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하이브리도마 배양을 위한 저혈청배지의 조성 결정

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Low Serum Medium for CH07E02 Hybridoma

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ABSTRACT

Up to now, 10% Fetal Bovine Serum(FBS(V/V)) was added to basal medium for the cultivation of hybridoma. For the cultivation of hybridoma cell line, CH07E02, against colon cancer, serum concentration was reduced to 3% FBS without influence on cell growth and maximum cell concentration. By the addition of cell growth promoting substances-insulin(I), pyruvate(P), oxaloacetate(O), Pluronic F-68(P) and 2-mercaptoethanol(2-ME)-to 1% FBS medium, a cell density higher than that with 1% FBS medium alone was achieved. FBS 3% medium was replaced by very cheap 2% Calf Serum(CS) medium without influence on cell growth rate and concentration. Cells grew vigorously in 0.5% CS+ IPOP medium. This composition was used during suspension culture and exhibited good viability and high specific growth rate.

INTRODUCTION

It is a well-known fact that serum is indispensable for animal cell culture. Although several kinds of serum free media have been developed recently, these media were not cheap because of the expensive growth factors added for cell growth. Serum consists of growth factors, hormones, lipids, minerals and proteins(1-3) and could be replaced by insulin, pyruvate, oxaloacetate, thiols, and transferrin in other studies(4-6).

In the current study, the serum level in the medium was minimized and the effects of adding several key components were investigated. The newly developed media could substantially reduce the cost of culture medium.

MATERIALS AND METHODS

Cell line

Mouse-mouse hybridoma, CH07E02, which is producing IgM against the surface antigen of SC-1, a human digestive organ cancer cell line, was

used. CH07E02 and SC-1 were obtained from college of medicine, Seoul National University.

Medium and serum

Basal medium used was RPMI-1640(#430-1800, Gibco), and fetal bovine serum(FBS) and calf serum(CS) were purchased from Gibco and Hyclone respectively.

Chemicals

Insulin, pyruvate, and oxaloacetate were purchased from Sigma(cell culture tested), and Pluronic F-68 from BASF and 2-mercaptoethanol from MERCK-Schuchardt, F. R. G..

Cell culture

Hybridoma was cultured in 36(±0.6)℃ CO_2 incubator and subcultured in D-100 dish(Green Cross Co., Korea) using 2% CS or 3% FBS medium. Cell concentration was estimated by dye exclusion method using 0.05%(v/v) trypan blue solution and haemocytometer. Suspension culture was conducted by a spinner flask(Bellco.) in CO_2 incubator.

Glucose concentration analysis

Glucose was quantified by Somogy-Nelson method, based on the measurement of absorbance at 660 nm using Spectronic-20(7).

RESULTS AND DISCUSSION

Minimization of serum concentration

CH07E02 was cultured in the medium containing 0, 3, 5, 7, and 10% FBS respectively. 0% means that basal medium alone was used without adding any other components and this was chosen as control. When inoculum size was 2×10^5 cells/ml in D-100 dish, there was little difference in the final cell concentration and the specific growth rate achieved in the range of 3-10% FBS (Fig. 1). In the dish without FBS, cell growth did not occur. From this, one can conclude that 3% FBS is sufficient for basic cell growth. The remaining 7% FBS out of the total 10% FBS

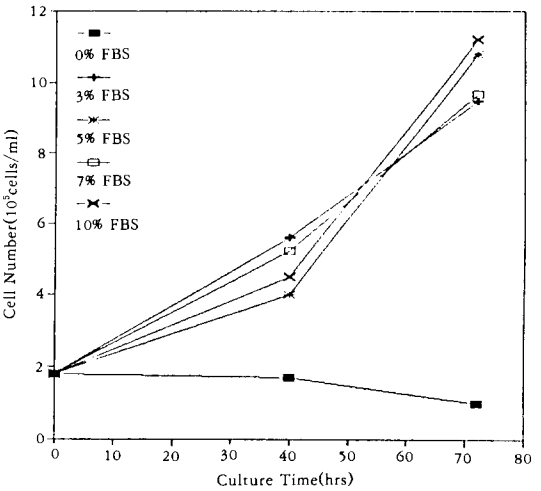

Fig. 1. Effect of serum concentration on cell growth. Balance was RPMI-1640.

added acts as the supply for additional nutrient only. Growth factors and hormones were sufficiently supplied by 3% FBS. Additional 7% FBS did not substantially enhance the cell growth. Cell death can be caused by the limitation of key substances or accumulation of toxic metabolites. The only difference between growth curves for 3% FBS and 10% FBS was death rate. In 10% FBS medium, cell death rate was slower than that in 3 % FBS medium. This could be the results from detoxification effect by certain enzymes or other substances present in FBS, or from the supply of substrates from additional FBS.

Below 3% FBS concentration, cell growth and possibility of substitution by cheap CS for FBS were investigated(Fig. 2). Above 3% FBS cells showed active proliferation, while in 0.5% or 1.0 % FBS medium very low growth was observed. Culture in 2% CS medium showed growth similar to that with 3% or 5% FBS medium. 5% CS medium showed still higher growth rate and cell concentration.

From the above data, one can conclude that CS is more effective than FBS for the growth of CH07E02. The decrease of cell growth with 0.5% or 1.0% FBS medium indicates that both FBS and CS have cell growth promoting factors in

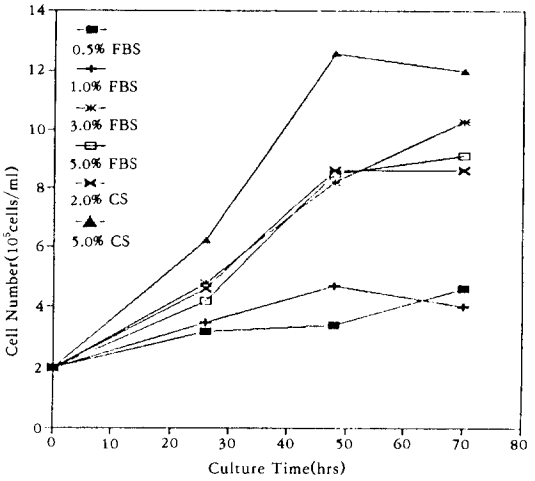

Fig. 2. Cell growth profiles with different kinds of serum at different concentrations during D-100 dish culture. Balance was RPMI-1640.

common. The minimum CS level for cell growth without components addition was 2%.

Serum substitution by components addition

Insulin(0.1mg/ℓ), pyruvate(110mg/ℓ), oxaloacetate(150mg/ℓ), pluronic F-68(1.0g/ℓ) and 2-ME(3.92mg/ℓ) were added to 1% FBS medium individually(Fig. 3). 2-ME showed growth promoting effect in initial phase but beyond 60 hours, cell concentration was decreased. One can conclude that 2-ME is not so effective or important for cell growth. This conclusion was further examined in another experiment(Fig. 4). Insulin was effective for cell growth and oxaloacetate was related to cell growth in late exponential phase. When FBS was substituted for by CS, and the four components-IPOP except 2-ME-were added, cell growth was considerably improved (Fig. 5). When IPOP was added to 2% CS medium, cell growth promoting effect was not observed. This result confirms the previous results that 2% CS is minimum concentration to support cell growth without any other components addition. In case of 1.0% and 0.5% CS, the addition of IPOP caused considerable increase of

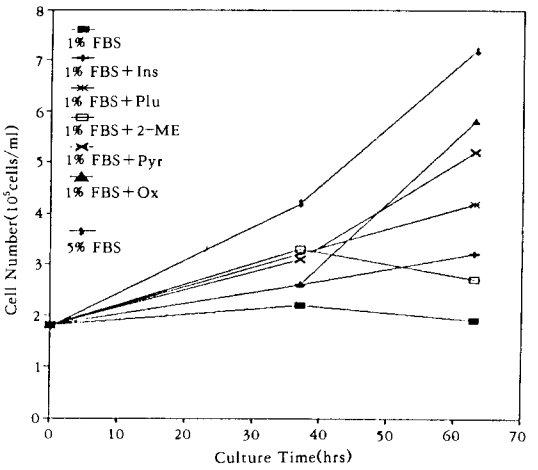

Fig. 3. Effect of the addition of five kinds of components on cell growth. Ins: Insulin; Plu: Pluronic F-68; 2-ME: 2-Mercaptoethanol ; Pyr: Pyruvate; Ox; Oxaloacetate.

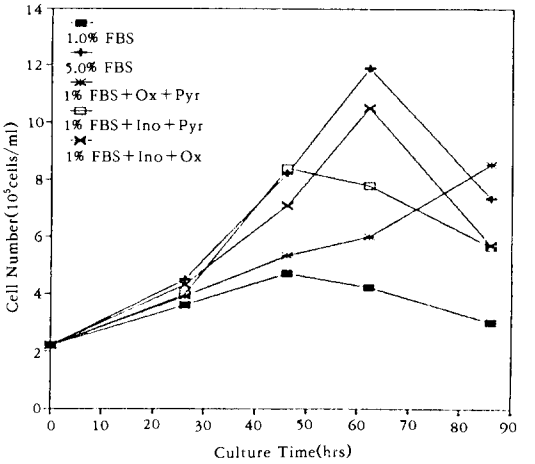

Fig. 4. Effect of components addition on cell growth in 1% FBS medium. Ins: Insulin; Pyr: Soudium Pyruvate; Ox: Sodium Oxaloacetate.

specific growth rate and cell concentration. The difference in maximum cell concentration is supposed to be caused by the limitation of substrates. In 5% and 2.0% CS media, serum could have served as both substrate and growth factor, but in 0.5% and 1.0% CS media, serum served

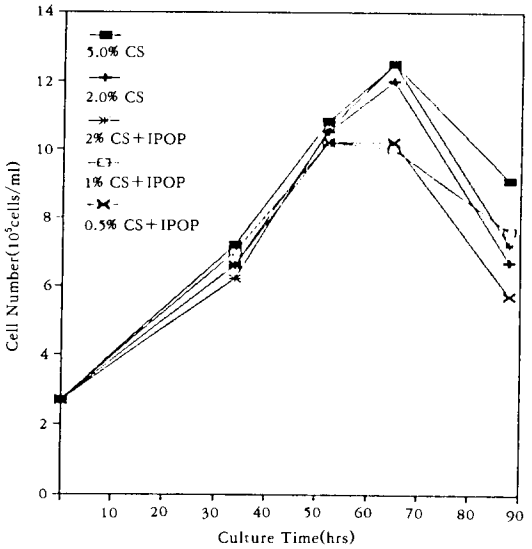

Fig. 5. Effect of components addition on cell growth at different serum concentration levels. IPOP: Insulin, Pyruvate, Oxaloacetate, Pluronic F-68.

only as growth factors. The composition of the medium finally chosen was 0.5% CS+IPOP. When cells were not in active proliferation state, 2% CS medium was chosen.

Suspension culture

In suspension culture, one should consider cell growth inhibition by shear damage(8, 9) which need not be considered in stationary culture. Serum protects cells from shear damages and promotes cell growth when low inoculum size is used. 0.5% CS+IPOP was the medium employed in suspension culture(Fig. 6). Lag time was about 20 hours and maximum cell concentration was 1.2×10^6 cells/ml with very high viability. At maximum cell concentration, the glucose concentration in the medium was 0.5g/ℓ. This concentration was not sufficient for cell growth. Therefore, viability decrease was caused by substrate limitation, especially glucose or glutamine. With this medium, specific monoclonal antibody(MAb) productivity was not decreased(data not shown). This medium was suitable for suspension culture at 40~70rpm without shear damage. In the fed-

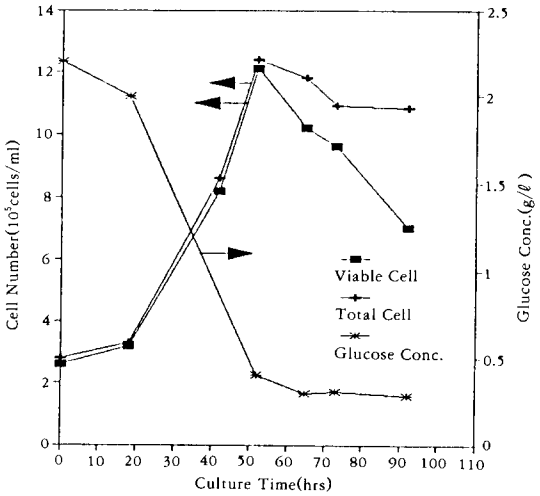

Fig. 6. Cell growth and glucose concentration profiles in 250ml apinner flask culture. Medium: 0.5%CS+IPOP in RPMI-1640.

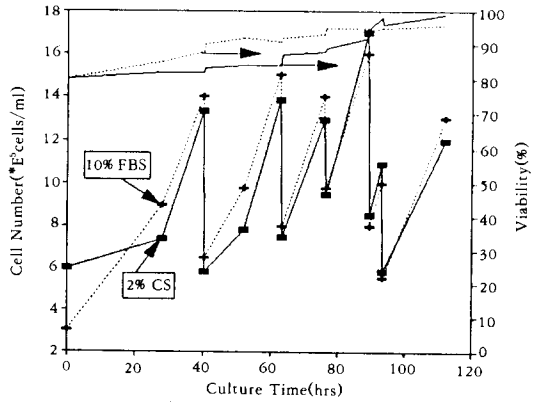

Fig. 7. Cell growth and viability profiles in spinner flask culture; fed-batch operation. Media were supplemented by 10% FBS or 2% CS.

batch culture carried out as a subculture for perfusion culture, the viability, the specific growth rate and the maximum cell concentration were similar to those in 10% FBS medium(Fig. 7) although, in 10% FBS medium, death rate was slow and activation was fast. Media cost was reduced to 10% compared with 10% FBS medium. In late exponential phase, the limitation of glucose can be avoided by glucose feeding. Improved low cost medium can be very attractive for large-scale high density culture(10).

To increase antibody productivity, investigation about specific component addition, selection of high activity cell line and development of new operation system should be done in future study.

요 약

하이브리도마 배양을 위해 첨가한 기존의 혈청농도는 10% FBS였으나, 이 농도는 3%까지 줄여도 세포의 비성장속도와 최고세포농도에 차이가 없었다. 세포성장을 촉진한다고 알려진 물질들을 1% FBS 배지에 첨가하였을 때 insulin, pyruvate, oxaloacetate, pluronic F-68 등에 의해 세포성장이 촉진되었다. 3% FBS는 2% CS로 대치할 수 있었고, 혈청의 농도는 IPOP의 첨가시 0.5% CS에서도 active proliferation을 보였다. 최종 조성의 배지가격은 기존의 10% FBS 배지의 1/10 정도였으며 배지조성을 cell line에 따라 달리 결정함으로써 배지의 가격은 현저히 낮출 수 있음을 알았다.

ABREVIATION

FBS : Fetal Bovine Serum; CS : Calf Serum; IPOP : Insulin, Pyruvate, Oxaloacetate, Pluronic F-68; 2-ME : 2-Mercaptoethanol

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REFERENCES

1. G. H. Rothblat and V. J. Cristofalo(1972), "Growth, Nutrition and Metabolism of Cell in Culture," **1**, 49, Academic Press, London.
2. R. J. Sugasawara(1988), *Bio/Tech.*, **6**, 895.
3. R. Freshney(1986), "Animal cell culture : a practical approach," 24, IRL Press, N. Y.
4. M. C. Glassy, J. P. Tharakan and P. C. Chau (1988), *Biotech. Bioeng.*, **32**, 1015.
5. K. Sugama, Y. Namba, M. Hatanaka and M. Hanaoka(1987), *J. Cell Physiol.*, **131**, 23.
6. B. W. Murhammer and C. F. Goochee(1985), *Bio/Tech.*, **6**, 1411.
7. K. H. Jung(1987), *M. S. Thesis, Dept. Chem. Tech.*, S. N. U.
8. K. T. Kunas and E. T. Papoutsakis(1989), *Biotech. Lett.*, **11**(8), 525.
9. G. M. Lee, T. K. Huard and B. D. Pallson (1990), *Biotech. Bioeng.*
10. B. Griffiths(1986), *Trends in Biotechnology*, **8**, 268.