PROCESS DEVELOPENT OF A SERUM-FREE AND SCALABLE LENTIVIRAL VECTOR MANUFACTURING PLATFORM FOR CELLULAR IMMUNOTHERAPIES

Carme Ripoll Fiol, Department of Biochemical Engineering, University College London, United Kingdom
carme.fiol.19@ucl.ac.uk
Dale Stibbs, Department of Biochemical Engineering, University College London, United Kingdom
John Welsh, Department of R&D, Pall Life Sciences
Qasim Rafiq, Department of Biochemical Engineering, University College London, United Kingdom

Key Words: iCELLis bioreactor, lentiviral vectors, serum-free production, scalable process

Lentiviral vectors (LV) have played a critical role in gene delivery for *ex vivo* gene-modified cell therapies, including T cells and NK cells; however, a large-scale manufacturing process is needed to cope with the increasing demand from the cell and gene therapy sector. Single-use fixed-bed bioreactors, such as the iCELLis bioreactor, provide a promising automated, scalable platform for the generation of large volumes of GMP-grade LV. Fixed-bed bioreactors have already been shown to generate high LV titers; nevertheless, most of the work currently undertaken in the field relies on serum-containing medium formulations. This is largely because to date serum-containing media produces higher viral titer yields and an increased LV stability during the down-stream processing of the product. However, relying on serum-based processes adds significant supply chain and manufacturing risk to the process.

This work focuses on the development of a scalable serum-free manufacturing platform for the production of 3rd generation lentivirus. Using serum-free will reduce process cost, decrease batch-to-batch variability and avoid the presence of animal-derived components in the final product. In this work, the LV production relies on the transient transfection of human embryonic kidney (HEK) 293T cells, cultured in serum-free medium for the whole duration of the culture and the production, including the viral supernatant collection 48h post-transfection.

To characterize the process and maximize LV titers, some initial small-scale experiments were performed. The experimental parameters that were optimized initially were cell seeding density on the inoculation day, total plasmid DNA used and day of transfection. The results from these studies showed that with constant seeding density and total plasmid DNA for the transfection, 72h was the optimal time point to transfect the cells. LV titers achieved with the serum-free medium were above 2.8x10^6 TU/cm^2 (*Figure 1*).

In an effort to further increase the yields in a serum-free process, supplementary small-scale experiments were performed to explore different chemically defined media and the addition of some medium additives post-transfection. The chemically defined medium and serum-free optimized process was then transferred to the iCELLis Nano bioreactor, where a 1L production was completed under serum-free conditions. The process was further optimized to decrease seeding density to as low as 5x10^3 cells/cm^2, as seeding fewer cells at inoculation presents significant advantages when moving to larger-scale systems.

The work presented shows the feasibility of generating LV in serum-free medium in the iCELLis Nano bioreactor, an effective scale-down manufacturing platform which can be scaled up to the larger iCELLis 500 bioreactor. The outcome of this work will support the development of a scalable and consistent process for the production of high LV titers without the presence of animal-derived components, which together with the optimized total plasmid DNA for transfection, will result in a significant cost reduction.

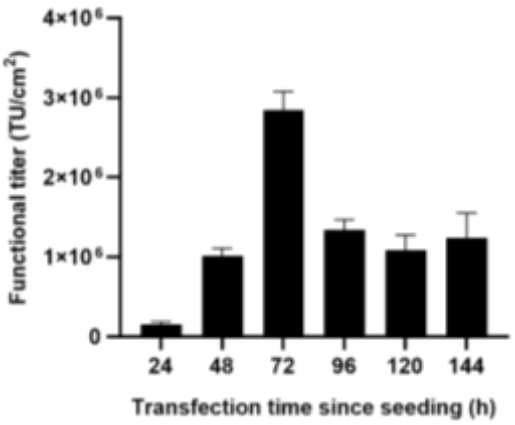

Figure 1 - Functional titers (TU/cm^2) obtained in small-scale experiments when the seeding density and the total plasmid DNA remained constant. Data shows mean ± SD for n=3 technical replicates.