Towards Integrated HEK293T Expansion and Transfection in the Osilaris Bioreactor for Lentiviral Vector Manufacturing

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INTRODUCTION

Current viral vector manufacturing still relies on traditional 2D monolayer cultures, which are insufficient to meet the growing demand in cell and gene therapy. 2D culture is expensive and difficult to scale, requires extensive manual handling, and involves open steps that increase contamination risk and process variability.

To address these limitations, we developed a novel microcarrier-based HEK293T expansion protocol in the Osilaris™ bioreactor platform (Figure 1). This approach enables cell growth within a closed, automated environment, reducing handling while supporting scalable and reproducible processing. This study compares the performance of the Osilaris bioreactor with conventional spinner flask and 2D monolayer cultures, while also assessing its potential to integrate transfection within the same platform as a step towards more streamlined lentiviral vector manufacturing.

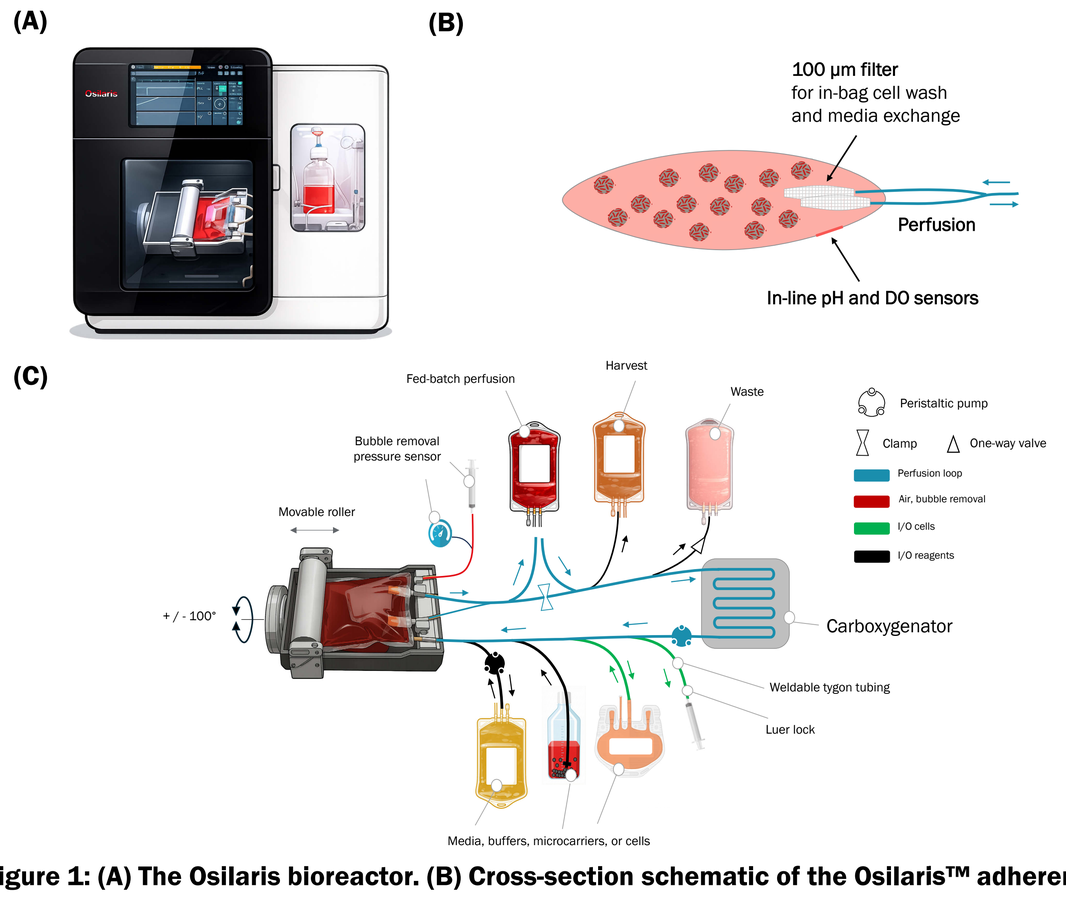

Figure 1: (A) The Osilaris bioreactor. (B) Cross-section schematic of the Osilaris™ adherent culture bag with microcarriers. (C) Schematic of the fluidic pathway in the Osilaris cabinet.

MATERIALS AND METHODS

Figure 2 summarizes the *in-situ* expansion strategy. Lenti-X-293T cells were seeded on Cytodex 1 microcarriers (3 g/L) at 2 × 10^4 cells/cm² in 10% FBS-supplemented medium and cultured in the Osilaris bioreactor (n = 2) at 200 mL under 90° rocking at 90°/s, with spinner cultures run as controls. After 6 days, cells were dissociated using 100 mL Hyrtryp, fresh microcarriers were added, and cultures were expanded to a final volume of 1.2 L until day 11.

To evaluate transfection, separate bioreactor and spinner cultures were seeded at 5 × 10^4 cells/cm² and transiently transfected with a GFP plasmid using Transporter 5 (4:1 ratio). GFP expression was assessed by flow cytometry 48 h post-transfection.

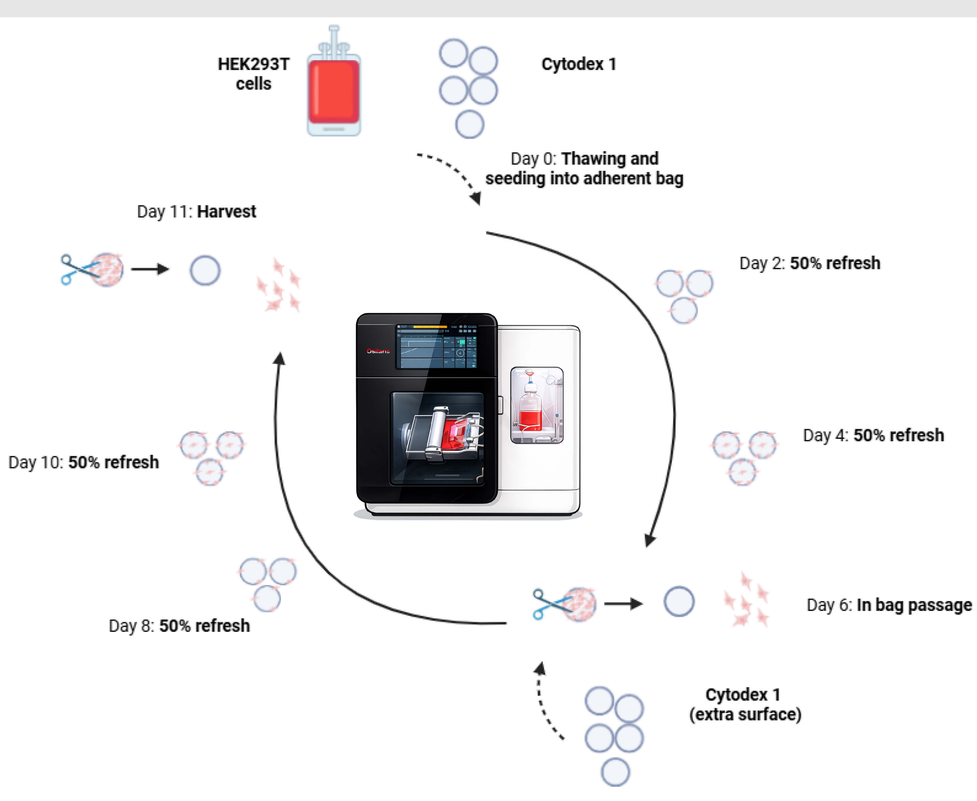

Figure 2: Process overview of HEK293T expansion in the Osilaris bioreactor. HEK293T cells are thawed and directly seeded into an adherent bag containing Cytodex 1 microcarriers. Cells attach and proliferate on the microcarriers, with media refreshed every 2 days. On day 6, in-bag dissociation is performed via the bag's tubing system: the medium is replaced with Hyrtryp to detach cells from the microcarriers, followed by the addition of fresh microcarriers to enable reseeding onto an expanded surface area. By day 11, the expanded HEK293T cells are harvested for downstream analysis.

RESULTS

Full microcarrier coverage was achieved by day 11 (Figure 3A), with a mean cell density of ~2.9 × 10^6 cells/mL (Figure 3B), demonstrating comparable expansion performance to spinner flasks. Notably, due to the scalability of the system, the Osilaris bioreactor enabled a total cell yield exceeding 3.5 × 10^9 cells, while maintaining a closed process without open handling steps.

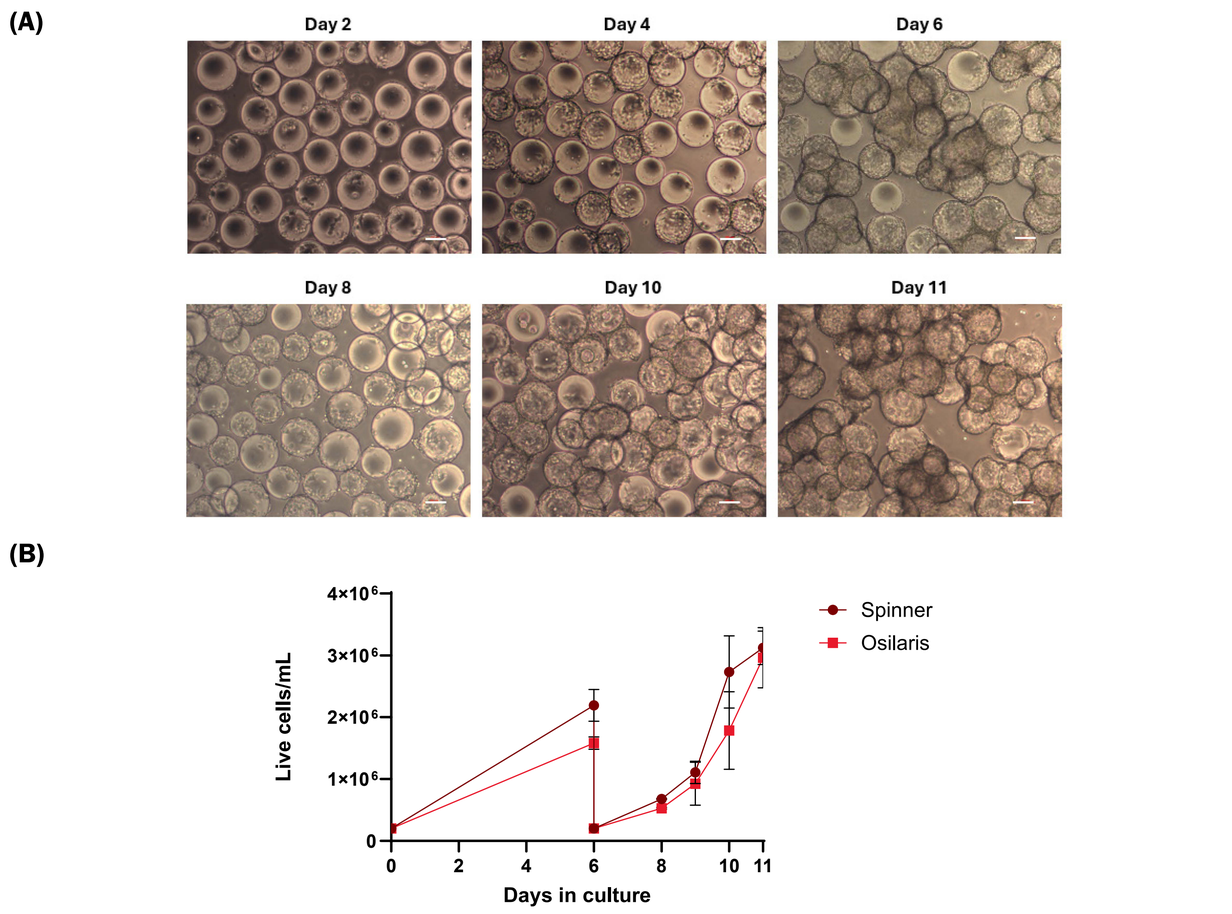

Figure 3: (A) Representative images of HEK293T cells cultured on Cytodex 1 microcarriers in the Osilaris bioreactor over an 11-day expansion (scale bar: 100 µm). (B) Live cell density of HEK293T cultures in the Osilaris bioreactor and spinner flasks throughout the 11-day culture (N=2, n=2, error bars represent ± SD).

Transfection and GFP expression

At 48 h post-transfection, HEK293T cells from the Osilaris bioreactor, spinner flasks, and monolayer control were harvested, and GFP expression was assessed by flow cytometry (Figure 4). Monolayer cultures showed the highest transfection efficiency. Microcarrier-based systems, the spinner flasks and Osilaris bioreactor, exhibited moderate and comparable levels of GFP expression, demonstrating similar performance between the two platforms.

These results establish a proof of concept for transfection within the Osilaris system, showing similar outcomes to conventional spinner flasks while enabling operation in a closed, scalable, and automated system. This provides a clear advantage for GMP-compliant manufacturing by reducing contamination risk and supporting the integration of expansion and transfection in a single platform.

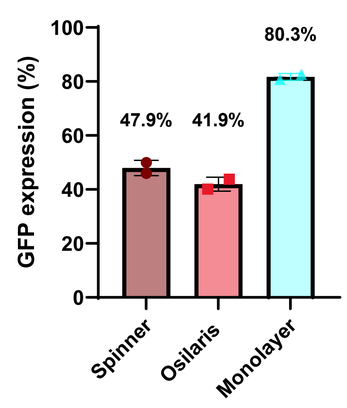

Figure 4: Comparison of GFP expression in HEK293T cells transfected in monolayer, spinner, and Osilaris bioreactor (N = 1, n = 2; error bars represent ± SD)

CONCLUDING REMARKS

This study demonstrates a microcarrier-based HEK293T expansion strategy in the Osilaris bioreactor, featuring a streamlined harvest and reseeding approach that enables robust, high-yield expansion while reducing manual handling in a closed and scalable system. By maintaining cells on microcarriers throughout the process, the workflow is simplified, improving process efficiency and consistency. The ability to perform both expansion and transfection within the same platform paves the way for a more streamlined manufacturing by reducing process complexity, minimizing open handling steps, and improving overall scalability and reproducibility. Together, these results position the Osilaris bioreactor as a GMP-compatible and efficient alternative to conventional systems, with future work focused on further optimization and advancing toward integrated, end-to-end lentiviral vector production workflows.

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