CLOSED-SYSTEM MICROCARRIER-BASED MASTER CELL BANK GENERATION FOR LENTIVIRAL PRODUCTION

Javier Olmos Becerra[1,2] (Javier.olmos@scinus.com), Stella van Bergen [2] (s.d.van.Bergen@umcg.nl), Orsolya Frittmann[2] (o.frittmann@umcg.nl), Edwin Bremer[2] (e.bremer@umcg.nl) and Ruud Das[1] (ruud.das@scinus.com)

1. Scinus Cell Expansion Netherlands B.V., Bilthoven, Netherlands
2. Department of Hematology, University of Groningen, University Medical Center Groningen (UMCG), Groningen, Netherland

INTRODUCTION

HEK293T cells are widely used in biopharmaceutical production, particularly for viral vector manufacturing. We developed a Good Manufacturing Practice (GMP)-compliant, microcarrier-based process for generating a reproducible HEK293T Master Cell Bank (MCB) using a closed system bioreactor (Osilaris). The resulting cells were tested for stability over several passages and successfully produced lentiviral vectors, confirming their suitability for viral manufacturing in Point-of-Care (PoC) applications, particularly for CAR-T therapies.

METHODS AND MATERIALS

Cells were cultured in an Osilaris bioreactor on collagen-coated dissolvable microcarriers (9 cm^2/mL) for 15 days, starting with an initial seeding density of two million cells in 120 mL of medium. New microcarriers were added during culture to increase the available surface area through bead-to-bead transfer. General culture conditions are summarized in Table 1.

To evaluate the stability of the MCBs, cells were expanded over 10 passages in T-flasks under standard adherent culture conditions. A control group of cells from the original seed stock was expanded in parallel for growth comparison.

All conditions were transfected with Lenti-Helper plasmids using PEI Max.

After 48 hours, the lentivirus-containing supernatant was harvested to transduce Jurkat cells. Transfection efficiency was evaluated by flow cytometry. Transduction efficiency was assessed through physical and infectious titers: physical titer quantified by qPCR, and infectious titer determined by flow cytometry ,based on GFP-positive Jurkat cells.

Table 1 : *Overview of Cell Culture Conditions in the Osilaris bioreactor*

General Osilaris settings	
DO control	75%
pH control	7.3
Rocking angle	90 °
Rocking velocity	90 °/s
Vertical hold	10 seconds
Rocking time	15 minutes
Horizontal hold	2 hours

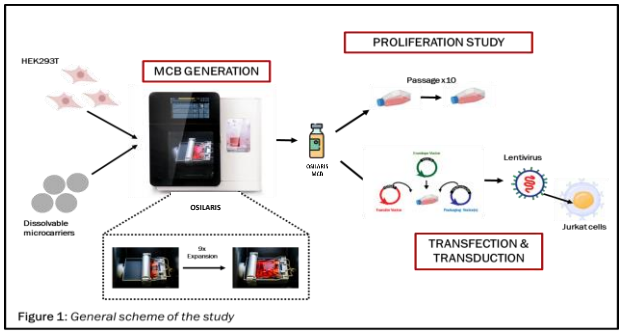

Figure 1: *General scheme of the study*

RESULTS

Robust cell yields and viabilities in the Osilaris bioreactor

Over 1 billion cells were harvested in all the runs, with yields ranging from 1.1 to 1.4 billion cells and viabilities between 80.0 % and 93.4%, demonstrating the system's ability to produce high-density cultures in two weeks (Figure 2).

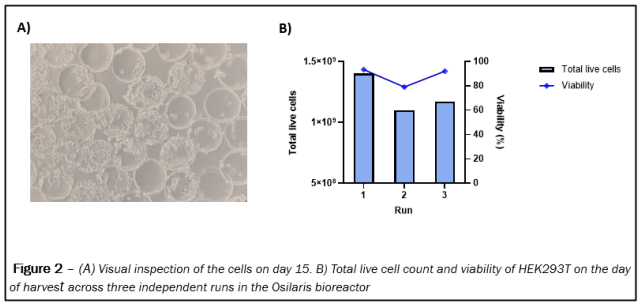

Figure 2 – *(A) Visual inspection of the cells on day 15. B) Total live cell count and viability of HEK293T on the day of harvest across three independent runs in the Osilaris bioreactor*

Comparable expansion rates in Osilaris and control cultures

Cells from all four culture conditions showed consistent logarithmic cell expansion over 10 passages (Figure 3A). Population Doubling Times (Figure 3B) showed no statistically significant differences ($p > 0.05$), indicating comparable proliferative capacity.

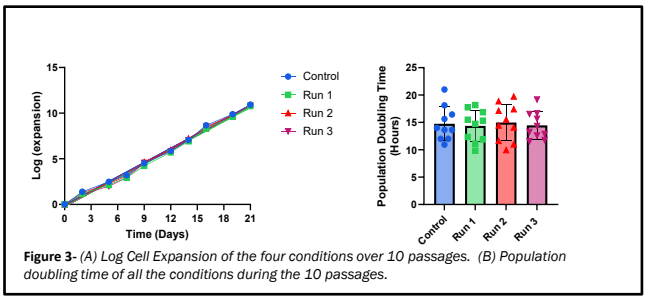

Figure 3- *(A) Log Cell Expansion of the four conditions over 10 passages. (B) Population doubling time of all the conditions during the 10 passages.*

High transfection and lentiviral production across Osilaris runs

Transfection efficiency (Figure 4A), assessed by GFP expression, remained consistently high across all groups (92% to 95%) with no significant differences. Infectious titers (Figure 4B) ranged from 8.7×10^6 to 1.3×10^7 Transducing Units per mL (TU/mL), and physical titers (Figure 4C) ranged from ~1.5×10^8 to 2.0×10^8 Viral Particles per mL (VP/mL). While some variation was observed, none of the differences between groups reached statistical significance. These results demonstrate that the Osilaris bioreactor system supports the generation of HEK293T cells that can be efficiently transfected and produce lentivirus at levels comparable to the standard methods.

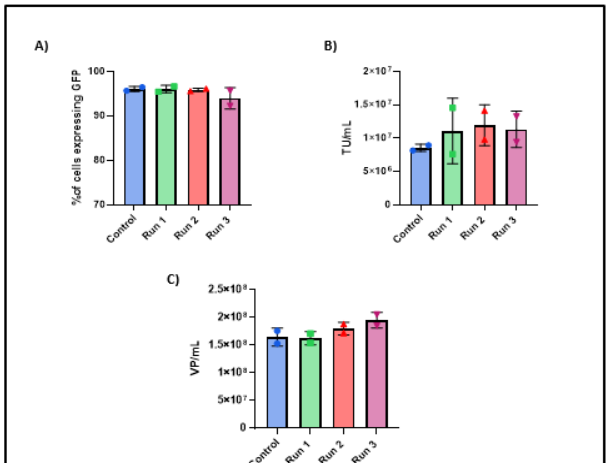

Figure 4- *(A) Transfection efficiency of cells derived from the control group and the three independent Osilaris bioreactor runs. (B) Infectious titer of the lentivirus produced in cells derived from the four conditions. (C) Physical titer of the lentivirus produced in cells derived from the four conditions.*

CONCLUDING REMARKS

This study confirms that the Osilaris bioreactor yields viable, proliferative, and transfectable HEK293T cells, supporting PoC manufacturing. Further testing will ensure MCB quality compliance. The process will now be implemented in GMP manufacturing for clinical use.

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