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Platform Technology for Therapeutic Protein Production

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1.1 Introduction

In 1987, the human tissue plasminogen activator (trade name: Activase®) was the first therapeutic protein produced in Chinese hamster ovary (CHO) cells to receive US Food and Drug Administration (FDA) approval, which triggered the emergence of mammalian cell culture for production of biopharmaceuticals [1]. Therapeutic proteins are effective drugs for many diseases including diabetes, rheumatoid arthritis, clotting disorders, and cancers because of their highly specific functions with reduced side effects and no immune response [2, 3]. With the increasing number of therapeutic proteins, the biopharmaceutical market has expanded dramatically over the past few decades. The global market value of therapeutic proteins reached $140 billions in 2014, and AbbVie's Humira® (adalimumab), one of the profitable drugs in the biopharmaceutical industry, generated worldwide sales of $13.9 billions in 2015 [4, 5].

From 2011 to 2015, 40 novel therapeutic proteins were approved by the FDA, and nearly 70% of therapeutic proteins are produced in mammalian cells, particularly CHO cells, because of their capability for humanlike post-translation modification (PTM) including glycosylation and protein folding [6]. Notably, 7 out of 10 top-selling blockbuster therapeutic proteins were produced in mammalian cells in 2015 (Table 1.1), and this trend of the prominence of mammalian manufacturing platforms over microbial manufacturing platforms will continue with the steady increase in the proportion of complex molecules in the pipeline at both the qualitative and quantitative levels [5].

Therapeutic protein production, however, requires time-consuming and complicated processes. In a mammalian manufacturing platform of therapeutic proteins that includes the cloning of a target gene into an appropriate expression vector, the selection of a suitable host cell line for the target product, and final

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Cell Culture Engineering: Recombinant Protein Production,
First Edition. Edited by Gyun Min Lee and Helene Faustrup Kildegaard.

Table 1.1 The 10 top-selling therapeutic proteins in 2015.

Ranking	Product (active ingredient)	Product category	Target[a)]	Therapeutic indication	Company	Approved/ expiry (EU, US)	2015 Sales ($ millions)	Host
1	Humira (adalimumab)	Human mAb	Anti-TNF	Rheumatoid arthritis	AbbVie & Eisai	2003, 2002/ 2018, 2016	13 932.00	CHO
2	Enbrel (etanercept)	Fusion protein (receptor – IgG fragment)	Anti-TNF	Rheumatoid arthritis	Amgen, Pfizer, Takeda Pharmaceuticals	2000, 1998/2015, 2028	9008.70	CHO
3	Rituxan/ Mabthera (rituximab)	Chimeric mAb	Anti-CD20	Non-Hodgkin's lymphoma	Biogen-IDEC, Roche	1998, 1997/ 2013, 2016	7395.00	CHO
4	Lantus (insulin glargine)	Peptide	rh insulin analog	Diabetes mellitus	Sanofi	2000, 2000/ 2014, 2014	7095.40	*E. coli*
5	Avastin (bevacizumab)	Humanized mAb	Anti-VEGF	Metastatic colorectal cancer, glioblastoma, metastatic renal carcinoma	Roche/ Genentech	2005, 2004/ 2019, 2017	7014.20	CHO
6	Herceptin (trastuzumab)	humanized mAb	Anit-HER2	Breast cancer, gastric cancer	Roche/ Genentech	2000, 1998/ 2014, 2019	6862.60	CHO
7	Remicade (infliximab)	Chimeric mAb	Anti-TNF	Crohn's disease	J&J, Merck & Mitsubishi Tanabe Pharma	1999, 1998/ 2015, 2018	6826.10	Sp2/0
8	Neulasta (pegfilgrastim)	Peptide	PEGylated rh G-CSF	Chemotherapy-induced neutropenia	Amgen	2002, 2002/ 2015, 2014	4715.10[b)]	*E. coli*
9	Eylea (aflibercept)	Fusion protein (receptor – IgG Fc)	Anti-VEGF	Neovascular (wet) age-related macular degeneration	Regeneron, Bayer	2012, 2011/ 2020, 2021	4089.00[b)]	CHO
10	Lucentis (ranibizumab)	Humanized IgG fragment	Anti-VEGF	Neovascular (wet) age-related macular degeneration	Roche/ Genentech, Novartis	2007, 2006/ 2016, 2016	3580.00[b)]	*E. coli*

a) In the case of peptide products, other general names of products, not generic and trade names, are described.
b) Full-year 2015 financial reports of Amgen, Regeneron, Bayer, Roche/Genentech, and Novartis.
Source: Adapted from Morrison 2016 [4] and Walsh 2014 [5].

processing for commercialization, many resources are required to ensure quality control at every step [6]. Furthermore, the mammalian cell culture that involves CHO cells is considered to be difficult because of low yield, complexity, price of media, and obstacles to optimization of culture conditions. Traditionally, various parameters in the production processes have had to be independently optimized for each target product because of clonal variability and product dependency. The effect of each parameter, such as the type of the host cell line, expression vector design, screening and selection methods, media composition, feed media, and culture conditions, including temperature, pH, and agitation speed, on protein productivity and product quality is highly dependent on the specific cell lines [7, 8].

Along with the technical advances in the upstream process development, specific productivity of over 20 pg/cell/day and product titer of over 10 g/l have been reached in many cases in the biopharmaceutical industry [8, 9]. The improvement of specific productivity and final yield has been achieved not only through expression vector and clone selection methods but also through the enhancement of commercial culture media and optimization of operational conditions. Today, the focus in mammalian cell culture process development has changed from higher productivity to proper and consistent quality with higher productivity at all developmental stages and at large scales [10].

In the following sections, we provide a general overview of platform technology for therapeutic protein production that has been commonly used in mammalian cell culture. Because of the complexity and diversity of the field, there is limited room to cover all the details in this chapter. Rather, we include references for more detailed information, and we devote special attention to general guidelines and considerations for bioprocess development. Then, we introduce the trends in platform technology development that are applied recently in this field (Figure 1.1).

1.2 Overall Trend Analysis

1.2.1 Mammalian Cell Lines

Recombinant therapeutic proteins are mainly produced in mammalian host cell lines, including NS0 murine myeloma, CHO, and human embryonic kidney (HEK) 293 cells. Humans and other mammals share a closer evolutionary lineage compared to microorganisms such as *Escherichia coli* (*E. coli*), which means that mammalian cells are suitable for the generation of complex and highly valuable humanlike proteins [11, 12].

Murine NS0 cells were initially used in the production of therapeutic antibodies in the biopharmaceutical industry. NS0 cells lack endogenous glutamine synthetase (GS) enzyme activity, which makes them suitable for the use of the GS/methionine sulfoximine (MSX) amplification system. Although high

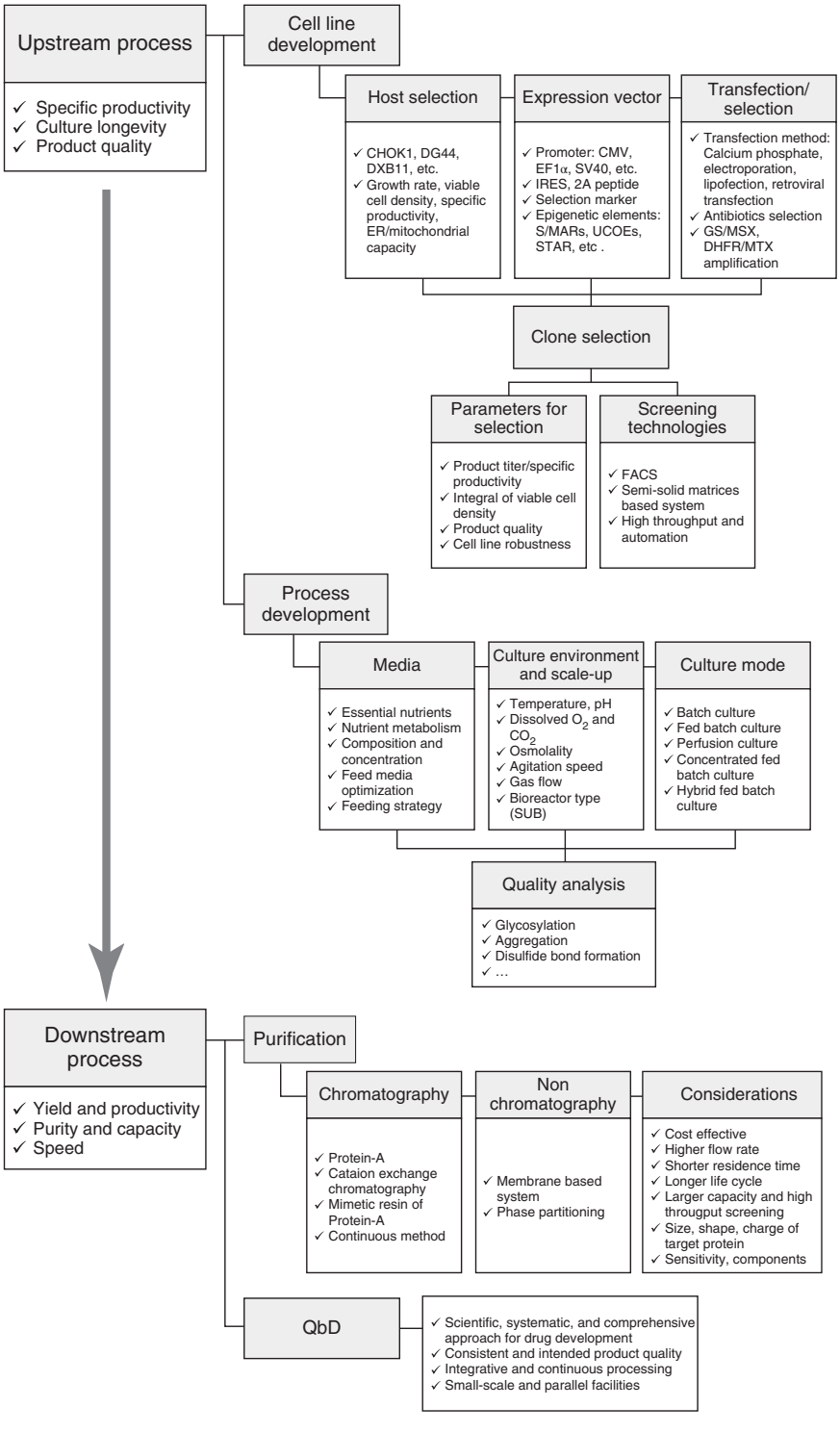

Figure 1.1 Optimization parameters in upstream and downstream process.

antibody productivity has been achieved in GS-NS0 cells, *N*-glycolylneuraminic acid-bound proteins produced from NS0 cells led to an immunogenicity concern in humans. Therefore, NS0 cells have limited use in therapeutic protein production today [8, 13].

Human cell lines including HEK293 have the ability to produce proteins mostly like natural human products, which is their main advantage over other expression systems. Recently, several therapeutic proteins produced from HEK293 cells have been approved by the FDA or the European Medicines Agency (EMA). A major concern with the use of human cell lines is low productivity and the risk of viral infection [14].

For these reasons, CHO cells are the most predominantly used mammalian host cell lines in the production of various therapeutic proteins, including monoclonal antibodies (mAbs), cytokines, and fusion proteins. Nearly 70% of all recombinant therapeutic proteins produced today are made in CHO cells because of several key advantages over other host cells, such as safety regarding human pathogenic viruses, ease of growth in a large-scale suspension culture, and the ability to express humanlike proteins along with humanlike PTMs. Furthermore, CHO cells have strong gene amplification systems such as dihydrofolate reductase (DHFR)/methotrexate (MTX) and GS/MSX to improve protein production, and various genetic manipulation strategies have been developed to improve protein production and product quality [15, 16].

1.2.2 Brief Introduction of Advances and Techniques

With the expansion of biopharmaceutical markets, the improvement of mammalian cell lines is a key challenge to meet the higher demand for therapeutic proteins. Because the biopharmaceutical industry pursues inexpensive and high-yield manufacturing processes to maximize production yields at low cost, several strategies have been developed and implemented in recombinant mammalian cell line generation and cell culture processes.

Strategies to improve therapeutic protein production in mammalian cells can be divided into two major categories: (i) increasing cell mass and (ii) increasing specific productivity. Increasing cell mass through a fast growth rate, maximum viable cell density, and/or longer culture duration has been achieved by process and media optimizations and genetic manipulation of several pathways involved in proliferation, apoptosis, autophagy, and cellular metabolism [13, 15]. Improving specific productivity by genetic manipulation has also been successfully implemented with CHO cells, such as the engineering of secretion, chaperone, cell cycle, transcription, and translation-related genes [15]. Protein quality, including glycosylation, is also a critical factor to determine the efficacy and stability of therapeutic proteins, which has been improved by the optimization of culture conditions, media, and feeding strategies and genetic manipulation of genes related to protein folding and glycosylation [17].

In 2011, the CHO-K1 genome was first sequenced by Xu et al., enabling more detailed and accurate bioinformatics analyses [18]. Previously, researchers had to infer genome information from other published mammalian genomes such as mouse and human genomes, raising inaccuracy issues regarding the CHO

genome sequence. Since 2011, draft genomes of not only Chinese hamster but also six CHO cell lines including CHO-K1, DG44, and CHO-S have been sequenced and published [19]. Additionally, transcription start sites, expressed gene profiles, miRNA profiles, and secreted and host cell proteomes were serially identified and published, which has facilitated better understanding of CHO cells and substantially supported research efforts in cellular engineering [19, 20].

1.3 General Guidelines for Recombinant Cell Line Development

The production of therapeutic proteins can be achieved by either a transient or stable gene expression system in mammalian cells. Given the fact that stable gene expression remains the preferred choice for the large-scale production of therapeutic proteins in the biopharmaceutical industry, we emphasize stable cell line generation in this section.

A typical process of recombinant cell line development (CLD) for high-level therapeutic protein production includes the introduction of the exogenous gene of interest (GOI) into host cell lines in the form of an expression vector and the selection of stable and high-producing clones. Selected high producers are further evaluated in downstream processes with regard to a sustainable high production level with proper product quality within the acceptable range. There have been significant advances in host cell selection and engineering and expression vector engineering toward increased productivity and robust clone selection, as described in detail in the following sections.

1.3.1 Host Selection

Within selected mammalian expression systems among various manufacturing platforms, genetic and phenotypic diversity exists. Chinese hamster ovary cells have a family of cell lines, referred to as K1, DG44, DXB11, CHO-Toronto, CHOpro3-, and CHO-S, with distinct genomic backgrounds and physiological diversity (reviewed in [21]). Genetically divergent host cell lines have shown phenotypic differences with regard to growth rate, viable cell density, specific production rate, and ER/mitochondrial capacity [22, 23]. To date, DHFR-deficient CHO host cell lines, CHO-DXB11 and CHO-DG44, and CHO-K1 cell lines have been preferred for the production of therapeutic proteins in the industry because of their well-established gene amplification systems, referred to as DHFR and GS systems, respectively.

The individual host cell line itself exhibits phenotypic heterogeneity within the cell population, i.e. clonal variation, which can be derived from either the inherent genomic plasticity of immortalized mammalian cell lines or nongenetic functional diversity such as stochastic gene expression [24, 25]. The heritable nature of functional properties including the specific growth rate and surface glycan content emphasizes that it is possible to screen the host cell population for the isolation of clonal derivatives with desirable attributes for biomanufacturing [26].

1.3.2 Expression Vector

The GOI encoding model proteins in the expression vector is delivered into the host cells. Mammalian expression vectors typically contain separate gene expression cassettes – one for expression in mammalian cells and the other for plasmid replication in bacteria. Within the cassettes for mammalian gene expression, selectable marker gene(s) and target product gene(s) are driven by promoters/enhancers such as cytomegalovirus (CMV), elongation factor α (EF1α), or simian virus 40 (SV40) promoter and terminated by 3′ polyadenylation signal sequences such as SV40 or bovine/human growth hormone polyadenylation sequence [8]. Based on this basic vector configuration, expression vector engineering has led to increased productivity and stability of production cell lines through modulation of the transcriptional activity of either GOI or marker genes (reviewed in [2]). The coexpression of GOI and marker genes via internal ribosome entry site (IRES) elements or self-cleaving 2A peptides allows for the selection of producers devoid of false positive survivors without expressing GOI [2, 27]. Additionally, selection marker attenuation through the use of a weak promoter, deoptimization of the marker gene, or insertion of mRNA/protein destabilizing elements weakens the selection marker, resulting in high selection stringency and the selection of high producers [2, 28]. Some cis-acting DNA regulatory elements have added value in vector engineering because of augmented attention directed to the epigenetic regulation of GOI in CLD (reviewed in [29]). The inclusion of epigenetic elements including scaffold/matrix attachment regions (S/MARs), ubiquitously acting chromatin opening elements (UCOEs), and the stabilizing and antirepressor (STAR) element can not only promote gene expression by remodeling the chromatin landscape so that it is favorable to high transgene expression but also allow for stable expression in long-term cultures because of its antisilencing effect [2, 15, 29]. Recent advances in promoter engineering efforts include either modifying natural promoters, e.g. mutation of methylation-prone CpG sites or insertion of the methylation-resistant core CpG element for enhanced stability [30, 31], or constructing synthetic promoters through the bottom-up assembly of several sequence elements such as transcription factor regulatory elements (TFREs) to core promoters. This effort may drive the tailored control of recombinant gene transcription for the next generation of mammalian cell factories (reviewed in [32]).

1.3.3 Transfection/Selection

The choice of how to introduce vector DNA into mammalian cells, i.e. transfection, is usually determined by its efficiency and toxicity. In contrast with the transient gene expression, in which many factors, including cost effectiveness vs. transfection efficiency and the cytotoxicity of the transfection reagent, must be considered for efficient large-scale transfection, the stable gene expression system allows an easy choice of transfection methods, as it merely requires small-scale transfection in one shot followed by selection of the transfected population. Among several transfection methods, such as calcium phosphate, electroporation, lipofection, and retroviral transfection, nonviral gene transfer

methods, such as lipofection and electroporation, are commonly used to generate stable cell lines.

Selectable marker genes in the expression vector enable the selection of the transfected population and the subsequent subpopulation that harbors stably integrated GOI in the chromosome, owing to cell growth and survival advantages upon the expression of marker genes in the presence of selection pressure. A variety of metabolic and antibiotic selectable markers are used, where a single or double selection approach can be applied [8]. The metabolic selection system, including GS and DHFR systems coupled with MSX and MTX addition, respectively, is frequently used in the biopharmaceutical industry. These systems exploit the complementation of glutamine or nucleoside precursor (hypoxanthine and thymidine) auxotrophy by transfected GS or DHFR encoding genes. In combination with the removal of glutamine or nucleoside precursors in the media, the addition of GS and DHFR inhibitor (MSX and MTX, respectively) not only improves the selection stringency but can also result in increased productivity through one step or stepwise-increased gene amplification [16].

1.3.4 Clone Selection

The selection process generates stable cell lines, more accurately, a stable pool of cells harboring GOI at random locations in the genome. Randomly integrated transgenes confer highly variable expression levels, possibly because of the different chromosomal context of integration sites, transgene copy-number variation, and disruption of the genome by gene amplification. It necessitates considerable screening effort to select production clones with the desired clone attributes (see Section 1.4.1). Several clone screening methods have been developed to accelerate CLD while increasing the predictability of clone assessments and lowering the number of clones to be assessed (see Section 1.4.2). Typical selection strategies start with a few hundred clones and end with a small number (~10–20) of candidate production cell lines through each assessment stage [33].

1.3.4.1 Primary Parameters During Clone Selection

The recombinant clone screening process is aimed at the isolation of the "right" candidate production clones through the evaluation of several features early in CLD for the large-scale production of therapeutic proteins in stirred tank bioreactors. Such parameters include (i) a high product yield, i.e. a product titer that is a function of high specific productivity (q; above 20 pg/cell/day to meet industrial demand) and/or the time integral of viable cell concentration (IVCC), (ii) cell line stability, which refers to maintaining the production capability over an extended period during subculture and scale-up, (iii) the desired product quality (e.g. glycosylation, proteolytic processing, molecular integrity, and aggregation) that meets predefined criteria with high consistency and comparability, (iv) cell line robustness, including acceptable cell growth with high viability and the preferred metabolism, such as low lactate synthesis, that fits the final large-scale production process [8, 16, 33].

1.3.4.2 Clone Screening Technologies

When it comes to screening a large number of clones, significant progress has been made in clone screening technologies, pursuing efficient high-throughput screening methods, apart from the traditional time-consuming and labor-intensive limiting dilution cloning. Most high-throughput methods rely on both automation of the cloning step and capture of the product secreted by the clones. Fluorescence-activated cell sorting (FACS) and semisolid matrix-based systems such as ClonePix FL™ or CellCelector™ allow the rapid and high-throughput isolation of high-producing cell lines with a high level of confidence in "clonality" (reviewed in [2, 28]). These are fluorescence-based systems, necessitating conversion of the amount of secreted recombinant protein into a fluorescent signal. FACS, which was adopted originally to detect fluorescent cells, can be used to isolate high producers when combined with a labeling strategy: (i) capturing the secreted target protein on the cell surface or in close proximity to the individual clonal population stained with a fluorescent antibody or (ii) measuring the expression of surrogate reporters genetically linked to the GOIs, which include fluorescent proteins or surface marker proteins labeled with a fluorescent antibody [2, 28]. The use of semisolid media enables to limit the diffusion of secreted proteins while supporting cell growth and thereby facilitates the isolation of high-producing cell lines in a high-throughput manner when coupled with automated detection and clone picking [34, 35]. A complete automation system from clone selection to cell culture provides the highest throughput for the isolation of high-producing cell lines by employing the aforementioned screening methods and robotic systems [36, 37].

Despite the adoption of effective productivity screening technologies in the early stage of CLD, there is still the challenge of the performance consistency of candidate clones that have been adapted from static to suspension growth (in the case of the use of semisolid media) and scaled up to a large volume, manufacturing relevant production platform (see Section 1.4) [38]. Various scale-down models utilizing miniaturized systems with analysis capabilities have been developed in an attempt to simulate the large-scale performance of clones and to streamline the CLD [28].

1.4 Process Development

Over the past few decades, a more than 100-fold improvement of titers in mammalian cells has been achieved by advances in CLD and selection techniques as well as the optimization of media and culture processes. Over 10 g/l of antibody concentrations in the fed-batch or perfusion process has been reported in many cases. Although the selection of the most suitable clone for the stable production of therapeutic proteins is one of the most important steps in the upstream process, cell performance, including productivity, product quality, and metabolic profiles, depends strongly on cell culture conditions such as the media, environmental parameters, culture mode, and scale-up processes [7, 8].

1.4.1 Media Development

Early mammalian cell culture media contained bovine serum and animal-derived raw materials that were a complex mixture of unknown components. Because of safety concerns, serum-free media containing non-animal-derived hydrolysates such as soy, wheat, and yeast instead of animal-derived materials were developed and commercially available in the 1970s and 1980s [39, 40]. Nowadays, to avoid lot-to-lot variation, fully chemically defined media without any unknown components have been developed and implemented in small-scale as well as in large-scale culture processes [41, 42].

Media are a critical factor for improving cell growth, productivity, and product quality, so the optimization of culture media is necessarily considered in the early stage of CLD because of the clonal variation of metabolism, nutrient consumption, and interactions with components among production cell lines. A traditional approach for media optimization is based on the titration of individual components, but it is labor-intensive and time-consuming to evaluate the effect of numerous components in the media. To reduce the experimental efforts, a combination of statistical design of experiment (DoE) approaches with a high-throughput scale-down method is commonly used in industrial processes. DoE is useful not only in media optimization steps but also in the development of feed media for fed-batch culture [7, 8].

1.4.2 Culture Environment

Optimization of culture environmental parameters, such as culture temperature, pH, agitation speed, dissolved oxygen and carbon dioxide, gas flow rate, osmolality, and more, is also required for a high yield of therapeutic proteins with reliable product quality. As with culture media, these parameters must be optimized for the specific production cell line because the effect of each parameter on culture performance, productivity, and product quality varies significantly from clone to clone [43, 44]. For example, culture temperature is the most commonly and easily adjustable culture parameter. To extend culture longevity and improve productivity, culture temperature is often lowered from 37 °C to 30–35 °C at 48–72 hours post inoculation, depending on the production cell line [45]. The effect of each parameter on culture performance and product quality has been well summarized previously [8].

A typical stirred tank bioreactor is equipped with temperature, agitation, pH, dissolved gas, and sometimes osmolality controllers. A traditional method of culture environment optimization was performed based on the control of individual parameters in a bench-scale bioreactor. Nowadays, a combination of statistical DoE approaches with a multiparallel microscale bioreactor system such as Ambr® can unlock the bottleneck in process development.

1.4.3 Culture Mode (Operation)

The mode of mammalian cell culture is classified as a batch, fed-batch, or continuous (chemostat and perfusion) culture based on the mode of feeding the bioreactors. In the batch culture, the bioreactor is fed only once at the beginning of

the culture with a media containing all nutrients and no more feeding, except for oxygen, after that. It is convenient to set up and maintain, and it is relatively safe against contamination. However, the culture duration is relatively short because of limiting nutrients or the accumulation of toxic by-products, resulting in relatively low productivity. As an alternative, fed-batch and perfusion cultures have been commonly used in industrial scales.

In the fed-batch culture, a fresh volume of selected nutrients that are depleted during cell culture is added to the bioreactor to improve cell growth, culture longevity, and productivity. To improve the efficiency of fed-batch culture, components of the feed media and feeding strategies must be optimized. For example, glutamine is an essential component as a main nitrogen source as well as an energy source in mammalian cell cultures. During the culture, glutamine is metabolized into ammonia, which is known to reduce cell growth, protein production, and product quality. The accumulation of ammonia was significantly reduced in fed-batch cultures of CHO cells where glutamine concentration was maintained at a low level by feeding with the necessary amount of glutamine [46]. Currently, fed-batch cultures are most widely used for the large-scale commercial production of therapeutic antibodies [47].

In perfusion culture, cells are maintained at a much higher concentration over even months by feeding fresh media and simultaneously removing spent media while keeping cells in the bioreactors using cell retention devices. Perfusion culture has some drawbacks, such as complex and expensive equipment, risk of contamination, and regulatory uncertainties. Nevertheless, perfusion culture is used for low titer or unstable products such as recombinant blood clotting factors and enzymes because of the short retention time of the product in the bioreactor. Numerous biopharmaceutical companies have started to use the perfusion technology along with disposable equipment and cell retention devices such as alternating tangential flow (ATF). Using perfusion culture, they can achieve much higher cell density and product yield than with fed-batch culture, achieving considerable cost savings [48, 49].

1.4.4 Scale-up and Single-Use Bioreactor

The mammalian cell culture process is usually performed in bench-scale bioreactors (1–2 L) and then scaled up to larger bioreactors (10 000–20 000 L) for commercial production purposes [50]. The aim of scale-up is to produce larger quantities of therapeutic proteins with equivalent product quality. Process scale-up, however, remains a challenging task because of difficulties in maintaining agitation efficiency, avoiding hydrodynamic shear and bubble stress, efficient oxygen and carbon dioxide transfer, etc. Therefore, a systematic approach for improving scale-up activities is necessary [50].

Today, single-use bioreactor systems are being increasingly used in mammalian cell culture as a new trend. As the product titers in mammalian cell cultures have been increased significantly over the past decade, a traditional bioreactor over 10 000 L may not be necessary in manufacturing therapeutic proteins. The scale of single-use bioreactors reaches up to 2000–2500 L. They have the advantages of lower investment and operational costs, flexibility, higher process replication,

and reduced contamination rates compared to traditional bioreactors. However, there is still some work to be done to improve and optimize single-use bioreactor systems, particularly mixing and aeration. In addition, the amount of disposable materials generated in a single-use bioreactor system is a concern [7].

1.4.5 Quality Analysis

Maintaining consistent and comparable product quality is one of the most important and challenging parts of therapeutic protein production because product quality significantly affects the safety and efficacy of the drugs. Measurement of the safety and efficacy of drugs is subdivided into biological activity, pharmacokinetics, pharmacodynamics, immunogenicity, and overall safety/toxicity. Quality attributes have also been subdivided into product-related impurities containing aggregation, fragmentation, glycosylation, disulfide bond formation, oxidation, deamidation, C- and N-terminal modifications, and so on, as well as process-related impurities containing DNA, host cell proteins, and raw materials. These quality attributes are highly affected by the cell line, culture media, and process conditions, and these factors must be optimized for specific cell lines and products [51, 52].

Among the several quality attributes, glycosylation is an important factor determining the quality of the therapeutic proteins. Glycosylation is easily affected by upstream process parameters such as the host cell type, glucose level, glutamine level, cell viability, culture temperature, and pH. Various approaches including host cell engineering and process development based on high-throughput and DoE methods have been applied to achieve the desired product quality [17].

1.5 Downstream Process Development

Dramatic improvement in the productivity in large-scale processes has shifted the bottleneck from production to the purification step in therapeutic protein production. A key challenge in the purification step is the development of efficient and cost-effective systems with higher yield and purity. Many recent advances have been achieved in downstream process through the implementation of a high-throughput process, improved platform technologies, and unit operations based on quality by design (QbD) and DoE experimental optimization. In particular, QbD, a new concept for regulatory needs, has resulted in a noticeable change in the perspective on the development of downstream processing strategies in the biopharmaceutical industry.

1.5.1 Purification

Traditionally, two major methods, chromatographic and nonchromatographic separation, have been used for protein purification. The chromatographic method includes affinity, ion exchange (IEX), hydrophobic interaction (HIC), size exclusion, and mixed mode chromatography [7]. The most common affinity

chromatographic process is protein-A method, which has been used for the capture and purification of mAb for over a decade. The protein-A resin has a dynamic binding capacity ranging from 15 to 100 g mAb/l with a high flow rate. However, the protein-A method has some drawbacks, such as resin leaching, nonspecific binding of impurities, including host cell proteins and DNA, and high price [53, 54].

Cation exchange (CEX) chromatography and mimetic resin of protein-A have been applied as an alternative to the traditional protein-A method. Subsequently, IEX and HIC are frequently used to purify non-mAb target proteins that are not tagged with a purification motif or to improve the purity of mAb because they have higher resolution in differentiating among related protein variants. Although these separation methods are much more cost-effective than protein-A, they suffer from limited capacity and elution issues because of the high affinity between the displacer and resins. As alternative methods, several optimization strategies for the resins, elution conditions, and operation modes were tested based on the DoE and modeling approaches [53, 55]. Recently, continuous chromatography methods such as multicolumn countercurrent solvent gradient purification have been implemented for the purification of recombinant streptokinase, mAb, and antibody fragments, along with cost savings and better productivity [7, 56–58].

Nonchromatographic separation includes a membrane-based system and phase partitioning; it is an alternative method to reduce or exclude chromatographic operations in the downstream process [59, 60]. The membrane-based system, which depends on the size, shape, and/or charge of the target proteins, has the advantages of low cost and ease of scaling up. The phase partitioning method, which is based on mixing two aqueous solutions of structurally different components, has the advantages of low cost, implementation of high-throughput screening, and combination of concentration and purification in a single step with a large scale. In the biopharmaceutical industry, the membrane-based system has been studied with regard to high permeability, capacity, sterility, and purification of large biomolecules, while the phase partitioning method has been studied to deal with issues of reduction and sensitivity caused by the complex interaction of multiple components and the feed stream variability [7, 56, 57].

1.5.2 Quality by Design (QbD)

With an evolved understanding of the interactions between process parameters and product quality in mammalian cell cultures, a new concept, QbD, has been implemented in the biopharmaceutical industry. The aim of biopharmaceutical development is to design a quality product to meet patient needs and to consistently deliver the intended product performance. Because developmental strategies for therapeutic proteins differ from company to company and from product to product, more systematic approaches, such as the integration of prior knowledge, the relationship between a process and the quality attributes of the product using DoE, and quality risk management, have become necessary to enhance the desired quality of the product and help regulatory agencies to understand the strategies of a company. The QbD concept pursues a more

scientific, systematic, and comprehensive approach to discovering, developing, and manufacturing pharmaceutical products.

If the quality of the product is measured only at the final stage of manufacturing, it would be inefficient. Product quality should be monitored throughout the manufacturing process by implementing process design. For the successful implementation of QbD concepts, cooperation across a multitude of company teams from R&D to manufacturing to quality assurance and regulatory affairs is required. In 2003, the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use created a vision for a future pharmaceutical quality system that includes QbD concepts, and the FDA has started assessing the implementation and effectiveness of process design from development to manufacturing [61–63].

1.6 Trends in Platform Technology Development

1.6.1 Rational Strategies for Cell Line and Process Development

There exists a strong desire to understand molecular mechanisms underlying high productivity and protein quality in mammalian cells. With the recent emergence of both Chinese hamster and several CHO cell line genome sequences, various efforts have accelerated the development of next-generation CHO cell factories, which can be categorized into two areas: novel target/marker discovery and targeted approaches to CLD (Figure 1.2). Multiomic approaches including transcriptomics, proteomics, metabolomics, and more recent lipidomics data sets provide insights into physiological differences across production hosts and clones while suggesting potential engineering targets associated with desired attributes [64, 65]. Recently developed genome-scale models of CHO

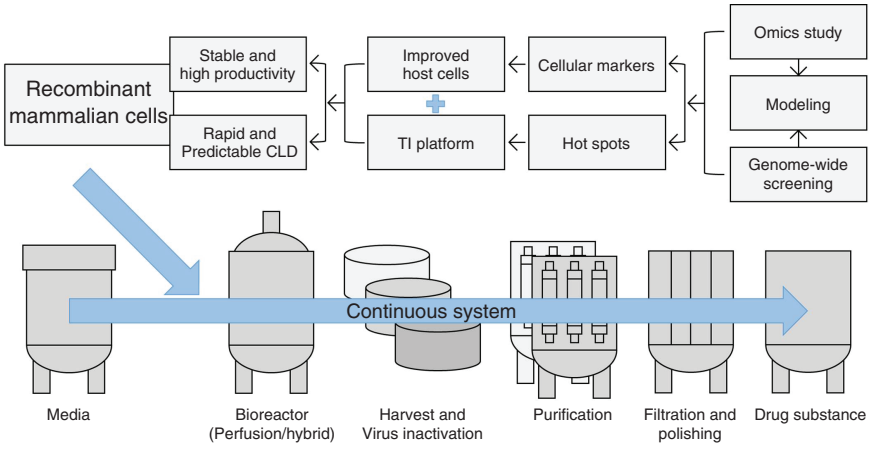

Figure 1.2 Schematic representation of trends in platform technology development encompassing the concepts from new CLD technology to continuous system.

metabolism have demonstrated the integration of high-throughput omics data sets and are capable of simulating experimentally observed phenotypes [66]. To identify a larger set of novel engineering targets, genome-wide screening methods employing RNA interference (RNAi) or genome editing tools such as a regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system can also be implemented [67, 68].

Such advances in systems biology approaches will result in the translation of knowledge gained to improve CLD and bioprocessing in mammalian cells through targeted engineering strategies. Candidate cellular markers identified from high producers or altered bioprocessing may serve as not only cell engineering targets but also readouts for the assessment of process development. The preselection of host cells or engineered host cells harboring desired marker attributes can expedite CLD with little effort for process development. Additionally, these markers will facilitate the generation of process analytical technology with the implementation of real-time monitoring and data analysis. Accumulation of the information from the iterative bioprocess can be translated for better process understanding and process control, ultimately leading to the successful settlement of QbD concepts.

Combined with improved host cells, targeted integration (TI)-based CLD offers new possibilities to shorten the CLD process with highly predictable gene expression. First-generation TI tools are based on recombinase-mediated cassette exchange (RMCE) systems, among which Cre/Lox and Flp/FRT systems have been widely adapted in CLD [2, 15, 16, 37]. The prerequisite platform cell lines are made through the traditional CLD process where reporter genes flanked by recombinase targeting sequences are integrated in highly transcribed chromosomal loci, the so-called hot spots. Afterward, the introduction of recombinase and targeting vectors with GOIs exchanges the reporter genes for GOIs, thereby allowing the use of hot spots for expressing therapeutic proteins. The advent of genome editing tools has enabled the more direct integration of GOIs into designated genomic sites in mammalian cells [68, 69]. The introduction of site-specific DNA double-strand breaks facilitates the integration of expression cassettes at precise locations by major DNA repair mechanisms, nonhomologous end joining (NHEJ), and homology-directed repair (HDR) [68]. The TI of transgenes allows stable and reproducible transgene expression between clones, suggesting the potential use of such TI platform technologies to overcome the limitations of clonal variation during CLD [69]. In the same context of target discovery, the systems biology-aided identification or prediction of hot spots will facilitate the implementation of this new CLD technology, which may allow the construction of high and stable production cell lines in a short time.

1.6.2 Hybrid Culture Mode and Continuous System

The fed-batch culture of mammalian cells for therapeutic protein production has been dominantly used in the biopharmaceutical industry for more than two

decades. However, fed-batch cultures also have some drawbacks, such as the accumulation of by-products and increased osmolality during the cultures. In addition, therapeutic proteins that have a growth inhibitory effect or are subject to degradation in culture conditions cannot be produced in fed-batch cultures because of the prolonged culture duration. Therefore, there is increasing interest in using the perfusion culture process in the biopharmaceutical industry.

With the trend of increased use of smaller equipment, an intensive and simple continuous process can provide operational flexibility and support process development, production for clinical study, and commercial manufacturing [70] (Figure 1.2). Furthermore, the low residence time of therapeutic proteins in a continuous system that includes perfusion culture facilitates the production of not only unstable proteins but also stable proteins such as mAbs [70, 71]. To advance this continuous system, stable cell lines with high productivity and the improvement of media performance are highly required. In addition, further development in downstream process is needed to meet commercial expectations [72].

Recently, a new culture method, referred to as a hybrid perfusion/fed-batch process, was developed to take advantage of both perfusion and fed-batch modes. The cell culture starts with the perfusion process for a few days to provide high cell density and then the operational mode changes to a fed-batch process for the remaining time. This process has shown a significant increase in productivity with short-duration cultures and low cost [73].

1.6.3 Recombinant Human Cell Line Development for Therapeutic Protein Production

Even though CHO cells have been dominantly used in therapeutic protein production because of the ability of humanlike glycosylated protein production, antigenic glycans such as *N*-glycolylneuraminic acid and α-galactose that are not presented in human-derived proteins are synthesized in CHO cells. The presence of antigenic glycan structures may result in increased immunogenicity, reduced efficacy, and altered pharmacokinetics in humans. In addition, some cases have been reported in which therapeutic proteins such as human interferon and recombinant factor VIII produced from CHO cells showed lower activity than those produced from HEK293 cells [74]. Therefore, the production of therapeutic proteins in human cell lines is expanding, and the FDA and EMA recently approved five drugs produced from HEK293 cells [14].

Two major concerns regarding the use of human cell lines are the risk of viral infection and low productivity. The current manufacturing process using human cells has multiple viral inactivation and clearance steps that may provide more effective viral clearance than CHO cells. However, the low productivity of human cells is still a concern. To overcome this issue, the implementation of a gene amplification system may be an efficient option. The recent TI-based CLD can also be applied to human cell lines [14, 74]. Because therapeutic protein production using stable human cell lines is a beginning step, more experience and research will be needed.

1.7 Conclusion

Health care systems are facing tremendous costs associated with the increasing demand for therapeutic proteins to address unmet medical needs. The increasing demand for therapeutic proteins has been a driving force for the development of platform technologies that can be applied for a variety of products in the same way. Successful platforms help to streamline upstream and downstream process development, enhance predictability and efficiency during CLD and manufacturing, and accelerate time lines to deliver high-value recombinant therapeutics. On the basis of the established platform, the process of fine tuning in the area of cell line engineering, such as manipulation of PTMs, changes in media composition and culture parameters will provide greater flexibility with less resource and effort expenditure in bioprocess development to produce diverse product lines from stable, easy-to-express proteins to labile, difficult-to-express proteins. Integrating emerging trends in CLD and the process control tool box, including real-time process monitoring and control, automation, and scale-down single-use bioreactors, promises the advent of continuous cell culture bioprocessing, which will lead to a decrease in infrastructure with greater cost efficiency and high productivity with consistent product quality. The advancements in robust platforms will facilitate biopharmaceutical drug discovery and development and contribute to disease treatment through the high accessibility of therapeutic proteins.

Acknowledgment

The authors thank the Novo Nordisk Foundation and Danish Council for Independent Research – Technology and Production Sciences (FTP) for funding.

Conflict of Interest

The authors declare no conflict of interest.

References

1 Jayapal, K., Wlaschin, K., Hu, W., and Yap, G. (2007). Recombinant protein therapeutics from CHO cells-20 years and counting. *Chem. Eng. Prog.* 103: 40–47.
2 Lai, T., Yang, Y., and Ng, S.K. (2013). Advances in mammalian cell line development technologies for recombinant protein production. *Pharmaceuticals (Basel)* 6 (5): 579–603.
3 Wurm, F.M. (2004). Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat. Biotechnol.* 22 (11): 1393–1398.

4 Morrison, C. and Lähteenmäki, R. (2016). Public biotech in 2015 – the numbers. *Nat. Biotechnol.* 34 (7): 709–715.

5 Walsh, G. (2014). Biopharmaceutical benchmarks 2014. *Nat. Biotechnol.* 32 (10): 992–1000.

6 Wells, E. and Robinson, A.S. (2017). Cellular engineering for therapeutic protein production: product quality, host modification, and process improvement. *Biotechnol. J.* 12 (1): https://doi.org/10.1002/biot.201600105.

7 Gronemeyer, P., Ditz, R., and Strube, J. (2014). Trends in upstream and downstream process development for antibody manufacturing. *Bioengineering* 1: 188–212.

8 Li, F., Vijayasankaran, N., Shen, A.Y. et al. (2010). Cell culture processes for monoclonal antibody production. *MAbs* 2 (5): 466–479.

9 Butler, M. and Meneses-Acosta, A. (2012). Recent advances in technology supporting biopharmaceutical production from mammalian cells. *Appl. Microbiol. Biotechnol.* 96 (4): 885–894.

10 Kelley, B. (2009). Industrialization of mAb production technology: the bioprocessing industry at a crossroads. *MAbs* 1 (5): 443–452.

11 Huang, Y.M., Hu, W., Rustandi, E. et al. (2010). Maximizing productivity of CHO cell-based fed-batch culture using chemically defined media conditions and typical manufacturing equipment. *Biotechnol. Progr.* 26 (5): 1400–1410.

12 Birch, J.R. and Racher, A.J. (2006). Antibody production. *Adv. Drug Delivery Rev.* 58 (5–6): 671–685.

13 Kunert, R. and Reinhart, D. (2016). Advances in recombinant antibody manufacturing. *Appl. Microbiol. Biotechnol.* 100 (8): 3451–3461.

14 Dumont, J., Euwart, D., Mei, B. et al. (2016). Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives. *Crit. Rev. Biotechnol.* 36 (6): 1110–1122.

15 Kim, J.Y., Kim, Y.G., and Lee, G.M. (2012). CHO cells in biotechnology for production of recombinant proteins: current state and further potential. *Appl. Microbiol. Biotechnol.* 93 (3): 917–930.

16 Noh, S.M., Sathyamurthy, M., and Lee, G.M. (2013). Development of recombinant Chinese hamster ovary cell lines for therapeutic protein production. *Curr. Opin. Chem. Eng.* 2: 391–397.

17 Hossler, P., Khattak, S.F., and Li, Z.J. (2009). Optimal and consistent protein glycosylation in mammalian cell culture. *Glycobiology* 19 (9): 936–949.

18 Xu, X., Nagarajan, H., Lewis, N.E. et al. (2011). The genomic sequence of the Chinese hamster ovary (CHO)-K1 cell line. *Nat. Biotechnol.* 31 (8): 759–765.

19 Lewis, N.E., Liu, X., Li, Y. et al. (2013). Genomic landscapes of Chinese hamster ovary cell lines as revealed by the *Cricetulus griseus* draft genome. *Nat. Biotechnol.* 31 (8): 759–765.

20 Fischer, S., Handrick, R., and Otte, K. (2015). The art of CHO cell engineering: a comprehensive retrospect and future perspectives. *Biotechnol. Adv.* 33 (8): 1878–1896.

21 Wurm, F. (2013). CHO quasispecies—implications for manufacturing processes. *Processes* 1: 296–311.

22 Golabgir, A., Gutierrez, J.M., Hefzi, H. et al. (2016). Quantitative feature extraction from the Chinese hamster ovary bioprocess bibliome using a novel meta-analysis workflow. *Biotechnol. Adv.* 34 (5): 621–633.

23 Hu, Z., Guo, D., Yip, S.S.M. et al. (2013). Chinese hamster ovary K1 host cell enables stable cell line development for antibody molecules which are difficult to express in DUXB11-derived dihydrofolate reductase deficient host cell. *Biotechnol. Progr.* 29 (4): 980–985.

24 Frye, C., Deshpande, R., Estes, S. et al. (2016). Industry view on the relative importance of "clonality" of biopharmaceutical-producing cell lines. *Biologicals* 44 (2): 117–122.

25 Sanchez, A. and Golding, I. (2013). Genetic determinants and cellular constraints in noisy gene expression. *Science* 342 (6163): 1188–1193.

26 Davies, S.L., Lovelady, C.S., Grainger, R.K. et al. (2013). Functional heterogeneity and heritability in CHO cell populations. *Biotechnol. Bioeng.* 110 (1): 260–274.

27 González, M., Martín-Ruíz, I., Jiménez, S. et al. (2011). Generation of stable *Drosophila* cell lines using multicistronic vectors. *Sci. Rep.* 1: 75.

28 Priola, J.J., Calzadilla, N., Baumann, M. et al. (2016). High-throughput screening and selection of mammalian cells for enhanced protein production. *Biotechnol. J.* 11 (7): 853–865.

29 Harraghy, N., Calabrese, D., Fisch, I. et al. (2015). Epigenetic regulatory elements: recent advances in understanding their mode of action and use for recombinant protein production in mammalian cells. *Biotechnol. J.* 10 (7): 967–978.

30 Moritz, B., Becker, P.B., and Göpfert, U. (2015). CMV promoter mutants with a reduced propensity to productivity loss in CHO cells. *Sci. Rep.* 5: 16952.

31 Mariati, Yeo, J.H., Koh, E.Y. et al. (2014). Insertion of core CpG island element into human CMV promoter for enhancing recombinant protein expression stability in CHO cells. *Biotechnol. Progr.* 30 (3): 523–534.

32 Brown, A.J. and James, D.C. (2016). Precision control of recombinant gene transcription for CHO cell synthetic biology. *Biotechnol. Adv.* 34 (5): 492–503.

33 Porter, A.J., Racher, A.J., Preziosi, R., and Dickson, A.J. (2010). Strategies for selecting recombinant CHO cell lines for cGMP manufacturing: improving the efficiency of cell line generation. *Biotechnol. Progr.* 26 (5): 1455–1464.

34 Caron, A.W., Nicolas, C., Gaillet, B. et al. (2009). Fluorescent labeling in semi-solid medium for selection of mammalian cells secreting high-levels of recombinant proteins. *BMC Biotechnol.* 9: 42.

35 Hou, J.J., Hughes, B.S., Smede, M. et al. (2014). High-throughput ClonePix FL analysis of mAb-expressing clones using the UCOE expression system. *New Biotechnol.* 31 (3): 214–220.

36 Lindgren, K., Salmén, A., Lundgren, M. et al. (2009). Automation of cell line development. *Cytotechnology* 59 (1): 1–10.

37 Estes, S. and Melville, M. (2014). Mammalian cell line developments in speed and efficiency. *Adv. Biochem. Eng. Biotechnol.* 139: 11–33.

38 Porter, A.J., Dickson, A.J., and Racher, A.J. (2010). Strategies for selecting recombinant CHO cell lines for cGMP manufacturing: realizing the potential in bioreactors. *Biotechnol. Progr.* 26 (5): 1446–1454.

39 Fletcher, T. (2005). Designing culture media for recombinant protein production: a rational approach. *Bioprocess Int.* 3: 30–36.

40 Kim, S.H. and Lee, G.M. (2009). Development of serum-free medium supplemented with hydrolysates for the production of therapeutic antibodies in CHO cell cultures using design of experiments. *Appl. Microbiol. Biotechnol.* 83 (4): 639–648.

41 Rouiller, Y., Périlleux, A., Collet, N. et al. (2013). A high-throughput media design approach for high performance mammalian fed-batch cultures. *MAbs* 5 (3): 501–511.

42 Kishishita, S., Katayama, S., Kodaira, K. et al. (2015). Optimization of chemically defined feed media for monoclonal antibody production in Chinese hamster ovary cells. *J. Biosci. Bioeng.* 120 (1): 78–84.

43 Butler, M. (2005). Animal cell cultures: recent achievements and perspectives in the production of biopharmaceuticals. *Appl. Microbiol. Biotechnol.* 68 (3): 283–291.

44 Gawlitzek, M., Estacio, M., Fürch, T., and Kiss, R. (2009). Identification of cell culture conditions to control N-glycosylation site-occupancy of recombinant glycoproteins expressed in CHO cells. *Biotechnol. Bioeng.* 103 (6): 1164–1175.

45 Yoon, S.K., Song, J.Y., and Lee, G.M. (2003). Effect of low culture temperature on specific productivity, transcription level, and heterogeneity of erythropoietin in Chinese hamster ovary cells. *Biotechnol. Bioeng.* 82 (3): 289–298.

46 Wong, D.C.F., Wong, K.T.K., Goh, L.T. et al. (2005). Impact of dynamic online fed-batch strategies on metabolism, productivity and N-glycosylation quality in CHO cell cultures. *Biotechnol. Bioeng.* 89 (2): 164–177.

47 Pollock, J., Coffman, J., Ho, S.V., and Farid, S.S. (2017). Integrated continuous bioprocessing: economic, operational, and environmental feasibility for clinical and commercial antibody manufacture. *Biotechnol. Progr.* https://doi.org/10.1002/btpr.2492.

48 Lehr, B. and Lyons, D. (2016). Perfusion in the 21st Century. Quality, flexibility, and cost savings are driving use of perfusion technology in biosimilars manufacturing. *Biopharm. Int.* 29 (8): 24–26.

49 Tapia, F., Vázquez-Ramírez, D., Genzel, Y., and Reichl, U. (2016). Bioreactors for high cell density and continuous multi-stage cultivations: options for process intensification in cell culture-based viral vaccine production. *Appl. Microbiol. Biotechnol.* 100 (5): 2121–2132.

50 Heath, C. and Kiss, R. (2007). Cell culture process development: advances in process engineering. *Biotechnol. Progr.* 23 (1): 46–51.

51 Eon-Duval, A., Broly, H., and Gleixner, R. (2012). Quality attributes of recombinant therapeutic proteins: an assessment of impact on safety and efficacy as part of a quality by design development approach. *Biotechnol. Progr.* 28 (3): 608–622.

52 Torkashvand, F. and Vaziri, B. (2017). Main quality attributes of monoclonal antibodies and effect of cell culture components. *Iran Biomed. J.* 21 (3): 131–141.

53 Liu, H.F., Ma, J., Winter, C., and Bayer, R. (2010). Recovery and purification process development for monoclonal antibody production. *MAbs* 2 (5): 480–499.

54 Tarrant, R.D., Velez-Suberbie, M.L., Tait, A.S. et al. (2012). Host cell protein adsorption characteristics during protein A chromatography. *Biotechnol. Progr.* 28 (4): 1037–1044.

55 Ghose, S., Hubbard, B., and Cramer, S.M. (2006). Evaluation and comparison of alternatives to protein A chromatography mimetic and hydrophobic charge induction chromatographic stationary phases. *J. Chromatogr. A* 1122 (1–2): 144–152.

56 Cramer, S.M. and Holstein, M.A. (2011). Downstream bioprocessing: recent advances and future promise. *Curr. Opin. Chem. Eng.* 1 (1): 27–37.

57 Saraswat, M., Musante, L., Ravidá, A. et al. (2013). Preparative purification of recombinant proteins: current status and future trends. *Biomed. Res. Int.* 2013: 312709.

58 Steinebach, F., Ulmer, N., Decker, L. et al. (2017). Experimental design of a twin-column countercurrent gradient purification process. *J. Chromatogr. A* 1492: 19–26.

59 Fröhlich, H., Villian, L., Melzner, D., and Strube, J. (2012). Membrane technology in bioprocess science. *Chem. Ing. Tech.* 84: 905–917.

60 Giese, G., Myrold, A., Gorrell, J., and Persson, J. (2013). Purification of antibodies by precipitating impurities using polyethylene glycol to enable a two chromatography step process. *J. Chromatogr. B* 938: 14–21.

61 Brunner, M., Fricke, J., Kroll, P., and Herwig, C. (2017). Investigation of the interactions of critical scale-up parameters (pH, pO_2 and pCO_2) on CHO batch performance and critical quality attributes. *Bioprocess. Biosyst. Eng.* 40 (2): 251–263.

62 Rathore, A.S. and Winkle, H. (2009). Quality by design for biopharmaceuticals. *Nat. Biotechnol.* 27 (1): 26–34.

63 Sommeregger, W., Sissolak, B., Kandra, K. et al. (2017). Quality by control: towards model predictive control of mammalian cell culture bioprocesses. *Biotechnol. J.* https://doi.org/10.1002/biot.201600546.

64 Kildegaard, H.F., Baycin-Hizal, D., Lewis, N.E., and Betenbaugh, M.J. (2013). The emerging CHO systems biology era: harnessing the 'omics revolution for biotechnology. *Curr. Opin. Biotechnol.* 24 (6): 1102–1107.

65 Zhang, Y., Baycin-Hizal, D., Kumar, A. et al. (2017). High-throughput lipidomic and transcriptomic analysis to compare SP2/0, CHO, and HEK-293 mammalian cell lines. *Anal. Chem.* 89 (3): 1477–1485.

66 Hefzi, H., Ang, K.S., Hanscho, M. et al. (2016). A consensus genome-scale reconstruction of Chinese hamster ovary cell metabolism. *Cell Syst.* 3 (5): 434–443.

67 Xiao, S., Chen, Y.C., Buehler, E. et al. (2016). Genome-scale RNA interference screen identifies antizyme 1 (OAZ1) as a target for improvement of recombinant protein production in mammalian cells. *Biotechnol. Bioeng.* 113 (11): 2403–2415.

68 Lee, J.S., Grav, L.M., Lewis, N.E., and Faustrup Kildegaard, H. (2015). CRISPR/Cas9-mediated genome engineering of CHO cell factories: application and perspectives. *Biotechnol. J.* 10 (7): 979–994.

69 Lee, J.S., Kallehauge, T.B., Pedersen, L.E., and Kildegaard, H.F. (2015). Site-specific integration in CHO cells mediated by CRISPR/Cas9 and homology-directed DNA repair pathway. *Sci. Rep.* 5: 8572.

70 Croughan, M.S., Konstantinov, K.B., and Cooney, C. (2015). The future of industrial bioprocessing: batch or continuous? *Biotechnol. Bioeng.* 112 (4): 648–651.

71 Daszkowski, T. (2013). Continuous processing in biotech production: an alternative to a modern single use, batch, facility? *Integrated Continuous Biomanufacturing Conference* (20–24 October 2013), Barcelona, Spain.

72 Konstantinov, K. and Cooney, C. (2015). White paper on continuous bioprocessing. *J. Pharm. Sci.* 104 (3): 813–820.

73 Hiller, G.W., Ovalle, A.M., Gagnon, M.P. et al. (2017). Cell-controlled hybrid perfusion fed-batch CHO cell process provides significant productivity improvement over conventional fed-batch cultures. *Biotechnol. Bioeng.* 114 (7): 1438–1447.

74 Gugliotta, A., Ceaglio, N., Raud, B. et al. (2017). Glycosylation and antiproliferative activity of hyperglycosylated IFN-α2 potentiate HEK293 cells as biofactories. *Eur. J. Pharm. Biopharm.* 112: 119–131.