SUPERCELL™ - The Perfect CHO Cell

I. Overview

Titers have drastically improved as a result of a focused effort in developing plasmid technologies, transfection technologies, and media/feed technologies. Over the last 10 years, average antibody titers of clonal cell lines developed by leaders in the bioprocessing industry have improved >5 fold, from an average of 0.5 g/L to 3 g/L. Although substantial, these benefits have had much more limited effects on expression of fusion proteins, bispecifics, antibody fragments, complex glycoproteins, and other difficult to express proteins. The relatively poor metabolic characteristics of CHO cells pose a challenge in scaling up high titer processes and negatively impacts product quality attributes such as glycan, charge variants, aggregation, oxidation and protein fragmentation/cleavage.

These growing challenges that principally affect next generation, complex glycoproteins cannot be solved using the classical methods of CHO cell production optimization. At Celltheon Corporation, we have systematically applied genetic engineering techniques to create a novel CHO cell line we call SUPERCELL™. SUPERCELL™ has been developed to reduce the harmful metabolic byproducts that hinder protein expression and product quality and has also been engineered to improve protein folding of hard to express proteins. Specifically, SUPERCELL™ has been designed to reduce ammonia and lactate by >90% yet maintain high bioproductivity, with clone titers reaching >6-8 g/L with no additional process optimization. Reduced metabolic waste and improved protein folding results in high quality protein with >99% of protein produced by SUPERCELL™ in monomeric form by SE-HPLC.

II. Lactate

Lactate is a difficult to control metabolic byproduct produced by CHO cells during cell growth, thus posing a particularly difficult challenge to long term/continuous culture[1]. Lactate inhibits cell growth and excessive accumulation results in lowered intracellular and extracellular pH[2]. To combat the toxic effects of rising lactate in bioproduction processes, a base is generally added to compensate for pH reductions caused by lactate accumulation. Base addition results in unwanted effects on osmolality, cell viability, and nutrient density. Lactate itself also has a profound effect on product quality, particularly at large scales where metabolic and pH balance issues are exacerbated. To combat these challenges Celltheon engineered SUPERCELL™ to reduce lactate accumulation by >90%, allowing for simple and consistent scaleup of all clones generated from the SUPERCELL™ cell line.

Celltheon's SUPERCELLs™ are the only commercially available engineered CHO cell line for reduced lactate accumulation. Figures 1 and 2 compare the average lactate profiles of SUPERCELL™ derived cell lines with CHO-s derived cell lines. This reduced lactate profile has additional beneficial impacts on protein quality as will be described further in Section IV.

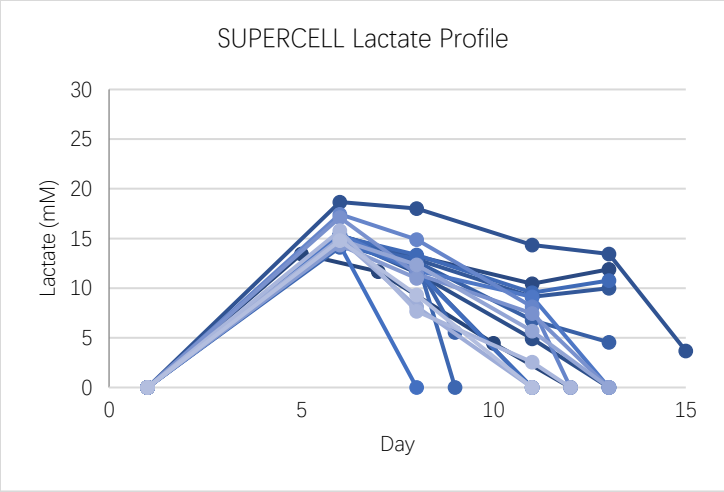


Figure 1. Lactate profile of top 20 clones from a representative subcloning experiment.

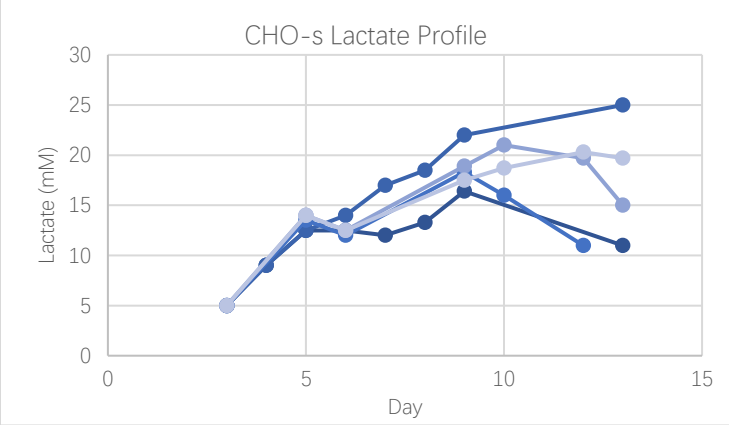


Figure 2. Published data from Zagari et al (2013) showing typical lactate profiles in CHO-s cells.

III. Ammonia

Ammonia is also a harmful metabolic byproduct common to CHO cell culture. Ammonia accumulation decreases cell specific growth rate, increases glucose consumption rates, increases glutamine consumption rates, decreases protein titer, increases intracellular pH, affects protein glycosylation, and reduces maximal cell densities[3,4,5]. To truly alleviate the harmful effects of the CHO cell metabolism on protein production, we simultaneously engineered SUPERCELLs™ to mitigate ammonia in addition to engineering reduced lactate accumulation. We again were able to reduce ammonia levels by >90%.

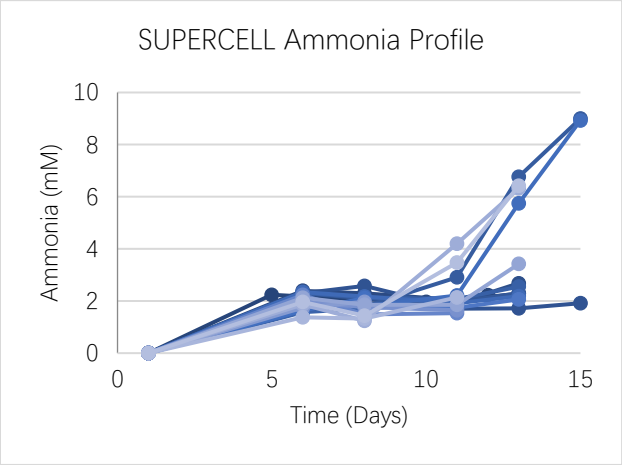


Figure 3. Ammonia profile of 20 top clones from representative subcloning experiment. 80% of SUPERCELL™ clones have ammonia levels 90% lower than competing CHO platforms.

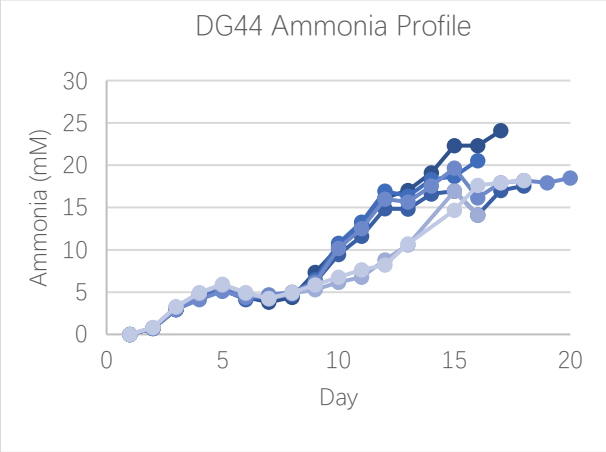


Figure 4. Published data from Reinhart et al (2015) showing typical DG44 ammonia accumulation profiles. CHO-s ammonia accumulation profile is comparable to the DG44 profile shown here.

IV. Product Quality

Proteins produced by SUPERCELL™ are produced at higher quality and titer than comparable CHO cell lines, partially due to the reduction in ammonia and lactate accumulation overtime. SUPERCELL™ has been further engineered to produce such high-quality product for all proteins, regardless of complexity (Figure 6a-f). The additional cell engineering employed to achieve such consistent expression of hard to express proteins is described in further detail in Section 5. After simple one column affinity purification, SUPERCELL™ produced proteins show profiles of >99% monomer (Figure 5 and 6a-f).

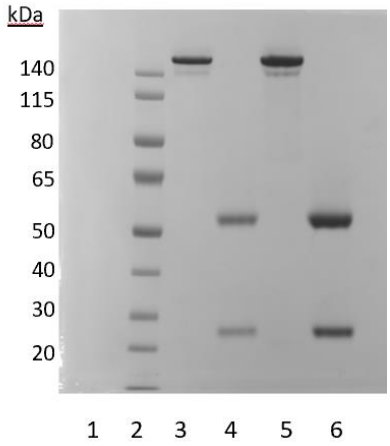


Figure 5. Model antibody run on SDS-PAGE after one column, affinity purification step. Without any additional purification, antibody is already >99% monomer. (Lane 1- blank, Lane 2- Ladder, Lane 3- 2ug nonreduced, Lane 4- 2ug reduced, Lane 5- 5ug nonreduced, Lane 6 – 5ug reduced)

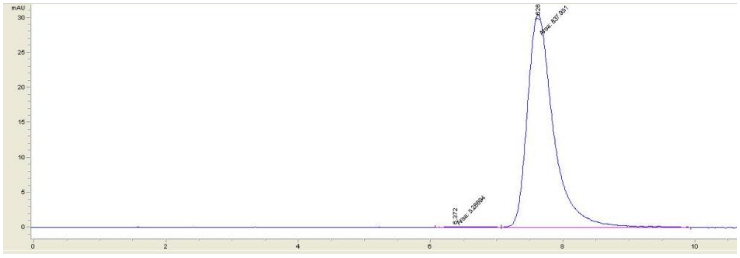
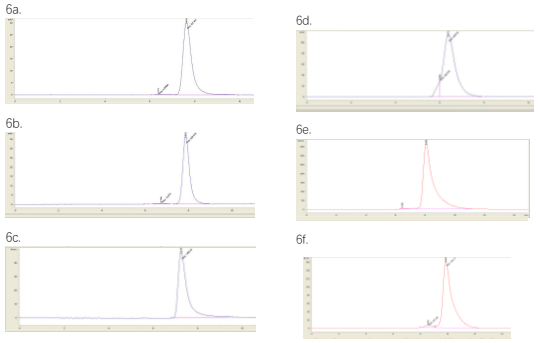


Figure 6a. Same material shown in Figure 5 characterized by SE-HPLC after one column affinity purification. Premonomer- 0.6% & Monomer- 99.4%



Molecule	Fig.	Pre-Main Peak	Main Peak	Post-Main Peak
Conventional mAb	6a	0.6%	99.4%	0%
HEP mAb	6b	1.6%	98.4%	0%
HMW Antibody Fusion	6c	0%	100%	0%
Oligomeric Cytokine + Fc	6d	7.1%	92.9%	0%
HEP Transmembrane Protein + Fc	6e	0.8%	99.2%	0%
HEP Transmembrane Protein	6f	2.7%	97.3%	0%

Figure 6a-f. SEC profiles of 5 hard to express proteins and 1 conventional mAb (6a) produced in SUPERCELL™ after minimal 1 or 2 column purification processes. Aggregation and molecule cleavage is minimal, regardless of protein.

V. HEP Expression

To date there have been no reports of a generalizable cell engineering method that consistently improves titers of the previously described, diverse group of hard to express proteins (HEPs). In a post-genomics era, many have concluded that there is no single genetic engineering solution to the ailment of low titer glycoprotein expression due to the complex, multifaceted nature of protein expression. However, Celltheon has again used its understanding of genetic engineering to push the envelope in the field of protein expression. Celltheon has further engineered the SUPERCELL™ cell line for improved expression of difficult to express proteins.

Using an in-depth knowledge of protein folding and stress pathways, Celltheon engineered SUPERCELL™ to have an enhanced ability to handle ER stress (ERS) and unfolded protein response (UPR). A representative set of hard to express mAbs, fusion proteins, and membrane proteins with reported titers of <100mg/L were expressed in SUPERCELL™ to confirm that improved UPR/ERS response translates to higher HEP titers. The reported titers were achieved 4 weeks from the date of transfection with no additional process development or gene amplification. Titers were assessed in a 2-week fed batch assay, shown in Figure 7. Impressively, multigram titers were achieved with an easy to express, conventional mAb at the pool stage, while hard to express proteins were of relatively comparable titer. The most difficult protein, a transmembrane protein was expressed at 75 mg/L in SUPERCELL™ while a comparable CHO platform was only able to express this protein at 20 mg/L after significant process optimization. All other reported HEPs expressed >2-5 fold higher in SUPERCELL™ as compared to other commercially available and proprietary CHO expression platforms.

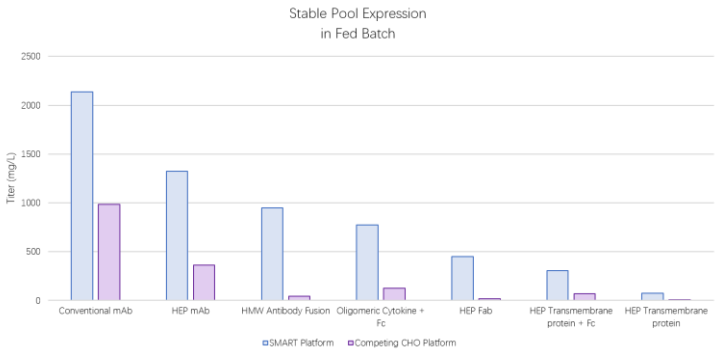


Figure 7. Titers achieved using Celltheon's SUPERCELLs™ for a range of hard to express proteins in a standard 2-week fed batch process as compared to competing CHO expression platforms. No gene amplification or process optimization was performed to achieve the reported titers.

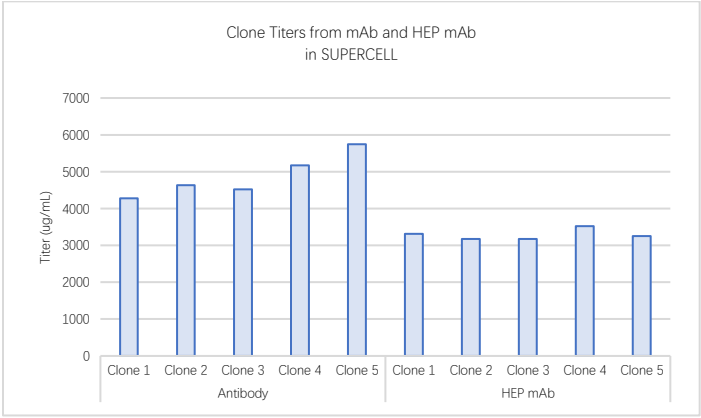


Figure 8. Titers of top 5 clones of the conventional and HEP mAb from Figures 6 and 7 under a simple, unoptimized base process. Expression is consistently high across all clones.

VI. Conclusion

The novel Celltheon SUPERCELL™ cell line has been fully integrated into an off-the-shelf bioproduction platform for stable cell line development. Full cell line

lineage, mycoplasma, virus, and sterility testing has been performed on SUPERCELL™ and derived cell lines, in addition to scalability in a 200L STR bioreactor. Celltheon's SUPERCELL™ is a novel CHO cell line suitable for large scale, economical production of complex glycoproteins, antibody fragments, and other difficult to express proteins. Furthermore, SUPERCELL™ produces easy to express proteins at higher purity and titers than comparable mammalian cell lines. Thus, SUPERCELL™ may be considered as a preferred CHO cell line for fast, simple, high quality production of any recombinant therapeutic protein.

References:

(1) Zagari et al. New Biotechnology Volume 30, Issue 2, 25 January 2013, Pages 238-245.

(2) Pereira et al. Biotechnology Journal, Volume 13, Issue3, March 2018.

(3) P. Chen, S. W. Harcum, Metab. Eng. 2006, 8, 123.

(4) M. Schneider, I. W. Marison, U. Von Stockar, J. Biotechnol. 1996, 46, 161.

(5) D. Reinhart, L. Damjanovic, C. Kaisermayer, R. Kunert, Appl. Microbiol. Biotechnol. 2015, 99, 4645.