Optimization of Cell Culture Media and Culture Conditions for HEK293 for Improved Cell Growth and Reduced Cell Clumping for the Effective Production of Viral Vectors

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Overview

- **Background:** In HEK293 bioprocess runs with different commercially available HEK293 cell culture media, we recognized that in certain media HEK 293 cell line used (ATCC HEK-293 CRL-1573) formed cell clumping. With REBEL analysis, this behavior was noticed to correlate with cell culture media choline levels: low-level choline medium had severe cell clumping.
- **Project Goals:** We aimed to investigate the correlation of choline and cell cluming, with series of experiments to showcase the effects of different cell culture media, added choline and other media components.
- **Summary of results:** Choline addition in shake flask scale did not reduce the size of cell clusters or appear to affect amino acid metabolism. However, the overall number of cell clumping were reduced with choline addition, while maintaining viable cell density.
- **This study demonstrate the power of at-line cell culture analyzers such as the REBEL to gain process understanding quickly to adjust media components for improved cell culture performance.**

Initial study and experimental set-up and findings

Methods: HEK293 cells were cultured for 6 days in a 3L bioreactors (Yokogawa BR1000) in either FreeStyle F17 Expression Medium (Thermo Fisher Scientific), CDM4HEK293 medium (Cytiva), or BalanCD HEK293 medium (FujiFilm/IrvineScientific). Samples were analyzed daily on the REBEL and BioProfile FLEX II.

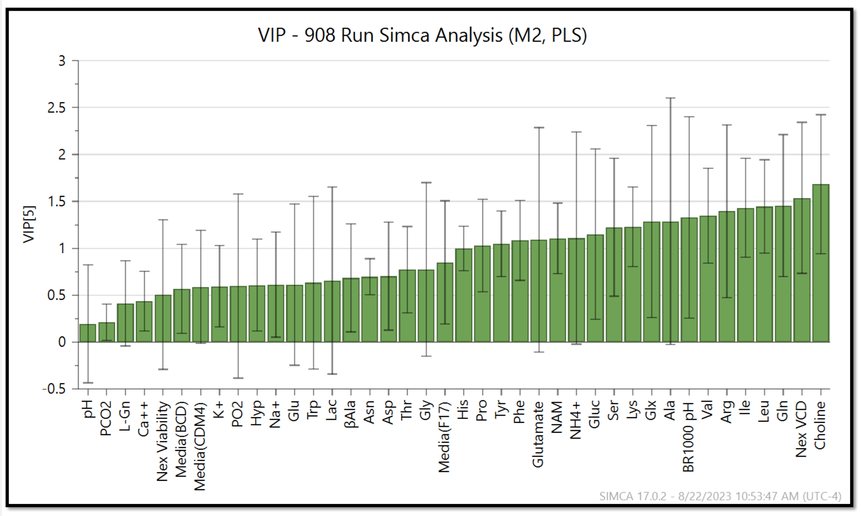


Figure 1. ***Variable Importance Plot using SIMCA*** *(Sartorius), which ranks variables based on importance in the PLS model for describing the batch evolution, suggested choline was present at significantly different concentrations in the 3 different media and that lower levels of choline correlated with increased cell clumping.*

Results: In this bioreactor experiment, the three cell culture media used showed different levels of cell clumping. Media component levels assessed by REBEL showed that choline was present at significantly different concentrations in the 3 media and was depleted in one medium by day 4. SIMCA analysis showed a correlation between lower levels of choline (the most significant difference between the cell culture media) and increased cell clumping.

Main study - Methods

Media	Choline Strategy
Viral Production + 5 mM GlutaMAX	No choline addition
Viral Production + 5 mM GlutaMAX	Choline added on Day 0 to target concentration of 1.2 mL
FreeStyle F17 + 5 mM GlutaMAX	No Choline Addition
FreeStyle F17 + 5 mM GlutaMAX	Choline added on Day 0 to target concentration of 1.2 mL

Both media (Viral Production medium and FreeStyle F17) are products of Thermo Fisher Scientific

Two different media were used to culture HEK-293 CRL-1573 (ATCC). Culture performance was assessed by cell growth, viability, and visual assessment of cell clumping using image analysis. REBEL at-line analyzer was used to analyze media component levels and component consumption levels from spent media.

The REBEL at-line cell culture media analyzer: Actionable information of your bioprocess at the point of need

AA concentrations were measured from the fresh and spent media using the REBEL (908 Devices Inc.), a capillary-electrophoresis (CE) - mass spectrometry (MS) -based device for AA analysis (**Fig 2**). HEK293 were removed via centrifugation / 400xg for 5 minutes, and the cell-free supernatant was diluted 10x and 200x with manufacturer-provided diluent. Automated quantitation of AAs for each sample was achieved using embedded calibrations. Two biological replicates of each sample were analyzed in triplicate using the REBEL. The nutrient compositions of the spent media were analyzed and plotted using 908 Devices Add-in for JMP software.

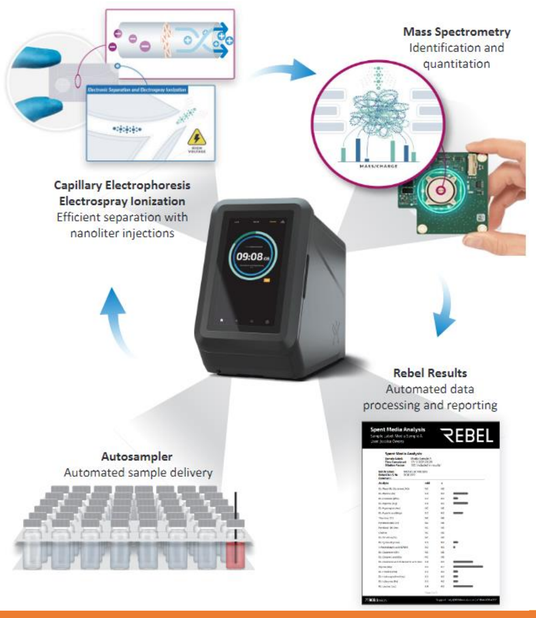


Figure 2

REBEL Spent Media Analysis Kit

- Minimal sample requirement as low as 10 µL
- Simple sample prep: spin or filter and dilute
- Integrated analyzer includes autosampler, separation, detection, analysis and reporting
- Analysis run-time ~10 min per sample
- Consumable kit optimized for 200 analyses

REBEL analyte panel

Amino Acids						
Alanine	Asparagine	Glutamic Acid	Histidine	Lysine	Proline	Tryptophan
Alanyl-Glutamine	Aspartic Acid	Glutamine	Isoleucine	Methionine	Serine	Tyrosine
Arginine	Cystine	Glycine	Leucine	Phenylalanine	Threonine	Valine
Vitamins etc.						
Choline	Nicotinamide	Pyridoxal	Pyridoxine	Thiamine	Betaine	
Amines						
ß-Alanine	Citrulline	GABA	Hydroxy-proline	Methyl-Histidine	Sarcosine	

Choline spent media trends

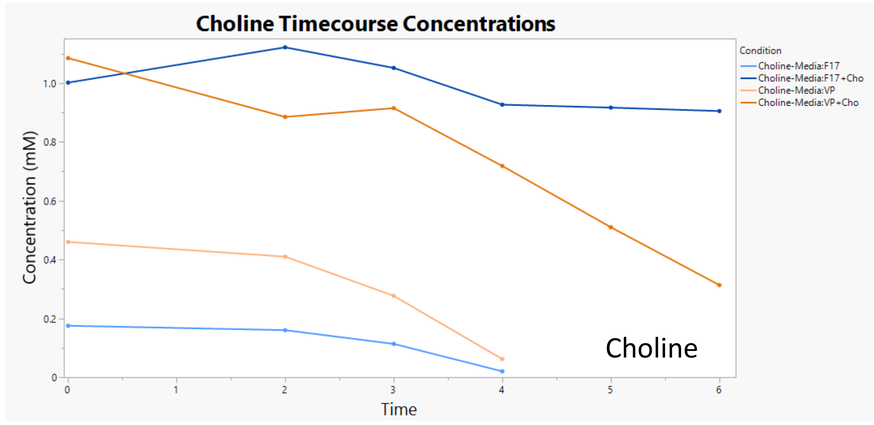
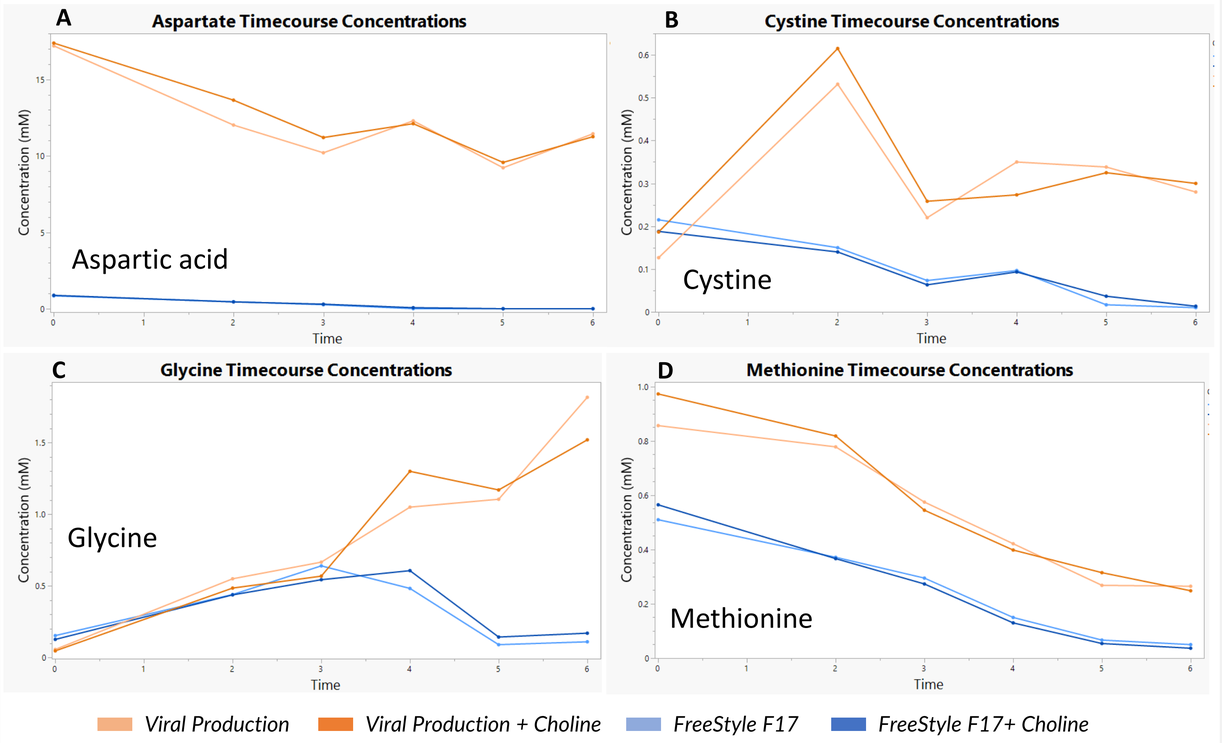


Figure 3. *Time course choline REBEL data for the four culture conditions tested: two different media (Viral Production or FreeStyle F17), with and without choline addition.*

Viral Production
Viral Production + Choline
FreeStyle F17
FreeStyle F17+ Choline

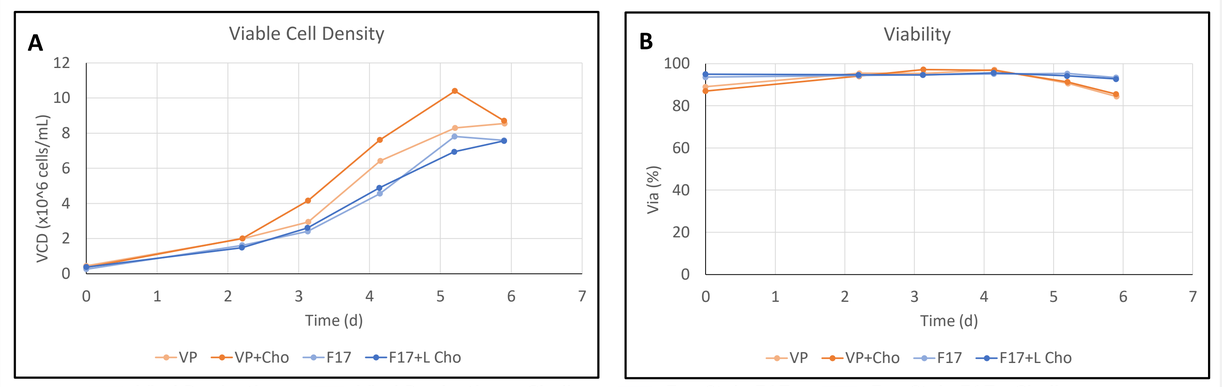
Choline addition impact on amino acid metabolism



Viral Production *Viral Production + Choline* *FreeStyle F17* *FreeStyle F17+ Choline*

Figure 4. *A) Aspartate, B) Cystine, C) Glycine, and D) Methionine concentration profiles for the four culture conditions tested: two different media (Viral Production or FreeStyle F17), with and without choline addition. 21 analytes were measured using the REBEL. No statistically significant differences in nutrient metabolism were observed upon addition of choline, however differences in metabolism between media was observed.*

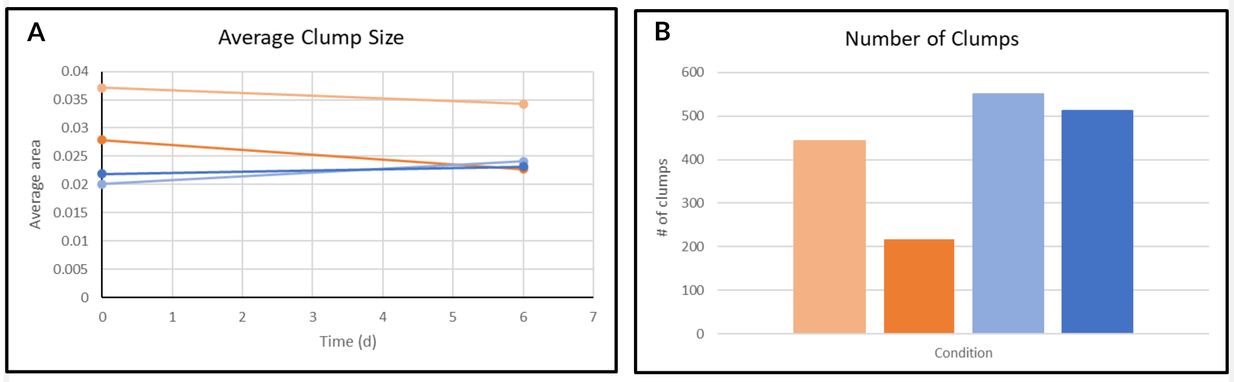
Choline addition impact on growth and viability



Viral Production *Viral Production + Choline* *FreeStyle F17* *FreeStyle F17+ Choline*

Figure 5. *A) Viable Cell Density and B) Viability profiles for the four culture conditions tested: two different media (Viral Production or FreeStyle F17), with and without choline addition. Slightly higher VCD was observed when choline was added to the Viral Production medium.*

Cell Clumping Analysis



Viral Production *Viral Production + Choline* *FreeStyle F17* *FreeStyle F17+ Choline*

Figure 6. ***A)*** *Average clump area and* ***B)*** *total number of clumps for the four culture conditions tested: two different media (Viral Production or FreeStyle F17), with or without choline addition. The clump size does not significantly differ between the conditions; however, the number of clumps is reduced with choline addition to Viral Production medium.*

Conclusions

REBEL was able to determine analyte concentrations in multiple media with different degrees of observed clumping. With REBEL input and SIMCA analysis, choline was determined to be present at statistically different concentrations. Shake flask experiments were conducted to further evaluate the impact of choline addition on the extend of cell clumping. Though the average size of the clumps did not decrease with choline additions, the number of clumps in the Viral Production medium decreased in the presence of higher choline concentrations with slight increased peak VCD. This demonstrates that in some media, choline additions could be an effective way to decrease the extent of clumping in HEKT293 cultures. More experiments are ongoing to further establish this observation.