Maximizing Productivity and Data Relevance with a Scalable, Non-viral Cell Engineering Technology.

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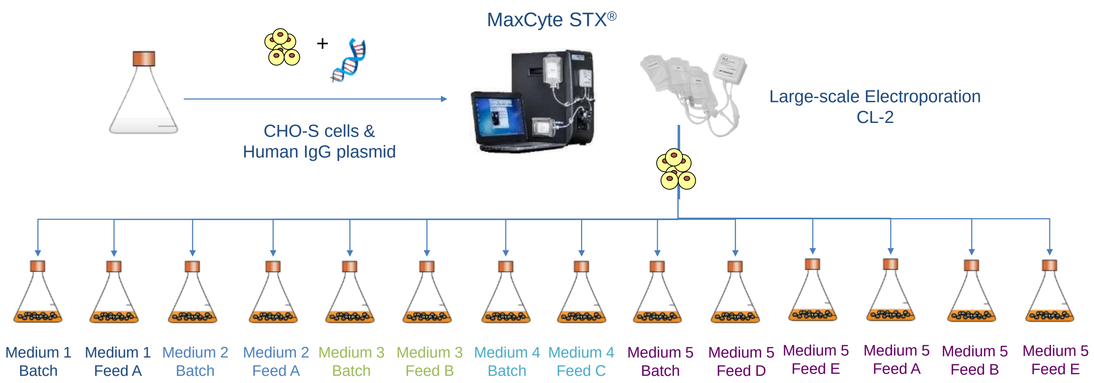
Abstract

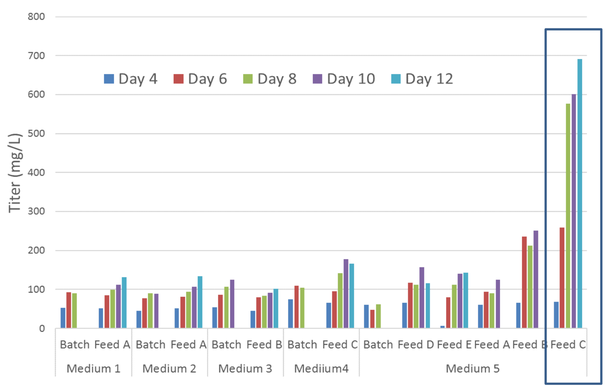
Protein-based and cellular therapeutics have revolutionized the treatment of cancer and other diseases. However, the need to produce complex biologics with limited resources and shortened timelines has created a demand for technologies that can express multiple therapeutic moieties in a range of cell types and culture processes. This poster describes the use of MaxCyte's flow electroporation-based delivery platform for rapid, transient production of monoclonal mouse and human antibodies, Fc-fusions and other proteins in a range of host cells. Emphasis will be placed on the use of large-scale transient transfection for optimizing media and feed strategies in batch fed cultures using multiple CHO cell lines. Data on protein titers and cell viability will be shared to illustrate how a flexible transfection platform enables the development of optimized production processes.

High Titer, CHO-S IgG Production Using Media of Choice

Media & Feed Optimization Produces 7x Improvement in Antibody Titers



	Initial Vol.	Total Feed	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Batch	**30mL**	**0**	Large-scale MaxCyte STX Run							
Fed-batch	**30mL**	**10.5mL**		Feed 1 (10%)		Feed 2 (10%)		Feed 3 (10%)		Feed 4 (5%)



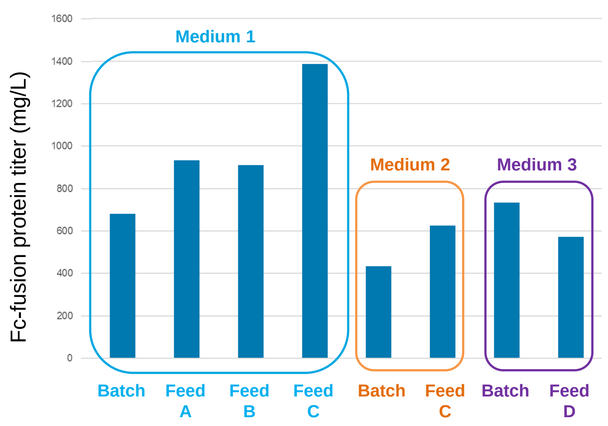
Medium 5, Feed C produced optimum results

- Increasing titer throughout 12 day culture
- Titer >250mg/L by Day 6
- Titer **>675mg/L** by Day 12

Figure 1: 7x Improvement in Titer Upon Optimization of Media and Feed. 2e9 CHO-S cells were transfected with a human IgG plasmid via a single large-scale electroporation. 1.2e8 cells were seeded in each of 14,125mL flask. 30mL of media (Medium 1, 2, 3, 4 or 5) was added per flask bringing the initial seeding density to 4e6 cells/mL. Cells were either grown as batch or fed-batch cultures following the feed schedule indicated in the table. Antibody titers, viability (data not shown) and metabolic profiles (data not shown) were analyzed on Days 4, 6, 8, 10 and 12 post transfection.

Media Flexibility Enables Significant Cost Savings

1.8x Increase in Fc-fusion Titers With a 75% Decrease in Media Cost



- Media 1, Feed C produced Fc-fusion protein titers ~1400mg/L
- Previous lipid-based titers = 800mg/L
- Enabled reduction in media cost from $316/L down to $81/L while increasing titers

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Batch (0.5% Pluronic-F68)	Large-scale MaxCyte STX Run	Temp shift to 32°C								Harvest
Fed-batch (0.5% Pluronic-F68)		Temp shift to 32°C Feed 1 (10%)		Feed 2 (10%)			Feed 3 (7.5%)			Harvest

Figure 2: Significant Reduction in Media Costs While Increasing Fc-fusion Production. 2e9 ExpiCHO cells were transfected with an Fc-fusion protein expression plasmid via a single large-scale electroporation. 1.5e8 cells were seeded per 125mL flask with 25mL of one of three commercially available media and one of four feeds. Cells were either grown as batch or fed-batch cultures following the feed schedule indicated in the table. Fc-fusion protein titers were analyzed on Days 9 post transfection.

Media & Feed Flexibility for Glyco-pattern Engineering

Production of Differently Glycosylated IgG from CHOZN Cells

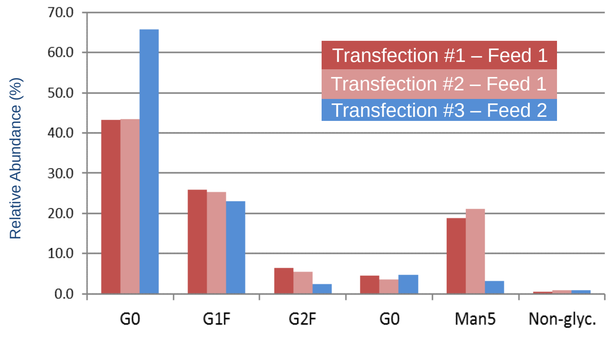


Figure 3: Media and Feed Flexibility Post Electroporation Allow Customization of Therapeutics. CHOZN® GS-/- cells were transfected with an hIgG expression plasmid via 3 independent electroporations using the MaxCyte STX. Cells from independent electroporations were cultured post electroporation in ExCell CD Fusion medium using two different feed supplements. Antibody glycosylation patterns were analyzed from 8 day fed-batch cultures.

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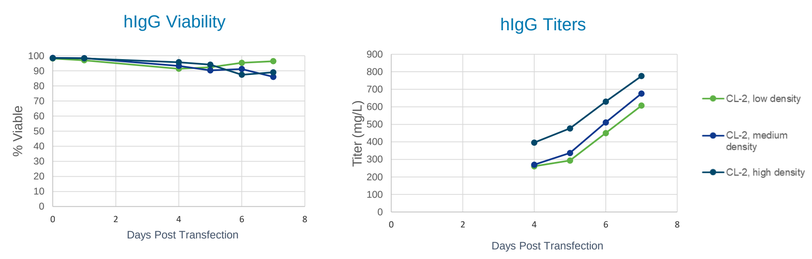
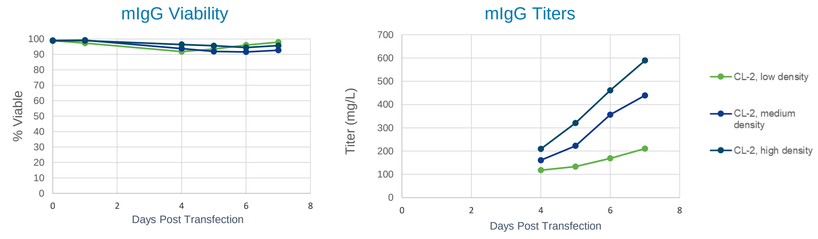
Increasing Laboratory Productivity Using Flow Electroporation Technology & Higher Seed Densities

4x to 28x Titer Improvements for Human & Mouse IgG While Cutting Production Time in Half



Human IgG

- Low density seed titer >600 mg/L
- High density seed titer >775 mg/L
- Previous lipid-based titers = 200 mg/L and required 14 day culture



Mouse IgG

- Low density seed titer >200 mg/L
- High density seed titer >575 mg/L
- Previous lipid-based titers = 20 mg/L and required 14 day culture

	Day 0	Day 1	Day 4
Low Density (4e6 cells/mL)		Feed at 20%	Feed at 20%
Medium Density (8e6 cells/mL)	Spike media with 5% feed	Feed at 35%	
High Density (1.2e7 cells/mL)	Spike media with 10% feed	Feed at 30%	

Figure 4: Increased Productivity Through Seed Density Optimization. 6e9 ExpiCHO cells were transfected with either a human or mouse IgG plasmid via a single large-scale electroporation. Transfected cells were seeded in 125mL flasks at three different densities: low (4e6 cells/mL), medium (8e6 cells/mL) or high (1.2e7 cells/mL). Cells were fed as indicated in the table. Antibody titers and cell viability were analyzed on Days 4, 5, 6 and 7 post transfection. Results were compared to the customer's established ExpiCHO process using ExpiCHO medium.

Seed Density Optimization for Improved Antigen Titers

2.5x Higher Titers Using Flow Electroporation Technology & Increased Cell Densities

Seed Density	Final Titer (mg/L)
6e6 cells/mL	470
1.0e7 cells/mL	750
1.4e7 cells/mL	810

Previous lipid-based titer = 330 mg/L

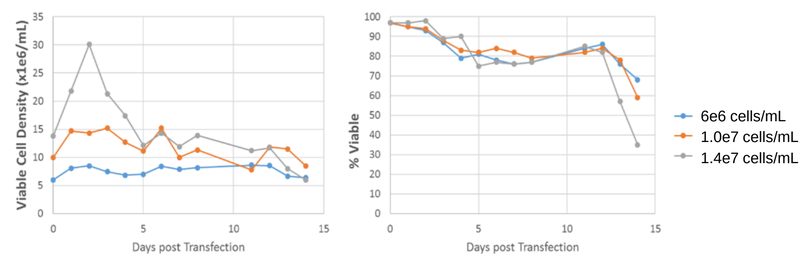
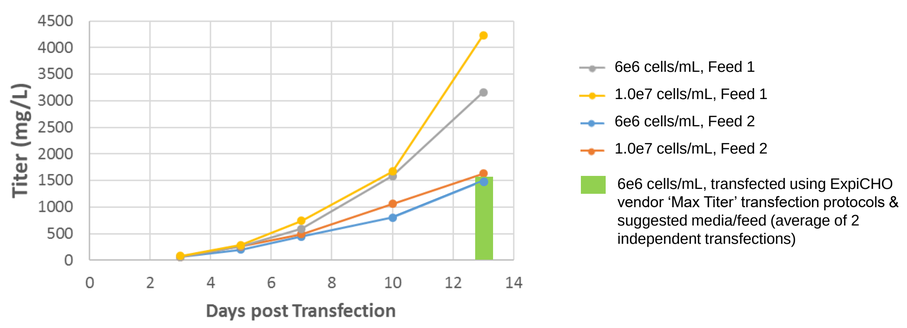


Figure 5: Higher Antigen Expression Levels Upon Switching to MaxCyte Electroporation & Optimizing Seed Density. 2e9 ExpiCHO cells were transfected with an antigen expression plasmid via a single large-scale electroporation. Transfected cells were seeded in CD OptiCHO medium at three different densities: low (6e6 cells/mL), medium (1.0e7 cells/mL) or high (1.4e7 cells/mL). Cells were fed on Day 5 and Day 9 with commercially available supplements. Antibody titers and cell viability were analyzed at various times over the course of a 14 day culture. Results were compared to the customer's established ExpiCHO process using ExpiCHO medium.

Increased Productivity Using Lower Cost Media & Feed

IgG Titers Improved to >4g/L



- Optimized conditions using MaxCyte transfection & lower cost media/feed resulted in titers >4g/L
- >2.5x increase using optimized conditions compared to ExpiCHO vendor reagents & 'Max Titer' protocol
- MaxCyte transfection & media/feed optimization enabled post transfection seed densities of 1e7 cells/mL resulting in improved productivity and lower consumable costs

Figure 6: Higher IgG Titers Using Flow Electroporation Technology & Lower Cost Media and Feed. ExpiCHO cells adapted to CDM4MAB media prior to transfection. 2e9 cells were transfected with a IgG expression plasmid using a single large-scale electroporation. Transfected cells were seeded at either 6e6 cells/mL or 1e7 cells/mL in CDM4MAB media. Cultures were supplemented every other day with specified feeds and additional glutamine. Antibody titers were determined at various times throughout a 13 day culture. Two independent ExpiCHO transfections were performed using vendor reagents and cultured and fed according to vendor's 'Max Titer' protocol.

Summary

- MaxCyte's Flow Electroporation™ Technology enables high efficiency, high viability transfection of a variety of CHO cell lines, including ExpiCHO cells.
- MaxCyte scalability allows the use of a single large-scale electroporation for in-depth process development, eliminating multiple transfection as a study variable.
- The MaxCyte platform allows the use of any media or culture supplements enabling:
  - significantly lower consumable costs (75% reduction in price enabled) 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
- MaxCyte transfection enables seed densities >1e7 cells/mL post electroporation while maintaining high antibody expression, thereby improving laboratory productivity.
- Process optimization enabled substantially higher expression levels which allows shorter production runs.
- High viability and transfection efficiency results in strong expression of more difficult-to-express proteins such as Fc-fusion proteins and mouse IgGs.