The Development of a Robust Chemically Defined Media (EX-CELL™ CD NS0) for NS0 & Myeloma Cell Lines

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Abstract

With 30 years of successful recombinant protein and antibody expression, the NS0 cell line continues to be an excellent choice for mammalian gene expression. To support the need for higher cell densities, viability and protein productivity, a new NS0 formulation was developed. Chemically defined (CD) formulations from the SAFC Biosciences media library were screened for growth and productivity with four NS0 clones expressing a recombinant human IgG. The best media from this screening was then optimized through multiple rounds of component group titrations. The additional rounds of media optimization increased the productivity of all four clones and reduced the variable levels of productivity observed between clones from 5-fold to 2-fold. The resulting EX-CELL™ CD NS0 is a chemically defined media containing recombinant proteins that surpasses the current SAFC Biosciences catalog offering as well as a competitor's CD Hybridoma media in growth, viability, culture longevity and productivity for the NS0 clones utilized in these experiments.

Using EX-CELL™ CD NS0 media peak viable cell densities of >6X10^6 viable cells/mL were routinely measured by Cedex counting compared to the competitor's CD Hybridoma reaching 3X10^6 viable cells/mL. The cell viabilities were 80% or better through day 6 of a batch cell culture growth curve and the final protein productivity was 3-4X that of the competitor's CD Hybridoma media.

In conclusion, we have developed a new CD media for NS0 cell lines exhibiting improved cell growth, improved viability, increased culture longevity, improved productivity and ease of adaptation from serum containing media. The new EX-CELL™ CD NS0 media supports both GS and non-GS myeloma/hybridoma cell lines and is available in both liquid and dry powder forms.

Materials and Methods

Cell Line & Vector Development

The NS0 parental cell line and vectors (pEE12.4 and pEE6.4) were licensed from Lonza Biologics. The heavy and light chain genes from an anti-rabies humanized IgG (SO47) were used to create the final plasmids following the Lonza protocol for construction. These plasmids were electroporated into the NS0 parental cell line using a Single-pulse Square Wave Setting set at 225 V and 30 msec (BTX Electroporator). Cells were allowed to recover in Dulbecco's Modified Eagle's media (SAFC Biosciences, Product Number 51435) with GS supplement (Sigma Aldrich, Product Number G9785) and 10% FBS (SAFC Biosciences). Expressing cells were then selected by incubating in L-glutamine free media +/- 7.5 mM MSX (Sigma-Aldrich, Product Number M5379) for 3 weeks.

Cell Line Screening

The transfected Masterwell cell lines were expanded from 96 well plates using Dulbecco's Modified Eagle's media (SAFC Biosciences, Product Number 51435) with GS supplement (Sigma Aldrich, Product Number G9785) and 10% FBS (SAFC Biosciences) to 24 well plates and tested for productivity using the Guava PCA-96. Those cell lines that continued to grow and produce were expanded and the serum concentration was reduced to 1% as the cell lines were scaled up to 125 mL shake flasks. Four cell lines were subcloned using limiting dilution and four subclones with diverse growth and productivity characteristics were studied as stated above. The subclones were adapted to serum free media and scaled up for characterization in EX-CELL™ NS0 (SAFC Biosciences, Product Number 14650C). Cholesterol (Sigma-Aldrich, Product Number S5542) was added to a final concentration of 2X as the serum was decreased. All studies were run at 37 °C in a humidified incubator at 5% $C0_2$.

Media Screens

Nineteen in-house CD and ACF media supplemented with GS (1X) and Cholesterol (2X) were used in the initial screening with the four NS0-GS cell lines. The studies were performed using TPP tubes in triplicate in a Kuhner shaking incubator (Appropriate Technical Resources, Laurel MD) at 200 rpm. Typically 30 mL of media was used per TPP tube seeded with 3x10^5 cells/mL. EX-CELL™ NS0 (SAFC Biosciences, Product Number 14650C) and a competitor's hybridoma media were used as controls. Samples were pulled daily for growth, viability and NOVA analysis. Productivity and spent media analysis samples were pulled beginning on Day 6 and continuing until the viability was 50% or below. Once the base media was identified the optimization of various component group titrations commenced. The final formulation chosen (EX-CELL™ CD NS0) was CD and ACF containing recombinant proteins.

Bioreactor and Feed Studies

Bioreactor runs were conducted in 3 L stirred tank bioreactors (Applikon, Inc., Schiedam, Holland THE NETHERLANDS). Bioreactors were seeded at 3x10^5 cells/mL in EX-CELL™ CD NS0 and Competitor's media. Dissolved oxygen (DO) and pH were monitored online and controlled using air, O_2, N_2, CO_2 and 7.5% $NaHCO_3$, respectively. Temperature was maintained at 37 °C using a heating blanket. Samples (5 mL) were collected daily to monitor cell density, viability and metabolic consumption/production (data not shown). All IgG titers were determined using a HPLC by injecting 25uL of spent cell culture media into a 2.1mm × 30mm POROS A20 Protein A Column (Applied Biosystems, CA, USA) connected to an Alliance HPLC with PDA detection (Waters, MA, USA).

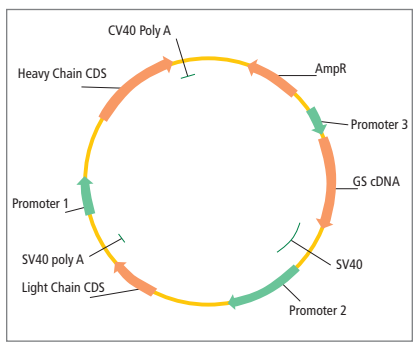

Figure 1. Anti-rabies GS Expression Vector

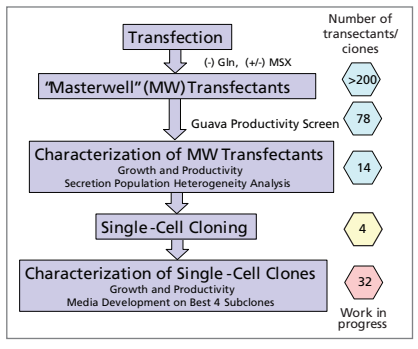

Figure 2. Cell Line Generation Work Flow

Results

Secretion Population Heterogeneity Analysis of the Anti-Rabies NS0-GS Single-Cell Clones

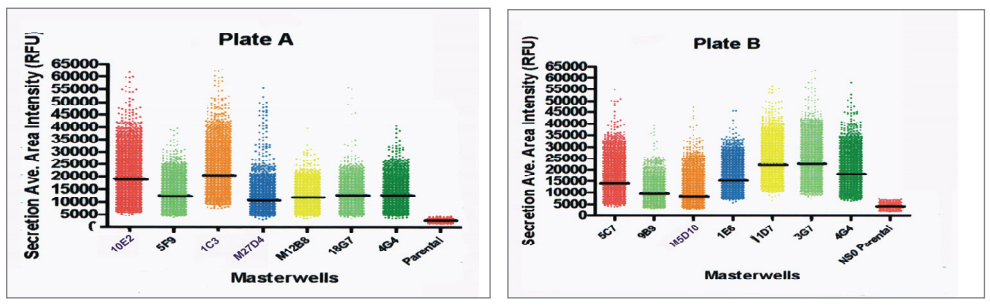

Figure 3. Cell Xpress secretion heterogeneity analysis results from 14 single-cell clones derived from two "Masterwell" transfectant populations. Parental NS0 cells were included as control. Mean values are indicated by black. Secretion Average Area Intensity is normalized to parental NS0. The LEAP system (Laser-Enabled Analysis and Processing, Cyntellect, San Diego, CA) is a unique laser-based cell processing system that combines cell imaging and laser-mediated cell manipulation in an automated and high-throughput manner via large field-of-view optics and galvanometer steering. It allows the user to efficiently identify, select and monitor expansion of high recombinant protein secreting clones. It also enables analysis of IgG-secretion heterogeneity and purification of transfected populations by eliminating non-producers

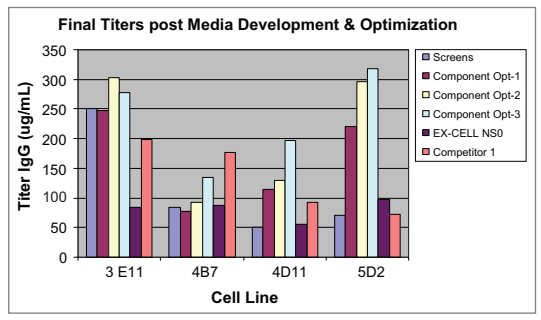

Figure 4. Protein Productivity results of 4 NS0-GS subclones over the course of the Media Development project for the chosen base media and the optimization of that base media. All cells lines were passaged several times in each media (19 in-house CD and ACF formulations) to allow for adaptation before each study. Various components and groupings of components were tested in triplicate. After passaging, the cell lines were seeded in TPP tubes at 3x10^5 cells/mL. Cell viability and density measurements were taken daily. While each cell line had unique growth and productivity characteristics, improvements in these properties were made for each cell line. The new optimized media (EX-CELL™ CD NS0) showed significant improvement in growth, viability (not shown) and productivity over EX-CELL™ NS0 and a Competitor's hybridoma's media.

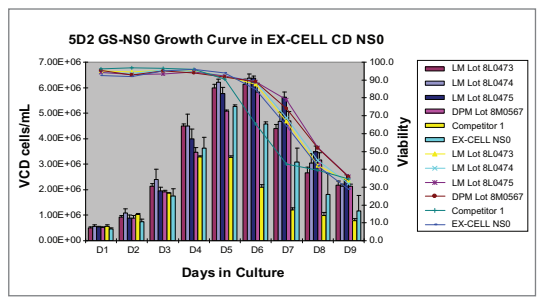

Figure 5. Growth Curve of cell line 5D2 in 3 lots of EX-CELL™ CD NS0 (LM), one lot of dry powder media (DPM), EX-CELL™NS0 and Competitor 1 hybridoma media. 5D2 cells were passaged several times in each media to allow for adaptation. After passaging, 5D2 cells were seeded in 125 mL shake flakes at 3x10^5 cells/mL. Cell viability and density measurements were taken daily. 5D2 cells reached significantly higher peak VCD in EX-CELL™ CD NS0 and demonstrated greater longevity as compared to Competitor1. 5D2 cells in EX-CELL™ CD NS0 reached a maximum cell density of approximately 6x10^6 cells/mL on Day 6. 5D2 cells in Competitor 1 media reached maximum cell density of 3.2x10^6 cells/mL on Day 4 and then rapidly declined. Data represents the mean viable cell density for triplicate flasks.

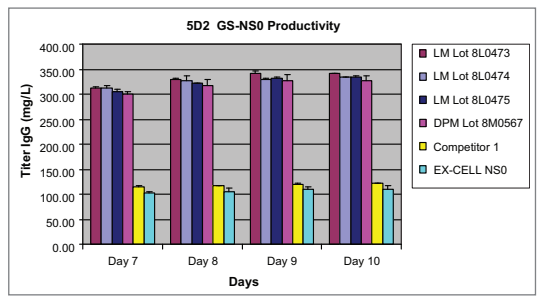

Figure 6. Productivity results from Growth Curve of cell line 5D2 in 3 lots of EX-CELL™ CD NS0 (LM), one lot of dry powder media (DPM), EX-CELL™ NS0 and Competitor 1 hybridoma media (**Figure 5**). Minimal lot to lot variation was observed for the liquid media (LM) and dry powder lot. The productivity for all lots of EX-CELL™ CD NS0 were 3 times greater than EX-CELL™ NS0 or a Competitor's hybridoma media. Data represents the mean productivity levels for triplicate flasks. See Figure 5 for Growth Curve.

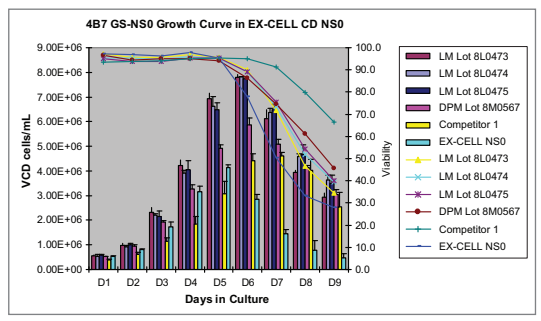

Figure 7. Growth Curve of cell line 4B7 in 3 lots of EX-CELL™ CD NS0 (LM), one lot of dry powder media (DPM), EX-CELL™ NS0 and Competitor 1 hybridoma media. 4B7 cells were passaged several times in each media to allow for adaptation. After passaging, 4B7 cells were seeded in 125 mL shake flakes at 3x10^5 cells/mL. Cell viability and density measurements were taken daily. 4B7 cells reached significantly higher peak VCD in EX-CELL™ CD NS0 and demonstrated greater longevity as compared to Competitor 1. 4B7 cells in EX-CELL™ CD NS0 reached a maximum cell density of approximately 7.8x10^6 cells/mL on Day 6. 4B7 cells in Competitor 1 media reached maximum cell density of 4.62x10^6 cells/mL on Day 7. Data represents the mean viable cell density for triplicate flasks.

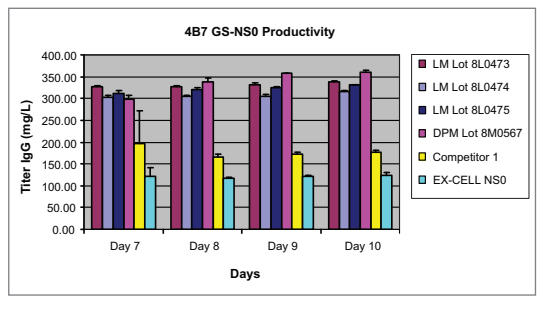

Figure 8. Productivity results from Growth Curve of cell line 4B7 in 3 lots of EX-CELL™ CD NS0 (LM), one lot of dry powder media (DPM), EX-CELL™ NS0 and Competitor 1 hybridoma media (**Figure 7**). Minimal lot to lot variation was observed for the liquid media (LM) and dry powder lot. The productivity for all lots of EX-CELL™ CD NS0 were 3 times greater than EX-CELL™ NS0 and the productivity was 1.6 – 2 times greater than the Competitor's hybridoma media. Data represents the mean productivity levels for triplicate flasks. See Figure 7 for Growth Curve.

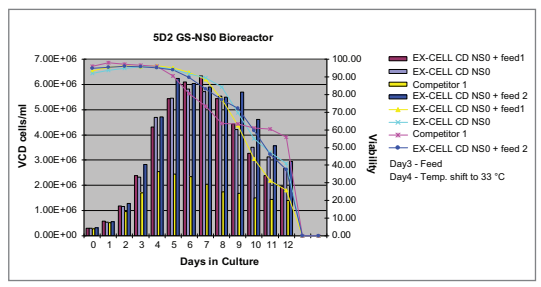

Figure 9. Batch and fed batch Bioreactors using the NS0-GS 5D2 cell line performed in duplicate. 5D2 cells were expanded in one lot of EX-CELL™ CD NS0 or the competitor's media. Bioreactors were inoculated with 3x10^5 cells/mL. The fed batch cultures were fed on Day 3 with one of two proprietary feed blends. All the bioreactors were temperature shifted on Day 4 to 33 °C. Samples were collected daily for growth, viability and metabolite consumption. Productivity samples were collected beginning on Day 8. 5D2 cells reached significantly higher peak VCD in EX-CELL™ CD NS0 and demonstrated greater longevity as compared to Competitor 1. 5D2 cells in EX-CELL CD™ NS0 reached a maximum cell density of approximately 6x10^6 cells/mL on Day 6. 5D2 cells in Competitor 1 media reached maximum cell density of 2.5x10^6 cells/mL on Day 4.

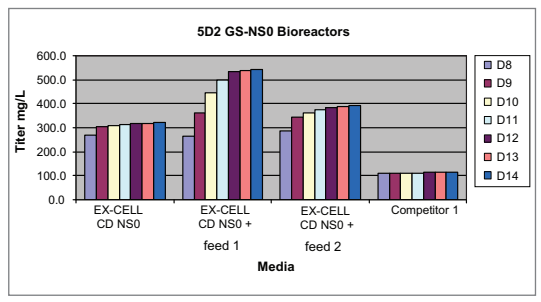

Figure 10. Productivity results from batch and fed batch Bioreactor studies performed with cell line 5D2 in 1 lot of EX-CELL™ CD NS0 (LM) and Competitor 1 hybridoma media in duplicate. The productivity of EX-CELL™ CD NS0 batch was 3 times greater than Competitor's hybridoma media. With Proprietary Feed 1 the productivity was 5 times that of the Competitor's hybridoma media and with Proprietary Feed 2 the productivity was 4 times that of the Competitor's hybridoma media. See (**Figure 9**) for Growth Curve.

Conclusions

The new product EX-CELL™ CD NS0, offers the following advantages over other products:

- Ease of adaptation from SAFC Biosciences media formulations or Competitor's media into EX-CELL™ CD NS0
- Growth was consistently higher in EX-CELL™ CD NS0 compared to current market offerings in a variety of vessels
- Productivity in EX-CELL™ CD NS0 was 1.7-3.8 times higher than in either the NS0 control media (EX-CELL™ NS0) or the Competitor's CD Hybridoma media
- Demonstrated consistency and equivalency of the liquid media formulation to the dry powder formulation for EX-CELL™ CD NS0

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