Streamlined Production of Complex Proteins Using MaxCyte's Delivery Platform for Cell Engineering

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Abstract

Complex proteins present a range of production challenges which can include low expression levels, degradation, aggregation, a high degree of post translational modifications, the need to use multiple or large expression plasmids or producing multi-subunit proteins with correct folding and subunit ratios. The MaxCyte delivery platform for cell engineering facilitates the production of complex proteins within days of transfection in your cell line-of-choice, at the scale you need, across your biotherapeutic and vaccine development and bioproduction pipelines. Case studies are presented in this technical note demonstrating diabody expression, gram-scale IgG production in CHO cells, multi-subunit ion channel expression, insect cell virus-like particles (VLP) production, and large-scale lentivirus production using MaxCyte's flow electroporation-based delivery platform. We highlight the platform's key attributes including its flexibility, performance, scalability, and cGMP-compliance that enable high yield, cost-effective production of complex proteins.

Introduction

Small molecule, biotherapeutic, vaccine development, and biomanufacturing depend on the expression of recombinant proteins ranging from simple antigens to complex, more difficult-to-express antibodies, antibody-like molecules, and VLP. Stable cell lines have been the standard for protein production for over two decades; however, their creation is costly, time-consuming, labor-intensive, and is unfeasible for some proteins.

Transient expression generally offers a rapid means of protein production, but not all transient methods meet protein quality standards as well as the performance, flexibility, scalability, and regulatory requirements for producing proteins across the pre-clinical and clinical development pipeline.

Transient transfection technologies have evolved from simple chemical carriers into sophisticated, highly engineered methodologies. Chemical carriers like PEI, while inexpensive, often yield inconsistent results, are challenging to scale-up and have lower expression levels even when using optimized protocols. In contrast, virus delivery, lipid-mediated transfection and electroporation generally provide enhanced performance and are more reproducible, but differ significantly in performance, scalability, time requirements, regulatory compliance, and cost.

Second generation lipid-based technologies have relatively high transfection efficiencies, but require re-optimization during scale-up and can quickly become cost-prohibitive.

Viral delivery methods are scalable, however, the creation of viral vectors and production of viral stocks require a high level of user knowledge, are labor- and time-intensive, have limited cell type flexibility, can negatively impact yield due to purification challenges, and face further regulatory hurdles later in the development pipeline.

MaxCyte's Flow Electroporation™ Technology is a universal, regulatory-compliant delivery platform that rapidly produces large quantities of membrane-bound and secreted proteins including antibodies, antibody-like molecules, virus-like particles (VLPs), and viral vectors while overcoming the flexibility, scalability and regulatory limitations associated with other transient transfection methods.

Flow Electroporation™ Technology: A Universal, High-Performance Delivery Platform

Flow Electroporation Technology efficiently (co)transfects a wide range of cells including CHO, HEK, Vero, insect cells, and other cell lines commonly used for protein expression with DNA, RNA, proteins, and cell lysates (Figure 1).

MaxCyte electroporation uses a single chemically defined, animal product-free, protein-free buffer for all cell types and does not require specialized constructs, engineered cells, media additives, or chemical reagents. It consistently results in high levels of transfection efficiency and cell viability that enable gram-scale production of proteins such as mono- and multi-specific antibodies, VLP's, antigens, and other recombinant proteins within days of transfection.

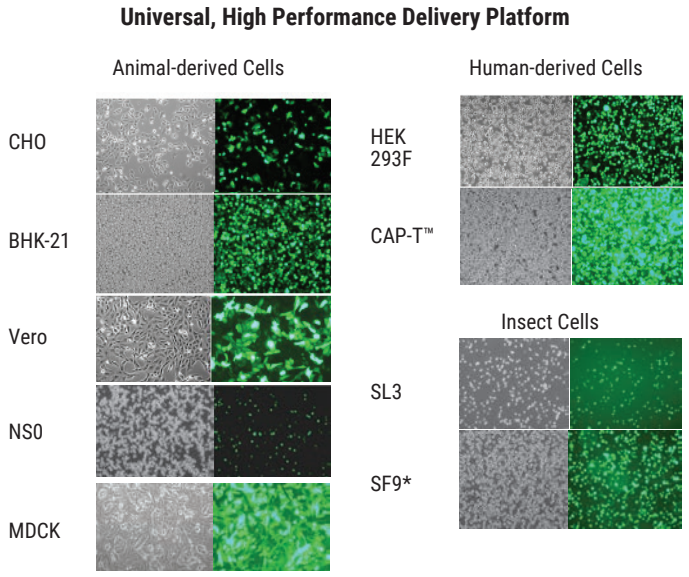


Figure 1: High Efficiency Transfection of Cell Types Commonly Used for Protein Production. *Cells were transfected with 2 µg/1e6 cells of pGFP DNA using the appropriate MaxCyte STx protocol. Cells were examined for GFP expression using fluorescence microscopy 24 hrs post electroporation. *SF9 cells examined at 72 hrs post electroporation.*

The MaxCyte delivery platform has the capacity to transfect up to 2e11 cells in under 30 minutes, taking you from the production of milligrams of protein for early-stage development through production of multiple grams of protein and generation of high-yield stable cell lines.

MaxCyte offers two benchtop, flow electroporation-based systems covering the full range of protein production needs — the MaxCyte STx® and MaxCyte VLX® Scalable Transfection Systems. Both systems are supplied with a pre-loaded library of electroporation protocols optimized for a wide variety of cell types including cells commonly used for protein production. These simple push-button systems result in highly reproducible, quality transfections. Additionally, MaxCyte systems are ISO certified and cGMP-compliant with a Master File on record with the USFDA and Health Canada.

Superior Production of High Quality Antibody-like Molecule

Transfection Method	Purified Protein Concentration	%HMW	% Monomer
STx Electroporation	**173 mg/L**	5.6	94.3
Lipid-based Reagent	7.3 mg/L	7.2	92.8

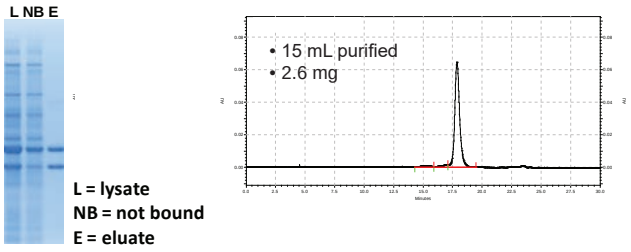


Figure 2: Production of Quality Bispecific Antibodies. *CHO-S cells were transfected with a bicistronic expression plasmid encoding the components of a bispecific diabody via static electroporation in an OC-400 processing assembly or using a standard lipid-based reagent protocol. Diabodies were purified and subjected to analysis via chromatography.*

Case Study 1: Antibody-like Protein Production

A diversity of antibody-like molecules including a host of bispecific antibody types, tri-functional antibodies, various antibody fragments (Fabs, scFv, hetero-dimerization domains), and IgG-fusions have been bioengineered in hopes of improving the therapeutic potential of standard antibodies. The efficacy of many of these formats has been demonstrated in pre-clinical and clinical studies and, thus, antibody-like molecules are of particular interest for continued development. These molecules can, however, be difficult to express at higher yields from mammalian cells. The proven high cell viability and transfection efficiency of MaxCyte electroporation enable production of a variety of antibody-like proteins and peptides.

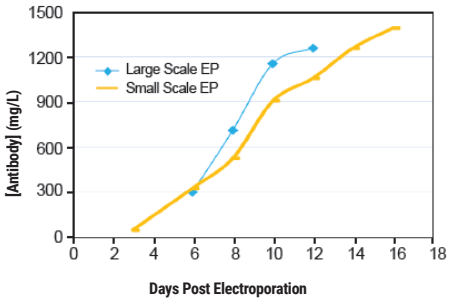
Production of Quality Bispecific Antibodies

MaxCyte Flow Electroporation Technology was compared to a lipid-based reagent system for expression of a diabody via CHO cell transient transfection (Figure 3). Diabody titers were more than twenty fold higher using MaxCyte electroporation. Analysis of the purified diabodies showed that greater than 94% of the MaxCyte produced protein was in the desired monomeric form. A single MaxCyte electroporation produced a total of 2.6 mg of purified diabody from a 15-mL culture equating to production of 1.7 grams of diabody from a 10-L culture.

Case Study 2: Early Stage Development in Biomanufacturing Host Cell - Gram-Scale CHO Antibody Production

While stable CHO cell lines remain the regulatory standard for manufacturing of clinical-grade biotherapeutics, industry has looked to CHO-based transient expression to reduce reliance on stable cell line generation for early- and mid-stage development activities. Initial transient expression activities were limited by poor transfection efficiencies, cell viabilities, and production of insufficient quantities of antibodies. MaxCyte electroporation enables high efficiency, high viability CHO cell transfection routinely resulting in secreted antibody titers >400 mg/L, which can exceed >1 g/L upon optimization of post electroporation culture conditions.

Rapid, Gram Scale Antibody Production



	Culture Volume	EP Volume	# of Cells	[IgG]	Total IgG Produced
Small Scale	20mL	0.4mL	8E7	**1.40 g/L**	28mg
Large Scale	2.8L	50mL	1E10	**1.22 g/L**	**3.42 g**

Figure 3: High Titer Antibody Production with Seamless Scalability. *CHO-S cells were transfected with an antibody expression plasmid (2 µg DNA/1e6 cells) via small-scale (8e7 cells) or large-scale (1e10 cells) MaxCyte STx electroporation. Cells were seeded at 6e6 cells/mL post electroporation. 1 mM sodium butyrate was added to cultures and the temperature lowered to 32°C 24 hours post electroporation. Cultures were fed daily with a media optimized for antibody production. Secreted IgG titers were measured via ELISA on various days post transfection and total IgG production calculated.*

The amount of antibody required throughout the biotherapeutic development process varies from low milligram to multi-gram quantities. Ideally, the transient transfection system of choice will have the scalability to simply and rapidly produce the full range of antibody quantities needed. Many alternative transfection technologies require multiple small-scale transfections, re-optimization of transfection protocols, and/or bulk usage of costly transfection agents for production scale-up.

Rapid, Scalable Protein Production

MaxCyte Flow Electroporation Technology has unmatched scalability, able to transfect as few as 5e5 cells up to as many as 2e11 cells without protocol re-optimization, allowing the progression from gene to gram-scale quantities of proteins within days. Comparable cell viabilities and antibody titers were observed upon small- and large-scale transfection using the same electroporation parameters and general cell handling methods on the MaxCyte STx (Figure 3). Both transfections led to increasing antibody titers that peaked at greater than 1.2 g/L during the 14-day study. Greater than 3 grams of antibody were produced from less than 3 liters of CHO culture following a single transfection. Additional studies have confirmed high levels of cell productivity for over 21 days, thus increasing the production potential.

Increased Yield and Laboratory Productivity

Significant efforts have been made to increase CHO cell antibody productivity from both transient and stable expression systems. A range

of factors have been identified that affect productivity including the use of specific CHO cell lines and various cell culture parameters such as cell density, feeding conditions, media additives, and culture temperature. We examined a variety of post transfection parameters including three different feed strategies and found the combination of a hypothermic temperature shift, the use of sodium butyrate, and more frequent nutrient replenishment significantly improved antibody titers with optimum titers > 2.7 grams/L (Figure 4 and data not shown). While these specific parameters increased productivity in our laboratory, MaxCyte technology is universal in nature, providing researchers the flexibility to use their expression system and cells of interest, as well as lab-specific cell handling methods.

Improved Yield Through Process Optimization

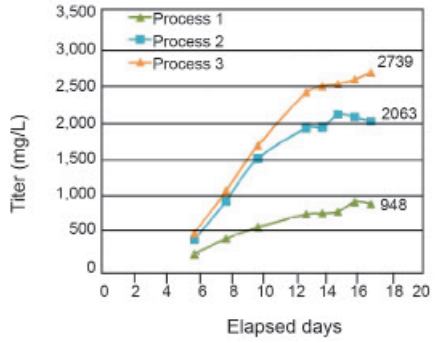


Figure 4: Post Electroporation Culture Process Optimization Produces CHO-S Antibody Titers >2.7 g/L. *CHO-S cells were transfected with an antibody expression plasmid (1µg DNA/1e6 cells) via small scale electroporation. Transfected cells were cultured for 17 days in media using different additives and feed strategies. IgG titer was verified by both ELISA and Protein A capture assays.*

Case Study 3: Insect Cell VLP Production

VLP are complex, multi-protein structures of self-assembling viral antigens commonly produced in insect cells. Although recombinant baculovirus platforms are commonly used for insect cell protein expression, MaxCyte's delivery platform offers a more streamlined means of protein production.

Baculovirus-mediated protein production remains an extended, multi-stage process, despite development of specialized media and baculovirus vectors aimed at simplifying gene cloning and virus stock production. In contrast, Flow Electroporation Technology directly transfects Sf9, Sf21, and SL3 cells with >90% cell viability and transfection efficiency levels allowing for high level protein production within days of transfection (Figure 1 and data not shown).

Sf9 cells transfected via MaxCyte electroporation with an expression construct encoding three VLP antigens resulted in significant secretion of the VLP within 48 hours post transfection (Figure 5). In tandem, a baculovirus expression system was used to produce VLPs containing the identical three antigens. SDS-PAGE analysis of cell supernatants shows the presence of the three VLP antigens in all electroporation and baculovirus samples; however, baculovirus protein contaminants were also present in supernatants from baculovirus-infected cells. This is consistent with literature, which documents the propensity for baculovirus protein contamination, creating purification challenges, and yield loss when using these expression systems. These results demonstrate the extremely rapid and high quality nature of direct insect cell transfection using MaxCyte's delivery platform, which streamlines protein production by eliminating the need for baculovirus use.

Case Study 4: Multi-subunit Ion Channel Expression

Historically ion channel assays have relied heavily on the use of stable cell lines, which can be challenging to create due to the multi-subunit nature of many ion channel targets. The need to use multiple selection agents during ion channel cell line creation often impairs cell health and proliferation making the production of a large number of quality, assay-ready cells difficult. Furthermore, ion channels can be toxic when expressed at high levels, necessitating the use of an inducible expression system, which adds an additional layer of complexity.

MaxCyte's delivery platform offers an attractive alternative. Multiple plasmids can rapidly be co-transfected at specific stoichiometric ratios to create highly defined functional ion channel complexes using Flow Electroporation Technology. Delivery of specific plasmid ratios is difficult using other transient transfection methods and often leads to sub-optimal or non-functional ion channel expression.

To demonstrate this feature, four cDNAs encoding the pore-forming alpha subunit of the voltage-gated calcium channel Cav2.2, the modulatory β subunit, the modulatory α2δ subunit, and an inward rectifier potassium channel (Kir2.1) were co-transfected into HEK293 cells using the MaxCyte STx and assayed using the FLIPR® calcium influx assay (Figure 6). High transfection efficiency and cell viability enabled strong expression of the desired multi-subunit ion channel. Strong calcium flux was evident in cells transfected with all four plasmids, but not in cells transfected without the inward rectifier plasmid or in untransfected control cells. Inclusion of the inward rectifier allowed modulation of resting membrane potential by external potassium and was essential for the FLIPR assay.

Sf9 VLP Production Without the Need for Baculovirus

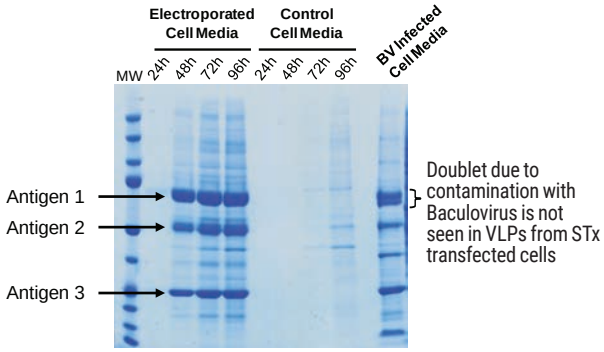


Figure 5: Sf9 VLP Production Using MaxCyte Electroporation: Plasmid to Protein in Two to Four Days. *Sf9 cells were transfected via small scale electroporation with a single plasmid encoding three antigens that co-assemble into VLPs. Culture media was collected at various times from cells post electroporation or following Baculovirus infection and analyzed using SDS PAGE.*

The Cav2.2 channel was inhibited by omega-conotoxin, a known calcium channel specific antagonist, illustrating the functionality of the channel and the applicability of transiently transfected cells for compound screening of ion channels. These data demonstrate the ability of Flow Electroporation Technology to express functional cell surface receptors even when multimeric in nature enabling high throughput drug discovery screening.

Case Study 5: Streamlined Scale-up of Lentivirus Production

Lentiviruses, a subclass of retroviruses, are popular in vivo gene delivery vectors due to their unique ability to integrate into the host genome of non-dividing cells, the minimal immune response they induce, their reduced risk of insertional mutagenesis, and the long-term nature of their expression. Lentiviral vectors are developed by removing all the viral genes except those

required in cis in order to complete a single round of replication. All other viral components are provided in trans during lentivirus stock production.

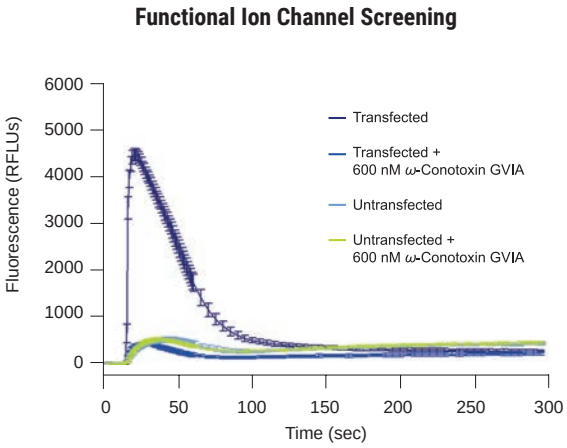
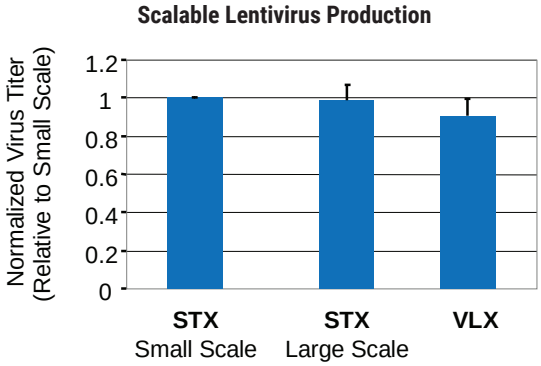


Figure 6: Expression of Functional, Multi-subunit Calcium Channel. *HEK293 cells were transfected in bulk with multiple cDNA constructs encoding 3 calcium channel subunits ± a plasmid expressing the inward rectifier potassium channel, Kir2.1. Calcium influx FLIPR assays were conducted 24 hr post transfection. Dye was added in a low potassium, low calcium solution and loaded for 30 min. Vehicle control or antagonist (600 nM ω-Conotoxin GVIA) was added for an additional 30 min. Cells were depolarized with high external K+ (up to 135 mM).*



Absolute virus titers ranged from 4.28 to 5.98E+05 TU/mL

Figure 7: Robust, Scalable Transfection for Lentivirus Production. *Suspension-adapted HEK 293FT cells were suspended with a mixture of plasmids encoding lentiviral vector components (0.4 µg of DNA/1e6 cells), and cells were transferred to sterile OC-400, CL-2, or VL2 processing assemblies. Cells in the OC-400 and CL-2 were transfected by static and flow electroporation, respectively, using the STx instrument; cells in the VL2 were transfected by flow electroporation on the VLX. Lentiviral titers were measured after 24-48 hrs in culture and normalized against small-scale STx electroporation.*

Lentivirus stocks are historically produced by transfecting a stable packaging cell line (PCL), commonly HEK293 cells which express the virion proteins and reverse transcriptase required for a single round of infection, with the shuttle vector encoding the gene of interest. While PCLs are a cGMP-compliant method of lentivirus stock production, their generation can take several months, gene silencing by vector components can negatively impact production titers over time, and their use in large-scale production is problematic. MaxCyte Flow Electroporation Technology offers a rapid, fully-scalable, and cGMP-compliant alternative to PCL generation by co-transfecting cells with shuttle and multiple helper plasmids encoding the required viral components.

In scalability experiments, HEK293FT suspension cells, which are better suited to large-scale lentivector manufacturing relative to comparable adherent cells, were co-transfected with a 4 plasmid lentiviral system using small-scale electroporation on the MaxCyte STx, and large-scale electroporation on the MaxCyte STx and MaxCyte VLX. Identical electroporation parameters were used for all electroporations. The experiments highlight the seamless scalability of MaxCyte's delivery platform as small- and large-scale transfection produced nearly identical high-titer viral stocks with absolute virus titers ranging from 4.28 to 5.98E+05 TU/mL (Figure 7).

The MaxCyte delivery platform has the scalability to produce lentivirus vectors in cultures ranging in size from T-flasks and roller bottles, to 10-tier cell factories and Wave bioreactors. Large-scale production studies using a 4 plasmid lentiviral system using Flow Electroporation Technology have been published. These studies demonstrate the consistent, highly-reproducible nature of MaxCyte electroporation (Table 1). Titers of approximately 1e8 infectious units (IU)/mL were achieved in three pilot qualification lots manufactured at a cGMP facility. Overall, MaxCyte's delivery platform has the safety, scalability, and performance allowing for scalable production of lentiviral vectors for clinical and commercial applications.

Consistent Large Scale Production of Lentiviral Vector

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

Table 1: Titers of Large-scale Lentivirus Production in 10-L Cellbags. *HEK 293FT cells (4.8–7.4e9 cells) were electroporation via flow electroporation with a mixture of plasmids encoding lentiviral vector components. Following a 20-minute recovery period, electroporated cells were inoculated into Cellbags at a final volume of 2.1 to 2.3 L. Virus was harvested 48 hours post transfection and infectious titers determined. See Human Gene Therapy. 23, 2012, p243-249 for full methods.*

Conclusions

MaxCyte's delivery platform is a proven means of rapid, high-performance transient (co)transfection of mammalian and insect cells supporting large-scale production of antibodies, antibody-like molecules, recombinant antigens, VLPs, and vaccines. High transfection efficiencies and cell viabilities enable production of gram to multi-gram quantities of proteins within days of a single transfection. Additionally, the MaxCyte delivery platform can be used to generate stable pools and cell lines further increasing its utility and streamlining of development pipelines. Flow Electroporation Technology has unmatched quality, flexibility, reproducibility, and scalability creating a single, cost-effective delivery platform that supports the full range of biotherapeutic and vaccine development activities from candidate identification and development through biomanufacturing.

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