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# De-risking AAV scale-up: process comparability data from a 50 m² fixed-bed model

Dylan Knutson, Xiang Cui, and Akash Khorran

**What you will learn:**

- How a Quality by Design framework guided scaling strategy and parameter selection across a five-run scale-down study in adherent AAV manufacturing
- What batch-level data revealed when process parameters were compared between a 50 m² scale-down model and 500 m² manufacturing scale
- How biomass capacitance enabled real-time detection of a seeding deviation before downstream impact

**Key findings:**

- pH, dissolved oxygen, and metabolite profiles were well aligned between the 50 m² and 500 m² fixed-bed systems across all five runs
- Bulk harvest titers from the 50 m² system fell within the interquartile range of 500 m² manufacturing batches when scaled by 10×
- An unplanned shift in transfection conditions may have led to an increase of 48% in bulk harvest titer, identifying a potential avenue for yield improvement not anticipated at the outset of the study

**Summary:**

- A 50 m² fixed-bed bioreactor reliably reflected 500 m² manufacturing-scale behavior across key process parameters
- The scale-down approach enabled 3–5 runs per single manufacturing run at significantly lower capital and material cost
- Specific operational findings – including PID tuning outcomes and a transfection deviation that may have led to a yield increase of 48% – provide practical guidance not available from pre-study modelling alone

**Q&A highlight:** From an MS&T perspective, what was the most valuable aspect of running batches in the 50 m² system?

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## SCALE-DOWN MODELLING AS A TOOL FOR AAV PROCESS DEVELOPMENT

AAV manufacturing processes, particularly adherent systems, do not always scale linearly, as critical behaviors such as oxygen transfer, hydrodynamics, and transfection complex dynamics can change substantially with scale. Scale-dependent effects are frequently identified late in development, when process flexibility is limited and modifications carry significant cost and regulatory risk, making earlier characterization through scale-down modeling extremely valuable. Fixed-bed bioreactor systems offer a single-platform approach spanning development through to manufacturing, enabling scale-down studies within a geometrically consistent system. Here, we describe a study conducted at Novartis examining whether a 50 m² fixed-bed system could serve as a reliable scale-down model for a 500 m² manufacturing process, using a Quality by Design (QbD) framework to guide parameter selection and comparability assessment.

## STUDY DESIGN & SCALING APPROACH

The objective of this study was to evaluate whether the iCELLis™ fixed-bed bioreactor platform, at 50 m² scale, could serve as an effective scale-down model for 500 m² manufacturing.

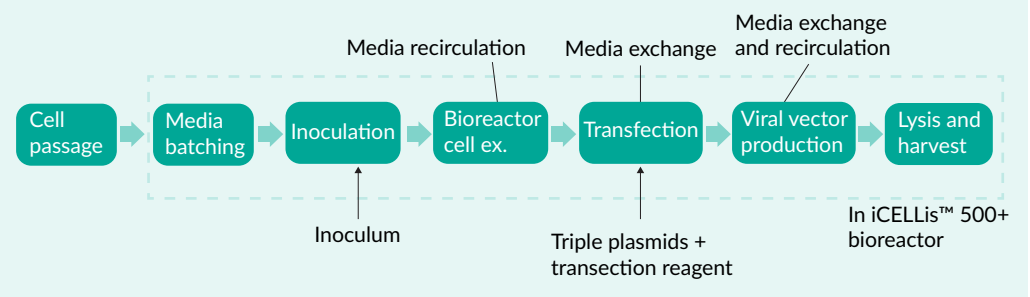
The study began with 25 quality attributes. A hazard analysis was performed, which identified 15 critical quality attributes common to AAV manufacturing. These were then examined in greater detail using literature and empirical studies to identify process parameters that influenced them. Literature-derived critical process parameters (CPPs) were used together with application knowledge to guide the scaling strategy.

Five runs were performed on a 50m² fixed-bed system and process parameters including pH, dissolved oxygen, metabolites, titers, key quality attributes, and impurity attributes were compared.

A simplified version of the upstream AAV process is shown in Figure 1. Cells are expanded through the seed train, inoculated into the bioreactor, and grown to the target cell density. Following the cell growth phase,

## FIGURE 1

**Upstream AAV process.**



adhered cells are transfected with three plasmids to form full AAV capsids containing the transgene. Two media exchanges were performed to aid the transfection process and capsid production. At harvest, the cells are lysed *in situ* within the bioreactor, enabling release of the AAV capsids.

To maintain comparability with manufacturing, the seed train and reagents used were manufacturing grade. Operation setup and process timing were kept the same, and medium volume per unit surface area was matched in cases where reagent concentration was an important factor. Linear speed was also matched to manufacturing conditions, as were pH and dissolved oxygen (DO) set points. Falling film height was adjusted where necessary to help maintain DO control in the growth and production phases.

## SCALING PARAMETERS & PRIORITIZATION

Linear speed profiles were aligned between manufacturing scale and the 50m$^2$ fixed-bed system, to maintain consistent hydrodynamic conditions across scales. Typical linear speeds ranged from 0.7 to 1.3 cm/s.

Due to geometric constraints in fixed-bed systems, it is not possible to perfectly scale both falling film and volume-to-surface area simultaneously. At each step, the more critical parameter must be prioritized. When reagent concentration is a CPP, such as during transfection, preserving the volume-to-surface area is essential. When oxygen transfer is the primary concern, falling film becomes the more important parameter to maintain.

Table 1 shows how these two parameters were prioritized across five runs. FF+5 indicates that the falling film height was increased by 5 cm to maintain DO.

## PROCESS PARAMETER ALIGNMENT ACROSS SCALES

### pH profiles

A comparison of pH trend between the 50 m$^2$ fixed-bed system (colored lines) and the 500 m$^2$ system (gray lines) is shown below (Figure 2). The pH set point was ≤7.3. Across the runs, pH process data and timing were well aligned, which was primarily achieved through carbon dioxide regulation. Small deviations in pH were observed during the growth phase; these differences were due to the chosen deadband rather than a biological difference.

## TABLE 1

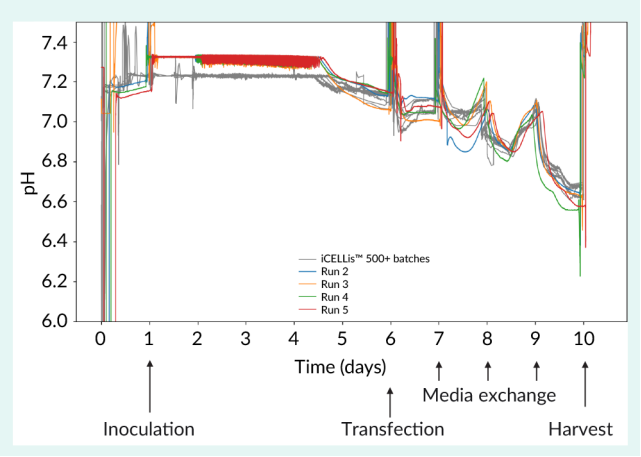
**Prioritization of falling film and volume-to-surface area parameters across five runs of the 50 m$^2$ fixed-bed system compared to manufacturing scale.**

| Parameter | Run 1 | Run 2 | Run 3 | Run 4 | Run 5 | iCELLis™ 500+ runs (*n*=10) |
|---|---|---|---|---|---|---|
| Conditioning | FF | FF | FF | FF | FF | FF |
| Inoc | FF | FF | FF | FF | FF | FF |
| Growth | FF | FF | FF + 5* | FF + 5* | FF + 5* | FF |
| Transfection | V/A | V/A | V/A | FF + 5 | V/A | V/A |
| Production | FF | FF + 5* | FF + 5* | FF + 5* | FF + 5* | FF w/ LS increases for DO |
| Harvest | V/A | V/A | V/A | V/A | V/A | V/A |

*In some cases, the falling firm was raised by 5 cm to maintain DO.

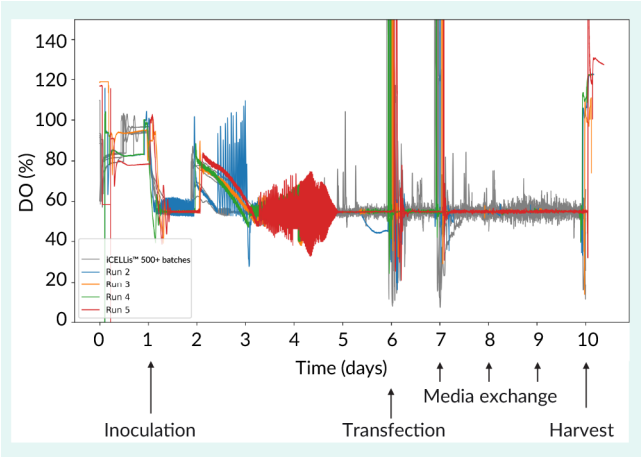
## FIGURE 2

**Comparison of pH trend between the 50 m² and 500 m² fixed-bed bioreactors.**




## FIGURE 3

**Comparison of DO trend between the 50 m² and 500 m² fixed-bed bioreactors.**

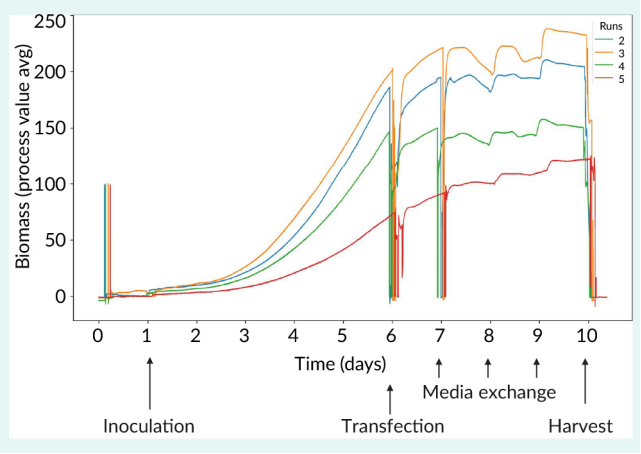



### Dissolved oxygen trend

DO trends can serve as an indicator of variability in operator technique. Four runs conducted with the 50 m² fixed-bed system were overlain with two runs of the 500 m² system for comparison (Figure 3). DO set point was ≥50%. Overall, DO behavior was similar between the two systems. However, Runs 1 and 2 were conducted using a prototype proportional–integral–derivative (PID) tuning protocol as well as a prototype recipe. The control strategy used for Run 2 mimicked a manufacturing scale approach, including use of a constant total gas flow method. The increase in DO demand meant that falling film height had to be increased to maintain DO. This is an important operational adjustment that can be utilized in fixed-bed systems.

## FIGURE 4

**Comparison of biomass capacitance between the 500 m² and 50 m² fixed-bed bioreactors.**



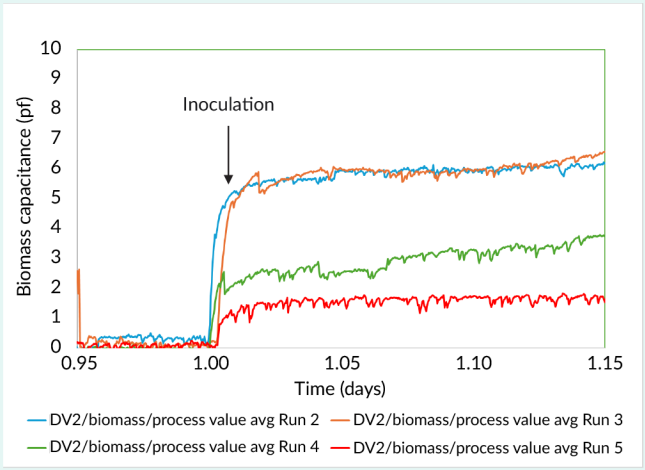

### Biomass capacitance as a process monitoring tool

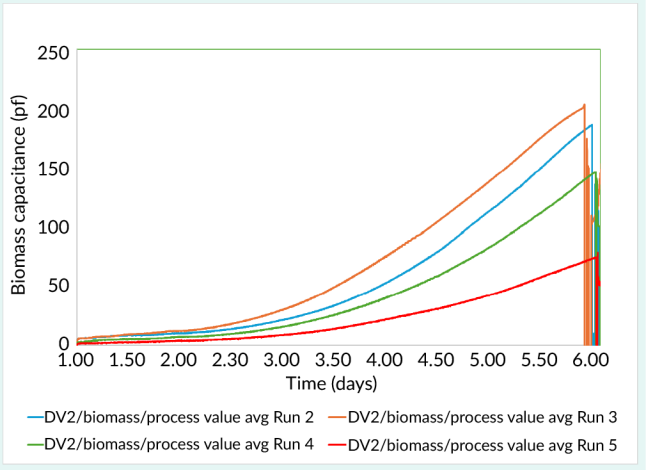
Biomass capacitance provides critical high-frequency insight by measuring the volume of viable living cells. Biomass capacitance was measured across multiple runs of the 50 m² fixed-bed system (Figure 4). In scale-down studies, integrated biomass measurements provide continuous process data throughout the run. The shape and amplitude of these trends offer insight into how the process progresses through the growth, transfection, and virus production phases. These profiles can show whether the run is behaving as expected and reveal deviations in process performance.

Biomass capacitance was also evaluated during the inoculation and growth phases. Notably, Run 5 (red line) was seeded differently, which was detected by the biomass

## FIGURE 5

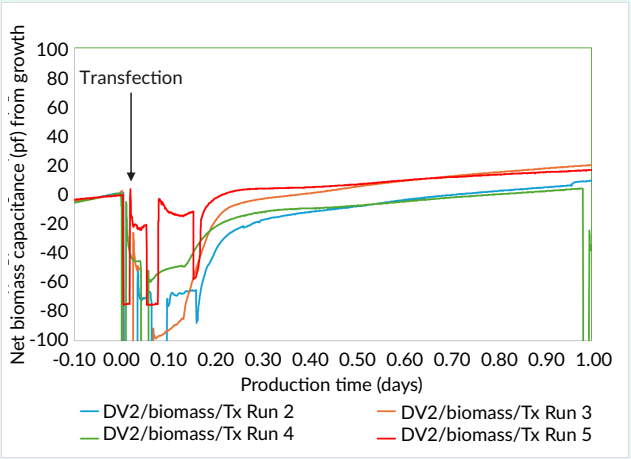
**Biomass capacitance measured during the A: inoculation and B: growth phases.**

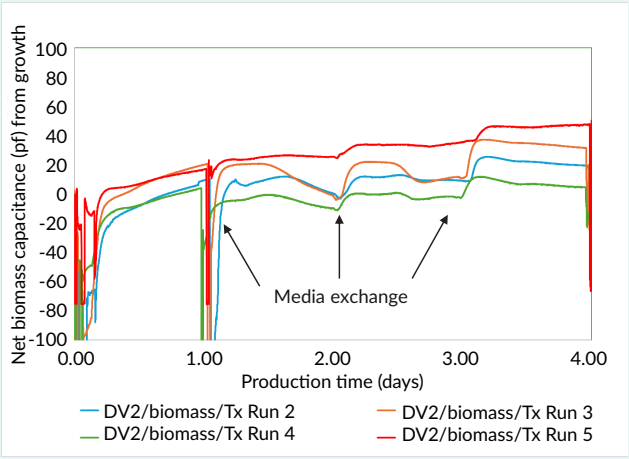



## FIGURE 6

**Biomass capacitance measured during the A: transfection and production and B: media exchange phases.**




signal early in the process (Figure 5). This is a useful finding as it demonstrates that seeding-related differences can be identified early, before they potentially lead to more significant issues later in the run.

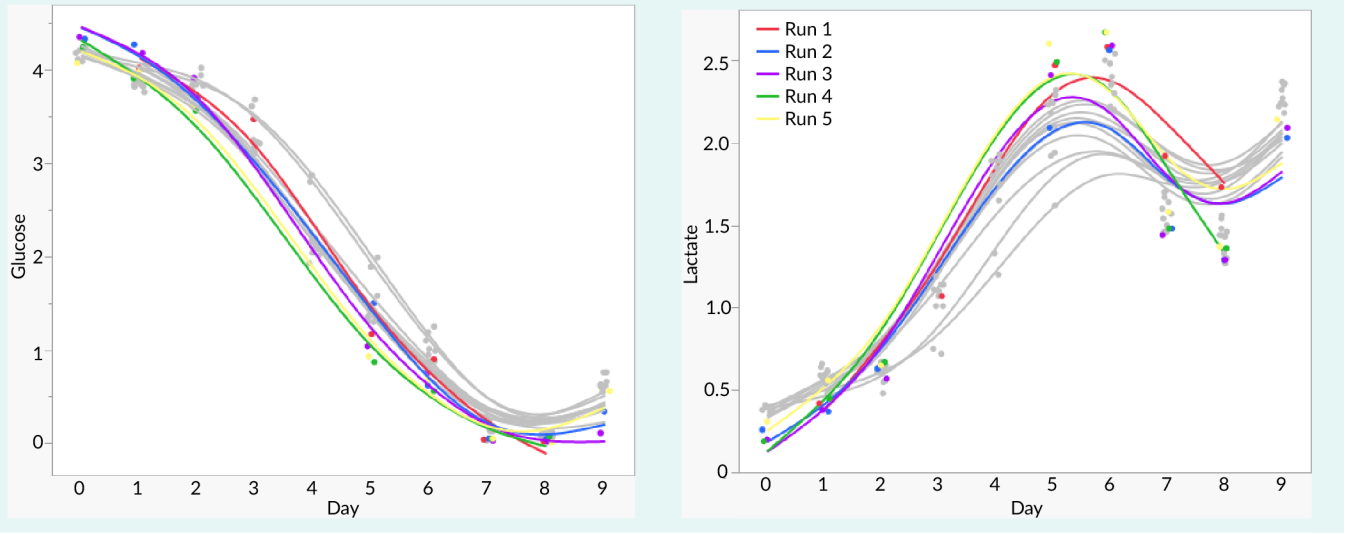
Biomass capacitance was also measured during the transfection phase and production and media exchanges. Although there is run-to-run variability in the signal, it is the trend and shape of the signal that is the real indicator of performance, rather than a perfect overlay of the signal (Figure 6). These signals show whether there is consistency during key steps, and also whether runs show a distinct pattern that correlates with other process values or outcomes.

### METABOLITE ALIGNMENT BETWEEN SCALES

Offline markers such as metabolites can serve as an indicator of process performance.

## FIGURE 7

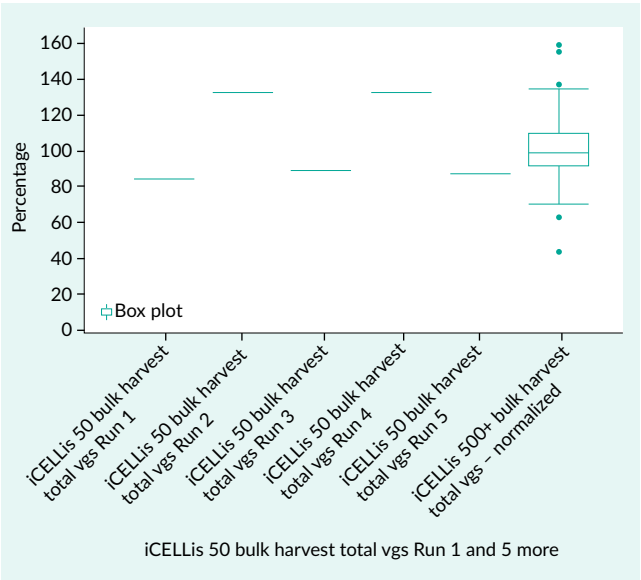
**Comparison of metabolite measurement between the 50 m² and 500 m² fixed-bed bioreactors.**




The graph below shows glucose and lactate profiles that are well aligned between the different scales (Figure 7). Despite metabolite sampling not being performed on some days for the 50 m² fixed-bed system, the results are similar to that of a larger system demonstrating that the conditions within the scale-down system are still able to achieve metabolic behavior comparable with a manufacturing-scale system.

## FIGURE 8

**Comparison of bulk harvest titer between the 50 m² and 500 m² fixed-bed bioreactors**



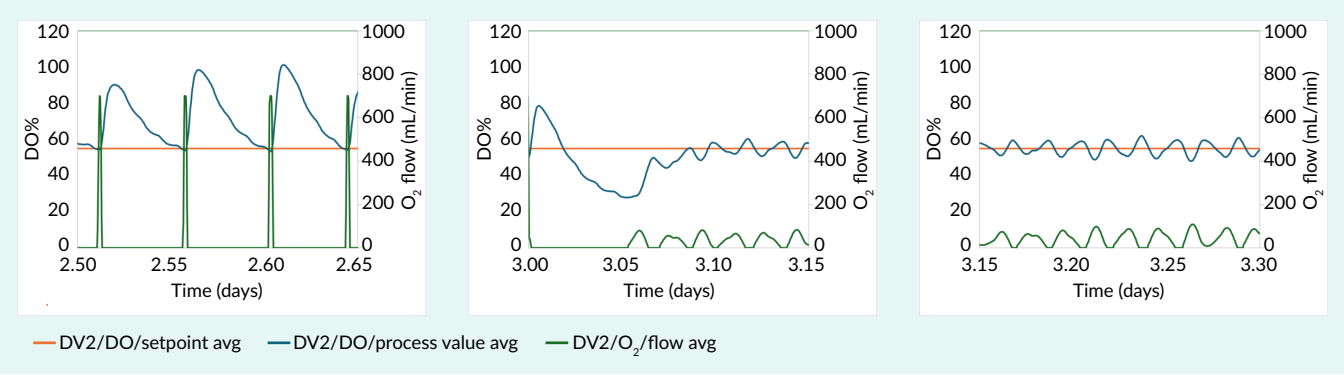

## BULK HARVEST TITER RESULTS

Taken together, the above results demonstrated that critical process values were consistent across scales. Comparisons were then performed to determine whether that consistency translated into comparable titer outcomes. The graph below shows the titer for the 50 m² fixed-bed system, scaled by 10× and then normalized to the average of the 500 m² system (shown by box plot; Figure 8). The five bars represent the titer from five individual runs of the 50 m² system. Titers from the 50 m² system fell within the interquartile range of the 500 m² batches, demonstrating comparability across scales. Of note, digital droplet PCR was used as a quantification method, which caused a slight elevation in titer values in the 50 m² system. Adjusting for this difference may bring the titer range into even closer alignment with that of the 500 m² system.

The similarity in manufacturing-scale process trajectory and titer outcomes make

## FIGURE 9

**DO PID loop tuning demonstrated that specific controller settings produced distinct response profiles.**




A: overshoot; B: undershoot; or C: some overshoot.

the scale-down system a useful tool to explore CPPs. Currently, work is ongoing to assess empty/full capsid ratios, hcDNA, hcprotein, and residual pDNA, which will be presented at the European Society of Gene and Cell Therapy in October 2026.

### RUN 2: DISSOLVED OXYGEN TUNING

A Ziegler–Nicholas closed-loop test was used to tune the DO control loop. During the test, PV values were used to determine minimum and maximum values and to propose new control settings. The procedure was performed in process and required approximately 1 day. Several combinations of proportional gain (Kp) and integral time (Ti) were evaluated and the resulting overshoot and undershoot responses were quantified. Although the 50 m² fixed-bed system was preloaded with default PID parameters, the tuning test demonstrated that specific parameters produced either overshoot, undershoot or controlled overshoot (Figure 9). The latter being the ideal situation as this is the most desirable for stable DO regulation.

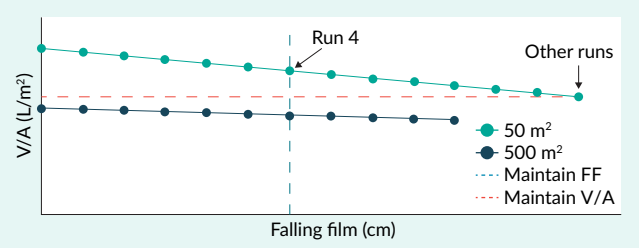
By transitioning from a prototype level control strategy to parameters that more closely reflect manufacturing behavior, it is possible to improve loop stability and reduce the need for operational intervention.

### RUN 4: AN UNPLANNED TRANSFECTION DEVIATION REVEALS A YIELD OPPORTUNITY

During Run 4 of the transfection step, the volume of the 50 m² fixed-bed system was inadvertently set to match falling film height rather than the intended scaled-down volume-to-surface ratio. Although the absolute addition amount of PEI/plasmid complexation was correct, the PEI/plasmic complex-to-volume ratio was lower than intended. Notably, Run 4 yielded a bulk harvest titer 48% higher than Run 3 (Figure 10). This increase may be due to the altered complex-to-volume ratio. Although this finding is based on a single data point, this observation warrants further investigation of parameters that have

## FIGURE 10

**Titer volume of Run 4 compared to other runs with the 50 m² and 500 m² fixed-bed systems**



the potential to increase yield at later manufacturing stages.

## TRANSLATION INSIGHT

Results showed that the 50 m² iCELLis™ fixed-bed system reliably reflected manufacturing-scale behavior on the 500 m² iCELLis™ platform. By defining appropriate CPPs and scaling these, it was possible to mimic large-scale process operations and process values within the batch (e.g., pH, DO, and metabolic profiles). Biomass capacitance was also a useful measure as it provided high-resolution, continuous process data, which will be useful for manufacturing science and technology (MS&T) studies. Importantly, manufacturing-scale outputs were achieved at significantly lower capital and material costs using the scale-down approach.

Although falling film is not a CPP, it was increased to maintain DO or to preserve the volume-to-surface area during transfection and harvest. In contrast, DO control at manufacturing scale is typically achieved by increasing linear speed. This illustrates how in a scale-down system, a different operational adjustment may be required to protect the same CPPs.

This study identified several opportunities for future optimization of the 50 m² fixed-bed system:

- Explore the effect of DO differences and titer
- Further refine transfection vessel volumes and cocktail dilution
- Understand the impact of pH setpoints on yield
- Evaluate biomass data across other scales to better understand how these signals correlate with process performance and outcomes

Overall, this work supports the 50 m² fixed-bed system as a robust, flexible, scale-down model, which enables learning optimization and risk reduction before commitments are made at manufacturing scale.

# Q&A

**Akash Khorran** (left), **Xiang Cui** (center), **Dylan Knutson** (right)

**Q** How did you introduce transfection complexes into the vessels?

**AK** The transfection step is one of the most critical steps in AAV production. The transfection complexes were added to the 50 m² fixed-bed system using a hand pump, as the required volume is relatively small. With the 500 m² system, the complex was added using a peristaltic pump. However, the timing and duration of the complex addition was maintained as consistently as possible across both scales.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**Q** From an MS&T perspective, what was the most valuable aspect of running batches in the 50 $m^2$ fixed-bed system?

**XC** The 50 $m^2$ fixed-bed system is a powerful and more representative model of the 500 $m^2$ system. The nano is limited in several aspects. For example, control of falling film height is not possible at the nanoscale. Also, gas control strategies are more effectively achieved on the 50 $m^2$ system. In terms of cost, for each run of the 500 $m^2$ system, we can perform 3–5 runs of the 50 $m^2$ system, while still achieving scale-representative data and significantly de-risking any scale-up parameters. This shows how useful this intermediate scale system is. It is a giant leap forward for us.

**Q** What were your observations around filming in the 50 $m^2$ fixed-bed system?

**AK** Filming was minimal during the cell growth phase when the media level was maintained at 10 cm falling film. In contrast, we did see increased filming during the transfection step, as the reagent concentrate was intentionally held constant. During transfection, the falling film height exceeded the 10 cm falling film height, which very likely contributed to the decreased film formation. Overall, more film is noticed in a large-scale system compared to a smaller-scale system. This is likely due to the larger volume of media used.

**Q** You mentioned that falling film was increased to maintain some parameters to scale-up. Does it impact the linear speed and how do you calculate falling film?

**XC** Using the 50 $m^2$ system it was not possible to maintain the DO at the same level of the target of the 500 $m^2$ system, especially during the exponential cell growth phase. The compromise was made to increase the falling film height to have more oxygen available to the cells, and this had an impact on linear speed. However, we have accepted this strategy for our intermediate scale system. In our CPP defining document, we use the RPM rather than linear speed for the large-scale production.

**DK** We can always match linear speed in these systems across different scales. We know that there is not a lot of evidence showing falling film to be a CPP, especially during growth and production phases. We opted to increase the falling film for ease of production, so we will not have any worries regarding DO control or raise RPM, which is usually done at manufacturing scale.

**Q** What is a typical yield for AAV for the 50 $m^2$ system, and how many production runs are required to demonstrate a well-controlled process from an FDA perspective?

**XC** That is the million-dollar question! We are unable to share the exact titer yield but I can say that from the five runs we performed on the 50 $m^2$ system, we saw a comparable yield to manufacturing scale; applying a 10× scale multiplier gave almost the exact average yield observed in a large-scale system.

In terms of how many runs you need to demonstrate to the FDA, I would say that this would be the same for any typical biological process. For us, we designed 5 runs for the 50 $m^2$ system, as this was a beta test and we were expecting some trial and error. We were

hoping for 3 runs to be representative. I am confident that four out of the five runs showed good representation of the scale-down system, except for some minor deviations. As seen from the data, the titer is comparable across scales, but we are still waiting on the impurity data.

## BIOGRAPHIES

**Akash Khorran** is an Upstream MS&T Engineer at Novartis Gene Therapies, based in Durham, North Carolina. In this role, he is the acting SME for Upstream GMP and lab-scale production of AAV. Akash has experience supporting gene therapies across the development stage and manufacturing scale. He holds a bachelor's degree in biology from the University of Florida.

**Akash Khorran**, Upstream Engineer, Manufacturing Science and Technology, Novartis Gene Therapies

**Xiang Cui** is a Senior Manager at Novartis Gene Therapies based in Durham, North Carolina. In this role, he is responsible for overseeing MS&T lab which supports GMP production of AAV. Xiang has experience supporting gene therapies and vaccines across development stage and manufacturing scale. He holds an MS degree in bioprocess engineering from North Carolina State University.

**Xiang Cui**, Senior Manager, Manufacturing Science and Technology, Novartis Gene Therapies

**Dylan Knutson** is an Upstream Field Application Specialist with extensive hands-on experience supporting viral vector manufacturing workflows. He works closely with customers to implement, and optimize iCELLis™ fixed-bed bioreactor platforms, bridging process development, scale-up, and real-world manufacturing challenges. His focus is on translating upstream science into robust, scalable solutions for gene therapy applications.

**Dylan Knutson**, Upstream Field Application Specialist, Cytiva

## AUTHORSHIP & CONFLICT OF INTEREST

**Contributions:** The named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

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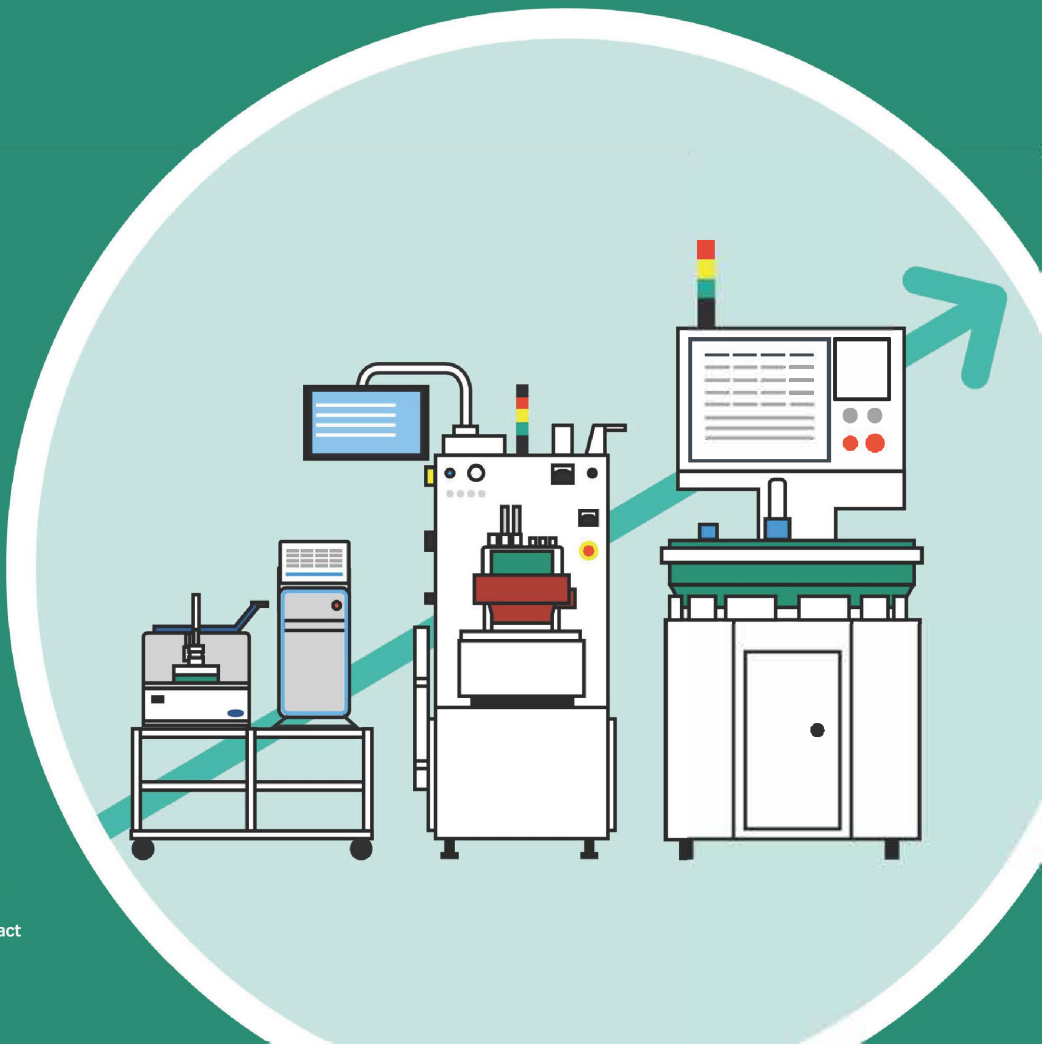

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