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An Overview on Mammalian Cell Protein Expression: Biopharmaceutical Production

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ABSTRACT: ***Along with its ability to perform post-translational modifications and assemble human protein-like molecular structures, mammalian cell expression has become the most popular recombinant protein production method for therapeutic purposes. While expression and manufacturing have been fully developed, and Chinese hamster ovary cells would be used in the majority of devices on the market and in clinical development, significant progress has been reported in the last several years in developing and engineering new cell lines, as well as introducing novel genetic mechanisms in expression, gene silencing, and gene targeting. More importance has been given to product quality, particularly glycol profile, as a result of the newest analytical techniques development, which leads to a better understanding of the effect of cells were grown during production. Furthermore, the transient gene expression technology platform is particularly essential in the early phases of biopharmaceutical development. The emphasis of this study was on the most recent developments in the field, particularly in active areas such expression systems, glycosylation impact factors, and transient cell proliferation.***

KEYWORDS: ***Biopharmaceutical Development, Cell Protein, Mammalian Cell, Manufacturing, Protein Expression.***

1. INTRODUCTION

Clinical reagents, vaccines, and medicines made using contemporary biotechnology for in vivo diagnostic, preventative, and therapeutic purposes are known as biopharmaceutical products. Insulin was the first recombinant protein to be authorized by the FDA. The US Food and Drug Administration has authorized more than 100 novel recombinant protein therapies, including many noteworthy monoclonal antibodies, as well as more than 300 non-recombinant biopharmaceuticals, such as vaccines and blood products, since its successful launch. Engineering biological systems using recombinant DNA and hybridism technologies produces[1]:

1. Recombinant versions of natural proteins, derivatives of natural proteins, and live systems.
2. Natural proteins and living systems derivatives
3. In-vivo diagnostic and therapeutic monoclonal antibodies, viral vectors, plasmid vectors, and tiny interfering RNAs that transport genes or genetic information for immunization or gene therapy.

1.1 System of expression:

Chinese hamster ovary cells, murine myeloma lymphoblastoid like cells, Human Embryonic Kidney 293 cells, and baby hamster kidney cells have all been used to make biopharmaceutical products. To improve output in mammalian cells, several expression methods have been explored[2]. Vector design and construction, codon optimization, gene amplification techniques, host cells, transfection methods, and screening tools have all improved dramatically in the past two decades. CHO cell lines for recombinant proteins and Mabs, and murine myeloma cell lines for Mabs, are two of the numerous available systems. Suitable vector design, which includes the

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use of a strong promoter, a proper signal peptide, chosen introns, product gene codon optimization, and the use of transcription regulatory sections, may improve mammalian expression[3]. Gene amplification triggered by a selective marker such as dihydrofolate reductase or glutamine synthetase is a common method for creating cell lines for the synthesis of therapeutic proteins. Vectors with strong promoters, such as the CMV promoter, induce high amounts of messenger RNA transcription to produce high levels of gene expression. Codon adjustment for the target cell type, balancing the GC/AT ratio, and signal sequence optimization have all been demonstrated to enhance mRNA processing and secretion. To enhance final product output, gene-targeting technologies, chromatin opening elements, and attachment areas have been integrated into vector optimization.

1.2 Cell line PER.C6:

The biopharmaceutical industry has found the retina-derived Per.C6 cell line, a human cell line with human glycosylation and other post-translational modification machinery, to be very appealing for generating therapeutic proteins and Mabs. There is no need for gene amplification or a selection marker in this cell line. Within a few months, high-producing stable clones may be produced. To maintain steady and effective protein expression, a modest copy number is required. Using the extreme-Density continuous method, the cell line has shown its ability to produce N2 g/L of recombinant protein in fed-batch culture. Using a suspension culture of PER.C6, both cells and product are maintained in a stirred-tank bioreactor in this method. PER.C6 was recently utilized to produce several antibodies in a single cell. A mixture of plasmids carrying genes for three distinct antibodies with identical light chains were transfected into the cells. Triple-positive clones have been discovered[4].

1.3 UCOE Expression System:

Gene amplification techniques are commonly used in mammalian cells to produce huge amounts of recombinant proteins. Current expression methods depend on a high number of clones being screened. However, due to the wide range of expression and unpredictability of expression in transfected cells, considerable clone screening is needed to find appropriate high producers. These methods are typically extremely time-consuming, despite the fact that they promise very high yields. Furthermore, the resultant high-producing clones may be unstable owing to the massive genomic re-arrangements that often occur during amplification. Modifications such as methylation of CpG DNA regions, histone deacetylation, and chromatin condensation may cause a cell line's instability by silencing an external gene[5]. The utilization of unmethylated CpG Island segments from housekeeping genes in plasmid vectors, known as ubiquitous chromatin opening elements, was developed for improved transgenic expression stability. UCOE vectors include non-tissue specific chromatinopening elements that allow for fast protein production without requiring integration. In contrast to conventional non-UCOE carrying vectors, efficient expression may be obtained from a single copy of an integrated gene site, resulting in a greater proportion of cells expressing the marker gene in the chosen pool. UCOE technology has the potential to be a helpful tool for producing proteins quickly[6]. The fast synthesis of approximately 300 mg of recombinant antibody proteins in less than one month after transfection was first reported in conjunction with a serum-free and suspension adapted parent cell line.

- *Gene targeting*

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To produce desirable cell lines for sustained high-level production of recombinant proteins, random integration connecting genomic amplification is frequently employed. Because of the randomized integration site, the expression level is uncertain. Flp/FRT, a site-specific recombinase recognition sequence, has been investigated for gene targeting. The gene targeting method was used to evaluate many proteins, including tissue plasminogen activator, secreted alkaline phosphatase, and erythropoietin, and several of them exhibited consistent high expression. When compared to random transfection, luciferase expression was found to be 60 times greater when utilizing this recombination method. The use of modified chromosomes has been explored to enhance the transfection process. For the targeted transfection of cells carrying mammalian-based artificial chromosomes with numerous recombination acceptor sites, an artificial chromosome expression system was utilized. This ACE System removes the requirement for random integration into native host chromosomes by allowing for the targeted transfection of single or multiple gene copies. Several case studies involving the creation of CHO cell lines producing monoclonal antibodies have shown the usefulness of utilizing artificially altered mammalian chromosomes, particularly the ACE System. Piggyback and sleeping beauty transposable elements have been demonstrated to help recombinant genes integrate into cultured mammalian cells. In the absence of selection, the cell lines exhibited steady expression for up to 3 months.

- *Other advancements*

In the E. coli expression system, high-efficiency expression controlled by lambda phage PL has been widely utilized. A thermolabile repressor product of the gene suppresses the promoter activity of PL at low temperatures, but it may be activated by heat induction. It wasn't until recently that similar temperature-sensitive promoters were discovered in a mammalian expression system. At a lower temperature, a new, endogenous, and highly active gene promoter derived from CHO cells demonstrates conditionally inducible gene expression. It had greater promoter activity than SV40, with the potential to be enhanced further by duplication of a core promoter region. This feature is especially useful for processes that have minimal expression during cell development and then a spike in expression during the manufacturing phase at low temperatures. Gene transfer through LV was shown to be a viable alternative to plasmid transfection. In the absence of MTX, DHFR was silenced, resulting in higher-producing clones with more consistent expression. Lactate dehydrogenase, an enzyme that catalyzes the conversion of glucose-derived pyruvate to lactate, was down-regulated in CHO cells generating human thrombopoietin by an expression vector of short interfering RNAS[7]. Other techniques, like as homologous recombination and antisense mRNA, have less of an impact than siRNA. The methods may be utilized to mute apoptosis-related gene expression, protein glycosylation-related gene expression, a cellular metabolism gene involving lactate dehydrogenase, and other genes involved in gene amplification. All of them, however, are part of a single targeting strategy that is heavily reliant on identifying the key target gene to downregulate. Only then can silencing be employed to stably affect cellular activities in mammalian cells by down-regulating target protein expression. Future RNAi methods may be used to silence numerous targets engaged in various cellular processes, as well as targets linked to microRNA molecules for cellular self-regulation, to alter global gene regulation in cells[8].

1.4 Engineering of cell lines:

Currently, there are approximately 200 recombinant biopharmaceutical drugs on the market, with hundreds more in clinical trials. Glycosylated proteins account for more than half of them. It would

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be ideal to develop an expression system that allows for the efficient production of high-quality glycoproteins. In order to sustain a lengthy residence time in circulation, glycoproteins must be sialylated for therapeutic application. Depending on the product, the host cell line, and the growth circumstances, the degree of sialylation varies. The production of sialic acid, the availability of nucleotide-sugars, and the CMPsialic acid transporter and sialyl-transferase are all limiting stages in sialylation[9].

When compared to fucosylated IgG, non-fucosylated IgG enhanced binding to human FcRIII by 50 times. In the presence of plasma IgG, nonfucosylated anti-CD20 had much greater ex vivo B-cell depletion efficacy than fucosylated anti-CD20. As a result, non-fucosylated IgG1 has a high therapeutic potential in people in vivo due to substantially increased ADCC at low dosages. A FUT8 deletion cell line was established in a CHO host cell line to manufacture afucosylated antibodies via transient transfection. Human IgG production titers were similar to the wild-type after transfection with the cationic liposome DMRIE-C. To obtain comparable expression levels to the wild-type, the cell line may potentially be co-transfected with the exostosin-1 gene to enhance heparin sulfate content[10].

1.5 Process development:

Engineering host cells and expression vectors, optimizing culture medium with additions, different feeding techniques, and improving process control such as temperature, pH, and osmolality have all been explored as ways to enhance production output. A simple technique for regulating lactate buildup in suspension cultures of CHO cells depending on the culture's pH was devised to reduce lactate's detrimental effect. When glucose levels fall below a certain threshold, cells begin to absorb lactic acid from the culture media, causing the pH to rise. A nutrition feeding technique for delivering a concentrated glucose solution was developed based on pH control. During the development phase of a fed-batch CHO cell culture, it was shown that high-end pH-controlled glucose supply may significantly decrease or eliminate lactate buildup. From a bench size to a huge 2500 L scale, the technique was scaled up. Furthermore, this technique has been shown to be applicable to the majority of CHO cell lines that produce monoclonal antibodies and other therapeutic proteins, with final titers for eight cell lines tripling.

Overexpression of suitable glycosyltransferases may improve glycan quality. For example, in CHO cells, overexpression of a galactosyltransferase and a sialyltransferase increased the galactose and sialic acid content of produced recombinant therapeutic protein. The impact of various culture settings, bioreactors, procedures, medium, and nutrients on the architectures of N-linked glycans bound to antibodies or therapeutic proteins has been studied extensively. However, owing to the complexities of glycan structure and glycosylation, there is no agreement on a culture system that would produce a product with a desired glycol profile. A systematic study would be the answer to understand each factor's effect on the end result for each unique expression system, cell line, and product. Glycan profiling assays have recently been developed, including a high-throughput screening assay to quantify major glycan species in crude mammalian cell culture samples for monoclonal antibodies, which should make antibody glycan profiling easier during cell culture expression, clone selection, and process optimization. Hundreds of crude cell culture samples can be analyzed in a few hours to identify the relative amounts of high mannose, fucosylated, and galactosylated glycan species in the Fc domain.

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- *Cell line and bioreactor:*

Detailed comparative studies were conducted to determine the effect of bioreactors and cell type on product glycosylation. In roller bottles and in suspension in a stirred tank, adherent cells were grown. There were no significant changes in glycosylation. When grown in a STR, SP2/0 galactosylated IgG to a greater degree than when cultured in a hollow fiber reactor with a shifted of in hollow fiber bioreactor in STR. CHO was often grown on a serum-free medium, while SP2/0 frequently needs serum for development. Minor quantities of sialylated product were found in SP2/0.

- *The pace of growth:*

The glycoprotein transferring secreted by confluent and subconfluent cultures includes different amounts of biantennary oligosaccharides, according to Hahn and Goochee. The biological activity of the biantennary glycoprotein was higher. Subconfluent cultures generate less active transferrin than confluent cells. Growth is required for oligosaccharide synthesis, according to Hahn and Goochee. As a result, the glycosylation pattern will change in conventional batch culture, where the growth rate fluctuates during the fermentation. The rates of protein synthesis and the resultant protein glycosylation were studied. The glycosylation site occupancy of recombinant protein generated by C127 murine cells was enhanced by lowering the protein synthesis rate using cycloheximide. Studies on TPA production in CHO cells, on the other hand, showed that the protein

- *Nutrients and medium:*

The cell culture medium influences the cell growth environment and physical parameters, all of which have a significant effect on cell growth, productivity, and product quality, including glycosylation. Sugar feeding, nucleotide feeding, oxygen sparging, amino acid additions, and serum components are all examples of nutrient supplements.

- *Serum:*

For decades, bovine serum has been utilized in mammalian cell culture as a nutritional supplement and to protect cells against pH changes and shear pressures. Serum includes growth hormones that help cells develop and lipids that help cells withstand shear. Serum, on the other hand, includes waste products and proteases that may harm the cell and glycoprotein products. In comparison to cultures with serum, a monoclonal IgG1 generated by mouse hybridoma in serum-free medium exhibited greater amounts of terminal N-acetylneuraminic acid and Gal, while terminal Gal was higher in CHO cells grown in serum-containing media.

- *Glucose:*

To see whether glycosylation was reliant on medium components, researchers looked examined glucose-limited chemostats. CHO cells with two distinct glucose concentrations and a constant dilution rate. They were able to show that when glucose was not restricted, completely glycosylated IFN-gamma was produced more easily. The physiological condition of the cells was shown to be the cause of this impact. The addition of glucose to the growth medium increased galactosylation somewhat. Minor differences in the ratio between galactosylated structures occurred from the use of various mediums for synthesis. A more completely galactosylated N-

glycan profile may be aided by galactose feeding. Glutamate and glucose levels below, respectively, resulted in reduced sialylation profiles and an increase in hybrid and high mannose type glycans, according to studies of CHO fed-batch cultures generating IFN-gamma.

1.6 Recombinant protein production by transient gene expression:

Over the last decade, researchers have been focusing on transient gene expression. When compared to stable cell line development, the method has the benefit of a faster development period and cheaper development expenses. Furthermore, the quality of TGE products is appropriate for preclinical evaluation, speeding up the "Proof of Principle" stage, when major biopharmaceutical firms test numerous drug ideas before moving them forward into the official research pipeline. Technically, all of the methods used to maximize expression in the creation of a stable cell line may be utilized and tested in TGE to determine their potential before investing substantial resources in the production of a stable cell line. TGE is utilized as the initial stage in screening expression strategy in terms of construction design and molecular candidates because of its fast turnaround and cheap cost. Transfection of DNA into a mammalian cell includes utilizing high-voltage electric shock "electroporation" or chemical mediators such as calcium-phosphate or lipofection to force nucleic acids into cells. Calcium phosphate is a well-known, low-cost, high-efficiency DNA delivery agent. Unfortunately, it does not function well with CHO cells, and the transfection protocol's time-sensitive nature makes large-scale adoption difficult. Lipofectamine-based techniques and procedures showed excellent transfection efficiency when plasmid DNA was introduced into CHO and other cells. Lipofection transfection reagents, on the other hand, are typically expensive. As a result, making gram-scale amounts of product with these chemicals during pilot scale production is not economically viable. Similarly, electroporation devices work well for transferring DNA into milliliters of cell culture, but they're only practical for small-scale operations. As a result, transfection reagents and electroporation are only suitable for laboratory usage and cannot be used on a wide scale.

2. DISCUSSION

The production of gram amounts of Mabs for preclinical research was described utilizing an alternate approach based on stable transfection pool technology. At the 200 L scale, Mabs may be expressed at quantities ranging from 100 to 1000 mg/L. Furthermore, there are structural and heterogeneous issues about DNA-cationic complexes or lipoplexes, while much basic research is focused at gaining a better understanding of their components. Fortunately, a variety of analytical methods, such as dynamic light scattering, analytical ultracentrifugation, gel electrophoresis, circular dichroism, and fluorescence spectroscopy, are being used in basic science labs to study lipoplexes. These may lead to the creation of a manufacturing process that is repeatable and consistent. Recent advances in TGE technology development, such as cell line engineering, plasmid preparation, media and additives development, procedural simplification, and process automation, are likely to contribute to the realization of large-scale biopharmaceutical manufacturing using TGE platforms for preclinical and early clinical development in the near future.

3. CONCLUSION

Mammalian cell protein expression has been the most common recombinant protein production method for therapeutic applications in the past two decades, accounting for more than half of all

biopharmaceutical products on the market and hundreds of candidates in clinical trials. Significant progress has been made in the development and creation of new cell lines, as well as the introduction of novel genetic processes in expression, gene silencing, and gene targeting. Understanding glycosylation has been a major emphasis, and the transient gene expression technology platform is becoming more essential in biopharmaceutical production. This review summarizes the most recent advances in mammalian production of recombinant proteins for biopharmaceutical research, with a focus on active areas including expression systems, glycosylation effect factors, and transient gene expression.

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