MONITORING THE PRODUCTION OF AAV VECTORS IN INSECT CELLS BY FLUORESCENCE SPECTROSCOPY

Daniel AM Pais, ITQB-NOVA - Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa ; iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
dpais@ibet.pt
Rui MC Portela, ITQB-NOVA - Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa ; iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Inês A Isidro, ITQB-NOVA - Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa ; iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Paula M Alves, ITQB-NOVA - Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa ; iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

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Adeno-associated viruses (AAV) are among the most promising viral vectors for gene therapy, since they can transduce non-dividing cells from several tissues while maintaining a long-term gene expression. Besides, AAVs possess low immunogenicity compared to other viral vectors, and are physically resistant, which makes them resilient to industrial manufacturing conditions, long-term storage, and in vivo administration. One of the systems available for large scale production of AAVs is the insect cell-baculovirus expression vector system (IC-BEVS). Insect cells grow in suspension to high cell densities with modest growth requirements and without the need of serum supplementation. Consequently, scaling up the production in order to achieve the large number of AAV needed for clinical trials is more straight-forward than with transfection-based systems.
However, methods for online monitoring of AAV production are still lacking. Such methods would allow determination of the best time of harvest in real-time, thus allowing recovery of AAV as soon as its concentration medium was higher.

Here we apply Fluorescence Spectroscopy to baculovirus-infected insect cell cultures producing adeno-associated virus vectors, correlating the spectra to critical process parameters like cell concentration, viability and AAV concentration. Sf9 cells were co-infected with two baculovirus (expressing AAV rep and cap and a CMV-GFP transgene) at low or high multiplicities of infection (MOI), and the culture was followed by Fluorescence Spectroscopy *in situ* through a bioreactor probe.

After an exploratory calibration using data from only one bioreactor, we attested the aptitude of this technique to capture overall data trend: using a 3 component PLS model, we have obtained a calibration NRMSE of 2.9% for total AAV particles per cell, 5.9% for viable cell density and 0.9% for viability). Additional bioreactor productions using different infection parameters (CCI, MOI, time of infection) allowed testing the robustness of fluorescence monitoring to process variability. With this dataset, we tested several pre-treatment methods for the raw spectra, as well as different regression algorithms in order to establish a good predictive model. Ultimately, fluorescence spectroscopy provides a simple tool for online monitoring of key process variables in baculovirus-infected insect cell cultures.

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