**Project Proposal**

**CELL CULTURE PROCESSING (BIO08045)**

**ATU Sligo**

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| **Student Name:** | Justyna Skiba |
| **Student Number:** | S00274779 |
| **Project Title:** | Cell Line Development for Biopharmaceutical Manufacturing: A Review of Recent Advances and the Productivity Gains Achieved |

**Research questions:**

* How have recent advancements in cell line development impacted the efficiency and productivity of biopharmaceutical manufacturing processes?
* What are the key challenges and opportunities in cell line development for the future of biopharmaceutical manufacturing?

**Methods to be used:**

Literature Review

**Breakdown of project:**

**1. Introduction**

Overview of biopharmaceutical manufacturing, and importance of cell line development in the process.

Thesis statement: *Recent advancements in cell line development, such as gene editing, advanced cell culture, and high-throughput screening, have significantly improved the efficiency and productivity of biopharmaceutical manufacturing processes.*

**2. Literature Review**

2. 1 Historical context of cell line development

2. 2 Traditional methods and their limitations

2. 3 Emergence of modern cell line engineering techniques:

2. 3.1 Gene Editing Technologies

* CRISPR: the mechanism, applications in cell line development, and its impact on productivity.
* Base Editing and Prime Editing: advanced gene editing techniques and their potential to further improve cell line engineering.

2. 3. 2 Advanced Cell Culture Systems

* Perfusion Culture: the principles, advantages, and its role in enhancing cell growth and productivity.
* 3D Cell Culture: the benefits of 3D culture systems, such as improved cell physiology and protein production.

2. 3. 3 High-Throughput Screening and Automation

* Automated Clonal Isolation - process explained and its impact on accelerating cell line development.
* Robotics and Artificial Intelligence - role of automation in improving efficiency and reproducibility.

2. 3. 4 Omics Technologies

* Genomics, Transcriptomics, Proteomics, and Metabolomics: how these technologies can be used to optimize cell line performance.

2. 3. 5 Synthetic Biology?

* Design and Construction of Synthetic Genetic Circuits: the potential of synthetic biology to create novel cell lines with enhanced properties.

2. 4. Productivity Gains

* Increased Product Yields: how advancements have led to higher protein expression levels and optimized cell growth conditions.
* Reduced Time-to-Market: the impact of these advancements on accelerating cell line development and regulatory approval processes.
* Improved Product Quality: how recent advancements have contributed to consistent product quality, reduced risk of contamination, and enhanced product potency.

2. 5 Challenges and Future Directions

* Challenges of regulatory approval for genetically engineered cell lines.
* Intellectual Property Issues: the importance of patent protection for innovative cell line development.
* Scale-up Challenges: the challenges of scaling up cell line production to meet commercial demand.
* Future Trends: emerging technologies, such as microfluidic devices and organ-on-a-chip systems, and their potential impact on cell line development.

**3. Conclusion**

* Restated thesis statement and the key findings of the analysis.
* Answer to the research questions.
* Future potential of cell line development and its impact on the biopharma industry.
* Areas for further research: identification of emerging trends.

**2. Literature Review**

2. 1 Historical context of cell line development

According to Maloy and Hughes (2023) a cell line is a constant established cell culture that will multiply indefinitely in optimum medium. Cell lines can be distinguished from cell strains as they become immortalized (Maloy and Hughes, 2013).

IN 2015, six of the top 10 drugs with sales of USD 59 bil lion were recombinant protein biopharmaceuticals manufactured in animal cells1. Expiry of patents on biopharmaceuticals along with increasing clarity on regulatory pathway for clearance of biosimilar versions in Europe and USA has generated significant interest in the manufacturing of such recombinant protein therapeutics world-wide. Unlike small molecule pharmaceuticals, recombinant protein therapeutics are larger, more complex molecules which cannot be chemically synthesized. As an illustration of the difference in size, aspirin has 21 atoms with a molecular weight of 180 Da, while a monoclonal antibody, currently the fastest growing class of recombi nant protein therapeutics, has ~20,000 atoms with a molecular weight of ~150,000 Da. Manufacturing these therapeutics requires harnessing the synthetic capability of living cells and is largely carried out in prokaryotic cells like E. coli, or in eukaryotic cells like animal cells. A significant factor governing the choice of the type of cell used to produce a particular protein is the capability of cell type to perform any required post-translational modifications (PTMs). PTMs are chemical modifications of a protein, which can widen the range of functionality of the protein. PTMs observed in context of therapeutic proteins include glycosylation, carboxylation, hydroxylation, sulphation, amidation, etc. with glycosylation being the most common modification2,3. Proteins requiring PTMs like glycosylation for their therapeutic effect need to be expressed in animal cells, since E. coli and yeast are unable to provide appropriate PTM. There have been some efforts to engineer yeast to provide human-like glycosylation profiles, but there are no glycoproteins yet in the market using such a platform4. Animal cells remain the predominant platform to manufacture recombinant glycoprotein therapeutics. Upstream process development for manufacturing recombinant proteins in animal cells comprises several steps outlined in Figure 1. Briefly, it starts with the selection of appropriate host cell line, which may be engineered to incorporate desirable features for production. During stable cell line development for production, the host cell is transfected with transgene encoding the protein of interest. Of the millions of cells transfected, selection and screening of single cell clones is carried out to identify the best possible clone with suitable growth and productivity attributes and showing stable expression of protein over a period of 2–3 months. Medium and process conditions are optimized for the identified cell line to in crease culture longevity and productivity of the cell line. Biosimilars development may have an added goal to achieve a product quality attribute similar to the innovator. Production has historically been carried out in stainless steel stirred tank reactors, but advances in single use bioreactors have provided the option of utilizing single use technology with reduced capital cost. This article aims at providing a birds’ eye view of upstream process development for animal cell culture processes, with a focus on advances relevant to the development of proc esses for biosimilars. An important area especially for biosimilar manufacturing not covered in this review is the application of high resolution analytical techniques to compare biosimilar products to innovator molecules.

Hamster and mouse cell lines have been widely used industrially as host cells for recombinant protein production and include Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, mouse myeloma (NS0) and hybridoma (SP2-0), with CHO cells being the most commonly used. Aspects such as PTM on recombinant protein and host cell impurity profile can differ depending on the host cell species. For example, nature of glycosylation of a recombinant glycoprotein can vary with the host  (Ebscohost.com, 2017)

T he production of protein therapeutics is a fast-growing field as it allows for the generation of sophisticated molecules with high specificity and activity in humans1–4. Even though the Chinese hamster ovary (CHO) cell line is a successfully used mammalian platform for the production of advanced recombinant proteins with the need for proper protein folding and post translational modifications, there is an increasing demand for improved and more efficient bioproduction platforms. With an increasing number of difficult-to-express proteins entering clinical development, including bispecific antibodies and antibody–drug conjugates, alternative or engineered expression hosts are being explored. Extensive omics profiling of CHO cells has been carried out during recent years5–12, which has paved the way for cell line engineering efforts aiming to improve bioproduction efficiency and product quality13–15. Moreover, human production cell lines, such as HEK293, have served as convenient expression hosts for proteins with specific requirement for human post-translational modifications16,17. T he human cell line HEK293 is the most commonly utilized human cell line for expression of recombinant proteins for a multitude of research applications. This cell line originate from the kidney of an aborted human female embryo and was originally immortalized in 1973 by the integration of a 4 kbp adenoviral 5 (Ad5) genome fragment including the E1A and E1B genes, at chromosome 1918,19. The expression of E1A and E1B enable continuous culturing of HEK293 cells by inhibiting apoptosis and interfering with transcription and cell cycle control pathways20. In addition, E1A and E1B are essential helper factors for adeno associated virus (AAV) pro duction, which makes HEK293 cells attractive production hosts for recombinant AAV particles21. HEK293 cell lines have been reported to have a pseudotriploid genome with the adenoviral DNA inserted on chromosome 1919,22,23. The organization of the HEK293 genome is continuously evolving through the events of chromosomal translocations and copy number alterations, suggesting that long-term cultivation and subcloning of cells result in karyotypic drift22,24. Such abnormalities and genomic instability is, however, characteristic for immortalized cells and have also been reported for CHO cells25–28 (Ebscohost.com, 2020).

References:

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