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Reduction of FBS Concentration through Adaptation Process in Mammalian Cell Culture and Addition of Silkworm Hemolymph in Insect Cell Culture

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Abstract Animal cell culture media are usually supplemented with fetal bovine serum (FBS); however, the use of FBS presents certain problems including high cost. By using an adaptation process and the addition of silkworm hemolymph, the FBS concentration can be reduced without causing a significant decrease in cell growth.

Key words: Fetal bovine serum, animal cell, silkworm hemolymph, adaptation

There have been several significant advances in recent bioprocessing research that have aided the maturation of animal cell-based manufacturing technology one of which includes culture medium development [3]. FBS has been widely used as a medium supplement for the culture of animal cells since it contains a large number of different growth-promoting activities. However, the use of FBS presents certain problems such as high cost, nonreproducibility due to lot-to-lot variation, undefined composition, increased contamination risk from mycoplasma, and the complication of downstream processing due to a high protein concentration [7]. Many attempts have been made to develop low serum or serum-free media and some of them have been commercialized; however, in most cases, the medium is still supplemented with 10% FBS.

This work is concerned with the reduction of FBS concentration in animal cell culture media by using an adaptation process and the supplement of silkworm hemolymph. Silkworm hemolymph is an inexpensive insect serum while FBS is a costly component, accounting for about 90% of the total cost when the medium is supplemented with 10% FBS [6]. Silkworm hemolymph can be easily collected since silkworm is a domesticated insect which has large volume.

Chinese Hamster Ovary (CHO K1) cells and *Spodoptera frugiperda* (*Sf*9) cells were used in this work. CHO and *Sf*9 are the most widely used cell lines in mammalian and insect cell cultures, respectively [4, 5]. Both cell lines were cultured in a 25 cm^2 tissue culture flask (Nunc) containing 6 ml of medium. The CHO cells were grown in a CO_2 incubator at 37°C while the *Sf*9 cells were cultured in an incubator at 28°C. Dulbeccos modified Eagles medium (DMEM/F12, Gibco) supplemented with 1.2 g/l $NaHCO_3$ and antibiotics-antimycotics (Gibco) was used for the CHO cells while Graces medium (Gibco) supplemented with 0.35 g/l $NaHCO_3$ and antibiotics-antimycotics (Gibco) was used for the *Sf*9 cells. Various concentrations of FBS and silkworm hemolymph were also added to the media.

Silkworm hemolymph was collected from the 5th instar larvae by clipping the side of an abdominal leg. The collected hemolymph was heat-treated at 60°C for 30 min, then chilled and centrifuged [2]. The supernatant was then used for supplementing the medium. Ten or more culture flasks were initially prepared for one set of experiments. Each day, a sample for an assay of cell concentration was taken from one culture flask; that flask was then discarded and the next one was used. Cell concentration was measured using a hemocytometer.

Figure 1 shows the growth of the CHO cells in the medium with various concentrations of FBS. The specific growth rate and final cell concentration decreased as the FBS concentration was reduced. These decreases could be overcome by use of an adaptation process. The adaptation was carried out by transferring the cells to the medium supplemented with less FBS, culturing them until the stationary phase, and then successively transferring them to a fresh medium containing a lower concentration of FBS. Figure 2 shows the CHO cell growth after adaptation. The maximum specific growth rate and final cell concentration before and after adaptation are summarized in Table 1. Although the cell concentrations were vastly recovered through the adaptation process, these were still somewhat lower than in media with a higher concentration of FBS. Silkworm hemolymph was, therefore, added to the medium containing a lower amount of FBS to determine if it could be used as a substitute for FBS. However, it did not

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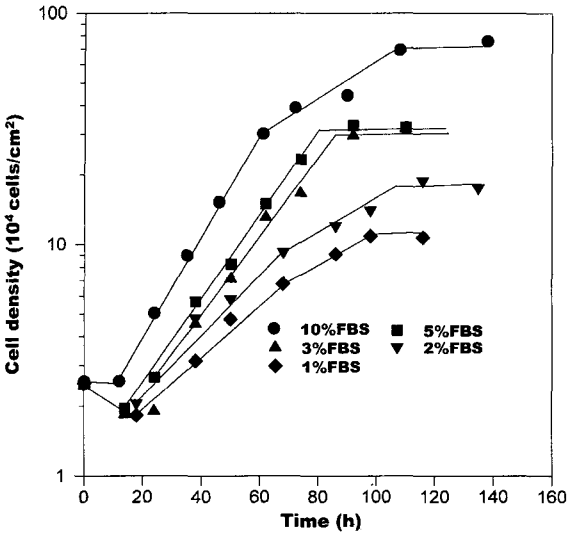

Fig. 1. CHO cell growth before adaptation at various concentrations of FBS.

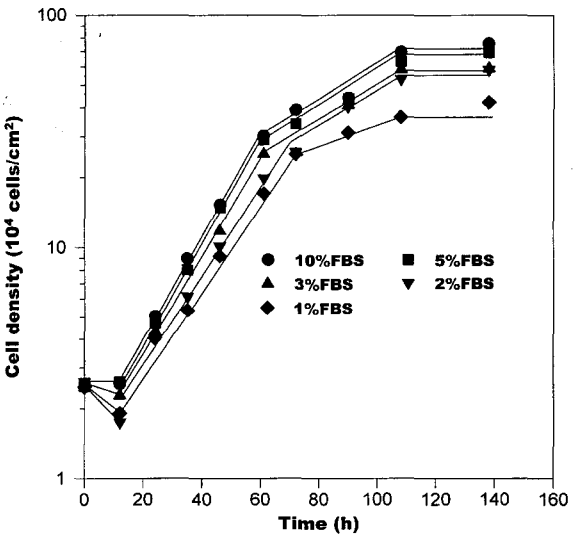

Fig. 2. CHO cell growth after adaptation at various concentrations of FBS.

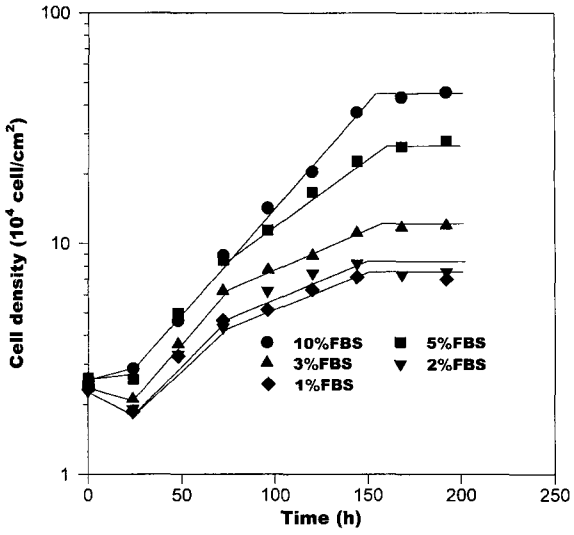

Fig. 3. *Sf*9 cell growth at various concentrations of FBS.

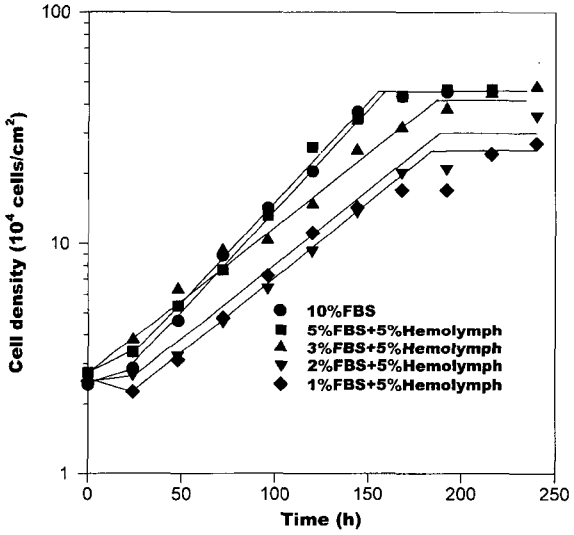

Fig. 4. *Sf*9 cell growth with the addition of 5% silkworm hemolymph at various concentrations of FBS.

increase the specific growth rate or the final cell concentration any further (data not shown).

Similar experiments were conducted for the *Sf*9 cells. The growth of the *Sf*9 cells in the medium with various concentrations of FBS is shown in Fig. 3. A lower FBS concentration resulted in a lower specific growth rate and lower final cell concentration as in the case of the CHO cells. Adaptation was carried out using the same method as

Table 1. Effect of FBS concentration on CHO cell growth.

FBS concentration	Before adaptation		After adaptation	
	Maximum specific growth rate (1/h)	Final cell concentration (10^4 cells/cm^2)	Maximum specific growth rate (1/h)	Final cell concentration (10^4 cells/cm^2)
10%	0.045	75.42	0.045	75.42
5%	0.039	32.35	0.045	66.09
3%	0.035	30.62	0.045	58.60
2%	0.034	18.17	0.044	55.57
1%	0.033	10.79	0.043	39.28

Table 2. Effect of FBS concentration on *Sf*9 cell growth.

FBS concentration	Without hemolymph		With 5% hemolymph (after adaptation)	
	Maximum specific growth rate (1/h)	Final cell concentration (10^4 cells/cm^2)	Maximum specific growth rate (1/h)	Final cell concentration (10^4 cells/cm^2)
10%	0.020	44.25	0.020	44.25
5%	0.020	27.06	0.020	46.26
3%	0.020	11.86	0.016	45.84
2%	0.011	7.44	0.016	30.04
1%	0.011	7.08	0.016	25.62

described earlier to observe if the growth could be recovered by this process. The results were negative. The growth was not recovered using adaptation and the cells showed no signs of growth in the third or fourth subculture during the adaptation process (data not shown).

Silkworm hemolymph was added to the medium with a low concentration of FBS as a substitute for FBS, and the adaptation process was performed again. This time positive results were recorded. The cell growth was recovered by the addition of 5% silkworm hemolymph as shown in Fig. 4. The maximum specific growth rate and final cell concentration with and without the addition of silkworm hemolymph are summarized in Table 2. Silkworm hemolymph has also been reported to increase the production of recombinant protein in *Sf*9 cells [1].

The lower FBS concentration in the medium resulted in a lower specific growth rate and lower final cell concentration in both cell lines. In the case of the mammalian cells (CHO), the growth characteristics in a low FBS concentration could be basically recovered through the adaptation process; however, the addition of hemolymph exhibited no effect. Conversely, the insect cell (*Sf*9) growth in a low FBS concentration was not improved by the adaptation process yet was restored by the addition of hemolymph. These results imply that CHO cells have an adaptation ability to low serum medium while *Sf*9 cells do not. However, the insect cell growth in low FBS medium can be recovered by the addition of insect serum.

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