MEDIUM- AND HIGH-CELL-DENSITY PRODUCTION OF ADENO-ASSOCIATED VIRUS SEROTYPE 6 AND THE MITIGATION OF CELL DENSITY EFFECT VIA MEDIUM SUPPLEMENTATION

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Cellular immunotherapy is a promising approach for the treatment of a variety of neoplastic diseases, especially lymphomas and leukemias. Several gene transfer platforms have been developed and are available to genetically modify T cells. In most cases, the transgene is inserted into the genome of the cell in a semi-random fashion, which raises safety concerns. On the other hand, adeno-associated virus (AAV) can be effectively used to transduce target cells with a transgene that remains in an episomal state. No case of human disease has been reported following the use of non-integrating vectors like AAV. Furthermore, AAVs are stable for long-term storage at -80°C, making them especially interesting as a commercial product. Among the different AAV serotypes, serotype 6 (AAV6) is well-known for its efficiency to transduce T cells. However, the multiplicity of infection (MOI) needed can reach 10^6 viral genomes (VG) per cell, meaning that the large-scale production of AAV6 is a challenge that needs to be addressed.

In this study, triple transient transfection of suspension HEK293SF cells at medium and high cell densities was evaluated for the production of AAV6. First, 4 million viable cells per mL, in batch mode, were transfected with plasmids (pAdDeltaF6, pRep2Cap6, and pAAV-CAG-GFP) on either a volumetric basis (VB - 1 µg/mL) or cell basis (CB - 1 µg/10^6 cells) and the viral titer (VG/mL) was assessed every 24 hours. At 48 hours post-transfection (hpt), the titer of the medium-cell-density (MCD) production with DNA delivered on a cell basis was 3.8 times the control (1 × 10^6 cells/mL), reaching 8.6 × 10^9 VG/mL, an almost linear increase in correlation with cell density. There was also an increase in functional titer (Enhanced Transducing Units (ETU)/ml) of up to 3.9 times depending on the harvest time. The delivery of DNA on a volumetric basis resulted in 4.9 × 10^9 VG/mL (a 2.2-fold increase from control). To achieve a higher cell density before transfection, a perfusion-like mode of operation was devised in 50-mL TubeSpin bioreactors, with a working volume of 10 mL and medium exchange of 1 vessel volume per day (VVD) both before and after transfection. Cells were transfected at a density of 10 × 10^6 cells/mL, with plasmid DNA delivered on a cell basis, and viral titer was assessed every 24 hours. AAV6 yield peaked at 72 hpt. The high-cell-density (HCD) perfusion-like mode of operation resulted in 9.3 × 10^9 VG/mL, a 4.6-fold increase when compared to the low-cell-density (LCD) batch control. Even though the yield was higher, this result shows that the titer does not increase linearly with the increase in cell density at the time of transfection. Looking at the cell-specific virus yield, this difference between LCD batch and HCD perfusion becomes even more evident. At 72 hpt, the batch control yielded 1.8 times more than the HCD perfusion. To try to alleviate this Cell Density Effect (CDE), medium supplementation was evaluated. As a result, when the HCD culture was done with a medium supplemented with 15% Cell Boost 5, the CDE was alleviated. The cell-specific titer was similar to the LCD control, resulting in an almost 10-fold increase in viral genomes (from 2.02 × 10^9 to 1.49 × 10^{10} VG/mL). There was an increase in functional titer when HCD production was conducted with supplemented media. Cell-specific functional titer also increased depending on the supplementation used, but it was 2 times inferior when compared to the LCD control, in all cases. This demonstrates the importance of quantifying both viral genome titer and functional titer of the produced virus during bioprocess optimization.

Our results show that cell-specific production is maintained at medium cell density when plasmid DNA is delivered on a cell basis. Medium supplementation alleviates the Cell Density Effect and restores the cell-specific yield of viral genomes, in high-cell-density production. The functional titer is susceptible to the Cell Density Effect and further research is necessary to understand the limitations imposed by high cell densities in functional AAV production.