Scale-up of a Perfusion-Based AAV Manufacturing Process

Authors: Becci Gunn, Ashley Vey, Florian Dziopa

MEIRAGTx

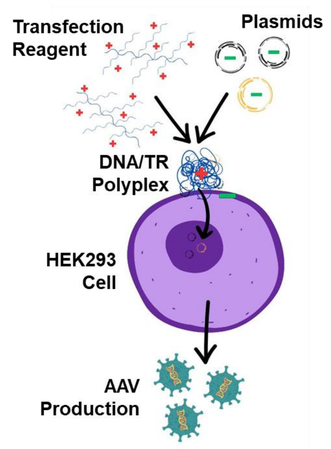
MSAT Upstream Process Development, MeiraGTx, London, UK

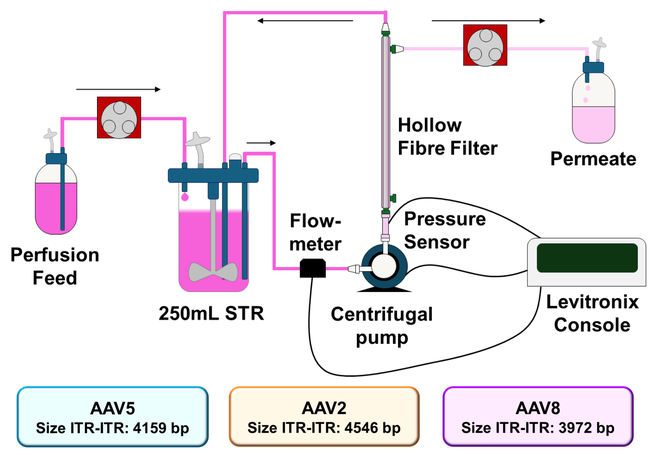
Introduction

MeiraGTx's perfusion-based adeno-associated viral vector (AAV) manufacturing platform process was previously optimized and modulated through the choice of transfection reagents, AAV production enhancers, and transfection parameters, in 250mL stirred tank reactors.

The work presented here shows the scale-up of our perfusion-enhanced AAV process to the 40L production scale, with a focus on the translation of perfusion parameters.

Perfusion-Enhanced HEK293 Triple-Transfection Platform





AAV5 Size ITR-ITR: 4159 bp

AAV2 Size ITR-ITR: 4546 bp

AAV8 Size ITR-ITR: 3972 bp

- Up to 2.2X Increase in VG titre
- Improved Quality Profile
- 33-50% Reduction in pDNA Usage
- Up to 2.2X Reduction in COGs per Dose

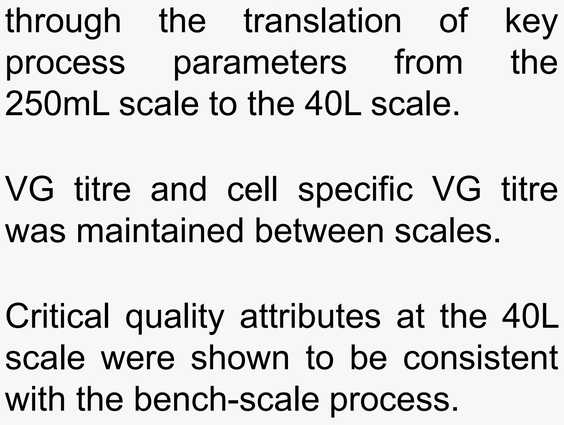
Methods

Perfusion Culture

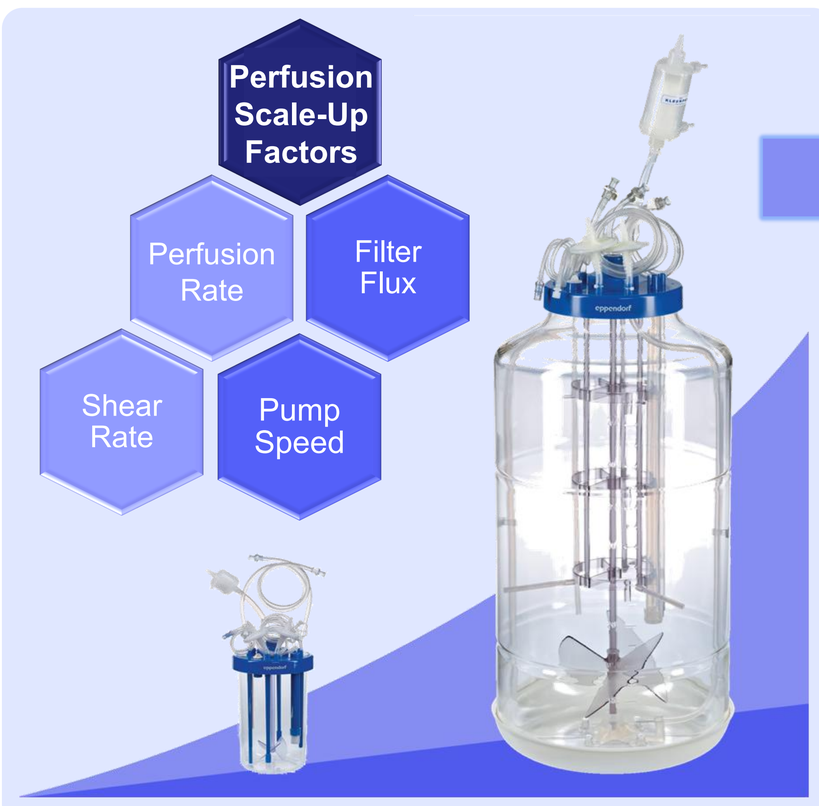
Cells were recirculated through a hollow fibre filter at constant flow rate using a centrifugal pump (Levitronix, Switzerland). A total of 2 vessel volumes of medium was perfused over ~10h prior to transfection. HEK293 cells were transfected at a target VCD range of 4.5-5.0x10^6 VC/mL. Product was harvested 3 days post-TFX. Harvest culture was clarified, then purified via affinity chromatography (AAVX).

Analytics

Viral genome (VG) titre and residual pDNA (KanR) were determined in lysate using qPCR. % full capsids and hcDNA were measured in AAVX eluate by AUC and qPCR, respectively.

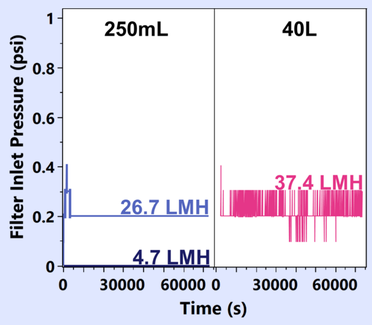


Scale-Up to 40L Manufacturing Scale



- Scalable Perfusion Process
- VG Titre in Line with Bench-Scale
- AAV Quality Profile Maintained at 40L Manufacturing-Scale

Perfusion Filter Scale-Up



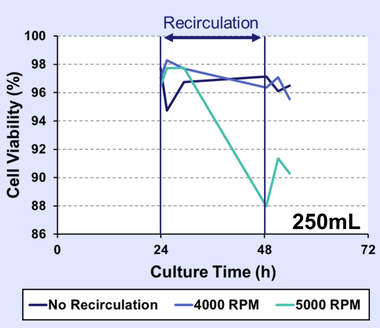
$$Flux\ (\mathrm{LMH}) = \frac{Permeate\ Flow\ Rate\ (\mathrm{L/h})}{Filter\ Surface\ Area\ (\mathrm{m^2})}$$

Bioreactor Volume (L)	0.213		34	
Filter Surface Area (cm^2)	85	15	3,900	1,800
Permeate Flow Rate (mL/h)	40		6,732	
LMH	4.7	26.7	17.3	37.4

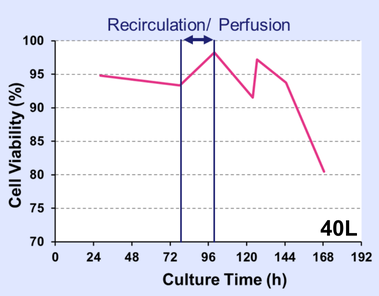
Two options for scale-up

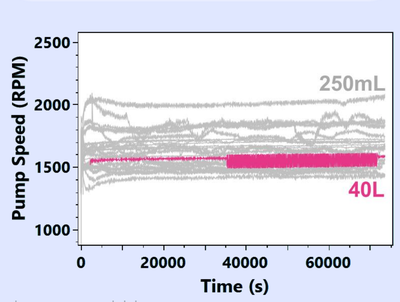
- With 15cm^2 filter at 250mL scale, negligible pressure at filter inlet (0.2 psi) with no increase, indicating minimal fouling.
- With 1,800cm^2 filter at 40L scale, inlet pressure remained below 0.3 psi and profile was comparable to 250mL, confirming suitable filter size.

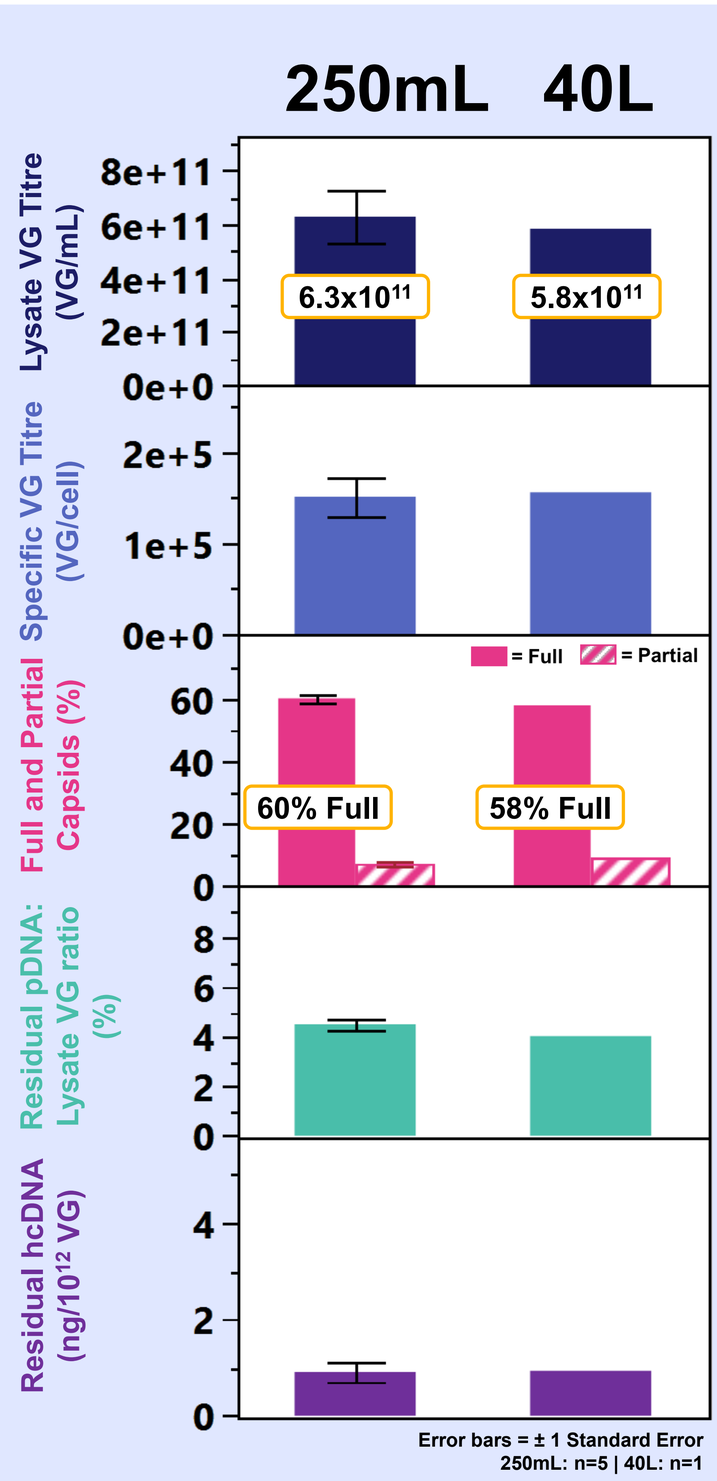
Centrifugal Pump Scale-Up



- Negative impact of shear was found ≥ 5000 RPM at 250mL.
- Pump size was scaled to maintain pump speed between scales.
- Cell viability did not drop during recirculation at 40L scale, and pump speed was in line with 250mL process, confirming suitable pump size.







- VG titre and cell specific VG titre at the 40L manufacturing scale was in line with the 250mL bench scale.
- AAV produced with a scaled-up 40L perfusion process maintained high product quality, equivalent to the 250mL scale.

Conclusions

- MeiraGTx's optimized perfusion platform was successfully scaled up from bench-scale to the 40L manufacturing scale, demonstrating a scalable perfusion process.
- Process efficiency was maintained through the translation of key process parameters from the 250mL scale to the 40L scale.
- VG titre and cell specific VG titre was maintained between scales.
- Critical quality attributes at the 40L scale were shown to be consistent with the bench-scale process.
- Residual DNA levels were maintained at an adequate level for patient safety across production scales.