COMPARISON BETWEEN BATCH AND FED-BATCH CULTURES OF CHINESE HAMSTER OVARY-DG44 CELL USING MULTI-WELL PLATE

ABDULKARIM ABDULKADER YOUSFI

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DEDICATION

This dissertation is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

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ABSTRACT

The biopharmaceutical proteins, especially monoclonal antibodies have become of great significance in the drug manufacturing and development industry during the last two decades. This significance can be attributed to their effectiveness, selectivity and the wide range of diseases and health problems cured by them. Consequently, the demand for these proteins has increased greatly. To meet this increasing demand for these proteins, optimization and development of the production process is required. This optimization is done through intensive screening of different culture options which requires an effective screening tool. The traditional screening methods such as shake flask system are slow and cost-ineffective. Multi-well plate is an option to accelerate the process of development with a rational cost. The goal of this study is to evaluate the usage of the multi-well plate as a screening tool in the bioprocess development. To achieve this goal, 24-multi-well plate was used to compare between batch and fed-batch cultures of CHO-DG44 cells. Cell density, cell viability, cell morphology and confluency, glucose concentration, and total protein concentration were determined in both batch and fed-batch cultures. Cell counting was done using trypan blue excluding method with a haemocytometer, while the glucose concentration was determined using 3, 5-dinitrosalicylic acid (DNS) assay. On the other hand, total protein concentration was determined using Lowry assay. It has been observed that feeding nutrients during the fed-batch culture enhanced the cell density and viability compared to the batch culture. On day 7, the cell density and viability of the cells in batch culture were (3.667±1.04) $\times 10^4$ cell/ml and (45.453±4.54) % respectively, while in fed-batch culture the cell density and viability were (5.167±0.76) $\times 10^4$ cell/ml and (70.416±1.11) % respectively. Moreover, the protein production of the cells was prolonged during the fed-batch culture. This study revealed that the multi-well plate system can be used as a small-scale screening tool for optimization of CHO-cell culture.

ABSTRAK

Protein biofarmaseutikal, terutamanya antibodi monoklonal telah menjadi sangat penting dalam industri pembuatan dan pembangunan dadah sejak dua dekad kebelakangan ini. Kepentingan ini boleh dikaitkan dengan keberkesanan, pemilihan dan keupavaan dalam mengubati pelbagai penyakit dan masalah kesihatan. Oleh yang demikian, permintaan untuk protein-protein ini telah bertambah tinggt. Bagi memenuhi permintaan yang semakin meningkat terhadap protein ini, pengoptimuman dan pembangunan proses pengeluaran diperlukan. Pengoptimuman ini dilakukan melalui pemeriksaan intensif pilihan kultur yang berbeza dan memerlukan alat pemeriksaan yang berkesan. Kaedah pemeriksaan tradisional seperti sistem kelalang goncang adalah lambat dan kos yang tidak berkesan. Piring berbilang telaga adalah pilihan untuk mempercepatkan proses pembangunan dengan kos yang rasional. Matlamat kajian ini adalah untuk menilai penggunaan piring berbilang telaga sebagai alat penyaringan dalam pembangunan bioproses. Untuk mencapai matlamat ini, 24-piring berbilong telaga digunakan untuk membandingkan antara kultur ‘batch’ dan ‘fed-batch’ sel CHO-DG44. Ketumpatan sel, daya tahan sel, morfologi dan konfluens sel, kepekatan glukosa, dan jumlah kepekatan protein ditentukan dalam kedua-dua ‘batch’ dan ‘fed batch’ kultur. Penghitungan sel dilakukan menggunakan kaedah trypan biru tidak termasuk dengan hemasitometer, manakala kepekatan glukosa ditentukan dengan menggunakan asid 3, 5-dinitrosalicylic (DNS). Sebaliknya, jumlah kepekatan protein ditentukan menggunakan ujian Lowry. Dari pemerhatian, memberi nutrient semasa kultur fed-batch meningkatkan ketumpatan dan daya tahan sel berbanding dengan kultur ‘batch’. Pada hari ke-7, ketumpatan sel dan daya maju sel-sel dalam kultur ‘fed-batch’ adalah (3.667±1.04) $\times 10^4$ sel/ml dan (45.453±4.54) %, manakala dalam kultur fed-batch, ketumpatan dan daya tahan sel adalah (5.167±0.76) $\times 10^4$ sel/ml dan (70.416±1.11) % masing-masing. Tambahan pula, pengeluaran protein sel-sel telah berlanjutan semasa kultur fed-batch. Kajian ini mendedahkan bahawa sistem piring berbilang telaga boleh digunakan sebagai alat pemeriksaan berskala kecil untuk mengoptimumkan kultur sel CHO.

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LIST OF ABBREVIATIONS

ADAs	-	Anti-Drug Antibodies
ADCC	-	Antibody-Dependent Cellular Cytotoxicity
ATP	-	Adenosine Triphosphate
BHK-21	-	Baby Hamster Kidney
BSA	-	Bovine Serum Albumin
CDC	-	Complement-Dependent Cytotoxicity
CDR	-	Complementary-Determining Region
CHO	-	Chinese Hamster Ovary
CO2	-	Carbon Dioxide
DHFR	-	Dihydrofolate Reductase
DMSO	-	Dimethyl sulfoxide
DNS	-	3, 5-dinitrosalicylic acid
ELISA	-	Enzyme-Linked Immunosorbent Assay
EPO	-	Erythropoietin
Fab	-	Fragment Antigen Binding Domain
FBS	-	Fetal bovine serum
Fc	-	Fragment Crystallizable
FcRn	-	Human Neonatal Fc Receptor
FDA	-	United States Food and Drug Administration
GHT	-	Glycine, Hypoxanthine, and Thymidine
GOI	-	Gene of Interest
GS	-	Glutamine Synthetase
HCPs	-	Host Cell Proteins
HEK 293	-	Human Embryonic Kidney 293
HPLC	-	High-Performance Liquid Chromatography
IgG	-	Immunoglobulin G
LDH	-	Lactate Dehydrogenase Enzyme
MSX	-	Methionine Sulfoximine
MTX	-	Methotrexate
NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen

PBS	-	Phosphate-Buffered Saline
PER-C6	-	Human-Retina-Derived
pH	-	Potential of Hydrogen
PPP	-	Pentose Phosphate Pathways
RPMI	-	Roswell Park Memorial Institute
scFv	-	Single-Chain Variable Fragment
TCA	-	Tricarboxylic Acid
tPA	-	Tissue Plasminogen Activator
UPR	-	Unfolded Protein Response
(v/v)	-	Volume per Unit Volume

LIST OF SYMBOLS

°C	-	Temperature in Degree Celsius
h	-	Hour
g	-	Gram
mg	-	Milligram
ml	-	Millilitre
nm	-	Nanometre
L	-	Litre
µl	-	Microlitre
µ	-	Specific growth rate

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The biopharmaceutical therapeutic proteins have become an important part in the world of medicine. This importance can be attributed to the effectiveness of these proteins, their selectivity and the wide range of diseases and health problems that can be treated by them (Chaturvedi *et al.*, 2014; Ho *et al.*, 2013; Li and Zhu, 2010). Consequently, the market of these proteins has been rapidly developing; thus, the revenue of these products is increasing by 10 – 20% annually worldwide (López-Meza *et al.*, 2016; Zhu, 2012). Among the various recombinant therapeutic proteins, monoclonal antibodies are the most rapidly growing group (Ecker *et al.*, 2015; Elgundi *et al.*, 2017; Liu, 2014; López-Meza *et al.*, 2016).

Traditionally, various expression systems have been used for the production of therapeutic proteins such as bacteria and fungi (Birch and Onakunle, 2005). *Escherichia coli* (*E. coli)* is the most well-known bacteria strain and it has been used to produce several important recombinant therapeutic proteins such as human insulin and growth hormone (Agrawal and Bal, 2012). Despite the advantages of the bacterial expression system such as low cost, short term production cycle, and the high productivity, bacteria are not able to perform post-translational modifications like glycosylation. Post-translational modifications affect the kinetic and the biological activity of the therapeutic proteins in human (Mahmoud, 2007; Zhu, 2012). On the other hand, the post-translational modifications of the yeast (the most well-known fungi) expression system are different from human post-translational modifications which lead to decrease the efficiency of the protein, lower the half-life and cause an immunogenic reaction in human (Lam *et al.*, 2007). Conversely, mammalian cells like Chinese hamster ovary (CHO) cells perform post-translational modification similar to human post-translational modification. Mammalian cells have the capacity for

appropriate folding and assembly and they are able to produce complex therapeutic proteins with higher efficacy and half-life (Chadd and Chamow, 2001; Warnock and Al-Rubeai, 2006; Wurm, 2004; Zhang, 2010). As a result, the mammalian expression system has become the most preferred system for the production of recombinant therapeutic proteins and more than 50% of the approved recombinant proteins on the market are produced using mammalian expression system (Matasci *et al.*, 2008; Zhu, 2012).

Chinese hamster ovary (CHO) cell is known as the model cell for the mammalian cells, similar to *E. coli* in bacterial cells (Jayapal *et al.*, 2007). CHO cells are the most well-known and most preferred cell line for monoclonal antibodies production due to their high adaptability, capacity for production of high concentration of recombinant proteins, good growth profile, and stability (Browne and Al-Rubeai, 2009; Kelley, 2007). In order to increase the viable cell density and recombinant protein production of CHO cell culture and other mammalian culture, most of biopharmaceutical companies use fed-batch or perfusion system in place of the traditional batch culture (Birch and Racher, 2006; Rouiller *et al.*, 2013; Toussaint *et al.*, 2016). In the fed-batch system, nutrients are supplied to the culture during the incubation time to prolong the culture duration and increase the recombinant protein production (Altamirano *et al.*, 2004; Birch and Racher, 2006; Chu and Robinson, 2001; Matasci *et al.*, 2008).

Various strategies have been used to meet the demand for the recombinant proteins by enhancing the production of these agents through improving cell performance and viability. The optimization of media composition and strategy of feeding in fed-batch culture are found to be the most efficient strategies to enhance the process of the cell culture (Chaturvedi *et al.*, 2014; Rouiller *et al.*, 2013). In order to perform these developments, intensive screening of feeding strategies, components and many elements of the culture is needed. The application of multi-well plates as a high-throughput screening system is an option to study the effects of a large range of the culture conditions and different concentration of a large number of components. These studies cannot be efficiently done by the traditional methods, such as shake flask or spin tube cultures because these traditional methods are slower and require more

efforts and materials (Chaturvedi *et al.*, 2014; Rouiller *et al.*, 2013). In this study, the performance of the CHO-DG44 cells in batch and fed-batch cultures were evaluated using the 24-multi-well plate to assess the performance of the multi-well plate system as a screening tool for bioprocesses development.

1.2 Problem Statement

The bioprocesses developments such as cell line development and selection, optimization of the media composition and feeding strategy are the key factors to improve the performance of the culture and thus increase the production of the recombinant proteins. These developments require intensive screening of different options and need to be done in small scale bioreactors before initiating the large-scale bioreactors. However, the traditional screening methods for bioprocesses development such as shake flasks and spin tube are slow and require a relatively high amount of materials (Chaturvedi *et al.*, 2014; Rouiller *et al.*, 2013).

Therefore, a faster and more effective screening method is needed to meet the demand of the market at an appropriate time, affordable prices and with excellent quality. The use of multi-well plates as high-throughput screening system is an option for bioprocesses development with more time and cost-effectiveness and higher level of throughput (Betts and Baganz, 2006; Chaturvedi *et al.*, 2014; Duetz *et al.*, 2001; Lye *et al.*, 2003; Rouiller *et al.*, 2013). In this study and in order to evaluate the multi-well plate as an alternative screening method for bioprocess development, CHO-DG44 cell was cultivated in 24-multi-well plate using batch and fed-batch systems. Cell viability, cell density, culture morphology, cell metabolism and protein production were monitored and compared between batch and fed-batch system.

1.3 Objectives of the Study

The main aim of this study was to evaluate the use of multi-well plate system as a screening tool in process development of animal cell culture.

The objectives of the research are:

(a) To study the growth profiles of CHO-DG44 cells during batch and fed-batch cultures in 24-multi-well plates.

(b) To study the glucose concentration of the CHO-DG44 cells during batch and fed-batch cultures in 24-multi-well plates.

(c) To determine the total protein concentration during the batch and fed-batch cultures of CHO-DG44 cells in 24-multi-well plates.

1.4 Scope of the Study

The 24-multi-well plates system was evaluated throughout this study. CHO-DG44 cells were cultivated in 24-multi-well plates for the batch and fed-batch cultures system. The growth profile and glucose concentration were determined and studied in both systems. Furthermore, the protein production of CHO-DG44 cells in batch and fed-batch cultures were compared and the total protein concentration was determined.

1.5 Significance of the Study

The substitution of the traditional screening methods by multi-well plates can reduce time and cost of the bioprocess development and increase the level of throughput and parallelism (Betts and Baganz, 2006; Chaturvedi *et al.*, 2014; Duetz *et al.*, 2001; Lye *et al.*, 2003; Rouiller *et al.*, 2013). The use of multi-well plate as a high-throughput screening system in bioprocess development could accelerate the

development process and make the pharmaceutical companies able to meet the demand of the market at the desired time and at a more affordable price. Silk *et al.* (2010) mentioned that the use of multi-well plate as a high-throughput screening system has resulted in an approximately 50-fold reduction in medium requirements compared to the traditional culture systems used now in early-stage cell culture process improvement such as shake flask system. The reduction in costs and time also allows more investigation and development to be carried by the researchers in order to increase the accuracy in choosing the most proper culture option.

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