FASTFACTS

Scalable cell culture and transient transfection for viral vector manufacturing

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The acceleration of clinical cell and gene therapy programs is creating a growing worldwide demand for lower-cost and higher-yield viral vector manufacturing, especially for adeno-associated virus (AAV) and lentivirus. Increasing capacity by improving cell densities and cell numbers per batch could address this need, whether by a scaled up suspension process, or a scaled out adherent process. However, it's not enough to simply grow more cells; the production process, including transfection methods, must also be optimized to maintain product quality while minimizing operating costs. This poster will explore two key factors affecting scalability and yield: choice of cell culture platform, and optimizing transient transfection.

CHOICE OF CELL CULTURE PLATFORM

The cell culture platform used is a key determining factor in the capacity of viral vector manufacturing. Suspension culture is more easily scaled; however, transfection of adherent cultures remains a tried-and-true method with proven protocols and an extensive knowledge base.

Table 1 compares the number of vessels needed and the total surface area achieved for the major Corning bioprocess vessels. The best system for a given process depends on many factors: there is no one-size-fits-all solution. For example, if footprint is a key consideration, the Corning® CellCube® system is an obvious choice. However, the CellCube requires a bioreactor, whereas vessels such as the Corning CellSTACK® culture chambers, require only incubators.

Table 1. Adherent culture platform comparison.

Platform	No. of Vessels	Total Surface Area (cm^2)	Media Volume (L)	Required Equipment
Polystyrene Microcarrier 40L Bioreactor[1]	1	403,000	40	Bioreactor, controlle
Dissolvable Microcarrier 40L Bioreactor[2]	2	400,000	40	Bioreactor, controller
Corning® CellCube® 100-layer module warm room	4	340,000	32	Controller, oxygenator,
Corning HYPERStack® 36-layer vessel	20	360,000	78	Incubators
Corning HYPERFlask® vessel	209	359,480	117	Incubators
Corning CellSTACK® 40-layer vessel manipulator	14	356,160	38 to 45	Incubators/ warm room
Corning CellSTACK 10-layer vessel	56	356,160	38 to 45	Incubators/ warm room
Roller bottle	400	340,000	51	Racks, warm room

[1]Assumes 28 g/L of Polystyrene microcarrier, 1400 g total Polystyrene microcarriers used
[2]Assumes 2 g/L of Dissolvable microcarrier, 100 g total Dissolvable microcarriers used

OPTIMIZING TRANSFECTION

Another critical aspect for successful viral vector production is transfection, with transient transfection the most efficient means of producing viral vectors. Best practices for transient transfection include:

- Spend sufficient time optimizing your culture, ensuring you have healthy, actively dividing cultures.
- Choose transfection reagents that have been optimized for your processes and are scalable, such as PEIpro® from Polyplus.
- Develop a robust protocol with the transition from pilot-scale to production-scale in mind, whether the final production scale is intermediate or large, adherent or suspension.
- Don't assume viral titer will correlate with transfection efficiency.

PROOF-OF-CONCEPT: SCALABLE TRANSFECTIONS IN AN ADHERENT PLATFORM

We carried out two proof-of-concept studies of green fluorescent protein (GFP) transfections, to act as a starting point for customer optimization. **Figure 1** shows transfection in a Corning CellSTACK 10- chamber vessel. HEK293 cells were seeded at 5,000 cells per cm^2 with PEIpro and allowed to grow to roughly 50% confluence or transfection. The transfection mix was added directly to the CellSTACK 10-chamber vessel, mixed, and incubated until harvest, with no medium exchange before or after transfection. Two days after transfection, the cells were harvested and a GFP transfection efficiency assay was performed. There were 83% GFP-positive viable cells, typical for this protocol.

We achieved very similar results with the CellCube 25-chamber system **(Figure 2)**, with 70% to 80% GFP-positive viable cells. The only difference in protocol is that the CellCube must be rotated to ensure transfection of both sides.

Figure 1. Corning CellSTACK culture chamber PEIpro transfection. Left: Top chamber of CellSTACK vessel, showing cell confluence on day of transfection. Middle: CellSTACK 10-chamber vessel transfected with GFP (top) and negative control (bottom). Right: GFP-positive cells in the CellSTACK vessel.

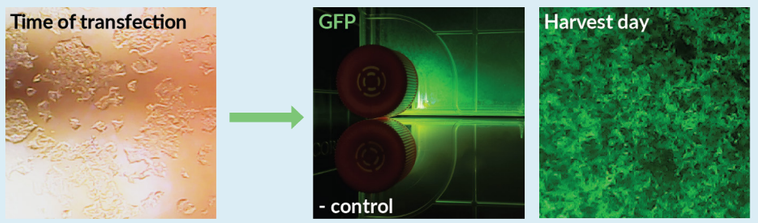
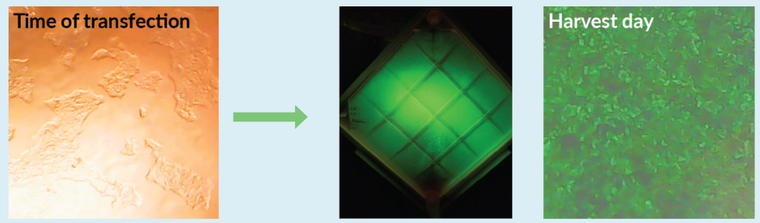


Figure 2. Corning CellCube system PEIpro transfection. Left: Cell confluence on day of transfection. Middle: CellCube 25-chamber vessel transfected with GFP. Right: GFP-positive cells in the CellCube vessel.



CONCLUSIONS

Many cell and gene therapy applications are dependent upon viral vectors for recombinant gene delivery. Efficient transfection of a large biomass of cells represents a significant challenge for transient viral vector production systems. However, identifying a scalable adherent cell culture platform for high-density cell culture helps to streamline the transfection process. Further, utilizing transfection reagents that are optimized for transfection at scale ensures robust and reproducible transactions from process development through to large-scale manufacturing.

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