ENABLING PAT IN INSECT CELL BIOPROCESSES: A MONITORING TOOLBOX FOR AAV PRODUCTION

Inês A Isidro, iBET and ITQB NOVA, Portugal
iaisidro@ibet.pt
Daniel AM Pais, iBET and ITQB NOVA, Portugal
Rui MC Portela, iBET, Portugal
Anastasiya Kryzhanska, iBET, Portugal
Paulo Galrão, iBET, Portugal
Manuel JT Carrondo, iBET , Portugal
Paula M Alves, iBET and ITQB NOVA, Portugal

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Recombinant adeno-associated viruses (rAAV) are promising delivery vectors for gene therapy and, to meet demand from ongoing clinical trials, there is an urgent need for more efficient production platforms. Insect cells combined with baculovirus expression vectors constitute a well-established production platform for viral products, both for gene therapy and vaccination. However, as a lytic production system, with released proteases likely to impact product quality attributes, the ability to monitor culture progression becomes essential to ensure high product titers and quality.

In this work, we explored different techniques to monitor, in situ and in real-time, the production of rAAV in the insect cell-baculovirus system. Fluorescence spectroscopy was combined with artificial neural network models and a genetic algorithm to fine-tune spectra pre-processing. Important process variables, namely cell concentration, cell viability and rAAV titer, were predicted for four independent batches, with normalized root mean squared errors (nRMSE) of 4 to 7% in leave-one-batch-out cross-validation. Sensitivity analysis highlighted how each of these process variables is correlated with different spectral regions and, consequently, associated with distinct metabolic signatures. Online digital holographic microscopy was employed to calculate over 30 cell-related optical attributes, which were used to predict process variables using multiple linear regression models with stepwise variable selection. Cell concentration, viability and rAAV titer were predicted with an nRMSE between 4 and 8% for 3-fold cross-validation (two batches). Importantly, we found specific optical attributes which correlate with baculovirus infection and production onset and are thus especially relevant for process decisions.

This work establishes a toolbox that can be leveraged to detect deviations, improve process understanding and support the critical time-of-harvest decision. Such monitoring tools will ultimately enable a knowledge-based approach to bioprocess development and control, in line with process analytical technology (PAT) principles, which can be applied to other biopharmaceuticals produced in insect cells.