High-level Transient Production of IgGs, Bi-specific T-cell Engaging (BITE) Molecules & Fc Fragments with the Quality, Glycosylation & Functionality Required for Use as Surrogates for Stably Produced Proteins.

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Abstract

Companies are turning to transient protein production during early development to delay stable cell line generation, accelerate timelines, and reduce costs. A key factor for the success of this approach is the production of high quality, functional proteins with a high degree of similarity to stably produced proteins. MaxCyte’s delivery platform is a high performance, electroporation-based technology that can rapidly generate milligram to gram quantities of protein in the cell line of choice. In this poster, data are shown including protein quality, glycosylation and functionality that support the unique ability of MaxCyte Flow Electroporation™ Technology to accelerate early to mid-stage biotherapeutic development via transient protein expression. Specifically, we present data on transient expression of high quality bi-specific antibodies, bi-specific T-cell engaging (BITE) molecules, tribodies, and full IgGs. Protein quality and glycosylation pattern analysis demonstrate that transiently produced proteins mimic product qualities of stably produced proteins. Furthermore, we expand on the high quality of transiently expressed BITEs and Fc fragments by illustrating their functionality through tumor cell-specific cytotoxicity, tumor cell binding and anti-inflammatory activity. In summary, we illustrate that MaxCyte’s flow electroporation delivery platform can expand the footprint of transient transfection by delaying stable cell line generation while maintaining the integrity of candidate selection during biotherapeutic development.

Transient Expression of Functional Antibody-like Molecules

High Expression of Functional Bispecific Tandem scFvΔs Targeting CD19 x CD3 and Her2 x CD3

A. Affinity Purification

B. Specific Killing of Tumor Cells by Immune Effector Cells Mediated by BITE Molecules

Figure 3: Expression of functional bispecific T cell engaging (BITE) molecules in CHO cells. A) Conditioned media samples from CHO-S cultures transiently expressing CD19 x CD3 or Her2 x CD3 bispecific molecules were assayed in in vitro binding buffer, and proteins were enriched using Hi-NTA agarose. After dialysis against PBS, proteins were assayed by capillary electrophoresis. B) Tumor cells were co-cultured with non-stimulated T-cells for 20 hours. In the presence of [3H]TdR or CD19 x CD3 BITE molecules and cell lysate measured by a chromium release assay. Data Courtesy of Dr. Matthias Peppe, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts University.

High Expression of Tribody Targeting Her2 x CD16

A. Affinity Purification

B. High, Specific Binding of Enriched Tribodies to Tumor Cells

Figure 4: Binding of affinity enriched tribodies to tumor cells. A) Following co-transfection of CHO-S cells with plasmids encoding a Her2 x CD16 tribody, proteins were enriched from conditioned media samples using CH1-specific agarose beads. Proteins were assayed by capillary electrophoresis under reducing and non-reducing conditions. Assays indicate bands of the expected sizes for single chains and intact tribody. B) SKBR-3 cells (derived from human breast cancer) were incubated with [3H]TdR x CD16 tribody or with CD19 x CD3 BITE molecules. FACS analysis showed binding of the tribody to Her2 antigens on SKBR-3 cells, whereas binding was not observed using the negative control CD19 x CD3 bispecific molecules. Data Courtesy of Dr. Matthias Peppe, Division of Stem Cell Transplantation and Immunotherapy, Christian-Albrechts University.

Expression of Functional Fc Fusion Protein

A. Fc Fusion Protein Gel Analysis

B. Stimulation of Donor Cell as Measured by 1Tb Secretion

Figure 5: Purified Fc fusion protein has anti-inflammatory activity. A) CHO2ZNF6 cells were transfected with an expression plasmid encoding an Fc fusion containing a recombinant form of the naturally occurring human protein, alpha 1-antitrypsin. Cultured samples were loaded onto a 1µm Protein A FastFlow HiTrap column (GE) at room temperature. Gel analysis of purified proteins expressed by transiently transfected cells showed quality and size attributes consistent with those of a reference protein and that produced in a customer lab. B) Two different donors’ T-cells were challenged with varying inflammatory substrate either in the presence or absence of various quantities of the purified Fc fusion protein. Transiently produced Fc fusion protein had the expected anti-inflammatory activity as measured by an overall decrease in IL1β secretion. Data Courtesy of MAnogenigma.

Summary

• MaxCyte Flow Electroporation Technology can be used to transiently express proteins with the quality and functional activity required to conduct early development of therapeutics against a variety of diseases.

• The MaxCyte delivery platform can produce high titers of antibodies and antibody-like molecules using a variety of CHO cell lines, including CHO-S and CHOZN® cells, providing researchers the ability to use their CHO cell line of choice.

• Flow Electroporation Technology is universal in nature and can produce a variety of high quality protein types including IgG, bi-specifics, tribodies and Fc fusion proteins.

• MaxCyte transient production produced IgG with glycosylation patterns similar to stably produced protein, supporting the use of transiently produced proteins in early-stage discovery efforts delaying the need to generate stable cell lines.

• A variety of transiently produced protein types exhibited functional activity including tumor binding, tumor lysis and anti-inflammatory activity.

MaxCyte Scalable Transfection Systems

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

• High efficiency & high cell viability

• True scalability requiring no re-optimization

• Closed, computer-controlled instruments

• GMP-compliant & CE marked

• Master file with US FDA & Health Canada

Transient vs Stable Expression Protein Attributes

Equivalent Antibody Quality & Glycosylation

Similar IgG Produced via MaxCyte Transient Transfection & Stable Cell Lines Generated Using MaxCyte Transfection

Figure 1: IgG Quality & Glyco-form Comparison - Transient vs Stable Expression. A human IgG molecule was expressed transiently in CHO-S cells via electroporation with the MaxCyte STX™. A stable cell line (S17) was also generated by extending transient transfections to cell lines to antibiotic selection, followed by limited dilution cloning. A) Glycoform analysis showed highly consistent patterns of post-translational modification between transient and stable proteins. B) SDS-PAGE gel analysis (reducing and non-reducing) data in SDS-PAGE gel analysis indicate equivalent quality (i.e aggregation or degradation) of antibodies produced via transient or stable transfection.

Gel Analysis of Protein Quality and Titer Compared to Stable Reference

5%Ha Heavy Chain and the 250kDa Light Chain Are Clearly Seen

Figure 2: Titers, SDS PAGE, and glyco-form analysis of transiently expressed IgGs. CHO-S cells were electroporated using the MaxCyte STX™ - four independent runs. 1 of the 4 transfections were cultured in simple glucose media, while cells from the remaining transfections were cultured in glucose depleted media. Samples were collected on days 5-8 and analyzed for IgG titers. Samples from day 8 post EFW were run on a Novex 4-20% SDS PAGE Trio Glycogen gel and stained with Coomassie Blue G-250. Bands of the correct size for high light and heavy chains are clearly evident on a reducing gel loaded with unpurified media samples and Protein A purified samples. No additional bands are evident in the purified samples, indicating good protein quality. Glycoform analysis was performed via mass spectrometry on the protein samples produced transiently with glucose or complex media fed from a reference stable cell line cultured in a bioreactor with slightly optimized growth conditions. The largest changes in glycoform were associated with changes in media rather than transient vs stable protein production. Data Courtesy of MAnogenigma.