EVALUATION OF MULTI-WELL BASED SYSTEM FOR ANTIBODY PRODUCTION USING CHINESE HAMSTER OVARY CELL

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ABSTRACT

The increased use of antibodies and antibody fragments for human therapy is driving the search for rational approaches to accelerate bioprocess development. However, screening of the operating conditions of process parameters in large scale bioreactors for biopharmaceuticals industry is not cost effective. Using microwell plate as the 'microreactor' for screening process would be a solution to this. Hence, this study was carried out to evaluate multi-well based system by determining the growth rate and antibody expression of the Chinese hamster ovary (CHO) cells. In this study, two types of CHO cells have been seeded with different concentrations which were 7×10^5 cell/ml and 1×10^6 cell/ml. Wild type CHO cell and CHO DG44 were used in this study. The wild type CHO cell was used as a reference cell as it is a stable cell. The cell number was calculated by using trypan blue exclusion method while glucose concentration was determined by 3, 5-Dinitrosalicyclic acid (DNS) assay. On the other hand, antibody production was determined by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. For the cell growth rate and doubling time, CHO DG44 (1×10^6 cell/ml) has recorded the highest growth rate and shortest doubling time with (0.3301 ± 0.1536)/h and (2.61 ±1.66)h. Besides that the highest glucose concentration recorded by wild type CHO cell (1×10^6 cell/ml) and CHO DG44 (7×10^5 cell/ml) was on day 0 of incubation with (1.817±0.1187)mg/ml and (1.819±0.9331)mg/ml. Meanwhile, CHO DG44 (1×10^6 cell/ml) has recorded the highest glucose concentration on day 1 with (1.611±0.1273)mg/ml. Lastly, the antibody expression by CHO DG44 was verified. Both batches of CHO DG44 have successfully expressed human IgG on day 2 until day 10. In conclusion, the CHO cell is possible to be cultured in the multi-well plate.

ABSTRAK

Peningkatan penggunaan antibodi untuk terapi manusia mendorong pencarian untuk pendekatan rasional untuk mempercepatkan pembangunan bioproses. Walaubagaimanapun. penyaringan awal bagi kondisi parameter proses untuk bioreaktor berskala besar bagi industri biofarmaseutikal memakan belanja yang besar. Penggunaan plat multi-well sebagai 'mikroreaktor' mungkin dapat menyelesaikan masalah ini. Oleh itu. kajian ini dijalankan untuk menilai sistem berasaskan multi-well melalui penentuan kadar pertumbuhan dan antibody ekspresi yang dihasilkan oleh sel ovari hamster China (CHO). Di dalam kajian ini. dua jenis sel CHO telah dikultur dengan kepekatan yang berbeza iaitu 7×10^5 sel/ml dan 1×10^6 sel/ml. Sel CHO jenis bukan mutan dan CHO DG44 telah digunakan di dalam kajian ini dimana sel CHO jenis bukan mutan digunakan sebagai rujukan kerana ia merupakan sel yang stabil. Bilangan sel telah dikira menggunakan kaedah penyisihan trypan biru manakala kepekatan glukosa ditentukan dengan ujian 3. 5-Dinitrosalicyclic (DNS). Seterusnya. penghasilan antibodi ditentukan dengan menggunakan elektroforesis gel polyacrylamide natrium dodecyl sulfate (SDS-PAGE) dan blot Barat. Bagi kadar pertumbuhan dan masa penggandaan sel, CHO DG44 (1×10^6 sel/ml) telah merekodkan kadar pertumbuhan tertinggi dan masa penggandaan tersingkat dengan (0.3301 ± 0.1536)/jam dan (2.61 ±1.66)jam. Selain itu, kepekatan glukosa yang tertinggi yang telah direkodkan oleh CHO sel bukan jenis mutan (1×10^6 sel/ml) dan CHO DG44 (7×10^5 sel/ml) adalah pada hari 0 dengan nilai (1.817±0.1187)mg/ml dan (1.819±0.9331)mg/ml. Sementara itu, CHO DG44 (1×10^6 sel/ml) telah merekodkan kepekatan glukosa tertinggi pada hari pertama inkubasi dengan nilai (1.611±0.1273)mg/ml. Akhir sekali, antibodi eskpresi oleh CHO DG44 telah disahkan. Kedua-dua CHO DG44 telah berjaya mengekspresi antibodi bermula pada hari kedua hingga hari kesepuluh. Secara keseluruhan, CHO sel berkemungkinan untuk dikultur di plat multi-well.

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LIST OF ABBREVIATION

BHK	Baby Hamster Kidney
CHO	Chinese Hamster Ovary
pH	Potential of Hydrogen
CO_2	Carbon Dioxide
DHFR	Dihydrofolate Reductase
DMSO	Dimethyl Sulfoxide
EU	European Union
GS	Glutamine Synthetase
HEK-293	Human Embryonic Kidney
mAbs	Monoclonal Antibodies
MSX	Methionine Sulphoximine
MTX	Methotrexate
NS0	Mouse Myeloma
PER-C6	Human Retina-derived
Rpm	Rotational per Minutes
US	United States
v/v	Volume per Unit Volume
w/v	Weight per Unit Volume

LIST OF SYMBOL

°C	Temperature in Degree Centigrade
g	gram
gL^{-1}	Gram per Litre
h	Hour
mg	Miligram
mL	Mililitre
nm	nanometer
μ	Specific growth rate

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Emerging technologies on therapeutic protein production has set a new level of development in medical research. The production of therapeutic protein focuses on finding the cure for diseases such as cancer and autoimmune disease. In biopharmaceutical industry, various new monoclonal antibodies (MAbs) and recombinant proteins are estimated to enter preclinical and clinical development every year (Durocher & Butler, 2009).

Birch and Okunle (2005) stated that various sources such as bacteria and fungi have been used in traditional production of recombinant protein. *Escherichia coli* is one of the sources used to produce human insulin and growth hormone (Agrawal & Bal, 2012). The usage of bacteria as the producer of recombinant antibody production is said to be the most economics in expenditure. This is due to its robust characteristic, short term production cycle, low cost in strain establishment and higher productivity compared to the mammalian cell. However, bacteria do not able to perform posttranslational protein modification unlike mammalian cell line such as Chinese hamster ovary (CHO) cell and baby hamster kidney (BHK) cell (Dingermann, 2008). Thus, mammalian cells are the suitable alternative for the

monoclonal antibody production. The protein expressed by the mammalian cells proving that the glycosylation has taken place where carbohydrates (glycan) has been added to the synthesized protein (Butler, 2005).

The mammalian cell expressed antibody is a better option compared to the bacteria. In the biopharmaceutical industry, the ability of the cell to produce in high titer is very important. Many researches have been done on the optimization of the bioprocessing in mammalian cell culture in order to achieve high antibody titer at lower cost. This study focuses on evaluating the multi-well based system which is an alternative to the flask cultures in the upstream processing. The multi-well plate is one of the tools that applies multi-well based system and will be evaluated in the study. The evaluation was determined by the growth rate of cell, cell metabolism and antibody expression of CHO cell cultured in 24 multi-well plate.

1.2 Problem Statement

In the biopharmaceutical industry, it is crucial for the company to be able to produce large amount of therapeutic protein in order to fulfill its high demand. Thus, many companies build large scale manufacturing plant that consists of large cell culture bioreactors as the response to the high demand.

However, large scale production is not cost-effective as the focus has shifted from controlling product quality and process consistency towards producing larger capacity of therapeutic protein (Li *et al.*, 2010). Thus, higher cost is needed to provide more material in order to pursue higher titer. In the end of the day, the quality of product produced might be not up to par to the standardized quality of the therapeutic protein as the cell screening is hard to do in large scale. Therefore, cell line generation and selection followed by process and media optimization need to be done in small scale before going to the larger scale which requires more funding.

Multi-well based system is one of the useful scale down of larger reactors (Girard *et al.*, 2001). This system is making the job in generating and screening the cell line easier due to its small scale. Multi-well based system also able to produce high throughput in a miniature scale. Thus, it is cost-effective as it only needed small amount of materials. Thus, this study will evaluate the multi-well based system according to the growth rate of CHO cell and antibody expression.

1.3 Objective of Study

The aims of this study are:

1. To determine the growth rate of CHO cell in 24 multi-well plate.
2. To determine the glucose content of two types of CHO cell.
3. To verify the antibody expression of CHO DG44 cell.

1.4 Scope of Study

In this study, the multi-well based system was evaluated. Two types of CHO cells which are the wild type CHO cell and CHO DG44 was seeded into the 24-well microplate with different seeding density. The growth rate of the CHO cell and glucose content was analyzed. Besides that, the antibody expressed by CHO DG44 was determined by analyzing its presence in the media proving that it is successfully expressed and secreted in the media. Lastly, multi-well based system was concluded whether it is suitable for the cell growth and works as 'microreactor'.

1.5 Significance of Study

Through this research, one of the multi-well based systems which are multi-well plate was evaluated. The outcome of this study would provide foundation for the future bioreactor and animal tissue culture research on the production of monoclonal antibody. Besides that, multi-well plate can be applied as an alternative to the flask culture. In addition, multi-well plate can be used as 'microreactor' and works as one of the high throughput technology that be able to produce high antibody titer in small scale. In this system, only small amount of raw material needed thus reducing the cost and time consumption needed. Thus, helps to improve the upstream processing for the monoclonal antibody production in biopharmaceutical industry besides promoting safer, low cost and time efficient system for high production of monoclonal antibody. Lastly, this study helps to fulfill market demand on therapeutic protein for wide population of patient.

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