Transfection to Manufacturing: Reducing Timelines for High Yielding GS-CHO Processes

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Introduction

Chinese hamster ovary (CHO) cell lines are widely used by the bio-pharmaceutical industry for therapeutic protein manufacture. The rapid construction of high yielding recombinant CHO cell lines are a key economic consideration. Gene amplification is widely used to create high yielding CHO cell lines, however this can substantially increase the length of a cell line development programme. As a further consideration, adaptation to single cell suspension culture and to protein-free (or chemically defined) media can be problematic, and impact project timelines; one solution is to use a host cell line pre-adapted to the desired culture conditions.

The work reported here describes the creation of high yielding GS-CHO cell lines for use in a chemically-defined fermentation process. This was achieved though improvements to our cell line construction process, focusing on the development of a pre-adapted host cell line, and the optimisation of the transfection and selection stages.

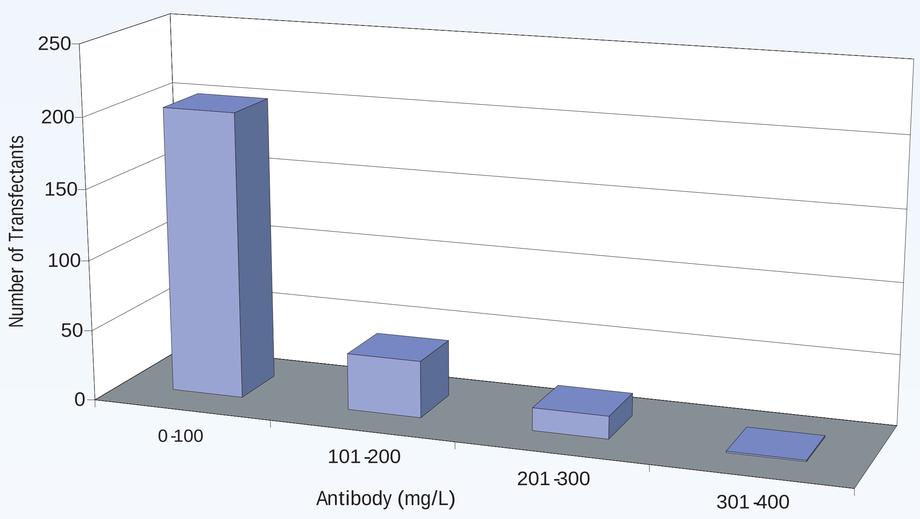
Methods

- Used a host CHO cell line pre-adapted to suspension culture and chemically-defined medium (CHOK1SV). This was evaluated against the original host cell line (CHOK1).
- Host cells, were transfected with genes coding for the antibody cB72.3 linked to the glutamine synthetase (GS) selectable marker.
- Transfectants were selected in serum containing glutamine-free medium.
- Successive productivity assays were performed by ELISA to select the highest yielding cell lines.
- High yielding cell lines were progressed to suspension cultures in a chemically-defined, animal component free medium.
- Productivity was assessed in shake flask cultures.
- Growth and productivity kinetics were evaluated in a platform chemically-defined GS-CHO fermentation process utilising a 10 L airlift bioreactor operated in fed-batch mode.

Results

CHOK1SV and CHO K1 host cells were transfected with a GS expression vector encoding the genes for the antibody cB72.3. Glutamine independent transfectants were selected and supernatants were screened by ELISA for the presence of assembled antibody. A total of 341 transfectants were initially screened, all of which produced antibody. From these, 259 transfectants were selected and expanded in static culture. The transfectants were allowed to overgrow and the supernatants were assayed for antibody (Figure 1). Antibody concentrations up to 315 mg/L were obtained.

Figure 1 Distribution of antibody productivities from a screen of 259 GS-CHO transfectants.



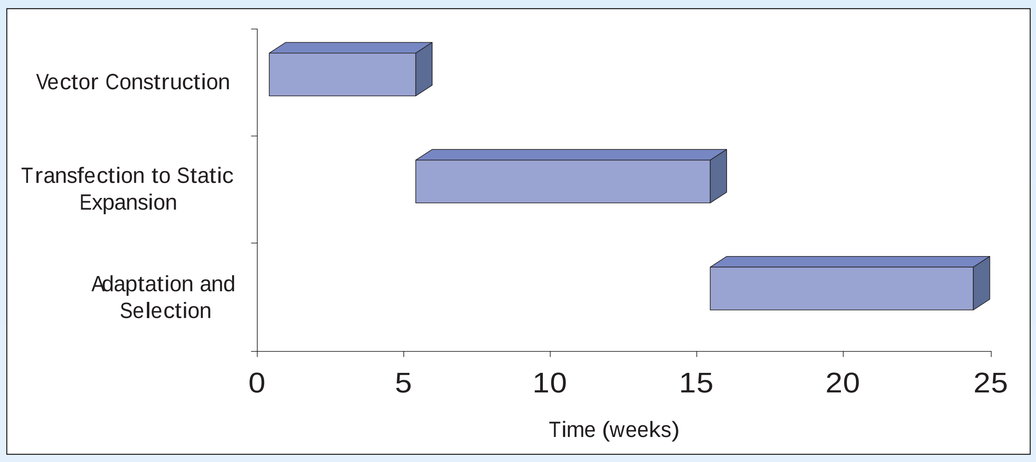
Thirty of these cell lines were selected based on their antibody concentration in static cultures and adapted to suspension culture in protein free medium. Selected cell lines were evaluated in shake flask culture (Table 1). Antibody concentrations up to 1150 mg/L were obtained. These cell lines did not require gene amplification.

The development of the pre-adapted CHOK1SV host cell line has resulted in a substantial timeline reduction. High yielding GS-CHO cell lines adapted to chemically-defined medium can be created within six months (Figure 2).

Table 1 Growth and productivity in shake flask suspension cultures using protein free medium.

Culture ID	Maximum Viable Cell Concentration (10^6/mL)	Product Concentration at Harvest (mg/L)
C6	8.2	422
C7	8.2	514
C11	3.5	641
C12	2.4	632
C01	2.9	417
C18	8.5	378
C23	2.4	957
LB01	14.1	1150

Figure 2 Timeline showing various steps performed to select high yielding GS-CHO cell lines adapted to a protein free medium. Use of the pre-adapted CHOK1SV host has resulted in a substantial reduction in the timeline. High yielding GS-CHO cell lines can be created within six months.



Two model cell lines: 22H11 (original CHOK1 host) and LB01 (created using the new CHOK1SV host), were grown in 10 L airlift bioreactors using successively improved versions of Lonza Biologics' platform GS-CHO fermentation process. These processes used chemically-defined medium and feeds. Medium and feed optimisation strategies enabled extension of culture duration, increased viable cell density and increased specific cellular productivity.
In figures 3 and 4, cell growth and productivity data are shown comparing the performance of the 22H11 and LB01 cell lines in conjunction with improvements to the generic GS-CHO fermentation process. The data here represent typical fermentation cultures. Using the LB01 cell line with the improved process (V2.0), the antibody concentration at harvest was elevated to 2.8 g/L.

Figure 3 GS-CHO chemically-defined fed batch bioreactor culture process and cell line optimisation: cell growth

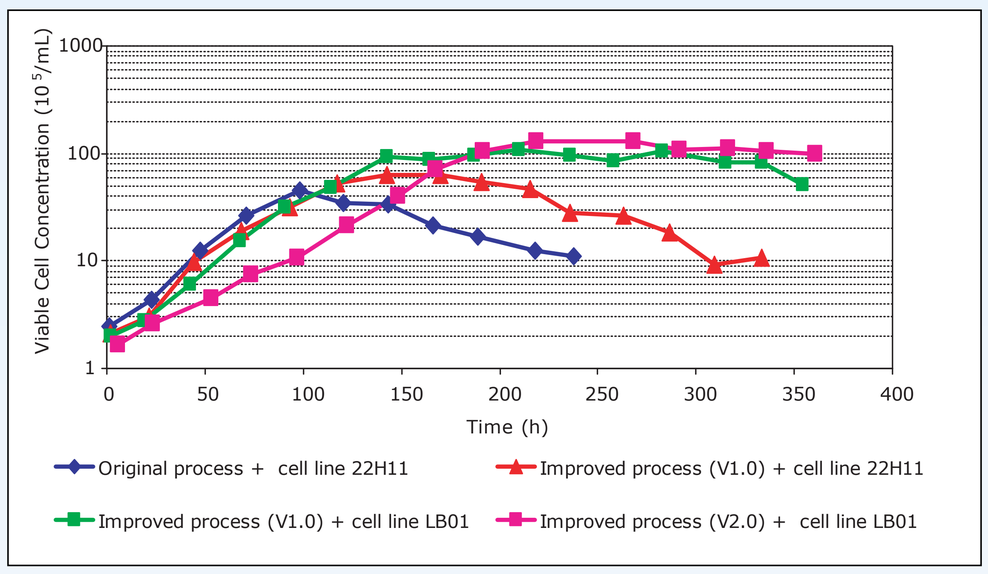
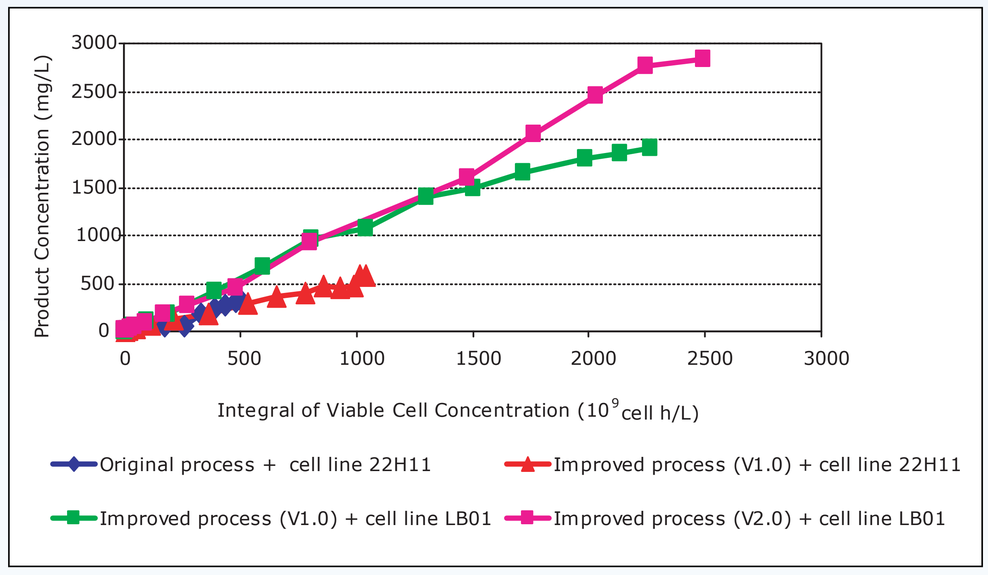


Figure 4 GS-CHO chemically-defined fed batch bioreactor culture: productivity improvement by process and cell line optimisation



Summary

- CHOK1SV derived transfectants rapidly adapted to suspension culture in protein free medium.
- Antibody concentrations up to 2.8 g/L were achieved in fed batch bioreactor culture.
- We have shown that we can develop high yielding GS-CHO cell lines for use in a fully chemically-defined, animal component free process in less than 6 months.
- Gene amplification was not required.
- Productivity and timeline improvements were the result of changes made at all stages of the cell line construction programme.

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