Simplifying Progress

Application of PATfix® Valve Switch Analytics in Media Screening for AAV8 Production

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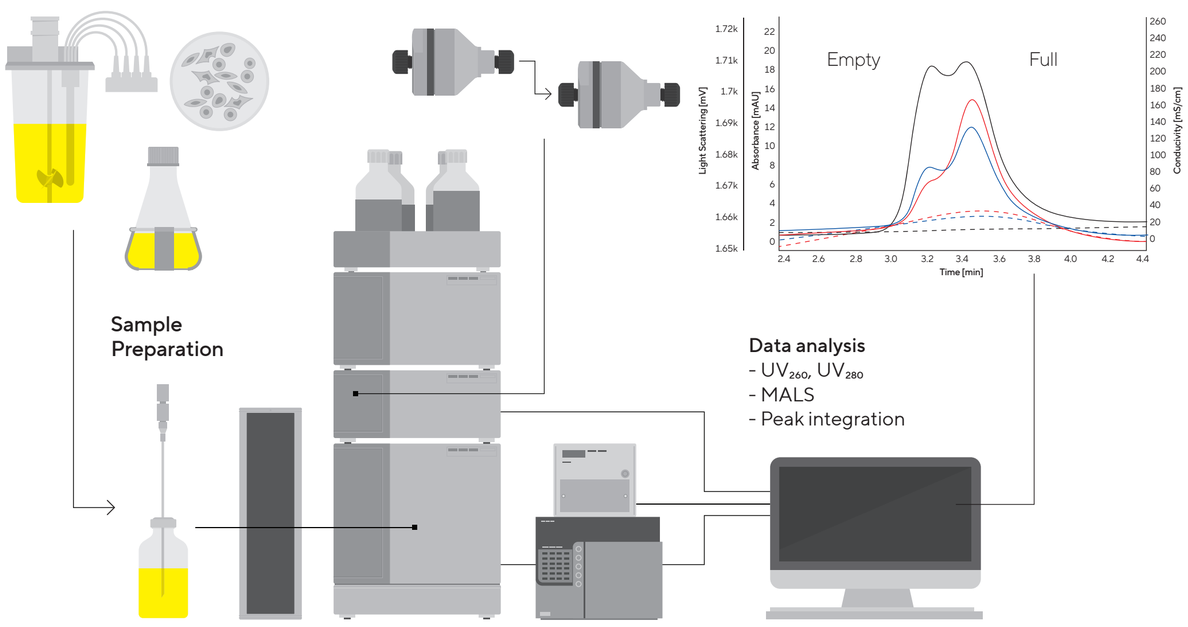
Introduction

Recombinant adeno-associated viral (rAAV) vectors are the leading gene delivery tool for the treatment of a variety of diseases. While several rAAV-mediated therapies have been approved so far, and many more are in clinical trials, rAAV production still faces many challenges. The key goal of rAAV upstream process development is achieving high viral titer together with a sufficient percentage of full capsids. However, the analysis of complex upstream harvest samples can be challenging. Classical analytical methods such as ddPCR | ELISA offer limited information due to differences in sample preparation and basic principles for detecting empty and full capsids. The methods are also time-consuming and therefore less useful for following rAAV production processes in real-time. To overcome these limitations, we developed a PATfix® Valve Switch analytical method that is based on ion-exchange high-pressure liquid chromatography (IEX-HPLC) and can be successfully applied for analysis of empty | full ratios in crude upstream samples.

PATfix® Valve Switch Analytical Tool

The PATfixTM Valve Switch system enables empty|full analysis of rAAV capsids in harvests. The sample is first lysed and, in case of high cell density harvests, diluted. The next step is acidification, followed by clarification with a benchtop centrifuge and a 0.45 µm syringe filter. The sample is loaded on a CIMac™ SO3 column, where the virus is captured, concentrated, and partially purified. The sample is then eluted and re-directed through the valve to the CIMac™ AAV Full | Empty column, where empty and full rAAV capsids are separated along the salt gradient. PATfix® is supplied with multiple detectors, such as UV260, UV280, and multiangle light scattering (MALS). The key element of the system is a novel MALS detector, which enables selective quantification of empty and full capsids in relatively impure samples with better sensitivity than MALS detectors used in the field so far.

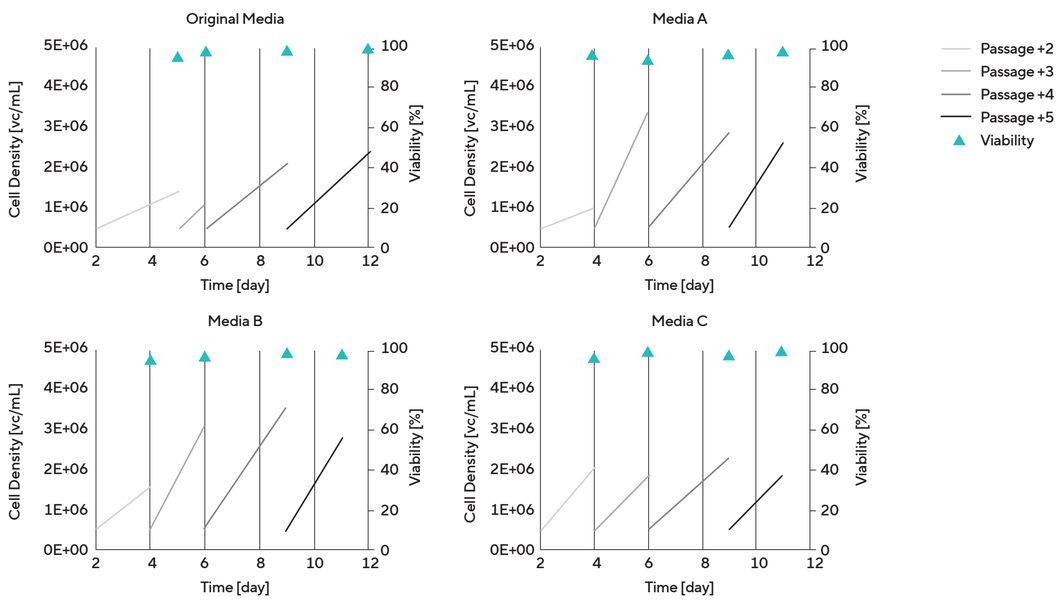
Figure 1: *Schematic Diagram of the PATfix® Valve Switch Method Setup and Sample Characterization Output, Including an Example of a Zoomed in View of the Salt Gradient on CIMac™ AAV Full | Empty Column*



Testing Media for AAV8 Production in HEK293 Cells

We tested a panel of three chemically defined, animal component-free media developed to support viral vector production in HEK293 (HEK) suspension cells. Cells were thawed in the original media and adapted to corresponding media for three passages, as recommended by producers. All media were supplemented with 1× GlutaMAX™. For the purpose of this experiment, suspension HEK cells were seeded in shake flasks at a density of 1E+06 viable cells (vc)/mL one day before transfection. Cells were triple plasmid transfected and mixed with polyethylenimine transfection reagent at a 1:3 ratio. Where indicated, 12% (v/v) of media feeds were added 24 hours post-transfection (hpt). 72 hpt samples were collected, lysed, and frozen.

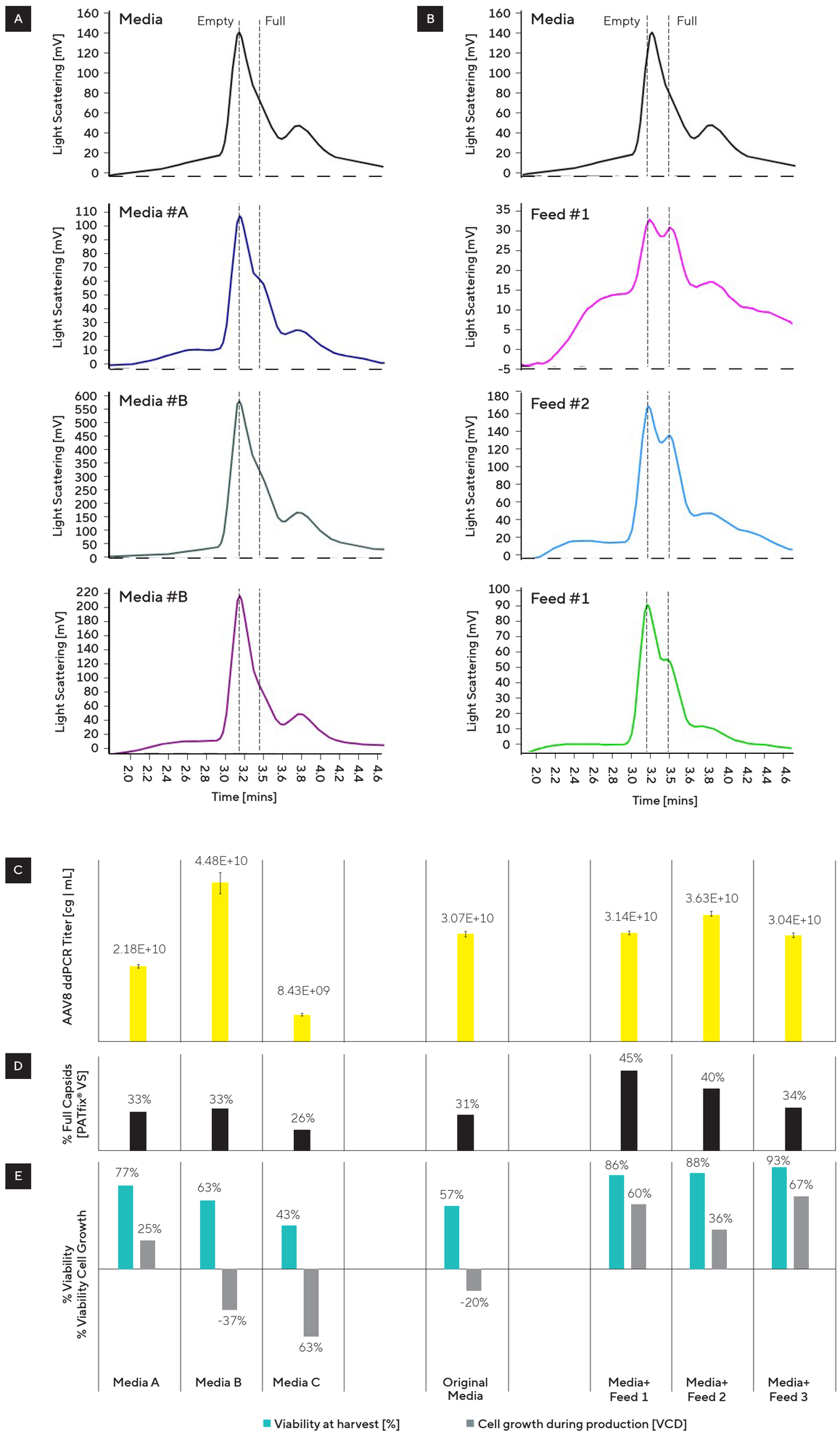
Figure 2: *Cell Growth in the HEK Media Panel Compared to the Original Media as a Control*



Note. HEK cells were thawed in the original media and passaged in the new media for a minimum of three passages to allow cell adaptation. Cell growth was monitored by measuring cell density (grey lines) and viability (yellow triangles). All the represented media indicate healthy cell growth and a high percent of viable cells. Media showing poor doubling time and viability were eliminated from the study (data not shown).

Results: AAV8 Production in Batch and Fed-Batch Mode

Figure 3: *Analysis of AAV8 Production in Different Media*



Note. A) Zoomed-in view of AEX column elution gradient for harvests in batch mode from cells adapted to different media. B) Zoomed-in view of elution gradient on PATfix® VS AEX column. Samples represent AAV8 harvests from fed-batch cultures alongside harvests from cells cultured in basal media in batch mode. C) AAV8 titer determined by ddPCR (dark grey). D) Percentage of full AAV8 capsids calculated by MALS peak integration. E) Percentage of viable cells at harvest (blue) and cell growth during production measured as the ratio of cell density at transfection to cell density at harvest (light grey).

VS = valve switching, ddPCR = droplet digital PCR, vg/mL = vector genome per millilitre

Conclusions

In this work, we demonstrate that PATfix® Valve Switching analytics can be used as the main analytical tool in upstream process optimization:

- It can be used to improve the percentage of full capsids.
- It provides fast analytics for complex upstream samples.
- Cell growth and viability at harvest were not correlated with the obtained AAV8 titers.
- In our experiments, some of the fed-batch approaches resulted in an increased percentage of full AAV8 capsids compared to batch runs with different media tested.