UNLOCKING OPTIMAL CONDITIONS FOR HEK293T CELL EXPANSION USING SCINUS BIOREACTOR TECHNOLOGY

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INTRODUCTION

HEK293T cells are a versatile cell line that can, among others, be used a producer cells for lentiviral vectors (LV) for cell and gene therapy. In particular, HEK293T can be used for LV production for Chimeric Antigen-Receptor T cell (CAR-T) therapy. Innovations in CAR-T therapy might include those that facilitate point-of-care production, instead of logistically challenging centralized production. Microcarriers are an appealing culture substrate due to their efficient surface-to-volume ratio, but efficient HEK293T culture in agitation systems is challenging because of their sensitivity to shear stresses. In this study we explore the ability to employ microcarrier-based HEK293t cultivation for use in closed bioreactor technology (SCINUS bioreactor) for point-of-care LV production.

METHODS AND MATERIALS

Design-of-experiments for HEK293 culture on Cytodex-1

A bag-in-bag system was developed, to test multiple conditions with the agitation approach of the SCINUS system. A two-level factorial design was created for three parameters: rocking speed, seeding density and static pause. PDT (day 1-3) was used as a response.

	Low	Center	High
Rocking speed (deg/sec)	60	190	320
Seeding density (cells/mL)	5×10^4	1.25×10^5	2×10^5
Static interval (H)	1	2.5	4

Expansion and bead to bead transfer

The ability of HEK293 cells to proliferate under agitated conditions and subsequently display bead-to-bead transfer was investigated using two different microcarrier cultures in spinners. Cells were seeded at various seeding densities (50 – 200k cells/mL). Volume and microcarrier content were increased 9-fold on day 3. Growth and bead-to-bead transfer were then assessed on day 7.

SCINUS culture and transfectability test

HEK293T cells were culture in the SCINUS bioreactor using LDC-DMC. After 8 days of culture, cells were harvested and then seeded for transfection. Transfectability of the cells was evaluated by using a GFP plasmid and then examining the expression through flow cytometry.

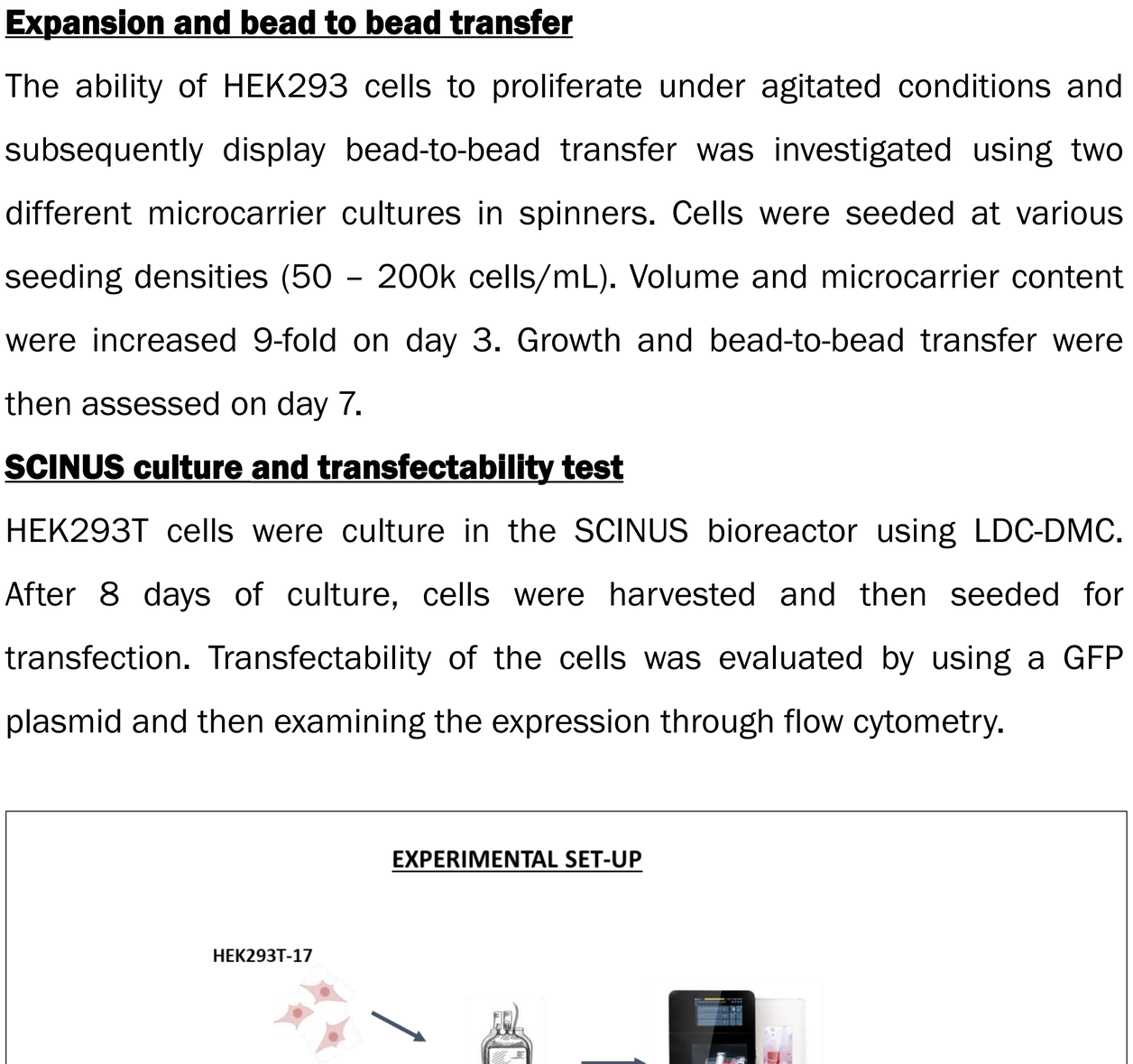

Figure 1 – *General scheme of the experiments.*

RESULTS

DoE experiment

No significant interaction was found between the studied parameters. Response surface models (Figure 2) indicated that a lower seeding density (p=0.0002) and a lower initial static pause (p=0.0162) resulted in faster cell growth. Distribution was comparable with the spinner cultures (Figure 3)

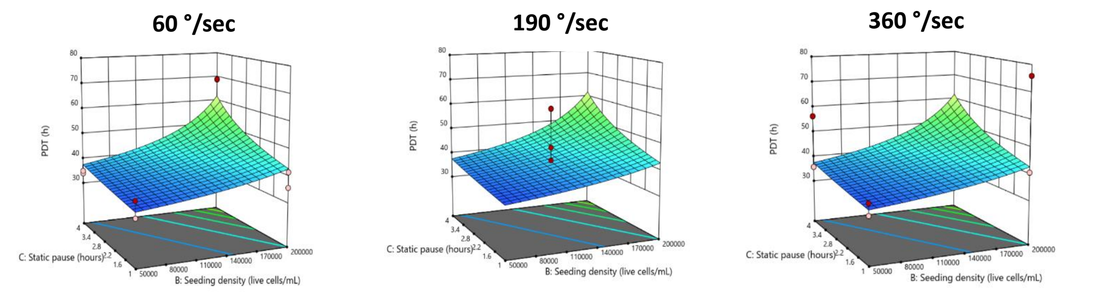

Figure 2 – *Response surface model for the three different rocking speeds.*

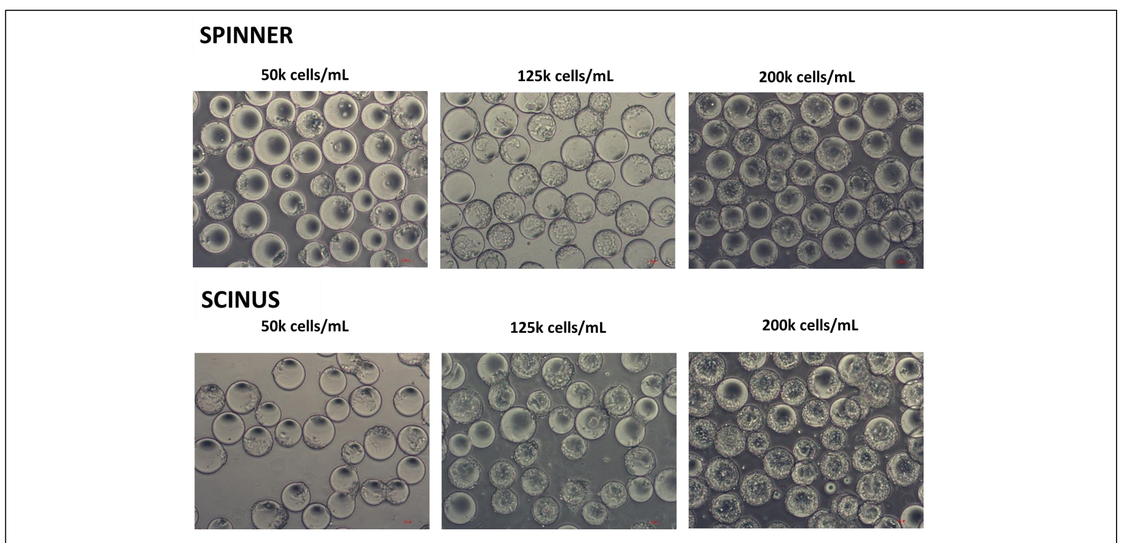

Figure 3 – *Visual inspections at day 3 of HEK293T cells on Cytodex 1 (3 g/L) cultured at different seeding densities using spinners and the Scinus bioreactor.*

Bead-to-bead transfer

Both microcarriers supported the growth of HEK293T cells, but Cytodex1 showed clumped growth on old microcarriers, while in LDC-DMC cells were well distributed, showing bead-to-bead transfer (Figure 4).

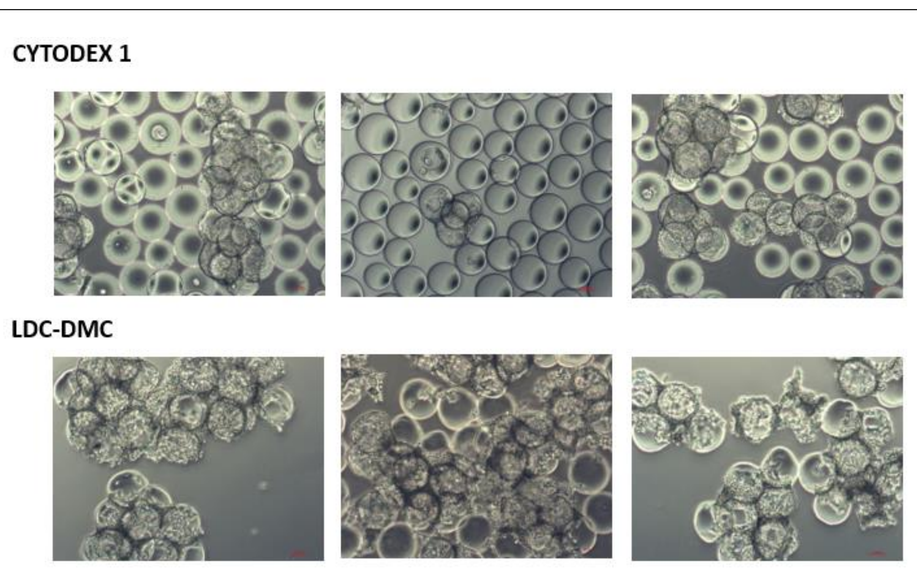

Figure 4 – *Visual inspections at day 7 (harvest day) of HEK293T-17 on cytodex1 and LDC-DMC.*

Expansion in SCINUS and transfection

HEK293T-17 cells demonstrated the ability to proliferate in the SCINUS bioreactor, obtaining >800 million cells in 8 days. Aggregation was observed (Figure 5A) but harvested and transfected cells showed high level of GFP expression (Figure 5B), comparable to the monolayer control (p=0.089) .

Figure 5 – *(A) Visual inspections at day 8(harvest day). (B) GFP expression levels after 48h.*

CONCLUDING REMARKS

This study highlights the integration of microcarrier-based HEK293T culture into the SCINUS bioreactor, laying the groundwork for point-of-care lentiviral vector production for advanced therapies, such as CAR-T cell therapy.

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