Strategies for Improved Protein Expression, Production Timelines and Laboratory Productivity Using CHO Cell Transient Expression.

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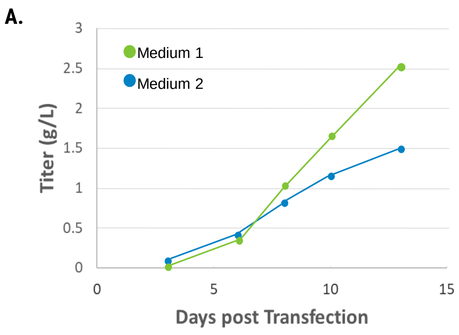
Abstract

Although protein biotherapeutics continue to have clinical success, companies are challenged to produce new clinical candidates with fewer resources and shortened timelines. Maximizing transient protein production and laboratory productivity play central roles in meeting those challenges, both of which rely on high performance, flexible CHO cell transfection for implementation. This poster highlights upstream process development strategies, including media/feed composition, feed schedules and seed density optimization, that can be used to increase titers, shorten timelines, simplify feed strategy and/or lower production costs. These data will illustrate the critical roles of transfection efficiency, scalability, and flexibility in enabling the development of streamlined production processes.

Case Study #1 - Media/Feed Composition and Schedule Flexibility Provide Avenues for ExpiCHO Protein Expression Optimization

Striking the Balance of Titer, Timing & Cost to Meet Your Goals



	Seed Density	Day 0	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
Medium 1	6 x 10^6 cells/mL		10% feed + glutamine		15% feed + glutamine		15% feed + glutamine		10% feed + glutamine			10% feed + glutamine			Harvest
Medium 1	1 x 10^7 cells/mL	10% feed + glucose	15% feed + glutamine		15% feed + glutamine		10% feed + glutamine					10% feed + glutamine			Harvest
Medium 2	6 x 10^6 cells/mL	20% feed + glucose	1 mM Na Butyrate			10% feed				10% feed					Harvest
Medium 2	1 x 10^7 cells/mL	20% feed + glucose	1 mM Na Butyrate			10% feed				10% feed					Harvest

Figure 1: Effects of Media Composition, Feed Schedule & Seed Density on Antibody Production Titers and Timelines. ExpiCHO cells were adapted to two different commercial medium (Medium 1 = $131/L, Medium 2 = $95/L). Cells were resuspended at 2e8 cells/mL and transfected with a human IgG plasmid via large-scale (CL-2 processing assembly) electroporation using the MaxCyte STX®. Transfected cells were seeded into 30 mL of Medium 1 or Medium 2 at 6e6 cells/mL. Cells were fed using two feed strategies as indicated. 24 hours post electroporation all cultures were shifted to 32°C. Antibody titers were analyzed on Days 3, 6, 8, 10 and 13 post transfection via ELISA and viability measured on Days 1, 2, 3, 6, 7, 8, 10 and 13.

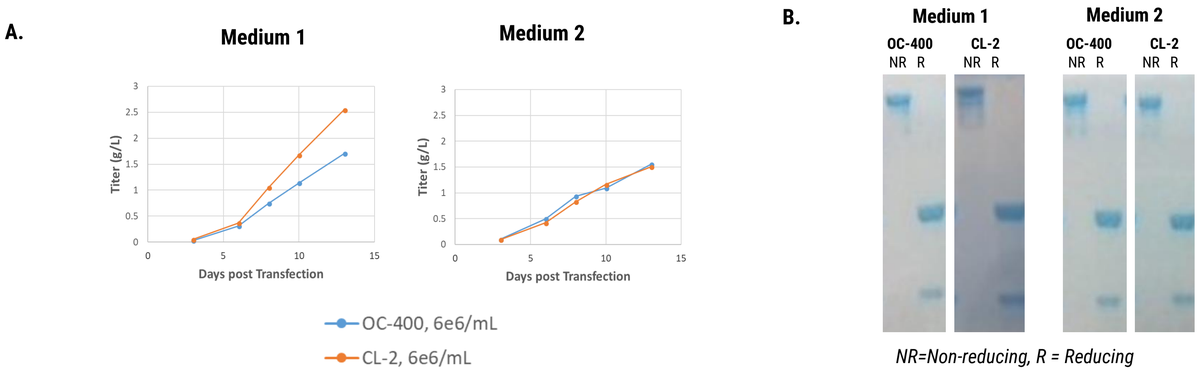
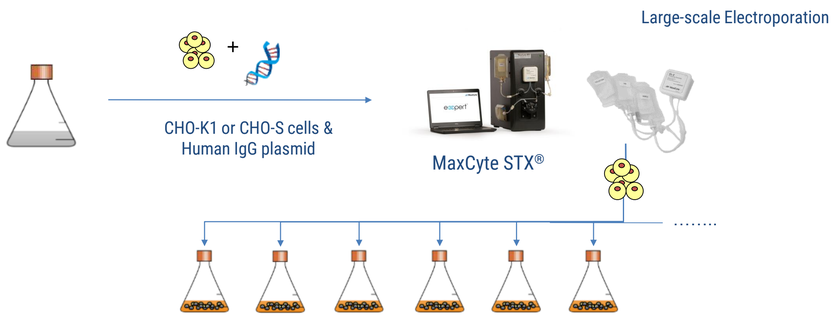


Figure 2: Rapid Transfection Scale While Maintaining Protein Titers and Product Quality. ExpiCHO cells were transfected with a human IgG plasmid at large-scale (CL-2 flow electroporation with 2e9 cells) or at small scale (OC-400 static electroporation with 2e8 cells) and seeded into 30 mL of Medium 1 or Medium 2 at 6e6 cells/mL. Cells were fed as indicated in Figure 1 table. **A).** Antibody titers were analyzed on Days 3, 6, 8, 10 and 13 post transfection. **B).** IgG was purified from Day 13 samples and run on reducing and non-reducing gels to assess final product quality. High quality IgG was produced for all samples.

Case Study #2 – Improving CHO-K1 and CHO-S IgG Production

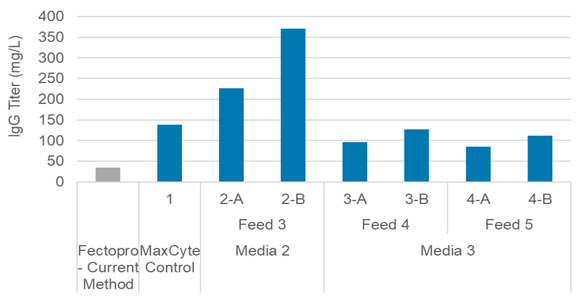
Maximum Titer Effected by Cell Type, Media, and Vessel Type



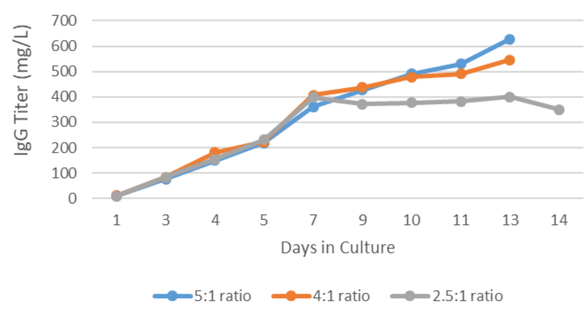
Parameters Examined:
- Transfection Method (FectoPRO® vs MaxCyte)
- CHO Cell Type (CHO-K1, CHO-S)
- Media/Feed (4 tested)
- Culture Vessel (3 tested)

Culture Assessment:
- Gas & Electrolytes
- Nutrients, Metabolites & Osmolality
- Cell Viability & Viable Cell Density
- IgG titers

A. Effect of Media Type on CHO-K1 IgG Harvest Titers



B. Effect of Vessel to Culture Ratio in CHO-S Cells



C. Superior Transfection Boosts IgG Harvest Titers

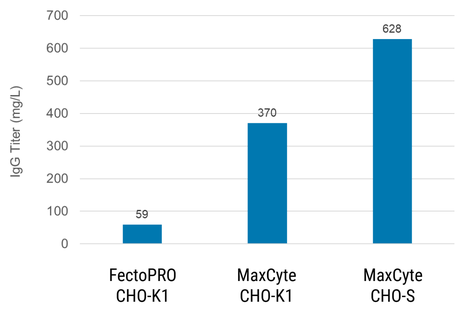
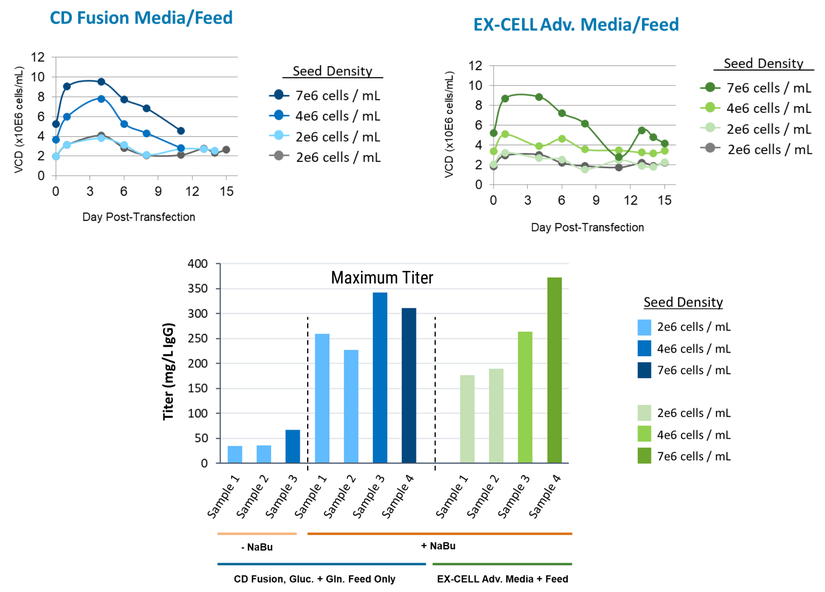


Figure 3: Full Process Optimization in CHO Cell Line of Choice for Titer Improvements. CHO-K1 or CHO-S cells were transfected with a human IgG plasmid via a single large-scale electroporation using the MaxCyte STX or FectoPRO transfection. Transfected CHO cells were cultured in 4 different media (CHO-S and CHO-K1) and three different vessel:culture ratios (CHO-S only). Temperature was shifted to 32°C and 1mM sodium butyrate (HDAC inhibitor) added 24 hours post transfection. **A).** Significant effects of media/feed on IgG titers at the time of harvest for CHO-K1 transfected cells. **B).** MaxCyte-transfected CHO-S seeded at various vessel:culture ratios exhibit differences in antibody production for cultures older than 1 week. **C).** Antibody titers at the time of harvest were >6x higher for CHO-K1 cells transfected via MaxCyte electroporation compared to client's established FectoPRO method. MaxCyte-transfected CHO-S cultures produced roughly 2x higher titers than MaxCyte-transfected CHO-K1 cells.

Case Study #3 – Boosting CHOZN® Cell Productivity

Media/Feed & Seed Density Optimization

A. Effects of Media Composition & Seed Density on Cell Growth & Antibody Titers



B. Protein Quality Unaffected by Media Choice

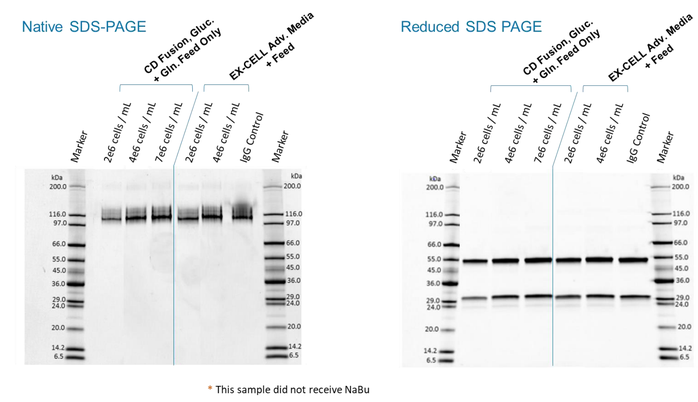


Figure 4: CHOZN-based Production of High Quality Antibody. CHOZN cells were transfected with an antibody plasmid via MaxCyte electroporation. Transfected cells were seeded in either CD Fusion medium or Ex-Cell Advanced (Sigma Aldrich Cat # 14365C) medium at three different densities: 2e6 cells/mL, 4e6 cells/mL or 7e6 cells/mL. 24 hours post electroporation culture temperature was lowered to 32°C and sodium butyrate (NaBu) added (except where indicated in Panel A). **A).** CD Fusion media was supplemented with glucose and glutamine at time of feed. Antibody titers (HPLC) and cell viability were analyzed on Days 1, 4, 6, 8, 11, 13, 14 and 15 post transfection. **B).** Purified antibodies were run on reducing and non-reducing gels to assess quality.

Case Study #4 – Reducing Production Timelines

Seed Density Optimization Boost Human IgG4 Titers by 4-6x

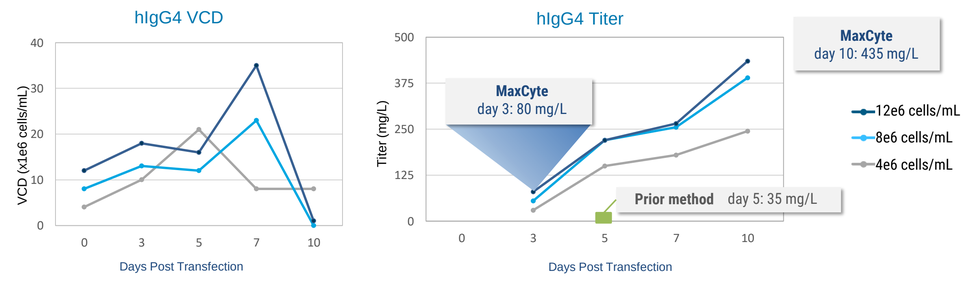


Figure 5: Higher ExpiCHO IgG4 Titers Using Flow Electroporation Technology. ExpiCHO cells were transfected with a human IgG4 expression plasmids using MaxCyte electroporation. Transfected cells were seeded at 4e6 cells/mL, 6e6 cells/mL, or 1.2e7 cells/mL using ExpiCHO medium and feed. Cell viability and antibody titers were determined at Days 3, 5, 7 and 10 post transfection.

Summary

- MaxCyte's Flow Electroporation® Technology provides for high efficiency, high viability transfection of a variety of CHO cell lines, including CHO-S, CHOZN and ExpiCHO cells.
- MaxCyte's high transfection performance and universal nature provide for full, post transfection culture conditions enabling laboratories to run full optimization studies to reach their quantity, quality, cost and timeline goals.
- The MaxCyte platform allows the use of any media or culture supplements enabling:
  - significantly lower consumable costs with equal to or improved protein titers in the cell line of choice
  - use of specialty media such as those used to direct glycosylation patterns
- Full process optimization results in higher protein expression levels which can significantly shortens production run times.
- MaxCyte transfection supports high seed densities post electroporation while maintaining high antibody expression and product quality, thereby improving laboratory productivity.
- High viability and transfection efficiency result in strong expression of more difficult-to-express proteins such as human IgG4 antibodies.

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