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Improvement of mammalian cells performance by addition of glucose for the expression of erythropoietin with 2 additional link in CHO-DG44 cells

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Abstract. The use of recombinant proteins for therapeutic use, currently, have become a standard procedure to fight many diseases and this reality have a great impact on the biotechnology industry. To produce this drug, established mammalian cell lines, especially CHO cells, have become a standard system for the production of such proteins. The main goal in recombinant protein production using Chinese hamster ovary (CHO) cells is to achieve both high specific productivity and high cell density. These cells demonstrate a high consumption of glucose and this condition causes rapid depletion this nutrient in the medium. The loss of nutrient such as glucose can result in the limitation of cell growth and viability and finally the loss of quality of the protein. In relation to the previous knowledge, in this work the effect of glucose on cell density and viability of CHO-DG44 cell capable of producing erythropoietin (EPO) was studied. With this in mind 3 mg/liter of glucose was added to the medium at day 4 and 7. The cells were cultured for total 10 days in orbital shaker at the speed of 130 rpm at 37°C and 5% CO₂ condition and samples were taken every 2 days. The results showed significantly that the addition of glucose to the medium with the time and concentration mentioned above increased the density and viability of the cell.

1. Introduction

Cultured mammalian cells has been widely used and become the main workhorses in the production of recombinant DNA derived protein therapeutics and viral vaccines due to their capacity for correct protein folding, assembly, and post translational modification [1] [2]. The performance of these cell culture processes, in terms of both productivity and product quality attributes, is significantly influenced by cellular metabolism.

In the last few decades, the productivity of CHO cell in protein production have increased from 100 mg/L in the initial processes commissioned to about 5-10 g/L in the current processes [4] [5][6]. Some of the progress was partly due to advances in media optimization, screening and cell line development and by process control improvements. However, the progress that have been achieved in mammalian cell is still 10-100 fold lower than what can be achieved using microbial host systems [5]. Despite the enormous potential of established cell lines, these production systems have inherent technological limitations: low specific growth rate, low cell concentration (Claudio et al., 2013). In addition, the production of therapeutic r-proteins in CHO cells is expensive and presents insufficient production yields for certain proteins [2]. Additional bottlenecks include accumulation of waste product, inconsistent glycosylation and low cell densities [7] [4] [8]. Therefore the main target in cell line development development for mammalian cell cultures is the enhancement of product yield to achieve economical and competitive product titers.

It was known that in a majority of the mammalian cell lines, glucose serves as the primary carbon source required for growth and maintenance. Several important metabolic pathways such as glycolysis,



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pentose pathway and TCA cycle are all involved in the catabolism of glucose. Key intermediates for the synthesis of cellular components such as lipids, sugar chains and nucleic acids are generated during glycolysis [9]. Glucose is the major carbon source for cellular biosynthesis and energy generation [1]. Thus, to some degree glucose play roles in cellular productivity. In this study we attempted to develop cell line capable of producing erythropoietin and found out that the addition of glucose to the culture make a big difference in cell density and viability.

2. Material and Method

2.1. Cell Culture and Reagents

The CHO DG44 cell line was obtained from Life Technology (NY, USA). Since originally the cell line was DHFR- negative, this cell was cultured in CD DG44 Medium containing hypoxanthine and thymidine. Following transfection with the gene of interest which cloned in pOptiVEC-TOPO containing DHFR gene, the cell was grown in CD OptiCHO medium (Life technologies, NY, USA). For this study the cell was cultured in 125-ml polycarbonate disposable sterile Erlenmeyer flasks were ordered from Corning. L-glutamine, glucose, Pluronic F68 and Freestyle Max (Life technologies, NY, USA). Suspension cultures were shaken at 130 rpm in CO₂ incubator which maintained at 37°C and humidified 5% CO₂ atmosphere. Non-ionic surfactant, Pluronic F-68(Life technologies, NY, USA) which causes multi-functional effects that enhance cell yield in agitated cultures and control shear forces was applied in the culture.

2.2. Transfection cells

Initially, before transformation was grown in CD DG44 medium supplemented with hypoxantne and thymidine (Life Technology). Trasfection was performed using Freestyle Max reagent as described by manufacturer (Life Technology). Following transfection, for MTX screening work, the cells were grown in CD OptiCHO in the presence of MTX with 2000 nm concentration. The cell was cultured in an incubator in orbital shaker at 130 rpm with the condition of 5% CO₂ and 37°C temperature. Samples were taken for analysis every 2 days for total 10 days period.

3. Result and Discussion

Mammalian cells are the preferred production organisms which can produce recombinant proteins in their native, fully glycosylated form. However, the overall cellular productivity is limited, when compared to yeasts and prokaryotes. Consequently, cell line development work is focused on the enhancement of product yield to achieve economical and competitive product titers.

One of the steps in cell line development is to study the growth of the cell which includes its density and viability. Following screening of the cell candidate that was expected to have the capacity to produce the protein of our interest at good yield, assessing productivity was performed by culturing the cell in 30 ml media in 125 ml flask. To observe the growth profile of the cell, samples were taken every 2 days for total 10 days period for cell density and viability analyses. To study whether the addition of glucose at specific time of cell growth has an impact on the density and viability of the cell, glucose was added in assessing productivity step. As much as 3 mg/liter of glucose concentration glucose was added to the culture at day 4 and 7. Since during this cell line development study involved the use of MTX for gene amplification, besides the addition of glucose and in order to maintain the high expression protein of interest, 2000 nM of MTX was also added to the medium. As seen in Figure 1, the cell density of both cells with and without glucose increased sharply from day 2 and peaked at day 6 at about $7-8 \times 10^6$ cells/ml medium. After day 6, cell density without addition of glucose drop sharply even almost reached zero. Meanwhile, the cell density in the presence of glucose decreased only slightly which was about 6×10^6 cell/ml medium. Anaysis of cell viability showed similar result where the presence of glucose in the medium gave positive effect of viability Figure 2. In the presence of glucose, cell viability, from day 2 to day 10, was about 80 – 100%. The opposite

result was obtained when the cell was cultured without glucose. After day 6, the cell viability decreased significantly to almost approximately 5%.

To verify whether the positive effect of glucose was consistent and also observed in another clone (clone 193 4.3). In addition, whether the presence of MTX has the effect in the result of the previous experiment Figure 1, the second experiment Figure 3 was performed in the absence of MTX. As previously shown in experiment 1 in Figure 1 and 2 the presence of glucose in experiment 2 in Figure 3 also increased the cell densities significantly from day 2 to day 10 both in clone 193 2.3 and clone 193 4.3. The cell densities reached the peak at day 10 with the densities about 14×10^6 cells/ml medium. As seen in experiment 1, in this second experiment in Figure 3 the cell viability of clone 193 2.3 and clone 193 4.3 was also improved by the presence of glucose. From day 2 to day 10, the cell viability of both clone remained around 90 – 100%.

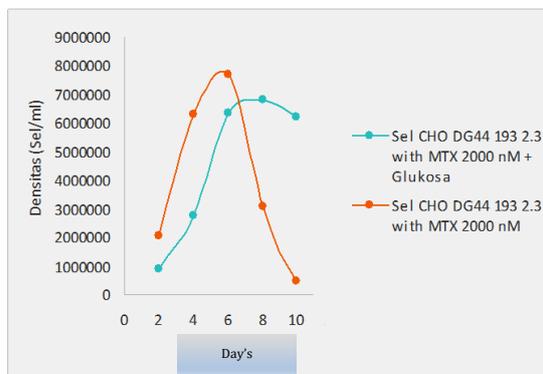


Figure 1. Cell density of cell clone number 193 2.3 in the presence and absence of glucose.

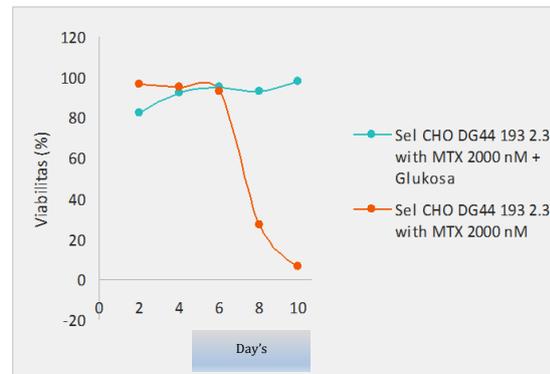


Figure 2. Cell viability of cell clone number 193 2.3 in the presence and absence of glucose.

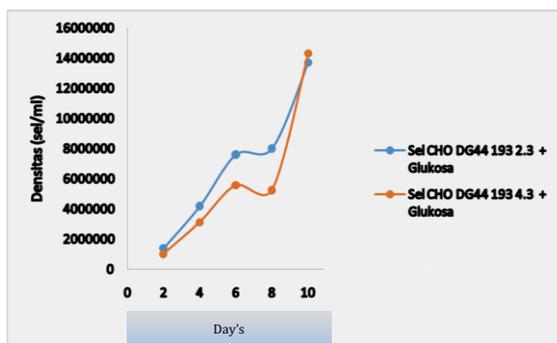


Figure 3. Cell density of cell clone number 193 2.3 and 193 4.3 in the presence and absence of glucose.

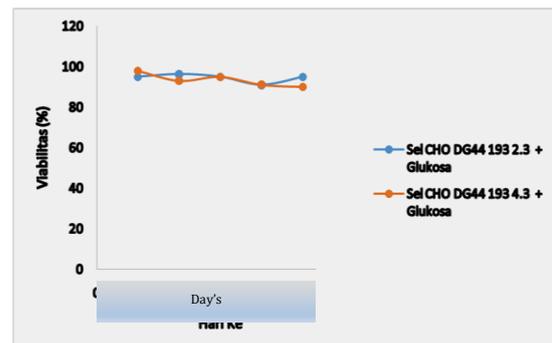


Figure 4. Cell viability of cell clone number 193 2.3 and 193 4.3 in the presence and absence of glucose.

Development of cell line has been one of ongoing priorities in the life sciences, especially in the production of biopharmaceuticals. The quest for better productivity and product quality in protein based therapeutic drug is constantly seeking to improve the performance of production cells. Given that both research and industrial concerns are demanding better cells, it should come as no surprise that that cell-line development has become one of the backbones in biopharmaceutical industry.

Current optimization methods in cell line development mainly optimization of media, feed, and processes plus improved expression vector and selection workflows [10].

As part of cell development work, in this study, the effect of glucose on the growth profile of CHO-DG44 that is capable of producing erythropoietin was analyzed. With regard to the previous knowledge [11] [9] and expectation that the growth profile of CHO-DH44 understudy can be improved, glucose was added to the medium. As seen in Figure 1, 2, 3 and 4, it was revealed that the addition of glucose in the medium indeed clearly increased cell density and viability. Previous study has stated that for most mammalian cell lines, glucose serves as the primary carbon source required for growth and maintenance. Glucose metabolism is also central to several key intermediates for the synthesis of cellular components such as lipids, sugar chains and nucleic acids are generated during glycolysis [11] [9]. The finding of our study turned out to be supportive to this previous study where glucose increased the cell density starts from day 2 to day 10. The cell viability was also in good condition where the viability remained at about 90 – 100%.

Besides the fact that glucose and also glutamine serve as the primary carbon source required for growth and maintenance. However, these source of carbons are not without consequences. Incomplete oxidation of this carbon source can lead to high generation of undesirable metabolites (mainly lactate and ammonium), limiting the life-span of the culture and the quality of the protein of interest. These limits result in low productivity for the production process [13] [17] [14] [15] [16]. However, since in our study the growth profile of our cell was good we assume that the addition of glucose can still be tolerated and has positive impact to stimulate the increase density and viability of the cell.

4. Conclusion

From this research it can be concluded that glucose can increase cell density and reach its peak around the 6-10 days with a density of about 8-14 x 10⁶ cells / ml. The presence of MTX does not affect the increase in density. Besides density, the presence of glucose also increase cell viability where until the tenth day cell viability remains high at around 90-100%.

5. Acknowledgement

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