Transient lentiviral vector production using a packed-bed bioreactor system

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Introduction

- Lentiviral (LV) vectors are promising tools for gene and cell therapy applications.
- Two-dimensional culture technologies currently used for transient LV production cannot sustain growing market demands.
- Developing scalable LV production methods is imperative to translation of gene and cell therapies to the clinic.
- A packed-bed bioreactor system offers a potential scale-out solution for producing LVs.
- In this study a single-use packed-bed bioreactor vessel was successfully used to produce LVs.

Methods

Packed-bed bioreactor LV production

- HEK 293T cells were expanded in T-flasks to yield ~7 x 10^8 cells.
- Packed-bed vessel was operated in batch mode with 3 L volume.
- BioFlo® 320 control station (Eppendorf) was used to regulate temperature, agitation, pH, and dissolved oxygen (Table 1).
- Three-days post seeding the cells were transiently transfected.
- A 5-plasmid, second generation LV plasmid system consisting of an EF1α-3XFLAG-fLUC-F2A-eGFP vector and VSV-G envelope were used. Polyethylenimine (25 kDa, linear) to DNA ratio was 3:1.
- Viral supernatant was harvested at 48 and 72 hours post-transfection.
- Purification and concentration were performed using anion-exchange chromatography and ultracentrifugation.

Titering

- Functional titres were determined using flow cytometric detection of GFP-positive cells.
- Physical titres were determined using an ELISA for p24 HIV-1 capsid protein.

In vivo assessment of packed-bed produced vector

- Anaesthetised Sprague Dawley rats were intubated with a 16 G cannula to guide tracheal fluid delivery.
- Airways were conditioned with 200 µL of our standard 0.1% lysophosphatidylcholine (LPC).
- One hour following LPC, 250 µL (4 x 10^7 TU) LV was delivered.
- Bioluminescence imaging was performed one week following gene transfer (IVIS machine).

Table 1. Operational setpoints used for packed-bed bioreactor runs.

Operational parameter	Setpoint
Temperature	37 °C
Agitation	80 RPM
pH	7.2 (0.1 deadband)
Dissolved oxygen (DO)	50% air saturation
Gas (sparger)	Auto 3-gas mix control (Air, O2 and CO2)
Total gas flow rate	0.006 – 0.05 SLPM

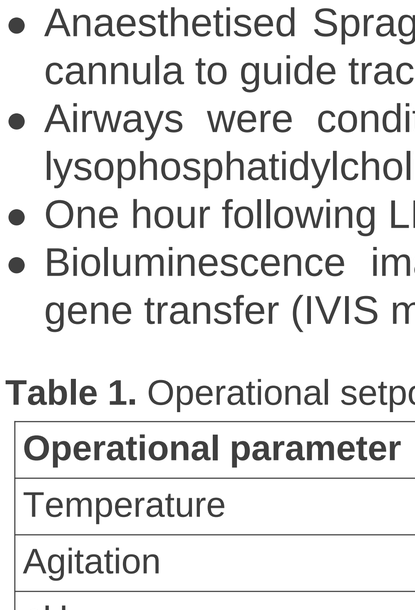

Figure 1: BioBLU® 5p single-use packed-bed bioreactor vessel. The vessel contains Fibra-Cel® disks, which provide a substrate for the attachment of cells. The total surface area of the vessel is 18 m^2.

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Results

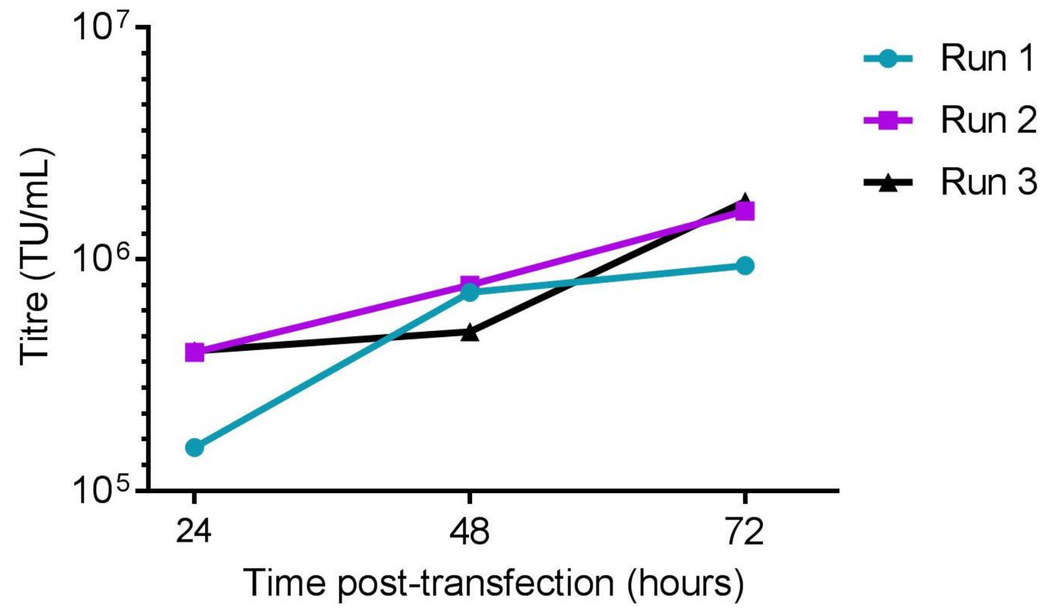

Figure 1. Functional titres (TU/mL) of unconcentrated LV supernatant collected at 24, 48 and 72-hours post-transfection.

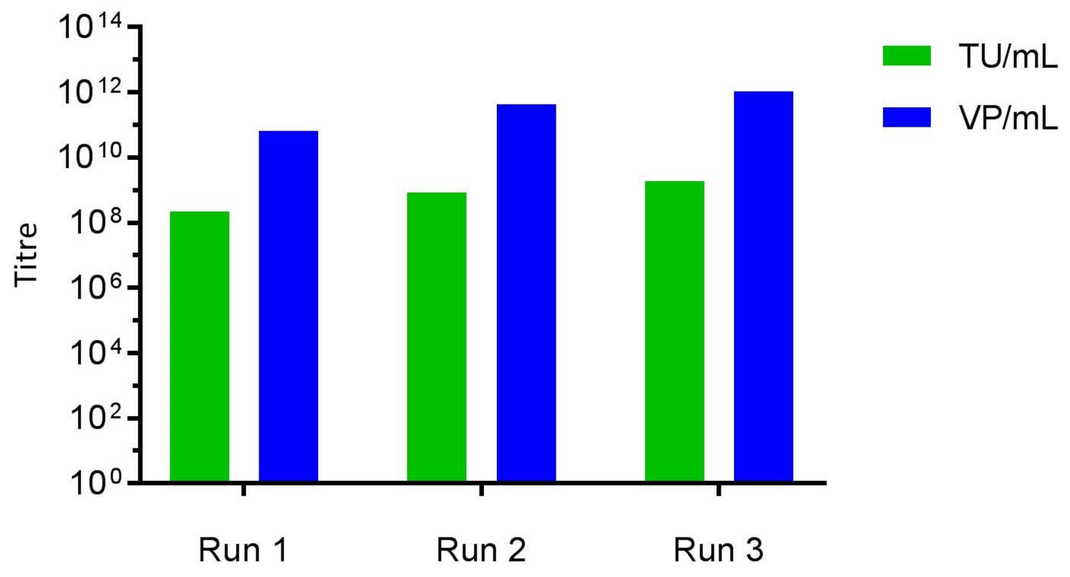

Figure 2: LV yields from each packed-bed run following purification and concentration. Both functional LV titres (TU/mL) and physical vector particles (VP/mL) are displayed.

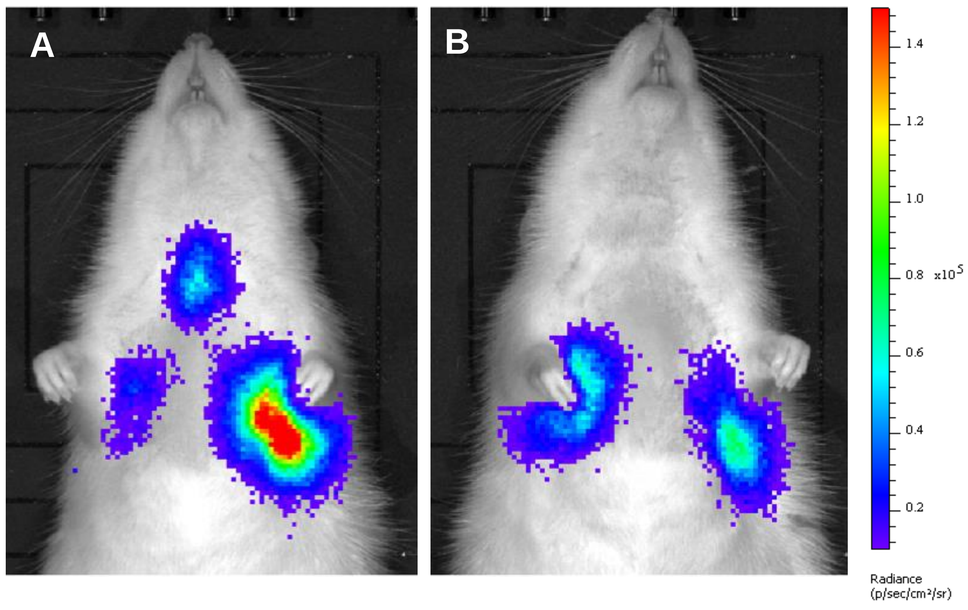

Figure 3: Airway gene transfer performed in rats to assess the *in vivo* efficacy of packed-bed produced LV. Rat **(A)** shows luciferase gene expression in the trachea and lungs, while rat **(B)** only shows expression in the lungs.

Conclusions

- LVs can be transiently produced using a packed-bed vessel.
- Unconcentrated titres in the 10^6 TU/mL range and concentrated titres of up to 10^9 TU/mL were attainted.
- Further optimisation of bioreactor conditions are expected to improve titres.
- A packed-bed system could offer a potential solution to meeting the ever-growing demands for scalable LV production.