Optimization of Transient Transfection of CHO cells. A comparison between transfection efficiency utilizing PEI vs. an electroporation transient transfection system.

Richard H. Archer, Jeanette R. Doerr, Teri Taylor and Connie Chang
Avid Bioservices Inc., Tustin, CA

Abstract

Transient gene expression in CHO cells is a difficult enterprise to obtain high titers of recombinant antibody proteins. Conducting a transient transfection in the same host as our stable cell lines allows similar post translational modification of the expressed protein or antibodies, such as glycosylation patterns. The polycation compound polyethylenimine (PEI) transient transfection is economical but results in poor protein yields. AVID Bioservices has recently tested an electroporation transient transfection system using their small scale transfection set up side by side with PEI transient transfections to compare final yields of either mouse chimeric or fully human recombinant antibodies in CHO cells. PEI experiments were conducted comparing different PEI: DNA ratios, growth temperatures and culture feeds. Electroporation transfections were conducted examining the parameters of different DNA concentrations, temperature shift and proprietary feed conditions. Optimized electroporation resulted in an increase of 20- 60 fold in expression titers compared to PEI expression yields with up to 1 gram/ L obtained using the electroporation based transfection system. The electroporation system can greatly increase the overall yield of transiently expressed antibody constructs and shorten the time required to obtain gram amounts of protein for in vitro characterization and in vivo tumor model studies. This system will also be utilized to generate transient and stable material concurrently.

Introduction

Transient gene expression in mammalian cells is a valuable tool to determine the efficacy of recombinant proteins in a short amount of time. This method is an alternative to produce large amounts of protein within weeks instead of 2-6 months required for stable transfection and expansion of isolated clones. The polycation compound polyethylenimine (PEI) is one of the most utilized reagents for small to large scale transient transfections of CHO cells as it is simple to use and very cost effective (1). The major drawback is that PEI yields very low amounts of product (usually less than 10-30 mg/L) so that very large cultures are required to obtain the milligram to gram amounts of protein required for biochemical, biophysical and ultimately in vivo animal tumor model studies.

Recent advances in chemical (liposomal) transfection systems have yielded higher titers of recombinant proteins but become cost prohibitive with large scale cultures. Higher titers have been obtained using the HEK293 cell line and associated transfection kits but products derived from the HEK293 cells differ in glycosylation patterns compared to CHO derived products (2).

The MaxCyte® STX electroporation transfection system tested here has optimized the transient transfection of CHO cells to yield very high cell viabilities with the ability to expand to a flow electroporation protocol with culture volumes up to 4L. This system can also be utilized for stable transfections.

Experimental Design

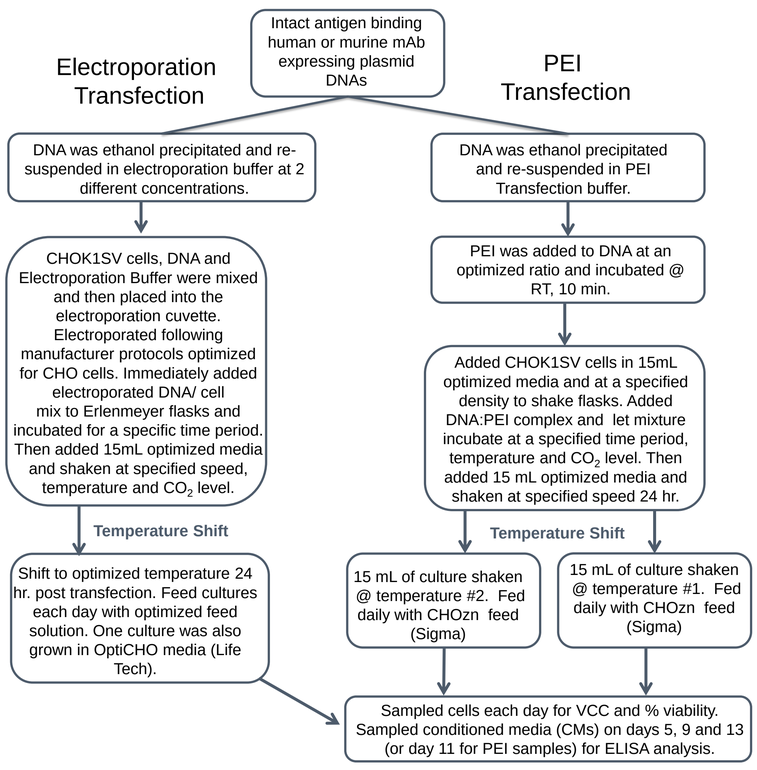


Figure 1. Experimental protocol scheme comparing PEI and MaxCyte® **STX** electroporation transient transfection techniques..

Figure 2. MaxCyte® STX electroporation transfection apparatus. Pictured is the apparatus set up for 100mL flow electroporation and expansion to a 4L culture. The experiments described here utilized the 0.4mL cuvette and 15mL culture volumes.

RESULTS

Fig. 3a. MaxCyte® STX Electroporation Transfection-Viable Cell Density

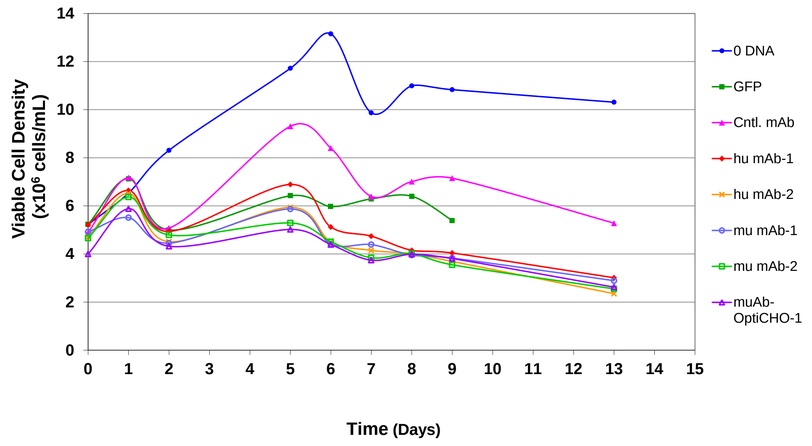


Fig. 3b MaxCyte® STX Electroporation Transfection- Viability

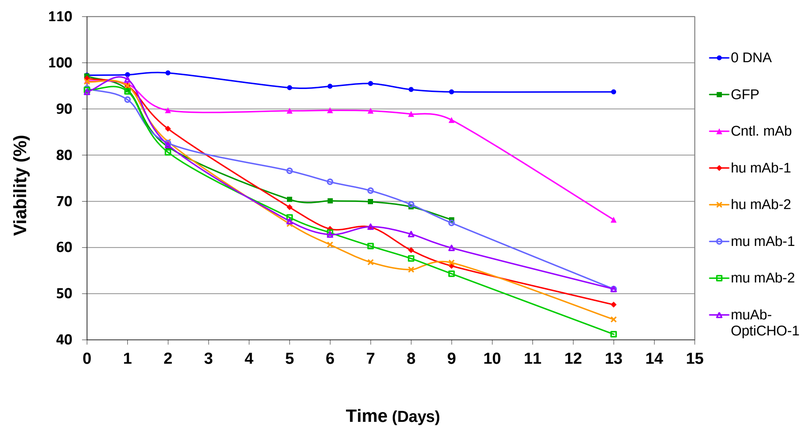


Figure 3. Growth characterization of post electroporation transient transfection cultures. (3a.) Viable cell counts and (3b.) cell viability were assayed on the days indicated and measured by a ViCell cell counter (Beckman Coulter).

Fig.4a PEI Transfection Viable Cell Density

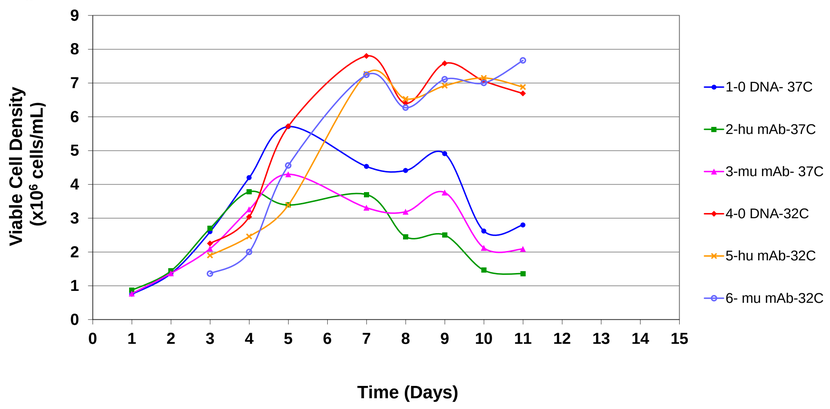


Fig.4b PEI Transfection Viability

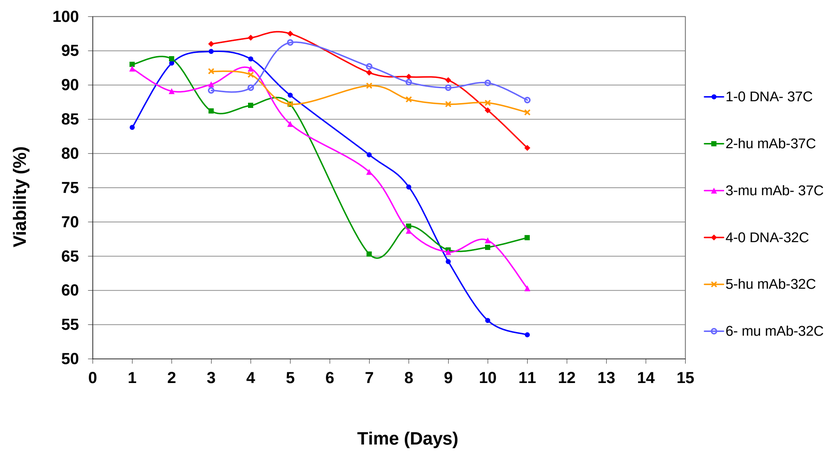
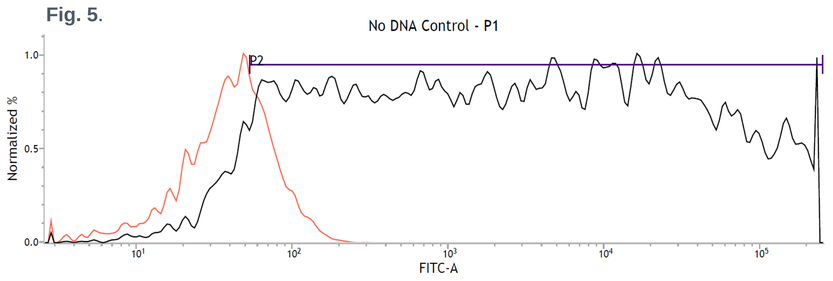


Figure 4. Growth and cell viability of transient transfection cultures conducted using PEI as the transfection reagent. (4a) Viable cell density and (4b) cell viability as measured on a ViCell XR cell counter (Beckman Coulter).

Fig. 5.



Statistics

Name	Events	% Parent	% Grandparent	% Total	FSC-A Mean	SSC-A Mean	FITC-A Mean	FITC-A Geo Mean	FITC-A Median	FITC-A Mode
No DNA Control:P1	8,949	89.49	***	89.49	96,042	53,091	46	34	42	0
GFP Test:P1	7,563	75.63	***	75.63	97,537	57,022	32,409	2,880	3,027	0
GFP Test:P2	6,819	90.16	68.19	68.19	97,840	56,665	23,029	3,060	3,199	0
No DNA Control:P2	2,862	31.98	28.62	28.62	114,594	70,116	80	77	71	0

Figure 5. CHO cell Transfection Efficiency and Cell Viability Using MaxCyte® **STX** Electroporation Transient Transfection. CHOK1SV cells were transfected with a plasmid encoding green fluorescent protein using small the scale (static) electroporation apparatus. GFP expression and viability were measured by flow cytometry (FACS) 24 hours post electroporation.

Fig. 6a Antigen binding/ titer ELISA- MaxCyte® STX Electroporation Transient Transfection (CHOK1SV) CMs

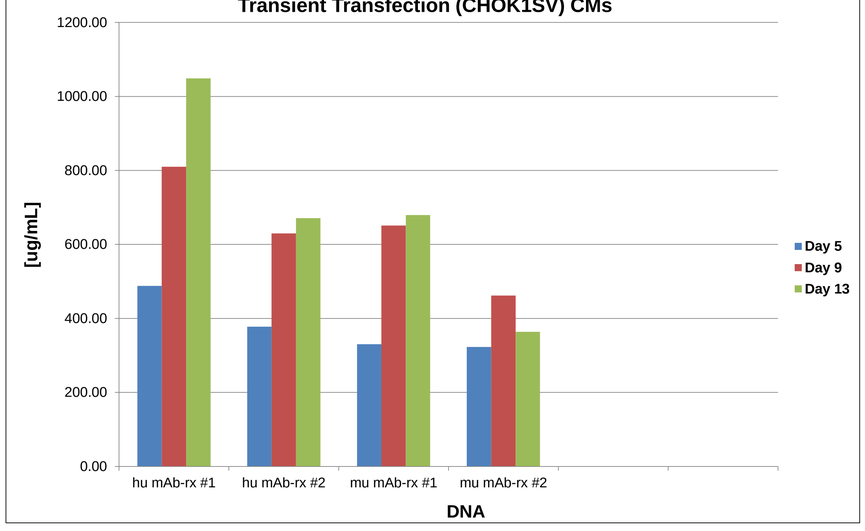
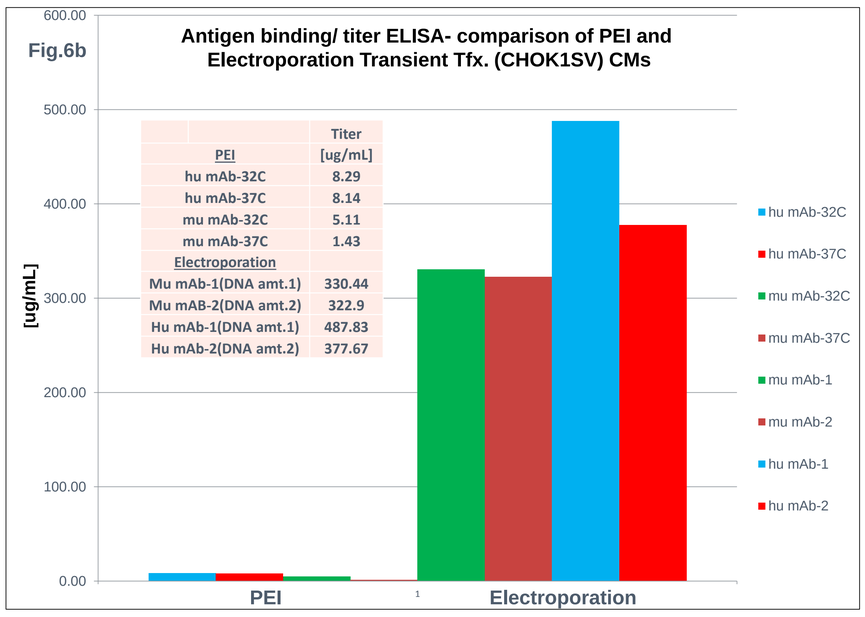


Fig.6b Antigen binding/ titer ELISA- comparison of PEI and Electroporation Transient Tfx. (CHOK1SV) CMs



	Titer
PEI	[ug/mL]
hu mAb-32C	8.29
hu mAb-37C	8.14
mu mAb-32C	5.11
mu mAb-37C	1.43
Electroporation	
Mu mAb-1(DNA amt.1)	330.44
Mu mAB-2(DNA amt.2)	322.9
Hu mAb-1(DNA amt.1)	487.83
Hu mAb-2(DNA amt.2)	377.67

Figure 6. PEI vs. MaxCyte® STX electroporation transfection efficiency determined by antigen binding/ titer ELISA of transfection conditioned media (CM). (6a.) Electroporation transfection CMs were collected on days 5, 9 and 13. (6b.) Comparison of PEI vs. electroporation transfection antibody titers for day 9 CMs. Inset table shows actual antibody titer levels depicted in the associated graph.

Fig. 7.

1	BioRad Dual Color Std.
2	Cntl. huAb PP
3	Hu mAb-1 harvest
4	Hu mAb-1 Eluate PP
5	Hu mAb-2 harvest
6	Hu mAb-2 Eluate PP
7	Mu mAb-1 harvest
8	Mu mAb-1 Eluate PP
9	Mu mAb-2 harvest
10	Mu mAb-2 Eluate PP

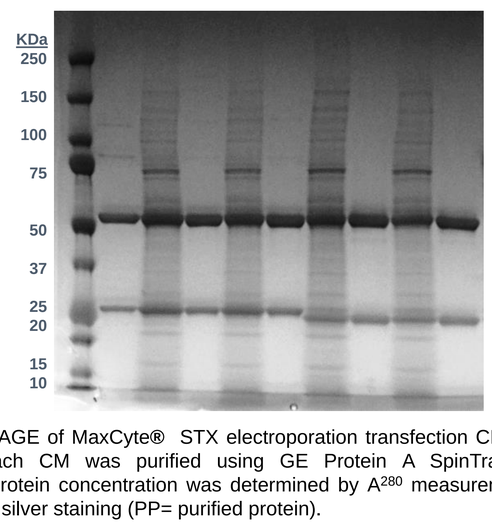


Figure 7. Reducing SDS PAGE of MaxCyte® STX electroporation transfection CM harvests and purified proteins. Each CM was purified using GE Protein A SpinTrap column chromatography. Purified protein concentration was determined by A^{280} measurement . The proteins were visualized by silver staining (PP= purified protein).

CONCLUSIONS

1. Evaluation of these transfection systems for cell growth (VCD) and % viability post transfection demonstrated slightly higher VCD and % viability for the MaxCyte® STX electroporation transfections over time then when using PEI as the transfection reagent.
2. Transfection efficiency, comparing overall yield of secreted protein at different time points measured by antigen binding ELISA assays, confirmed that the MaxCyte® STX electroporation transient transfection system is far superior to transfections using PEI. Using 2.7 fold higher initial VCD seeding and only 1.6 fold higher DNA input, antibody titers obtained from the electroporation transfection system were up to **50- 60 fold higher** than achieved using PEI as the transfection reagent for CHO cells.
3. Protein A spin column purified antibodies analyzed by reducing SDS-PAGE demonstrated the expected size and integrity of the heavy and light chains.
4. This protein yield, calculated from the volume of culture CM processed through the column and concentration calculated by A^{280} after column purification was in the range of 200- 400ug/mL, only slightly lower than the quantitation of titer ELISA results.

References

1. Bollin F., et al. Design of experiment in CHO and HEK transient transfection condition optimization. (2011) Protein Expression and Purification 78: 61-68.
2. Croset, A. et al. Differences in the glycosylation of recombinant proteins expressed in HEK and CHO cells. (2012) J. Biotechnology 161(3): 336-48.

ACKNOWLEDGEMENTS

We would like to thank the Process Science team for their technical, intellectual and editorial support. We would also like to thank Steve King for his support. MaxCyte is a registered trademark of MaxCyte, Inc.