AN EFFICIENT MICROCARRIER BASED ADENO-ASSOCIATED VIRUS PRODUCTION METHOD

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Adeno-associated viruses (AAV) are reported to have a great potential for gene therapy. However, a major bottleneck for these kinds of therapies are efficient methods for viral production.

The purpose of the present study was to explore how a production-cell culture attached to microcarriers in a stirred tank bioreactor could provide a solution for efficient AAV production. Microcarriers can provide an accelerated process development platform where an already high product yielding adherent cell clone can be directly transitioned to suspension production. Higher cell specific AAV yields are often reported for adherent cell systems compared to cells in suspension, and microcarrier-based culturing is well established for anchored cells in larger scale. Taken together, using microcarriers as an AAV production platform has the potential to increase cell specific yields and increase volumetric productivity thus lowering the total cost of goods for AAV based therapies.

In the present study, HEK293T cells were grown and expanded in suspension, offering the flexibility this type of operation provides. The cells were then anchored onto cytodex microcarriers in order to proceed with a tri-plasmid transient transfection for AAV expression. The process was developed with a scaled-up commercial application in a bioreactor in mind, and the culture was studied in terms of limitations brought on by shear force and oxygenation in a stirred tank vessel. The HEK293T cells when attached to the microcarriers were able to withstand an agitation environment corresponding to a Kolmogorov eddy length of 2/3 of the diameter of the microcarrier. Production of up to 10e11 capsids/mL was observed with a 10x higher cell specific yield compared to suspension cells. Furthermore, amenability to a continuous process was studied and a continuous microcarrier inoculation system was developed. The present investigation provided a proof-of-concept of a process based on microcarriers, in view of a readily scalable solution for the production of AAV at commercial scale.