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Transient Lentiviral Vector Production in HEK 293T Cells Using the BioFlo® 320 Control Station with a BioBLU® 5p Single-Use Packed-Bed Vessel

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Abstract

Lentiviral vectors (LVs), which are especially applicable to gene therapy, are promising vector types for the clinical trials of such treatments. Current bottlenecks in the production of LVs are caused mainly by the disadvantages of classical two-dimensional culture forms. Switching to bioreactors can eliminate those disadvantages and offer the benefits of process automation, tight regulation of production conditions, and reduced labor input. This application note describes the first successful experimental setup to cultivate LVs in HEK 293T cells adherently grown on Fibra-Cel® disks in a BioBLU 5p Single-Use Vessel. Functional unconcentrated titers (TU) between 10^5 - 10^6 TU/mL and physical vector particles (VP) of 10^8 - 10^9 VP/mL were achieved, with no significant difference in quality compared to production on conventional cell culture plates, demonstrating the great potential for LV production in stirred-tank bioreactors on Fibra-Cel disks.

Introduction

A well-established method to produce LVs is the transient transfection of adherent human embryonic kidney (HEK) 293T cells. LVs are gaining more and more attention as candidates for gene and cell therapies, resulting in an increasing demand. Multi-layer cell factories have often been used, but are limited by their poor scalability, high cost, labor intensiveness, and high batch-to-batch variability of production. Using bioreactors, most of the problems of the classical two-dimensional culture methods can be solved. Stirred-tank bioreactors allow us to optimize cell growth and achieve higher cell densities. To further increase the cell density, and, at the same time, protect cells from shear forces, they can be grown on Fibra-Cel disks [1]. Fibra-Cel disks are made of polypropylene and polyester non-woven fiber to which the cells adhere. Fibra-Cel has already been successfully used for the production of retroviral vectors in rocking bag bioreactors, and also in a packed-bed bioreactors [2, 3]. The packed-bed basket technology, developed by Eppendorf, provides a low-shear environment for the cultivation of animal cells to very high cell densities [4]. An Eppendorf BioBLU 5p single-use packed-bed bioreactor packed with 150 g of Fibra-Cel disks provides a growth surface area of 180,000 cm^2, which is the equivalent of 29 10-layer, stacked-plate, cell-culture vessels [5]. Instead of increasing the size of a bioreactor, scale-out strategies using multiple small bioreactors may be used to increase the production yield. Such systems have the advantage that the whole production lot is not lost if a single bioreactor is contaminated. Additionally, scaling-out (or scaling-in) offers greater flexibility when larger (or lesser) amounts of the product are needed.

This study demonstrates that LVs can be transiently produced in single-use BioBLU packed-bed bioreactor vessels equipped with Fibra-Cel disks. LV titers were analyzed and compared to classical cell factory vector production.

Material and Methods

Fig. 1: BioBLU 5p Single-Use Vessel packed with Fibra-Cel disks. Fibra-Cel disks serve as a growth matrix for the cells and provide a shear-free environment for the cultivation of animal cells to very high densities.

Bioreactor and control software

A BioFlo 320 control station was used together with a BioBLU 5p single-use packed-bed vessel with macrosparger for HEK 293T cell growth and parameter monitoring. Temperature, agitation, pH, and dissolved oxygen (DO) were measured throughout the run. DO was measured using a polarographic ISM® DO sensor (Mettler Toledo®, Switzerland) and the pH was measured with an optical pH sensor (Mettler Toledo). The program parameters are listed in Table 1. To optimize a DO concentration of ~50% the gassing parameters were manually adjusted subsequent to each run by increasing the maximum O_2 concentration while reducing the maximum air concentration.

Cell maintenance and inoculum preparation

HEK 293T cells were maintained in Dulbecco's modified Eagle medium (DMEM, Gibco®) supplemented with 10% fetal calf serum (FCS, Gibco) and 1% penicillin/streptomycin (PenStrep, Gibco) in a humidified incubator at 37 °C and 5% CO_2. Cells were initially expanded in T-75 and T-175 flasks for bioreactor inoculation. The number of viable cells were counted using a haemocytometer and trypan blue exclusion.

Cell culture in bioreactors

The three experimental trials were performed using the packed-bed vessel in batch mode. The BioBLU 5p single-use packed-bed vessel was inoculated with 4 x 10^3 cells/cm^2 to a working volume of 3.75 L.

Table 1: Operational parameter

Operational parameter	Setpoint
Temperature	37 °C
Agitation	80 rpm
pH	7.2 (0.1 deadband, directional control with CO2 only)
Dissolved oxygen (DO)	50 % air saturation
Gas (supplied by sparger)	Auto 3-gas mix control with manual limits (air, O_2 and CO_2)
Total gas flow rate	0.006 - 0.05 SLPM

Vector production in a cell factory

Cells were grown in a cell factory to a density of ~ 1.65 x 10^5 cells/cm^2 and transfected using calcium-phosphate co-precipitation and the same plasmid ratios as described below for the packed-bed bioreactor. The medium was changed to OptiPRO™ medium (1% PenStrep, 4 mM L-glutamine) eight hours post-transfection. Harvest and purification of the vector supernatant was conducted 48 hours post transfection. Concentration was performed as described below for the packed-bed bioreactor preparation.

Transient transfection of cells grown in the bioreactor

The cells were transiently transfected three days following seeding with a 5-plasmid, second generation LV plasmid system and polyethylenimine (PEI). A plasmid DNA to PEI ratio of 1:3 was used (4.77 mg DNA and 14.31 mg PEI). At eight hours post-transfection the medium was replaced with OptiPRO serum-free medium (Gibco) supplemented with 1% PenStrep and 4 mM L-glutamine.

The complete supernatant was harvested 48-hours post-transfection followed by a transfer of fresh 3 L OptiPRO medium into the vessel. The procedure was repeated 72-hours post-transfection. Residual cell debris was removed by centrifugation and filtration prior to analysis. The entire 6 L of vector supernatant was purified using a Mustang®Q XT5 anion-exchange column (PALL), followed by ultracentrifugation. LVs were additionally produced using a standard 10-layer cell factory method, as described above, to compare virus titers between the different cultivation methods.

Lentivirus titering
Functional titers were determined using flow cytometric detection of GFP-positive cells. The proportion of GFP-positive cells was used to calculate functional titers (TU/mL). Physical vector particles (VP)/mL were determined by measuring HIV-1 p24 capsid protein by ELISA (Abcam). Calculations were based on the previously reported estimation of 10,000 physical LV particles per 1 pg of p24.

Quality determination of the vector production methods
To compare the quality between both production methods, the VP/TU ratio of the supernatant was calculated for each preparation method. The lower the ratio, the lower the amount of non-functional particles in the preparation.

Results and Discussion

Scalability is a major hurdle in LV production for commercial gene and cell therapies. This study tested whether HEK 293T cells can be grown on Fibra-Cel disks in a stirred-tank bioreactor to successfully transfect the cells for lentiviral vector production. The cells were attached to the Fibra-Cel disks and could not, therefore, be counted directly. The steadily decreasing DO over the course of each run indicated an increase of cell biomass (Figure 2).

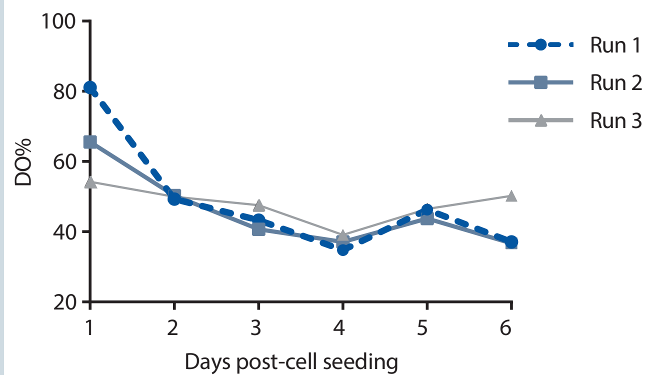

Fig. 2: Average daily DO readings followed six days post cell seeding .

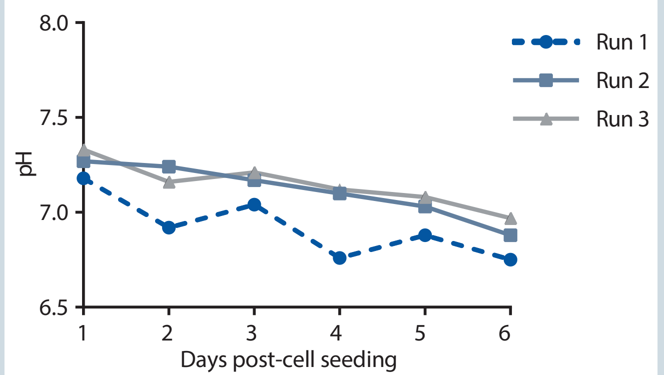

Fig. 3: Average daily optical pH readings followed six days post cell seeding.

The efficiency of the DO control over time increased with each run, due to the manual adjustment of the gassing parameters as mentioned in the methods section. The pH dropped slightly from 7.2 to an average of 6.9. CO_2 sparging was used to control the pH in the basic range, but no control (by the addition of a base) was performed in the acidic range (Fig. 3). Mildly acidic conditions have been shown to increase the expression of viral components and improve viral titers [6]. The unconcentrated functional LV titers were in a range of 10^5 – 10^6 TU/mL and physical particles in the range of 10^7 – 10^9 VP/mL (Fig. 4). The observed LV production was comparable to previously described methods, as indicated by an increasing titer over time, with peak production at 72 hours post-transfection. An average titer of 1.4 x 10^6 TU/mL and 4.3 x 10^8 VP/mL were achieved across the three runs. The average functional titer was 1 x 10^9 TU/mL, with an average particle number of 5.3 x 10^{11} VP/mL after purification (Fig.5).

In comparison to the standard 10-layer cell factory production method, the unconcentrated titers and physical titers were lower for the packed-bed bioreactor approach with 3.5 x 10^4 TU/mL vs. 4.5 x 10^5 TU/mL, and 1.0 x 10^7 VP/mL vs. 1.2 x 10^8 VP/mL, respectively. The lower cell concentration at the time of transfection in the bioreactor approach could be one explanation for the lower yield. Comparing the quality of the different preparation methods, determined by the VP/TU ratio (281 VP/TU compared to 266 VP/TU respectively), revealed a comparable quality for both methods (Fig. 6), which was additionally confirmed by *in vivo* analysis in rats (data not shown).

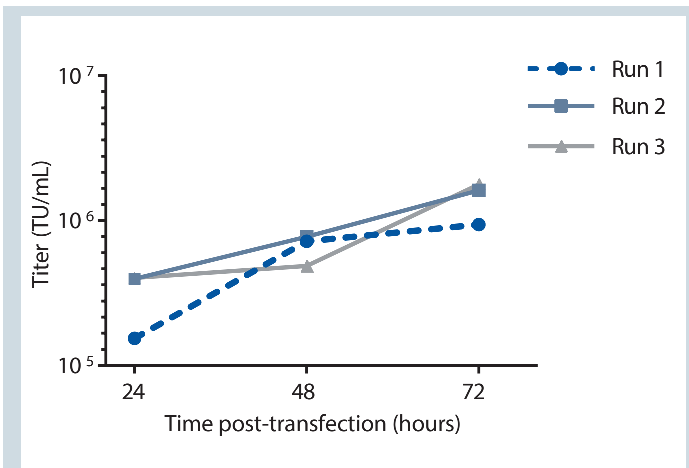

Fig. 4: Functional titres (TU/mL) of the unconcentrated LV supernatant determined by using flow cytometric detection of GFP-positive cells.

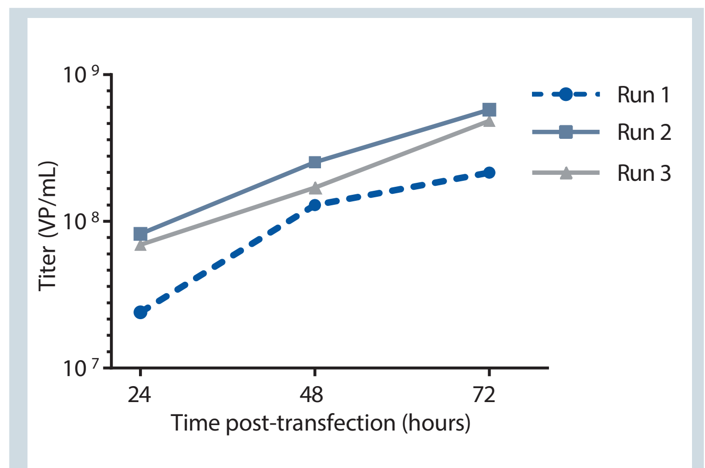

Fig. 5: Physical LV vector particles (VP/mL) of the unconcentrated LV supernatant determined by an enzyme-linked immunosorbent assay for HIV-1 p24 capsid protein.

Conclusion

This study demonstrated the great potential of the Eppendorf BioBLU 5p Single-Use Vessel pre-packed with Fibra-Cel disks in the production of LV. Due to the low cell densities at time of transfection, the achieved titers in this study were lower compared to the standard cell factory-based method with comparable quality. Keeping in mind that the substantially higher productivity potential of the bioreactor due to the 28-times greater surface area (18 m^2 vs. 0.632 m^2) was not exploited in this study, these are promising preliminary results. For future titer optimization, it may be necessary to optimize to higher cell densities prior to transfection. BioBLU 5p cell densities during the culture can be calculated based on previous published method using daily glucose consumption rate [7].

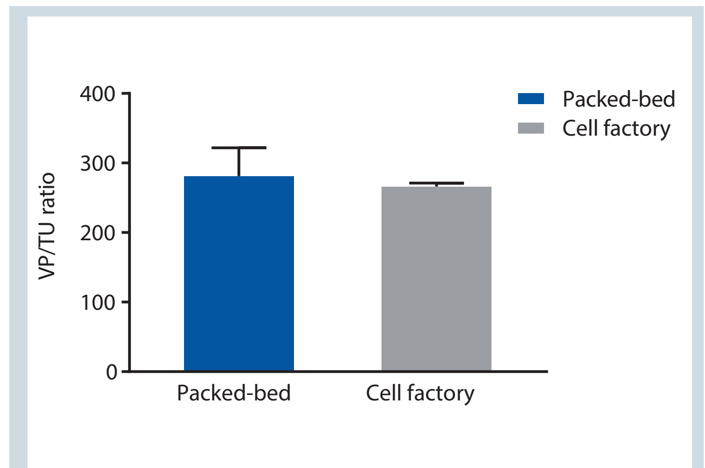

Fig. 6: The VP to TU ratio demonstrates comparable vector preparation quality between the packed-bed and cell factory production methods.

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Ordering information

Description	Order no.
BioFlo® 320, base control station	1379963011
Single-Use Vessel Bundle, for BioFlo® 320, for BioBLU® 5p	M1379-0323
Packed-bed Impeller Kit, for BioFlo® 320, 3 L	M1379-1141
Macrosparge	M1287-9476

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