Biotechnol. Bioprocess Eng. **1997**, 2, 73-76

Encapsulated Animal Cell Culture for the Production of Monoclonal Antibody (MAb)

Sung-Koo Kim*, Jeong-Hwa Son, and Sun-Hee Yu

Department of Biotechnology and Bioengineering, Pukyong National University, Pusan 608-737, Korea

Biopolymer membrane was prepared using two oppositely charged natural biopolymer. The biopolymer membrane was used for the encapsulation of two hybridoma cell lines (ATCC CRL-1606, ATCC BH-8852) to produce monoclonal antibodies. In order to reduce the down stream steps, the pore size of the membrane was controlled to retain the monoclonal antibodies in the capsules based on the diffusion experiments with standard proteins. T-flask culture showed cell densities of 8×10^7 cells/mL and 3×10^7 cells/mL, and MAb concentrations of 506 μg/mL and 109 μg/mL for encapsulated ATCC CRL-1606 and HB-8852, respectively. Two liter perfusion culture with encapsulated ATCC HB-8852 was performed to enhance the MAb production. The MAb production of the encapsulated hybridoma increased considerably comparing to the culture using silicone tubing for oxygen transfer.

Key words: encapsulation, MAb, biopolymer membrane, hybridoma

INTRODUCTION

Membranes serve numerous utilities both industrial and biomedial areas [1]. One of the major functions of the membrane is to control the permeation of proteins. This allows a selective permeation of some components while preventing others through membrane. Therefore, the pore size of the membrane is a critical parameter for its applications.

Biopolymer membrane can be formed by the ionic interactions between polycationic polymers and counter ionic polymers and encapsulates biological or cells [2, 3]. Since encapsulation using natural polymer is carried out under mild condition, it is suitable for biological products, and thus has a unique advantage and various potential applications [4-7].

In this study, the two hybridoma cell lines were encapsulated using chitosan, β-1,4 linked glucosamine and alginate, the copolymer of guluronic acids and mannuronic acids and the increase of MAb titer in the capasuls was tried. The productivity and cell growth of MAb using 2 liter culture with encapsulated hybridoma were determined and compared to conventional cell culture system.

MATERIALS AND METHODS

Cell Line

Hybridomas producing MAb against human fibronectin (ATCC CRL-1606) and bovine lactoferrin (ATCC HB-8852) were used for the encapsulated cell culture study. The medium used for the cell culture was Delbulco's Modified Eagles Medium (DMEM) supplemented with 10% fatal calf serum.

* Corresponding author
Tel: 051-620-6188 Fax: 051-627-1096
e-mail: skkim@dolphin.pknu.ac.kr

Preparation of Solution

Chitosan (0.5% w/v) was dissolved to the glutamic acid solutions (0.5%, w/v) and stirred overnight, then filtered to remove insoluble fractions using Whatman No. 1 filter paper and 0.1 M $CaCl_2$ was added to the solution. pH of chitosan solution was adjusted to 6.0 by adding NaOH. Alginate (0.5% w/v) was dissolved in deionized water. Glycerol solution (0.25%, w/v) was prepared for washing the capsules. For the cell culture the chitosan and alginate solution under UV for five hours, and the glycerol solution was autoclaved.

Encapsulation Procedure

The procedure for preparing the capsules is shown in Fig. 1. A triple concentric orifice was used for the encapsulation of hybridoma in order to minimize the contact time between cells and chitosan solution. The air flow rate of outer air blowing orifice (ID=2.033 mm) was adjusted to 6 L/min for the preparation of capsules (2 mm diameter). After 3 minutes, the prepared capsules were collected by straining on mesh screen and washed three times with glycerol solution (0.25% w/v). Then, the capsules were transferred to the medium (DMEM+10% fatal calf serum) for incubation. The encapsulation procedure was performed under an aseptic condition.

Diffusion Experiment with Standard Proteins

Diffusion experiments using β-lactoglobulin (MW= 32,000, long axis=38Å), ovalbumin (MW=45,000, long axis=114Å), bovine serum albumin(MW=66,000, long axis=140Å), conalbumin(MW=76,000, long axis= 170Å), γ-globulin (MW=156,000, long axis=220Å), and fibrinogen (MW=400,000, long axis=700Å) was carried out to determine the pore size of capsule with the parameter of pH of chitosan solution. The overall

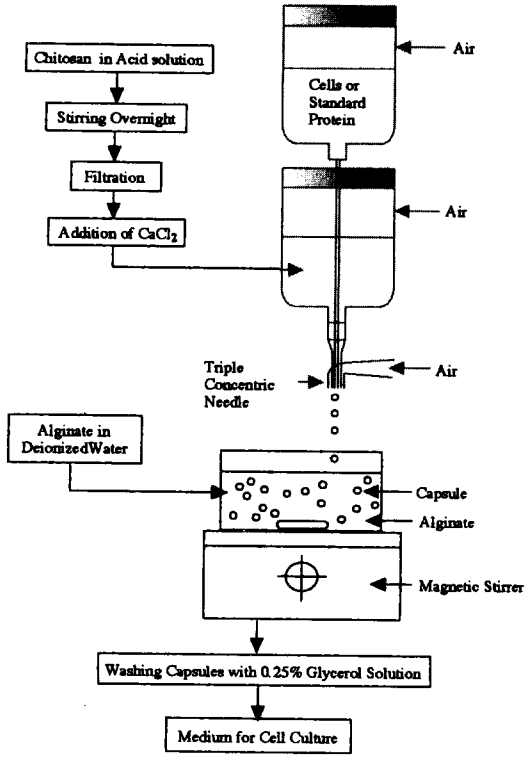

Fig. 1. Encapsulation procedure.

permeabilities were measured for the standard proteins.

T-flask Culture

Hybridoma cells (ATCC CRL-1606, ATCC HB-8852) were encapsulated and the viability of cells, specific growth and production rates were monitored. Forty mL of wet capsules were prepared during 3 minutes of encapsulation. Four mL of the capsules were transferred to each of ten T-flasks, which contained 30 mL of medium and stored in the CO_2 controlled 37°C incubator without changing medium. One T-flask among ten, was taken out from the incubator every 24 hours. Cell viability was measured by trypan blue dye methods. The micrographs of capsules were taken by inverted optical microscope with a large format instant camera (Olympus Corp., Model CK 2. Lake Success, NY and Polaroid Type 52 film).

Semi-Perfusion Culture

In order to increase the productivity of the encapsulated hybridoma a semi-perfusion culture system was carried out using a 2 L fermenter [Sparging: (0.2 VVM) w/marine type impeller 200 rpm (tip speed=40 cm/s) with 50 ppm antifoam type AF emulsion (Dow coning)] The free cell culture using the silicone tubing (O.D.=1.96 mm, wall thickness=0.25 mm) fermenter [8] was run as a control to compare the results of encapsulated system.

Counting of the Cell Number

The capsules were collected by draining the medium, then the capsules were transferred to a petri dish and cut open with a surgical knife and the core liquid was released. The core liquid was diluted with the same amount of trypan blue dye and loaded onto a hemocytometer, and the cell number was counted.

Evaluation of Product Formation

The concentration of monoclonal antibodies against fibronectin or lactoferrin were measured by ELISA. Fibronectin or lactoferrin solution was poured into each well and incubated overnight at 4°C. Fibronectin or lactoferrin was discarded and 0.5% BSA or skim milk was plated and incubated for 3 hours. After discarding the BSA or the skim milk, the plate was washed with 0.5% BSA or skim milk solution. Samples with proper dilutions and standard samples were loaded and incubated for 2 hrs. After washing the plate 3 times, peroxidase linked IgG (Sigma cat. no. A12028) was poured into each well. After 10 minutes the reaction was terminated by adding 50 ml of 5% hydrogen peroxide. Absorbance at 420 nm was read by a microtiter plate reader. The standard curves of every plate were prepared for calculating the concentration of monoclonal antibody.

RESULTS AND DISCUSSION

Pore size Determination of Biopolymer Membrane

Overall permeabilities obtained from the diffusion tests of standard proteins are shown in Table 1. Among the parameter for pore size of the membrane, pH of chitosan solution influenced at the highest extent. Increase in pH of chitosan solution to prepare the capsule enhanced the permeation of proteins. The overall permeability decreased with the decrease of pH of chitosan. The barrier between permeation and non-permeation was determined by the overall permeability of standard proteins. The pore size of capsule membrane was determined by taking the middle point of the size of non-permeating and permeating standard proteins. Therefore, as shown in the Table 1, the pore size of capsule varied from 130Å to 210Å by changing pH of chitosan solution from 3.2 to 6.0. The reason for not lowering pH lower than 3.0 and not raising pH higher than 6.0 is that there were precipitates at these range of pH. Therefore, pH of chitosan solution for the encapsulation was adjusted to 6.0. At that pH, the most of antibody can not penetrate the membrane and can be retained in the capsule.

Hybridoma Cell Culture

The pH of the chitosan solution was adjusted to 6.0 to obtain the pore size of 210Å in the membrane of the capsule. With this pore size, it was predicted that the cell could proliferate by providing nutrients and removing the metabolic wastes such as lactic acid and ammonia through the membrane. Most importantly, the product, monoclonal antibody could be retained in the capsules.

Fig. 2. shows the growth curves of ATCC CRL-1606 cells. The maximum cell density (8.2×10^7 cells/mL) obtained in the encapsulated culture was approximately 2 orders of magnitude higher than that of the free cell culture (7×10^5 cells/mL). Fig. 3. shows that the concentration of monoclonal antibody produced by encapsulated ATCC CRL-1606 increased rapidly from the 5th day of incubation. The maximum monoclonal antibody con-

Table 1. Overall permeabilities* of proteins through the membrane prepared with chitosan solution at different pH

Proteins	Size (Å)	Overall permeability ($\times 10^{-5}$ cm/sec.)		
		pH of Chitosan solution		
		pH 3.2 (P=127Å)	pH 4.5 (P=155Å)	pH 6.0 (P=205Å)
β-lactoglobulin (MW=32,000)	38Å	15.3±1.2	14.2±1.0	20.6±2.9
Ovalbumin (MW=45,000)	114Å	11.5±2.0	25.6±1.8	32.7±2.1
Bovine Serum Albumin (MW=66,000)	140Å	1.1±0.1	21.7±2.1	30.2±2.8
Conalbumin (MW=76,000)	170Å	2.3±0.2	3.0±0.3	25.6±1.5
β-Globulin (MW=156,000)	220Å	1.0±0.1	1.1±0.1	1.4±0.2
Fibrinogen (MW=400,000)	700Å	0.9±0.1	1.0±0.2	1.0±0.2

*The overall permeability(U) was calculated according to:

$$U = w/(A \cdot \Delta C_{lm})$$

where, U=overall permeability (cm/min)
w=mass of materials passing through the membrane (g/min)
A=total membrane area (cm^2)
ΔC_l=the log mean of the concentration (g/ml)
$\Delta C_{lm}=(\Delta C_1 \cdot C_2)/2.303 \cdot \log(\Delta C_1/\Delta C_2)$
where, ΔC_1=the initial concentration difference between the inside and the outside of the capsule
ΔC_2=the final concentration difference between the inside and outside of the capsule

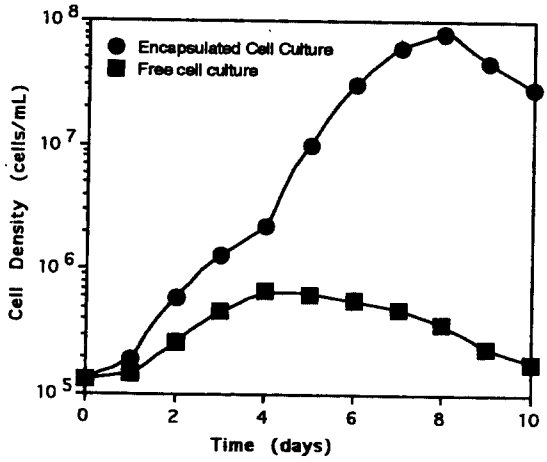

Fig. 2. Growth curve of encapsulated hybridoma (ATCC CRL-1606) for the production of MAb against human fibronectin in T-flask (w/o medium change).

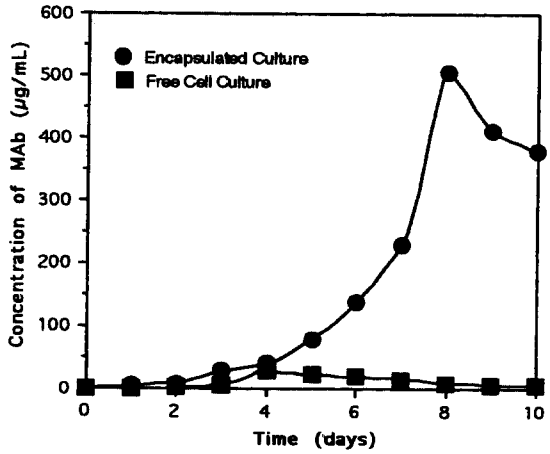

Fig. 3. Production of MAb against human fibronectin (ATCC CRL-1606) from the encapsulated hybridoma in T-flask (w/o medium change).

centration in encapsulated culture was 506 μg/mL on the 8th day of incubation. The concentration of the monoclonal antibody in the capsule was about 2 orders of magnitude higher than that in the free cell culture by

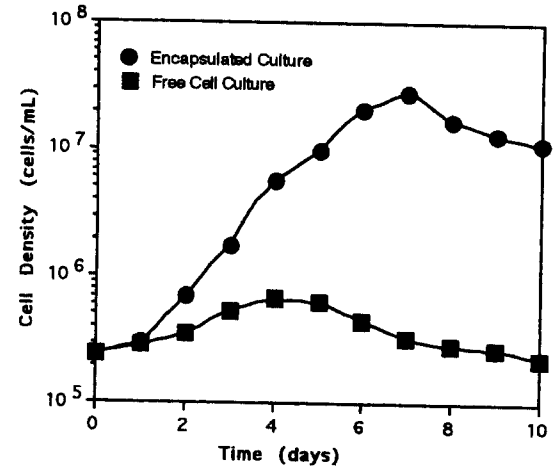

Fig. 4. Growth curve of encapsulated hybridoma (ATCC HB-8852) for the production of MAb against bovine lactoferrin in T-flask (w/o medium change).

retaining the monoclonal antibody in the capsules. The specific monoclonal antibody production rate of the encapsulated ATCC CRL-1606 was 8.2 pg/cell · day. Fig. 4. shows the growth curve of encapsulated ATCC HB-8852. The maximum cell density (2.8×10^7 cells/mL) was observed on the 7th day of incubation. The maximum production of the encapsulated ATCC HB-8852 showed a rapid increase from the 5th day of incubation. The maximum concentration of MAb in the capsule observed on the 7th day of incubation and reached 106 μg/mL (Fig. 5). The specific antibody production rate for encapsulated ATCC HB-8852 was 4.0 pg/cell · day. Also, the antibody produced by hybridoma was retained in the capsules and the concentration of antibody was about 20 times higher than that of free cell culture. The colony appeared after three days of incubation and grew with culturing time. The capsule became mostly black (colony) due to the cell growth after the 7th day of culture for encapsulated ATCC CRL-1606 and HB-8852.

Semi-Perfusion Culture of Hybridoma (ATCC HB-8852)

Based on the T-flask culture results, perfusion and

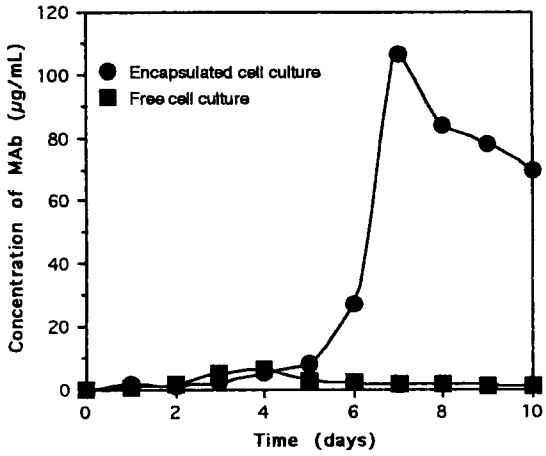

Fig. 5. Production of MAb against bovine lactoferrin from the encapsulated mouse-mouse hybridoma (ATCC HB-8852) in T-flask (w/o medium change).

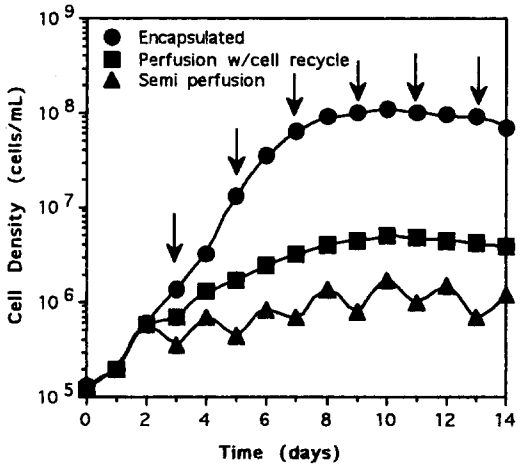

Fig. 6. Growth curve of encapsulated hybridoma (ATCC HB-8852) in direct sparged and aerated 2 L fermenter by feeding DMEM+10%FCS. Free cell culture as control was oxygenated by silicon tubing.

semi-perfusion cultures with 2 L fermenter were carried out for the enhancement of the cell density and the titer of MAb for encapsulated ATCC HB-8852. The free cell culture using the silicon tubing fermenter were run as controls to compare the results of encapsulated system. Fig. 6. shows that the maximum cell densitied of the free cell culture reached to 5×10^6 and 10^6 cells/mL for cell recycle and semi-perfusion culture, respectively. However, the cell density reached to 10^8 cells/el when hybridoma were encapsulated. The monoclonal antibody production also increased to mg quantity which was about 100 times higher than free cell cultures (Fig. 7).

These results indicate the effectiveness of encapsulation for the hybridoma cell culture. The monoclonal antibody production as well as preconcentrating effect were achieved with encapsulation in which membrane pore size was controlled. Further study on th primary cell culture with encapsulation technique is carried out for the application to synthetic organ formation.

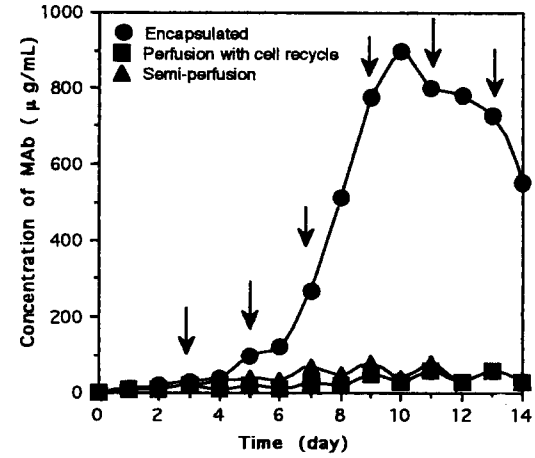

Fig. 7. Production of MAb against bovine lactoferrin fro: the encapsulated mouse-mouse hybridoma (ATCC HB-8852) in direct sparged and aerated 2 L fermernter by feeding DMEM+10%FCS. Free cell culture as a control was oxygenated by silicon tubing.

Acknowledgment This study was supported by the academic research fund (96-B(3)-4) from the Ministry of Education, Korea.

REFERENCES

[1] Kim, S. K. (1994) Cell immobilization using encapsulation technique and its applications, *Biotechnolgy News* 1(3): 28-32.

[2] Rha, C. K. and D. Rodriguez-Sanchez (1988) Process for Encapsulation and Encapsulated Active Material System, *US patent*, 4,774,937.

[3] Lee, J. S. and S. K. Kim (1996) Preparation and Applications of Synthetic Fish Capsules from Marine Polysaccharides, *Korean Journal of Life Science* 6(1): 34-39.

[4] Yu, S. H., J. H. Son, and S. K. Kim (1997) The Growth of Encapsulated Human Hepatoma, *Korean J. Biotechnol. Bong.* 12(4): 413-416.

[5] Kim, S. K. and C. K. Rha (1989) Transmembrane Permeation of Protein in Chitosan Capsules, *Chitin and Chitosan-Sources, Chemistry, Biochemistry, Physical Properties and Applications, (Skjak-Braek, G., Anthonsen, T., and Sandford, P., Elsevier Science Pub., New York)*, 636-642

[6] Kim, S. K. and C. K. Rha (1989), Chitosan for the Encapsulation of Mammalian Cell Culture, *Chitin and Chitosan-Sources, Chemistry, Biochemistry, Physical Properties and Applications, (Skjak-Braek, G., Anthonsen, T., and Sandford, P., Elsevier Science Pub., New York)*, 644-652.

[7] Gupta, S., S. K. Kim, R. P. Vemure, E. Aragona, P. R. Yerneni, R. D. Burk, and C. K. Rha (1993) Hepatocyte transplantation:an alternative system for evaluating cell survival and immunoisolation, *The International Journal of Artificial Organs*, 16(3): 155-163.

[8] Fleischaker, R. J., Jr. and A. Sinskey (1981) Determination of O2 transfer rate using silicone tubing in cell culture, *Eur. J. Appl. microbiol. Biotechnol.* 12: 193-197.