A single, chemically-defined culture medium supporting a wide-range of development activities for the production of biologics

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Abstract

In the production of biologics, various development activities require different medium requirements, calling for multiple media. Transitioning from one medium to another typically poses a certain level of risk. BalanCD™ CHO Growth A was designed as a high-performance CHO production medium for batch and fed-batch processes, however its ability to support other cell line development activities was untested. In this study, the potential of this medium in supporting cell line development activities and scalability to large-scale production was evaluated. The results demonstrate the additional utility of this medium as a cloning medium and proved its scalability from the 96-well plate all the way to the 2000L scale. Its compatibility and superior performance in supporting clonal cell selection and expansion assures a seamless transition from one stage of development to the next. The ability to use a single, chemically-defined medium mitigates the risk involved with switching media throughout development, and shortens development time. With the multitude of commercially available production media and relative scarcity of subcloning media, BalanCD CHO Growth A represents a unique, chemically-defined, and platform-ready solution for the production of biologics.

Materials & Methods

Automated Single-Cell Cloning: ClonePix™

BalanCD CHO Growth A was evaluated for single-cell subcloning using a ClonePix FL imager. Semi-solid media was prepared by mixing equal parts 2x liquid concentrate BalanCD CHO Growth A and CloneMatrix© and supplementing with 6mM L-glutamine and CloneDetect™ FITC. Cell density and viability of a 2-day old CHO-M culture was determined using a GUAVA® (EMD Millipore) cell counter. The medium was inoculated at 200 cells/mL. 30mL of the cell suspension was plated into 2 x 6-well plates with 2.5mL/well. Plates were incubated at 37°C, 5% CO_2, in a humidified incubator. ClonePix images were evaluated on days 10 and 15 to determine colony growth. High secreting colonies were picked and transferred to 96-well plates. IgG titer was quantified on day 7 by ELISA.

Traditional Single-Cell Cloning: Limiting Dilution Assay

BalanCD CHO Growth A was evaluated for single-cell subcloning in a limiting dilution assay. Using three IgG expressing CHO lines (CHO-M, CHO-S, and CHO DXB11), cells were seeded in 96-well plates at a calculated 1 cell/well in each test medium. Plates were incubated at 37°C, 5% CO_2, in a humidified incubator. Plates were evaluated on day 14 using light microscopy and scored for colony growth.

Clone Selection and Verification

Using CHO-S clones isolated from the limiting dilution assay, high producing clones were selected for scale-up from 96-well plates, to 6-well plates, to 50mL bioreactor tubes, and finally to 125mL shake flasks. Once in shake flasks, the resulting clones were evaluated in fed-batch mode using BalanCD CHO Growth A and fed with BalanCD CHO Feed 1. Cultures were fed 7 x 5% (of the initial working volume) on days 2, 4, 6, 8, 10, 12, and 15. Cultures were maintained at 37°C, 5% CO_2 in a humidified incubator and agitated at 120rpm on an orbital shaker. Cultures were inoculated at $2x10^5$ cells/mL. Cell counts were determined using a Vi-Cell™ XR Cell Viability Analyzer (Beckman Coulter). 6g/L glucose was fed to all cultures when glucose fell below 2g/L. Glucose levels were monitored using a BioProfile® FLEX (Nova Biomedical). Titer was quantified using an octet® QKe (fortéBIO).

Results

Automated Single-Cell Cloning in a Semi-Solid Medium

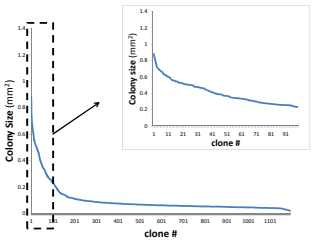


Figure 1a. Day 10 Colony Size Ranking Plot

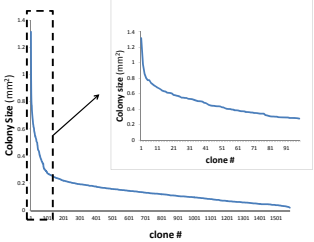


Figure 1b. Day 15 Colony Size Ranking Plot

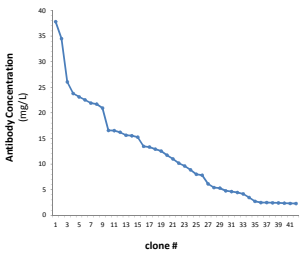


Figure 1c. Titer Distribution Ranking Plot

CHO-M cells plated as single cells readily formed colonies in BalanCD CHO Growth A. When evaluated on day 10 and day 15, 1193 and 1585 colonies were observed, respectively. The ranking plots (Figures 1a & b) depict the resulting colony size distribution. The inset plots depict the size distribution of the 100 largest colonies. The ranking plot of antibody concentration (Figure 1c) depicts the titer distribution of the 42 highest producing clones. **This evaluation demonstrates that BalanCD CHO Growth A is effective for automated single-cell cloning in a semi-solid medium.**

Traditional Single-Cell Cloning

Limiting Dilution Assay

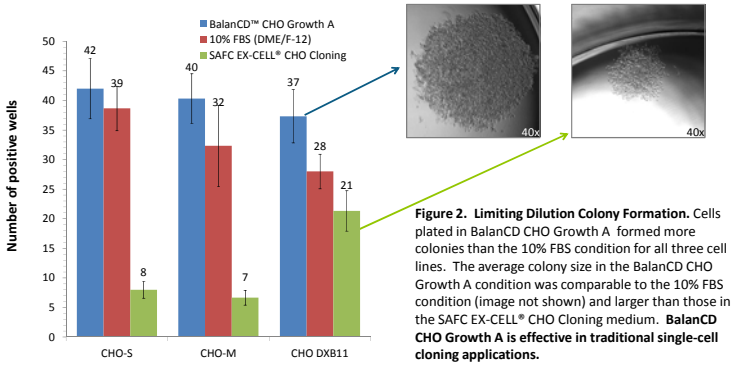
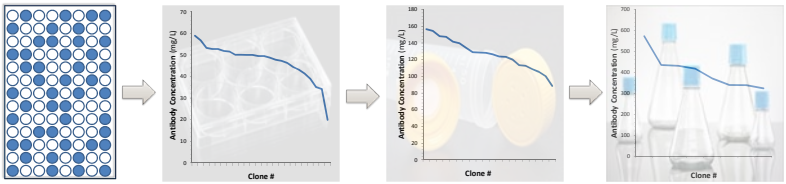


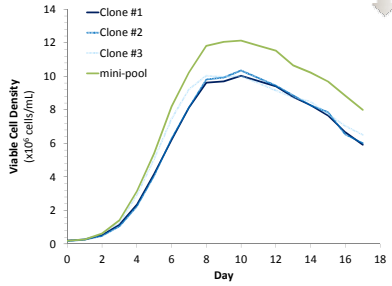
Figure 2. Limiting Dilution Colony Formation. Cells plated in BalanCD CHO Growth A formed more colonies than the 10% FBS condition for all three cell lines. The average colony size in the BalanCD CHO Growth A condition was comparable to the 10% FBS condition (image not shown) and larger than those in the SAFC EX-CELL® CHO Cloning medium. **BalanCD CHO Growth A is effective in traditional single-cell cloning applications.**

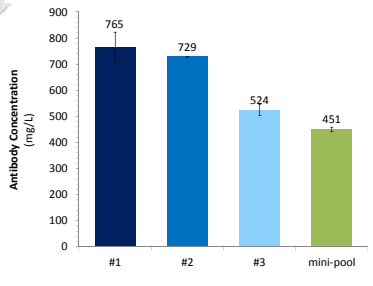
Results (continued)

Clone Selection and Verification

Figure 3a. Clone Selection: Titer Distribution. From the limiting dilution assay, the highest producing CHO-S clones were selected for scale-up to shake flasks in BalanCD CHO Growth A. The SAFC EX-CELL CHO Cloning medium failed in scale-up as it did not support normal density cultures. Expansion in a serum-containing medium would be unacceptable.







Figures 3b & c. Clone Verification: Growth Curves and Titer. Performance of the top three clones was evaluated relative to the original CHO-S mini-pool (from which all subsequent CHO-S clones were derived) in fed-batch using BalanCD CHO Growth A and BalanCD CHO Feed 1. **Although growth was slightly reduced relative to the mini-pool, the highest producing clone (#1) achieved a 70% improvement in titer over the mini-pool, demonstrating successful clone selection and expansion in BalanCD CHO Growth A.**

Scalability Study

In a separate study, scalability of the BalanCD CHO Growth A and BalanCD CHO Feed 1 platform were evaluated at the 10, 50, and 2000L scale. **The resulting growth and titer profiles prove the successful scale-up of these media** (Figures 4a & b).

	10L	50L	2000L
Agitation: rpm	105	88	110
Agitation: P/V (W/m^3)	38	49	46
Agitation: Tip speed (cm/s)	75	101.5	95
Maximum Aeration: slpm	0.1	0.5	20
Maximum Aeration: VVM	0.01	0.01	0.01

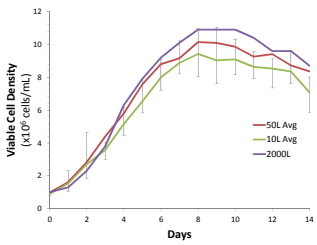


Figure 4a. Growth Profiles at Multiple Scales

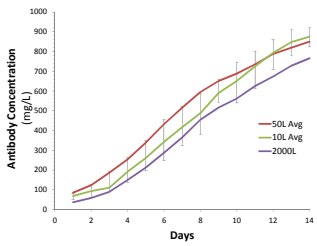


Figure 4b. Titer Profile at Multiple Scales

Summary

1. BalanCD CHO Growth A was designed as a high-performance CHO production medium for batch and fed-batch processes however its ability to support other cell line development activities was untested.
2. This study proved the value of BalanCD CHO Growth A in supporting clonal cell selection and expansion.
3. The scalability of BalanCD CHO Growth A ensures predictable scale-up of your process from bench-scale to full-scale manufacture.
4. Use of a single, chemically-defined medium throughout development ensures a seamless transition from one stage of development to the next.

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