rAAV Large-Scale Manufacturing using BEVS Technology: Scale-Up to 500L Single-Use Bioreactor

Juan Pablo Labbrozzi, Jacek Lubelski, Jeroen Verbruggen, Sherwin Francisco, Danica Blakslee, Nicholas Dion, Mirjam Hein, Scott McMillan

ABSTRACT

Recombinant Adeno Associated Virus is becoming a vector of choice for a variety of human gene therapy applications. This leads to an increased demand for AAV vectors of high quality and quantity to advance new therapies to large patient populations. In order to exploit the full potential of the Baculovirus Expression Vector System uniQure initiated the development of a Single-Use Stirred Tank Reactors (STR) process for rAAV manufacturing. Here we demonstrate similarity between the small-scale (50L) wave-based and STR processes. Furthermore, the feasibility of scaling to 500L scale is shown.

INTRODUCTION

A wave bioreactor based cell culture process is conventionally used at uniQure as the upstream process (USP) platform for production of rAAV. In this process, the expansion of *expres*SF^{+} cells followed by triple infection is performed in 50 L wave bioreactors. Although this process has been successfully used for (pre)clinical and commercial manufacturing of several products, wave bioreactor based processes have an inherently poor scalability. In order to meet the demand of large patient population, a scalable single use (SU) stirred tank reactor (STR) based process has been developed as the new USP platform. As a part of this new platform, uniQure developed a bench-top (2L), pilot-scale (50L) and GMP production scale (500L) processes based on SU STR.

OBJECTIVES

The objective of this study was to perform a comparability assessment based on overall process performance and critical quality attributes (CQA's) of the drug substance obtained from various upstream processes.

RESULTS

The criterion established for bioreactors scale-up was to keep the power input per volume constant and maintain a linear correlation with the volumetric gas flow rate. These parameters were employed to define optimal operational conditions at pilot-scale and final-scale. Appropriate supply of oxygen was determined by the gassing out method to determine the volumetric mass transfer coefficient (k_La). The work presented here enables consistent cell growth performance in all scales developed (**Table 1**).

Table 1. Cell growth performance in Wave Bioreactor and all scales of STR

System	μmax (h^{-1})	t_d (h)	Max. Cell Density
50L Wave Bag	0,031	22,3	Reference
2L SU STR	0,032	21,5	<
50L SU STR	0,034	20,4	=
500L SU STR	0,036	19,2	=

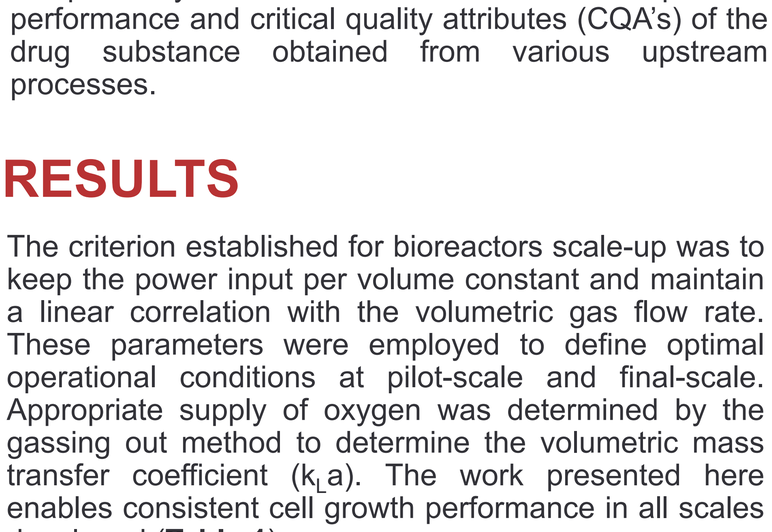

Figure 1 – Outline of the experimental design for the assessment of comparability for both systems.

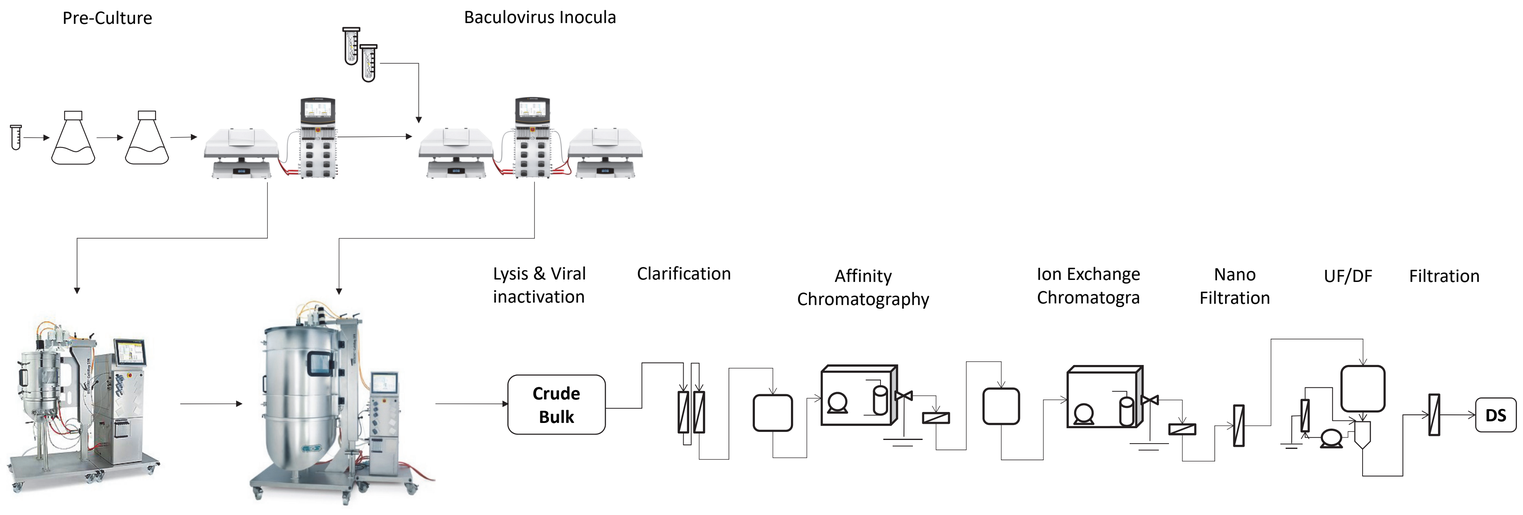

Figure 2 – Outline of the 500L scale process for rAAV production.

ASSESSMENT OF COMPARABILITY

The pilot-scale 50L SU STR system was developed and assessed in parallel to the wave–based platform process (control) (**Figure 1**). Cell growth performance and kinetics of rAAV production after triple infection was assessed confirming the optimal timepoints for process actuators (TOI & TOH) based on productivity and quality criteria (**Figure 3 & 4**). Both bulk materials were further downstream processed for CQA's analysis of the Drug Substance (DS) (**Table 2**).

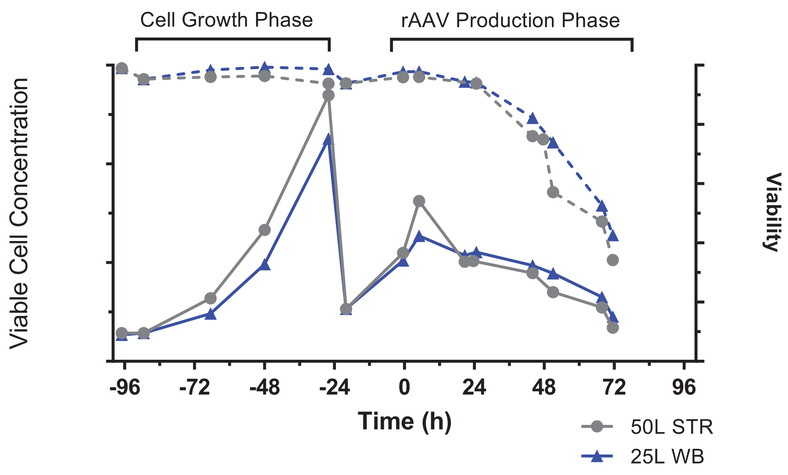

Figure 3 – Comparison of the cell growth profiles and rAAV productions after triple infection of both 50L STR system and 50L (25L working volume) wave system.

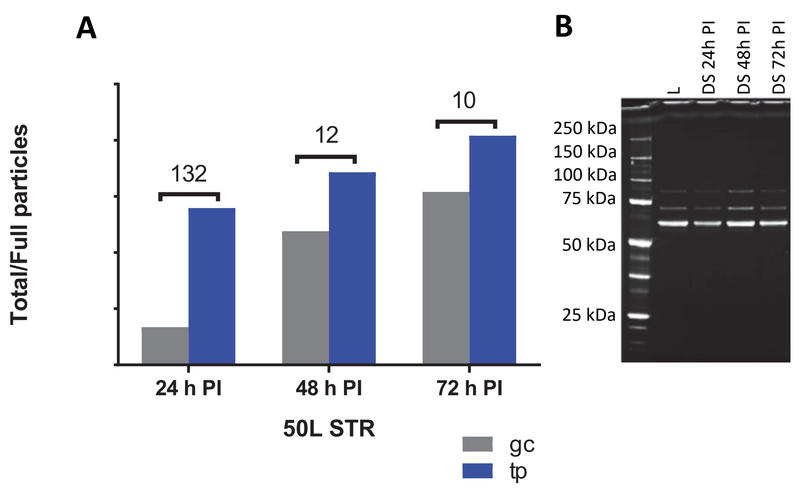

Figure 4 – (A) rAAV particles distribution analysis for optimal Time of Harvest based on Yield-CQA's criteri. (B) Results of polyacrylamide gel electrophoresis (NuPAGE).

- rAAV particles distribution study show comparable dynamics between the two systems after infection (**Figure 4**).
- Based on appropriate comparison of relevant quality attributes, drug substances produced in 50L STR were comparable for biological activity, content, and product purity to current wave bags –based platform (**Table 2**).

Table 2. Drug Substance quantitative and qualitative characterization

Parameters	rAAV Wave-based	rAAV 50L STR
DS [Gc/mL]	2,8e11	2,8e11
DS Total/Full ratio	12,1	12,9
DS Infectivity [Gc/ip]	31	25
DS DNA Comp.	Comparable WS	Comparable WS
DS Protein Comp.	Comparable WS	Comparable WS

500L STR SHAKE-DOWN BATCH

After the confirmation at pilot-scale of DS comparability combined with bioengineering characterization of the 500L STR; a "shake-down" run has been performed to assess the feasibility of producing the same product at the 500L scale.

- For the 500L -scale process, modifications to the preculture and seed expansion strategy (introducing wave bioreactors for the baculovirus inocula production) were introduced (**Figure 2**).
- A comparative growth behavior was observed for the 500L STR during cell expansion with all USP systems and all parameters were within expected ranges.
- Baculovirus inocula material produced in 10L wave systems fulfilled all the quality criteria.
- Overall process performance, based on volumetric productivity and CQA's of DS, was comparable to current wave –based platform (**Table 3**).

Table 3. DS quantitative and qualitative characterization of 500L STR process

Parameters	Evaluation Criteria	rAAV 500L STR
DS [Gc/mL]	≥0,7e13	1,7e13
Total Particles [Tp/mL]	≥5,0e13	1,6e14
DS Total/Full ratio	≤20	9,2
DS Infectivity [Gc/ip]	≤72	54,6
DS DNA Comp.	Comparable WS	Comparable WS
DS Protein Comp.	Comparable WS	Comparable WS

CONCLUSION

uniQure's rAAV manufacturing process at 500 liter scale has shown to be comparable to current wave bioreactor platform. Based on this proof of concept, the next generation of commercial products could be produced using stirred tank reactors at higher scales generating the desired product quality at commercial volume and acceptable costs of goods

uniQure abstract presented at ESCGT 2018

1. "Surgery and bleed management in patients receiving AMT-060 in a Phase I/II trial: evaluation of the safety of exogenous FIX treatment after gene transfer". **P026**.
2. "Comprehensive comparative evaluation of the qualitative attributes of AAV5 batches produced in mammalian and insect cells". **P454.**
3. "uniQure downstream purification process shows excellent viral clearance capabilities". **P478**