ANIMAL COMPONENT FREE CELL CULTURE MEDIA DEVELOPMENT: THE APPROACH FROM THE NORTH

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Cell therapies have been recognized as a promising treatment for various diseases including a wide range of malignancies. Hundreds of studies investigating the efficacy of cell infusions in terms of response level, duration and survival are currently in Phase1-2 clinical trials. Several research groups have developed successful protocols for the ex vivo expansion of therapeutic progenitor cells. However, upon the establishment of good manufacturing practice (GMP) standards, most established protocols are problematic mainly due to the presence of xenogeneic or human-derived components which introduce another level of product variability and regulatory complexity. At CCRM, we established a top down process for the development of cGMP-compliant, animal component free (ACF) cell culture media using a combination of mass spectrometry based multi-omics media interrogation and a DOE based, fully automated, high-throughput cell culture screening. Briefly, our media development pipeline can be described in three phases. Firstly, we define the solution space by interrogating a complete, fully supportive, serum-containing cell medium. Mass spectrometry based, open profile proteomics and metabolomics analyses is what allows us to identify the components that are available to the cells. We then reduce the solution space by utilizing the same multi-omics approach on depleting media during a time series interrogation of batch cell cultures. Based on the concentration fluctuations of the different media components, we identify the ones that are possibly correlated with key outcomes, such as cell growth. Finally, once we identify the correlated components, we design a multifactorial DOE based high-throughput screening (HTS) experiment that allows us to pick the critical media components and optimise their concentrations using a cutting edge, entirely enclosed, liquid handling robot. A few HTS iterations are usually necessary for the identification of the optimal medium formulation. For the validation of our approach we developed an in-house ACF formulation able to support the ex vivo expansion of a primary cell type at a similar level as the serum containing gold standard in the field. During the first phase of the media development we identified 1000s of unique media components that we utilised to construct an internal library for the interrogation of the time series multi-omics data. In the second phase and during the multivariate statistical analyses, multiple Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) models indicated that a total of 48 identified components were correlated with cell growth. Finally, after an exhaustive literature mining, 28 components were promoted as potential key factors that could support cell growth in the absence of serum. Based on a custom 2 level, 28 factor, fractional factorial DOE model, we designed and ran a fully automated, high-throughput cell culture screening assay which allowed us to monitor the growth of over 2000 cell cultures in parallel. Using the total cell number as the model's response (Figure 1), we promoted 15 factors for a second HTS iteration upon which, 6 factors had a significantly positive effect and were promoted for a final HTS iteration. Based on a mixed, 2-3 level, 6 factor, fractional factorial design we were able to optimize the concentrations of the final 6 media components. Three variations of the top performing formulation were chosen to be validated across 5 donors and up to a 1L scale bioreactor. Our fully chemically defined, ACF media formulation was consistently able to support cell growth at a comparable level to serum supplemented media while maintaining the same cell phenotype. To our knowledge, this is the first time that a top-down, multi-omics media interrogation is combined with high level automation and cell culture HTS towards the media development for a cell therapy.

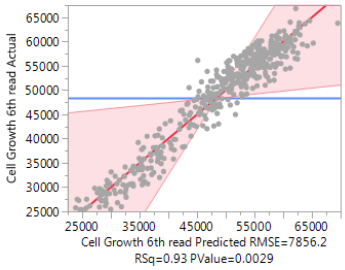

Figure 1 – Multiple Regression Model Fit on Growth Data of 1910 Cell Cultures