rpm. 10 ml of immobilized cells was inoculated into 90 ml of RPMI 1640 media containing 10% FBS. Medium was changed daily. Cell cultures were performed at 37°C in a $CO_2$ incubator (5% $CO_2$). Cell growth was monitored using photomicrography.

# RESULTS AND DISCUSSION

The ENCAPCEL process begins with the suspension of hybridoma cells in a sodium alginate solution. This suspension is extruded dropwise into a $CaCl_2$ solution. The $Ca^{2+}$ ions react instantly with the negatively charged alginate molecules thereby forming gelled microbeads containing the entrapped cells. Next, the cell-containing microbeads are treated with a PLL solution possessing complementary charges to those of alginate. In this membrane-forming step, the positively charged amino groups of the polyamino acid replace the surface layer of calcium ion in the gel beads. This treatment results in the formation of a polysalt hydrogel membrane on the surface of the microbeads. This polyelectrolyte complex is more

Table 1: Phosphate and calcium concentration in media (Freshney *et al.*, 1987).

| Components (mg/l) | RPMI 1640 | Eagle's MEM | Dulbecco's modification | Ham's F12 | CMRL 1066 | 199 | L15 | Fischer's | IMDM |
|---|---|---|---|---|---|---|---|---|---|
| $CaCl_2$ | - | 200 | 200 | - | 200 | - | - | - | - |
| $CaCl_2 \cdot 2H_2O$ | - | - | - | 44 | - | 186 | 186 | 91 | 219 |
| $NaH_2PO_4 \cdot H_2O$ | - | 140 | 125 | - | 140 | - | - | 78 | - |
| $Na_2HPO_4$ | - | - | - | - | - | 47.5 | 190 | 60 | 109 |
| $Na_2HPO_4 \cdot 7H_2O$ | 1512 | - | - | 268 | - | - | - | - | - |
| $KH_2PO_4$ | - | - | - | - | - | - | 60 | 60 | - |

stable than the $Ca^{2+}$ alginate gel. Finally, the interior calcium alginate gel is liquefied by removal of calcium ions with a mild chelating agent such as aqueous sodium citrate. Since only the interior of treated gel beads is $Ca^{2+}$ alginate, the sodium citrate treatment creates capsules with a liquid core surrounded by a PLL- alginate gel membrane.

The final step of reliquefying the calcium alginate gel can be eliminated by cultivating entrapped cells in RPMI 1640 medium. As shown in Table 1, phosphate concentration in RPMI 1640 (1512 mg/l) is much higher than that of any other basal media. Phosphate is one of the calcium chelating agents and its concentration in RPMI 1640 medium is high enough to dissolve the calcium alginate gel. In addition, there is no calcium chloride in RPMI 1640 medium which would stabilize the calcium alginate gel. Therefore, after the membrane (PLL-alginate) forming step, beads containing the cells can be directly transferred to RPMI 1640 medium without liquefying the interior calcium alginate gel, which is required when the other basal media are used.

As shown in Figure 2, cells were cultivated in beads and the beads became microcapsules within one day of presence in RPMI 1640 medium. Cells reached close to packing density inside the capsules after 3 weeks of cultivation. Since beads are more rigid than microcapsules, it is easier to handle beads than microcapsules for transfer and start-up of a reactor. RPMI 1640 medium is known to be as good as other basal media in the respect of cell growth and MAb production (Adamson *et al.*, 1987). For instance, S3H5/γ2bA2, the cell line used in this experiment, showed higher specific growth rate in RPMI 1640 medium than in Iscove's Modified Dulbecco's Medium (IMDM) (Savinell *et al.*, 1989).

In conclusion, the step of citrate buffer treatment required for reliquefying the interior alginate gel can be eliminated by cultivating the cells in RPMI1640 medium.
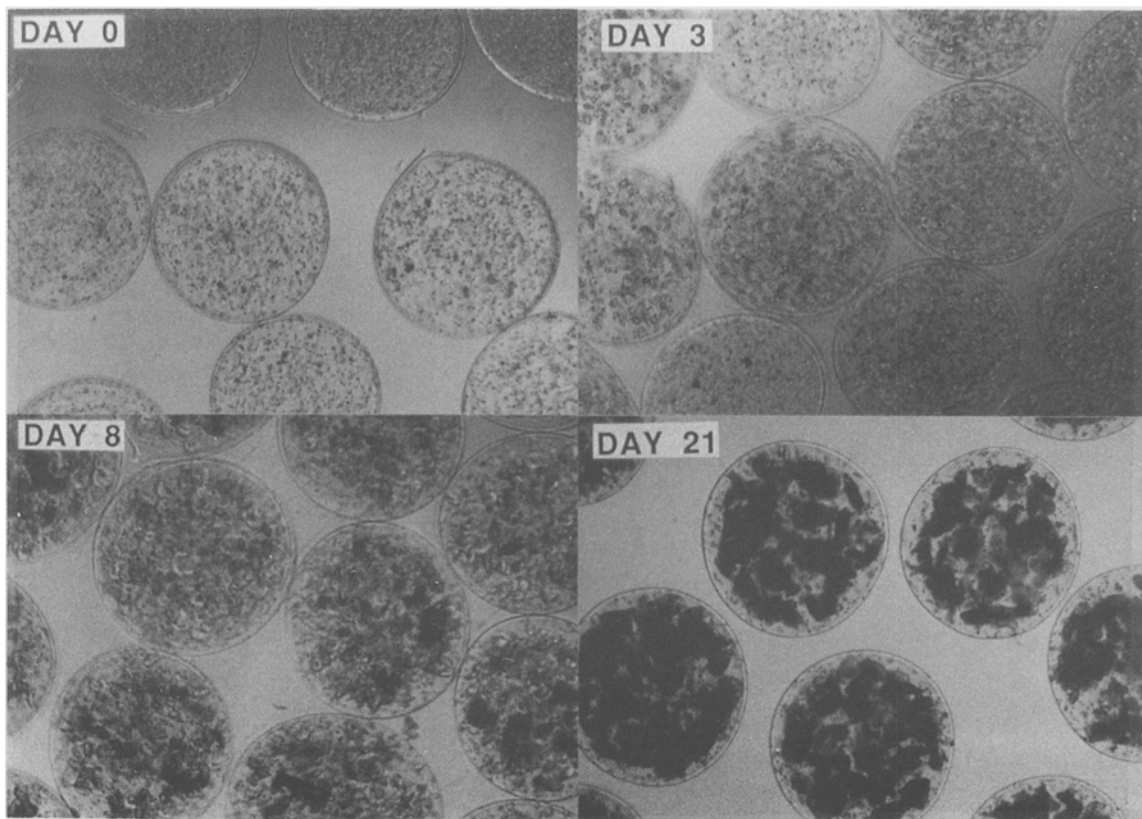
Figure 2: The Photomicrographs of hybridoma cell growth in capsules

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