Enhanced Growth and Productivity of CHO through rHSA Media Supplementation

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Abstract

As the workhorse of biopharmaceutical production, Chinese Hamster Ovary (CHO) cells are constantly being pushed to achieve greater productivity and higher titers of valuable life saving drugs. A number of methods have been developed toward this end including process control optimization, feeding strategy, genetic engineering, and media supplementation. Media supplementation with fetal bovine serum (FBS) is known to improve cell growth but is nonideal for cGMP manufacturing due to risks of contamination from animal sourced raw materials. Here, we tested two animal-free media supplements: recombinant lactoferrin (rLf), a milk protein with antimicrobial properties that can also aid in iron absorption by cells, and recombinant human serum albumin (rHSA), an anionic serum protein that aids in cell uptake of nutrients. We evaluated their effect on CHO cell growth and product yield.

InVitria Media Supplements

InVitria manufactures recombinant animal-free supplements

Lacromin

- Recombinant lactoferrin
- Iron chelator that aids cellular iron delivery without releasing free radicals
- Replaces more expensive products like transferrin

Cellastim

- Recombinant human serum albumin
- Binds and delivers nutrients to cells and may protect cells from toxins. May also serve as shear protectant
- Replaces FBS

Objective

Previous work has shown benefits from supplement concentrations of 1 g/L or more in plates and t-flasks, as an FBS replacement for serum-dependent lines.

- Study effectiveness of supplements at lower concentrations as cost of using supplements at 1 g/L or more for large scale could be prohibitive.
- Study utility of supplements in scale up experiments of shaker flasks and small bioreactors on CHO cells already adapted to serum-free media

Experimental Design

CHO DP-12 cells producing recombinant human anti IL-8 were grown in batch and fed-batch (one feed on day 7) modes in 30 mL shake flasks. Shake flasks contained either supplemented or unsupplemented media. Prior data showed Lacromin alone had only modest effects on cell growth and productivity (data not shown). Hence, three different media formulations were used for supplemented media.

Batch Shake Flasks	Replicates
Control (unsupplemented)	3
125:125 mg Cellastim:Lacromin	3
250 mg Cellastim	3
500 mg Cellastim	3

Fed-Batch Shake Flasks	Replicates
Control (unsupplemented)	3
125:125 mg Cellastim:Lacromin	3
250 mg Cellastim	3
500 mg Cellastim	3

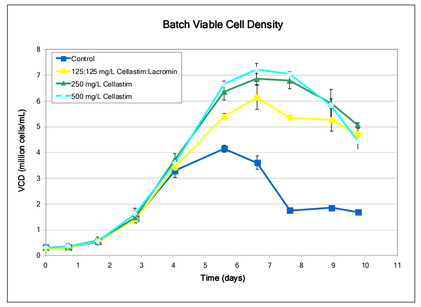
Preliminary bioreactor experiments were also performed using one of the supplemented formulations:

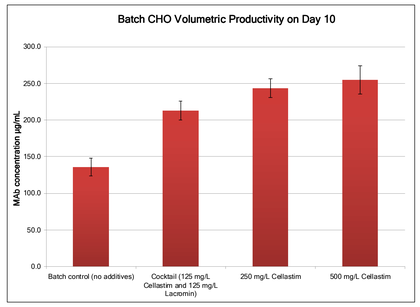
Bioreactor	Replicates
Control	2
250 mg Cellastim	3

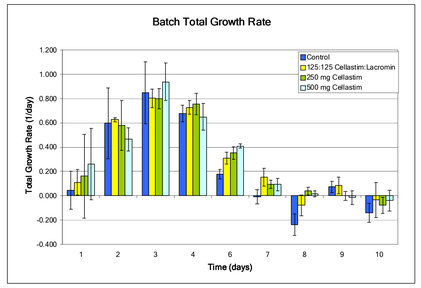
In all culture experiment, viabilities and cell densities were determined daily using a ViCell cell counter (Beckman Coulter). Titers were determined by measuring protein concentration in the culture supernatant using ELISA without any other processing steps.

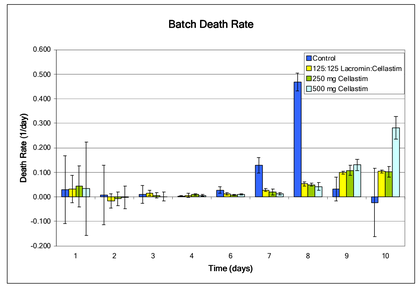
Shake Flask Results

Batch Experiments



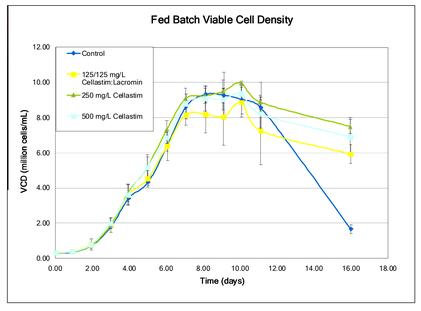


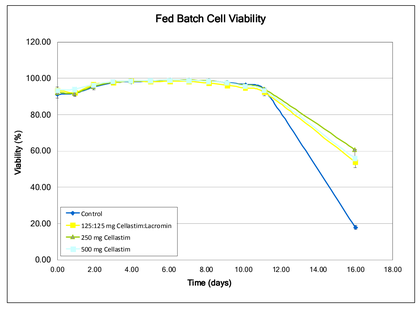


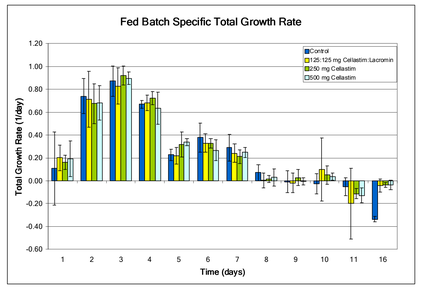


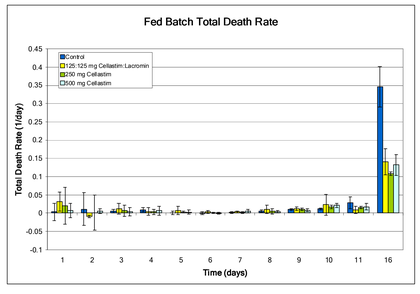
Batch shake flask studies show media supplementation yields higher viable cell densities with delayed death phase compared to control. Error bars represent two standard deviations on either side of mean

Fed-Batch Experiments



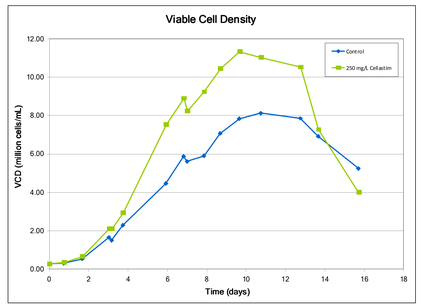


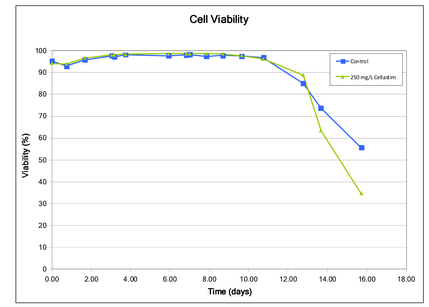


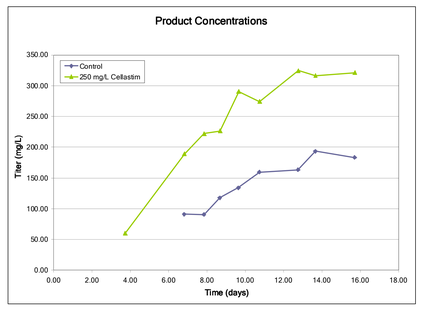


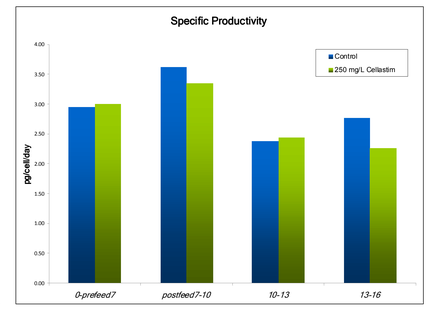
Fed-batch shake flask data also showed supplementation resulted in a delayed death phase compared to control, consistent with batch experiments. Error bars represent two standard deviations on either side of mean

Bioreactor Results



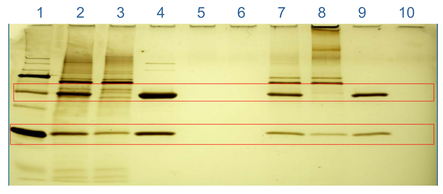






Bioreactor data was consistent with shake flask studies. 250 mg Cellastim was chosen for these experiments because they yielded high VCDs with only marginal improvement seen with higher supplement concentrations. Error bars represent two standard deviations on either side of mean

Effects on Downstream Purification



1	Protein Marker
2	Supernatant from 250 mg/L Cellastim
3	Flow-through fraction (time = 10-11 min) of #2 through Protein A Column
4	Eluent of #2 from Protein A Column
5	Waste Fraction (time point = 59-60 min) of #2 from Protein A Column
6	Supernatant from Protein A Column after column disassembly
7	Supernatant from 125/125 mg/L Cellastim/Lacromin
8	Flow-through fraction (time = 10-11 min) of #7 through Protein A Column
9	Eluent of #7 from Protein A Column
10	Waste Fraction (time point = 59-60 min) of #7 from Protein A Column

Discussion and Conclusions

Our data indicates that media supplementation with rHSA improves CHO cell culture performance. Results indicate that rHSA delays onset of death phase to achieve higher integrated viable cell days while having no effect on specific productivity. The higher titers observed in our studies, therefore, appear to be a result of higher IVCD and not of enhanced specific productivity. Moreover, rHSA did not interfere with protein A purification and may sometimes increase yields in low load scenarios (data not shown).

The manufacturer recommended concentration is 1 g/L; however, we saw higher cell densities and titers by adding even 250 mg/L of rHSA. We found that higher concentrations may yield better performance, although not in a linear fashion. Ideal concentrations, therefore, will need to be evaluated for individual processes and will depend on cell line and product attributes.

Future work will need to evaluate effectiveness of rHSA supplementation on higher volume bioreactor cultures as well as on other cell lines.