Assessment of Bioreactor Systems for Up-Scaling Lentivirus Production

Alexandra McCarron, Nathan Rout-Pitt, Chantelle McIntyre, Martin Donnelley, David Parsons

1 Adelaide Medical School, Discipline of Paediatrics, University of Adelaide, Adelaide SA, Australia
2 Respiratory and Sleep Medicine, Women's and Children's Hospital, North Adelaide, SA, Australia
3 Robinson Research Institute, University of Adelaide, Adelaide, SA, Australia.

Background

As gene therapy research progresses from *in vitro* and small animal studies into pre-clinical and clinical phases, there is a growing demand for large volumes of lentiviral (LV) vector, and accordingly, the means to produce such quantities. In this study, three bioreactor systems were evaluated as potential approaches for up-scaling transient production of a HIV-1 based LV vector.

Methods

Trial 1: CelliGen® BLU stirred-tank vessel (1.4 L)

The BioBLU® 3c vessel with pitched-blade impeller was operated in batch mode using the conditions outlined in Table 1. The 1.4 L culture was seeded with suspension HEK 293FT cells in FreeStyle™ 293 expression media at 5 x 10^5 cells/mL. When a density of ~ 1 x 10^6 cells/mL was achieved, the culture was transfected with a pHIV-1 vector expressing LacZ, VSV-G envelope plasmid, and three helper plasmids using polyethylenimine (PEI) (Table 3). After 48 hours, samples were harvested and clarified using 0.45 µm syringe filtration.

Trial 2: CelliGen® BLU packed-bed basket vessel (3.75 L)

The BioBLU® 5p packed-bed basket with Fibra-cel® disks was operated in batch mode using the parameters outlined in Table 1. The 3.75 L culture was seeded with adherent HEK 293T cells in DMEM/10%FCS. At a cell density of ~ 3 x 10^4 cells/cm^2, the culture was transfected with pHIV-1-LacZ, VSV-G envelope plasmid, and three helper plasmids using PEI (Table 3). 8 hours later the media was changed to OptiPRO™ SFM. At 48 hours post-transfection, the supernatant was harvested and processed using anion-exchange chromatography and ultracentrifugation. The viral pellets were resuspended in a final volume of 1 mL.

Table 1: Operational parameters for CelliGen® BLU stirred-tank and packed-bed runs

	Stirred-tank (1.4 L)	Packed-bed (3.75 L)
Temperature	37°C	37°C
pH	7.2	7.2
DO	50%	50%
Agitation	65 RPM	80 RPM
Gas supplied	2-gas mix control (Air, CO_2 for pH control)	2-gas mix control (Air, CO_2 for pH control)
Gas flow (sparge)	Cascaded range: 0.015 - 0.1 L/min	Cascaded range: 0.0375 - 0.1 L/min

Trial 3: ReadytoProcess WAVE™ 25 (0.5 L)

The 0.5 L culture was seeded with suspension HEK 293FT cells at a density of 5 x 10^5 cells/mL in FreeStyle™ media and was operated as a batch run using the conditions outlined in Table 2. The system exhibited poor cell growth, and consequently, the cell density required for transfection (1 x 10^6 cells/mL) was not achieved.

Table 2: Operational parameters for ReadytoProcess WAVE™ 25 run

Temperature	37°C
pH	7.2
DO	50%
Rocking speed	30 rocks/min
Rocking angle	7°
Gas supplied	2-gas mix control (Air, CO_2 for pH control)
Gas flow	0.1 L/min

Titering

LV titres were determined using a hemocytometer based LacZ assay. Each sample was assayed in triplicate.

Methods

Table 3: Plasmid and PEI quantities used for transient transfection of bioreactor cultures

	1.4 L stirred-tank (µg)	3.75 L packed-bed run (µg)
pHIV-1-MPSvnls-LacZco	6530	17290
pcDNATat101ml	121	321
pHCMSRevmlwhvpre	121	321
pHCMVgagpolmllstwhvpre	77	203
pHCMV-G	303	803
PEI (25 kDA, linear)	21461	56820

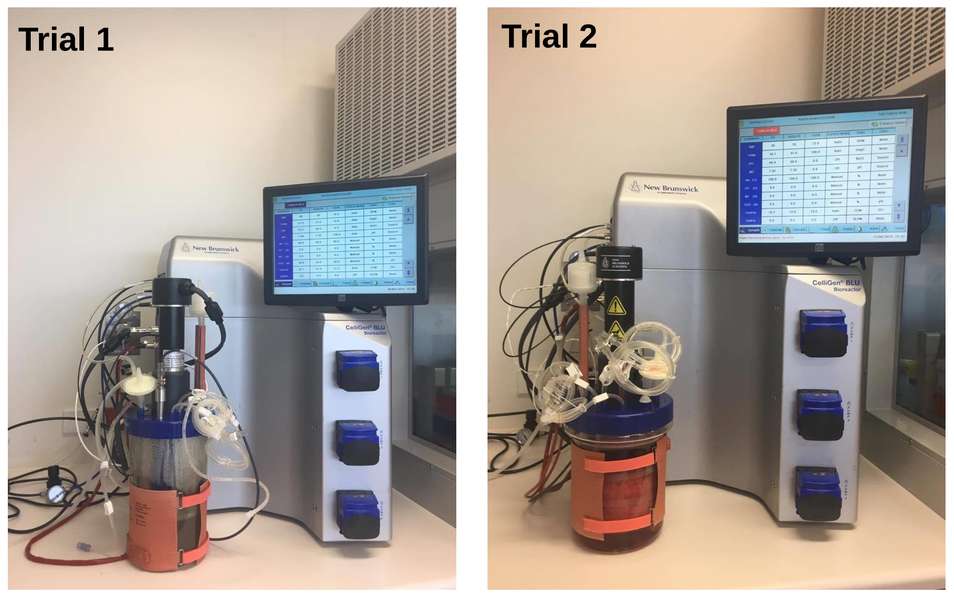

Figure 1: Bioreactor setup for each trial

Results

The titre of the unconcentrated LV supernatant from the stirred-tank trial was 2 x 10^4 TU/mL, while the packed-bed was 3 x 10^5 TU/mL (Figure 2), this compares to standard cell factory method where 10^5 – 10^6 TU/mL is typically achieved. When concentrated in 1 mL, the titre of the packed-bed preparation was 5 x 10^8 TU/mL. LV was not produced in the WAVE™ bioreactor due to insufficient cells for transfection.

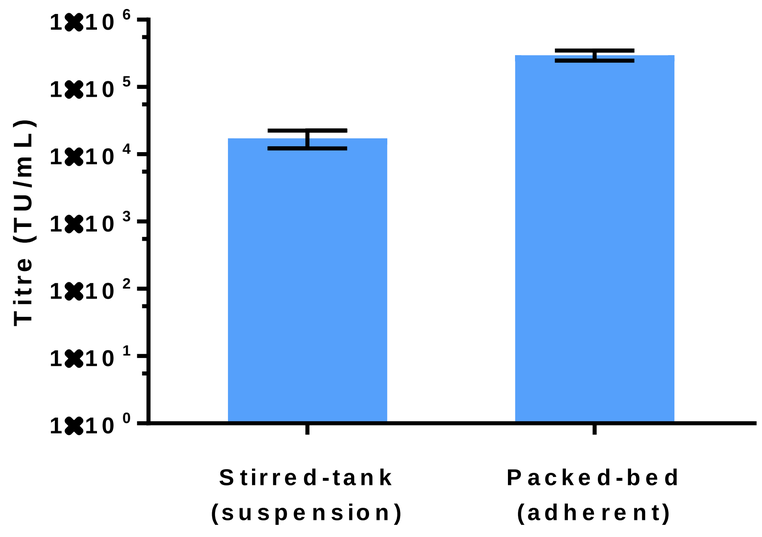

Figure 2: Unconcentrated titres from the stirred-tank and packed-bed runs. Data represents the mean of 3 technical replicates ± SD).

Conclusion

The WAVE™ is unlikely to be suitable for us at this stage due to poor cell growth. The stirred-tank and packed-bed approaches show potential for up-scaled LV production however, optimisation is necessary to maximise the capabilities of these systems.

Acknowledgments: Cure4CF Foundation, NHMRC and WCH Foundation.
Thanks to Eppendorf and GE Healthcare for providing trial equipment and support.