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Cellevat3d® nanofiber microcarriers designed for improved upstream productivity of viral vector biomanufacturing

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Background and Aim

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

Material and Methods

HEK293T cells were seeded on Cellevat3d® nanofiber microcarriers in shake flasks or spinner flasks with an inoculum density of 1.5x10^5 cells/ml. Cell growth studies were performed for 72-96h at 37°C, 5% CO_2. Plasmid transfection (Helper, RC and ZsGreen) for production of human AAV serotype 2 (AAV2) vectors was performed during the inoculum of HEK293T cells (1x10^6 cells/ml) on Cellevat3d® nanofiber microcarriers in shake flasks (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 both cell lysates and supernatants.

Conventional transfection of HEK293T cells in two-dimensional (2D) format was conducted as a parallel comparison and using the same number of cells and culture volume. Analysis of transfection efficiency was performed by flow cytometry and microscopy, while viral titers were assessed using quantitative polymerase chain reaction (qPCR) analysis. In a benchmarking study for the production of human AAV serotype 9 (AAV9) vectors, Cellevat3d® nanofiber microcarriers were compared with current standard microcarriers in parallel experiments using the same transfection mixture. A total of 20x10^6 HEK292T cells were transfected with plasmids (Helper, RC, EGFP) either at inoculum for nanofiber microcarriers or after 24h of cell growth on standard microcarriers beads according to manufacturer's protocol, using PEI as a transfection agent. Viruses were harvested from cell lysates 72h post-transfection and the AAV9 particles were passed through 0.45 µm filters to remove cell debris and fibers/beads, prior to digital droplet PCR analysis.

Results I

Data showed that HEK293T cells adhering to the Cellevat3d® nanofiber microcarriers establish homogeneous 3D cultures exhibiting high cell viability (>95%) and viable cell density in 72h, with a 13-fold change over seeding density **(Figures 1A,B).** The scalability of the microcarriers from milliliter to liter scale demonstrated consistent cell growth curves, achieving yields of up to 3x10^6 cells/ml **(Figure 1C).** Additionally, the generated spheroids increased in size over time while reaching an average diameter of 179 µm after four days of culture **(Figure 1D).**

Cellevat3d® nanofiber microcarriers for 3D cell culturing and scale-up

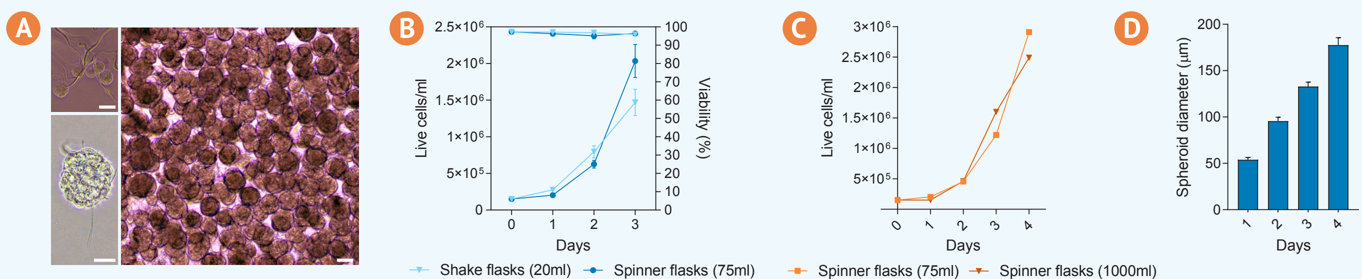

Figure 1. HEK293T cell growth on Cellevat3d® nanofiber microcarriers in different culture formats and volumes. A Bright field microscope pictures of HEK293T cells adhering to nanofiber microcarriers at inoculum and growing as homogeneous 3D spheroids shown after 72h in culture. Scale bars 25 µm (left top), 50 µm (left bottom) and 100 µm (right). B Cell growth curves displaying a similar growth trend in two different cell culture formats reaching a viable yield up to 2x10^6 cells/ml in 3 days, while retaining viability above 95% throughout the culture. C Scalability from ml to L scale in spinner flasks with retained cell growth curves reaching a yield up to 3x10^6 cells/ml in 4 days. D Nanofiber microcarrier cultures generate 3D spheroids that gradually increase in size over time reaching an average diameter of 179±8 µm in 4 days (n=50 measurements per timepoint). Data are presented as either mean or mean ± SEM.

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Viral vector production using Cellevat3d® nanofiber microcarriers

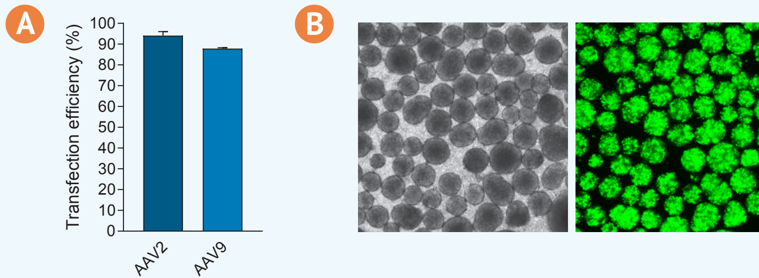

Figure 2. Transient transfection of HEK293T cells on Cellevat3d® nanofiber microcarriers. (A) Plasmid transfection efficiency (%) measured by flow cytometry for AAV2 and AAV9 (n=3-6 biological replicates). (B) Fluorescence microscope images of ZsGreen-expressing transfected 3D spheroids at 72h post-transfection. Data are presented as mean ± SEM.

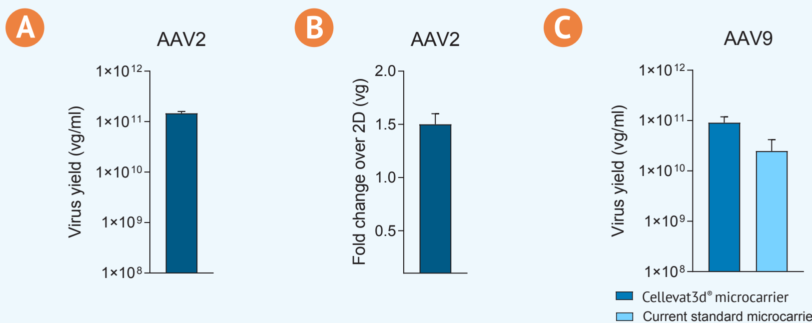

Figure 3. Volumetric productivity of AAV vectors using Cellevat3d® nanofiber microcarriers. (A) AAV2 titer of $1.5x10^{11}$ vg/ml measured by qPCR (n=4 biological replicates) from 20ml culture. (B) Increased product yield (1.5-fold) over parallel AAV2 production using 2D (culture flasks) with same number of cells. (C) Benchmarking data showed 2.7-fold increased in AAV9 yield when using Cellevat3d® nanofiber microcarriers compared to current standard microcarriers (n=5-6 biological replicates). Data are presented as mean ± SEM.

Results II

Transient plasmid transfection of adherent HEK293T cells on Cellevat3d® nanofiber microcarriers showed high transfection efficiency (88–94%) for both AAV2 and AAV9 **(Figure 2A,B).** AAV2 vectors yielded high volumetric productivity (10^{14} vg/L) **(Figure 3A)** and exhibited higher product yield compared to parallel 2D cultures **(Figure 3B).** Latest benchmarking results performed by UCL, London, UK showed 2.7-fold increase of AAV9 vector yield for Cellevat3d® nanofiber microcarriers compared to current standard microcarriers **(Figure 3C).**

Conclusions

Cellevat3d® nanofiber microcarriers provide a sustainable and scalable solution for upstream bioprocessing, enabling homogeneous 3D cell cultures with high product yields. The large surface area and lightweight nature of these microcarriers facilitate high cell densities and reduced shear forces in bioreactors. Our data demonstrate high transfection efficacy and volumetric productivity of AAV vectors using Cellevat3d® nanofiber microcarriers. Most importantly, benchmarking results showed 2.7-fold increase of AAV9 vector yield for Cellevat3d® nanofiber microcarriers compared to current standard microcarriers. These findings support the potential of Cellevat3d® nanofiber microcarriers as a scalable platform for adherent cell cultures in gene therapy applications, advancing bioprocessing strategies to enhance yield, reduce costs, and thereby increase accessibility to gene therapeutics. Cellevat3d® nanofiber microcarriers will be available from November 2024.

References

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