Flow Electroporation for Vaccine Development and Production: From Subunit Vaccines to *Ex Vivo* Immunotherapy

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Abstract

MaxCyte flow electroporation is a universal, clinically validated transient transfection platform for rapid, high-quality cell transfection. Flow electroporation enables (co)transfection of a wide range of cells with DNA, RNA, proteins, or cell lysates using single-use processing assemblies for cGMP, "plug-and-play" manufacturing of recombinant proteins and vaccines. This technology combines superior performance, broad applicability, and ease of use with the capacity to transfect up to 2E11 cells in under 30 minutes, creating a fully scalable, highly reproducible, and regulatory-compliant transfection method. No other single expression system—stable cells, baculoviruses, lipid- or chemical-based transient transfection—offers the ability to develop and manufacture the full complement of next-generation vaccines, including therapeutic antibodies and antibody-like molecules, subunit vaccines, virus-like particles (VLPs), virus-like replicon particles (VRPs), and viral vectors, as well as *ex vivo* cellular immunotherapies such as CAR engineering and dendritic cell loading. This technical note reviews MaxCyte flow electroporation and its applications in the development and production of vaccines and cellular immunotherapies.

Introduction

Vaccination has proven to be a successful and cost-effective public health intervention. It has eradicated many illnesses (e.g., smallpox) and provided drastic reductions in others (e.g., polio). Yet, vaccine development and production is often a lengthy and costly process, as exemplified by the 2009-2010 swine H1N1 flu outbreak in which egg-based manufacturing was unable to provide sufficiently large numbers of vaccine doses in a timely manner (1). In response to the need for rapid and reliable vaccine production, 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 (2-4). Newer, engineered vaccine modalities range from therapeutic antibodies, subunit vaccines, VLPs, and VRPs to virus-mediated gene therapy and *ex vivo* cellular immunotherapies such as dendritic cell loading and chimeric antigen receptor T-cell targeting.

Each of these engineered vaccine types—whether a simple therapeutic protein or complex modification of patient cells—requires the introduction of recombinant nucleic acids into cell lines or *ex vivo* patient-isolated cells via transfection and/or the creation of stable cell lines. While the downstream processing is vaccine-type dependent, a single unifying platform for upstream cell transfection could significantly reduce development timelines and production costs, if it fulfills the overarching needs for safety, flexibility, and scalability.

For more than two decades, stable cell lines have been the standard for biotherapeutic protein production; however, their creation is a costly, time-consuming, and labor-intensive process and is not feasible for all vaccine applications. In response, researchers have looked to transient gene expression (TGE) as a means of more cost-effective protein production, particularly during early development and preclinical stages (5, 6). While TGE generally offers a means of rapidly expressing proteins, not all transient expression methods fulfill the necessary requirements for broad use throughout vaccine development and production.

Transient transfection technologies have evolved from simple chemical methods such as PEI to sophisticated methodologies such as lipid-based reagents, baculovirus expression systems, and electroporation. MaxCyte's proprietary flow electroporation technology is a universal, regulatory-compliant transient transfection platform that provides a practical solution to the time, labor, and cost challenges of developing stable cell lines and baculovirus-based expression while overcoming the flexibility and scalability limitations associated with other transient transfection methods.

MaxCyte Transient Transfection

MaxCyte flow electroporation efficiently (co)transfects cells with DNA, RNA, siRNA, proteins, and cell lysates without requiring specialized constructs, engineered cells, media additives, or chemical reagents. A single chemically defined, animal product-free, protein-free electroporation buffer is used for all cell types.

With more than a dozen adherent- or suspension-adapted cell lines currently in use by vaccine manufacturers, including both mammalian and insect cells, there is a need for the transfection platform of choice to provide cell type flexibility (2, 7). MaxCyte electroporation consistently results in high levels of transfection efficiency and cell viability for a wide range of cells including CHO, MDCK, BHK-21, Vero, NS0, insect cells, and other cell lines commonly used for protein expression (Figure 1). Moreover, cell immunotherapy using patient-isolated primary cells, most frequently hematopoietic cells, requires high transfection efficiency and low cell toxicity while meeting stringent sterility and safety needs. Originally developed for this application, MaxCyte electroporation has extremely high levels of cell viability and transfection efficiency for a range of primary cells (Table 1) using a closed-system, cGMP-compliant instrument.

MaxCyte flow electroporation has been commercially and clinically validated in the manufacturing of a cellular immunotherapy product currently marketed in Japan. It has also been validated for multiple instances of the manufacturing of engineered cell and gene therapy products for use in clinical trials in North America and Asia.

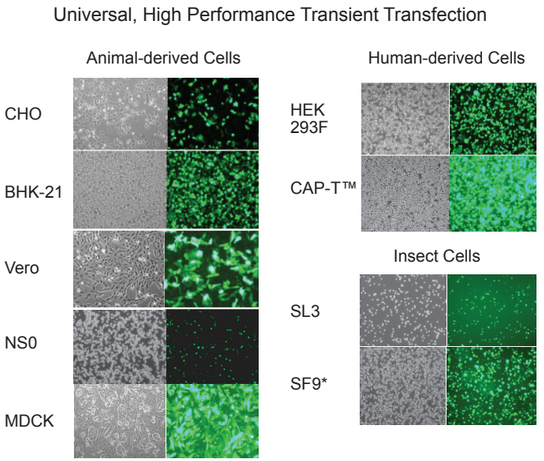


Figure 1: High-Efficiency Transfection of Cell Types Commonly Used for Protein and Vaccine Production. *Cells were transfected with 2 µg/1E6 cells of pGFP DNA using the appropriate MaxCyte STX protocol. Cells were examined for GFP expression using fluorescence microscopy 24 hrs post electroporation. *SF9 cells examined at 72 hrs post electroporation.*

Human Primary Cell Transient Transfection

Cell Type	Efficiency	Viability
Human Fibroblasts	95%	95%
Human Myoblasts	90%	90%
Human Mesenchymal Stem Cells	80%	80%
Human Dendritic Cells	50%	80%
Human Lymphocytes — B Cells	85%	90%
Human Lymphocytes — T Cells	50%	70%
Human HSC ($CD34^+$ cells)	60%	60%
Human MCL	40%	50%
Human CLL	50%	70%
Human NK Cells	50%	60%

Table 1: High-Performance Transfection of a Variety of Human Primary Cells. *Primary cells were isolated from patient samples and transfected with pGFP DNA using MaxCyte electroporation. Efficiency expressed as % cells GFP+ at 24 hours post electroporation; viability as % cells excluding propidium iodide.*

MaxCyte Instrumentation

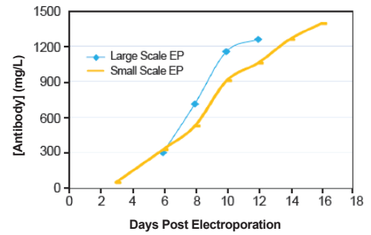
MaxCyte offers three benchtop flow electroporation systems: the MaxCyte STX® Scalable Transfection System, the MaxCyte VLX® Large Scale Transfection System, and the MaxCyte GT® Transfection System. These systems represent a universal platform for the development and production of vaccines ranging from simple recombinant antigens to *ex vivo* immunotherapy. The MaxCyte STX has the flexibility to transfect from 5E5 cells up to 2E10 cells, while the MaxCyte VLX is ideal for extremely large-scale transfections with the capacity to transiently transfect up to 2E11 cells. The large capacity and high performance of both these systems easily support the large-scale production of recombinant proteins, VLPs, VRPs, and viral vectors. The MaxCyte GT is a cGMP-compliant, clinical-grade instrument that enables the development of enhanced gene therapy and cell therapeutic applications by providing unparalleled safety, scalability, consistency, and cell-loading efficiencies of autologous and heterologous primary cells.

All MaxCyte systems are supplied with a pre-loaded library of cell type-specific electroporation protocols optimized for a wide variety of cell types—including cells commonly used for vaccine production and immunotherapy for the STX and VLX—simplifying process development while maximizing performance and reproducibility. All three MaxCyte systems are computer-controlled, "plug-and-play" systems that use single-use processing assemblies and are ISO certified and cGMP-compliant with a Master File on record with the USFDA and Health Canada.

Case Study 1: Gram-Scale CHO Antibody Production

Therapeutic monoclonal antibodies (mAbs) are the predominant component of biotherapeutic development pipelines for a range of clinical indications, including inflammatory disorders, cancers, and infectious diseases. mAbs can be used as prophylactic or therapeutic interventions via a variety of means ranging from targeted radioimmunotherapy to anti-idiotype immunization. Most biomanufacturing of clinical-grade therapeutic mAbs use CHO cells as the dominant choice because of the long history of use and regulatory hurdles and time delays that may be incurred for FDA approval of new cellular expression systems.

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 2: 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 protein required throughout mAb development varies from low milligram to multi-gram quantities. During early stage antibody development, hundreds of candidates may be under evaluation, and creation of stable cell lines for each candidate would require large investments of time and money. As a result, industry has looked to TGE to reduce reliance on stable cell line generation (5, 6, 8). Ideally, the transient transfection system of choice will have the scalability to simply and rapidly produce the full range of antibody types at sufficient quantities throughout the development process.

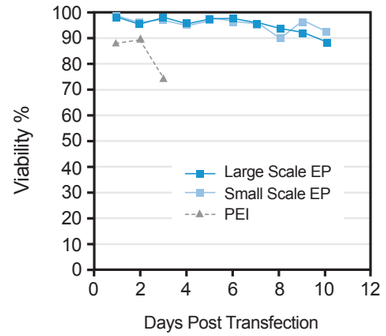
Despite advances *in trans* transfection methods and culture optimization, the majority of CHO-based TGE methods—including various PEI and lipid-based reagents and protocols—report antibody titers ranging from only 2 to 250 mg/L upon full optimization (9-13). These methods are also limited by a range of factors such as reproducibility, scalability, functional feasibility, and cost considerations as they require multiple small-scale transfections, re-optimization of transfection protocols, and/or bulk usage of costly transfection reagents during scale-up. With high-level performance and scalability, flow electroporation can be used for rapid scale-up and scale-down to align protein yields and resource usage with the stage of candidate development.

MaxCyte electroporation enables high efficiency, high viability CHO transient transfection that routinely results in secreted antibody titers >400 mg/L, which can exceed >2 g/L with optimization of post transfection culture conditions, such as cell density, feeding conditions, media additives, and culture temperature. This translates into the production of multi-gram quantities of antibodies within days of transfection. With post transfection optimization, MaxCyte electroporation resulted in antibody titers greater than 1.2 g/L, which produced approximately 3 grams of antibody from a CHO culture of less than 3 liters 12 days post transfection (Figure 2). MaxCyte STX scalability experiments demonstrated comparable small- and large-scale secreted antibody titers (Figure 2). Further scale-up to biomanufacturing using the MaxCyte VLX allows for transfection of up to 2E11 cells, which would equate to the production of over 50 grams of antibody within two weeks of a single transient transfection.

Case Study 2: High-level Subunit Vaccine Expression

Similar to inactivated virus vaccines, subunit vaccines are generally easy to administer, do not contain infectious material nor require a cold chain for distribution. However, unlike inactivated viruses, subunit vaccines contain only the antigen(s) that best stimulate a protective immune response. Subunit vaccine antigen(s) can be

Increased HIV gp145 Production Using the MaxCyte STX



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 3: Superior Cell Viability and Production of HIV-1 Envelope Protein Using MaxCyte Electroporation. *CHO-S cells were transfected with an HIV-1 gp145 expression plasmid via small-scale (static; 8E7 cells) or large-scale (flow; 2E9 cells) electroporation using the MaxCyte STX or using a standard polyethyleneimine (PEI) method. Cell viability was determined daily post transfection. gp145 production was assessed via ELISA on days 3, 7, and 10 post transfection. *PEI-tranfected cells did not have sufficient cell viability to maintain cultures for day 7 and 10 gp145 measurement.*

isolated directly from the pathogen, as is the case with the *Haemophilus influenzae* type b vaccine (shot form), or expressed recombinantly, like the current hepatitis B vaccine composed of the hepatitis B virus surface antigen (HBsAg). One advantage of recombinant expression is that the antigen(s) can be manipulated to increase immunogenicity, to elicit a more protective immune response, or be expressed as peptides or fusion proteins.

MaxCyte electroporation offers a rapid means of expressing recombinant antigens with superior performance compared with chemical transfection methods such as PEI (Figure 3). Following transfection, the viability of PEI-transfected cells drops off substantially by day 3, while >95% viability is maintained for 10 days after both small- and large-scale electroporation. Higher cell viability was reflected as a 6 times (6x) higher expression of the HIV-1 gp145 envelope protein on day 3 using MaxCyte electroporation. Additionally, gp145 expression was further increased to an average titer of >100 mg/L on day 10 post electroporation, while PEI-transfected cultures no longer secreted antigen due to the loss of cell viability.

Case Study 3: Rapid Response Vaccines Using Insect Cell VLP Production

Virus-like particles (VLPs) are a promising avenue of vaccination with an inherently higher level of safety compared to inactivated or live attenuated viral vaccines. These particles consist of one or more recombinantly expressed viral structural proteins that self-assemble into complexes that closely mimic the three-dimensional structure of native virus but lack the viral genome. VLPs have been produced for a variety of viruses, including influenza, and are an FDA-approved vaccine against human papilloma virus (1, 4, 14).

VLPs are manufactured using a variety of cells including mammalian and insect cells. Insect cells offer an attractive means of biomanufacturing as they post-translationally modify proteins in a manner similar to that of mammalian cells, yet are easy to culture with simplified cell growth that is readily adapted to high-density suspension. While both transient transfection and recombinant baculovirus platforms are commonly used methods for insect cell protein expression (15, 16), MaxCyte electroporation offers a more rapid and straightforward means of VLP 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. This 6- to 8-week process requires construction of expression plasmid(s), transfection of insect cells, viral stock preparation, and subsequent infection of insect cells, all prior to final production and purification of the recombinant protein(s) of interest. In contrast, MaxCyte electroporation directly transfects Sf9, Sf21, and SL3 cells with >90% cell viability and transfection efficiency levels, allowing for rapid, high-level protein production within 3 days (Figures 4 and 5).

Sf9 cells transfected with an expression construct encoding three VLP antigens via MaxCyte electroporation 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 the literature (17, 18), which documents the propensity for baculovirus protein contamination, creating purification challenges, and yield loss when using a baculovirus expression system. Overall, these results demonstrate the extremely rapid and high-quality nature of direct insect cell transfection using MaxCyte electroporation, which streamlines VLP production by eliminating the need for baculovirus usage.

Case Study 4: Increased Efficiency of Alphavirus VRP Production

Alphaviruses have a broad cellular tropism, but preferentially infect dendritic cells, thereby acting as a delivery vehicle to professional antigen-presenting cells (APCs), which in turn stimulate both humoral and cellular immune responses. Alphavirus-derived particles, called VRPs (virus-like replicon particles), represent a viable next-generation vaccine option, particularly against NIH Risk Group 3 viruses (such as H5N1, HIV, Ebola, etc.) as low biocontainment facilities can be used to produce the non-

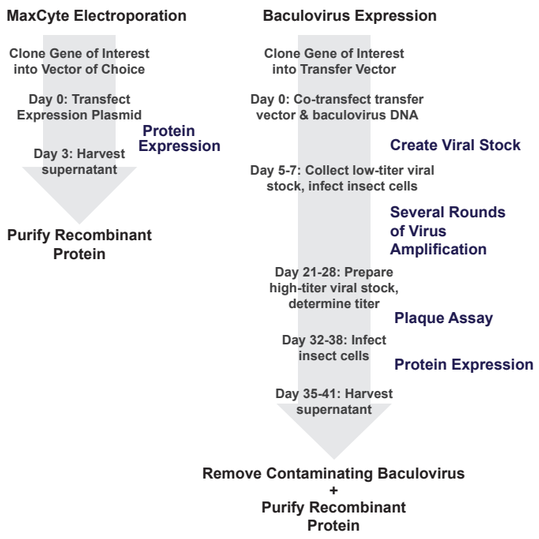


Figure 4: Streamlined VLP Production Using MaxCyte Electroporation

Rapid VLP Production Without the Need for Baculovirus

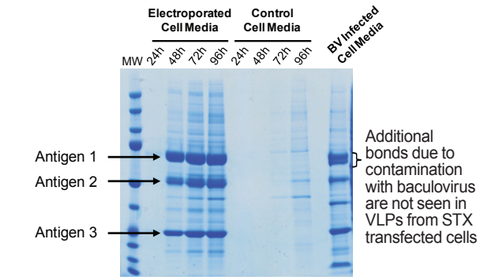


Figure 5: SF9 VLP Production Using MaxCyte Electroporation: Plasmid to Protein in 2–4 Days. *SF9 cells were transfected via small-scale electroporation with a single plasmid encoding three antigens that co-assemble into VLPs. Culture media was collected at various times from cells post EP or following baculovirus infection and analyzed using SDS-PAGE.*

pathogenic VRPs. VRPs against pathogenic agents as well as cancer immunotherapies have been evaluated and shown to be safe and induce protective immunity in a variety of animals and humans (19, 20).

Alphaviruses are positive-stranded RNA viruses that can be rapidly engineered to express high levels of heterologous proteins by replacing the viral coat protein open reading frame (ORF) with an ORF for an antigen(s) of interest. VRPs contain RNA and can direct the translation of large amounts of the antigen of interest upon host cell infection. While VRPs are capable of replicating their genome via a double-stranded RNA molecule, they cannot integrate into the host genome, nor can they propagate new virus particles unless the structural proteins are provided *in trans*. Thus, VRPs are considered single-cycle, propagation-defective vectors.

VRPs are produced by transiently (co)transfecting cells with an alphavirus vector expressing the antigen(s) of interest (in the form of mRNA or DNA plasmid) and helper RNAs or DNA plasmids expressing the alphavirus structural proteins, followed by harvesting of VRP-containing culture media. Many different cell lines are permissive to alphavirus infection, allowing a variety of cells to be used for VRP production. Vero and BHK are most common, but others including CHO and HEK have been used. Several VRPs produced via electroporation of Vero cells have entered into clinical studies (20).

MaxCyte electroporation is a proven means of high-efficiency (co)transfection of Vero cells with both mRNA and DNA. Alphavirus VRP experiments were conducted comparing the current 'best practice' method to MaxCyte electroporation and comparing RNA and DNA as vectors for the helper proteins. Production of two VRPs was assessed; one expressing GFP and the second expressing the HIV gag protein. For both transfection methods, RNA-based helper constructs supported higher levels of the VRP production, particularly for the MaxCyte electroporation-based method, which had 5x the VRP production rates using RNA (Figure 6). When both RNA replicons and RNA helpers were used, electroporation lead to 9x higher relative titers of both the GFP and HIV gag-expressing VRPs over the current best practice method.

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.

Lentivirus stocks are historically produced by transfecting a stable packaging cell line (PCL), commonly HEK 293 cells, which express the virion proteins and reverse transcriptase

Increased Efficiency of Alphavirus VRP Production

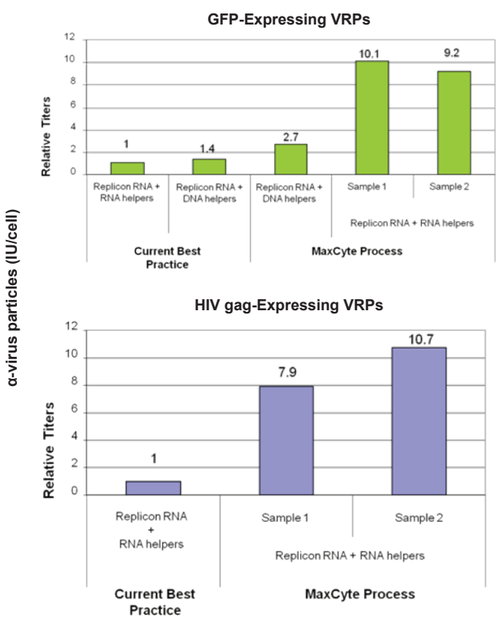


Figure 6. Significantly Higher VRP Production Using MaxCyte Electroporation. *Vero cells were suspended in MaxCyte electroporation buffer at a density of 1E8 cells/mL and mixed with mRNA and/or plasmid DNA encoding alphvirus replicon components. Cells and loading agents were transferred to single-use processing assemblies and electroporated with the MaxCyte STX instrument. Transfected cells were plated in standard tissue culture vessels, and secreted viral replicon particles were quantified using conventional titer assays.*

required for a single round of infection, with the shuttle vector encoding the gene of interest (21). 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 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, HEK 293FT 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 (static) electroporation on the MaxCyte STX, and large-scale (flow) electroporation on the MaxCyte STX and MaxCyte VLX. Identical electroporation parameters were used for all electroporations. The experiments highlight the seamless scalability of MaxCyte electroporation as small- and large-scale transfection produced nearly identical high-titer viral stocks (Figure 7).

MaxCyte electroporation 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 the 4-plasmid lentiviral system have been reported using the MaxCyte GT (22). These studies demonstrate the consistent, highly reproducible nature of MaxCyte electroporation (Table 2). Titers of approximately 40 transducing units of virus per cell were achieved in three pilot qualification lots manufactured at a cGMP facility. Thus, MaxCyte electroporation has proven safety, scalability, and performance allowing for scalable

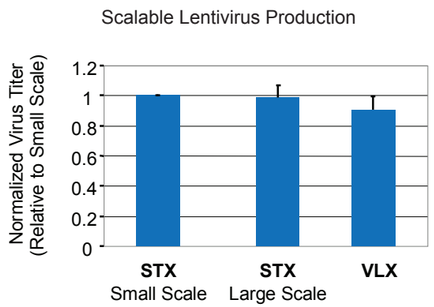


Figure 7: Robust, Scalable Transfection for Lentivirus Production. *Suspension-adapted HEK 293FT cells were suspended with a mixture of plasmids encoding lentiviral vector components (0.4 µg of DNA/1E6 cells), and cells were transferred to sterile OC-400, CL-2, or VL2 processing assemblies. Cells in the OC-400 and CL-2 were transfected by static and flow EP, respectively, using the MaxCyte STX instrument; cells in the VL2 were transfected by flow EP on the MaxCyte VLX. Lentiviral titers were measured after 24-48 hrs in culture and normalized against small-scale MaxCyte STX electroporation.*

Consistent Large-Scale Production of Lentiviral Vector

Production Number	Volume (ml)	Total Cells	IU	Productivity (IU/cell)	IU/ng of p24
1	2300	6.0E9	2.2E11	37	1.3E5
2	2300	4.8E9	2.0E11	42	1.4E5
3	2100	7.4E9	2.7E11	36	1.7E5

Table 2: 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. P24 was quantified using an HIV-1 p24 antigen capture assay. See* Human Gene Therapy. *23, 2012, p243-249 for full methods.*

production of lentiviral vectors for clinical and commercial applications.

Case Study 6: Efficacy While Maintaining Mitigated "On Target, Off Target" Toxicities Using mCAR Expression

MaxCyte flow electroporation overcomes many existing obstacles of cell therapy and enables the development and manufacturing of innovative immunotherapies, such as therapies using chimeric antigen receptor (CAR) expression, against a wide range of diseases (23-25).

CARs are engineered surface receptors that contain an extracellular tumor recognition domain of a single-chain antibody (scFv) and intracellular signaling domains for cell activation upon antigen stimulation. CARs confer non-MHC restricted specificity of T-cells or NK cells for antigen recognition. Loading of autologus patient cells with CAR-encoding RNA or DNA enables T-cells for target-specific cytotoxicity and is a clinically validated vehicle for adoptive immunotherapy.

Lenti and retroviral vectors and electroporation have been used clinically to mediate CAR gene transfer (26). While initial clinical trials using lentivirus vectors encoding anti-CD19, CAR demonstrated durable clinical responses, limited clinical benefit was observed against solid tumor due to 'on-target off-tumor' toxicities of viral-vector-modified CAR T-cells. Transfection of cells with mRNA encoding CAR via MaxCyte electroporation lessens toxicity to normal tissues, allows translation of CAR T-cell immunotherapy to solid tumors, and addresses potential safety and regulatory issues (25, 27).

T-cells electroporated using the MaxCyte GT with RNA encoding anti-mesothelin CAR that includes both the CD3-ζ and 4-1BB co-stimulatory domains expressed the CAR for as long as 7 days post transfection (Figure 8A). Adoptive transfer of these mesothelin-targeted CAR T-cells to mice was safe without overt evidence of off-tumor

CAR T-cell Expression via RNA Electroporation

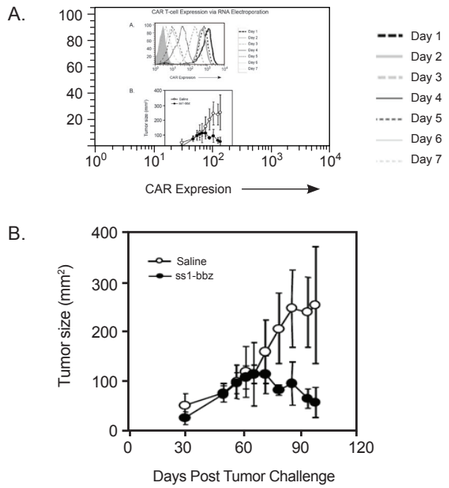


Figure 8: Sustained RNA CAR expression and Regression of Tumors in Mice Treated with RNA CAR T-cells. A. *Stimulated T-cells were electroporated with clinical-grade,* in vitro *transcribed RNA (10 µg RNA/100 µL T-cells) generated from pD-A.ss1.OF (meso-CAR RNA) and transgene expression analyzed via FACS daily.* ***B.*** *Flank tumors were established by M108 injection (s.c.) in NOD/scid/γc(−/−) (NSG) mice (n=6). Sixty-six days after tumor inoculation, mice were randomized to equalize tumor burden and treated with meso-CAR RNA-electroporated T-cells. The T-cells (1E7 to 1.5 E7) were injected intratumorally every 4 days for a total of four injections using the same healthy donor; mice treated with saline served as controls (n=3). Tumor size was measured weekly. Cancer Res. 70(22), 2010, p9053.*

on-target toxicity against normal tissues. Additionally, meso-CAR RNA electroporated T-cells injected in mice with established mesothelin-positive tumors were able to reduce tumor size, whereas progressive tumor growth was observed in the control group of mice (Figure 8B). In preliminary human studies, the injection of autologous T cells with transfection of mRNA encoding anti-mesothelin CAR was reported to be safe and resulted in measurable reduction in tumor burden in two patients (27). These published results support the safety and anti-solid tumor activity of T-cells electroporated using MaxCyte transfection with tumor-targeted CAR.

Case Study 7: Enhanced Dendritic Cell Antigen Presentation Efficacy

MaxCyte flow electroporation can be used to load cells not only with various nucleic acids, but also with proteins and tissue lysates. This feature allows patient immune cells, such as dendritic cells (DCs), to be loaded with tumor cell lysates, which act as a cancer vaccine when re-infused into patients.

DCs are professional APCs that elicit robust humoral and cellular immune responses and thus represent a key target for immunotherapy. The conventional avenue of loading DCs with tumoral antigens and/or whole cell lysates is through co-incubation/pulsing, which has been shown to elicit specific anti-tumor T-cell responses for a variety of cancers (28). However, MaxCyte electroporation of DCs results in significantly enhanced antigen uptake compared to co-incubation control (Figure 9). In contrast to co-incubation with tumor lysate in which antigen uptake is passive, the amount of antigen loading can be actively controlled using MaxCyte electroporation to deliver optimal concentrations of tumor lysate (29).

Side-by-side adoptive transfer studies demonstrate that DCs electroloaded with whole cell tumor lysate via MaxCyte electroporation elicit significantly stronger antitumor responses *in vitro* and *in vivo* (30), perhaps mediated by delivery of antigens directly into the cytoplasm that facilitates class I MHC, as opposed to antigen taken up during co-culture (macropinocytosis) that is associated with class II presentation. Using IFNγ production as well as specific tumor cell-killing assays (Figure 10), DCs electroporated with whole tumor lysate were capable of priming both naive and memory splenocytes *in vitro* more effectively than DCs co-incubated with whole tumor lysate. Additionally, in both a tumor challenge and a therapeutic metastatic tumor model, MaxCyte-electroporated DCs consistently blocked significant tumor growth *in vivo* to a larger degree than co-culture DCs. These results were observed for RENCA, B16 melanoma, and LLC, each considered to be poorly immunogenic and highly aggressive, with control mice routinely succumbing to disease within several weeks (30). Overall, these studies consistently demonstrate that MaxCyte electroporation offers considerable advantages over standard DC co-incubation protocolsby eliciting stronger T-cell responses while using considerably less tumor lysate.

Conclusions

MaxCyte electroporation is a clinically proven, transient transfection method that enables early risk reduction and accelerated development of antibodies, recombinant antigens, VLPs, VRPs, viral vectors, and cell immunotherapies thereby reducing cost and shortening timelines. Additionally, MaxCyte electroporation enables progression from gene to gram-scale quantities of proteins within days providing an attractive means of generating vaccines for pandemic, seasonal outbreaks, and biodefense applications. Flow electroporation has unmatched quality, flexibility, and scalability creating a single, cost-effective platform that supports the full range of biotherapeutic and vaccine development activities. The MaxCyte platform has the capacity to rapidly, simply, and cost-effectively scale-up and scale-down for use in early-phase candidate identification, late-stage pharmacology, stability, manufacturability studies, and process development and biomanufacturing activities.

Efficient Loading of Dendritic Cells With Proteins

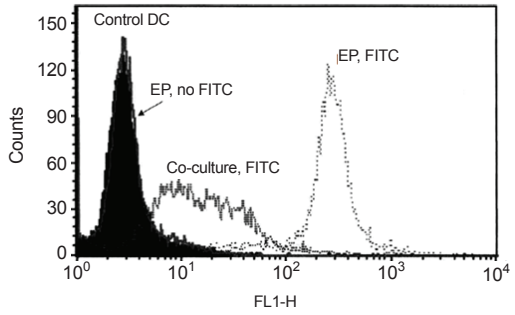


Figure 9: Efficient Electroporation-mediated Delivery of Macromolecule into Dendritic Cells. *C57BL6 mouse bone-marrow-derived DCs were either co-cultured or electroloaded with 0.5 mg/mL FITC-dextran (250 kDa). FACS analysis was performed 3 hrs after electroporation.* Methods in Molecular Biology. *Vol 423, 2008, 139-153.*

Improved Immune Induction Using Electroporation Loading

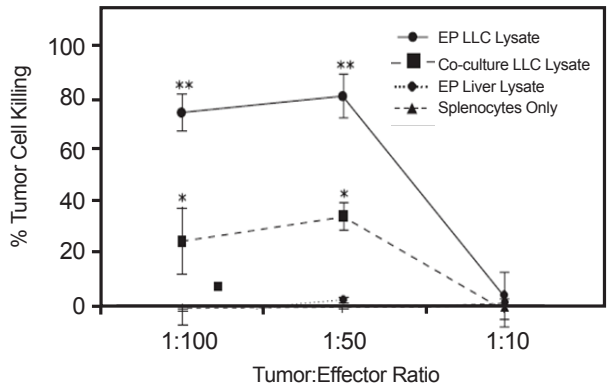


Figure 10: Whole Tumor Lysate Electroloaded DCs Elicit Anti-tumor Immune Response. *Splenocytes were isolated from C57BL6 mice that had previously received two intravenous administrations of 1E6 syngeneic DCs electroporated with Lewis lung carcinoma (LLC) lysate. They were divided into four groups and stimulated with previously processed DCs at a ratio of 1 DC to 10 T-cells as follows: 1). LLC lysate electroporated DCs; 2). LLC lysate co-cultured mDCs; 3). mDC electroporated with normal liver lysate; and 4). no mDC (T-cells only). The co-cultured cells were restimulated once/week for a total of three restimulations with appropriately prepared and cryopreserved mDC. The expanded LLC-tumor-specific T-cells were washed and incubated with 51Cr-labeled intact LLC tumor cells at various ratios of 50:1, 10:1, and 1:1 for a standard in vitro tumor killing assay.* J. Immunother. *28(6), 2005, p542-550.*

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