CASE STUDY

Animal component free (ACF) media development for cell therapies

ACF media achieved comparable performance to serum containing

CHALLENGE

To remove serum from media in a cost-effective manner without affecting cell performance.

PROCESS DEVELOPED

A fully automated and high-throughput pipeline for development and screening of 1000s of formulations.

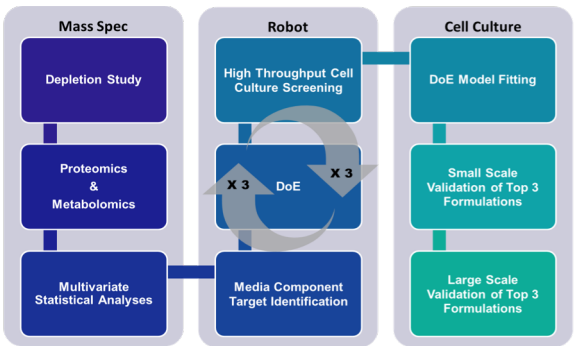
OUTCOMES

• ACF formulation in < eight months
• Comparable performance to serum containing media

INTRODUCTION

In the extremely fast-paced era of cell and gene therapies, the use of chemically defined (CD), ACF cell culture media is strongly encouraged by the regulators. However, the commercially available options are very limited or prohibitively expensive when available. Therefore, the leaders of the field are required to develop their own ACF media. At CCRM, we have developed a high-throughput, fully automated pipeline that allows us to formulate ACF and CD cell culture media fully tailored to the cell or gene therapy of interest.

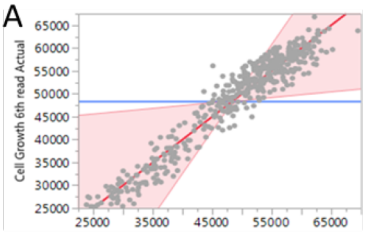
METHODS



RESULTS

The performance of a prototype basal ACF medium was initially evaluated demonstrating a ≈50% efficiency when compared to the serum supplemented control. A time series sampling strategy of the supernatant of batch, serum supplemented cell cultures was implemented to identify proteins and metabolites that correlate with cell growth.

A



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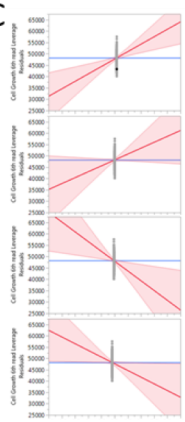

B

Summary of Fit	
RSquare	0.9312
RSquare Adj	0.4303
Root Mean Square Error	7856.18
Mean of Response	48426.71
Observations	457

Figure 1: *A) Reduced Multiple Regression Model B) Summary of Model Fit C) Standard Least Square Plots of 2 Positive & 2 Negative.*

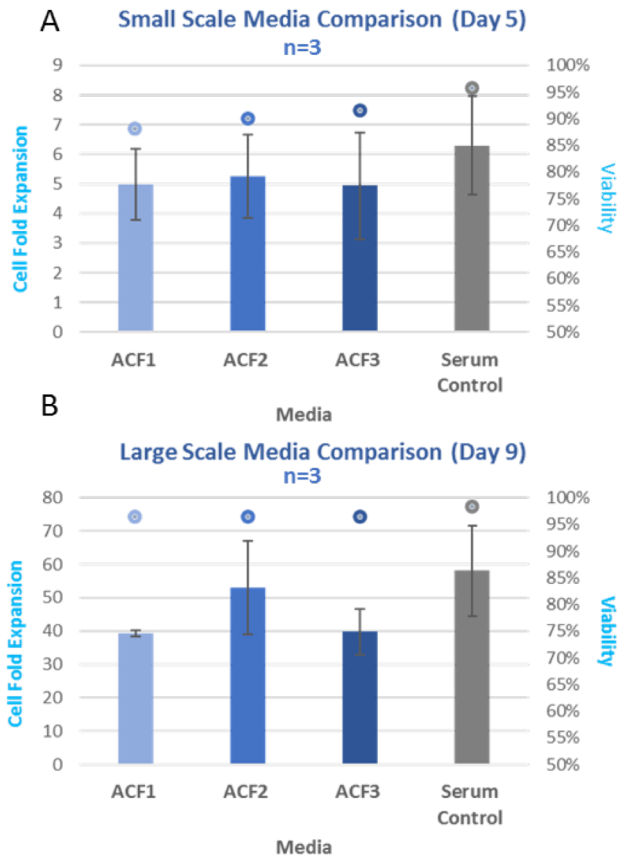

Figure 2: *Head-to-head comparison of cell fold expansion at A) small scale and static conditions on day 5 and B) large scale/bioreactor after nine days.*

Upon a mass spectrometry-based interrogation of the supernatants over time, 100s of molecular features were identified as potential candidates to form an ACF serum replacement. Following a thorough literature mining, 28 of them (proteins & metabolites) were promoted for testing. Based on a custom, 2 level, fractional factorial DoE model, we performed a side by side comparison of 1,910 individual media formulations. The cells were grown for seven days and cell counts were taken twice a day. Fitting of a multiple regression model (Figure 1) helped us minimize the solution space and, three iterations later, we had identified six media components that, when added to the media, could recapitulate serum supplementation. Three formulations were promoted as the top candidates. Small- and large-scale head-to-head comparisons across three donors showed that our ACF formulations matched the performance of the serum supplemented medium (Figure 2).

DISCUSSION

CCRM's cell and gene therapy media development pipeline described here (Figure 3) is, to our knowledge, one of a kind and is built upon three major pillars: 1) a group of scientists with rare skill sets, 2) exotic instrumentation designed explicitly to support this program and 3) a set of commercially available, as well as in-house designed, computer software packages.

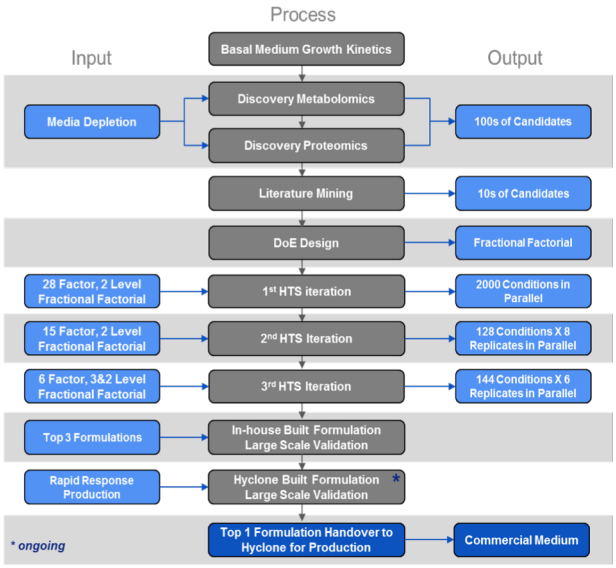

Figure 3: *CCRM's media development pipeline.*

SUMMARY

We used mass spectrometry based, multi-omics analytics and fully automated, high-throughput cell culture screening assays to develop an ACF medium formulation that performs as well as the serum supplemented control medium. To our knowledge, this is the first time this approach has been implemented successfully for media development.

REFERENCES

1. A robust and reproducible animal serum-free culture method for clinical-grade bone marrow-derived mesenchymal stromal cells. Laitinen A. et al. *Cytotechnology*. 2016 Aug;68(4):891-906.

2. Animal- cell culture media: History, characteristics, and current issues. Yao T. et al. *Reprod Med Biol*. 2017 Mar 21;16(2):99-117.

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