

Rapid production of antibodies by pooled CHO transfectants in disposable bioreactors

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INTRODUCTION

The biopharmaceutical industry is facing ever shorter timelines for initiating first-in-human studies. For animal cell expression systems, cell line development is often a critical path activity that limits the availability of material for starting other studies, e.g. formulation evaluation. The availability of antibody produced in the same host cell and cell culture process as the final cGMP manufacturing process has the potential to reduce a programme's duration. We report the development of a generic, chemically defined, animal component-free (CDACF) process for the rapid production of small quantities (5 to 50 g) of antibodies in CHO cells using pooled transfectants. In this process, antibody concentrations of 2 g/L were achieved within 9 weeks of transfection.

METHODS

Cell culture: The host cell line CHOK1SV (Lonza Biologics), is a derivative of the CHO-K1 cell line pre-adapted to CDACF media. Host cells were transfected by electroporation with the Glutamine Synthetase (GS) Gene Expression System (Lonza Biologics, UK): the expression vector encodes the genes for the chimeric antibody cB72.3 and the tightly linked GS gene, which is the selectable marker. Cryopreserved host cells were revived into the CDACF medium CD-CHO (Invitrogen) and the culture volume expanded. After transfection, the transfected pool was expanded using the selective medium CD-CHO/MSX. Cells were rapidly taken from static culture into suspension culture. Antibody production was carried out in a disposable bag bioreactor using a proprietary CDACF fed-batch process for GS-CHO cell lines.

Protein concentration assay: HPLC Protein A determination.

Product quality assessment: Reducing and non-reducing SDS-PAGE and IEF of protein A purified antibody.

ASSESSMENT OF MODEL ANTIBODY EXPRESSION BY POOLED CHO TRANSFECTANTS IN ERLERMAYER FLASK CULTURES

Transfections were performed in duplicate and cells derived from each transfection were divided between four T175 flasks. Following transfer into suspension, the productivity of the pooled transfectants was assessed in Erlenmeyer flask cultures operated in fed-batch mode. The antibody concentration at harvest, for Transfection A and Transfection B, was in the range of 430 to 2185 mg/L with a mean antibody concentration of 1031 ± 508 mg/L. The time taken from transfection to completion of these flask cultures was less than 49 days.

The productivity of cultures derived from the same transfection event was variable (Table 1). Cultures derived from Transfection A had a mean antibody concentration at harvest of 1232 ± 823 mg/L and a range of 554 to 2185 mg/L. Cultures derived from Transfection B had a mean antibody concentration at harvest of 829 ± 305 mg/L and a range of 430 to 1132 mg/L. The variation in product concentration appears to be due to variability in specific production rate (Q_p) rather than in cell growth. Each set of four flasks came from a single pool of transfectants, so a possible source of variation is the inherent heterogeneity of this pool, which leads to heterogeneity in Q_p .

Table 1: Summary of duplicate transfections performed.
Flasks A1-A4 were derived from the Transfection A and Flasks B1-B4 were derived from Transfection B.

	Flask ID	Day Post Transfection at Start of Culture	Maximum Viable Cell Concentration 10^6 /mL	Harvest Viable Cell Concentration 10^6 /mL	Viability at Harvest %	Culture Length days	Time Integral of Viable Cell Concentration 10^6 cell.h/mL	Specific Growth Rate 1/h	Antibody Concentration at Harvest mg/L	Q_p pg/(cell.h)	Mean Antibody concentration at Harvest mg/L	Standard Deviation
Transfection A	A1	34	12.2	8.8	66	15	2515	0.019	1262	0.502	1232	823
	A2	34	12.5	10.1	85	15	2755	0.020	2185	0.793		
	A3	34	14.3	1.8	12	15	2729	0.0195	554	0.203		
	A4	34	11.9	1.03	8	15	2279	0.017	926	0.406		
Transfection B	B1	31	13.5	2.9	21	15	2582	0.020	985	0.381	829	305
	B2	31	14.6	3.1	22	15	2847	0.020	1132	0.398		
	B3	31	13.2	3.8	31	15	2531	0.021	770	0.304		
	B4	31	14.9	3.1	20	15	3133	0.021	430	0.137		

DURATION OF MODEL ANTIBODY EXPRESSION BY POOLED CHO TRANSFECTANTS

The stability of product expression upon serial sub-culture of pooled transfectants was assessed. Cultures derived from Transfection A were maintained and fed-batch overgrow cultures were established at 61 days post-transfection. As shown in Table 2, cultures established at 61 days post-transfection had harvest antibody concentrations which ranged from 126 to 642 mg/L with a mean of 419 ± 194 mg/L. This represents a 66% decrease in expression over 27 days when compared to the productivity of cultures established 34 days post-transfection (Table 1). All cultures established at 61 days post-transfection had higher specific growth rates than cultures established at 34 days post-transfection. Three out of four cultures established at 61 days post-transfection had lower Q_p values than the earlier fed-batch cultures. The decline in antibody concentration with increasing cell age seen here, where a loss of Q_p is not off-set by improvements in cell growth, is similar to the behaviour observed with some cell lines.

Table 2: Summary of early and late fed-batch cultures of Transfection A established at day 61 post-transfection.

Flask ID	Day Post Transfection at Start of Culture	Maximum Viable Cell Concentration 10^6 /mL	Harvest Viable Cell Concentration 10^6 /mL	Viability at Harvest %	Culture Length days	Time Integral of Viable Cell Concentration 10^6 cell.h/mL	Specific Growth Rate 1/h	Antibody Concentration at Harvest mg/L	Q_p pg/(cell.h)	Mean Antibody concentration at Harvest mg/L	Standard Deviation
A1	61	16.6	2.8	21	11	1911	0.022	377	0.381	419	194
A2	61	15.9	2.8	21	11	1942	0.023	642	0.398		
A3	61	15.2	2.2	16	11	1788	0.024	126	0.304		
A4	61	15.8	2.4	17	11	1820	0.022	532	0.137		

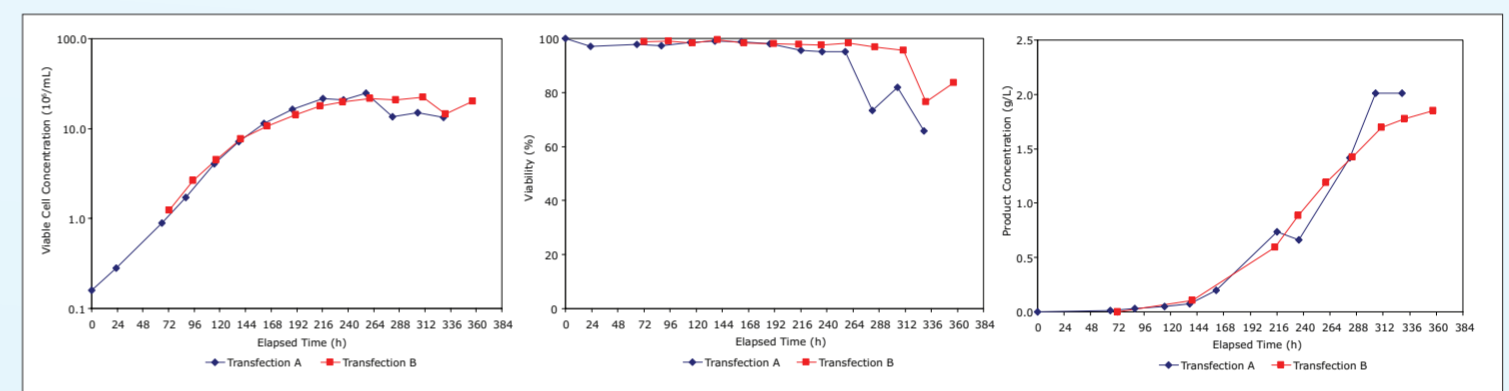
DISPOSABLE BIOREACTOR CULTURES OF POOLED CHO TRANSFECTANTS

The growth and productivity kinetics of pooled transfectants were assessed in a generic GS-CHO bioreactor process. Cultures derived from each transfection were pooled and expanded for inoculation of a disposable bioreactor. The bioreactor culture was operated in a fed-batch mode. Table 3 and Figure 1 show the results obtained.

Table 3: Summary of performance of Transfections A and B in disposable bioreactors

Run	Transfection	Day post-transfection at start of culture	Maximum Viable Cell Concentration 10^6 /mL	Harvest Viable Cell Concentration 10^6 /mL	Viability at Harvest %	Culture Length days	Time Integral of Viable Cell Concentration 10^6 cell.h/mL	Specific Growth Rate 1/h	Antibody Concentration at Harvest mg/L	Q_p pg/(cell.h)
A	Transfection A	49	24.81	13.4	66	14	3443	0.027	2010	0.584
B	Transfection B	45	22.4	20.1	84	15	4103	0.023	1856	0.452

Figure 1: Profiles of viable cell concentration, culture viability and antibody accumulation during bioreactor runs A and B.

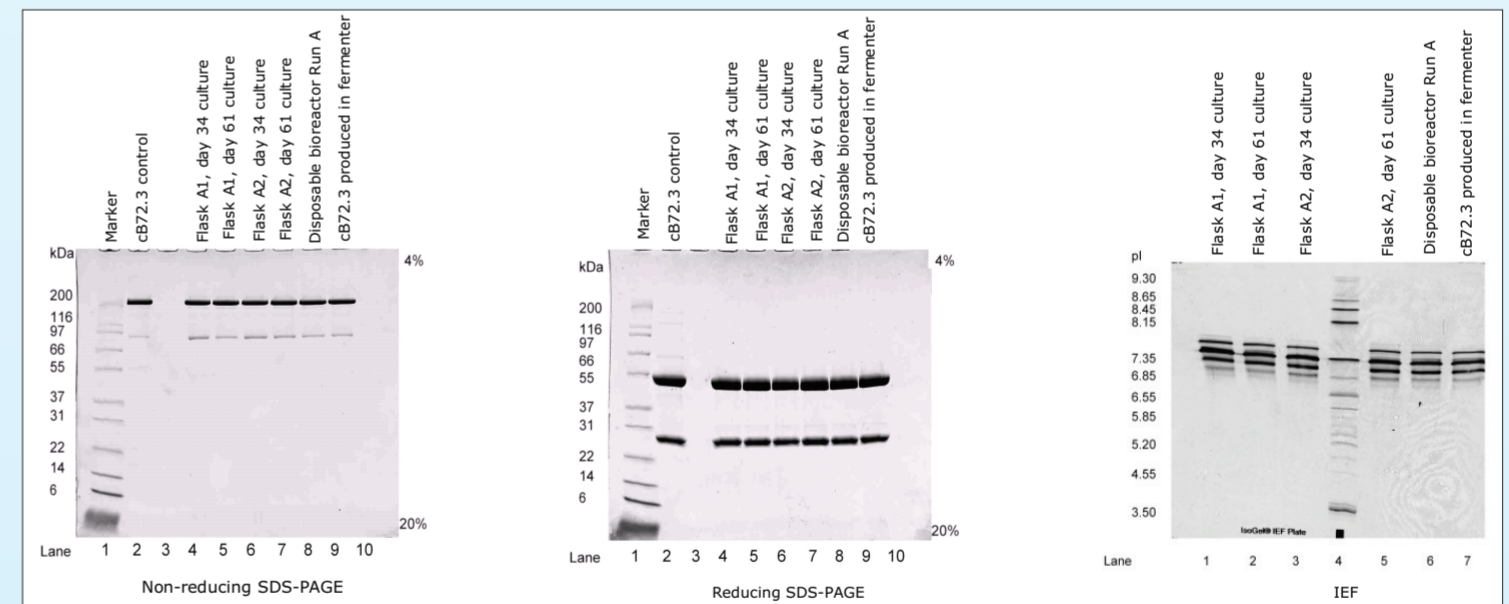


PRODUCT QUALITY ASSESSMENT

Preliminary analysis of protein characteristics was performed on the cB72.3 product from the early (day 34) and late (day 61) fed-batch cultures and disposable bioreactor run A. Samples were compared to cB72.3 produced by a stable cell line, LB01, in a bioreactor.

Differences in banding patterns were not observed upon analysis by SDS-PAGE (reduced or non-reduced) and IEF electrophoresis.

More detailed characterisation (eg oligosaccharide profile) data are not yet available.



CONCLUSION

- Antibody concentrations of 1031 ± 508 mg/L were achieved in Erlenmeyer flask cultures operated in fed-batch mode established up to 34 days post-transfection.
- Antibody concentrations of approximately 2000 mg/L were achieved in disposable bioreactor cultures operated in fed-batch mode established up to 49 days post-transfection.
- Time taken from transfection to completion of bioreactor culture was 9 weeks.
- Preliminary product quality assessment shows no marked differences between cB72.3 antibody produced by pooled transfectants in either Erlenmeyer flask cultures or a disposable bioreactor and cB72.3 antibody produced by stable GS-CHO cell line LB01 in a bioreactor.
- Pooled transfectants represent an alternative approach to transient gene expression in CHO cells using polyethylenimine or calcium phosphate. A 5 L culture would be required to produce 5 g of cB72.3 (assuming 50% loss on purification) using pooled transfectants compared to a 250 L culture using transient gene expression (assuming productivity of 40 mg/L).

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FOR FURTHER INFORMATION

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