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Effects of High Cell Density on Growth-Associated Monoclonal Antibody Production by Hybridoma T0405 Cells Immobilized in Macroporous Cellulose Carriers

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Abstract Relationship between monoclonal antibody (MAb) productivity and growth rate, and effects of high cell density on MAb production of hybridoma T0405 cells immobilized in macroporous cellulose carriers were investigated in continuous and batch cultures The results showing that the specific MAb production rate increased with increasing specific growth rate in both suspended and immobilized continuous cultures indicate a positively growth-associated relationship between MAb productivity and growth rate. Moreover, the specific production rate was higher in the immobilized cell culture than that in suspended one at all dilution rates. In order to clarify these phenomena, MAb mRNA expression and cell cycle distribution were investigated in batch cultures with immobilized cells and suspended cells. RT-PCR was used for observation of MAb mRNA expression and a two-color bromodeoxyuridine (BrdU)/propidium iodide (PI) flow cytometry method for determination of cell cycle distribution. The results revealed that MAb mRNA expression reached the peak during the exponential growth phase, suggest a positively growth-associated MAb production. And the immobilized cells continued the MAb mRNA expression until dead phase, which was longer than that in suspended cells. The cell cycle distribution patterns were observed almost the same for both immobilized and suspended cells Such results may imply that a high cell density state has positive influence on the mRNA expression and on growth-associated MAb productivity of T0405 cells.

Keywords: hybridoma, HBs monoclonal antibody, antibody production rate, cell cycle, continuous culture, immobilized culture, macroporous cellulose carrier

INTRODUCTION

High density culture of hybridoma has been recognized as one of the best method to get large amount of monoclonal antibody (MAb) in compact reactors. Many strategies for high density culture have been developed and cell immobilization is one of the methods that has gained much attention. In 1969, Van Wezel [1] firstly reported the immobilization method for the culture of anchorage dependent cells onto microcarrier. Many types of carriers with various configurations and shapes have been developed using different materials to provide larger surface area since then. Application of immobilization has been extended even to anchorage-independent cell cultures, in which gel beads [2,3] and macroporous carriers [4,5] were noted to support high density growth of hybridoma cells in culture. One of the main concerns for the extensive application of immobilization, however, is the possible mass transfer limitations in immobilized cell systems For example, Yanaji *et al* [6] tried batch culture of immobilized mouse myeloma cells in PVF porous carrier cut from sponge material and reported that the high density of cell growth near the carrier surface, but not in the inner pores, was due to oxygen and nutrient limitations. Nonetheless, many investigators have continued to pursue high density culture of immobilized hybridoma because its recognized advantages over free-cell suspension culture. Hybridoma cells are also known to be better protected from the damaging shear stress in immobilized cultures Besides higher reactor volumetric productivity due to high density in immobilized hybridoma culture [4], immobilization can provide better separation of cells from medium, also provide an easy way for medium change, and create the possibility of obtaining cell-free product in perfusion systems. Moreover, immobilization may enhance the specific productivity of hybridoma cells. Ray *et al.* [7] also observed that the specific MAb production rate of cultured hybridoma cells, immobilized in collagen microspheres in perfused culture, was significantly higher compared to cells grown in suspension. Likewise, Lee *et al.* [2,3] reported that the specific MAb production rate of hybridoma cells entrapped in calcium alginate gel beads was 3 folds of suspended cells.

The potential of immobilization to enhance the MAb

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productivity of hybridoma cells has been recognized but not sufficiently substantiated. Many researches on the relationship between cell growth and antibody production in batch and continuous suspended cell cultures have been reported, in which positive growth-associated production [8,9] and negative growth-associated production [10-12] were found to be largely depended on cell line. However, the research on the relationship between MAb productivity and cell growth in immobilized system is still lacking. In this work, the features of monoclonal antibody production of immobilized hybridoma T0405 cells and relationship between cell growth and MAb production in continuous culture were investigated. Moreover, batch cultures were performed to investigate the effects of immobilization on MAb mRNA expression and cell cycle distribution of T0405 cells.

MATERIALS AND METHODS

Cell line and Culture Medium

Mouse-mouse hybridoma T0405 was derived from cell fusion between NS-1 mouse myeloma and mouse spleen cell (prepared by the Institute of Immunology Tochigi, Japan) The hybridoma secretes IgG monoclonal antibody against hepatitis B surface antigen (HBs-MAb). Serum-free ERDF medium (Kyokuto Pharmaceuticals, Japan) was used as basal medium. The basal medium was supplemented with growth factor ITES (Kyokuto Pharmaceuticals, Japan) containing 200 IU/L insulin, 10 mg/L human transferrin, 10 mM/L ethanolamine and 20 nM/L Na-selenite Bovine serum albumin (BSA, 1 g/L, Sigma) and sodium bicarbonate (1.13 g/L) were also added to the serum-free medium.

Carriers

Macroporous cellulose carriers, CellsnowTM EX-S33 and Cellsnow P9-402 (Biomaterial, Japan), were used in this study. Both are cubic in shape (1 mm × 1 mm × 1 mm) with a pore size of 100 μm. The surface of the carriers was treated with polyethyleneimine to give an ion exchange capacity (IEC) of 0.16 and 1.03 meq/g, respectively, and then stabilized by cross-linking.

Continuous Culture

Free suspended and immobilized continuous cultures with partial cell bleeding were carried out in a 500-mL stirred glass reactor (250 mL working volume) equipped with control devices for pH, D O., temperature, and liquid level as shown in Fig. 1. An UF membrane with a molecular cut-off of 10,000 Daltons (Diaflo, YM10, Amicon) was fixed at the bottom of the reactor. Agitation was provided by a magnetic impeller operating at 50 to 55 rpm D.O. was controlled at 6% of air saturation by supplying air mixed with 5% CO_2 gas supplied into the vessel head space, and also through a 1-m porous Teflon tube (TP-32, 70% porosity, Sumitomo, Japan) for bubble-free aeration. Pure oxygen was supplied into the teflon tube, when necessary. The pH was maintained at 7 1 ± 0.1 with 0.5 M NaOH solution

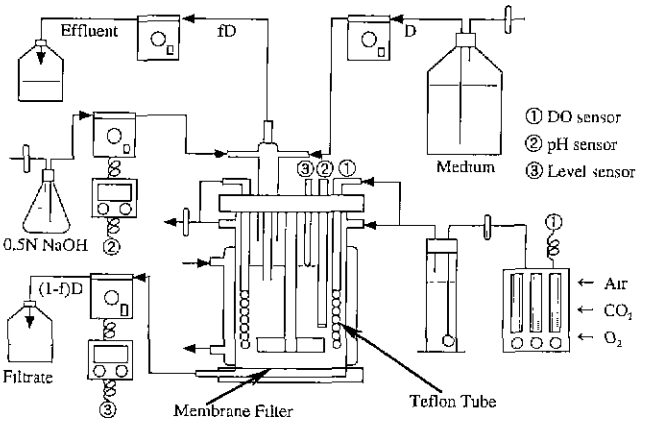

Fig. 1. Experimental set-up for continuous culture with partial cell bleeding

Continuous culture commenced by drawing out the reaction mixture through an effluent tube, and cell-free and MAb-free spent medium through the UF membrane at a dilution rate of *f*D and (1-*f*)D, respectively By varying the cell dilution rate *f*D, it was possible to obtain various steady-state data of cell growth and MAb production. To maintain the total dilution rate D, cell-free MAb-free spent medium was withdrawn through the UF membrane by means of a peristaltic pump controlled by a level sensor. Cell count and viability were initially determined by trypan blue exclusion using a hemocytometer

Batch Cultures

Free suspended and immobilized batch cultures were performed in 15 mL dishes and in 50 mL spinner flasks magnetically stirred at 60 rpm. Exponentially growing cells were inoculated to give an initial cell concentration of 1×10^5 cells/mL in dish culture and 2×10^5 cells/mL in spinner flask culture. In the case of immobilized batch culture, cells were immobilized by incubating with the carriers for 9 h with intermittent agitation (2 min stirring every 30 min). Cell count was determined by trypan blue exclusion using a hemocytometer and MTT method as described [13]. Many investigators confirmed that MTT assay is an efficient and realizable method for cell enumeration [6], and Nomura *et al.* [13] reported a convenient method using MTT for counting immobilized anchorage-dependet cells in porous carriers. MTT is a kind of tetrasodium salt that can be uptaken by viable cells into the mitochondria where dehydrogenase enzyme reduces MTT to formazan, a blue color pigment. Formazan is extracted from the cells using DMSO. The absorbance can then be correlated with cell concentration.

Glucose, Glutamine, Ammonia and Antibody Assay

Glucose and ammonia concentrations in the culture medium were determined by using Glucose-C II Test Kit and Ammonia Test Kit (Wako Pure Chemicals, Japan), respectively. Glutamine was analyzed by an Amino

Acid Analyzer HBs MAb activity was assayed by enzyme-linked immunosorbent assay (ELISA).

Cell Cycle Analysis

A two-color bromodeoxyuridine (BrdU)/propidium iodide (PI) flow cytometry method [14,15] was used to determine the fraction of population in each cell cycle phase Cells were simultaneously stained for measuring the DNA content using PI (a dye that interchelates and binds to DNA) and the uptaken amount of BrdU, a thymidine analog, using an anti-BrdU monoclonal antibody conjugated to a fluorescent molecule. Two-parameter contour plots were obtained by analyzing the cells through a FACS sort flow cytometer (Becton Dickinson, Mt View, CA, USA). The population fraction in each cell cycle phase as well as the relative amount of BrdU uptaken into the synthesized DNA of S phase cells were evaluated from the cell distribution on the plots. BrdU (Sigma) was added to each sample at a concentration of 5 mg/mL and incubated for 30 min at 37°C Cells were then harvested, washed with phosphate buffered saline (PBS) and then fixed with cold 70% ethanol and stored at 4°C until analysis. Prior to analysis, samples were acid hydrolyzed (4 M HCl) for 20 min, and resuspended in 1 mL of 0.1 M $Na_2B_4O_7$ (Wako) The cells were then pelleted by centrifugation, and resuspended in 1 mL of 0 5% Tween 20 in PBS (Nissui, Tokyo) until the pH of supernatant reached 7. Subsequent to repeated washing the cells with 0.5% Tween 20 in PBS, cells were resuspended in 50 μL of 0.5% Tween 20 in PBS, and 10 μL of fluorescent isothiocyanate (FITC)-conjugated antibody to BrdU (Sigma) was added to react with each sample. Then, cells were washed with PBS twice, and resuspended in 450 μL of PBS Finally, propidium iodide (Sigma, 5 μg/mL) was added to measure the relative amount of viable cells in a particular phase of the cell cycle

Construction of Primers for RT-PCR

Since DNA or mRNA of MAb from T0405 cells have not been fully sequenced yet, the primers for PCR were designed from available mRNA sequences of cells producing MAb against the same antigen (HBs) [16-23]. We designed the primers by firstly searching the high homology from the available sequences of mRNA for HBs antibody in various domains, and then designed and constructed 4 primers for RT-PCR, finally screened 2 out of 4 for the use in this study.

Analysis of MAb mRNA Expression

The mRNA expression level of HBS-MAb was detected by the reverse transcription polymerase chain reaction (RT-PCR) method. The mRNA was prepared from cells using a Quick Prep Micro mRNA purification kit (Pharmacia Biotech, Uppsala, Sweden) following the manufacturer's protocol. MAb mRNA from 2.5×10^5 viable cells was used for first strand cDNA synthesis in a total volume of 20 μL. The cDNA was synthesized with oligo (dT) 12-18 as a primer (0.5 mg), 5 × first strand buffer (50 mM Tris-HCl (pH 8 3), 75 mM KCl

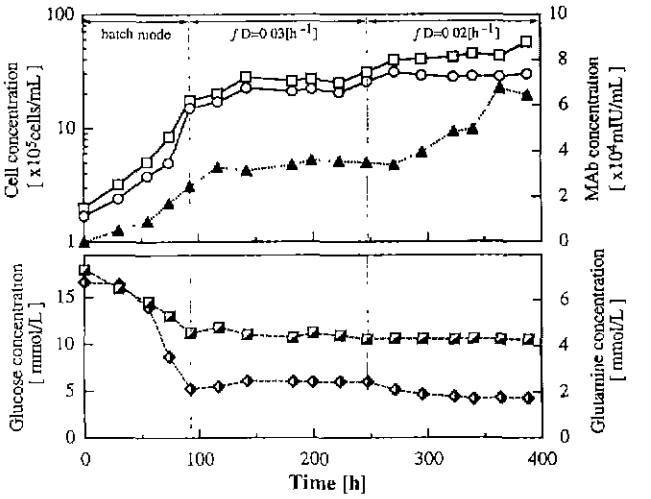

Fig. 2 A typical time course of perfusion culture of T0405 cells in membrane reactor The culture was operated in batch mode until 92 5 h When the cell concentration reached around 10^6 cells/mL, the supply of fresh medium into the reactor and cell bleeding were started. The dilution rate was fixed to 0.03 h^{-1} from 92 5 to 247 h, 0.02 h^{-1} from 247 h until the end of the culture. Symbols. ○, viable cell concentration, □, total cell concentration; ▲, MAb concentration; ◪, glucose concentration; ◆, glutamine concentration.

and 3 mM $MgCl_2$), dithiothreitol (DTT) (10 mM), deoxynucleotide triphosphate (dNTPs) (0.5 mM) and Moloney murine leukemia virus reverse transcriptase (Gibco BRL). The PCR was performed by the addition of a PCR reaction mixture [Tris-HCl (20 mM, pH 8.4), KCl (50 mM), $MgCl_2$ (1.5mM), and dNTPs (0.2 mM)] (Gibco BRL) containing primer 1 (0 5 mM, 5'TGGTACCAGCAGAAACCTGGC), primer 2 (0.5 mM, 5'CTTGGTCCCGCCGCCGAA), Taq DNA polymerase (Gibco BRL; 5 U), and 1 μL cDNA solution from the first strand reaction with 30 thermal cycles: 94°C for 30 sec, 59°C for 1 min and 72°C for 1 min. The last cycle was followed by an additional incubation period of 10 min at 72°C. 16 μL of the PCR products were analyzed using 2% agarose gel electrophoresis. The MAb mRNA band was observed at 204 bp.

RESULTS AND DISCUSSION

Continuous Culture

Fig 2 shows a typical time course of continuous culture of suspended hybridoma T0405 cells at cell dilution rates fD=0.03 and 0 02 h^{-1}. For suspended continuous culture, exponentially growing cells were inoculated in the bioreactor to achieve an initial concentration around 2×10^5 cells/mL. The culture was operated in batch mode until 92.5 h When the cell concentration reached 10^6 cells/mL, the supply of fresh medium into the reactor and cell bleeding were started at a constant feed rate. The dilution rate was fixed to 0.03 h^{-1} from 92.5 to 247 h, and 0.02 h^{-1} from 247 h until the end of the culture. When cell concentration is relatively con-

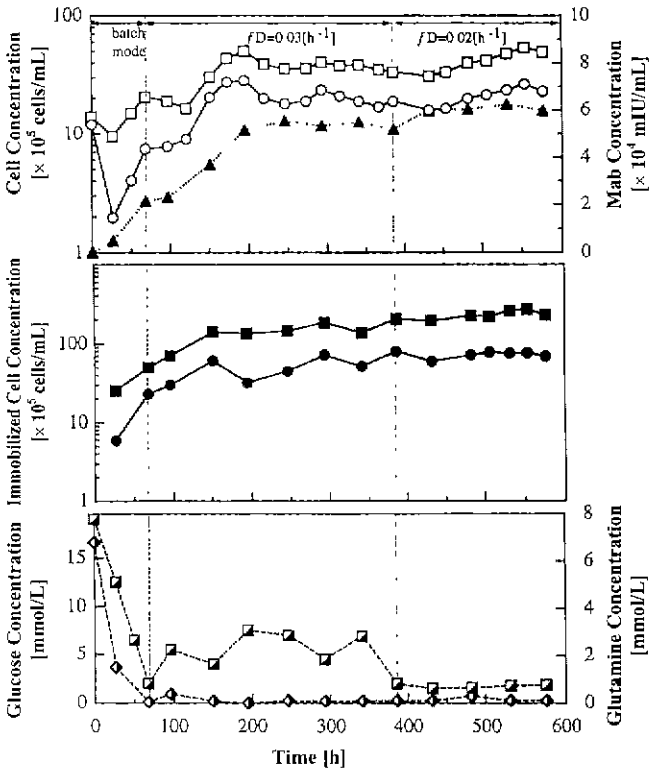

Fig. 3. A typical time course of immobilized continuous culture of T0405 cells in membrane reactor. After 68 h of batch culture, when the glucose concentration decreased to 1 mM, continuous culture was started. The dilution rate was fixed to 0.03 h^{-1} from 68 to 386 h, 0.02 h^{-1} from 386 h until the end of the culture. Symbols ○, suspended viable cell concentration; □, suspended total cell concentration, ▲, MAb concentration, ●, immobilized viable cell concentration; ■, immobilized total cell concentration, ◪, glucose concentration, ◆, glutamine concentration

sistent during 3 to 4 consecutive samplings, it is regarded as steady state in each dilution rate. We used the average value of these sampling points to calculate the specific growth rate and specific MAb production rate for that particular dilution rate. In other dilution rates, the similar steady-state conditions were obtained, respectively.

The cell concentration was observed to increase with the decrement of dilution rate and cell viability changed from 82% under dilution rate of 0.03 h^{-1} to 62% under dilution of 0.02 h^{-1}. Since it has been reported that the specific growth rate of T0405 cells increased with increasing dilution rate, and its specific death rate decreased with increasing dilution rate [24], it may suggest that the cell viability can be expected to decrease in low dilution rate. Moreover, MAb concentration in the culture medium was found to increase, and glucose / glutamine concentrations decreased with decreasing dilution rate.

Fig. 3 shows a typical time course of the continuous culture of immobilized hybridoma T0405 cells at two dilution rates fD (0.03 and 0.02 h^{-1}). In the case of immobilized continuous culture, a higher initial concentration of about 1.2×10^6 cells/mL was used to achieve complete immobilization. Cells were immobilized onto the carriers by incubating cells together with carriers under intermittent agitation (2 min stirring every 30 min) for 6 h. The decrease of the suspended cell concentration indicated that cells were immobilized onto the carrier after inoculation. When the glucose concentration decreased to 1 mM after 68 h of batch culture, cell culture was switched to continuous one as described above. The dilution rate was fixed to 0.03 h^{-1} from 68 to 386 h, and 0.02 h^{-1} from 386 hrs until the end of the culture. Steady state condition was achieved by the same methods as that described in the suspended continuous culture. The concentration of immobilized viable cells and total cells were consistently higher (2-4 times) than those of the suspended ones. These results clearly showed that the high density culture of hybridoma T0405 cells was achieved with macroporous cellulose carrier. Although the viability of suspended cells was maintained about 50% in all dilution rates, the viability of immobilized cells was about 30% during the continuous culture. The lower viability of the immobilized cells may be due to the rapid consumption of oxygen and nutrients by high concentration of suspended cells, before they reached the immobilized cells. MAb concentration in the culture medium increased and glucose concentrations decreased with the decrement of dilution rate. Glutamine in the culture medium was almost consumed through the continuous culture.

Specific Growth Rate and Specific Production Rate

The specific growth rate μ and specific production rate ηMAb were calculated from the following material balance for total cell concentration XT, viable cell concentration XV, and monoclonal antibody (MAb) concentration PMAb.

$$d\,XT / dt = \mu XV - fD\,XT$$
$$d\,PMAb / dt = \eta MAb\,XV - fD\,PMAb$$

where cell lysis was assumed negligible. At the steady state,

$$\mu = fD\,(XT / XV)$$
$$\eta MAb = fD\,PMAb / XV$$

Similarly, the specific production rate of immobilized cell ηI MAb was obtained from the following material balance equation:

$$V \cdot d\,PMAb / dt = VS \cdot NSV \cdot \eta MAb + VC \cdot NCV \cdot \eta I\,MAb - V \cdot fD\,PMAb$$

where V is the working volume, VC is the carrier volume, Vs = (V - Vc), NSV and NCV are concentrations of suspended viable cells and immobilized viable cells, respectively. At the steady state,

$$\eta MAb = (V \cdot fD\,PMAb - VS \cdot NSV \cdot \eta MAb) / VC \cdot NCV$$

Fig. 4 shows the relationship between the specific

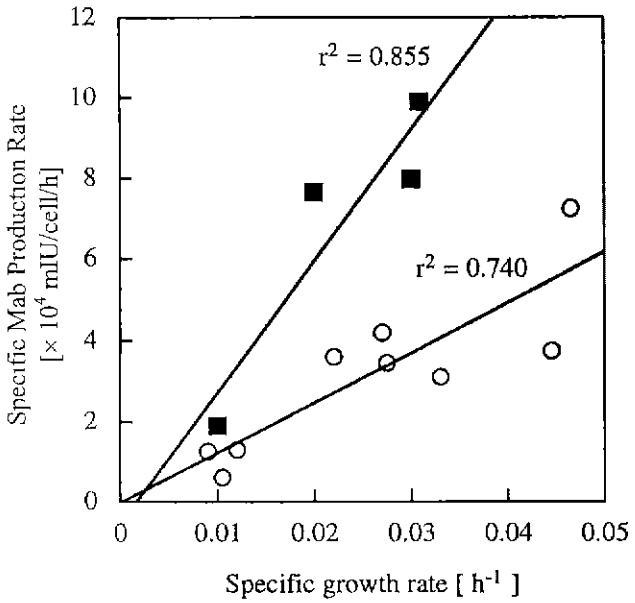

Fig. 4 Comparison of effect of specific growth rate on specific MAb production rate between suspended cells and immobilized cells in continuous culture Symbols O, suspended continuous culture; ■, immobilized continuous culture

growth rate and specific MAb production rate in suspended cells and immobilized cells. Since the specific production rate increased with specific growth rate, we concluded that the MAb production of T0405 hybridoma is positively growth associated in both suspended and immobilized cells, and MAb production may occur in growth phases (S, G2, M) of cell cycle. Interestingly, it is found that the specific production rate of immobilized cells was not only higher than that of suspended cells, but also increased with dilution rate by more than 2 times at 0 01 h^{-1} and about 3 times at 0.03 h^{-1}. Low *et al* [25] reported that loss of activity and decomposition of MAb by protease enzyme may take place at low dilution rate However, Harigae *et al* [24] reported that T0405 culture did not show any enzyme degradation of MAb in the medium, and the low specific production rate at low dilution rate is not caused by the presence of protease enzyme

Fig 2 clarified that the lower specific MAb production rate in suspended culture was not due to nutrient limitation nor metabolic waste inhibition, because, in suspended culture, both glucose and glutamine were not limited for cell growth in all dilution rates although both of them decreased with the decreasing dilution rate. Moreover, ammonia concentration was below inhibitory level (about 5 mM [26])(data not shown). Fig. 3 shows that both glucose and glutamine concentrations in the medium of immobilized culture were even lower than those in suspended culture, while a higher specific MAb production rate was obtained. The high glucose and glutamine consumption rates in immobilized culture may reflect the high specific MAb production rate If the MAb production of T0405 cells occurs in growth phase (S, G2, M) of cell cycle, the higher or improved specific MAb production rate of immobilized cells may be consistent to the growth phase of cell cycle We, therefore, performed the following experiments to clarify this assumption.

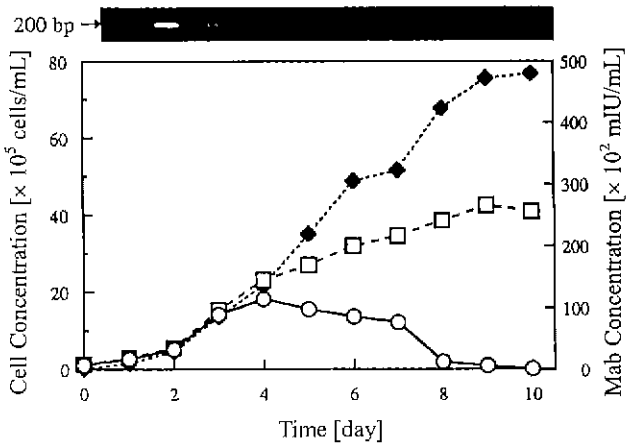

Fig 5 Time course of batch culture of T0405 cells. Agarose gel electrophoresis shows the amount of MAb mRNA in 1.25 × 10^4 viable cells Symbols O, suspended viable cell concentration; □, suspended total cell concentration; ◆, MAb concentration.

MAb mRNA Expression and Cell Cycle Distribution in Batch Cultures

In order to clarify the intracellular phenomena, batch cultures were performed to investigate the effects of immobilization on MAb mRNA expression and cell cycle distributions of T0405 cells. Batch culture has several advantages for this study: first, it dose not take a long time, and, second, it only needs very simple apparatus; Additionally, details of a cell cycle and accurate cell information can be obtained.

Fig 5 shows the time course of cell population, MAb concentration, and MAb mRNA expression in static batch culture. The viable cell concentration reached maximum after 4 days of culture, then decreased gradually, while the MAb concentration in the medium increased gradually and reached the maximum after 9 days, and no viable cell remained The mRNA expression lasted 5 days, and the mRNA expression was observed to the highest on the second day corresponding to the exponential phase, then decreased gradually and vanished on the 6th day. As to why suspended batch culture in spinner flask was not conducted to compare with the immobilized one, actually, we did try to culture T0405 cells in suspended batch conditions using spinner flask, but T0405 cells could not grow and survive in suspended batch culture in spinner flask (data not shown). The reason of this phenomena is not clear. It is likely that inhibitory factors such as metabolic substances might have fateful influence on the cell growth in batch conditions, which caused T0405 cells weak and could not tolerate the agitation in spinner flask. Under such a circumstance, we had no other choice but to use the static batch culture to compare with immobilized agitated batch culture.

Fig. 6 shows the time course of cell population, MAb

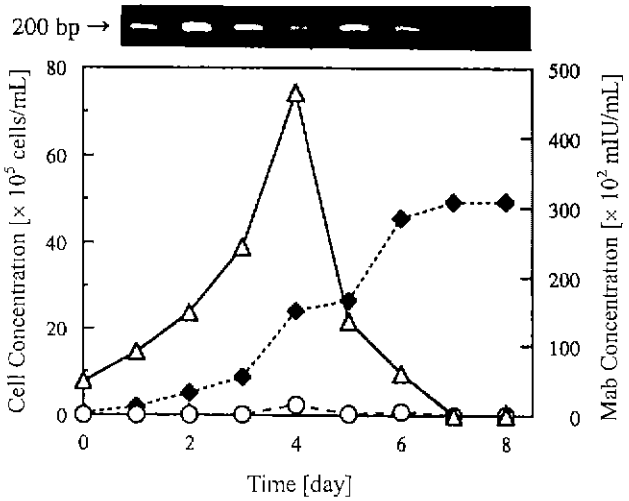

Fig 6 Time course of immobilized batch culture of T0405 cells Agarose gel electrophoresis shows the amount of MAb mRNA in 1.25×10^4 viable cells Symbols ○, suspended viable cell concentration, △, immobilized viable cell concentration, ◆, MAb concentration

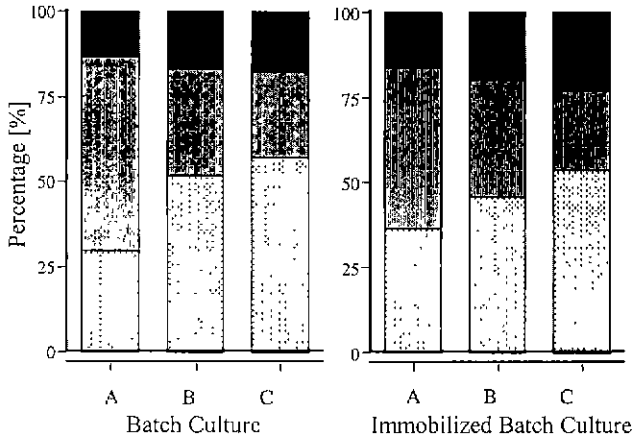

Fig. 7. Comparison of cell cycle distribution at each phase in batch culture between static culture and immobilized agitated culture. A: Exponential growth phase, B: Stationary phase, C: Dead phase Symbols □, G0/G1 phase; ▨, S phase; ■, G2/M phase.

concentration, and MAb mRNA expression in the immobilized spinner batch culture The immobilized viable cell concentration reached the maximum after 4 days and then decreased abruptly. The MAb concentration in the medium increased with cell exponential growth and the increment continued until death phase. Eight days later, immobilized viable cells were not detected, while the MAb concentration still remained constant, and the MAb mRNA expression was found lasted for seven days and its maximum expression on the second day It seems that specific MAb production rate is lower for immobilized cells than suspended culture, we need to be careful in interpreting the antibody concentration of the medium in batch cultures. Hybridoma cells are found to produce not only stimulatry factors but also inhibitory factors of antibody production [27] In batch cultures, no medium was exchanged. Under such a condition, these stimulatory factors are apt to accumulate in the duration of mRNA transcription stage Therefore, such factors will actually effect on antibody production because cells are maintained in high density in the immobilizd batch culture. However, in the duration of post-transcriptional stage, the high density cells will start secreting inhibitory factors which may cause negative effect on antibody production in immobilized batch culture. Such phenomena can hardly be found in suspended ones. Compared with static batch culture, the highest expression of mRNA was also observed during the exponential phase, which was in agreement with the result of the positively growth-associated MAb production in continuous cultures. On the other hand, the overall duration of MAb mRNA expression became longer in immobilized cells than that in suspended ones The facts that higher specific MAb production rate observed in the continuous immobilized culture and longer mRNA expression in immobilized batch culture may suggest that immobilization has positive effect on MAb production of T0405 cells.

Barford *et al* [28] pointed out the level of MAb production of individual cell was various. It was concluded that MAb production was not constant through all phases of cell cycle. Takahashi *et al.* [29] reported that MAb production of NK9 cells reached maximum in the S phase and gradually decreased from late S phase to G2, and dropped to minimum in M phase. Accordingly, the higher or improved specific MAb production rate of immobilized cells may be related to the cell cycle in the different cell phases. Because the MAb production of T0405 cells is growth-associated, it may be expected that MAb is produced in the growth phases (S, G2, and M) of cell cycle We, therefore, determined the cell cycle distribution of viable cells in different phases of batch cultures, and the results were showed in Fig. 7. The proportions of cells at S phase were high at exponential growth phase in both suspended and immobilized cultures. Other higher proportion of cells was also found at G0/G1 phase at dead phase. Additionally, continuous culture also revealed high cell cycle distribution in S phase at high dilution rate with high specification MAb production rate (data not shown). However, no significant difference was observed in cell cycle distribution between immobilized and suspended batch culture. Accordingly, it became clear that enhancement of MAb production by immobilization dose not involve the change of cell cycle distribution.

Moreover, Leno *et al.* [8] and Bibila *et al.* [30] reported that the stability of antibody mRNA changed through the batch culture and the half life of mRNA is longest in exponential phase and shortest in death phase. But Cowan and Milstein [31] investigated the stability of mRNA encoding the immunoglobulin light chain, and concluded that it is possible for the mRNA to survive in the cytoplasm longer than one cell cycle These results indicate that the change of the stability of MAb mRNA is possible to occur The longer expression of MAb mRNA observed during immobilized batch culture, then, may be due to the continuous transcription

of mRNA or the accumulation of more stable mRNA, which resulted from immobilization There are many reports describing that high specific MAb production rate in negatively growth-associated hybridoma was due to low growth rate [32-34]. Lee *et al.* [2] proposed that the higher specific MAb production in immobilized hybridoma was caused by the low growth rate of immobilized cells. But such hypothesis cannot interpret the higher specific MAb production of immobilized T0405 cells because our results showed that this cell line is positively growth-associated. Therefore, the enhanced MAb production in immobilized cells dose not relate to the growth rate.

In addition to the longer expression of MAb mRNA described above, other factors which may enhance the high MAb specific productivity in the immobilized cells under the condition of high cell density can be the metabolic change of cells. Zhang *et al.* [35] reported that the specific MAb production rate increased with increasing cell concentration in hybridoma perfusion culture. Shirai *et al* [36] described that oxygen consumption rate increased more in high density immobilized cells than in low density, and energy utilization efficiency was also found higher in high density than in low density culture [37]. High density cultures were reported to be less inhibited by ammonia compared to low density culture [38]. These facts imply that high density condition in the macroporous carriers is more advantageous for MAb production of T0405 cells. Furthermore, substances like autocrine growth factors secreted by hybridoma cells may stimulate other cell growth and MAb production [27] For example, interleukin-6, one of the autocrine growth factor, was reported to stimulate immunoglobulin production of lymphoid cells [39,40]. Taken together, these conditions in immobilized cells may result in the longer stable expression of MAb mRNA and improve the specific MAb productivity.

In conclusion, we demonstrated that the mass cultivation to high density of anchorage-independent cells like hybridoma can be immobilized onto macroporus cellulose carrier, and it was shown that immobilization not only resulted in higher cell density but also increased specific MAb production rate. Such facts may be interpreted by the longer stable expression of the MAb mRNA in immobilized cells than in suspended ones, and the advantage of high cell density as mentioned above High cell density via immobilization increased the duration of the expression of more stable MAb mRNA, and consequently achieved a higher specific MAb production rate.

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