Cellevat3d™ nanofiber-based microcarriers designed for improved upstream productivity of viral vector biomanufacturing

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Background and Aim

Gene therapy and viral vector manufacturing address important medical needs while challenged with high manufacturing costs [1,2]. Hence, the development of innovative cell culture techniques is vital for improving viral vector productivity, ensuring scalability while upholding high product yield, quality and process robustness. Adherent cell culture systems known for their ability to produce high yields of viral vectors are associated with scalability challenges [2,3]. To overcome this issue, microcarrier based cell culture systems offer opportunities for large scale anchorage-dependent adeno-associated virus (AAV) production [4,5]. In this regards, Cellevat3d™ proprietary nanofiber-based microcarriers, represent a novel and sustainable product format providing three-dimensional (3D) culture of adherent cells readily scalable in stirred tank (suspension) bioreactor systems. Such microcarriers offer promising avenues for scaling-up of adherent cells and improving viral vector productivity.

Material and Methods

HEK293T cells were seeded on Cellevat3d™ nanofiber-based microcarriers in Erlenmeyer flasks (E125) with an inoculum density of $1.5x10^5$ cells/ml in a working volume of 20 ml. Cell growth studies were performed for 72h at 37°C, 5% CO_2. Plasmid transfection (Helper, RC2 and GFP) for production of human AAV serotype 2 (AAV2) vectors was performed during inoculum of HEK293T cells ($1x10^6$ cells/ml) on Cellevat3d™ nanofiber-based microcarriers in Erlenmeyer flasks (E125, 20 ml working volume), using polyethylenimine (PEI) as a transfection agent. Cells were incubated at 37°C, 5% CO_2 for 72h before proceeding to virus harvest from cell lysates. Conventional transfection of HEK293T cells in two-dimensional (2D) format was conducted as a parallel comparison and using the same number of cells. Analysis of transfection efficiency was performed by flow cytometry and microscopy, while viral titers were assessed using quantitative polymerase chain reaction (qPCR) analysis.

Results

Data showed that HEK293T cells adhering to the Cellevat3d™ nanofiberbased microcarriers establish homogeneous 3D cultures exhibiting high cell viability (>95%) and viable cell density in 72h (Figure 1 and 2). Additionally, data demonstrated reproducibility of four independent microcarrier batches produced at different timepoints during one year.

Transfection of adherent HEK293T cells on Cellevat3d™ nanofiber-based microcarriers showed high (>90%) transfection efficiency (Figure 3) and yielded high volumetric productivity (10^{14} vg/L) of AAV2 vectors (Figure 4). Compared to 2D culture (T-flask) and current standard microcarriers, Cellevat3d™ nanofiber-based microcarriers exhibited higher product yield and a two-fold increase in specific productivity (vg/cell), respectively.

Cellevat3d™ nanofiber-based microcarriers for 3D cell culturing

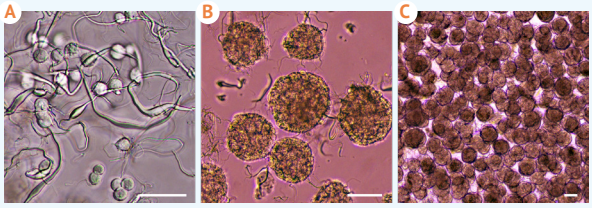

Figure 1. Bright field microscope images of HEK293T cells adhering to Cellevat3d™ nanofiber microcarriers at inoculum and growing as homogeneous 3D spheroids shown after 72h in culture. Scale bars: 50 µm A and 100 µm B C.

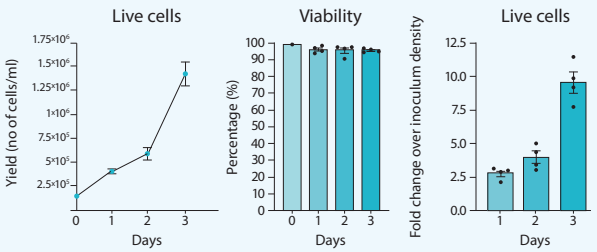

Figure 2. HEK293T cell growth on Cellevat3d™ nanofiber-based microcarriers presented for four independent microcarrier batches produced over 1 year. Data are presented as mean ± SEM.

Results continue >>

AAV2 vector production using HEK293T cells on Cellevat3d™ nanofiber-based microcarriers

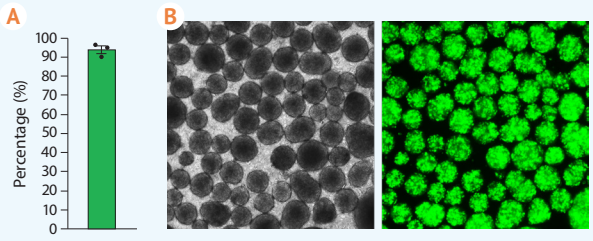

Figure 3. Transfection of HEK293T cells on Cellevat3d™ nanofiber-based microcarriers. Transfection efficiency (%) measured by flow cytometry A, and bright field and fluorescence microscope images images of GFP-transfected cells 72h post transfection B. Data are presented as mean ± SEM.

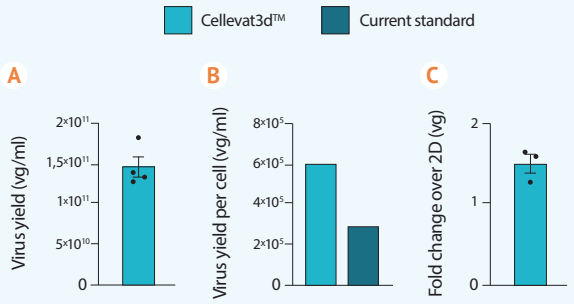

Figure 4. Volumetric productivity of AAV2 using Cellevat3d™ nanofiber-based microcarriers. Obtained virus titer of $1.5x10^{11}$ vg per ml of cell culture measured by qPCR A. Comparison to published data of current standard microcarriers showed two-fold increased productivity per cell B. Increased product yield (1.5-fold) over parallel AAV2 production using 2D culture (T-flask) with same number of cells C. Data are presented as mean ± SEM. Each data point represents a biological replicate.

Conclusions

Cellevat3d™ nanofiber-based microcarriers provide a sustainable upstream bioprocessing solution establishing scalable and homogeneous 3D cell cultures. The microcarriers' large surface area and lightweight nature allow for achieving high cell densities and reduced shear forces in bioreactors. Importantly, our data demonstrate high transfection efficacy and volumetric productivity of AAV2 vectors using Cellevat3d™ nanofiber-based microcarriers. These findings support the potential of Cellevat3d™ nanofiber-based microcarriers as a scalable platform for adherent cell cultures in gene therapy applications. Advancing bioprocessing strategies to enhance yield, reduce time, and lower costs improves upstream bioprocessing productivity, thereby increasing accessibility to these costly therapeutics. Cellevat3d™ nanofiber-based microcarriers will be available from November 2024.

References

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