THE EFFECT OF CELL DENSITY ON THE PLASMID UTILIZATION FOR THE PRODUCTION OF ADENO-ASSOCIATED VIRUS VIA THE TRIPLE-TRANSFECTION METHOD

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Adeno-associated virus (AAV) is one of the leading vectors in the field of gene therapy with several approved treatments currently on the market. Production of AAV via the triple-transfection method is highly flexible with the capsid and gene-of-interest (GOI) being readily exchanged, facilitating rapid development. This method, however requires large quantities of high-grade plasmid DNA. To facilitate scale up and reduce the cost-of-goods, the utilization of plasmid DNA should be increased. We have previously devolved a process using adherent HEK293T cells on microcarriers to take advantage of the higher cell specific productivity seen in adherent cells and the scalability of microcarriers[1]. However, this process was limited in the maximum cell density achievable due to several reasons, primarily shear stress. This limitation is not present in suspension cells with cell densities in excess of 80 x 10^6 cells/mL being achieved[2]. The increase in cell density and shift to a perfusion-based process requires the screening of a large number of conditions for transfection, necessitating a scale down model.

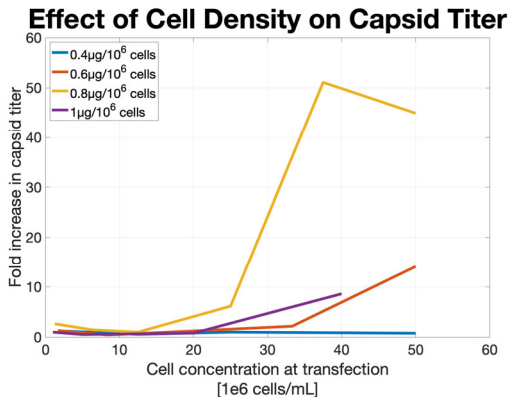

Figure 1: The effect of cell density at the time of transfection on the relative capsid titer of rAAV9 production through the triple transfection method

In this study a process intensification strategy was used to increase the transfection efficiency and product titer of AAV9 in suspension HEK293T cells, with a view for continuous production. A scale down model system, based on maximum power input, was used together with pseudo-perfusion to closely mimic the conditions in a stirred tank bioreactor in continuous production. Cell specific rates of glucose, glutamine, lactate, LDH, and ammonia were measured during cultivations. The AAV9 vector with green fluorescent protein (GFP) as the GOI was produced in suspension HEK293T cells via the triple-transfection method with polyethylenimine (PEI) as the transfection reagent. The impact of key transfection parameters on transfection efficiency and AAV9 titer was evaluated using flow cytometry, cell-based assays, ELISA, and qPCR. The scale down model showed a high degree of similarity to the stirred tank bioreactor with similar growth and cell specific rates in the measured metabolites being observed. Increasing cell density while maintaining a constant cell-to-DNA ratio greatly improved transfection efficiency and AAV9 production. The results of this study show that intensification of the transfection step in AAV production will increase the efficiency to which the plasmid DNA is used, thus lowering the cost-of-goods.

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