cell & gene

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Achieving rAAV Bioreactor Titer Of >1E15 vg/L With Novel Plasmid Transient Transfection Platform

Source: Oxford Biomedica

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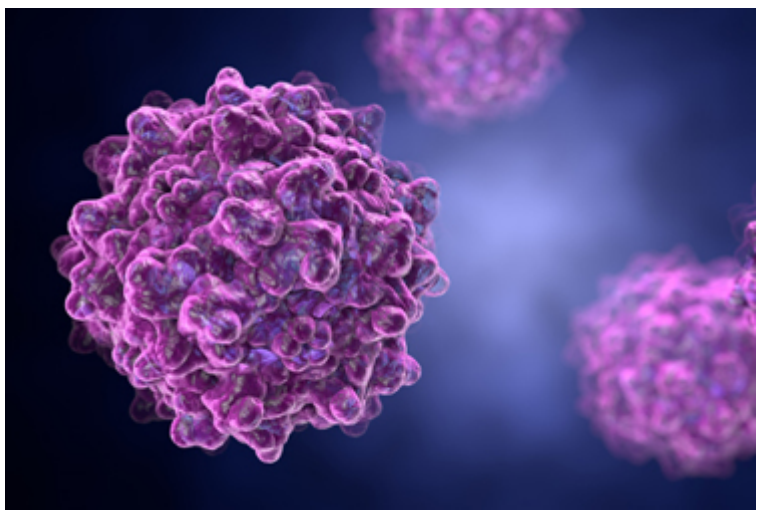

Recombinant adeno-associated virus (rAAV) vectors have become a popular gene therapy delivery platform due to their high safety profile, low immunogenicity, and capacity for transduction into different cell and tissue types. Furthermore, rAAV can be delivered through either intravenous injection (systemic delivery) or direct injection into the affected tissue (targeted delivery).

Transient transfection of plasmids into suspension HEK293 cells is the most common platform for producing rAAV, owing to its flexibility and speed in moving drug candidates into clinical trials. Despite this, its utility has been limited by its perceived low productivity and poor scalability when compared to other platforms (e.g., Baculovirus expression systems with insect sf9 cells, adenovirus-infected producer cell line systems, and Herpes simplex virus helper systems) [1]. Based on data from 136 ongoing clinical trials, 77% of systemically delivered rAAVs are dosed at 1E14 – 1E16 vector genomes (vg) or higher, whereas 59% of targeted delivered are dosed at 1E12 - 1E14 vg or higher [2]. The high level of rAAV dosage poses a major supply and cost challenge for manufacturers and gene therapy companies – in some cases of systemic delivery, one 200L bioreactor batch is insufficient to dose one patient.

During a typical transient transfection process, cells are grown to 1-2E6 viable cells/mL before plasmids are added to transfect those cells. When determining how to increase the titers for a prototypical transfection process, there are two primary considerations: the number of cells produced prior to transfection, and the productivity of the cells themselves in generating vector. By increasing both, manufacturers can achieve higher concentrations of viral genome. The concept of higher cell density transfection has been pursued by others, but this approach has often produced what is known as the “cell density effect”, defined as a decrease in rAAV productivity when cells are transfected at higher than 2E6 viable cells/mL [3].

When pursuing transient transfection, there are three key factors that can serve to inhibit a process: poor packaging, low productivity, and an inability to scale up. In conventional plasmid transfection processes, standard across the industry, bioreactor titers range from 5E13 to 2.4E14 vector genome per liter (vg/L) [4]. The pursuit of higher rAAV productivity for transient transfection has led to an increased focus on the factors that particularly influence the upstream processing. To improve bioreactor vector genome (vg) productivity, Oxford Biomedica Solutions (OXB Solutions) has developed a high cell density process with a novel transfection method. By homing in on medium additives, transfection cell density, vector and plasmid design, process optimization, plasmid transfection understanding, and equipment engineering and process control, OXB Solutions has been able to demonstrate bioreactor vg titer higher than 1E15 vg/L and successful scale-up to 2,000L bioreactor.

Overcoming the Obstacles to Achieving High Bioreactor vg Titer

OXB Solutions has circumvented issues with low titers by investigating cell and rAAV biology, as well as the nutrients and byproducts involved in its process, eventually identifying a novel medium additive that has enabled more consistent, scalable productivity through greater transfection efficiency and higher rAAV cell-specific productivity. A transfection efficiency analysis protocol has been established utilizing flow cytometry, particle size measurement, and rAAV-GFP technologies, and found that the “cell density effect” is likely related to the decrease in plasmid transfection efficiency, the depletion of nutrients for cells to produce rAAV, and the accumulation of cell culture by-products.

In optimizing its plasmid transfection and rAAV production, OXB Solutions took a systematic approach to optimizing total amount of plasmid DNA, ratio of plasmids, ratio of plasmid DNA to cells, amount of transfection reagent, ratio of plasmid DNA to transfection reagent, transfection solution mixing time, as well as equilibration time, and was able to identify a set of parameters optimal for its process. Additionally, several key bioreactor operating parameters have been optimized, such as agitation, pH, temperature, dissolved oxygen (DO), and dissolved CO_2 (pCO_2) for cell growth as well as rAAV production in 2L bioreactors.

Through its creative work, OXB Solutions was able to increase its bioreactor vg titer by nearly a log. With its three-plasmid system, 11 different AAV serotypes and capsid variants were tested and saw an average bioreactor vg titer of 5.3E14 vg/L (range of 3.0-8.9E14 vg/L) and an average %full vectors (coming out of the bioreactors) of 34% (range of 17-64%) (**Figure 1**). With OXB Solutions’ dual-plasmid system [5], it obtained an average bioreactor vg titer of 1E15 vg/L (range of 6.6E14 – 1.4E15) across 9 different AAV serotypes and capsid variants and an average %full vectors (coming out of the bioreactors) of 45% (range of 28-69%) (**Figure 2**).

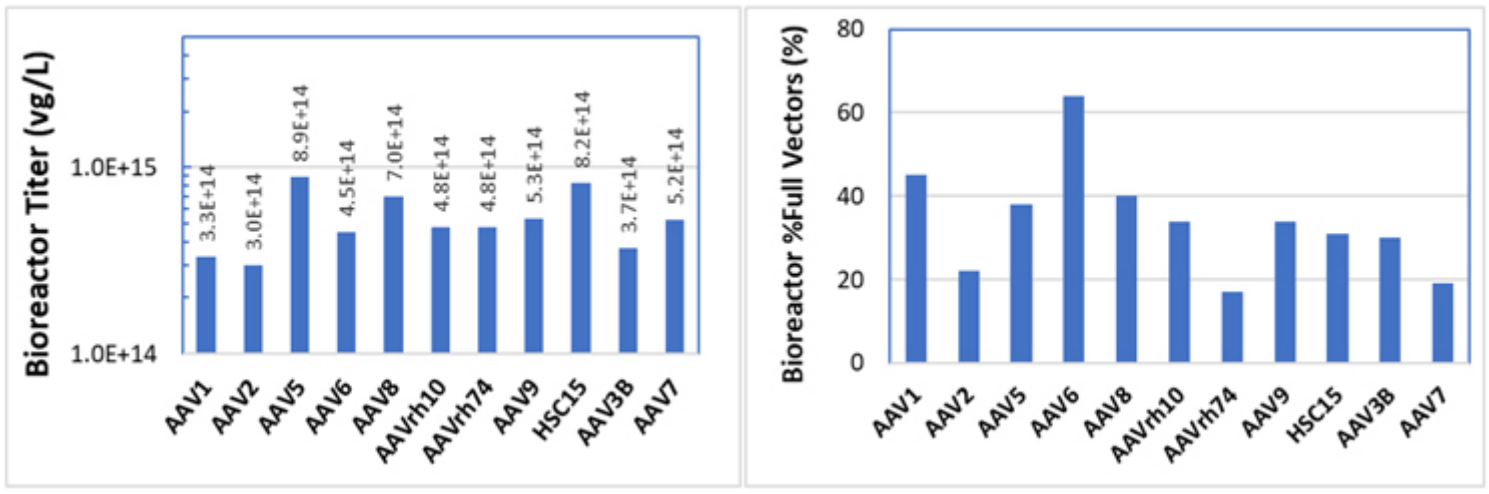

Figure 1. Productivity assessment of eleven rAAV serotypes and capsid variants with triple-plasmid transfection. Transfections were performed in 2 L bioreactors. Bioreactor %full vectors was calculated by dividing ddPCR vg titer by ELISA capsids titer.

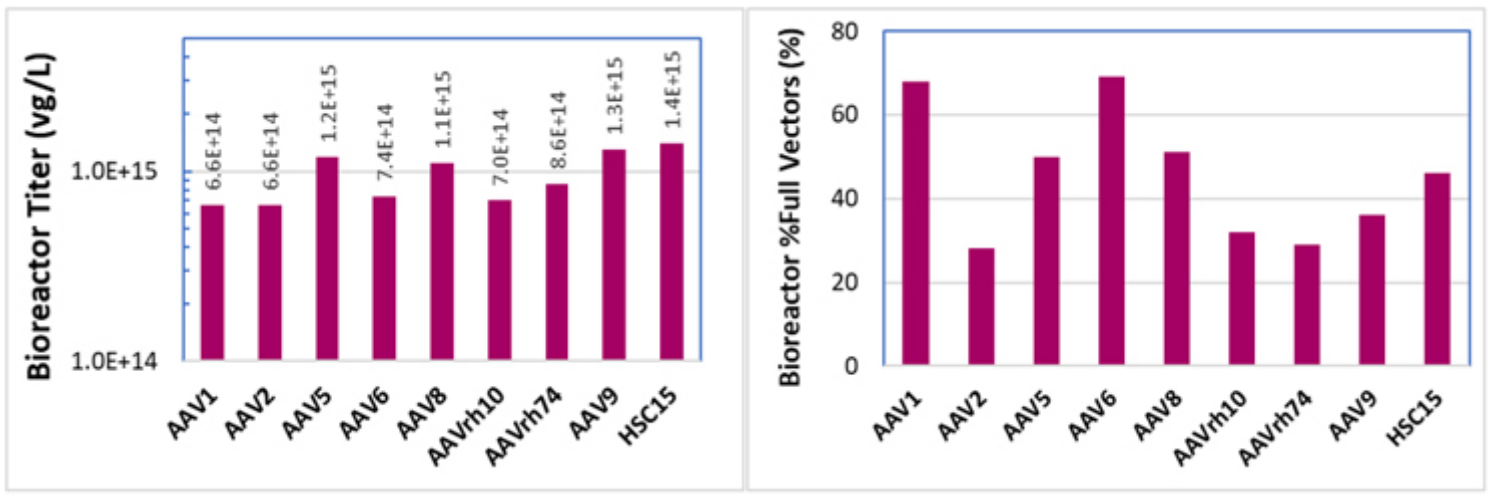

Figure 2. Productivity assessment of nine rAAV serotypes and capsid variants with dual-plasmid transfection. Transfections were performed in 2 L bioreactors. Bioreactor %full vectors was calculated by dividing ddPCR vg titer by ELISA capsids titer.

Scaling up to Decrease Manufacturing Cost

Scaling up transient transfection is a uniquely challenging proposition. This is chiefly because, in addition to the three factors that typically inform bioreactor scale-up for a product (mixing, mass transfer, and shear stress), transient transfection also requires developers to evaluate how the transfection process itself will scale in conjunction with these other variables. To scale up its rAAV plasmid transient transfection bioreactor process, OXB Solutions gave particular consideration to the preparation and addition of its novel transfection solution to ensure similar transfection efficiency between small and large scales.

Scientists and engineers at OXB Solutions used shake flasks and 2L bioreactors to define the process parameters and worked to identify the appropriate containers and pumps to support the addition of transfection mix into the bioreactor. Finally, OXB Solutions designed a comprehensive, small-scale satellite bioreactor strategy during scale-up to assess all critical process steps in order to pinpoint any differences between the small-scale and large-scale process. Through this systematic work, OXB Solutions has now successfully scaled up 3 different programs to the 2,000L scale with consistent bioreactor vg titer (**Figure 3**) and high product quality of drug substance with fully intact vectors close to or higher than 90%.

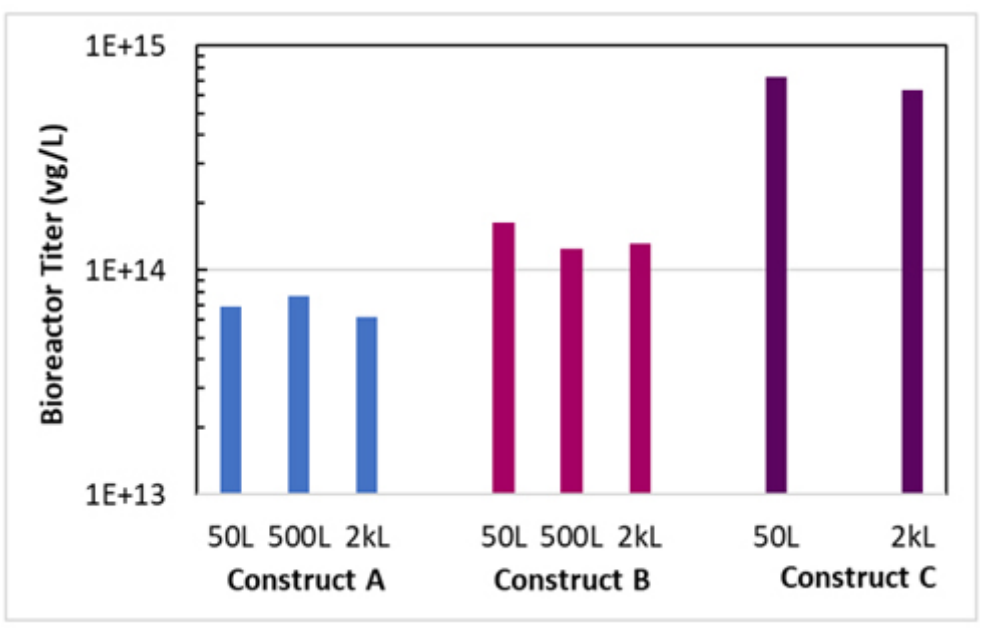

Figure 3. Bioreactor rAAV productivity comparison at 50L, 500L and 2kL scales for three rAAV Constructs. The three-plasmid system was used for Constructs A&B and the dual-plasmid system was used for Construct C.

Conclusion

OXB Solutions' new platform process is highly reproducible and allows for plug-and-play use, now tested in over 11 different AAV serotypes and capsid variants, typically resulting in 2L bioreactor vg titers close to or above 1E15 vg/L, as well as fully intact capsids coming out of the bioreactors close to or above 50 percent. The new platform process has also been successfully scaled up from 2L to

50L and now to the 2,000L bioreactor level, with consistent vg productivity and drug substance product quality, with 90 percent fully intact vectors.

High titer, high product quality, proven scalability, and accelerated development are the foundations of OXB Solutions' system, resolving some of the key manufacturing challenges faced by rAAV developers. With approximately 1E15 vg/L bioreactor titer in comparison to that of currently available in the field (5E13 to 2.4E14 vg/L) [4], this novel system is able to produce significantly more patient doses per batch and can help to significantly drive down the number of batches needed and the overall manufacturing cost for both clinical and commercial operations.

As smaller companies continue to innovate in the cell and gene therapy space, the utility of partners striving to innovate alongside them will prove invaluable in furthering novel science. At OXB Solutions, taking processes from concept to commercialization is core to its client offering; with decades of experience, a wealth of expertise, and a range of capabilities in the rAAV space, OXB Solutions can help partners achieve the necessary speed to market, quality, and scale to bring novel, life-changing therapies to bear. Its work improving productivity in particular could have important cost and time implications for many advanced therapies, ultimately improving the access and economics for patients who need them. OXB Solutions is continuing to innovate on the next generation of its platform technology, with plans to further increase its bioreactor transfection cell density to 10-20E6 cells/mL and introduce its perfusion process in order to facilitate even greater vg productivity.

References

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