CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Scaling AAV5 production from bench to industrial scale: strategies for efficient, de-risked manufacturing

Sara Krekels and Anne MacIntyre

Gene therapy is progressing quickly, but persistent manufacturing challenges still restrict its accessibility to reach patients in need. This article outlines how these barriers can be addressed by implementing more efficient, scalable production platforms. To overcome production limitations, advanced manufacturing systems can provide an integrated approach designed to improve efficiency, consistency, and scalability. The case study in this article illustrates how an advanced platform supports robust AAV5 production in adherent cells, presenting comparative data across three fixed-bed bioreactor scales. The results demonstrate a complete and practical scaling strategy from benchtop to large-scale manufacturing, highlighting the potential of advanced bioreactor systems to streamline gene therapy production and expand patient access.

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OVERVIEW OF GENE THERAPY'S PROGRESS, CHALLENGES, & ECONOMIC CONSIDERATIONS

Gene therapy is being recognized as a transformative force in healthcare. Since 2022, the pace of US FDA approvals has accelerated, with therapies such as LUXTURNA™, ZOLGENSMA®, and ELEVIDYS® paving the way. In particular, AAV vectors have been established as a leading platform for the *in vivo* delivery of therapeutic genes due to their safety and effectiveness. As a result, these therapies are now offering hope for conditions that were previously considered untreatable.

While the promise of gene therapy is evident, cost remains a significant barrier. Recent approvals have demonstrated prices of approximately $2.5–$3.5 million per dose. When compared with the lifetime cost of conventional treatment, where such treatment exists, these prices are often similar or lower. However, the current healthcare system is not structured to manage such substantial upfront costs, which has limited widespread access to these potentially curative therapies, particularly for ultra-rare diseases. Reducing

production costs is therefore essential in order to improve accessibility.

TRANSITIONING FROM TRADITIONAL FLATWARE PRODUCTION TO SCALABLE, AUTOMATED MANUFACTURING

The traditional upstream production method for viral vector-based therapies has relied on 2D flatware for cell growth and vector manufacturing. These processes require very large facilities to accommodate numerous multi-tray incubators, each of which must be seeded, transfected, and harvested individually. The approach is highly labor intensive, and every manual intervention introduces a potential contamination risk. In addition, cell cultures are difficult to monitor or control, which often results in substantial and poorly understood batch-to-batch variability. Further scale-up using this format is often not practical.

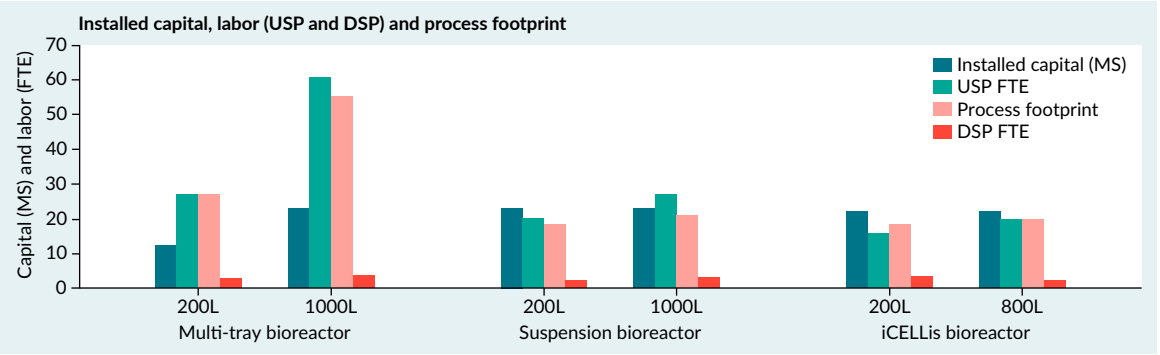
Advanced therapies can be produced more efficiently using single-use bioreactors. When compared with multi-tray platforms, bioreactors markedly reduce labor and space requirements while maintaining comparable capital costs (Figure 1). In addition, they enable fully closed process handling, batch automation, and live culture monitoring, which results in reduced variability, faster scale-up, and lower operational complexity.

Advanced systems, such as iCELLis™ technology from Cytiva, can be utilized for adherent cell and gene therapy manufacturing. The iCELLis bioreactors are equipped with GMP-compatible (21 CFR Part 11) software that enables process automation and incorporates integrated sensors (pH, dissolved oxygen (DO), temperature, and biomass) for real-time culture monitoring. Additionally, the platform enables scalability, beginning at 0.5 m^2 of cell growth surface area and extending to 500 m^2. The system is also widely used in clinical trial manufacturing and has already been applied in the production of six approved, commercially available therapeutics.

The iCELLis family includes three different bioreactor scales: iCELLis Nano, iCELLis 50, and iCELLis 500+. The benchtop-scale iCELLis Nano bioreactor provides 0.5–4 m^2 of cell growth surface area, the fully single-use iCELLis 50 bioreactor 6.6–50 m^2, and the industrial-scale iCELLis 500+ bioreactor 66–500 m^2. Together, these systems enable the iCELLis technology to support preclinical development, clinical trial manufacturing, validation runs, and full-scale commercial production.

FIGURE 1

Comparison of cost and labor requirements for upstream and downstream cell and gene therapy processing using multi-tray platforms, suspension bioreactors, and iCELLis fixed-bed adherent bioreactors.



Efficient scaling of gene therapy production requires movement beyond traditional manufacturing methods, and advanced technologies such as the iCELLis bioreactors can provide a scalable approach, designed to reduce production costs and operational complexity.

CASE STUDY: UTILIZING iCELLis BIOREACTOR TECHNOLOGY FOR SCALABLE AAV5 PRODUCTION

In a proprietary study, AAV5 production was performed across all three iCELLis bioreactor scales. Each bioreactor vessel used during testing was configured with low carrier compaction and a fixed bed height of 10 cm, resulting in cell growth surface areas of 2.65 m^2 for the iCELLis Nano bioreactor, 33 m^2 for the iCELLis 50 bioreactor, and 333 m^2 for the iCELLis 500+ bioreactor.

Several culture rounds were conducted, with multiple scales operated in parallel whenever possible. In total, six runs were completed using the iCELLis Nano bioreactor, three runs were completed using the iCELLis 50 bioreactor, and one run was performed using the iCELLis 500+ bioreactor. Each bioreactor was inoculated at a target density of 5,000 cells/cm^2, followed by 5 days of cell growth in a serum-containing medium.

Because the required total media volume exceeded the capacity of the bioreactor vessel, an external biocontainer was used to hold the remaining media, which was automatically recirculated in and out of the vessel throughout the growth phase. Immediately before transfection on day 5, a complete media exchange was performed to replace the serum-containing medium with serum-free medium. Triple transfection was then carried out using a complex containing 0.2 µg total plasmid DNA/cm^2 of cell growth area together with the transfection reagent PEI MAX.

Transfection was followed by 5 additional days of culture for AAV5 production in serum-free medium, again with recirculation. Harvesting included the collection of the spent supernatant, the lysate generated from an overnight lysis of the cells within the vessel, and a final PBS wash fraction. Collected samples were frozen and later analyzed for genomic titer and capsid titer by ddPCR and ELISA, respectively.

Because all three iCELLis scales were used, several key variables were considered to ensure consistent execution of the AAV process across scales. To reduce variability in input materials during parallel runs, all solutions, including media, transfection complex, and lysis buffer, were prepared as single batches and divided across scales immediately before use. For example, one transfection complex was prepared and incubated for 15 min. Afterwards, a defined portion was used for transfection of the iCELLis Nano systems, and the remaining defined volume was used for the iCELLis 50 or iCELLis 500+ vessels.

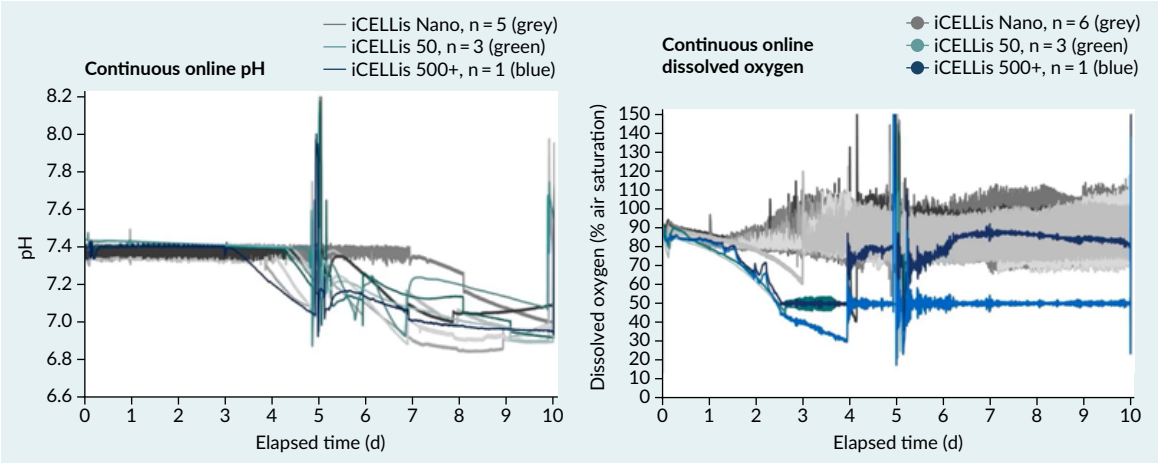
The linear speed of media flowing upward through the fixed bed, along with the ratio of media volume to cell growth surface area, can be critical for maintaining a homogeneous environment that supports consistent cell growth and productivity. The falling-film height, which describes the height of the downward-flowing media film, can influence the efficiency of low-shear gas exchange. Parameter selection for these variables should align with the priorities of the targeted process step.

EVALUATING pH & DO TRENDS IN CASE STUDY iCELLis BIOREACTORS

Based on the cell cultures conducted, the pH trends were similar across all three culture scales – iCELLis Nano, iCELLis 50, and iCELLis 500+ bioreactors **(Figure 2A)**. Control was maintained at the high end

FIGURE 2

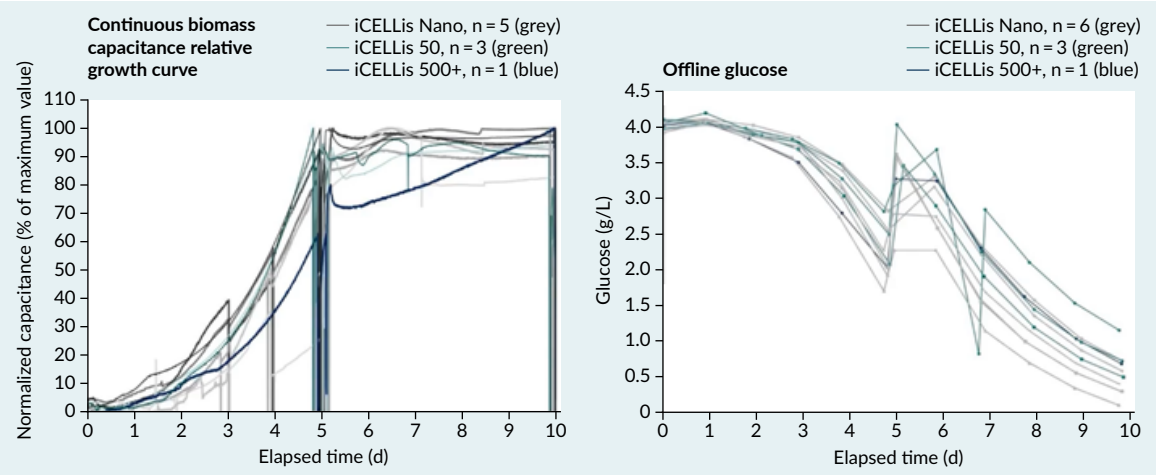
Examination of continuous pH (A) and dissolved oxygen (B) trends across all iCELLis bioreactor scales.



iCELLis Nano bioreactor runs appear in varying shades of gray, the iCELLis 50 bioreactor runs in green, and the iCELLis 500+ bioreactor runs in blue. (A) A brief deviation is visible around days 6 and 7 in one of the iCELLis 50 bioreactor runs, caused by an overnight recirculation malfunction. (B) DO set points differ across scales.

FIGURE 3

Trends in biomass capacitance and glucose concentration.



(A) Biomass capacitance trends, providing real-time cell growth monitoring across scales. Capacitance values were normalized to each batch's maximum, with spikes resulting from planned agitation stoppages during sampling and media exchanges. (B) Glucose concentration trends. Glucose is shown as a representative metabolite, as all measured metabolites displayed consistent patterns across scales, demonstrating effective process scalability. A brief deviation is visible around days six and seven in one of the iCELLis 50 bioreactor runs, caused by an overnight recirculation malfunction.

of the pH deadband during most of the cell growth phase, followed by a steady decline in pH immediately before and after the media exchange and transfection on day 5. Overall, the pH profiles were aligned within the expected variability across scales.

For DO control, the iCELLis Nano bioreactor was operated at a set point of 80%, while the iCELLis 50 and iCELLis 500+

bioreactors were operated at a lower set point of 50%, owing to the positioning of their DO sensors relative to the fixed-bed structure (Figure 2B). After a DO control correction was applied on day 4 in the iCELLis 500+ bioreactor, sensor measurements taken after the fixed bed (light blue lines) closely resembled those from the iCELLis 50 bioreactor, while measurements taken before the fixed bed (dark blue line) aligned with those from the iCELLis Nano. bioreactor. This pattern is consistent with the expected control strategy and reflects the influence of cellular oxygen consumption within the fixed bed.

In summary, pH trends were comparable across all scales, whereas DO profiles differed because each system required its dedicated control approach.

ASSESSING BIOMASS GROWTH PATTERNS & METABOLITE CONSISTENCY ACROSS iCELLis BIOREACTOR SCALES

Bioreactor biomass analysis showed a consistent increase in capacitance during the cell growth phase, followed by a plateau in values after transfection (Figure 3A).

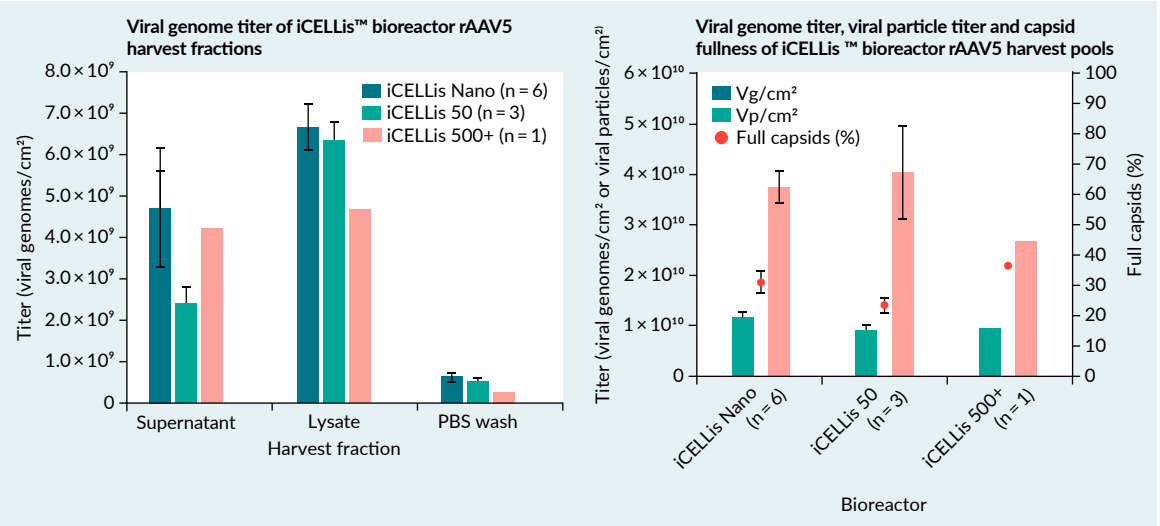
Glucose trends also served as an indirect indicator of cell growth for each run (Figure 3B). Fixed-bed carriers from the iCELLis Nano bioreactor were sampled prior to transfection, with day-5 cell counts ranging between 117,000 and 129,000 cells/cm² across production runs. The uniformity of the glucose profiles, together with the tight cell count range observed in the iCELLis Nano bioreactors, confirmed that cell densities were comparable across all systems at the time of transfection.

EVALUATING HARVEST YIELDS, VIRAL TITERS, & CAPSID CHARACTERISTICS ACROSS iCELLis BIOREACTOR SCALES

As noted earlier, the bioreactors were harvested after a 5-day production phase. The spent-media supernatant, cell-lysate fraction, and PBS-wash fraction were collected and combined to generate the final harvest pool per each culture. Harvest fractions were analyzed for genomic titer only,

FIGURE 4

Viral genome titers and capsid fullness across harvest fractions and bioreactor scales.



(A) Viral genome titers measured in each harvest fraction. (B) Total viral particles and viral genomes in the pooled harvest for all bioreactor scales. Full capsid percentages were calculated directly from viral genome and particle data.

whereas final harvest pools were evaluated for both genomic titer and capsid titer by ddPCR and ELISA, respectively.

All bioreactor systems exhibited their highest titers in the lysate fraction, representing approximately 60% of the total titer, with 35% in the supernatant fraction and minimal recovery observed in the PBS-wash fraction at approximately 5% of the total titer (Figure 4A).

Regarding total viral particles and genomes, although the relative contribution of each fraction varied somewhat between scales, the average genomic titers from final harvest pools remained consistent across scales (Figure 4B). Viral genome averages were approximately 1×10^{10} viral genomes/cm² for all scales. A lower viral-particle count was observed in the iCELLis 500+ bioreactor, accompanied by a higher proportion of full capsids. The source of this variability cannot be determined from a single production batch.

To summarize, similarities were observed in harvest function yields, viral particle counts, and full capsid percentages, with some variability. Genomic titers from the harvest pools remained consistent across all scales.

TRANSLATION INSIGHT

This case study demonstrates the scalability of the iCELLis platform and its ability to support a robust and consistent AAV production process. Consistent cell growth, metabolite profiles, and titer outputs were observed across all three scales. The iCELLis bioreactor system contributes to de-risking biomanufacturing processes by providing efficient and reproducible performance across scales. Overall, the implementation of advanced, fully closed manufacturing platforms can accelerate the broader adoption of gene therapies by enabling more efficient, scalable, and cost-effective production.

Q&A

Sara Krekels (left), **Anne MacIntyre** (right)

Q How do you introduce the transfection complex into the vessels?

AM Depending on the size of the transfection complex, we either used a bag or a large shake flask. In both cases, we ensured that the tubing, attached to either the bag or the shake flask, was sufficiently large to facilitate transferring the material from the bag into the vessel as quickly as possible. We then applied light pressure using a hand pump to either the bag or the shake flask and allowed gravity to complete the transfer. In this case study, we did not use any peristaltic pumps or similar equipment to introduce the material.

Q Was the overnight endonuclease lysis step performed inside the bioreactor or after harvest?

AM For the harvest, we added the lysis buffer into the vessel for the overnight lysis. We removed the supernatant and then added the lysis buffer for the overnight incubation. The solution within the vessel overnight is the lysis buffer, including the endonuclease.

Q Do you see a future for processes that rely on adherent cell cultures?

SK Yes—some people believe the future lies in suspension systems because adherent processes are viewed as less scalable. However, when using a bioreactor like iCELLis, industrial scales can be reached very easily. We are happy to support either approach, based on preference and intended application.

Additionally, in some cases, cells do not perform as well in suspension after being adapted from adherent culture, and the adaptation process itself can be time-consuming. Keeping cells adherent allows production to reach industrial scale, with cells held securely in the fixed bed. Media exchanges or perfusion become straightforward, without the need for tangential flow filtration or other cell-retention devices. Furthermore, harvests from adherent systems are often clearer than those from suspension bioreactors with less host-cell debris.

Q What were your observations around foaming in the iCELLis 50 bioreactor?

AM For anyone who has used the iCELLis platform, specifically the iCELLis 500+ bioreactor, we do observe foaming, especially when using media that contains serum during the process. When we originally operated the iCELLis 50 bioreactor, we kept this in mind because we were not exactly sure what we would see. However, it turned out that we observed very minimal foaming within the iCELLis 50 bioreactor.

BIOGRAPHIES

Sara Krekels is a field application specialist who supports biomanufacturing companies across Europe in their implementation, optimization, and scale-up of single-use bioreactor processes. With 9 years of experience on the iCELLis™ fixed bed bioreactor platform and a strong foundation from previous quality and product engineering roles, she bridges technical insight with practical application to accelerate biomanufacturing success.

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Anne MacIntyre is a Senior Scientist for Cytiva R&D supporting the advancement and implementation of cutting-edge products and processes for advanced therapy medicinal products. Having accumulated 20 years of industry insight into upstream process development across both adherent and suspension cell culture platforms, Anne now concentrates on gene therapy viral vector applications and technical support, including the iCELLis™ fixed-bed bioreactor. Her extensive experience has enabled her to provide effective solutions to a wide range of bioprocessing challenges.

Anne MacIntyre, Senior Scientist, Viral Vector R&D Upstream, Cytiva, Westborough, MA, USA

AUTHORSHIP & CONFLICT OF INTEREST

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