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# 하이브리도마 배양을 위한 lipids의 공급

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# Lipids Supply for Hybridoma Culture

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## ABSTRACT

BSA/acids component in serum free mdium(SFM) developed for the culture of hybridoma cell line, KA112, was replaced by acids/Pluronic F-68 emulsion. Protein content of SFM was minimized, and increased maximum cell density was obtained in serum-free lipids supplemented medium(SFLSM). Cell growth promotion effect of the emulsion was not affected by filtration with 0.2$\mu$m filter.

## INTRODUCTION

Serum is a rich source for the various lipids cultured cells generally need for survival and particularly for cell growth. Cell lines differ in their requirements for essential fatty acids, phospholipids, lecithin and cholesterol. For example optimum fatty acid/cholesterol/albumin balance varies radically for different haematopoietic lineages and for different maturation stages within a given lineage(1). Lipid is one of them. Therefore, lipids supply is essential for the cultivation of animal cell using serum-free medium(SFM). Lipids or lipid precursors, added to medium, are known as having influence on proliferation, differentiation and production of antibody(2). Growth promoting effect of lipids for animal cell differs in the methods of lipids supply. Recently, several methods-direct supply, lipids/BSA system etc. - to improve cell growth and antibody production have been investigated(3). Lipids are introduced into cell by direct uptake and are concerned with cell consisting materials and energy sources rather than with the generation of secondary messengers in cell. Lipids uptake by cell reduces the necessity of the production of lipids by cell itself(4).

In the present study, lipids adsorbed to BSA, BSA/acids, was replaced by lipids emulsion thus minimizing the amount of BSA, a protein added to medium as lipids carrier. This makes the monoclonal antibody(MAb) purification work much easier than before.

## MATERIALS AND METHODS

### Cell line

Mouse-mouse hybridoma, KA112, producing IgM against the surface antigen of *Chlamydia trachomatis* was used. This cell line was constructed in the senior author's laboratory(5).

### Medium

Basal medium was Dulbeco's Modified Eagle Medium(DMEM) with high glucose. Composition of serum-free medium(SFM) and low serum medium(LSM) were the same as those of Jeh(5).

### Cell culture

Hybridoma was cultured in 36(±0.6)℃ $CO_2$ incubator and subcultured in LSM. Actively proliferating cells were transferred to SFM and adapted for 2~3 days before inoculation. Cell concentration was estimated by dye exclusion method using 0.05%(v/v) trypan blue solution with haemocytometer.

### Chemicals

Linoleic acid and bovine serum albumin(BSA, Factor V) were purchased from Sigma(cell culture tested) and oleic acid from Junsei, Japan. As emulsifier. Pluronic F68 from BASF and Tween 80 from Junsei, Japan, were used.

### Preparation of emulsion

Emulsifier in deionized water and fatty acids in ethanol were filtered with 0.2 micron filter in laminar flow bench. Sterilized fatty acids in glass tube were added by emulsifier dropwise with vortexing. The concentration of emulsifier used was 10%(v/f)(6).

## RESULTS AND DISCUSSION

### Effect of oleic acid emulsion on cell growth

SFM control was SFM added BSA/acids. The concentrations of added acids to SFM were 1mg/ℓ oleic acid and 0.5mg/ℓ linoleic acid. In 1.0mg/ℓ oleic acid emulsion, cell growth was better than

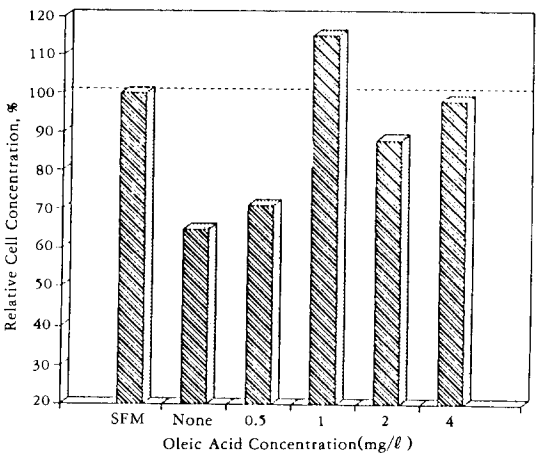


**Fig. 1. Effect of the concentration of oleic acid/pluronic F-68 emulsion on cell growth.**
**SFM: serum free medium as control culture;**
**None: SFM without BSA/acids.**

that in SFM control(Fig. 1). In the case of 0.5mg/ℓ of oleic acid, little difference with SFM without BSA/acids, (BSA/acids)(-), was observed. However, in the case of 2mg/ℓ or 4mg/ℓ of oleic acid, cell growth promoting effect existed but to less extent than with 1mg/ℓ. One can suppose that this repression was caused by emulsifier, added in excess with emulsion, or oleic acid. Further studies are needed about this point.

### Effect of linoleic acid emulsion on cell growth

Maximum cell growth was obtained at 0.5mg/ℓ linoleic acid emulsion and a little growth at 1mg/ℓ and very low growth at 2 and 4mg/ℓ was observed(Fig. 2). The reason for this phenomenon is considered to be same as in the oleic acid case.

### Effect of emulsifier change on cell growth

Emulsifier, pluronic F-68, was replaced by Tween 80 with the range of oleic acid concentration, 0.5~3.0mg/ℓ. A slower growth as compared with that of SFM control was observed (Fig. 3). With linoleic acid/Tween 80 emulsion, cell growth was promoted as compared with the dish without emulsion in the range of tested con-

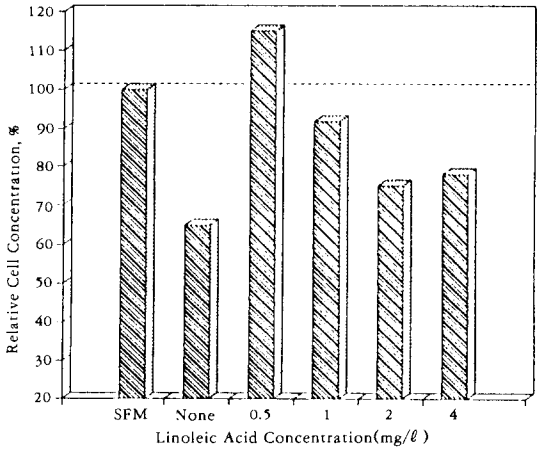


Fig. 2. Effect of the concentration of linoleic acid/ Puronic F-68 emulsion on cell growth.
SFM: serum free medium as control culture;
None: SFM without BSA/acids.

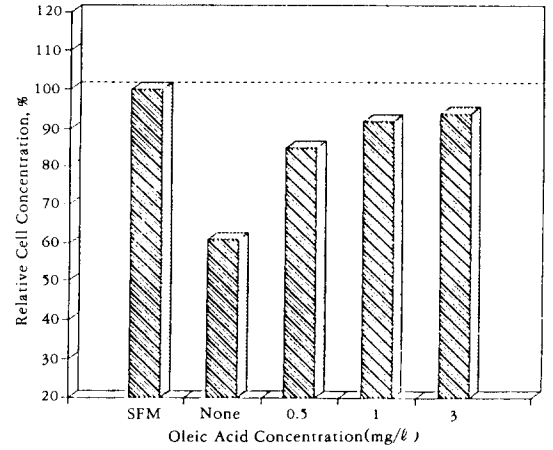


Fig. 3. Effect of the concentration of oleic acid/ Tween 80 emulsion on cell growth.
SFM: serum free medium as control culture;
None: SFM without BSA/acids.

centration. However, maximum cell concentration was lower than SFM control with BSA/acids (Fig. 4).

### Effect of acids/pluronic F-68 on cell growth

When both oleic acid and linoleic acid emul-

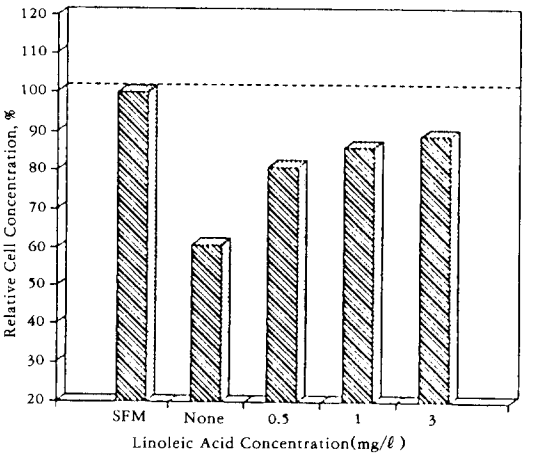


Fig. 4. Effect of the concentration of linoleic acid/ Tween 80 emulsion on cell growth.
SFM: serum free medium as control culture;
None: SFM without BSA/acids.

sions were added simultaneously, higher cell growth was observed than the case with separate addition(Fig. 5). However, acids(-) showed higher cell growth than SFM control. This is considered to be caused by lipids contaminants in

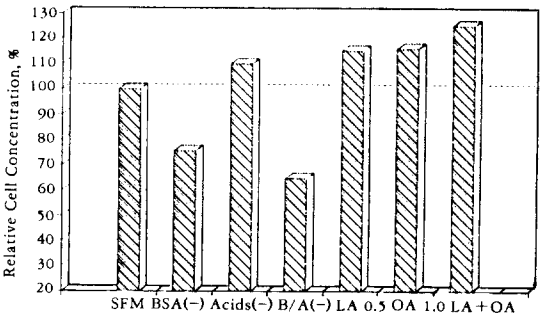


Fig. 5. Comparison of cell growth promoting effect at optimized acids concentration; acids/pluronic F-68 emulsion.
SFM: control culture: BSA(-): SFM without BSA: acids(-): SFM without acids; B/A(-): SFM without BSA/acids; LA 0.5: linoleic acid 0.5mg/ℓ emulsion; OA 1.0: Oleic acid 1.0mg/ℓ emulsion; LA+OA: Linoleic acid 1mg/ℓ plus oleic acid 1mg/ℓ emulsion.

**Table 1. The comparison of specific antibody production rate, lactate concentration and protein content in culture broth of four kinds of media.**

| Title | Specific Productivity | Relative Protein Content | Relative Lactate Concentration |
|---|---|---|---|
| Medium | (MAb/$10^6$ cells/hr) | (%) | (%) |
| 7% FBS | 0.0189 | 100 | 100 |
| LSM(1% FBS) | 0.0218 | 29 | 111 |
| SFM | 0.0313 | 31 | 80 |
| SFLSM | 0.0253 | 21 | 65 |

BSA. Because added BSA was factor V and contained some fatty acid contaminants. Lipids added to BSA did not have significant influence on cell growth(4). This supposition comes from the fact that BSA(−) and (BSA/acids)(−) show very low cell growth. Specific MAb productivity in SFLSM was higher than that of LSM and 7% FBS and lower than that of SFM(Table 1). Protein content was cut down to one fifth of that in 7% FBS medium, and production of lactate, a cell growth inhibitor, was reduced(Table 1). Final glucose concentration was similar among four kinds of medium, 7% FBS, LSM, SFM, and SFLSM. This result implies that the portion of glucose to lactate via pyruvate was decreased and the portion to cellular materials, for example, MAb, was increased in SFLSM. This opinion is related to previous MAb production result(Table 1).

### Effect of filtration of SFLSM on cell growh

We supplied lipids to cell by emulsion. This emulsion can be destroyed during filtration because animal cell medium was sterilized by filtation with 0.2~0.45$\mu$m filter paper. Therefore, we checked cell growth promotion effect of SFLSM before and after filtration(Fig. 6).

The above results indicate that lipid emulsion is a good method for lipid delivery into cell. Because of lower lactate production and higher specific MAb productivity, SFLSM is suitable to perfusion culture for the mass production of MAb. and, lower protein content in culture broth makes MAb purification work easier than other medium.

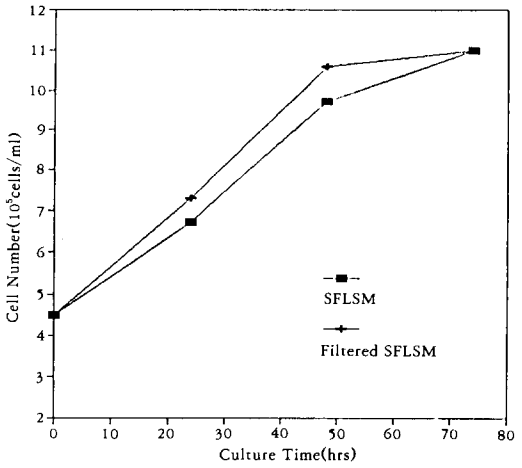


**Fig. 6. Comparison of cell growth before and after filtration of the mediu, SFLSM.**

For future works, in addition to oleic acid and linoleic acid, other lipids can be added to medium in the form of emulsion to enhance viability and productivity. MAb productivity can be increased by the addition of some cmponents to SFM amd SFLSM, or by the development of fully defined medium(2, 7, 8).

## 요 약

기존의 무혈청 배지에 첨가하던 BSA/acids를 acid/Pluronic F-68 emulsion으로 대치하여도 세포 성장에는 전혀 지장이 없었다. BSA를 제외함으로써 무혈청 배지에 첨가하는 단백질 함량을 최소화할 수 있었고, 이로 인한 세포농도 감소는 없었다. SFLSM은 0.2micron 필터로 여과 멸균하여도 SFLSM의 세포성장효과는 영향을 받지 않는다.

## ABBREVIATION

SFLSM : Serum Free Lipid Supplemented Medium ; SFM : Serum Free Medium ; LSM : Low Serum Medium ; BSA : Bovine Serum Albumin ; 7% FBS : 7% Fetal Bovine Serum Added Medium ; MAb : Monoclonal Antibody ; acids : oleic acid+linoleic acid ; (BSA/acids)(−) : SFM without BSA/acids ; BSA(−) : SFM without

BSA ; Acids(−) : SFM without acids, or BSA alone added SFM.

## ACKNOWLEDGEMENT

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