Cell Line Development for Biopharmaceutical Manufacturing: A Review of Recent Advances and the Productivity Gains Achieved

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**Abstract**

The provided project reviews advancements in cell line development, primarily focusing on Chinese Hamster Ovary (CHO) cells for biopharmaceutical production. It details a shift from traditional, random transgene integration methods to modern techniques like CRISPR-Cas9 gene editing, offering increased precision and efficiency. Further improvements are discussed through advanced cell culture systems, including 3D cultures and perfusion, alongside the use of "omics" technologies for comprehensive cellular analysis. High-throughput screening and automation are highlighted as key factors in accelerating the process, leading to increased product yields, faster time-to-market, and improved product quality. Ultimately, these advancements aim to streamline biopharmaceutical production, creating more efficient, cost-effective, and reliable processes.

**1. Introduction**

To fully appreciate the transition from traditional to modern biopharmaceutical production techniques, we must trace the journey back to the early 20th century, where the establishment of basic cell lines laid the groundwork for future advancements. The introduction of genetic engineering in the 1970s revolutionised the field, enabling the production of recombinant proteins. Initially, random transgene integration (RTI) was the primary method for cell line development. This technique, however, had several limitations. The location of gene integration in the host genome impacted expression levels. Multiple copies of the expression vector could integrate, leading to instability. Screening numerous clones for desirable traits was time and labour intensive. Endogenous gene disruption could occur leading to unpredictable phenotypic effects. Also limited control over transgene insertion leading to variability. Despite limitations, RTI, particularly with Chinese Hamster Ovary (CHO) cells, has been widely used, contributing to the successful production of biopharmaceuticals.

Advancements in selection systems, screening methods, and workflows have improved the efficiency of RTI-based processes. The emergence of modern cell line engineering techniques of Gene Editing Technologies like CRISPR-Cas9 have enabled targeted gene editing, providing greater control and precision for CHO cell line engineering. CRISPR-Cas9 allows for a wide range of applications, including gene knockout, targeted knock-in, glycan profile modification, enhanced cell performance, reduction of host cell protein (HCP) contamination, and increased viral resistance. Advanced Cell Culture Systems including 3D cell culture systems mimic in vivo conditions more effectively than traditional 2D cultures. Perfusion culture allows for higher cell densities and continuous product removal. Omics Technologies like genomics, transcriptomics, proteomics, and metabolomics provide a comprehensive understanding of cellular processes, enabling cell line optimisation. High-Throughput Screening and Automated platforms facilitate the screening of vast libraries for desired traits. Automated clonal isolation technologies, like FACS and ClonePix, have replaced traditional methods, allowing for the efficient isolation of high-performing clones. Robotics and AI play an increasingly important role in automating processes, optimising bioreactor conditions, and analysing large datasets, further enhancing efficiency and productivity.

These advancements collectively represent a paradigm shift in cell line development, significantly impacting the biopharmaceutical industry. They have paved the way for the development of more efficient, cost-effective, and robust bioprocesses, ultimately leading to improved therapies and faster access to life-saving treatments.

**2. Literature Review**

**2. 1. Historical context of cell line development**

The historical context of cell line development is marked by a fascinating journey of scientific discoveries, technological advancements, and an increasing demand for complex biopharmaceuticals. It began as a niche field and has evolved into a cornerstone of modern medicine, enabling the production of life-saving therapies like monoclonal antibodies, vaccines, and other recombinant proteins. The earliest cell lines were established in the early 20th century, primarily for research purposes. These cell lines were often derived from tumors or immortalized cells through spontaneous mutations, allowing them to grow indefinitely in culture (Maloy & Hughes, 2013). The advent of genetic engineering in the 1970s revolutionized cell line development. Scientists gained the ability to introduce specific genes into cells, paving the way to produce recombinant proteins. This breakthrough, coupled with the development of robust cell culture techniques, marked the birth of the biopharmaceutical industry (Esmaeili et al., 2023).

**2. 2. Traditional methods and their limitations**

Cell line development for biopharmaceutical production is connected to development in genetic engineering and growing understanding of cellular biology. Initially the process relied heavily on random transgene integration (RTI), where efforts dependent almost exclusively on RTI, a method where the gene of interest (GOI) was randomly inserted into the host cell genome. The area however has progressively moved towards targeted methodologies, driven by a need for improved efficiency, control, and predictability (Majumdar et al., 2024). The industry standard for RTI-based cell line development has been the Chinese Hamster Ovary (CHO) cell line (Horie et al., 2022). CHO cells are favoured for their ability to produce complex therapeutic proteins with human-like glycosylation patterns, grow in suspension culture, and have a long history of regulatory acceptance (Majumdar et al., 2024).

The biggest limitation of traditional cell line development is its primarily dependency on random transgene integration. This results in the transgene integrating at various locations within the host cell genome, leading to significant variations in expression levels between different clones. This is due to the influence of the surrounding chromatin environment, known as “position effects” on the transcriptional activity of the integrated transgene. A common outcome of RTI is the formation of concatemeric structures, where multiple copies of the expression vector integrate at a single locus. These repetitive sequences are prone to recombination events, leading to genetic instability and potential loss of transgene copies over time, ultimately impacting cell line stability and productivity. Due to the heterogeneity resulting from RTI, many clones need to be screened to identify those with desirable characteristics. This process is time-consuming, labor-intensive, and often requires sophisticated high-throughput screening platforms to effectively assess cellular productivity, product quality, and stability. The selection and amplification steps in traditional cell line development, particularly when using the DHFR system with methotrexate (MTX) gradients, can significantly prolong the process. This can add weeks or even months to the overall timeline, delaying the availability of stable cell lines for downstream development. RTI offers limited control over the number and location of transgene insertions. This lack of control can contribute to clonal variability and make it difficult to ensure consistent and predictable performance across different cell lines (Zeh et al., 2024). Despite these limitations, traditional methods based on RTI have been extensively used and optimized over the years, contributing significantly to the successful production of many biopharmaceuticals. Advancements like optimized selection systems (DHFR, GS), high-throughput screening methods, and streamlined workflows like ‘direct cloning’ have improved the efficiency of RTI-based processes (Noh et al., 2019, Zeh et al., 2024).

**2. 3. Emergence of modern cell line engineering techniques**

**2. 3. 1. Gene Editing Technologies**

Scientists developed several technologies as a response to overcome challenges linked to traditional cell line development. The advent of programmable nucleases like ZFNs, TALENs, and CRISPR-Cas systems has enabled targeted gene editing, offering more control and precision in CHO cell line engineering (Glinšek et al., 2023).

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and refers to DNA sequences found in bacteria that form the basis of the CRISPR-Cas gene editing system. This mechanism is based on Guide RNA (gRNA) a short RNA sequence designed to be complementary to a specific target DNA sequence. The gRNA directs the Cas9 nuclease to the desired genomic location. Cas9 nuclease is a protein derived from *Streptococcus pyogenes* that cuts DNA at the site specified by the gRNA. This cleavage requires the presence of a short protospacer adjacent motif (PAM) sequence (NGG for *S. pyogenes* Cas9) located next to the target sequence. The gRNA, through base pairing, guides Cas9 to the target DNA, ensuring site-specific binding. Cas9 then scans the DNA for the PAM sequence, which triggers its endonuclease activity, creating a Double-Strand Break (DSB) (Figure 1). This break initiates the cell's DNA repair mechanisms, which can be manipulated for various gene editing purposes (Dara et al., 2024, Shen et al., 2024, Zhou et al., 2024).

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Figure 1. A visual representation of CRISPR-Cas mechanism, gRNA - guide RNA, dsDNA- double-strand DNA (Wikimedia.org, 2022).

CRISPR-Cas has enabled a wide range of applications in cell line development, particularly for CHO cells used in biopharmaceutical production. It can efficiently disrupt gene function by introducing indels (insertions or deletions) at the DSB site. Gene knockout is valuable for studying gene function, eliminating undesirable traits, and creating cell lines resistant to viruses (Shen et al., 2024). By providing a donor DNA template along with the CRISPR-Cas9 components, researchers can introduce precise gene insertions. The cell can use the template for homology-directed repair (HDR), a more accurate repair pathway that copies the donor sequence into the genome at the DSB site. Targeted knock-in enables the integration of genes at specific locations, such as transcriptionally active "hot-spots," enhancing expression stability and predictability. CRISPR-Cas has been used to knock out genes involved in various glycosylation pathways, such as fucosylation, sialylation, and O-glycosylation, to engineer CHO cells that produce proteins with desired glycan profiles. These modifications can enhance the therapeutic potential of antibodies and other biopharmaceuticals. It can also enhance CHO cell performance by targeting genes that influence cell growth, viability, and protein production. For instance, knocking out genes involved in apoptosis (e.g., caspases, BCL-2 family members) can increase cell survival, extend culture longevity, and ultimately boost productivity. Gene editing allows researchers to fine-tune cellular processes related to protein production, leading to higher titres of therapeutic proteins with enhanced quality attributes, such as already mentioned glycosylation patterns and reduced HCP contamination. HCPs (Host Cell Proteins) are proteins produced by the host cell that can co-purify with the desired therapeutic protein, potentially affecting its quality and safety. CRISPR-Cas9 can be used to knock out genes encoding problematic HCPs, such as lipoprotein lipase (LPL) and cathepsin D, reducing contamination and improving product purity. Additionally, this systemis highly versatile and adaptable, enabling the production of complex therapeutic proteins, such as difficult-to-express (DTE) proteins and novel antibody formats, which are becoming increasingly important in the biopharmaceutical industry. Viral contamination is a significant concern in biopharmaceutical production. CRISPR-Cas9 can enhance the resistance of CHO cells to viruses by knocking out genes involved in viral entry or replication, improving the safety of cell lines and therapeutic products (Dara et al., 2024, Glinšek et al., 2023, Shen et al., 2024, Zhou et al., 2024).

Ultimately CRISPR-Cas9 technology has significantly accelerated the cell line development process. Targeted gene knock-in and knockout strategies enable faster identification of high-performing clones, reducing the time and resources required for screening. CRISPR-Cas technologies continue to evolve, with researchers developing new Cas variants, improved gRNA design tools, and innovative applications. These ongoing advancements promise to further enhance the capabilities of CHO cell line engineering, paving the way for the development of next generation biotherapeutics with improved efficacy, safety, and affordability (Dara et al., 2024).

**2. 3. 2. Advanced Cell Culture Systems**

3D cell culture systems have gained prominence due to their ability to provide an environment that more closely mimics the in vivo conditions compared to traditional 2D monolayer cultures. This has significant implications for cell physiology and protein production. 3D cultures inherently create gradients of nutrients and oxygen, much like those found in tissues. The spatial organization of cells in 3D cultures promotes cell-cell interactions, leading to a more physiologically relevant cellular response The 3D microenvironment and cell-cell interactions can influence protein folding and post-translational modifications, leading to proteins that are more like their native counterparts. This is crucial for therapeutic proteins, where proper folding and modifications are essential for biological activity. Dynamic 3D cultures have been shown to promote cell proliferation and reduce apoptosis, further contributing to increased protein production. Overall, 3D culture systems offer several benefits over traditional 2D cultures, particularly for studying complex cellular processes and producing therapeutic proteins. The improved cell physiology and the more realistic microenvironment provided by these systems can lead to more accurate and insightful results, with potential implications for drug discovery, tissue engineering, and regenerative medicine (Mastaneh Parchami et al., 202, Mohamadian et al., 2024, Wu et al., 2021).

Perfusion culture is a cell culture technique in which spent, cell-free medium is continuously removed and replaced with fresh medium while retaining the cells in the bioreactor. This technique allows for high cell density cultures and offers several advantages over traditional batch or fed-batch cultures. This method allows for precise control of key process parameters like temperature, pH, and dissolved oxygen, optimizing the culture environment for cell growth and product formation. Compared to fed-batch processes, perfusion cultures can achieve 2- to 5-fold higher viable cell densities (VCDs), sustain these densities for extended periods, and attain significantly higher volumetric productivities (Tachihana et al., 2023). Due to the continuous supply of nutrients and removal of waste products, system offer high control over the culture environment, leading to more consistent product quality attributes, including glycosylation profiles and charge variants. The continuous harvest in perfusion cultures reduces the product's residence time in the bioreactor, which is particularly advantageous for labile proteins (Iannacci et al., 2024, Romann et al., 2023, Tachihana et al., 2023, Zhang et al., 2024).

In general, perfusion culture is a powerful technique for enhancing cell growth and productivity. It offers several advantages over traditional culture methods, particularly to produce high-value biomolecules and therapeutic proteins. However, the increased complexity and media consumption associated with perfusion cultures need to be carefully considered during process development and scale-up.

**2. 3. 3. Omics Technologies**

Omics technologies like genomics, transcriptomics, proteomics, and metabolomics are powerful tools that can be used to optimise cell line performance. These technologies are a suite of techniques used to study the collective characterization and quantification of biological molecules and lead to comprehensive understanding of the cellular processes that are involved in protein production.

Genomics is the study of an organism's entire genome. In the context of cell line optimisation, genomics can be used to identify genes that are involved in protein production. Identify genetic modifications that can be made to improve protein production by creating cell lines with specific genetic traits (Peeters et al., 2024). For example, the CRISPR-Cas9 system can be used to knock out genes that inhibit protein production or knock in genes that enhance protein production (Majumdar et al., 2024).

Transcriptomics is the study of the complete set of RNA transcripts in a cell. This knowledge can be used to identify genes that are differentially expressed in high-producing cell lines and transcripts that can be used as biomarkers for cell line performance. It provides understanding the effects of genetic modifications on gene expression. For example, RNA sequencing can be used to compare the transcriptomes of high-producing and low-producing cell lines (Han et al., 2024).

Proteomics is the study of the complete set of proteins in a cell or organism. Proteomics can be used to identify proteins that are differentially expressed in high-producing cell lines or proteins that can be used as biomarkers for cell line performance and comprehend the effects of genetic modifications on protein expression. For example, mass spectrometry can be used to compare the proteomes of high-producing and low-producing cell lines (Peeters et al., 2024).

Metabolomics is the study of the complete set of metabolites in cells. Metabolomics can be used to recognize metabolites that are differentially expressed in high-producing cell lines, identify metabolites that can be used as biomarkers for cell line performance and understand the effects of genetic modifications on metabolism. Metabolomics can be used to identify nutrients that are limiting cell growth or to identify toxic byproducts that are accumulating in the culture medium (Dar et al., 2022).

The sources emphasise the importance of integrating multiple "omics" datasets to gain a holistic understanding of cell line performance. By combining data from genomics, transcriptomics, proteomics, and metabolomics, researchers can identify the key factors that contribute to high productivity and develop strategies to optimise cell line performance (Ramírez et al., 2024). It is also important to highlight the recent advancements in single-cell multi-omics technologies. These technologies allow researchers to measure multiple "omics" layers (e.g., genome, transcriptome, proteome) within the same cell, providing an unprecedented level of detail about cellular heterogeneity and function. This information can be used to identify rare subpopulations of cells that are high producers or to understand the molecular mechanisms that underlie cell line performance (Liang et al., 2023, Shi et al., 2023).

Despite the significant progress in "omics" technologies, there are still challenges that need to be addressed, such as: complexity of data analysis, high cost of some technologies, limited availability of standardized protocols. Future developments in "omics" technologies are expected to enhance sensitivity, accuracy and simplify procedures (Liang et al., 2023).

**2. 3. 4. High-Throughput Screening and Automation**

Automated platforms of High-Throughput Screening (HTS) facilitate the screening of vast libraries of compounds or genetic modifications to identify those that exhibit desired effects on cell behaviour or protein production. Techniques such as droplet microfluidics and microchamber array technology are mentioned for improving reaction efficiency and enabling simultaneous detection of multiple targets (Zhou et al., 2024).

Automated Clonal Isolation (ACI) refers to the application of technology that enables the isolation and screening of single cells in a high-throughput and automated manner. This approach has significantly impacted cell line development by making it possible to efficiently select and isolate high-performing clones for biopharmaceutical production. Traditional methods for clonal isolation, such as limiting dilution, are time-consuming and limited in the number of clones they can screen. Limiting dilution involves serially diluting cells until individual cells are isolated and expanded into clonal populations. While considered reliable, it requires two rounds to ensure monoclonality (originating from a single progenitor cell), as cells can clump together. The manual nature of this technique also limits its throughput. In contrast, ACI technologies allow for high-throughput screening of thousands of individual cells, leading to the identification of rare, high-producing clones in a shorter timeframe (Noh et al., 2019). Examples of Automated Clonal Isolation Systems:

Fluorescence-Activated Cell Sorting (FACS) separates cells based on fluorescence and can be used to isolate cells expressing high levels of a protein of interest.

ClonePix Systememploys image-based technology to select and isolate single cells based on user-defined criteria, such as colony morphology and fluorescence intensity.

Cyto-Mine System uses Pico droplet technology to encapsulate individual cells and screen for desired traits. It can then dispense single cells into individual wells for clonal expansion. Cellcelector is an automated rare single cell picking system that isolates single cells based on visual characteristics.

Single-Cell Printersystem combines cell printing and plate imaging to achieve greater efficiency and confidence in single-cell cloning (Majumdar et al., 2024).

The implementation of Automated Clonal Isolation has had a significant impact on cell line development. Those platforms can drastically reduce the time required for clone selection compared to traditional methods. This is particularly important for bringing new biopharmaceuticals to market quickly. It also allows researchers to increased screening capacity, screen significantly more clones, leading to the identification of rare high-producing and stable cell lines. ACI also reduces the risk of human error and ensures the isolation of true monoclonal cell lines, enhancing the reproducibility of the process. Those technologies allow researchers to process large numbers of samples with minimal manual intervention, increasing efficiency and reducing labour costs. It is not surprising that this tool has become an essential tool in modern cell line development for biopharmaceutical production (Luangphakdy et al., 2020, Majumdar et al., 2024, Nguyen et al., 2011, Noh et al., 2019).

The sources also highlight the significant role of robotics and artificial intelligence in improving efficiency and reproducibility within high-throughput screening and automation, particularly for biopharmaceutical production. Robotic platforms like the Cell XTM are used to automate tasks such as cell picking, weeding, passage, and expansion. This platform enables automated large field of view imaging, serial imaging, quantitative image analysis, media changes, and cell manipulation. The integration of robotics significantly increases the throughput and reproducibility of these processes (Luangphakdy et al., 2020).

Automation is also employed to optimise bioreactor conditions for cell growth and protein production. This includes controlling parameters such as temperature, pH, dissolved oxygen tension, and nutrient feeding. Microscale cell culture systems controlled by automated workstations are increasingly used for process optimisation in the industry.

AI and machine learning algorithms are being used to analyse large datasets generated from HTS and other automated processes. This data can be used to identify patterns and relationships between variables, develop predictive models for cell line performance, optimise bioprocess parameters in real-time and predict product yields and quality. These applications of AI are driving significant advancements in bioprocess control, cell line selection, and metabolic engineering, allowing more efficient and robust biopharmaceutical production processes.

While automation offers significant benefits, there are some challenges and considerations. The development of robust and efficient high-throughput undertakes is essential for accurately quantifying key parameters such as occlusion body production in insect cell lines. Maintaining the genetic stability of cell lines is crucial for consistent biopharmaceutical production. Strategies for enhancing stability include selecting clones with amplified genes in stable chromosomal regions and utilising gene editing techniques to modify host cell lines. Adapting clones from static culture to suspension culture for large-scale production requires optimising culture conditions to accommodate variations in aeration and nutrient distribution. Lastly implementing automated systems can be expensive, and integrating these systems effectively requires specialised expertise (Luangphakdy et al., 2020).

**2. 4. Productivity Gains**

**2. 4. 1. Increased Product Yields: how advancements have led to higher protein expression levels and optimized cell growth conditions**

Advancements in biopharmaceutical production, particularly using Chinese Hamster Ovary (CHO) cells, have focused on increasing productivity by enhancing protein expression levels and optimizing cell growth conditions. Developments in cell line engineering, such as using CRISPR-Cas9, can lead to significant increases in product yields. This is achieved by enhancing transgene expression, improving protein folding and secretion, and optimizing cell growth conditions. For instance, CRISPR-Cas9-mediated knockout of the lipoprotein lipase (LPL) gene in CHO cells resulted in a more than 95% reduction of LPL expression, leading to improved product stability. These improvements can result in faster production of the required quantities of biopharmaceuticals for clinical trials and commercialization (Glinšek et al., 2023, Zhan et al., 2020).

The composition of the cell culture media and the feeding strategy significantly impact cell growth and protein production. Advanced techniques for media and feed optimisation include shifting from static to dynamic cell culture conditions can significantly enhance cell proliferation. The use of flow bioreactors can improve oxygen and nutrient diffusion and promote cell growth (Mohamadian et al., 2024), Perfusion culture, a continuous cell culture process, allows for higher cell densities and sustained protein production (Figure 2) compared to traditional fed-batch cultures (Bausch et al., 2019, MacDonald et al., 2021).

Emerging 'omics' technologies are playing a key role in identifying new engineering targets and optimising CHO cell lines (Jerabek et al., 2022). Omics technologies have enabled significant advancements in microbial manufacturing process optimization. By analyzing genomic, transcriptomic, proteomic, and metabolomic data, researchers can fine-tune medium composition, fermentation parameters, and scale-up strategies (Figure 3), (Wan et al., 2023). Transcriptomics can be used to identify genes and pathways related to cell growth, productivity, and product quality. Proteomics, particularly sub-proteomics and spatial proteomics, allows for the characterization of specific cellular compartments and the identification of proteins involved in protein production and secretion.

A graph of a graph of a cell density

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Figure 2. Exemplary model of Fed-Batch and Perfusion processes. Graph illustrates viable cell density (VCD), product concentration (cP) and yield (Y). Equations indicate how space-time yield (STY) and volumetric productivity (VP) (Bausch et al., 2019).

Phosphoproteomics focuses on the study of protein phosphorylation, a crucial regulatory mechanism in cells. Identifying differentially phosphorylated proteins can reveal insights into cell signalling and metabolic pathways related to protein production. Secretome Analysis focuses on the proteins secreted by cells. By studying the secretome, researchers can identify and engineer proteins that affect product purity, integrity, and cell growth. Single-cell 'omics' technologies can offer a deeper understanding of cellular heterogeneity and enable the identification of high-performing cells for improved cell line development (Jerabek et al., 2022).

**A diagram of a scientific experiment

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Figure 3. Optimization strategies of protein production using 'omics' technologies (Wan et al., 2023).

**2. 4. 2. Reduced Time-to-Market: the impact of these advancements on accelerating cell line development and regulatory approval processes**

Traditional random transgene integration (RTI) methods often result in heterogeneous cell pools with variable transgene expression levels and stability. This necessitates extensive screening efforts to identify high-performing clones. Targeted integration technologies like CRISPR-Cas9 and recombinases allow for precise integration of transgenes into specific genomic locations, leading to more predictable and consistent transgene expression. This can significantly reduce the time and resources required for cell line development. Semi-Targeted Integration systems like Sleeping Beauty, PiggyBac, and Leap-In offer a semi-targeted approach for transgene integration, where the transgene is inserted into transcriptionally active regions of the genome. These systems offer high integration efficiencies and reduce the risk of gene silencing or positional effects. This leads to faster generation of stable cell pools with higher average productivities compared to RTI-based pools, potentially enabling the use of pools for early clinical studies (Zeh et al., 2024).

Advancements in high-throughput screening methods and automation have significantly accelerated the process of identifying high-producing clones. Techniques like fluorescence-activated cell sorting (FACS), automated cell imaging, and microfluidic platforms allow for rapid analysis and selection of cells based on productivity, product quality, and growth characteristics (Zeh et al., 2024).

Process Intensification and Continuous Manufacturing like perfusion culture enables continuous production of biopharmaceuticals, resulting in higher volumetric productivity compared to traditional fed-batch processes. Continuous manufacturing, in conjunction with advancements in process monitoring and control, can reduce batch-to-batch variability and improve process consistency. These are critical factors for regulatory compliance and ensuring a reliable supply of high-quality biopharmaceuticals (MacDonald et al., 2021).

The sources, suggest that a deeper understanding of cell biology and process parameters, enabled by omics technologies and advanced modelling tools, can enhance the predictability and control of bioprocesses. This can result in the generation of more robust and reliable data for regulatory submissions, potentially expediting the review process (Jerabek et al., 2022, Han et al., 2024).

It’s worth to mention the role of advancements in cell line engineering and bioprocessing in facilitating innovation and development of new therapies. the development of more complex and innovative therapies. This can lead to a faster approval pathway for novel treatments that address unmet medical needs. For instance, the development of high throughput screening (HTS) models for highly migratory cancer cells has the potential to expedite the development of therapeutics for aggressive tumours. There is strong evidence suggesting that these advancements have the potential to significantly contribute to reduced time-to-market, enabling quicker access to life-saving therapies for patients (Liang et al., 2023).

**2. 4. 3. Improved Product Quality: how recent advancements have contributed to consistent product quality, reduced risk of contamination, and enhanced product potency**

Optimizing cell culture conditions, such as using perfusion cultures, can lead to improved product quality. This includes achieving desired glycosylation patterns, reducing protein aggregation, and lowering levels of host cell protein (HCP) impurities. Perfusion culture, as discussed, allows for continuous removal of waste products and replenishment of nutrients, creating a more stable and controlled environment for cell growth and protein production. This, in turn, can lead to a more consistent product quality. Improved product quality can potentially streamline the purification process and reduce the risk of regulatory delays due to quality concerns (Zhang et al., 2024).

**3. Conclusion and Recommendations for Future Research and Development**

The advancements in cell line development for biopharmaceutical manufacturing have significantly increased productivity and product quality while reducing development timelines. Continued innovation in this field, driven by research and collaboration between academia and industry, is essential to meet the growing demand for biopharmaceuticals and accelerate the delivery of life-saving therapies to patients.

Continued advancements in genome engineering technologies, like CRISPR-Cas9 and transposases, are crucial for developing more efficient and versatile tools for cell line engineering. This includes refining target site selection, improving integration efficiency, and minimising off-target effects.

Deeper understanding of CHO cell biology and metabolism is essential for developing strategies to further enhance productivity and product quality. Omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, offer powerful tools for unravelling the complexities of CHO cells and identifying new engineering targets.

It is important to mention that development of novel selection and screening methods tailored for specific product types and production processes is needed. This includes developing high-throughput assays for product quality attributes, such as glycosylation patterns and aggregation propensity.

Further optimisation of perfusion culture processes is essential for maximising productivity gains and ensuring robust and scalable production. This includes developing strategies for cell retention, media optimisation, and process monitoring and control.

Integration of artificial intelligence (AI) and machine learning (ML) into cell line development workflows can accelerate the process of identifying high-producing clones and optimising culture conditions. AI and ML algorithms can analyse vast datasets from omics and process monitoring data to predict cell line performance and guide decision-making.

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