Exciting Trends in Bioprocessing

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Getting products onto the market as quickly as possible still remains a key driver in the development of recombinant therapeutic proteins. Any advance in bioprocessing is of particular interest to the industry if it significantly shortens the development timeline or improves the end product. Even better are advances that do both. In the monoclonal antibody (MAb) area, platform processes have enabled companies to standardize on specific mammalian cell lines, transfection approaches, process conditions and downstream processing to shorten the development timeframe. This white paper describes several interesting trends in two development areas, which have the potential to shorten the development timeline still further.

Production Cell Line Development

Any technique that could speed the selection of high product producing cell lines and enable more rapid identification of the best cell line from among many clones could offer a faster approach to generating a production cell line of commercial interest. An intriguing paper by Povey et al[1] describes the use of MALDI-ToF mass spectrometry fingerprinting and an *in silico* modelling method that could potentially reduce the clone selection timeline by 30 to 40%. The method, using intact CHO cells, provides a rapid high throughput characterization, screening and selection of cell phenotypes including the normally time-consuming genetic stability testing. Transfected CHO cells were isolated and expanded in 96 well plates and the resulting whole cell pellet from each well was analyzed by mass spectrometry.

An associated Partial Least Squares Discriminant Analysis method was used to predict the productivity of these recombinant cell lines at the 10 L bioreactor scale. The prediction of productivity at the 10 L bioreactor level involved the use of Lonza's large-scale (up to 20,000 L) fed-batch cell culture scaled-down process model. Using MALDI-ToF mass spectrometry on intact cells, this approach may allow for early productivity predictions and reduce the need to carry cells through an extended cell line construction process. This methodology is reported to be applicable for CLC/clone selection. The authors report that their rapid phenotyping selection process decreased the clonal selection timeline from 19.8 weeks to 12.4 weeks.

Rapid Process Development

Technical challenges and regulatory requirements are more easily met when process performance data and a good understanding of critical process parameters can be obtained early in development. BioProcess Technology Consultants[2] recently reviewed high throughput instrument innovations applied to clone screening, cell line selection and evaluation. This review focused on high throughput technologies and instruments for obtaining sufficient data to support cell culture process development and optimization. Several of the instruments enable rapid development of a robust mammalian cell culture processes and can be used to produce non-cGMP drug substance material.

One such system is the Advanced Microscale Bioreactor (ambr™) from Sartorius Stedim Biotech. The system consists of an automated workstation with 24 or 48 stirred-tank micro-bioreactors having working volumes of 10 mL to 15 mL. A newer model will extend the working volume of the individual bioreactors up to 250 mL. The bioreactors have individual monitoring to control temperature, DO, and pH.

In a recent paper, Rameez et al[3] compared the ambr™ system performance against conventional bioreactor systems for MAb production using a CHO cell line. The ambr™ system was found to produce cell culture profiles that matched across scales of 3 L, 15 L, and 200 L stirred tank bioreactors. The processes evaluated included complex feed formulations, perturbations, and strict process control within the design space, which were in-line with processes used for commercial scale manufacturing of biopharmaceuticals. The authors noted that, "Changes to important process parameters in ambr™ resulted in predictable cell growth, viability, and titer changes, which were in good agreement to data from the conventional larger scale bioreactors. Additionally, the miniature bioreactors were found to react well to perturbations in pH and DO through adjustments to the Proportional and Integral control loop."

The data reported in this and earlier studies demonstrate the utility of high throughput systems for cell culture development. It also demonstrates that conventional bioreactors can be adequately modeled using micro- or mini-bioreactors and that such systems allow for the investigation of culture conditions at greater statistical depth than can be performed with a conventional bioreactor. High throughput cell culture technologies can be an effective tool for the development and optimization of cell culture processes as well as in troubleshooting cell culture problems, and their use will continue to increase. Further, these systems offer significant time and materials cost advantages.

Product quality improvement

It has long been known that recombinant proteins produced by mammalian cells have differing post translational glycan patterns from the human protein. These patterns can differ based on mammalian cell type used for manufacture and differ between cell lines of the same cell type. Further, it is known that cell culture processing conditions can lead to protein product exhibiting a range of glycan patterns.

There is a growing appreciation of the role of post-translational glycan features associated with recombinant therapeutic proteins. It is known that these glycan patterns are associated with product efficacy, half-life, solubility, PK properties, and immune response potential. Further, from a regulatory standpoint, a molecule can be partially defined by its carbohydrate profile. A review article by Hossler[4] reviewed the various causes of glycoform variation in CHO cells. Among the factors found to affect glycoform heterogeneity were DO, pH, ammonia, cell hypoxia, manganese, pCO_2, cell viability, shear stress and process operations.

A recent paper by Liu, et al[5], showed that the depletion of glucose changed the pattern of macro- and micro-heterogeneity glycosylation of a MAb expressed by CHO cells. High density CHO

cell cultures were incubated with differing starting concentrations of glucose for 24 hours. Samples were taken at intervals to measure glucose concentration, the glycan profile of produced MAb and intracellular metabolic precursors associated with glycan formation. Their results indicate that periods of glucose depletion can cause the production of non-glycosylated MAb and a decrease in galactosylation and sialylation. They suggest that commercial fed-batch production strategies can result in periods of glucose depletion over the course of the manufacturing run and contribute to the overall heterogeneity of the MAb product. This work suggests that deliberately maintaining low glucose levels using a fed-batch approach during cell culture, likely leads to periods of glucose depletion and contributes to variations in glycan patterns and non-glycosylated MAb in the product.

CHO cells continue to be a predominate platform for MAb production and innovation. As our knowledge of the metabolic machinery of these cells increases in parallel with our understanding of the impact of recombinant techniques on these cells, we may be getting closer to controlling the heterogeneity in MAb products through a better understanding of the metabolic pathways of these cells, especially under manufacturing conditions. The goal would be the ability to produce more homogeneous MAb products using mammalian cells that have glycan patterns more closely resembling human MAbs. As our level of understanding cellular processes and metabolic controls progresses, we may, using the QbD approach, be in a position to define the critical process parameters (CPP) associated with manufacturing MAbs for human use that lead to more glycan uniformity in the harvested product.

Manufacturing Capacity

Clearly, the increases in cell density and viability, coupled with higher product titers has lowered the need for manufacturing capacity[6]. This trend will likely lead to less investment in manufacturing capacity with some of that savings being directed toward increased instrumentation and automation of next generation production systems. One can envision a number of key entities that ,with close control, would yield a more homogeneous product. For example, the continuous measurement and addition of glucose to avoid glucose depletion.

Smaller production volumes could also allow for more complete mixing and distribution of dissolved gases and nutrients, thus reducing concentration differences that subject cells to varying micro-environments during a production run. The goal would be to more tightly control the production environmental encountered by cells, which in turn could lead to a more homogenous final upstream product and simplify downstream processing.

References

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