HIGH YIELD TRANSIENT PROTEIN PRODUCTION IN CHO CELLS

Jenny Bang, Alex A. Jurisch, Catherine Nguyen, Elaine Nguyen, Tom Fletcher, and Jessie H.-T. Ni
Department of Research and Development, Irvine Scientific, Santa Ana, California, USA

Introduction

CHO cells are the dominant hosts for commercial biopharmaceutical production processes, therefore they offer obvious advantages as hosts for producing drug candidates using transient methods during early phase research and development. Unfortunately, the transient transfection in CHO cells is not as well developed compared to that in HEK293 cells. Here we demonstrate a high yield transient protein production method that achieved >0.7g/L of a bio-similar protein by PEI-mediated transfection in CHO cells.
A chemically-defined medium was developed specifically for CHO cells by optimizing crucial components that affected cell growth and transfection efficiency. The transfection protocol was then refined by optimizing transfection parameters which included the amount of DNA, ratio of DNA-to-PEI, and cell density at time of transfection. Further improvement in yield was observed by enhancing post-transfection cell growth by developing a supplement strategy. We will present results including transfection efficiency, cell growth, titer, and product quality along with a detailed protocol.

Background

Figure 1. Media Development Overview

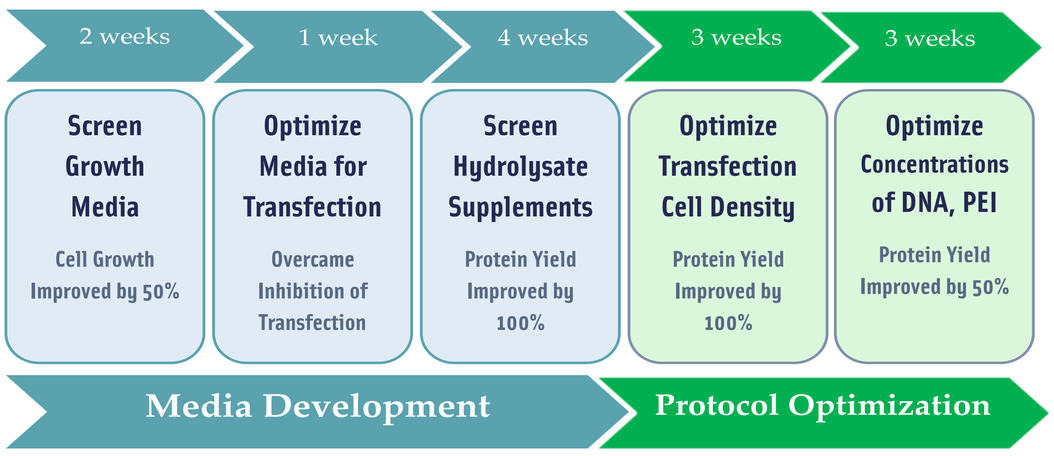
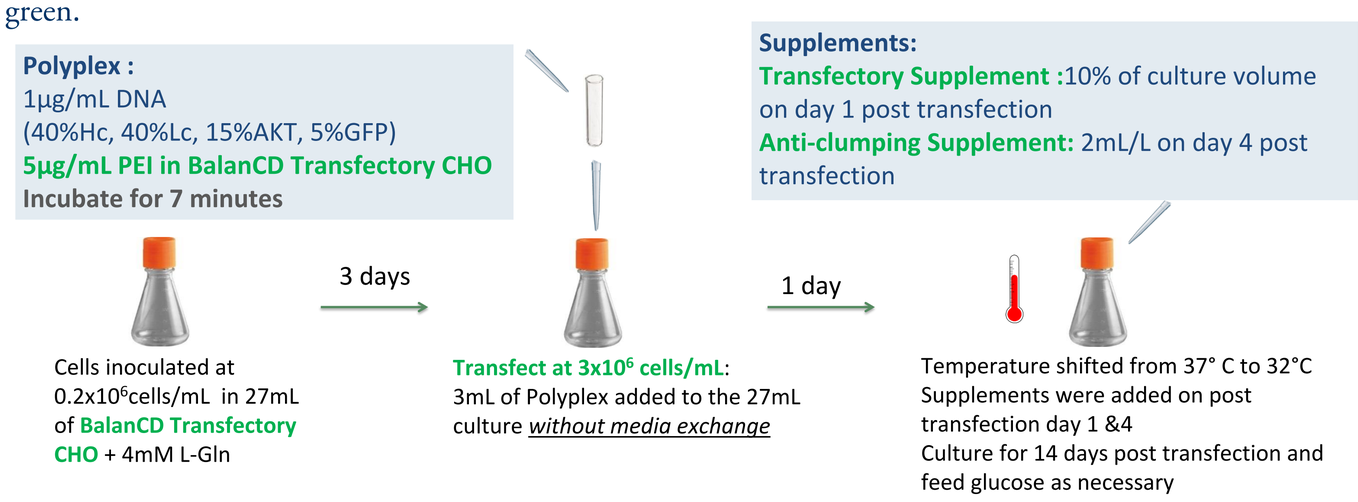
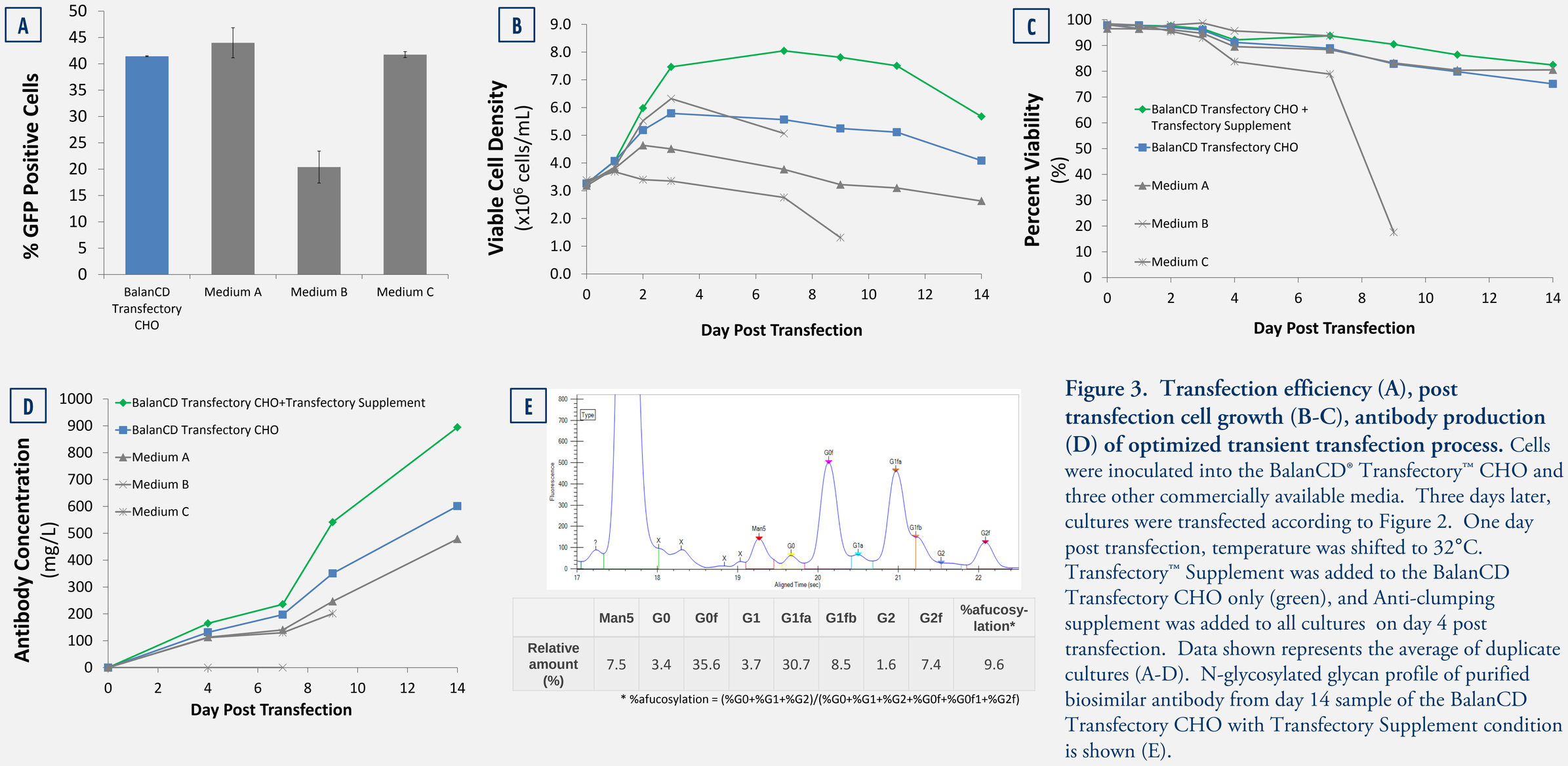


Figure 2. Optimized Transfection Protocol. Optimized media and transfection parameters shown in green.



Results



	Man5	G0	G0f	G1	G1fa	G1fb	G2	G2f	%afucosy-lation*
Relative amount (%)	7.5	3.4	35.6	3.7	30.7	8.5	1.6	7.4	9.6

* %afucosylation = (%G0+%G1+%G2)/(%G0+%G1+%G2+%G0f+%G0f1+%G2f)

Figure 3. Transfection efficiency (A), post transfection cell growth (B-C), antibody production (D) of optimized transient transfection process. Cells were inoculated into the BalanCD® Transfectory™ CHO and three other commercially available media. Three days later, cultures were transfected according to Figure 2. One day post transfection, temperature was shifted to 32°C. Transfectory™ Supplement was added to the BalanCD Transfectory CHO only (green), and Anti-clumping supplement was added to all cultures on day 4 post transfection. Data shown represents the average of duplicate cultures (A-D). N-glycosylated glycan profile of purified biosimilar antibody from day 14 sample of the BalanCD Transfectory CHO with Transfectory Supplement condition is shown (E).

Methods

Culture Parameters
- Cell Line: CHO-3E7
- Seeding Density: 0.2x10⁶cells/mL
- Passage: every 3-4 days using BalanCD Transfectory CHO + 4mM Gln
- 125mL Shake flasks with 30mL working volume
- Incubator: 37°C, 5% CO_2 120rpm agitation

Media and Reagents
- BalanCD® Transfectory™ CHO: Irvine Scientific (PN 91147)
- Transfectory™ Supplement: Irvine Scientific (PN91148)
- Anti-clumping supplement: Irvine Scientific (PN91150)
- PEIpro™ Polyplus (Catalog# 115-010)

Measurements
- Antibody Concentration: fortéBio, Octet® QKᵉ
- Cell density and viability: Beckman Coulter, Vi-Cell® XR Cell Viability Analyzer
- Glycan Analysis: Caliper Life Sciences, LabChip® GX II
- Antibody Purification: GE Healthcare Life Sciences, MabSelect SuRe™ LX

Summary

- By collectively optimizing the growth medium, hydrolysate supplement and transfection method, approximately 900mg/L titer was achieved
- This approach can be applied to multiple cell line and media combinations

Acknowledgments
CHO-3E7 model system provided by Dr. Yves Durocher of Canadian National Research Council
Product quality analysis performed by David T. Ho and David E. Ho of Irvine Scientific

Presented at BPI October 2015 (Boston, USA)