DEVELOPMENT OF A SCALABLE PLATFORM FOR AAV MANUFACTURING

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Introduction: AAV vectors are an appealing tool for both ex-vivo and in-vivo gene therapy. The number of AAV gene products entering early and late phase clinical trials is significantly on the increase. High cost of goods, low process yield, and poor product characterisation are all metrics that require substantial development and improvement within this emerging field. Currently most AAV vectors are manufactured using an adherent process and the demand currently outstrips capacity. Whilst switching from a classical 2D approach to a suspension process using single use bioreactors might be appealing to meet the requirement in term of doses and patient number for late stage of development (e.g Phase III study), this can come at the expense of laborious and costly comparability study. Hence, a reliable, low risk manufacturing platform delivering at the desired scale should be identified early on during development. Therefore, it becomes clear that there is a need to develop the next generation of upstream platform processes using a suspension cell line in STRs. Following Quality by design principles, we sought to develop this platform for AAV manufacturing. Using a scale down model, we investigated the impact of a broad range of process parameters using a design of experiment approach on AAV productivity. Scalability of the newly designed process as well as the impact of our USP on full capsid enrichment during our purification process has been investigated. Overall, our latest efforts in developing an end to end scalable suspension platform for AAV manufacturing will be presented.

Manufacturing challenges

Despite all efforts…

'Viral vector manufacturing capacity is a barrier that is limiting the development of therapies and places the industry at risk.

We've seen an improvement in capacity but demand has increased'

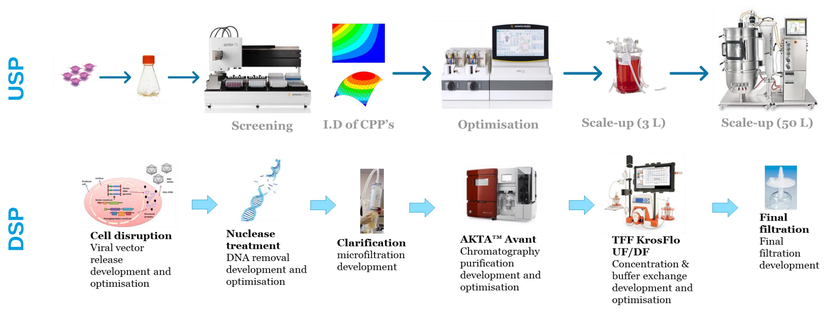
Manufacturing capacity
- –Limited capacity
- –Skills Shortage
- –Labour intensive/High Risks
- –Large Volume
- –Unit Operation selection

Processing challenges
- –Low process yields
- –Suboptimal unit operation
- –High cost

QC challenges
- –International standards
- –Variable Analytical assays
- –Labour intensive
- –Time consuming

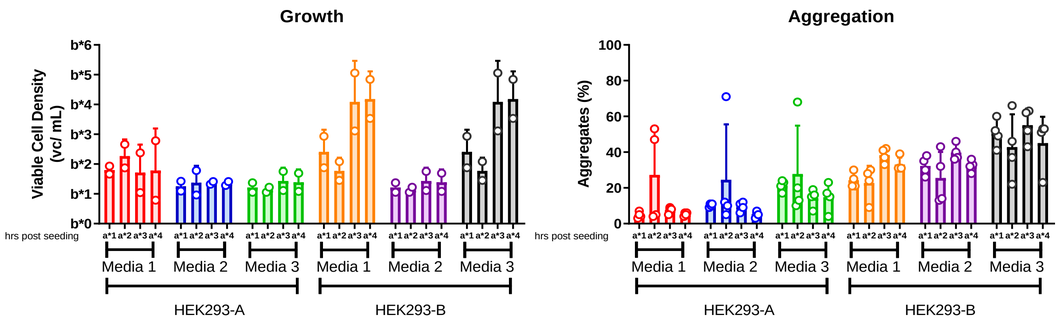
Regulatory Challenges
- –Automation
- –Continuous processing
- –PAT Implementation
- –AI

Our Approach for process optimisation

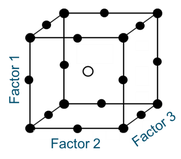


Cell line and media selection

Two cell lines were cultivated in three medias using historical culture parameters, on the Ambr® 15 system, transfection in triplicates.



USP Optimisation



Statistical design of experiments (DoE) was applied to determine the relationship between factors affecting the process and the output of that process. Transfection efficiency and AAV titre were selected as outputs of the process.

CPPs Identification
- Seeding density
- DNA quantity
- Transfection methods
- Physical parameters

Screening DoE #1

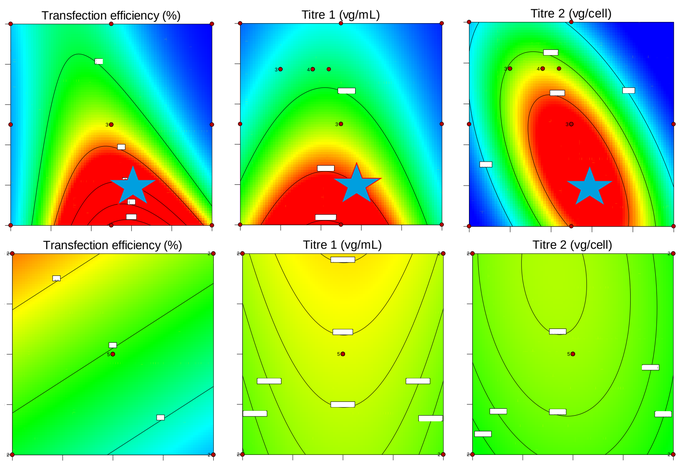
CPPs Identification Mapping Design Space
- Seeding density
- DNA quantity
- Transfection methods
- Physical parameters

Screening DoE #2

CPPs Optimisation
- Seeding density
- DNA quantity
- Plasmid Ratio
- Physical parameters

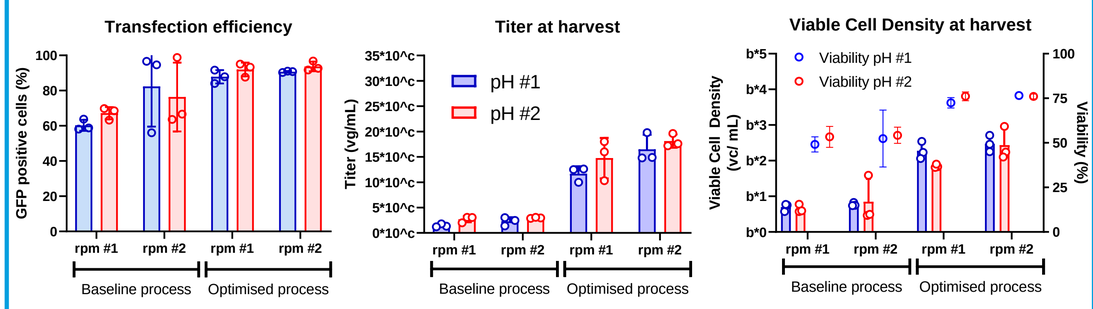
Screening DoE #3,4,5

Optimum *Optimum*



DoE #2
3D surface response of modelled outputs. Y axis represent input (1) and X axis input (2). Red colour indicates higher response and Blue lower. The stars show predicted best combination.

DoE #5
3D surface response of modelled outputs. Y axis represent input (1) and X axis input (2).

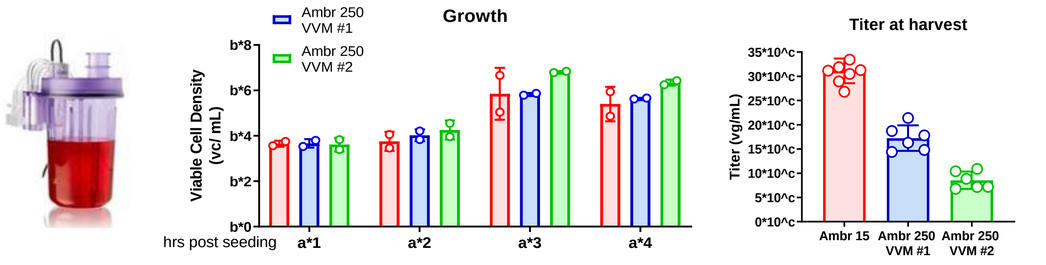


Optimised process led to a eight times titer increase while decreasing the cost.

USP Scale-up

Scale-up from Ambr® 15 to Ambr® 250
- Maintenance of geometric similarity
- Equal specific energy dissipation rates (volumetric power input P/V)

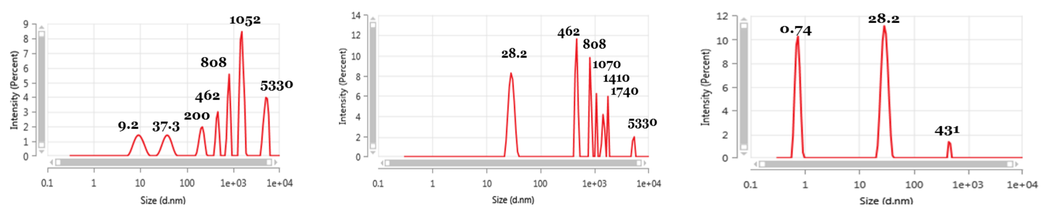
Screen Optimisation → **Optimisation Scale up** → **Scale up Proving Run**



Successful scale-up to 250mL using the optimised process developed at Ambr® 15 scale. Next steps: further optimisation at Ambr® 250 scale, and scale up to 2.0L UniVessels®.

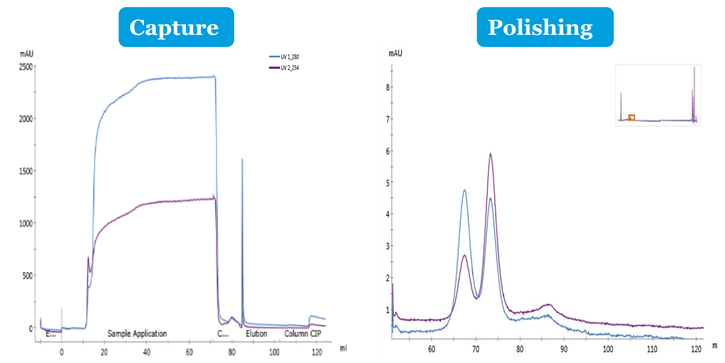
DSP optimisation

Load | **Post-capture** | **Post-polishing**



Multi-angle dynamic light scattering (MADLS®) from Zetasizer®. X axis represent size (d.nm) and Y axis represent intensity of the signal.

Capture | **Polishing**



Post capture (left) and post polishing (right) material purified on AKTA®.

Two-steps purification with overall yield >75% With 60% full capsids.

Conclusion

Conclusion: Here is presented a scalable AAV manufacturing platform using STRs bioreactors for production. Production process was defined and optimised at small scale and is now being scaled up at production scale. Purification and analytics are already enabling efficient end to end process but will be further optimised.

We work with Innovate UK

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