PROCESS DEVELOPMENT FOR IMPROVED CAR-T PRODUCTION UTILIZING AN AUTOMATED PERFUSION STIRRED-TANK BIOREACTOR

Tiffany Hood, Department of Biochemical Engineering, University College London, United Kingdom
tiffany.hood.19@ucl.ac.uk
Fern Slingby, Sartorius Stedim SAS, United Kingdom
Winfred Geis, Sartorius Stedim SAS, United Kingdom
Viktor Sander, Sartorius Stedim SAS, United Kingdom
Nicola Bevan, Sartorius Stedim SAS, United Kingdom
Quentin Vicard, Sartorius Stedim SAS, United Kingdom
Qasim A. Rafiq, Department of Biochemical Engineering, University College London, United Kingdom

Key words: CAR-T cell manufacturing, stirred tank bioreactor, perfusion culture, CAR-T phenotype

Ex vivo genetically-modified cellular immunotherapies, such as chimeric antigen receptor T-cells (CAR-T), have generated significant clinical and commercial outcomes due to their unparalleled response rates against refractory/relapsed blood cancers. However, the development and manufacture of these advanced therapies face a number of translational bottlenecks that must be addressed to ensure long-term commercial viability.

The cost and variability associated with these personalized advanced therapies presents a critical manufacturing and translational challenge. This work demonstrates the importance of determining critical manufacturing process parameters and incorporating automation into the process. The work builds off of previous proof of concept work completed in the group for the production of CAR-T cells in automated, stirred tank ambr250® bioreactors. These experiments utilized concepts of quality by design and design of experiments (DoE) to systematically determine the impact of process parameters on CAR-T production to further improve the initial proof of concept studies.

This work aimed to identify critical process parameters to improve overall CAR-T yield while maintaining cell quality. Based on a small-scale DoE study, it was found seed train time has a significant impact on CAR-T quality. This was then scaled up to the ambr250® bioreactor process. By decreasing the seed train time, the final cell yield was more than doubled in the stirred tank bioreactor (*Figure 1*). Additionally, the differentiation of the cells and the expression of exhaustion markers were significantly reduced. Therefore, both the yield and cell quality improved by implementing a shorter seed train.

The next stage of the work aimed to implement perfusion into the ambr250® process. Initial proof of concept studies showed CAR-T yield increased by about 50% by incorporating perfusion. Additionally, even with the increased cell densities, high cell quality in regards to differentiation, exhaustion marker expression, killing efficiency, and cytokine production was maintained. Further studies were then completed to optimize the perfusion process to maximize the yield benefit. To do this, a DoE of perfusion parameters was completed in the ambr250® bioreactor system. An optimized perfusion process was then identified that further doubled the final cell yield while still maintaining cell quality.

The combined process improvements in this work led to a 5X cell yield increase with significantly improved cell quality in regards to cell differentiation and exhaustion marker expression. This demonstrates the strong need for systematic process development and incorporation of automation for improved CAR-T production.

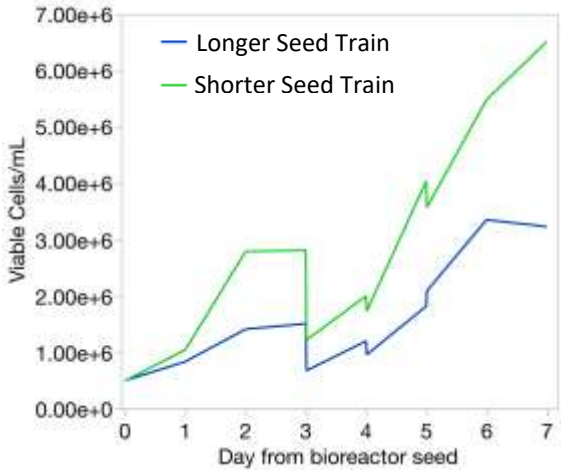

Figure 1 Shorter seed train improved CAR-T cell density in the ambr250®. Growth curves for the viable cell density over time in the ambr250® bioreactor comparing a longer versus shorter seed train prior to the bioreactor culture.