Editorial

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Cell culture processes for biologics manufacturing: recent developments and trends

As the number and quantity of biologics produced by mammalian cell cultures continues to increase, it is of great interest for the biopharmaceutical industry to continue to develop more efficient cell culture processes.

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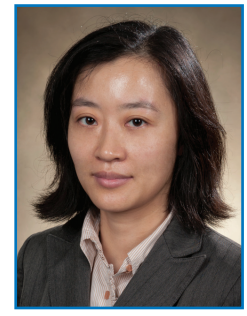

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The US sale of biopharmaceuticals, mainly therapeutic proteins or biologics derived from living cells to treat or cure diseases, is expected to increase to US$144 billion by 2016, with a compound annual growth rate of 11.2% [101]. In the last two decades, the biopharmaceutical industry has increasingly moved toward biologics manufactured by mammalian cell culture processes. Mammalian cells such as Chinese hamster ovary (CHO) cells are vital to the manufacturing of recombinant proteins with complex structures that require sophisticated post-translational modifications critical to their biological functions [1,2]. Over 60% of biopharmaceuticals currently in clinical trials or approved by the US FDA in the last few years are therapeutic proteins, especially antibodies for treating cancers and autoimmune diseases, produced by using mammalian cells. Therapeutic proteins, such as tissue plasminogen activator and erythropoietin, produced by CHO cells in the earlier cell culture processes, which generally had very low product titres (less than 100 mg/l) and productivity (less than 1 pg/cell/day or 10 mg/l/day), require a relatively low dosage (25–125 IU/kg) for disease treatment. However, for therapeutic antibodies treating cancers, the dosage and total amount of the protein required for treating each patient are much higher (4–6 mg/kg). It has been estimated that the annual demand of a single-branded therapeutic antibody product could reach 10,000 kg/year [3], which requires ten- to 100-fold higher production capacity compared with other therapeutic proteins such as tissue plasminogen activator. It is, therefore, imperative for the biopharmaceutical industry to develop advanced platform cell culture technology that can not only significantly shorten the development timeline, but also greatly increase production efficiency with a much higher product titer and yield. With the increasingly tightening FDA regulations on biologics and 'biosimilars' (generic or follow-on versions of biologics), it is also important to develop online process analytical technology (PAT) for better process and product quality control.

The development of a high-yield protein-producing cell line is the first step in developing an efficient cell culture process for the manufacture of a therapeutic protein, but it is time consuming. Several mammalian cell lines, including CHO, lymphoma (NS0, SP2/0) and human embryonic kidney, have been developed and used to produce various therapeutic proteins at high levels [1]. These cells typically contain a stable gene expression cassette with various key elements, including an mRNA transcription promoter/enhancer (such as MPSV, RSV, VISNA, EF1-α, SV40 and CMV), translation-enhancing sequences (PolyA, Kozak and IVS) and a selection marker gene (G418, hygromycin B, puromycin and Zeocin). Chemicals such as methotrexate and methionine sulfoximine can be used to select and amplify gene copy number and, thus, increase its expression level. The protein expression level can also be

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significantly improved by increasing the transcription and translation efficiencies of interested genes in the cells using novel elements for the expression vector [4–9]. For example, the epigenetic regulatory elements, such as selexic genetic elements, can be used to promote the homologous integration of expression plasmid and prevent transgene silencing [5]. Also, the ubiquitous chromatin opening element that can open up chromatin can be used to generate stable expression irrespective of chromosomal integration site [7,9]. Other vector elements, including special promoter, enhancer element, intron, chromatin modifier and insulator have also been used to develop cell line platforms with improved protein expression levels [10–12]. Transient gene expression systems have also been developed and used to shorten the development timeline for generating clinical-grade biologics. For example, an epi-CHO transient expression system was developed using *cis*-elements (such as OriP) to maintain the transfected plasmid episomally [13]. However, transient gene expression suffers from process instability and batch-to-batch variation and is not used in large-scale manufacturing processes.

Medium development and optimization is another critical step in developing a cell culture process for biologics manufacturing. Today, the industry is focusing on chemically defined media that are serum-free and animal-origin-free to reduce variation and contamination risk from undefined medium components. However, such a medium requires systematic evaluation of additives or nutrient supplements needed to support cell growth and optimize the protein expression. Advanced analytical and high-throughput screening (HTS) methods are, therefore, important to the development of medium used in a cell-culture process [14]. HTS using scale-down bioreactors, such as multitube microbioreactors and SimCell™, can shorten the timeline for developing customized media and accelerate process development [15]. Intracellular metabolic profiling and understanding extracellular medium component usage are essential to the rational design and development of cell line-specific medium and feeding nutrients. Product quality (such as glycan profile and charge variants) could also be improved or better controlled by using quality-by-design through scaled-down cell culture models [16]. For example, using the high-throughput process combined with design of experiments can facilitate rapid and parallel analyses of product quality. PAT, which uses online sensing methods such as near infrared spectroscopy to monitor critical process parameters affecting critical quality attributes, is widely used in controlling pharmaceutical processes and implementing quality-by-design.

Up to 60% of product development costs are associated with process development for producing clinical materials.

Up to 60% of product development costs are associated with process development for producing clinical materials. [17]. However, the depletion of critical nutrients in a fed-batch process could induce apoptosis and cell death, decrease productivity and reduce final protein quality. Therefore, it is important to develop appropriate feed formulation and feeding strategy through rational design based on cell metabolism and physiology. Productivity and product quality can also be increased or controlled through targeted changes to cell metabolism by timed addition of chemicals such as sodium butyrate, a technology called metabolic process engineering [18]. Metabolic process engineering can manipulate cell metabolism, growth and protein expression at the process level instead of through genetic engineering of the cell, which is done during cell line development. Feeding can be controlled based on the concentrations of glucose and lactate, or metabolic activities through the analysis of oxygen uptake rate, carbon dioxide evolution rate, or cell growth using online probes. Fed-batch processes are limited by the accumulation of toxic metabolites such as lactate and ammonia, which can be reduced by using a proper expression system (e.g., GS vector developed by Lonza) and optimizing feeding schemes. Perfusion to continuously refresh the culture medium can also be used to reduce end product inhibition and achieve a high volumetric productivity, thus allowing for integrated continuous manufacturing with greatly increased production capacity. However, the perfusion process requires cell-retention devices, is more difficult to control, and has limited flexibility.

The bioreactor plays the key role in the upstream cell culture process. Stainless steel stirred-tank bioreactors up to 20,000 l are commonly used. Cell culture process scale-up is limited

by the complex hydrodynamics that not only affect oxygen transfer to support cell growth but could also impose detrimental shear stress on cells. Aeration enriched with oxygen and through well-designed gas spargers could address oxygen supply issues. Cell damage caused by shear force can be addressed by appropriate impeller design and by the addition of shear-protecting agents. In the last decade, the industry has seen increased uses of single-use or disposable bioreactors, replacing stainless steel tanks in the seed culture train for cell expansion and even at the production stage, thanks to the increased product titer that now requires a smaller reactor with the same production capacity. Single-use bioreactors reduce the process downtime due to equipment cleaning and sterilization and have become the preferred choice at many contract manufacturing sites where the production timeline is tight and frequent process changeover is a norm.

As the number and quantity of biologics produced by mammalian cell cultures continues to increase, it is of great interest for the biopharmaceutical industry to continue to develop more efficient cell culture processes with higher product quality at lower manufacturing costs through the development of more efficient cell lines and the optimization of media formulation, feeding strategy, and bioreactor design and operating conditions. The recent advances in genomics, proteomics and metabolomics have provided additional powerful tools to increase understanding of cell metabolism and physiology, which could accelerate both drug discovery and bioprocess development [19].

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