Development of a Chemically Defined Growth Medium Specifically Designed for CHO DG44 Cells

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BACKGROUND

Chinese hamster ovary (CHO) cells are the most utilized cell type in the manufacturing of monoclonal antibodies and recombinant proteins. Many different CHO cell lines have been derived from the original cloned cell line, including CHO-S, CHO-K1, and CHO DG44 cells, each with their own specific metabolic attributes. Therefore, there is a need to design media for individual CHO cell types in order to maximize their growth and productivity. A new DG44-specific medium was developed with multi-stage design approach (Figure 1) to optimize critical components that affect both growth and protein production. Results of the media optimization were confirmed across multiple DG44 cell lines in fed-batch cultures utilizing the top 3 prototype candidates. These candidates showed significant improvement in cell growth and increased antibody titers 2-fold compared to benchmark medium. From there, the top candidate was developed into BalanCD CHO DG44 to achieve a final formulation with simplified hydration for upstream processing.

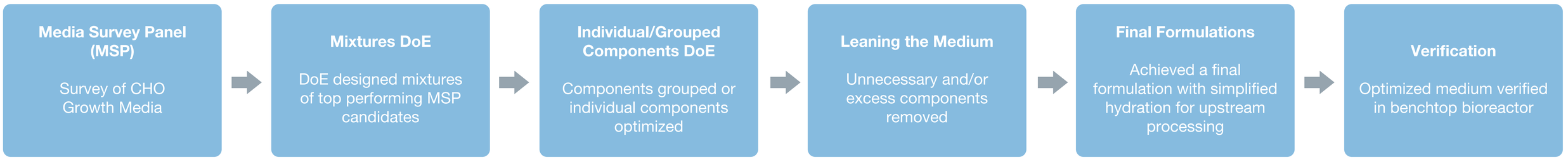
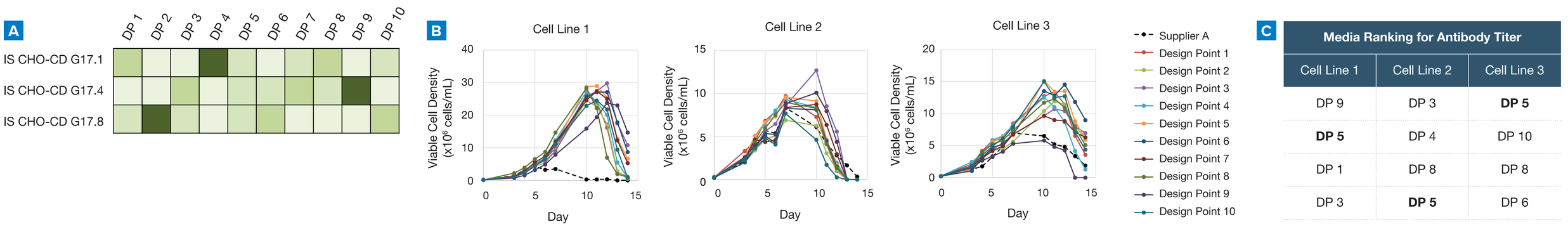


Figure 1. Media Development Approach

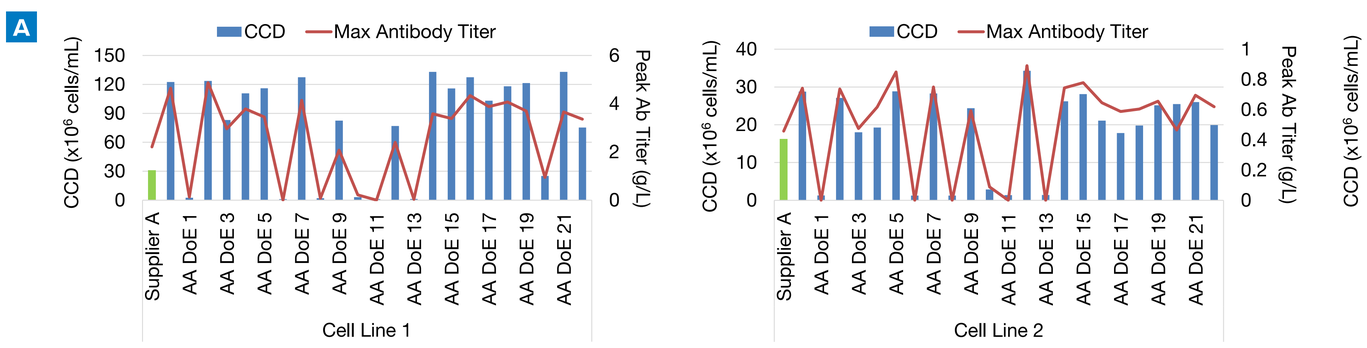
RESULTS



C

Media Ranking for Antibody Titer		
Cell Line 1	Cell Line 2	Cell Line 3
DP 9	DP 3	**DP 5**
DP 5	DP 4	DP 10
DP 1	DP 8	DP 8
DP 3	**DP 5**	DP 6

Figure 2. Mixtures Design of Experiment (DoE) assessing growth and titer of Media Survey Panel (MSP). A custom media panel using a DoE approach was conducted in 24-deep well plates using 3 different CHO DG44 cell lines with 16 panel media. The mixtures combined the top MSP media (A, y-axis) for each individual cell line from a prior experiment (data not shown). The heat map (A) depicts the composition of the combined 3 media in each design point. 3 distinct CHO DG44 cell lines were evaluated for cell growth (B) and ranked for antibody titer (C). Based on antibody titer ranking, Design Point (DP) 5 was selected for further optimization due to its consistent performance being in the top 4 across all 3 cell lines.



B

Amino Acid (AA)	Optimal Concentration from DoE Analysis
AA 1	1.09x
AA 2	0.5x
AA 3	1.65x
AA 4	0.5x

Figure 3. Medium optimization of amino acids using DoE. Amino acids were optimized utilizing the top MSP mixture from the previous experiment and evaluated in 24-deep well plates using 3 different CHO DG44 cell lines. (A) The total viable cells produced by the culture were calculated as cumulative cell density (CCD), and peak antibody titers were measured using biolayer interferometry. Various DoE conditions improved CCD and peak antibody titer across the 3 cell lines. (B) DoE analysis predicted an optimized increase in 2 amino acids and a decrease in 2 other amino acids when considering all 3 cell lines.

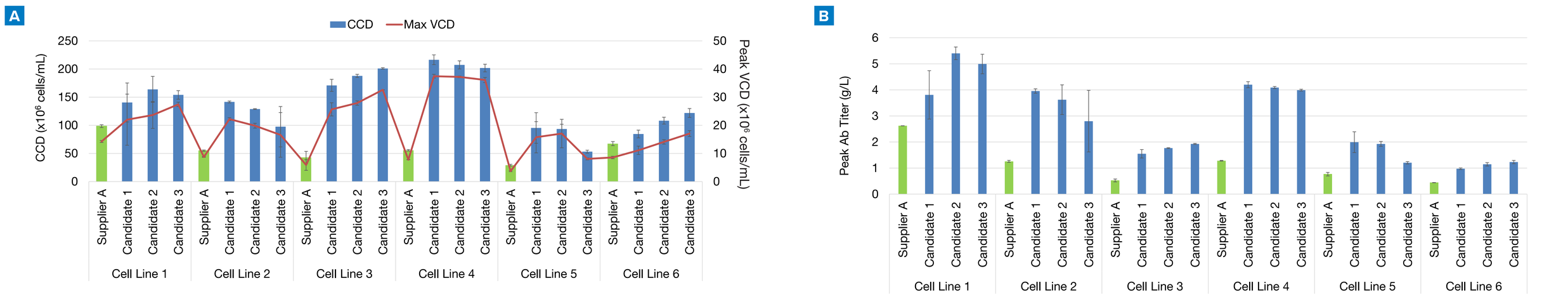


Figure 4. Verification of top 3 optimized candidate media in an Ambr®15 microbioreactor. Following additional rounds of DoE optimization (data not shown), the top 3 candidate media were evaluated for growth and titer as previously mentioned. (A) The 3 candidate media showed comparable or higher CCD and peak viable cell density (VCD) compared to Supplier A for all CHO DG44 cell lines tested. (B) Additionally, the peak antibody titer was improved by approximately 2-fold over Supplier A. Error bars represent experiments performed in duplicate.

MATERIAL AND METHODS

Fed-Batch Cultures
- Cell lines: CHO DG44
- Culture vessel: 24-deep well plates or 10 mL microbioreactor
- Working volume: 2.5 mL (deep well plate) or 10 mL microbioreactor
- Seeding density: 0.3x10^6 cells/mL
- Feed medium: BalanCD CHO Feed 4
- Supplier growth and feed media were used as directed

Cell Culture Analysis
- Viable cell density and cell viability
- Cell culture metabolites and gases
- Antibody titer: biolayer interferometry
- Design of experiment software

SUMMARY

- A mixtures DoE was utilized to formulate a Media Survey Panel for evaluation of multiple CHO DG44 cell lines
- Design Point 5 was selected for further optimization due to its consistent performance being in the top 4 across all 3 cell lines
- Multiple rounds of amino acid optimization led to a predicted optima which resulted in the top 3 candidates
- The top 3 candidate CHO DG44 media achieved superior cell growth and 2-fold antibody titer production in fed-batch, compared to commercially-available CHO growth medium
- The top final formulation, BalanCD CHO DG44, showed consistent improvement in cell growth and productivity across all DG44 cell lines tested, making it suitable for upstream fed-batch processes for mAb production