

Transient Transfection at Large Scale for Clinical AAV9 Vector Manufacturing

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INTRODUCTION

Adeno-associated virus (AAV) vectors are potent gene therapy vectors, used to deliver therapeutic transgenes to target tissues. Significant growth in gene therapy, fueled by positive clinical results and approved drugs often require high titer vector preparations to adequately deliver the therapeutic transgene, in great excess of research-level production utilized in many laboratories. AAV is often produced utilizing the transient transfection of adherent HEK293 cells with multiple plasmids.

A HEK293 cell seed train was utilized, using the Xpansion® 200 bioreactor to generate sufficient cell numbers for seeding the production large scale bioreactor. Based on conditions developed in the pilot iCELLis® Nano bioreactor system, we scaled this production to the large scale fixed-bed iCELLis 500 bioreactor (200 m² and 333 m² surface area) enabling large scale production of AAV in adherent HEK293 cells. For efficient scale-up transient transfection process, we have used a strategy of mixing large volume of plasmids with Polyplus PEIpro*, as a transfection agent, to create a large volume transfection complex that is delivered rapidly into the bioreactor without compromising on the complex quality. Following a production phase, >10¹⁶ vector genomes were isolated from crude harvest of the bioreactor.

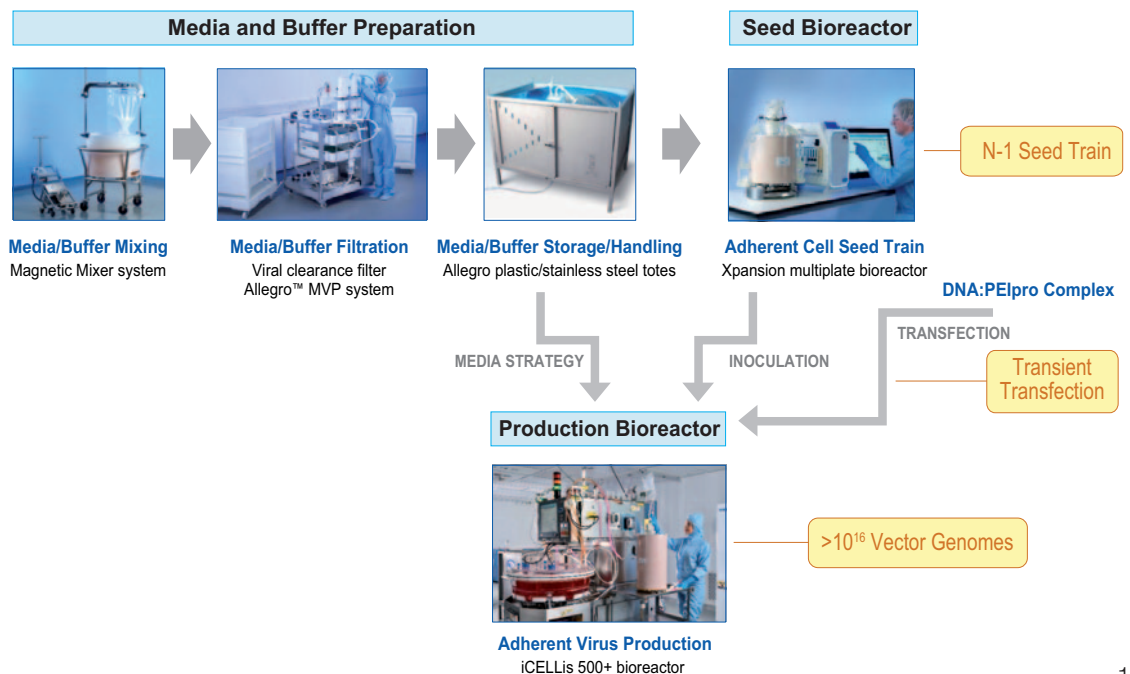
SCALABILITY CHALLENGES

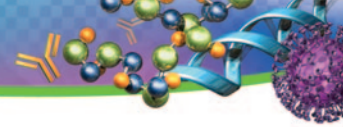
Manufacturing of Viral Vector in Adherent Cell

- ▶ The seed train (N-1) for large scale production with adherent cells requires multiple flatware culture vessels (e.g., roller bottles, multilayer flasks)
- ▶ Rapid delivery of large volume of shear sensitive DNA: PEIpro complex into the large scale production bioreactor
- ▶ Large scale AAV production by adherent HEK293 cells is labor-intensive and costly

Figure 1

Upstream platform (AAV): Scalability challenges





Scalability Challenge 1 – Adherent Cell Seed Train

Figure 2

Seed train and seeding strategy for iCELLis 500+ bioreactor – 500 m²

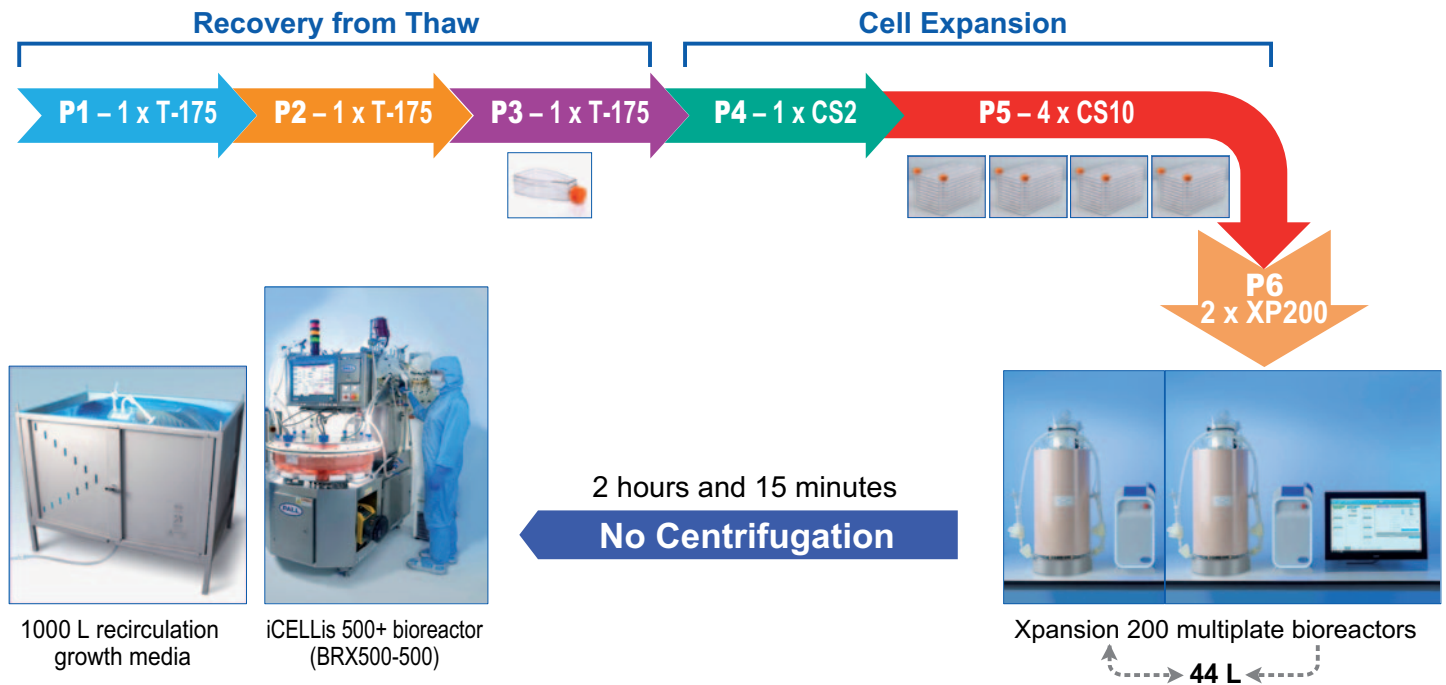
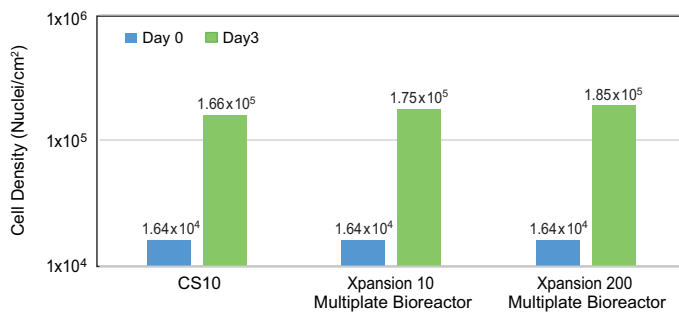


Figure 3

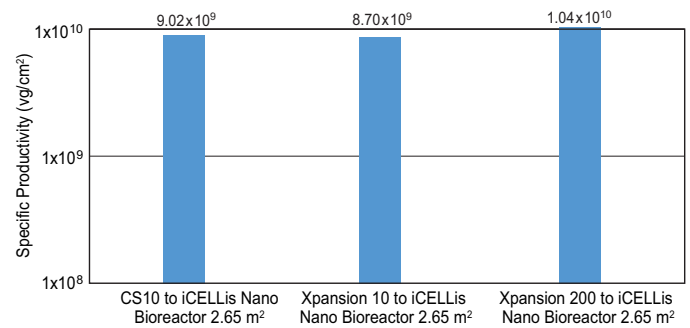
Growth comparability of HEK293 cell: Xpansion bioreactor versus flatware



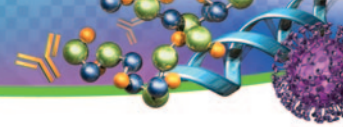
- ▶ Similar growth and cell doubling time were observed in planar vessels as in Xpansion 10 and Xpansion 200 bioreactors

Figure 4

AAV9 viral vector production comparability: Xpansion bioreactor versus flatware. Effect of seed train source on specific productivity: Flatware vs. Xpansion 10 and Xpansion 200 bioreactor



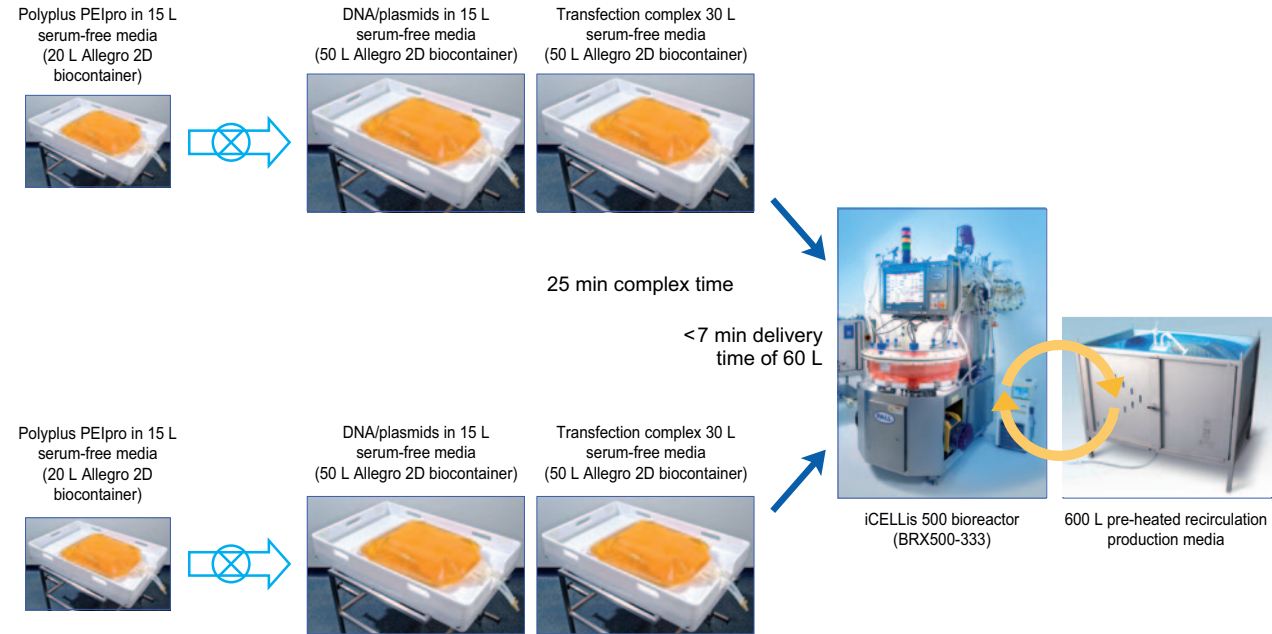
- ▶ Similar productivity in both iCELLis Nano bioreactor observed when using cells amplified by the simplified scalable seed train of Xpansion 200 bioreactor, as they were seeded by the manual seed train of planar vessels



Scalability Challenge 2 – DNA/Polyplus Complex Delivery

Figure 5

Transfection strategy into iCELLis 500 bioreactor

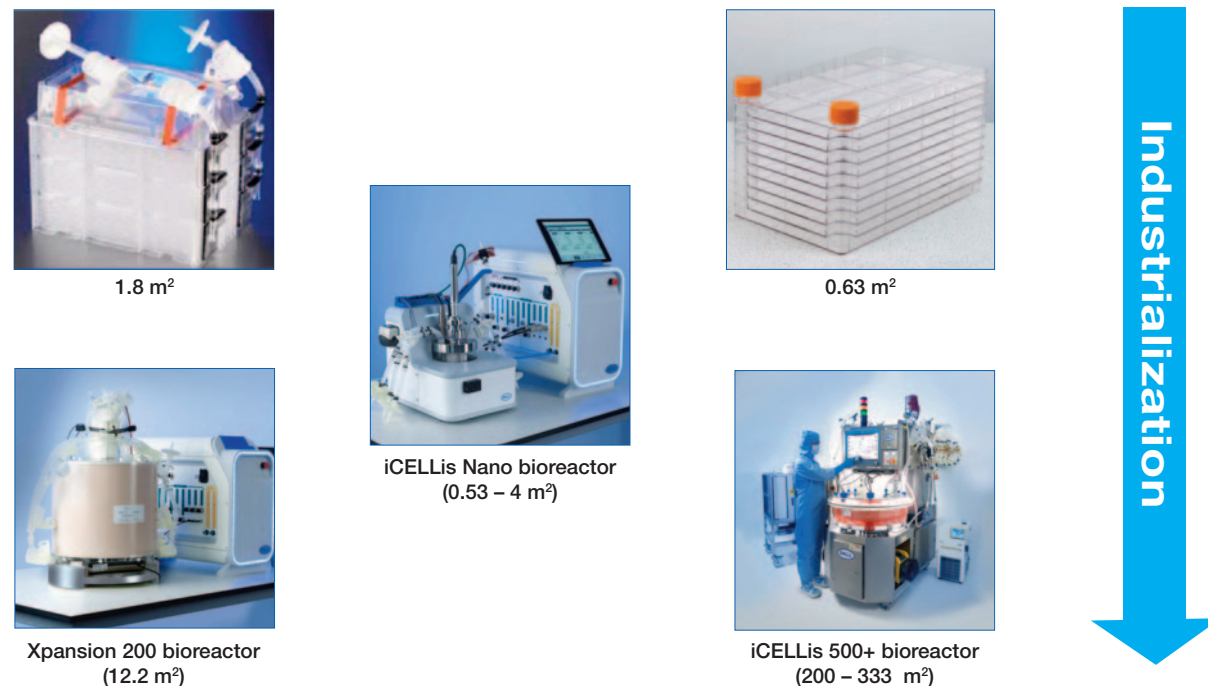


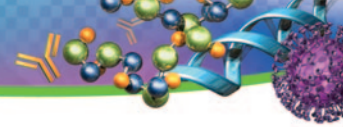
- ▶ The DNA/PEIpro complexes are shear-sensitive. Rapid transfer of the 60 L complexes into the production bioreactor is performed either by gravity flow or usage of low shear stress pumps, such as Levitronix pumps

Scalability Challenge 3 – Large Scale AAV Demand

Figure 6

Evaluating the iCELLis bioreactor for the scalable production of AAV utilizing an adherent HEK293





- ▶ **Technical transfer**
 - Transfer flatware process between sites
- ▶ **Proof of concept**
 - Translate flatware process to iCELLis Nano bioreactor
- ▶ **Process development and scalability in iCELLis Nano bioreactor**
 - Seeding density
 - Simplify seed train
 - Transfection strategy
 - Fixed bed compaction
 - Glucose control strategy
- ▶ **Reproduce**
 - Perform 3 runs with one virus to ensure reproducibility
- ▶ **Scale-up**
 - Scale-up to iCELLis 500+ scale bioreactor (2 runs)

Large Scale AAV Production by Adherent HEK293 Cells Using Commercial Scale iCELLis 500 Fixed-Bed Bioreactor

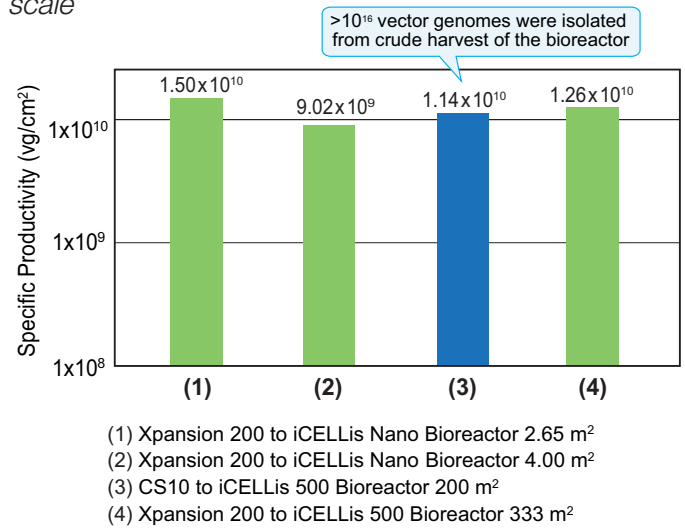
- ▶ **Performance**
 - High and consistent virus titers
 - Predictable scale-up performance from iCELLis Nano bioreactors
- ▶ **Usability**
 - Single-use bioreactor: No cleaning or cleaning validation
 - Reduced operator intervention
 - Reduced footprint: Up to 500 m² surface area ~790 10-layer vessels (CS10)
- ▶ **Process assurance**
 - Closed system
 - Automated and controlled
 - Validated control software



iCELLis 500+ bioreactor

Figure 7

Linear titer scalability: Benchtop to manufacturing scale



- ▶ Parameters determined in the iCELLis Nano bioreactor are transferable and scale linearly to the iCELLis 500 bioreactor

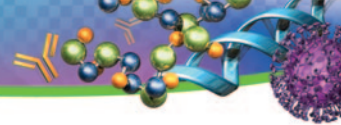
ENABLING

Successful development of an industrial scale upstream process for associated adeno virus manufacture

- ▶ This study demonstrates that a scalable closed manufacturing platform for AAV produced by adherent cells was developed using the iCELLis 500 fixed-bed bioreactor
- ▶ A HEK293 cell seed train was utilized, using the Xpansion 200 bioreactor to generate sufficient cell numbers for seeding the bioreactor
- ▶ Transient transfection reagents were scaled for achieving scalable efficient transfection
- ▶ Total harvest of an iCELLis bioreactor run at 333 m² yielded vector titer > 1 x 10¹⁶ vector genomes per batch

Reference

Kaspar B et al., Assessment of an adherent HEK293 cell transfection process for scalable AAV production in the iCELLis 500 fixed-bed bioreactors, 2019 ASCGT, USA



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