Rapid Vaccine Development & Biomanufacturing Using a Scalable, Non-viral Delivery Platform for Cell Engineering.

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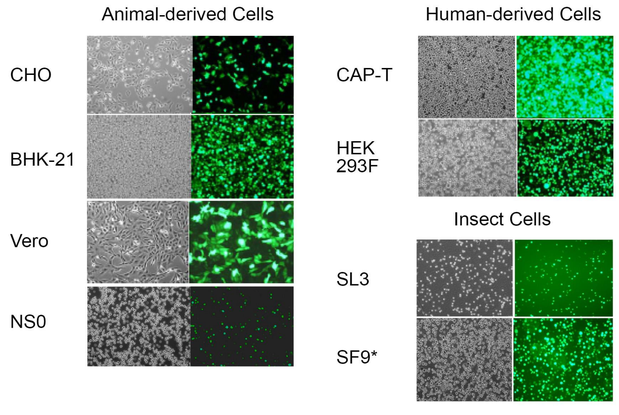
Abstract

Researchers have looked to recombinant technologies to develop innovative types of vaccines and new cell culture-based means of production that offer shorter lead times and greater production flexibility while maintaining vaccine safety. Transient transfection offers a means of rapidly producing an array of proteins, including antibodies, vaccines, viral vectors, and virus-like particles (VLPs). Although a variety of transient transfection methods are available, most do not meet the requirements of scalability, consistency, and cell type flexibility for use in vaccine development and manufacturing. MaxCyte's electroporation-based delivery platform reproducibly transfects a broad range of biorelevant adherent and suspension cell types with high cell viabilities & transfection efficiencies using single-use processing assemblies for cGMP, "plug-and-play" production of recombinant proteins and vaccines. In this poster we present data for large-scale production of antibodies, recombinant antigens, VLPs, and lentiviral vectors using the MaxCyte STX® Scalable Transfection System. Data are presented for high-efficiency transfection of cells commonly used in protein production including CHO, HEK293, and insect cells--without the use of baculovirus--with a timeline of just a few days from plasmid to gram quantities of protein.

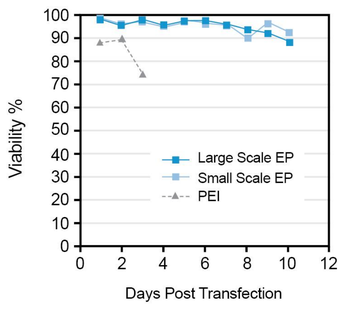


High Level Cell Viability and Transfection Efficiencies

Figure 1. High Efficiency Transfection of Cell Types Commonly Used for Protein and Vaccine Production. Various cells were transfected with 2 µg/1E6 cells of pGFP DNA using the appropriate MaxCyte STX protocol. Cells were examined for GFP expression using fluorescence microscopy 24 hrs post electroporation (EP).

Expression of Multiple Protein Types Using MaxCyte's Delivery Platform

High Cell Viability Leads to Strong HIV gp145 Expression



Day Post Transfection	gp145 (mg/L)		
	MaxCyte		PEI
	Small-scale	Large-scale	
3	27.8	23.1	4.5
7	67.2	75.1	-
10	95.2	113.5	-

Figure 2: Cell Viability and Protein Titer Data Following Transfection of CHO Cells with an HIV Envelope Protein Expression Plasmid. CHO-S cells were transfected with a gp145 expression plasmid via small scale EP (8e7 cells) and large scale EP (2e9 cells). Transfected cells were inoculated into shake flasks at the same density and cultured for 10 days. Cells from the small scale and large scale EPs yielded consistent titers and exhibited high viabilities. Viabilities and titers from the electroporated cells were much higher than corresponding values for a customer's optimized PEI process.

Multi-Gram, CHO-based Antibody Production

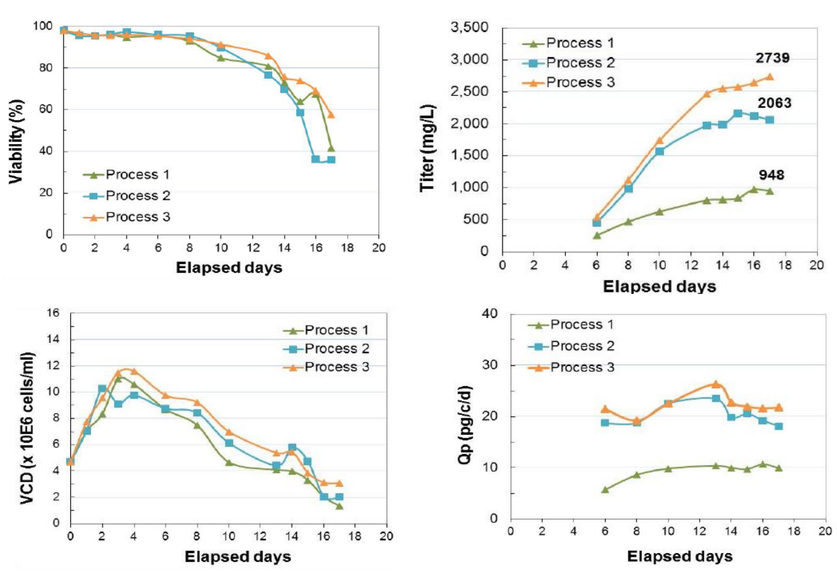


Figure 3: Post Electroporation Culture Process Optimization Produces CHO-S Antibody Titers >2.7 g/L. CHO-S cells were transfected with an antibody expression plasmid (1µg DNA/1E6 cells) via small scale electroporation on the MaxCyte STX. Cells were plated at approximately 4E6 cells/mL post electroporation. Transfected cells were cultured in media with different additives and culture conditions. Titer was verified by both ELISA and Protein A capture assays. The optimized process produced antibody titers of 2.74 g/L at day 17 post EP as a fed batch.

Rapid, More Efficient VLP Production Compared to Baculovirus Expression Systems

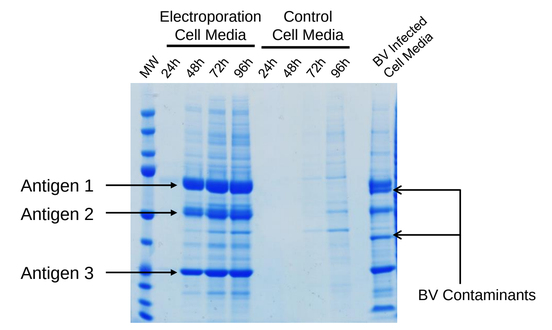


Figure 4. Sf9 VLP Production Using MaxCyte Electroporation: Plasmid to Product in Two to Four Days. Sf9 cells were transfected via static electroporation with a single plasmid encoding three antigens that co-assemble into VLPs. Culture media was collected at various times from cells post EP or following baculovirus infection and analyzed using SDS PAGE. VLP production was observed within 48 hours post electroporation.

Viral Vector Production: Seamless Scalability for Rapid Manufacturing

Consistent Large-scale Lentivirus Manufacturing Using Suspension Cells

Production Run	Volume (mL)	Total Cells	48 hr Titer (IU/mL)	Cumulative Titer (IU)	Productivity (IU/cell)
1	2300	6.0×10^9	9.8×10^7	2.2×10^{11}	37
2	2300	4.8×10^9	8.8×10^7	2.0×10^{11}	42
3	2100	7.4×10^9	1.3×10^8	2.7×10^{11}	36

Figure 5. Large-scale Lentiviral Vector Production. Suspension-adapted HEK 293FT cells were harvested, resuspended at 1E8 cells/mL, and co-transfected with 4 plasmids (HIV-based lentivector system) using flow electroporation. Cells were cultured post EP in 10-L Cellbag in a Wave Bioreactor in a final volume of 2.1 to 2.3 L. 48 hours post EP, media was collected and infectious units measured. Results for three independent production runs are shown. These data demonstrate the reproducibility of MaxCyte flow electroporation enabling large-scale, quality lentivirus production. *See *Human Gene Therapy* (2012) 23:243-249 for detailed methodology.

Seamless Lentivirus Scalability - MaxCyte STX to VLX

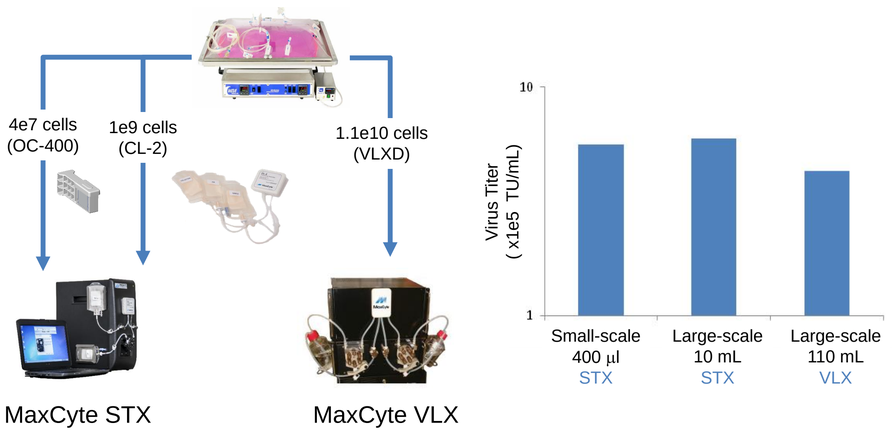


Figure 6. Scale Up of Lentiviral Vector Production from Small-Scale to Large-Scale Production Using the MaxCyte Platform. Suspension-adapted HEK 293FT cells were suspended in MaxCyteEP buffer at 1E8 cells/mL. A mixture of plasmids encoding lentiviral vector components was added to the cells (0.4µg of DNA/1E6 cells), and cells were transferred to sterile OC-400, CL-2 and VLXD processing assemblies. Cells in the OC-400 and CL-2 were transfected by static and flow EP, respectively, using the STX instrument; cells in the VLXD were transfected by flow EP on the VLX. Lentiviral titers were measured after 24-48 hrs in culture. Normalized titer data show seamless scalability of the MaxCyte transfection process.

High Titer AAV Production in HEK Cells

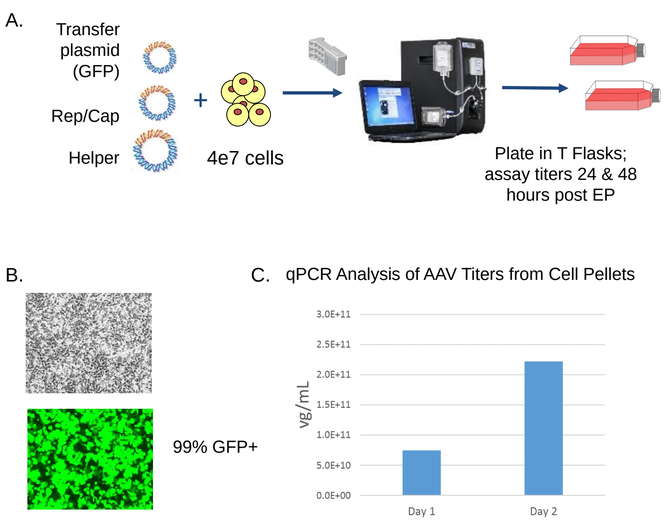


Figure 7: Production of AAV in HEK Cells. (A). Adherent HEK cells were transfected with three plasmids encoding AAV vector components (GFP transgene) via static electroporation using the MaxCyte STX. (B). Nearly 100% of the transfected cells exhibited robust transgene expression 48 hours post electroporation. (C). High AAV titers were detected in cell pellets via qPCR analysis.

Summary

- MaxCyte's electroporation-based delivery platform is fully scalable from 5E5 cells to 2E11 cells allowing for production of milligram to multi-gram quantities of proteins, viral vectors, and multi-protein complexes such as VLPs.
- MaxCyte electroporation is high performance means of transiently transfecting adherent and suspension cell lines commonly used during vaccine development and production such as CHO, HEK, and insect cells.
- MaxCyte transfection rapidly produces a variety of proteins including antigens, antibodies, lentiviral vectors, and VLPs more efficiently than chemical transfection methods and baculovirus expression systems.
- MaxCyte transfection of CHO cells can produce secreted antibody titers **>2.5 gram/L** with optimization of post transfection culture conditions.
- Insect cells rapidly express recombinant proteins, including VLPs, at high efficiency following MaxCyte electroporation, eliminating the need for baculovirus use.
- MaxCyte electroporation results in high-performance transfection of suspension-adapted HEK cells allowing for large-scale, highly reproducible production of viral vectors.
- Production scale-up from the MaxCyte STX to the MaxCyte VLX is seamless – maintenance of transfection performance without the need for reoptimization.

MaxCyte STX®
5E5 Cells in Seconds
Up to 2E10 Cells in <30 min

MaxCyte VLX®
Up to 2E11 Cells in <30 min

MaxCyte Delivery Platform

The MaxCyte STX® and MaxCyte VLX® Transient Transfection Systems use fully scalable flow electroporation for rapid, highly efficient transfection.

- High efficiency & high cell viability
- Broad cell compatibility
- True scalability requiring no re-optimization
- Closed, computer-controlled instruments
- cGMP-compliant & CE-marked
- Master file with US FDA & Health Canada

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