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A Multi-Scale Approach to CHO Media Benchmarking

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Abstract

The selection of optimal growth media and reproducible process conditions are important tasks in the generation of high-yield, high-quality biotherapeutics. The current industry standard is to produce these drugs using mammalian cells such as clonal CHO suspension cell lines. Chemically-defined cell culture media have become the gold standard of modern CHO production processes, facilitating regulatory approval. A well-chosen combination of production clone, process conditions and cell culture media can ensure optimum process performance with regard to product titer and product quality. Unless a platform process is already in place, this often necessitates finding suitable clone/media combinations before embarking on process development. In this context, media formulations reliably supporting robust cell growth and process scalability are critically important to assure reproducible quality and desired characteristics of the final drug product.

We applied a multi-scale approach for media benchmarking, making use of the Ambr® 15 mini-bioreactor platform for media screening of various clone/media combinations. The results suggest the Sartorius 4Cell® XtraCHO Media System delivers higher performance in terms of cell growth, viability, titer and glycoprofile compared to reference media.

Introduction

Competitive commercial CHO processes demand a balanced combination of media, process and production clone to achieve optimal performance. The aim of this study was to apply a multi-scale approach to efficiently screen combinations of chemically defined commercial media and various CHO production clones using benchtop bioreactor systems.

Methods

We cultivated four DG44 CHO clones, expressing IgG1 or IgG4 products, in fed-batch mode using commercial media and feed sets from five different suppliers. Before use in fed batch, we cultivated each clone in the respective suppliers' stock culture mediums for four weeks to ensure adaptation.

We tested all clones and media in shake flask mode at a culture volume of 25 mL and in parallel in the Ambr® 15 device at a culture volume of 14 mL.

We assessed performance in reference to cell growth, productivity and product N-glycosylation. The best clone media combinations were further characterized in 5L bioreactors, and we compared screening results against a more traditional shake flask approach.

Two selected clones and the two best-performing media were subsequently tested at 5L scale in a Univessel glass bioreactor. We determined product titers using the FortéBio Octet Qke system and employed a LabChip GXII (PerkinElmer) for product N-glycan profiles analysis in final samples. Raw data was processed to compute integral viable cell density (IVCC) as a measure of cell growth.

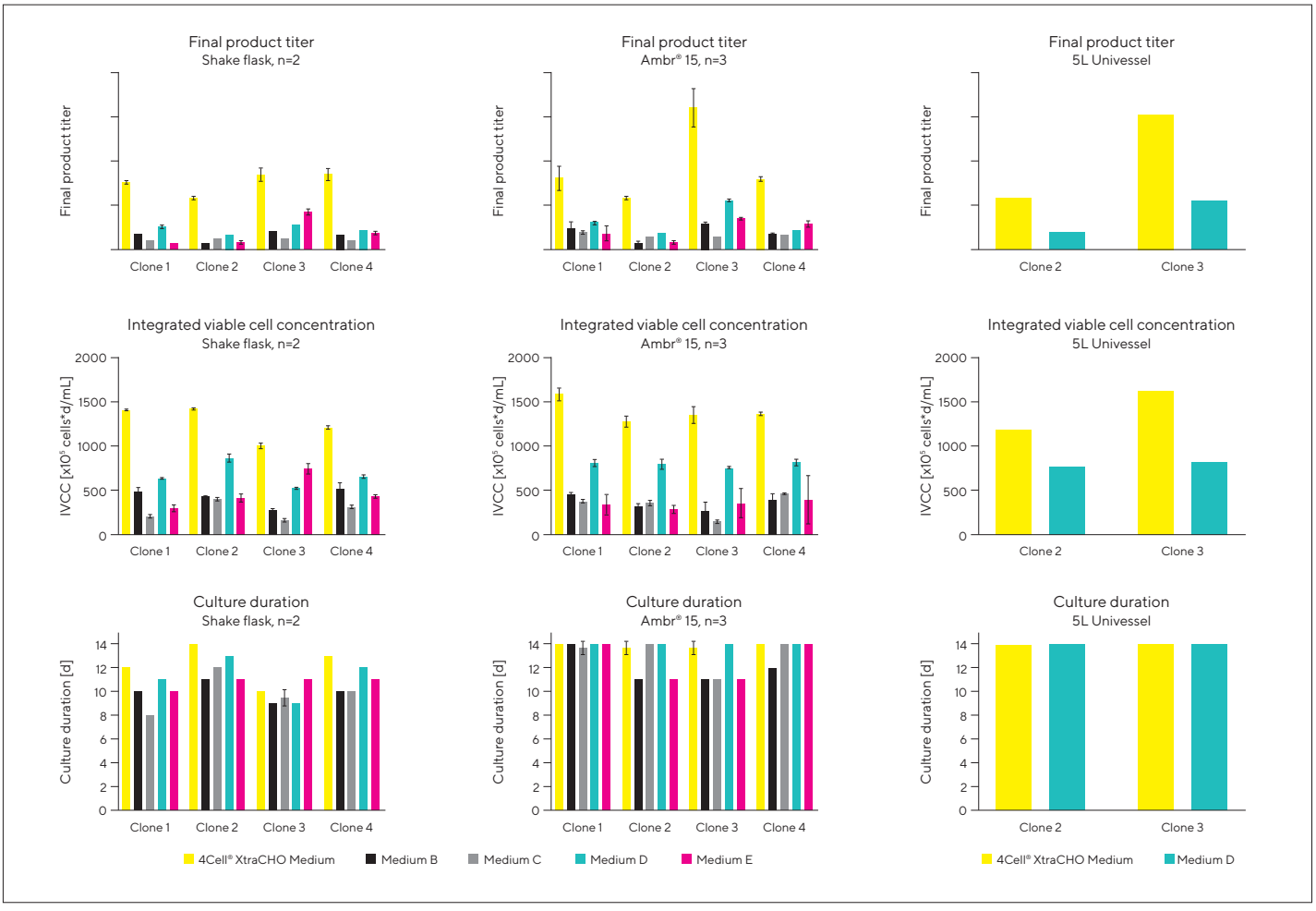

Figure 1: Product titers, integrated cell concentrations and culture duration in three cultivation systems, using four DG44 CHO cell clones and five sets of CHO culture media.

Results

Titer and growth performance in three culture systems
In fed-batch screening, the performance was superior using Medium A for all tested clones in regard to product titer and cell growth across all culture systems (Figure 1).

Medium D reproducibly performed second best, with the exception of Clone 3 in shake flasks. For media performance evaluation, consider that the maximum 14-day culture duration was achieved more reliably in the controlled cultivation systems (Ambr® 15 and 5L bioreactor) than in shake flask format.

For example, we observed a good correspondence between the titers of Clone 3 in 4Cell® XtraCHO Medium and Medium C in 5L and Ambr® 15 system, whereas the shake flask titer in both media was significantly lower and in line with the shorter process duration.

Moreover, Ambr® 15 cultures also correctly captured the improved growth (as judged by IVCC) of Clone 3 vs. Clone 2 at 5L scale).

This is the opposite of their respective growth in shake flask format. These findings illustrate that Ambr® 15 provides a better scale-down model for the present benchtop process than shake flask cultures and helps reduce unintended bias in media evaluation.

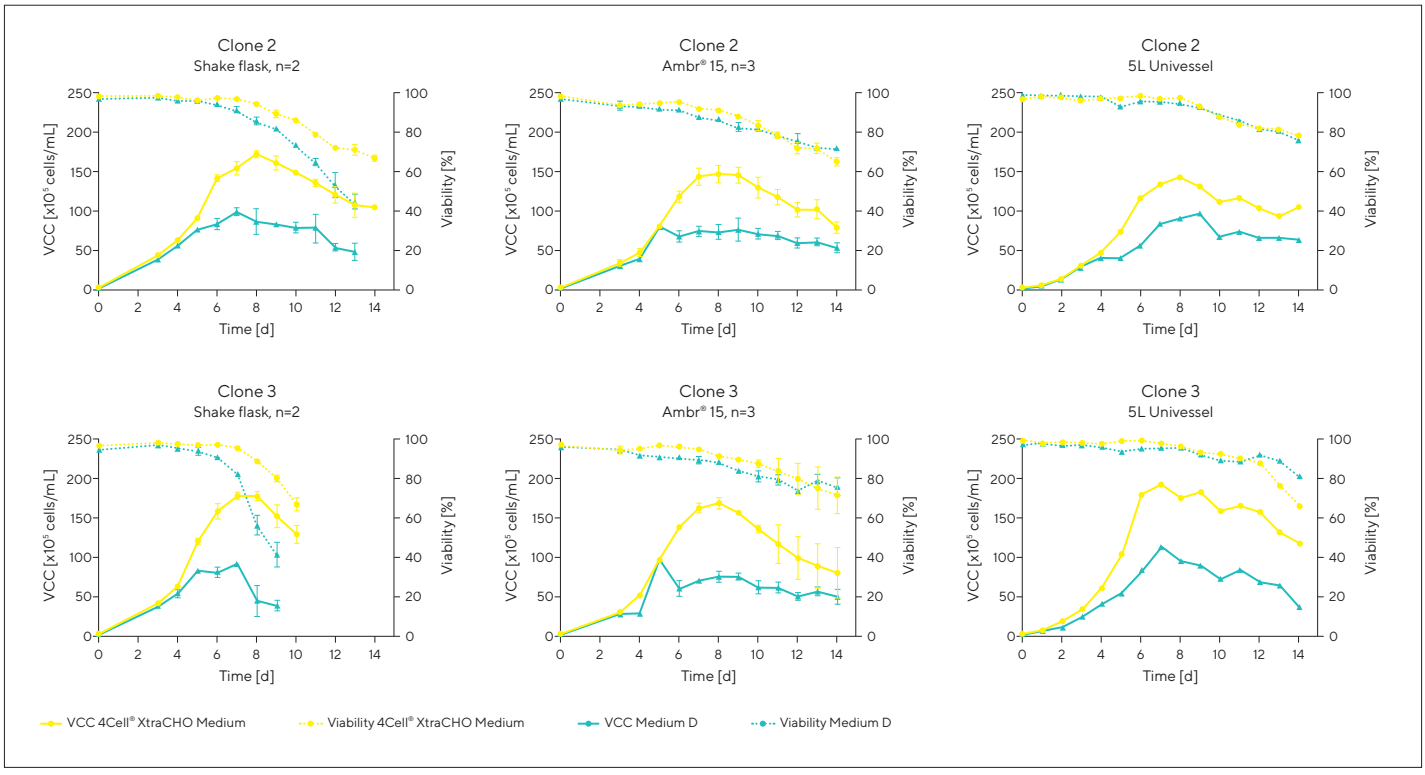

Figure 2: Profiles of viable cell concentration (VCC) and cell viability for two representative CHO clones in three cultivation systems for two of the culture media tested
Shake flask cultivations were performed in duplicate, Ambr® 15 runs in triplicate and 5L cultivations as single runs. Error bars signify standard deviations where applicable.

Growth and viability profiles are illustrated for representative clone/media combinations in three culture systems (Figure 2). We found differences between shake flask and Ambr® 15 cultivations were minor for some clone/media setups (e.g. Clone 2 in 4Cell® XtraCHO Media System); however, cell viability may drop below the harvest threshold one or several days earlier in shake flasks than in the controlled cultivation systems (Clone 2 in Medium C, Clone 3 in 4Cell® XtraCHO Media System and Medium C), leading to an earlier termination of culture and lower product yield. This makes it difficult to reliably predict bioreactor performance from shake flask data alone and may lead to wrongly excluding certain media for a given clone (e.g., Medium D instead of Medium C for Clone 3) due to the application of a sub-optimal culture model.

Product N-glycosylation profiles showed a high degree of comparability between Ambr® 15 and 5L bioreactor scales

To assess the impact of different media and culture scales on product quality, we analyzed final-day samples of Ambr® 15 and 5L bioreactor cultures with regard to product N-glycosylation profiles.

For all four proteins investigated, N-glycan distribution was product-specific, but we observed significant and reproducible trends in glycan patterns for all products when cultivating in different media (Figure 3). For example, cultures in 4Cell® XtraCHO Media System show the smallest fraction of G0f in every product, while the same glycan is very high in Medium C and Medium D. G1'f, G1f and G2f are consistently highest in 4Cell® XtraCHO Media System, followed by Medium A and Medium B, illustrating higher support of product galactosylation in the former.

For the two clones and two media investigated at 5L scale, we found glycosylation patterns to be highly similar to the respective Ambr® 15 cultures, underscoring the suitability of the controlled Ambr® 15 system for media screening purposes, including product quality.

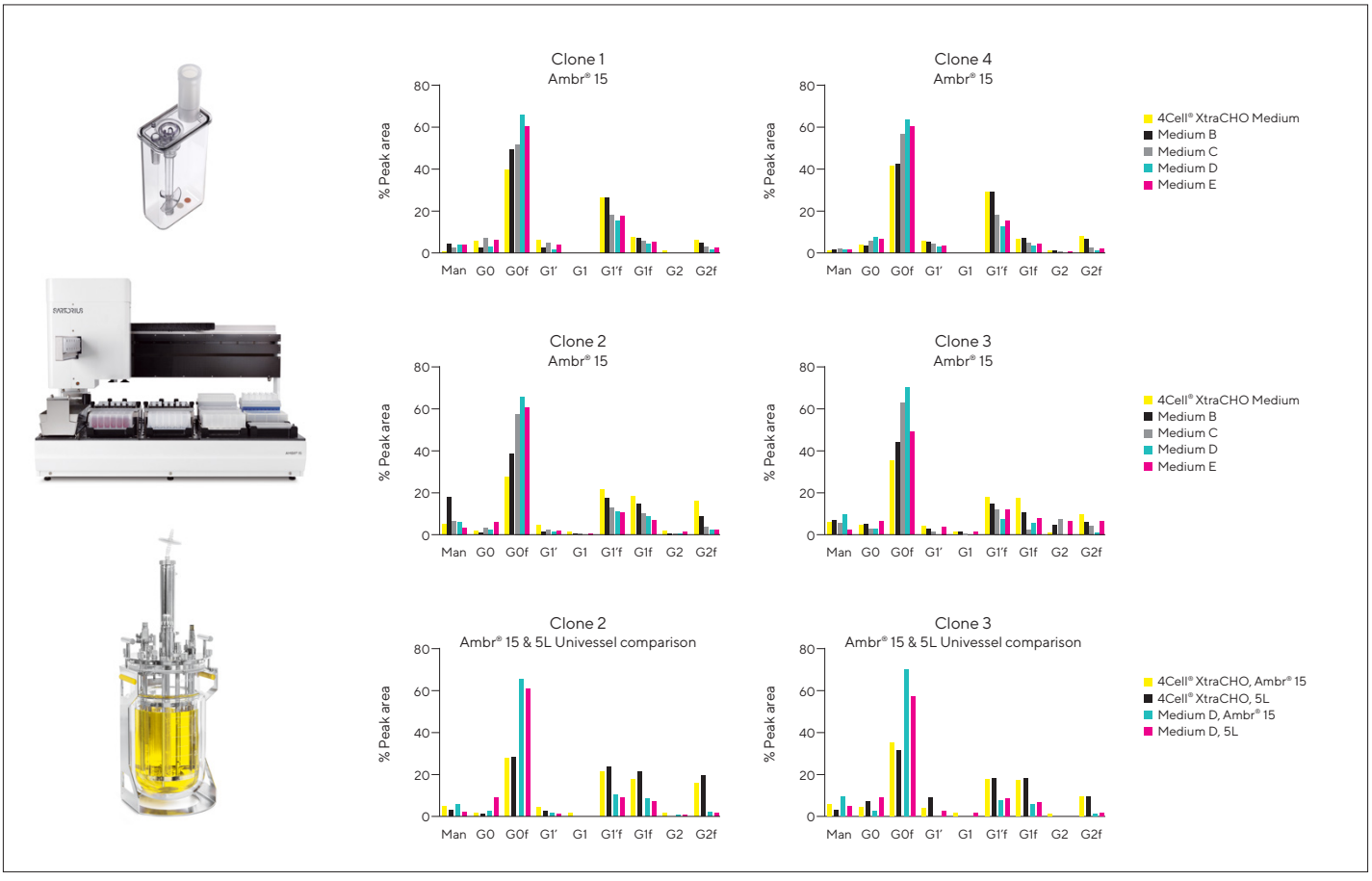

Figure 3: Product N-glycosylation profiles in final-day samples of Ambr® 15 and 5L bioreactor culture

Conclusion

With Ambr® 15, most fed-batch cultures are maintained at high viability for several days compared to shaker flask cultures, thereby enabling higher volumetric productivity and better production quality. The Ambr® 15 system provides a good small-scale model for predicting N-glycosylation patterns at 5L stirred bioreactor scale.

In sum, N-glycosylation patterns of product proteins show a clear and consistent trend for specific media across multiple product types. Media choice has a considerable impact on product quality and offers opportunities to improve it.

Reference

- Ritcacco FV, Wu, Khetan A (2018) Cell culture media for recombinant protein expression in Chinese hamster ovary (CHO) cells: History, key components and optimization strategies. Biotechnol Prog 34(6):1407-1426.
- DeJesus M, Wurm FM (2011) Manufacturing recombinant proteins in kg-ton quantities using animal cells in bioreactors. Eur J Pharm Biopharm 78(2):184-8.
- Velugula-Yelleta SR, Williams A, Trunfio N, Hsu CJ, Chavez B, Yoon S, Agarabi C (2018) Impact of media and antifoam selection on monoclonal antibody production and quality using a high-throughput, micro-bioreactor system. Biotechnol Prog 34(1):262-270.

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