Fusion of Enhanced Cell Performance with Improved Productivity: Development of a Robust Chemically Defined Formulation for Culture of Chinese Hamster Ovary Cells

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Introduction

Undefined components, such as protein hydrolysates, have been routinely used in the biopharmaceutical industry to boost the growth and productivity of recombinant protein-producing Chinese Hamster Ovary (CHO) cells in culture. Due to regulatory concerns and the variability associated with these components, a need for chemically defined (CD) basal formulations has emerged. SAFC Biosciences (SAFC) has offered CD formulations in the past and has now expanded on that knowledge and expertise to develop a novel chemically defined formulation that yields both good growth and productivity for cultured CHO cells. This most recent medium, EX-CELL™ CD CHO Fusion, has shown enhanced performance in batch culture alongside legacy SAFC formulations as well as competitive performance against formulations available from external vendors. In addition, EX-CELL™ CD CHO Fusion shows good performance in fed-batch culture utilizing various plant hydrolysates as well as proprietary chemically defined feeds.

Materials and Methods

Cell lines and media:

Four recombinant IgG-producing test cell lines (**Table 1**) were initially maintained in EX-CELL™ CD CHO Fusion (SAFC Biosciences) and supplemented appropriately.

CELL LINE	PARENTAL	MEDIA SUPPLEMENT
1	CHO-S Derived	6mM L-Glutamine
2	CHO-S Derived	6mM L-Glutamine
3	CHOK1SV*	25µM methionine sulphoximine
4	CHOK1SV*	25µM methionine sulphoximine

*Licensed from Lonza Biologics

All legacy SAFC Biosciences formulations compared to EX-CELL™ CD CHO Fusion (SAFC Biosciences) were chemically defined.

For competitor assays, five CD formulations were tested alongside EX-CELL™ CD CHO Fusion (SAFC Biosciences). All stocks were adapted over at least five passages in each test condition before initiation of growth and productivity assays.

In the fed-batch assays, EX-CELL™ CD CHO Fusion (SAFC Biosciences) was supplemented on days two and four of culture with 3 g/L glucose and either 10% initial culture volume of proprietary chemically defined feed or 1g/L hydrolysates, where appropriate.

For each assay, cultures were inoculated directly from stocks into TPP® (Techno Plastic Products AG) 50ml bioreactor tubes and conditions were averaged after being evaluated in duplicate. Cultures were seeded at 2-3 E5 cells per milliliter and terminated when viabilities decreased to less than 70%.

Productivity assay:

Human IgG: IgG concentrations were quantitated using the Octet QK Bio-Layer Interferometer (ForteBio).

Results

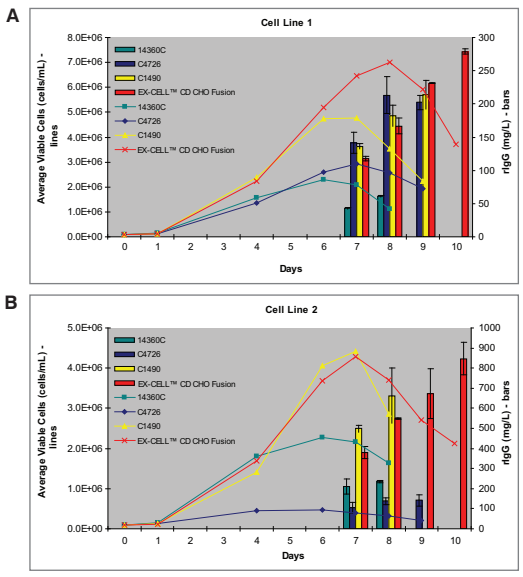

Figure 1. EX-CELL™ CD CHO Fusion compared to legacy SAFC Catalog formulations.

A) Cell Line 1, **B**) Cell Line 2. CHO cells were screened with all the chemically defined catalog formulations currently available from SAFC Biosciences. As seen from improvements in both growth and productivity, EX-CELL™ CD CHO Fusion demonstrates the progress that has been made in the development of chemically defined formulations that can produce high cell densities and IgG titers.

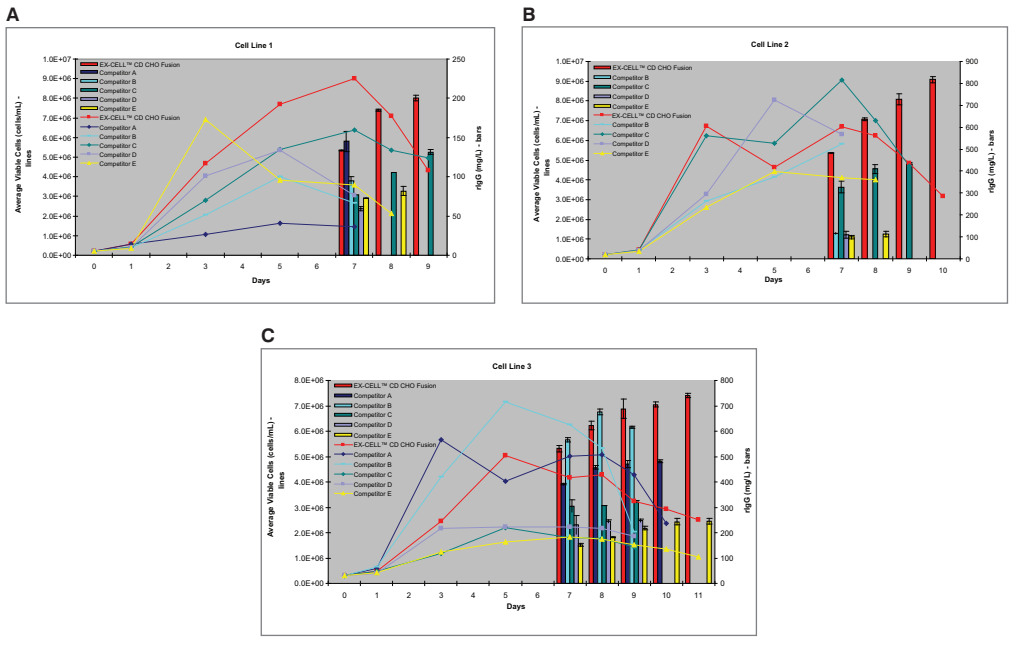

Figure 2. EX-CELL™ CD CHO Fusion compared to five competitor formulations.

A) Cell Line 1, **B**) Cell Line 2, **C**) Cell Line 3. Results show the differences in the viable cell densities and cumulative IgG productions of each cell line. In each experiment, EX-CELL™ CD CHO Fusion is competitive for cell growth and is superior for productivity. The overall performance of EX-CELL™ CD CHO Fusion demonstrates its versatility among cell lines and competitiveness with other formulations available for CHO cell culture. **Note:** Competitor A did not survive the adaptation process for Cell Line 2 so was not included in the assay.

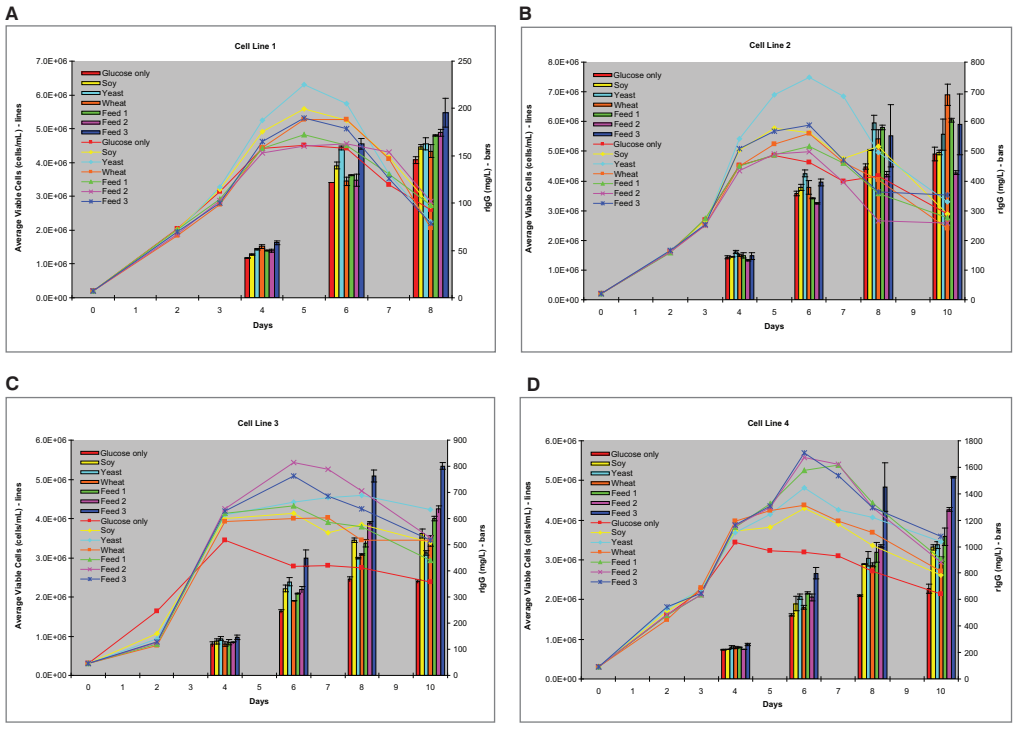

Figure 3. Fed-batch comparison in EX-CELL™ CD CHO Fusion using three plant hydrolysates and three chemically defined feeds.

A & B) Cell Lines 1 and 2, **C & D**) Cell Lines 3 and 4. While the degree to which feeds improve culture performance is cell line dependent, the data show that EX-CELL™ CD CHO Fusion can be used in combination with multiple defined and undefined feeds to enhance growth and productivity of CHO cells.

Conclusions

- SAFC Biosciences has developed a novel chemically defined formulation called EX-CELL™ CD CHO Fusion.
- EX-CELL™ CD CHO Fusion exceeds the overall performance of legacy SAFC chemically defined catalog offerings.
- When used alongside chemically defined competitor formulations, EX-CELL™ CD CHO Fusion achieves competitive growth and superior cumulative productivity.
- EX-CELL™ CD CHO Fusion can be used as a robust platform formulation that, when complimented with the appropriate CD feed, effectively replaces the need for undefined components to yield increased IgG production.

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