Cell Culture and Upstream Processing

Improving Process Development for Better Product Quality

by Maribel Rios

Cell culture processes are a vital part of manufacturing, yet the bioprocessing industry still needs optimized process development approaches. Increasing interest in chemically defined media, perfusion cell processes, and high-throughput approaches are driving the need for better understanding of cell culture characterization and process development for current and next-generation protein therapeutics.

Process Development

Optimizing the business process of cell line development involves creating competitive advantages with increased efficiency and reduced risk. “Intelligent business practices that support scientific processes are essential in today’s competitive environment,” says Steve Lang of Janssen Research & Development. His Wednesday morning presentation will include case studies and “lessons learned” examples in cell culture process development. The talk will focus on preclinical enabling strategies and decision processes.

Perfusion cell culture processes are gaining interest in the industry as manufacturers aim for higher productivities in monoclonal antibody (MAb) production. Marcella Yu of Genzyme (a Sanofi company) points out that perfusion cell culture platforms for MAb production has been developed by integrating continuous manufacturing concepts including continuous downstream purification. She will present a case study that focuses on the development and challenges that her company encountered in the upstream cell culture process as well as compare different cell separation devices.

Bioprocessing companies can apply the research and development aspects of lean process development approaches to product development systems from the pre-NME (new molecular entity) phase through process validation. As Ben Bulthuis of Janssen explains, “Improved development timelines result from cross-functional cooperation, combining high-level planning with much attention to detail and deployment of platform technology in both upstream production and downstream purification.” His Thursday presentation will describe a case study in which those principles were applied to improve development timelines.

One challenge in using perfusion mammalian cell culture is increasing production capacity and flexibility. Yuval Shimoni of Bayer HealthCare Production will describe a case study in which the company increased capacity of a continuous mammalian cell culture platform using perfusion rate reduction, recombinant-protein-product stabilizers, increased media strength, and working-volume adjustments of a bioreactor vessel/
Later, Jason Goodrick of Genentech will discuss process optimization and scale-up challenges in the development of a large-scale phase 3 manufacturing process. “Developing a phase 3/commercial cell culture process presents many challenges, including optimizing cell culture conditions to maintain quality and maximize process performance,” he says. Goodrick will discuss the development of a phase 3/commercial process for a CHO monoclonal antibody (MAb) product through process optimization and scale-up. During the development, researchers implemented a new chemically defined medium (CDM) formulation for the phase 3 process at large scale for the first time. They concluded that “the use of this CDM formulation resulted in highly consistent cell culture performance at the small scale and pilot scale.” However, during scale-up for clinical manufacturing, they identified additional challenges and evaluated potential process improvements to mitigate risk for future campaigns.

The final presentation of the session will focus on chemically defined (CD) media. Patrick Hossler of Abbott Laboratories will review two approaches for developing chemically defined feed media. One approach involved robotic liquid handling to generate hundreds of variants for high-throughput screening. The second used a novel media enrichment strategy. According to his presentation, both approaches increased antibody titers at gram per liter levels without effects in quality.

**CELL CULTURE CHARACTERIZATION AND PRODUCT QUALITY**

Product quality directly relates with the characteristics of a cell culture’s environment, as a series of talks on Thursday afternoon will illustrate the topic. First, Sang Taek Jung of The University of Texas at Austin will show that in IgG molecules, removing the glycan at Asn297 abolishes binding to FcγRs and effector functions mediated by leukocytes. “We have developed a robust screening platform for engineering a glycosylated full-length IgG with various FcγRs selectivity,” elaborates Jung. The presentation will describe the how a set of Fc-engineered versions of a glycosylated antibody was generated with “unique or improved” effector functions.

Melissa Mun of Genentech will demonstrate the optimization of productivity and product quality of a commercial cell culture process for a “high demand MAb product.” Her presentation highlights challenges and key achievements in developing a high-titer cell culture process under short timelines. “Initial platform process bioreactor studies yielded unexpectedly low titers,” explains Mun. “But a twofold improvement was obtained through mimicking the uncontrolled pH profile observed in shake flasks.” The study addressed several product quality challenges, including mitigating sequence variants and modulating charge variants.

Also with Genentech, Angela Meier will present a separate case study on quality by design (QbD) cell culture process characterization. The study involved a scale-down model of a cell culture process characterization for a protein production process. Researchers implemented a QbD approach to improve overall process understanding, which resulted in both univariate and multivariate acceptable ranges for each parameter tested. “Applicability of these study results to the manufacturing scale depends on the validity of the scale-down models that have been used,” explains Meier. That particular case study involved qualification of both small and pilot-scale models. “Evaluation of some product quality attributes required further downstream processing through scale-down purification models,” she says. “As a result, qualification of the cell culture model ultimately relies on qualification with the purification scale-down model as well.” Application of the models depends on both the product quality attribute being evaluated and the unit operation being characterized. Meier will present the scale-down model qualification approach, including challenges related to applying scale-down results to manufacturing scale.

Antibody structures such as specific glycan profile and charge variants are important quality attributes. Cell culture bioprocess conditions may have some influence over such attributes, so one challenge is to discover methods to keep those quality attributes consistent and successfully implement those methods in antibody process development. “It is considered more challenging to try to...
target a specific quality attribute of an antibody in development within a desired range with a flexible methodology that is not in conflict to the direction of developing higher productivity,” says Jerry Yang of Amgen. His presentation will demonstrate the application of a few methodologies for targeting specific antibody quality attribute profiles while maintaining or advancing productivity.

**Next-Generation Protein Therapeutics**

Friday’s sessions highlight cell culture process development for novel molecules and next-generation therapeutics. Gregory Zarbis-Papastoisis and Kathryn Golden of Eleven Biotherapeutics will talk about case study that involves the expression, purification, and characterization of an IL-1 therapeutic inhibitor.

Afterward, Timothy Johnson of Genzyme will talk about process and technology development for the continuous production of nonantibody therapeutic proteins. “The integration of bioprocessing steps into continuous operations is paving the future for streamlined and flexible biopharmaceutical production.” Nonetheless, companies must carefully optimize process, hardware, and associated control strategies to achieve overall robustness and product quality. Johnson will relate those issues to the development of a continuous nonantibody protein process.

**Keynote Address: QbD Implementation**

In Friday morning’s keynote address, Steven Meier of Genentech will discuss his company’s implementation of QbD in cell culture. He will review the tools and studies, and discuss lessons learned in applying QbD. As Meier observes, the expectations for cell culture process characterization have evolved during the past decade, and the integration of QbD principles is the latest example. “Several Genentech projects have applied various aspects of QbD in the past several years, and with each new project, we’ve built upon lessons learned.”

The company’s broadest QbD strategy consisted of a quality target product profile, other risk assessments to determine critical quality attributes (CQAs), risk assessments to determine what parameters to study, design of experiment (DoE) studies to investigate parameter effects on CQAs, a critical process parameter identification tool, more risk assessments related to control system definition, and life-cycle management. “Development and use of risk assessments and other tools have been the most challenging aspects of applying QbD to cell culture process characterization,” explains Meier. “At first glance, all the moving pieces that are part of QbD can appear quite complex, both to people within the company and to health authorities.”

Meier points out that although the strategy is complex, one main benefit is that it has provided a structured approach to document rationale for how the company executes and interprets its characterization studies. “As we’ve become more experienced with QbD, our study designs and tools have evolved.”

**High-Throughput Processes**

High-throughput processes (HTPs) offer several benefits in production time efficiency as manufacturers continue to look for ways of shortening initial development timelines. Some manufacturers are taking a “holistic approach” to HTP development that factors in process understanding. Hitto Kaufman of Boehringer Ingelheim, says his company has expanded its technologies around flexible small-scale model systems and the corresponding “miniaturized” analytical methods spanning the entire process chain. His Friday presentation will focus on how those successes will be critical to success for pipelines expanding toward classical IgG molecules.

The success of HTPs relies on its analytical support systems. Shashi Prajapati of Biogen Idec will discuss how cell line and process development play a major role in producing therapeutic proteins with high productivity and product quality. “To support this development, analyses of large number of samples generated from thousands of clones and process optimization are critical,” he points out. Analyzing large numbers of samples can create bottlenecks because conventional analytical assays are low-throughput. Prajapati will discuss various HTP analytical platforms to facilitate rapid and parallel analyses of product quantity. Such platforms include HTP protein quantitation and purification and product quality analyses. “With these analytical capabilities, we can assess product quality in the early stage of clone screening, as well as expedite the cell line and process development.”

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John Cesarek of Five Prime Therapeutics will discuss the advantages of integrating, within a single system, fully automated process workflows for both cell line and cell culture development. His case study covers both the development and implementation of an in-house, customized automation platform as well as software tools for data analysis. That approach increased efficiency and allowed for the processing of multiple cell line screening and cell culture development projects.

**Disposables in Cell Culture**

Single-use technologies can be implemented with HTP and automation technologies. Rachel Bareither of Merck will discuss a strategy for applying targeted automation tools to resolve resource constraints in upstream development. Technologies include automated spin tubes for clonal evaluations and a 250-mL single-use prototype bioreactor for robotic sampling automated feeding, and independent control.

Joseph Wood of Genentech will provide an update on difficulties associated with some disposable bags, including usage conditions that can reduce cell growth and product yield. His “lessons learned” presentation will updates the ongoing investigation presented by Masaru Shiratori at the 2011 BPI Conference & Exhibition. He will review current understanding of the root cause of the problem and recommendations for selecting disposables for future applications.

Rocking-type bioreactors can offer some benefits, depending on the particular cell culture process. Bert Frohlich of Shire Human Genetic Therapies will close Friday’s sessions by describing a novel two-dimensional rocking bioreactor. According to Frohlich, the rocking mechanism enables high oxygen transfer rates, and the single-use bioreactor bag offers a wide range of working volumes, eliminating labor-intensive multistage cell expansion trains.

*Maribel Rios* is managing editor of BioProcess International. Quotes not otherwise attributed are from presentation abstracts.