FET 2017 ۷ اسفند ۱۳۹۵ 25 February 2017
اولین کنفرانس ملی
آینده مهندسی و تکنولوژی
1st national Conference On Future Of Engineering And Technology
دانشگاه علم و فرهنگ
تهران - ایران
Iran - Tehran

Effects of protein, vitamins, essential amino acids and glucose on growth and proliferation of baby hamster kidney cells (BHK) in suspension culture

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Abstract:
The present study focuses on the effects of protein, glucose as a carbohydrate, essential amino acids and vitamins on the baby hamster kidney (BHK) cells growth and proliferation. BHK cells culture is of special importance in pharmaceutical and biological products, particularly animal drugs. BHK cells culture is very important in production processes. All experiments have been done under suspension culture conditions in a 1-liter bioreactor having blades and air mixing pores. Considering the final cell number, which is the most important factor in studying the state of cell culture under suspension conditions, the effect of protein, glucose, essential amino acids and vitamins on BHK cells has been investigated. The higher final number of cells means higher cell culture productivity. During the study, it was indicated that the glucose, protein, essential amino acids and vitamins have the greatest impact on BHK cells growth and proliferation, respectively.

Keywords*:* supplement, baby hamster kidney (BHK), suspension, growth, proliferation

Introduction:

Cell culture is a process in which cells taken from fetus or animal are grown by meeting their physical and chemical needs under the experimental conditions (Butler, 2003).

Cell culture is mainly used in biological sciences such as virus propagation, vaccine production, diagnosis, hormones production, physiological, biological, pharmaceutical studies, basic , applied researches including cell biology, physiology, pharmacology and toxicology, recombinant protein production and gene therapy, cancer research and production of drugs, antibodies, interferons, erythropoietin, coagulation factors and safety testing, and many other aspects. (Vester et al, 2010; Merten, 2006; Liu et al, 2013)

BHK cells are derived from baby hamster kidney. (Macpherson and stoker, 1961)

BHK cells are mainly used in animal products, particularly for FMD vaccine and rabies vaccine production. They are also used to produce recombinant proteins such as factor IIIV and ELISA antigen for Japanese encephalitis virus (JE) and to extract DNA from Pseudorabies virus. (Kallel et al, 2003; Aunin, 2010)

Keeping cell culture under the stirile conditions and achieving the highest possible number of cells are two important principles in production using cell culture, affected by many factors. (Kretzmer, 2002; Chu, 2001)

An overall culture medium is a combination of essential and non-essential amino acids, vitamins, mineral salts, glucose and serum.(Arora , 2015; Diego et al, 2010)

Progress in mammalian cell culture process is possible in two ways: First by improving the production process and second by improving the cell culture medium. (McKeehan, 1982)

In this study, the effects of proteins, vitamins, essential amino acids and glucose as supplements in the culture medium under the suspension culture in bioreactors were investigated. The effect of tested supplements was determined on the basis of the final number of cells.

Materials and Method:

BHK cells source:

BHK cells were obtained from foot and mouth disease (FMDV) department, Razi vaccine and serum research institute, Karaj, Iran.

Serum source:

Serum used in this research was obtained from foot and mouth disease (FMDV) department, Razi Vaccine and Serum research Institute, Karaj, Iran.

Preparation of the culture medium:

Cells were cultivated in the culture medium purchased from Razi Vaccine and Serum research Institute, FMD department and prepared on the basis of MEM medium. Of course, a set of proteins including lactalbumin, New Zealand casein, and peptone were added into this medium. The 5% calf serum treated with PEG was added to the culture medium.

BHK cells were cultivated in 1-liter glass bottle (bioreactors). Throughout the culture process, temperature was 36.5℃ , the amount of CO_2 was equal to 5% and stirring speed was between 130-120 rpm. For each cell culture, the initial seed was 350000 per ml. As well as, the culture medium used was 500 ml. Monolayer cells were used for suspension cell culture. First, the flasks containing the baby hamster kidney cells are placed in an incubator for 48 hours at a temperature of 36.5 ° C and 5%

CO2. After 48 hours flasks containing the cells are observed under a microscope and if the cells are in good condition and cover the surface of flasks, they are used for the next step which is the cell culture under suspension conditions. Preparation is such that liquid on their surface is first evacuated and about 2 mm Trypsin-versene is added to them and using trypsin the cells are washed for 1-2 minutes and then the flask is put into the incubator for 15 minutes. Trypsin-versene separates the cells from the flask surface. After this time, cells exit from the single layer state and medium is added to the flask in order for cells to be collected and transferred into the dishes prepared for suspension culture (bioreactors). Then the cells under the suspension and mentioned conditions passed three passages to enter the testing phase.

Cell counting:

For counting the suspension cells at the beginning and end of culture, a sample was taken and then 0.1 mL of the taken sample was mixed with 0.1 mL of 0.4% W / V% Trypan and injected on Neubauer slide such that it covered the entire the surface of the slide. Counting was done in accordance with the manufacturer instructions. For obtaining the number of cells per milliliter, the counted number of cells was multiplied at 10,000.

Results:

In order to perform experiments to study the effect of food supplements on the BHK cells, cells were cultivated in media such as MEM formulated without glucose, MEM formulated without protein, MEM formulated without essential amino acids, and MEM formulated without vitamin and with initial seed of $3.5*10^5$ and 5% calf serum. The highest number of cells after 48 hours of culture was in the medium MEM enriched with protein. The final number of cells in this medium was $1.35*10^5$. Then, the highest number of cells was in the medium without vitamin with the final number of cells $1.2*10^5$. This medium had the highest number of cells at the end of the cell culture process. After that, the final number of cells in protein- and glucose-free media was, $0.2*10^6$ and $0.15*10^6$, respectively.

Discussion and conclusion:

Most researches performed on BHK cells focus on the chemical parameters and nutrients such as glucose and glutamine. (Cruz et al, 2000a , 2000b)

The culture medium contains inorganic salts, carbohydrates, amino acids, vitamins, proteins, serum and other additives such as glutamine, and non-essential amino acids. (Arora , 2015;Anand et al, 2009)

The results of the conducted experiments on BHK cells in different media, each lacking a set of main components of the culture medium, are very interesting.

The greatest number of cells at the end of the culture process is in the perfect culture medium (MEM) containing protein, glutamine and all nutrients required for cells and the results of this experiment are as expected and according to the initial hypothesis.

BHK cells had the lowest growth in the medium without glucose. The reason is that the culture medium didn't contain carbohydrates and in particular glucose as the main sources of carbon and energy.

BHK cells did not grow in the culture medium without protein. However, their situation was better than the medium without glucose. But the results show that the final number of cells has decreased compared to the initial cell number.

Proteins act as carriers of molecules with low weight and cause adhesion. (Taub ,1990)

BHK cells grew and proliferated in the culture medium without essential amino acids, but this amount was not significant. Of course, it seems that absence of glutamine has the greatest impact among all essential amino acids on progressing the cell culture process. Amino acids are the basic building blocks of proteins. Essential amino acids must be added to the culture medium because the cells themselves cannot synthesize them.(Lanc et al ,1987)

The growth and proliferation of BHK cells in the culture medium without vitamin was very similar to their growth in the perfect culture medium. The results of this study show that the amount of vitamins synthesized by cells themselves and vitamins present in serum can greatly alleviate cells need for

survival and proliferation. Vitamins are precursors for co-factors. Many vitamins are essential for cell growth and proliferation. Despite that some believe that vitamins cannot be synthesized in sufficient quantities by the cells, but the experiments performed in this study showed that cells can grow and proliferate in the culture medium without vitamins.(Taub , 1990;Paranjape, 2004)

The results show that cells are not cultivated well without glucose and protein and this causes cell death. Moreover, presence of essential amino acids and vitamins is necessary to achieve optimal growth and proliferation of BHK cells. The highest number of cells in the experiments performed in this study was obtained when all components of the culture medium were available. In this study it was shown that none of the components of the culture medium, especially glucose and protein, can be deleted and replaced with 5% calf serum.

In future researches, it is suggested that the effects of this substance on the growth and proliferation of other cells capable of growing as suspension are studied. Moreover, some researches are suggested on other chemical factors forming the medium. In addition, physical factors affecting the growth and proliferation of baby hamster kidney cells or other cells can also be examined in future.

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